Targeted therapy in oncology: mechanisms and toxicity

Neeltje Steeghs

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General introduction and outline of the thesis

Cancer is one of the leading causes of death in developed countries, responsible for about 25% of all deaths. On a yearly basis, 0.5% of the population is diagnosed with cancer. Treatment options include surgery, radiotherapy and systemic therapies such as chemotherapy, endocrine therapy and targeted agents. These targeted anti cancer therapies include monoclonal antibodies and small molecules, for example tyrosine kinase inhibitors.

Conventional chemotherapeutical agents act by creating toxic effects on all dividing cells. This frequently results in in severe damage of normal tissues leading to side effects like myelosuppression, alopecia, and gastrointestinal problems. The optimum goal is to find a treatment modality that specifically kills malignant cells and causes little or no side effects.

This thesis focuses on targeted anticancer agents. An important class of these agents are the tyrosine kinase inhibitors (TKIs). One of the first steps in TKI treatment development is defining whether a specific type of cancer, for example the sarcomas in chapter 3 of this thesis, express the receptors that are targeted. Once a TKI is developed, phase I studies are conducted to characterize the safety and side effects of the drug when administered to patients. When relevant side effects emerge, studies investigating the underlying mechanisms leading to these side effects are called for. Also pharmacogenetic studies can be performed to investigate whether certain heritable genetic variations influence efficacy or safety of the drug. After the phase I studies have proven the drug to be safe, the drug can be further developed. This includes the investigation of the TKI when combined with other anticancer agents. Items of all the described steps in TKI development are described in this thesis.

In **Chapter 2,** recent developments of small molecule TKIs in the treatment of solid tumors are reviewed. These therapies were developed to target key elements that play a role in tumor development and tumor growth. Hormonal therapy in breast cancer is probably the oldest targeted therapy known in oncology. A more recent discovery is the class of drugs designated as tyrosine kinase inhibitors, developed to block intracellular signaling pathways in tumor cells, leading to dysregulation of key cell functions such as proliferation and differentiation.

In this chapter the following TKIs are reviewed: imatinib (Gleevec®/Glivec®), gefitinib (Iressa®), erlotinib (OSI-774, Tarceva®), lapatinib (GW-572016, Tykerb®, Tyverb®), canertinib (CI-1033), sunitinib (SU 11248, Sutent®), vandetanib (ZD6474, Zactima®), vatalanib (PTK787/ZK 222584), sorafenib (Bay 43-9006, Nexavar®), and Leflunomide (SU101, Arava®). Clinical studies with these new targeted agents in a wide range of tumor types and their future role in anticancer treatment is discussed.

Overexpression of the epidermal growth factor receptors EGFR and ERBB2 (Her2neu) is a negative prognostic factor in a variety of malignancies, including breast cancer, ovarian cancer, and lung cancer. These receptors constitute interesting drug targets. Indeed, drugs such as erlotinib, cetuximab and trastuzumab were developed specifically to inhibit these targets. In various subtypes of sarcomas, EGFR and ERBB2 overexpression has been reported and therefore drugs targeting these receptors may potentially be useful in the treatment of sarcomas. This is important because most sarcomas are relatively resistant to chemotherapy and novel treatments are urgently called for. Therefore, in **Chapter 3** we describe the construction of a tissue micro-array with 18 different types of soft tissue tumors to evaluate EGFR and ERBB2 expression.

The development and registration of new small molecule kinase inhibitors is proceeding remarkably fast. In this thesis, 2 phase I studies of new agents and 1 combination study of a new agent with a registered agent are described. The main objective of these studies is to evaluate the safety and tolerability of the new drug, with additional pharmacokinetic, pharmacodynamic and efficacy assessments. In **Chapter 4,** a phase I dose escalation study of telatinib (BAY 57-9352), a tyrosine kinase inhibitor of VEGFR-2, VEGFR-3, PDGFR-β and c-Kit, in patients with advanced or metastatic solid tumors is discussed. In **Chapter 9,** a phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib (PHA-739358) in similar patients is discussed. In **Chapter 8** the use of a targeted agent in combination with a conventional chemotherapeutic drug is investigated. This study aims at enhancing the efficacy of the combination compared to monotherapy with each of these drugs, without causing more toxicity. In this phase I dose escalation study, treatment with sunitinib in combination with ifosfamide is studied.

With the development of new drugs new side effects may emerge. Vascular endothelial growth factor (VEGF) inhibitors induce hypertension as a common side effect. The mechanisms leading to the increase in blood pressure during this anti-angiogenic therapy are not clear. We hypothesized that systemic inhibition of VEGF impairs vascular function and causes rarefaction, which then leads to the development of hypertension in patients treated with anti-angiogenic agents. Functional rarefaction (a decrease in perfused microvessels) or anatomic rarefaction (a reduction in capillary density) may be the underlying mechanism.

We performed blood pressure and vascular structure and function studies in patients treated with VEGF inhibitors in order to clarify the mechanism by which small molecule angiogenesis inhibitors cause an increase in blood pressure. In **Chapter 6** the blood pressure and vascular studies during treatment with telatinib, a small molecule VEGF in-

hibitor, are described. In **Chapter 7,** the underlying mechanisms of hypertension related to bevacizumab (Avastin®), a VEGF antibody, are investigated.

Many studies have been performed to individualize anticancer drug treatment aiming at decreasing side effects or optimizing efficacy. Pharmacogenomics is a very exciting and new field of today's medicine, promising a personalized, tailor-made medication strategy to improve drug response and decrease harmful adverse reactions. Pharmacogenomics, often used synonymously with pharmacogenetics, is defined as: 'the individualization of drug therapy through medication selection or dose adjustment based upon direct (e.g., genotyping) or indirect (e.g., phenotyping) assessment of a person's genetic constitution for drug response.'
The development of tailor-made pharmaceutics is especially useful in the field of oncology, since most anticancer agents have a very narrow therapeutic index. This sometimes leads to lack of any anti-tumor response or a high level of side effects. Heritable genetic variations (germline polymorphisms) in genes encoding for drug transporters, drug metabolizing enzymes or drug targets have been shown to influence the pharmacokinetics and pharmacodynamics of many drugs including drugs used in cancer therapy. There is a rapid development in the field of targeted anti-cancer agents, whereas the necessary accompanying pharmacogenetic research during drug development is lacking. It is important to conduct these studies for new anticancer agents to increase knowledge of variants in genes encoding for both drug metabolizing enzymes and drug targets, and to understand interindividual variability in pharmacokinetics and pharmacodynamics. Ultimately, this may lead to a better, tailor-made anticancer therapy with less side effects and more effective use of novel drugs in the future.

In **Chapter 5** the pharmacogenetics of telatinib (BAY 57-9352), a tyrosine kinase inhibitor of VEGFR-2, and VEGFR-3, used in patients with advanced or metastatic solid tumors is studied. **Chapter 10** describes the pharmacogenetic investigations of danusertib (PHA-739358), a small-molecule pan-aurora kinase inhibitor, used in similar patients.

A general discussion of the reported studies described in this thesis is presented in **Chapter 11**. Further, a summary of this thesis in both English and Dutch are provided.

2

Small molecule tyrosine kinase inhibitors in the treatment of solid tumors: an update of recent developments

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Abstract

Small molecule tyrosine kinase inhibitors (TKIs) are developed to block intracellular signaling pathways in tumor cells, leading to deregulation of key cell functions such as proliferation and differentiation. Over 25 years ago, tyrosine kinases were found to function as oncogenes in animal carcinogenesis; however, only recently TKIs were introduced as anti cancer drugs in human cancer treatment. Tyrosine kinase inhibitors have numerous good qualities. First, in many tumor types they tend to stabilize tumor progression and may create a chronic disease state which is no longer immediately life threatening. Second, side effects are minimal when compared to conventional chemotherapeutic agents. Third, synergistic effects are seen in vitro when TKIs are combined with radiotherapy and/or conventional chemotherapeutic agents. In this article, we will give an update of the tyrosine kinase inhibitors that are currently registered for use or in an advanced stage of development, and we will discuss the future role of TKIs in the treatment of solid tumors. The following TKIs are reviewed: Imatinib (Gleevec®/Glivec®), Gefitinib (Iressa®), Erlotinib (OSI-774, Tarceva®), Lapatinib (GW-572016, Tykerb®), Canertinib (CI-1033), Sunitinib (SU 11248, Sutent®), Zactima (ZD6474), Vatalanib (PTK787/ZK 222584), Sorafenib (Bay 43-9006, Nexavar®), and Leflunomide (SU101, Arava®).

Introduction

Conventional chemotherapeutical agents act by creating toxic effects on all dividing cells, frequently resulting in severe damage of normal tissues leading to side efects like myelosuppression, alopecia, or gastrointestinal problems. The optimum goal is to find a treatment modality that specifically kills malignant cells and causes little or no side efects. Targeted therapies were developed to target key elements that play a role in tumor development and tumor growth, with hormonal therapy in breast cancer being the oldest targeted therapy known in oncology. A more recent discovery are the tyrosine kinase inhibitors, developed to block intracellular signaling pathways in tumor cells, leading to deregulation of key cell functions such as proliferation and diferentiation. Over 25 years ago, tyrosine kinases were found to function as oncogenes in animal carcinogenesis. However, only recently, tyrosine kinase inhibitors were introduced as anti cancer drugs in human cancer treatment.[1–3]

Tyrosine kinases (TKs) are enzymes that catalyze the phosphorylation of tyrosine residues. There are two main classes of TKs: receptor TKs and cellular TKs. Receptor TKs have an extracellular ligand binding domain, a transmembrane domain, and an intracellular catalytic domain. The kinase is activated by binding of a ligand (mostly growth factors) to the extracellular domain, leading to dimerization of the receptors and autophosphorylation of the tyrosine residues of the intracellular catalytic domain. This results in an active receptor conformation and activation of signal transduction within the cell. Cellular TKs are located in the cytoplasm, nucleus, or at the intracellular side of the plasma membrane. Tyrosine kinases are involved in cellular signaling pathways and regulate key cell functions such as proliferation, diferentiation, anti-apoptotic signaling, and neurite outgrowth (Fig. 1).[4]

Unregulated activation of TKs, through mechanisms such as point mutations or overexpression, can lead to various forms of cancer as well as benign proliferative conditions.[5] These findings lead to the hypothesis that inhibitors of TKs could have antitumor effects, and many tyrosine kinase inhibitors (TKIs) were subsequently developed.[1,5] Today, there are two main mechanisms to block the activation of a tyrosine kinase. First, the TKI can block the ATP-binding side and prohibit the autophosphorylation of the tyrosine residues, and therefore prohibit the activation of the intracellular signal-transduction pathways. These drugs are usually referred to as small molecule tyrosine kinase inhibitors. Second, a monoclonal antibody can occupy the extracellular ligand domain of the receptor tyrosine kinase and prohibit binding of the actual ligand and, therefore, prohibit activation of the intracellular signal-transduction pathways.

In this article, we will focus on the small molecule tyrosine kinase inhibitors. The development and registration of new small molecule tyrosine kinase inhibitors is pro-

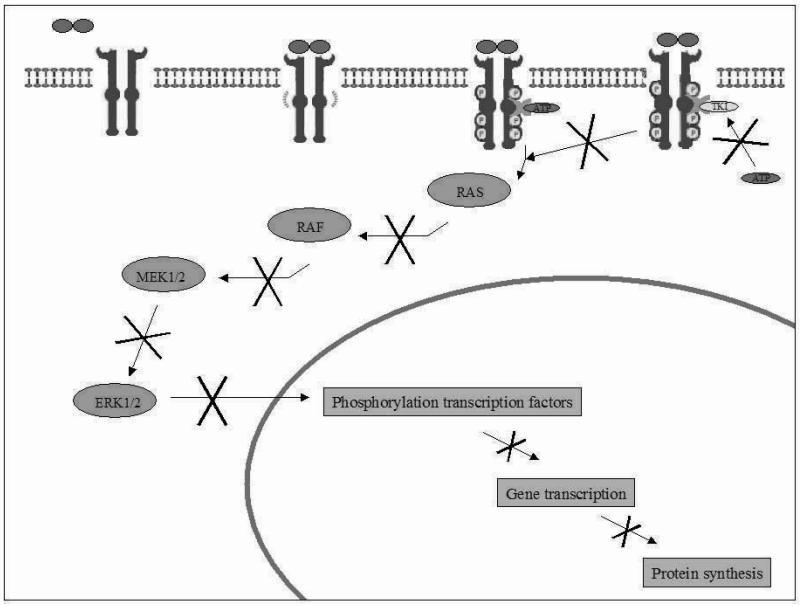

FIG. 1. Tyrosine kinase activation and the MAPK/Erk intracellular signaling pathway; mechanism of action of tyrosine kinase inhibitors (TKIs). The MAPK/Erk intracellular signaling pathway is an example of one of the pathways that can be activated by binding of a ligand (mostly growth factors) to the receptor tyrosine kinase. ATP binds to the tyrosine kinase and auto-phosphorylation takes place, resulting in activation of the MAPK/Erk intracellular signaling pathway. An activated Erk dimer can translocate to the nucleus where it phosphorylates a variety of transcription factors regulating gene expression. Tyrosine kinase inhibitors block the ATP-binding site of the tyrosine kinase and therefore inhibit the activation of the intracellular signaling pathway, resulting in blockage of protein synthesis necessary for proliferation and differentiation of the tumor cells.

ceeding remarkably fast. Therefore, frequent new updates of small molecule tyrosine kinase inhibitors are very relevant for physicians treating cancer patients. In this article, we will give an update of the tyrosine kinase inhibitors that are currently registered for use or in an advanced stage of development, and we will discuss the future role of TKIs in the treatment of solid tumors.

c-KIT Tyrosine Kinase Inhibitors

Imatinib (STI-571, Gleevec® (in US), Glivec® (in Europe))

Imatinib is a small molecule that reversibly competes with ATP for binding to the kinase domain of the c-KIT, c-Abl, and platelet-derived growth factor receptor-β (PDGFR-β) tyrosine kinases. Imatinib was the first commercially available as a small molecule tyrosine

kinase inhibitor, giving astonishing results in patients with chronic myelogenous leukemia (CML) by inhibiting the phosphorylation of the Bcr-Abl TK, and thereby suppressing the proliferation of Bcr-Abl expressing leukemic cells. A phase II study was performed in approximately 1000 patients with CML, with patients in the chronic phase receiving 400 mg of imatinib orally a day, and patients in accelerated phase or blast crisis receiving 600 mg/day. Complete hematological responses were seen in 91% of the patients in chronic phase CML, 53% of patients in accelerated phase CML, and 26% of patients in blast crisis. However, in the late-stages disease, the efects were short lasting, with a recurrence of imatinib-resistant cells within months.[6] In this article, we will focus on the results in solid tumors.

In gastrointestinal stromal tumors (GISTs), imatinib also showed remarkable results.[7,8] Imatinib blocks the c-KIT tyrosine kinase, which is constantly activated in 90% of GISTs by a gain-of-function mutation in the c-KIT proto-oncogene.[9] Approximately 30–50% of GISTs that harbour no c-KIT mutation do have PDGF mutations, and depending on the subtype of the PDGF mutation these GISTs are also sensitive to imatinib. The highest responses were seen in GISTs with exon 11 mutations and, the more rare, PDGF mutations.[9,10] Approximately 5–10% of GISTs are negative for both c-KIT and PDGF mutations. In a phase III trial reported in 2004, 946 patients were randomized for treatment with 400 mg imatinib once daily or 400 mg twice daily.[11] Complete responses were seen in 5 vs. 6%, partial responses in 45 vs. 48%, and stable disease in 32 vs. 32% of patients. At median follow-up of 760 days, 56% in the group receiving imatinib 400 mg once daily showed progression of the disease, compared with 50% of patients receiving 400 mg twice daily. Side effects were frequent but mostly mild. Anemia, edema, fatigue, nausea, pleuritic pain, diarrhea, granulocytopenia, and rash were the most common side effects. These were impressive results for a tumor type that, until recently, was poorly affected by chemo-or radiotherapy and for small molecule TKIs in general. Therefore, studies were initiated to explore the role of imatinib in the adjuvant setting in high risk patients with GISTs. Currently, the results of these studies with adjuvant imatinib in high and intermediate risk GIST are awaited. Resistance to imatinib in GISTs is a well known problem and can be caused by secondary mutations or c-KIT amplification. Therefore, other therapies for GISTs are being explored, like sunitinib (see chapter on sunitinib).[12]

Imatinib is also designated as orphan drug for the treatment of dermatofibrosarcoma protuberans (DFSP), based on case reports of this rare tumor type, in cases that can not be managed with surgery alone.[13,14] The cutaneous malignant mesenchymal tumor dermatofibrosarcoma protuberans is typically associated with a translocation between chromosomes 17 and 22, involving the platelet-derived growth factor-β (PDGF-β) gene, forming a ring chromosome. Imatinib inhibits the growth of these tumor cells by inhibiting PDGFR tyrosine kinase.

Imatinib's activity in advanced aggressive fibromatosis (desmoid tumor) and, to a lesser extent, in advanced chordoma may also be based on PDGFR-β inhibition. In a recently published article, 3 out of 19 desmoid patients demonstrated a partial response, with 4 additional patients showing stable disease for more than one year.[15] In a multicenter phase II trial, 51 patients with advanced aggressive fibromatosis were treated with imatinib 300 mg po BID. At the time of analysis, 45 patients were evaluable. Median time to treatment failure was 6.8 months. Remarkably, in only 1 of 22 available tumor specimens a PDGFR mutation was found.[16] In chordoma patients, the effect was often less clear on CT-scan, but in some cases clearly by subjective improvement of complaints.[17] Recent clinical studies suggest that there might also be an effect of imatinib in glioblastoma multiforme and malignant gliomas by inhibiting PDGFR tyrosine kinase.[18–21]

Imatinib inhibited growth of small-cell lung cancer (SCLC) cells in vitro by inhibiting c-KIT; however, there was no objective tumor response in SCLC patients in vivo. This was probably caused by the fact that there was no c-KIT mutation detectable in most of the patients.[22,23] This was also seen in other tumor types, like uterine leiomyosarcomas.[24]

EGFR/Her1 and Her2 Tyrosine Kinase Inhibitors

The Her-family of tyrosine kinases consists of four members: Her1 (Human Epidermal Growth Factor Receptor: EGFR, erbB1), Her2 (erbB2), Her3 (erbB3), and Her4 (erbB4). After binding of a receptor-specific ligand homodimeric or heterodimeric complexes are formed. Her-kinase activation deregulates growth, desensitises cells to apoptotic stimuli, and regulates angiogenesis.[25] Overexpression of EGFR and Her2 is a factor of poor prognosis in a variety of malignancies, including breast cancer, ovarian cancer, and lung cancer.[26,27]

Gefitinib (Iressa®)

Gefitinib was the first commercially available EGFR TKI and is now registered for use in Asia and the United States in second-or third line therapy for advanced non-small-cell lung cancer (NSCLC). Two phase II trials evaluated the efficacy of gefitinib in patients with advanced NSCLC: IDEAL (Iressa® Dose Evaluation in Advanced Lung Cancer)-1 and IDEAL-2. IDEAL-1 included 210 patients in Europe, Australia, South Africa, and Japan who had previously received one or two chemotherapy regimens, with at least one containing platinum. IDEAL-2 included 216 patients in the United States who had failed two or more prior chemotherapy regimens containing platinum and docetaxel. Patients were

randomized for continuous treatment with 250 or 500 mg gefitinib monotherapy once daily orally.

IDEAL-1 showed that gefitinib dosage of 250 and 500 mg/day were equally efective, with an objective tumor response of 18% and 19% respectively.[28] The objective tumor response rate in IDEAL-2 was 12% in the 250 mg/day gefitinib patients and 9% in the 500 mg/day patients.[29] The difference in response was most likely caused by the worse performance status in IDEAL-2, a higher number of previous chemotherapy regimens in IDEAL-2, and the Japanese origin of a subset of patients in IDEAL-1 (which later became correlated with an higher number of activating mutations in the EGFR gene).[30] Overall survival was 18.5 (IDEAL-1) and 16.3 (IDEAL-2) months in patients with complete or partial response, 8.5 and 9.4 months in stable disease, and 3.8 and 4.2 months in progressive disease. Most reported side effects were cutaneous and gastrointestinal complaints. Since the use of gefitinib became more widespread, a more serious side effect, pulmonary fibrosis, was noted in approximately 1% of patients.[31,32] The recommended dose for use was established at 250 mg/day while this was equally effective and better tolerated.

In large phase III studies, INTACT-1 and-2, gefitinib in combination with chemotherapy in previously untreated NSCLC patients did not show improved efficacy over chemotherapy alone.[33,34] A placebo-controlled phase III trial randomizing NSCLC patients in second-or third-line treatment for treatment with gefitinib 250 mg/day or placebo plus best supportive care also did not show any survival benefit.[35]

In the approval of gefitinib, the EGFR status of the tumor was not included in selecting patients for treatment. Patient characteristics that were associated with responsiveness to EGFR inhibitors were histologic features of adenocarcinoma, female sex, no history of smoking, and Asian ancestry. The EGFR level in immunohistochemical staining does not predict response to EGFR inhibiting therapies and does not correlate with poor survival.[36–38] Recent studies reported an activating mutation in the tyrosine kinase side of the EGFR gene in NSCLC that seemed predictive for response to gefitinib treatment.[39–41] For future use of gefitinib, it will be highly important to select those patients that are likely to benefit from this EGFR-TKI, while non-selection is probably the main cause of the disappointing results of gefitinib.

Phase II studies with gefitinib monotherapy or combination therapy have been conducted in many tumor types, including esophageal carcinoma, metastatic breast cancer, prostate cancer, head and neck cancer, colorectal cancer, renal cell carcinoma, and ovarian carcinoma.[42–53] In EGFR expressing colorectal cancer (CRC), the monoclonal antibody cetuximab has been proven active.[54,55] Therefore, beneficial effects of EGFR TKIs were expected. However, recent trials showed no effect of gefitinib in CRC patients. Of the 115 gefitinib treated patients, only one patient obtained a partial response, progression

free survival was 1.9 months, and median survival 6.3 months. No significant changes in biological indicator of EGFR pathway activation were detected.[52] However, a second phase II trial reported partial responses in 78% of patients treated with gefitinib in combination with fluorouracil and oxaliplatin (FOLFOX-4).[56] Many trials with gefitinib for various tumor types are still ongoing.

Erlotinib (OSI-774, Tarceva®)

Erlotinib is an EGFR TKI with proven efficacy in monotherapy phase II trials in NSCLC, ovarian cancer, pancreatic cancer, head and neck squamous cell cancer, and primary glioblastoma.[57–59]

A survival benefit of erlotinib compared with best supportive care was reported in previously treated NSCLC patients.[60] Patients with stage IIIB or IV NSCLC were randomly assigned in a 2:1 ratio to receive oral erlotinib, at a dose of 150 mg daily, or placebo. The response rate was 8.9 percent in the erlotinib group and less than 1 percent in the placebo group. Progression-free survival was 2.2 months and 1.8 months, respectively. In contrast to the trial with gefitinib,[35] the study comparing erlotinib with best supportive care[60] did show improved survival for erlotinib treated patients. The trials were similarly designed; however, the strict inclusion criterion describing refractory disease in the gefitinib trial may have resulted in a different patient population. After the publication of these trials, clinicians favored the use of erlotinib over gefitinib. However, a trial directly comparing the two drugs was never started.

In phase III trials (TALENT and TRIBUTE) in NSCLC patients, there was no additional benefit of erlotinib in combination with chemotherapy, compared to chemotherapy alone.[61,62] Erlotinib is registered for the second-and third-line treatment of patients with advanced NSCLC after failure of at least one prior platinum treatment.

Since late 2005, erlotinib is also registered for advanced pancreatic cancer. A Phase III trial in 569 chemotherapy-naıve patients with advanced pancreatic cancer reported an improval in 1-year survival from 17% to 24% when erlotinib 100 mg daily was added to gemcitabine 1000 mg/m^2/week, compared to gemcitabine alone.[63] Median overall survival increased from 5.9 months to 6.4 months. EGFR status was not an entry criterion; however, tumor samples are being evaluated for EGFR expression by immunohistochemistry. Current studies in pancreatic cancer patients focus on combination with chemotherapy, radiotherapy, and other targeted therapies, or on maintenance therapy of erlotinib.

A phase II study of erlotinib in patients with advanced biliary cancer showed a potentially beneficial efect of erlotinib. Progression free survival at 6 months was 17% and partial responses were seen in 3 of 42 patients.[64] Earlier, the same author reported

a phase II study of erlotinib in hepatocellular cancer patients. Progression free survival at 6 months was 32%, and partial responses were seen in 3 of 38 patients.[65] Phase II trials in metastatic colorectal carcinoma patients with erlotinib alone or in combination with chemotherapy showed promising results.[66,67] Erlotinib 150 mg orally daily, in combination with bevacizumab 10 mg/kg intravenously every 2 weeks, was evaluated in 63 patients with metastatic clear-cell renal carcinoma, which resulted in a median survival of 11 months and 1-year pro-gression-free survival of 43%. Treatment was well tolerated; skin rash and diarrhea were the most frequent treatment-related toxicities.[68]

The most frequent reported adverse events in erlotinib treatment are skin rash and diarrhea. The incidence of interstitial lung disease in patients receiving erlotinib was equal to that in gefitinib, approximately 1%.[69,70]

Lapatinib (GW-572016, Tykerb®)

Lapatinib is an EGFR and Her2 tyrosine kinase inhibitor.[71] Phase I studies in trastuzumab refractory breast cancer and NSCLC demonstrated clear tumor responses.[72] In a phase II study in 86 patients with metastatic colorectal cancer, effects of lapatinib were minor, with 1 partial response, 5 minor responses, and 5 patients with stable disease.[73] Reported adverse events were diarrhea and skin rash.

An international, multicenter, randomized, open-label phase III trial in patients with documented HER2 overexpressing refractory advanced or metastatic breast cancer treated with lapatinib in combination with capecitabine versus capecitabine alone was recently stopped after the interim analysis. At the time of interim analysis, 392 patients had been enrolled in the study, of which 321 were included in the analysis (161 in the combination arm and 160 in the monotherapy arm). Median time to progression in the combination arm was 8.5 months, compared with 4.5 months in the capecitabine alone arm.[74] The addition of lapatinib to capecitabine resulted in such a striking increase in time to progression that this combination will probably be used by clinicians as standard of care in patients with advanced HER2positive breast cancer that failed on trastuzumab. However lapatinib is not yet registered for use in this, or any, indication.

In a phase III trial, patients with advanced renal cell carcinoma (RCC) who failed prior cytokine therapy were randomized to receive oral lapatinib 1250 mg OD or hormone therapy. At the time of the analysis, 417 patients were randomized. In the general study-population, median time to progression and median overall survival did not difer between the two groups. In the EGFR overexpressing patients, median time to progression was 15.1 months in the lapatinib treated patients, vs. 10.9 weeks in the hormone therapy treated patients. The reported median overall survival was 46.0 vs. 37.9 weeks.[75]

Phase II results on the use of lapatinib in breast cancer patients with brain metasta-

ses, locally advanced squamous cell carcinoma of the head and neck, biliary carcinoma, and hepatocellular carcinoma have recently been reported at the 2006 ASCO Annual Meeting (http://www.asco.org).

Canertinib (CI-1033)

Canertinib is a tyrosine kinase inhibitor that non-selectively inhibits all members of the Her-family. This might result in a broader spectrum of anti tumor activity. In phase I studies, reported adverse events were diarrhea, rash, anorexia.[76] In a phase II study in patients with platinum-refractory or recurrent ovarian cancer, canertinib did not show activity in unscreened patients.[77] Studies in breast cancer and NSCLC are currently ongoing.

Vascular Endothelial Growth Factor Tyrosine Kinase Inhibitors

The Vascular Endothelial Growth Factor (VEGF) family belongs to the platelet-derived growth factor (PDGF) superfamily and consists of VEGF-A, -B, -C, -D, -E, and the placenta growth factor (PlGF). VEGF-A (normally referred to as VEGF) is the most potent endothelial growth factor. It contributes to tumor angiogenesis and presumably to tumor growth and haematogenous spread of tumor cells.[78] Moreover, VEGF-A protects endothelial cells from apoptosis and contributes to the maintenance of the vascular system.[79,80]

Most of the VEGF Receptor (VEGFR) kinase inhibitors under investigation inhibited multiple kinases not involved in angiogenesis, resulting in diverse side efects. New VEGFR kinase inhibitors are being developed to selectively target a small subset of protein kinases, and therefore minimalize the side-efects.

Sunitinib (SU 11248, Sutent®)

Sunitinib is an orally available inhibitor of VEGFR, PDGFR, c-KIT, and FLT-3 kinase activity. In a phase II study in patients with immunotherapy refractory metastatic renal cell carcinoma treated with sunitinib (6-week cycles: 50 mg orally once daily for 4 weeks, followed by 2 weeks of), 40% of patient showed a partial response and 27% stable disease.[81] When the results were combined with a second study with an identical patient population, the total evaluable patient population was 168 patients. Objective responses were seen in 42% and stable disease of 3 or more months in 24%. Median progression

free survival was 8.2 months.[82] These response rates were much higher than seen with any other systemic treatment in RCC. The main adverse effects were fatigue, diarrhea, nausea, dyspepsia, stomatitis, and bone marrow abnormalities. Motzer reported the results of a phase III study comparing sunitinib (6-week cycles: 50 mg orally once daily for 4 weeks, followed by 2 weeks off) to IFN-α (6-week cycles: subcutaneous injection 9 MU given three times weekly) as first line therapy for metastatic renal cell cancer patients. There was a statistically significant improvement in median progression free survival (47.3 vs. 24.9 weeks) and objective response rate (24.8% vs. 4.9%) for sunitinib over IFN-α.[83] Sunitinib might therefore now be considered the new standard first-line treatment for advanced kidney cancer.

In January 2006, sunitinib was not only approved by the FDA for advanced renal cell carcinoma, but also for imatinib-resistant and imatinib-intolerant GIST. This was based on the early results of a phase III trial in patients with documented progression of GIST on imatinib.[84,85] Patients were treated with a starting dose of 50 mg sunitinib once daily for four weeks, followed by 2 weeks off treatment, in repetitive 6-week cycles (N = 207) or placebo (N = 105). Due to the positive results found at a planned interim analysis, the trial was unblinded and all patients started treatment with sunitinib. Partial response was seen in 6.8% of sunitinib treated patients, compared to 0% in the placebo group. Stable disease for more than 22 weeks occurred in 17.4%, compared to 1.9%. Time to progression was significantly longer in the sunitinib treated patients, 27.3 weeks compared to 6.4 weeks. The most common non-hematological adverse events were fatigue, diarrhea, nausea, sore mouth, and skin discoloration.

From a biological point of view, continuous dosing of sunitinib seems more logical. A study in 28 patients with advanced imatinib-resistant GIST explored the continuous daily 37.5 mg dosing regimen, which was feasible and associated with similar tolerability as is seen with intermittent sunitinib dosing.[86]

Sunitinib showed a potentially beneficial efect in previously treated advanced NSCLC and unresectable neuroendocrine tumors in phase II studies.[87,88]

Zactima (ZD6474)

Zactima is an orally available, small molecule, dual VEGF receptor-2 (VEGFR-2) and EGFR tyrosine kinase inhibitor. Zactima has the potential to directly inhibit tumor cell proliferation and survival by blocking EGFR and inhibit tumor angiogenesis by blocking VEGF activity. Zactima inhibits VEGF signaling and angiogenesis in vivo and shows broad-spectrum antitumor activity in a range of histologically diverse tumor xenograft models.[89] In phase I trials, dose limiting toxicities were diarrhea, hypertension, thrombocytopenia, and prolongation of the cardiac QT interval. Phase II assessment of zactima is now in

progress in a variety of tumor types in single and combination regimens.[90,91] In early reports of two phase II studies of zactima in combination with docetaxel or carboplatin and paclitaxel for NSCLC, zactima did not significantly increase toxicity compared to chemotherapy alone.[92] In the study reported by Heymach, patients with locally advanced or metastatic (stage IIIB/IV) NSCLC after failure of first-line platinum-based chemotherapy were randomized to treatment with zactima 100 mg orally once daily plus docetaxel (75 mg/m^2 i.v. infusion every 21 days) (N = 42), zactima 300 mg orally once daily plus docetaxel (N = 44), or docetaxel alone (N = 41). Median progression free survival was higher in the combination therapy treated groups (19 vs. 17 vs. 12 weeks respectively).[93] This resulted in the initiation of a phase III evaluation of zactima plus docetaxel in second-line NSCLC.

In a double-blind, randomized phase II trial, 168 patients with NSCLC were randomized for initial treatment with zactima 300 mg or gefitinib 250 mg. Zactima demonstrated a significant prolongation of progression free survival versus gefitinib (11.0 vs. 8.1 weeks). Overall survival was not significantly diferent (median 6.1 and 7.4 months, respectively).[94]

Zactima shows also promising evidence of clinical activity in patients with hereditary medullary thyroid carcinoma. Of 15 evaluable patients, 3 had partial responses and 10 stable disease.[95]

Vatalanib (PTK787/ZK 222584 (PTK/ZK))

Vatalanib is an oral inhibitor of a number of kinases including VEGFR-1 and VEGFR-2 as well as the platelet-derived growth factor receptor (PDGFR). It clearly demonstrated an anti-tumor efect in several solid tumor types. Adverse events were lightheadedness, fatigue, transaminase elevation, hypertension, nausea, and vomiting.[96] Dynamic contrast-enhanced molecular resonance imaging (DCE-MRI) in patients with advanced colorectal carcinoma and liver metastases showed a vatalanib dose-dependent reduction of vascular permeability and blood flow in the liver metastases.[97] A phase III study (CONFIRM-1, Colorectal Oral Novel Therapy for the Inhibition of Angiogenesis and Retarding of Metastases in First-line) showed no beneficial effects of adding vatalanib to chemotherapy (oxaliplatin/5fluorouracil/leucovorin (FOLFOX4)) in metastatic colorectal cancer patients.[98] A second phase III study in 855 pretreated patients with metastatic colorectal carcinoma (CONFIRM-2, Colorectal Oral Novel Therapy for the Inhibition of Angiogenesis and Retarding of Metastases in Second-line) demonstrated a significant improvement in progression free survival when vatalanib 1250 mg qd was added to FOLFOX. Overall survival was the same in both treatment arms.[99] Combination and monotherapy trials are currently also conducted in other tumor types.

Sorafenib (Bay 43-9006, Nexavar®)

Sorafenib is a novel oral Raf-1 kinase, platelet-derived growth factor receptor (PDGFR) and VEGFR kinase inhibitor with antitumor efects in colon, pancreas and breast cancer cell lines and in colon, breast and non-small-cell lung cancer xenograft models.[100] A phase I study in 69 patients with refractory solid tumors reported promising results.[101] Dose limiting toxicities were hematological toxicity, diarrhea, fatigue, hypertension, and skin rash. In a recent phase II randomized discontinuation trial in patients with metastatic renal cell carcinoma, sorafenib showed anti-tumor activity and was well tolerated.[102,103] An interim analysis of a phase III trial randomizing 769 patients with advanced RCC to sorafenib 400 mg bid or placebo reported an improvement of progression free survival from 12 weeks to 24 weeks in sorafenib treated patients compared to placebo.[104] Updated results reported at the ASCO 2006 meeting showed a survival benefit for sorafenib over placebo (median overall survival of 19.3 months vs. 15.9 months).[105] Sorafenib was granted FDA fast track approval in December 2005.

Phase III trials in stage III or IV melanoma and in advanced hepatocellular carcinoma, and phase II trials in multiple tumor types are currently ongoing.

It has previously been suggested that rash commonly associated with EGF-pathway inhibitors could be predictive of treatment outcome, and that the onset of rash could be used for optimal dose titration.[106] This might also be effective in treatment with sorafenib, as it is an inhibitor of Raf kinase, which is a downstream effector molecule of the EGFR signaling pathway. A recent report combining data from four phase I trials supported this hypothesis. Patients receiving sorafenib dosed at or close to the recommended dose of 400 mg bid, and experiencing skin toxicity and/or diarrhea, had a significantly increased time to progression compared with patients without such toxicity.[107] Blood pressure has also been reported as a possible biomarker in patients treated with sorafenib and other VEGF inhibitors.[108,109]

Platelet-Derived Growth Factor Tyrosine Kinase Inhibitors

Platelet-derived growth factor (PDGF) and its tyrosine kinase receptor (PDGFR) have been implicated in the pathogenesis of a number of tumor types and play an important role in various cellular functions, including growth, proliferation, diferentiation, and angiogenesis.[110] Multiple PDGFR kinase inhibitors have been evaluated in human solid tumors; many are not specific for PDGF and act on a number of tyrosine kinase receptors. Examples are imatinib B-Raf, VEGFR, PDGFR), and leflunomide (SU101; PDGFR, EGFR, FGFR).[111]

Leflunomide (SU101, Arava®)

Leflunomide is a small molecule inhibitor of the PDGFR tyrosine kinase and partially inhibits EGFR and the fibroblast growth factor receptor (FGFR). Leflunomide is an immunomodulatory agent that is indicated in adults for treatment of active rheumatoid arthritis. It has demonstrated broad-spectrum antitumor activity in preclinical studies. A multicenter phase II study in hormone refractory prostate cancer patients treated with leflunomide showed partial responses in 1 of 19 patients, a prostate-specific antigen decline greater than 50% in 3 of 39 patients, and improvement in pain in nine of 35 evaluable patients. The patients received a 4-day i.v. loading dose of SU101 at 400 mg/m^2 for 4 consecutive days, followed by 10 weekly infusions at 400 mg/m^2. Despite the detection of PDGFR overexpression in 80% of the metastases and 88% of the primary tumors, these were disappointing results.[112] The most frequently reported side effects with leflunomide were asthenia, nausea, anorexia, and anemia.

A phase III randomized study of leflunomide versus procarbazine for patients with glioblastoma multiforme in first relapse, and a phase II/III randomized study of leflunomide with mitoxantrone and prednisone versus mitoxantrone and prednisone alone in patients with hormone refractory prostate cancer have just finished recruiting. Results have not yet been reported.

Tyrosine Kinases As A Target: Success Or Failure?

Imatinib (Gleevec®/Glivec®) was the first small molecule TKI that was successfully developed. The results of imatinib in GIST, a tumor that is poorly afected by chemotherapy and radiotherapy, were astonishing and lead to a boost in research of small molecule tyrosine kinase inhibitors in solid tumors. The results of these investigations in other solid tumors were not as astonishing, although substantial efects were seen in many diferent tumor types.

There are multiple reasons for this more modest efect in other solid tumors. First, most tumor cells harbor multiple genetic defects, and inhibiting one tyrosine kinase might not be sufficient. Second, inhibiting tyrosine kinases leads to a stop in cell division, and lack of further growth is therefore the maximum achieved goal. Third, inhibiting an intracellular signaling pathway by a TKI can be overcome by tumor cells by redirecting the signals through other pathways. Fourth, tumor cells can become resistant to TKIs, mostly due to new mutations in the tyrosine kinase, drug efflux mechanisms, receptor down-regulation, and loss of TK-inhibitory pathways.

However, TKIs do have numerous good qualities. First, in many tumor types, they tend to stabilize tumor progression and may create a chronic disease state which is no longer immediately life threatening. Second, side efects are minimal when compared to con-

Table 1. Tyrosine kinase inhibitors: currently registered or in clinical development for solid tumors

Agent	Target receptors	Development stage
Imatinib (STI-571, Gleevec®)	c-Abl, PDGFR-b, c-KIT	Licensed for GIST, (CML) Orphan drug request for DFSP
Gefitinib (Iressa®)	EGFR	Licensed for 2d- or 3rd line NSCLC (Asia, United States)
Erlotinib (OSI-774, Tarceva®)	EGFR	Licensed for 2d- or 3rd line NSCLC, advanced pancreatic cancer
Lapatinib (GW-572016, Tykerb®)	EGFR, Her-2	Phase I/II/III
Canertinib (CI-1033)	EGFR, Her-2, Her-3, Her4	Phase I/II
Sunitinib (SU11248, Sutent®)	PDGFR, VEGFR, KIT, FLT-3	Licensed for advanced RCC, and imatinib-resistant/-intolerant GIST
Zactima (ZD6474)	VEGFR, EGFR	Phase I/II/III
Vatalanib (PTK787)	VEGFR, PDGFR, C-KIT	Phase II/III (colorectal carcinoma)
Sorafenib (BAY43-9006, Nexavar®)	c-Raf-1, B-Raf, VEGFR, PDGFR	Licensed for advanced RCC, Phase II/III (melanoma, HCC)
Leflunomide (SU101, Arava®)	PDGFR (EGFR, FGFR)	Phase II/III (prostate cancer, GBM)

PDGFR: platelet-derived growth factor receptor, GIST: gastrointestinal stromal cell tumor, CML: chronic myelogenous leukemia, DFSP: dermatofibrosarcoma protuberans, EGFR: epidermal growth factor receptor, NSCLC: non-small-cell lung cancer, VEGFR: vascular endothelial growth factor receptor, RCC: renal cell carcinoma, HCC: hepatocellular carcinoma, FGFR: fibroblast growth factor receptor, GBM: glioblastoma multiforme

ventional chemotherapeutic agents. Third, synergistic efects are seen in vitro when TKIs are combined with radiotherapy and/or conventional chemotherapeutic agents.[113–117] If studies in vivo confirm these results, one should consider studying the effects of reducing chemotherapy dose, which might lead to fewer side effects with equal efficacy. One of the mechanisms of synergy between these drugs and chemotherapy is the increase of drug uptake due to decrease of interstitial fluid pressure by PDGF inhibition.[1–3]

The TKIs that are currently registered or in advanced stages of clinical development are shown in Table 1.

Future directions

The identification of patients who are likely to benefit from inhibition of specific tyrosine kinases will become highly important. An important issue is the high costs of small

molecule tyrosine kinase inhibitors, up to $30,000 per patient per year.[1] Patients should be selected based on genetics of their cancer cells, as is proven to be effective in NSCLC patients, where only patients with a mutation in the EGFR receptor showed a favorable response to gefitinib.

Alterations should be made to the conventional phases of drug-development. Maximum tolerated dose (MTD) can no longer be the only end-point in phase I studies, since TKIs have limited side efects and MTD might never be reached. Instead, phase I studies should aim at identifying the maximum biological active dose, i.e. the dose that creates the maximum target inhibition. In phase III studies, selection of the study population should be made based on biogenetics of the tumor, and investigations should also include pharmacodynamic analysis of target inhibition. In previous large phase III trials in unselected patients, TKIs were incorrectly judged to be inefective, and research of an efective drug has incorrectly been stopped. Instead of response rate, other endpoints should be chosen, like time to progression, while with tyrosine kinase inhibitors it might take some time before stabilization of the disease occurs.

Most small molecule tyrosine kinase inhibitors lack substantial benefit when given as monotherapy. Therefore combination therapies based on synergy, combining multiple small molecule TKIs (like gefitinib and sunitinib in RCC trials), combining a small molecule TKI with an antibody TKI (like erlotinib and bevacuzimab in CRC trials, and lapatinib and trastuzumab in breast cancer trials), or combining a TKI with conventional chemotherapy and/or radiotherapy are more likely to be efective.

In the near future, preclinical studies will hopefully be able to identify more activated tyrosine kinases, as overexpression of a target is not a guarantee for treatment success. Molecular markers for toxicity, response and survival, such as the various mutations in GISTs are needed. Future treatment regiments are likely to include multiple tyrosine kinase inhibitors, based on biogenetics of the tumor cells, in combination with chemotherapy, radiotherapy, and other anticancer agents. Hopefully, this will improve the prognosis of patients with several solid tumors by giving a complete or partial tumor response or by creating a chronic stable state in which the disease is no longer immediately life threatening.

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3

EGFR and ERBB2 expression in sarcomas: the search for new treatment options

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Abstract

Aims

Epidermal growth factor receptor (EGFR, Her1) and human epidermal growth factor receptor 2 (ERBB2, Her2) are members of the Her-family of transmembrane receptor tyrosine kinases. In various subtypes of sarcomas EGFR and ERBB2 overexpression has been reported. We studied different subtypes of sarcomas for EGFR and ERBB2 expression to evaluate possible candidates likely to benefit from EGFR and ERBB2 blocking therapies.

Methods

A tissue micro-array with 18 different types of soft tissue tumors was constructed, and immunohistochemical (IHC) analyzed for EGFR and ERBB2 expression.

Results

Positive membranous staining for EGFR was seen in various sarcoma subtypes, including liposarcomas (3/20), leiomyosarcomas (3/8), synovial sarcomas (4/5), malignant peripheral nerve sheath tumors (3/7), rhabdomyosarcomas (2/3), solitary fibrous tumors (1/2), and angiosarcomas (1/1). IHC staining for ERBB2 was negative in all subtypes.

Conclusions

Our results demonstrate that IHC staining for EGFR and ERBB2 shows cytoplasmatic staining in many subtypes of sarcomas, and membranous staining for EGFR in multiple subtypes of sarcomas. However, the immunohistochemical presence of growth factor receptors does not necessarily implicate that the subsequent pathway is activated, or is a potential subject to therapy. These results however open the possibility to study the effect of EGFR blocking therapies, and confirm previous results that ERBB2 is not a potential treatment target.

Introduction

Sarcomas are rare and complex malignant tumors of mesenchymal origin, with a broad histopathologic spectrum.[1] Sarcomas represent less than 1% of all malignancies. The overall prognosis of sarcomas depends on the possibility of complete surgical removal of the tumor, while in general the effects of radiation therapy and chemotherapy are limited. The search for new treatment modalities, especially in tumor types resistant to known cancer therapies, is focusing on identification and inhibition of molecular targets, such as growth factor receptors. Recent research has also focused on the Her-family of tyrosine kinases.

Epidermal growth factor receptor (EGFR, Her1) and human epidermal growth factor receptor 2 (ERBB2, Her2) are members of the Her-family of transmembrane receptor tyrosine kinases. Her-kinase activation deregulates growth, desensitizes cells to apoptotic stimuli and regulates angiogenesis. Overexpression of EGFR and ERBB2 is a factor of poor prognosis in a variety of malignancies, including breast cancer, ovarian cancer, and lung cancer.[2,3] Inhibiting EGFR with a tyrosine kinase inhibitor like erlotinib (Tarceva®) in non-small-cell lung cancer patients, with a monoclonal antibody like cetuximab (Erbitux®) in colorectal and head- and neck carcinoma, or blocking ERBB2 with a monoclonal antibody like trastuzumab (Herceptin®) in breast cancer patients are approved treatment options nowadays.

In breast cancer patients a higher level of membranous ERBB2 overexpression is a predictive factor for increased response to treatment with trastuzumab.[4] In normal clinical practice ERBB2 overexpression is classified as negative (0, 1+) or positive (2+, 3+) by immunohistochemical (IHC) staining. For EGFR the correlation between the level of EGFR expression with IHC staining and response to EGFR blocking therapies is not equally clear, sometimes demonstrating activity of EGFR inhibiting therapies in tumors that express low levels of EGFR.[5,6] Therefore EGFR levels are described as negative (0) and positive (1+, 2+, 3+).

In various subtypes of sarcomas EGFR and ERBB2 overexpression has been reported, however the number of studies are limited and sometimes contradictory.[7-18] For a better defining of the sarcoma subtypes that are overexpressing EGFR and/or ERBB2 and are therefore more likely to benefit from EGFR and/or ERBB2 blocking therapy, a tissue micro-array, with 19 different types of soft tissue tumors, was evaluated for EGFR and ERBB2 expression by IHC staining.

Methods

A tissue micro-array (TMA) with 18 different types of soft tissue tumors, was constructed at the Department of Pathology of the Leiden University Medical Center, and used for the

immunohistochemical (IHC) analyses. Triplicate tissue cores with a diameter of 0.6 mm, as selected by two pathologists (SR and PCWH), were taken from each specimen using a tissue arrayer (Beecher Instruments, Silver Springs, MD, USA) and arrayed on a recipient paraffin block, using standard procedures.[19] Table 1 shows the tumor types that are present on the TMA. All leiomyosarcomas were of deep soft tissue origin. Immunohistochemical staining was performed on 5 μm sections of the tissue array, using a paraffin sectioning aid system (Instrumedics Inc, Hackensack, NJ, USA).

Staining for EGFR was performed using the EGFR detection system (Zymed, San Francisco, California, USA), according to the manufacturer's instructions. The samples were dewaxed, rehydrated, and washed with phosphate-buffered saline (PBS). Antigen retrieval with pepsine was employed before incubation with the primary antibody, anti-EGFR (mouse monoclonal antibody, clone 31G7, 1:100). After washing with PBS, the samples were incubated with the biotinylated secondary antibody, followed by incubation with labeled streptavidin-biotin complex. After the final washing with PBS, staining was performed by means of 3,3'-diamino-benzidine (DAB), followed by counterstaining with Mayer–haematoxylin for 30 sec.

Staining for ERBB2 was performed using the ERBB2 detection system (Lab Vision Corporation Fremont, California, USA), according to the manufacturer's instructions. Microwave citric acid antigen retrieval was employed before incubation with the primary antibody, anti- ERBB2 (mouse monoclonal antibody, clone 3B5 1:1000).

As a negative control sections were stained without adding the primary antibody. Positive controls (placenta for EGFR and 3+ overexpressing breast carcinoma for ERBB2) were present on the TMA. Both EGFR and ERBB2 staining was scored as 0 (negative), 1+ (weak), 2+ (moderate) or 3+ (strong), according to the scoring system provided by the manufacturer. For EGFR levels were described as negative (0) and positive (1+, 2+, and 3+). For ERBB2 expression was classified as negative (0, 1+) or positive (2+, 3+).

Slides were examined and scored blind by two of the investigators (NS, SR) independently. Conflicting assessments were reviewed until final agreement was achieved. Where duplicate cores gave discordant results, the higher score was used.

Results

A tissue micro-array with 18 different soft tissue tumors, including 12 types of sarcomas, and 6 types of benign soft tissue tumors (table 1), was immunohistochemically (IHC) stained for epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (ERBB2, Her2). Not all cases were evaluable, because tissue cores may be lost from the slides. The IHC results are also shown in table 1.

Table 1. Contents of the tissue micro-array (evaluable tissue samples) and results of the immunohistochemically (membranous) staining for epidermal growth factor receptor (EGFR) and ERBB2.

Tissue type	membranous IHC staining		
	EGFR		ERBB2
	positive/ total number cases	positive cells (%)	positive/ total number cases
Liposarcoma	3/20		0/19
Myxoid liposarcoma	2/8	50-75	0/8
Pleomorphic liposarcoma	1/2	25-50	0/2
Dedifferentiated liposarcoma	0/2		0/2
Atypical lipomatous tumor	0/8		0/7
Leiomyosarcoma	3/8	50-75	0/8
MPNST	3/7	50-75	0/6
Synovial sarcoma	4/5	75-100	0/5
Rhabdomyosarcoma	2/3		0/3
Pleomorphic	1/1	75-100	0/1
Embryonal	1/2	0-25	0/2
Gastrointestinal stromal tumor	0/3		0/3
Myxofibrosarcoma	0/3		0/3
Solitary fibrous tumor	1/2	25-50	0/2
Myxoinflammatory fibroblastic sarcoma	0/2		0/2
Dermatofibrosarcoma protuberans	0/2		0/2
Angiosarcoma of soft tissue	1/1	25-50	0/1
Undifferentiated high grade pleomorphica sarcoma	1/4	0-25	0/4
Lipoma	0/5		0/4
Desmoid type fibromatosis	0/4		0/4
Myxoma	0/4		0/4
Schwannoma	0/2		0/2
Synovial chondromatosis	0/1		0/1
Diffuse type giant cell tumor of soft tissue	0/1		0/1

MPNST: malignant peripheral nerve sheath tumor

In all positive cases ERBB2 staining was diffusely present throughout the cell, consistent with cytoplasmatic rather than membranous expression. The positive controls showed clear membranous staining. Positive membranous staining for EGFR was seen in multiple malignant soft tissue tumor types and in one of the benign soft tissue tumors.

Figure 1 shows examples of positive cytoplasmatic staining in ERBB2 (rhabdomyosarcoma) and positive membranous staining in EGFR (solitary fibrous tumor, synovial sarcoma, angiosarcoma).

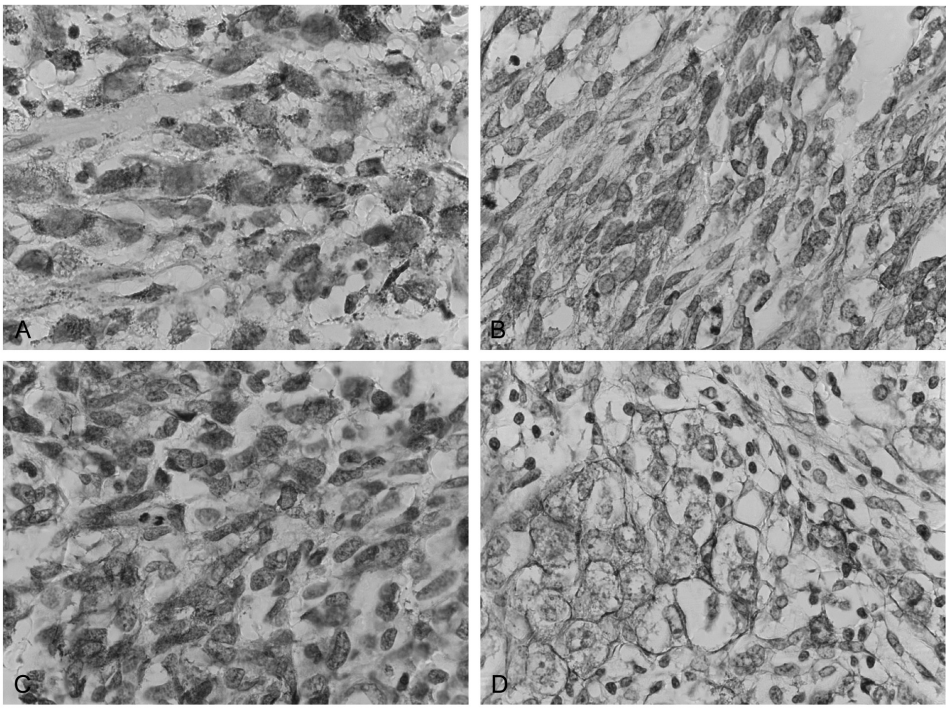

Figure 1. Epidermal growth factor receptor (EGFR) and ERBB2 immunostaining results. All original magnification 630X.
A Diffuse cytoplasmic staining for ERBB2 in rhabdomyosarcoma.
B Membranous staining for EGFR in solitary fibrous tumor.
C Membranous staining for EGFR in synovial sarcoma.
D Membranous staining for EGFR in angiosarcoma.

Discussion

Most sarcomas are resistant to radiotherapy and many commonly used chemotherapy agents, and therefore investigators are searching for other treatment options in sarcoma patients. In the oncology field over the last years there has been great interest in the growth factor receptor blocking therapies. Both the epidermal growth factor receptor (EGFR) inhibitors and ERBB2 inhibitors have been proven beneficial in respectively non-small-cell lung, head- and neck, pancreatic and colorectal cancer (all EGFR) and breast cancer (ERBB2). In sarcoma patients EGFR or ERBB2 inhibitors have never been evaluated systematically.

Our results show positive membranous staining for EGFR in a variety of sarcoma subtypes, including liposarcomas (myxoid and pleomorphic), leiomyosarcomas (intra-

muscular), synovial sarcomas, malignant peripheral nerve sheath tumors (MPNST), rhabdomyosarcomas (pleomorphic and embryonal), solitary fibrous tumors, and angiosarcomas (deep). Membranous IHC staining for ERBB2 was negative in all sarcoma subtypes. These results show that treatment of these EGFR positive subtypes of sarcomas with EGFR blocking therapies can theoretically be effective.

Published reports on EGFR and especially ERBB2 expression in sarcomas have been contradicting.[7-18] Positive IHC staining for EGFR in soft tissue sarcomas ranges from 50 – 100%, with the highest number of positive cases in synovial sarcoma, myxofibrosarcoma, MPNST, and so-called malignant fibrous histiocytoma.[7-9,12,13,20,21] Yang et al reported EGFR positive staining in 3 out of 4 angiosarcomas, which is consisted with our findings. Positive IHC staining for ERBB2 ranges from 0-60%.[7-12,14-16] The results of IHC staining depend highly on the types of antibodies used, the time-span and method of fixation, and the absence of a uniform scoring system leads to a high interobserver variability.[22] This, together with the low number of sarcoma samples investigated, may be the reason for the previously described contradicting results.

In our study EGFR was considered positive when membranous staining was present. In most cases membranous staining was focal rather than diffuse in all malignant cells. When IHC staining for a growth factor receptor is focal a beneficial effect of receptor inhibiting therapies is less likely, while staining is not present in all malignant cells of that patient. However in our study, EGFR and ERBB2 staining was also diffusely present throughout the cell, consistent with cytoplasmatic rather than membrane expression. Previous reports in osteosarcoma cell-lines describe that even in tumors that have no membrane pattern on IHC staining, EGFR and ERBB2 receptors are located on the cell membrane.[9] The difference is that receptor levels expressed are much lower than in the epithelial malignancies in which the IHC staining for EGFR and ERBB2 was first used, which can prevent a clear immunodetection of the protein on the cell surface. Other possible explanations for the cytoplasmatic immunostaining are that the antibody binds to precursor forms of the EGFR/ ERBB2 protein in the cytoplasm or that the activated antibody-EGFR/ ERBB2 complex is internalized.[23]

Previous reports show that EGFR expression is more frequently found in synovial sarcomas than in other soft tissue tumors.[7,17,18] This resulted in the initiation of a phase II trial treating patients with synovial sarcoma with gefitinib therapy (an EGFR small molecule tyrosine kinase inhibitor) by the European Organization for Research and Treatment of Cancer (EORTC). However in recent reports in IHC positive synovial sarcomas, and other soft tissue sarcomas, there was no EGFR gene amplification seen by FISH, and positive effects of gefitinib therefore seemed less likely.[20,24] The results of this Phase II trial were reported recently and no substantial benefit of gefitinib monotherapy compared to conventional chemotherapy was seen.[25]

Questions are still rising whether IHC staining for EGFR is the correct method to evaluate EGFR status.[5] It is possible that other markers, like the level of activated phosphorylated EGFR or the presence of activating EGFR mutations, are more important. In non-small cell lung cancer (NSCLC) total EGFR expression does not relate to other clinical prognostic indicators, and does not relate to clinical response to EGFR inhibitors like erlotinib or gefitinib.[26] Phosphorylated EGFR (p-EGFR), EGFR gene copy number, and EGFR activating mutations have been shown to be better markers then EGFR overexpression by IHC for prediction of poor prognosis in NSCLC.[27,28] Future research should focus on the determination of markers that can predict a favorable outcome, like the association between activating mutations in the ATP-binding site of EGFR and response on gefitinib (Iressa) in non-small-cell lung cancer patients, or the association between KRAS mutations and resistance to cetuximab (Erbitux®) in colorectal carcinoma patients.[29,30]

In breast cancer there is a correlation between ERBB2 gene amplification and ERBB2 protein overexpression.[2,31] Studies in other tumor types, including osteosarcoma, Ewing sarcoma and synovial sarcoma report a ERBB2 protein overexpression with positive, mainly cytoplasmatic, IHC staining without amplification of ERBB2 gene by fluorescence in situ hybridization (FISH) evaluation, indicating that ERBB2 overexpression can be independent from gene amplification.[16,17] There was no effect of trastuzumab (ERBB2 inhibition) in osteosarcoma and Ewing sarcoma cell-lines.[15,17] Further studies are required to increase insight in the role of IHC and FISH evaluation of ERBB2 as predictive markers for response to ERBB2 inhibiting therapies in sarcomas.

Our results demonstrate that IHC staining for EGFR and ERBB2 shows cytoplasmatic staining in many subtypes of sarcomas, and membranous staining for EGFR in multiple subtypes of sarcomas. Therefore there is a possibility that sarcoma patients may benefit from EGFR inhibiting therapies. Benefit from ERBB2 blocking therapies is highly unlikely.

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4

A phase I dose escalation study of telatinib (BAY 57-9352), a tyrosine kinase inhibitor of VEGFR-2, VEGFR-3, PDGFR-β and c-Kit, in patients with advanced or metastatic solid tumors

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Abstract

Purpose

Telatinib (BAY 57-9352) is an orally available tyrosine kinase inhibitor of VEGFR-2, VEGFR-3, PDGFR-β, and c-Kit. This phase I dose escalation study was conducted to evaluate the safety and tolerability of telatinib, with additional pharmacokinetic, pharmacodynamic and efficacy assessments.

Patients and methods

Patients with solid tumors refractory to standard therapies or with no standard therapy available were enrolled. Doses of continuously administered telatinib were escalated from 20 mg od to 1500 mg bid.

Results

Fifty-three patients were enrolled. Most frequently observed drug-related adverse events were nausea (26.4%, grade ≥3: 0%) and hypertension (20.8%, grade 3: 11.3%, grade 4: 0%). Two DLTs were observed: one poorly controlled hypertension (600 mg bid), and one grade 2 weight loss, anorexia, and fatigue (1500 mg bid). A formal MTD was not reached. Telatinib was rapidly absorbed, with median t_{max} <3 hours post-dose. Nearly dose proportional increase in exposure was observed with substantial variability. Telatinib half-life averaged 5.5 hours. Biomarker analyses showed dose-dependent increase in VEGF levels and decrease in sVEGFR-2 levels, with a plateau at 900 mg bid. A decrease in tumor blood flow (K_{trans} and $IAUC_{60}$) was observed with DCE-MRI. Best tumor response was stable disease, observed in 50.9% of patients.

Conclusions

Telatinib was safe and well tolerated up to 1500 mg bid. Based upon pharmacodynamic and pharmacokinetic endpoints, telatinib 900 mg bid is the recommended dose for subsequent phase II studies.

Introduction

The vascular endothelial growth factor (VEGF) and its receptors (VEGFRs) play a pivotal role in tumor-related angiogenesis, and the VEGF/VEGFR pathway is an important target for anti-angiogenic drug development and tumor therapy.[1-8]

Telatinib (BAY 57-9352) is an orally available, potent inhibitor of VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR-β), and c-Kit tyrosine kinases. Telatinib inhibits VEGFR-2 autophosphorylation in a whole-cell assay of receptor autophosphorylation with an IC_{50} of 19 nM. Telatinib also inhibits VEGF-dependent proliferation of human umbilical vein endothelial cells (HUVECs) with an IC_{50} of 26 nM and PDGF-stimulated growth of human aortic smooth muscle cells with an IC_{50} of 249 nM. Telatinib demonstrates anti-tumor activity in various cancer models. Formation of the N-glucuronides of telatinib is identified as the major biotransformation pathway in man. Telatinib is metabolized by various CYP isoforms and UGT1A4.[9,10]

We performed a phase I, pharmacological, and biomarker study of telatinib. Objectives were to (1) determine maximum tolerated dose (MTD) and define dose-limiting toxicities (DLT), (2) characterize safety, (3) pharmacokinetics, and (4) biomarkers of biological activity, including serum markers and dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) results, and (5) evaluate anti tumor activity.

Patients and Methods

Eligibility criteria

Patients with histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 were eligible. Other inclusion criteria were: evaluable or measurable disease by RECIST; age ≥18 years; life expectancy ≥12 weeks; adequate bone marrow, liver, and renal function (hemoglobin ≥9.0 g/dl; absolute neutrophil count ≥1,500/mm^3; platelet count ≥100,000/mm^3; total bilirubin ≤1.5x the upper limit of normal (ULN); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5x ULN, (liver metastases AST/ALT <5x ULN); alkaline phosphatase ≤ 4x ULN; PT-INR and PTT <1.5x ULN; serum creatinine ≤1.5x ULN). Exclusion criteria were: history of cardiac disease; HIV, hepatitis B or C infection; active infection; serious non-healing wound, ulcer, or bone fracture; symptomatic metastatic brain or meningeal tumors unless >6 months from definitive therapy without evidence of tumor growth, and clinically stable; seizure disorder requiring anticonvulsant medication; history of organ allograft; pregnancy or breast-feeding; history of any condition that could endanger the safety of

the patient; anticancer treatment <4 weeks before the first dose; previous anti-angiogenic therapies/VEGFR-2 inhibitors.

Written informed consent from all patients and approval from the institutional review boards was obtained.

Drug Administration and Dose Escalation Procedure

Telatinib was administered orally, once daily (od) or twice daily (bid), on a continuous basis. Based upon toxicological data, pharmacokinetic data, and a parallel phase I study with telatinib administered in a "14 days on / 7 days off" schedule, the starting dose was 20 mg od. The formulations used in this study were: solution formulation (20 mg od cohort), 25 mg telatinib mesylate tablet formulation (75 mg od cohort), and 150 mg telatinib mesylate tablet formulation (bid dosing cohorts). For the purpose of analysis, one cycle was defined as 21 days of administration.

Doses were doubled for subsequent cohorts if no drug-related toxicity in cycle 1 was observed. When DLT had been observed or following toxicity ≥grade 2 in ≥2 patients, subsequent dose increments were 33-66%.

DLT was defined as grade 4 neutropenia ≥7 days, febrile neutropenia, grade 4 thrombocytopenia, grade 3 thrombocytopenic bleeding, and any drug-related grade 3 or 4 non-hematological toxicity excluding alopecia, nausea and vomiting not refractory to anti-emetics, and hypertension not refractory to anti-hypertensive medication during the first cycle.

If DLT was observed in one patient, three additional patients were recruited at that dose level, with dose escalation proceeding if <2 of 6 patients exhibited DLT. Because pharmacokinetic results of the initial 2 cohorts showed significant inter patient variability, all subsequent cohorts were expanded to a minimum of six patients. If DLT was observed in ≥2 of 3 or ≥2 of 6 patients, the maximum-tolerated dose (MTD) had been exceeded, and additional patients were recruited at the next lower dose level. The MTD was defined as the highest dose level that could be given to 6 patients with <1 patient experiencing DLT. If a patient experienced a drug related DLT, telatinib was withheld for up to 3 weeks. If toxicity resolved to .grade 1, the dose of telatinib was reduced to the next lower dose level. Otherwise, the patient was withdrawn from the study. Administration of telatinib was continued until disease progression or unacceptable toxicity.

One additional cohort of 4 patients was enrolled (as part of a larger group in a companion study) to evaluate the bioavailability of a new 300 mg mesylate tablet formulation in comparison to the 150 mg mesylate tablet formulation. Patients received a single dose of 900 mg using the 300 mg tablet and continued with 150 mg tablets.

Pre-treatment Evaluation and Safety Assessment

Pre-treatment evaluation consisted of a complete medical history, physical examination, ECOG performance status assessment, vital signs, baseline 12 lead ECG, blood sample for complete blood count (CBC), coagulation analysis, biochemistry analysis, sample for urinalysis, serum pregnancy test, plasma and urine sampling for biomarkers, baseline tumor measurements, and DCE-MRI.

On days 1 and 14 of each cycle evaluation consisted of a brief history and physical examination, vital signs, blood samples for CBC, biochemistry, and coagulation analysis, urinalysis, 12-lead ECG. Response evaluation was performed every 2 cycles and was assessed according to RECIST.[11] Patients were evaluated weekly in the first cycle and every 1 or 2 weeks in additional cycles for adverse events and toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0.

Pharmacokinetic Evaluation

Pharmacokinetic (PK) evaluation was performed by collecting blood samples on days 1 and 14 of cycle 1, and day 14 of cycles 2 and 4 via an indwelling intravenous catheter. In cycle 1, a 5 mL sample was collected pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12 hours post-dose. An additional sample was collected at 24 hours post-dose for once daily regimen. In cycles 2 and 4, an abbreviated sampling schedule was used. Pharmacokinetic parameters C_{max}, t_{max}, $AUC_{0\text{-}tn}$, $AUC_{0\text{-}24}$ (for od regimen), $AUC_{0\text{-}12}$ (for bid regimen) and half-life for telatinib and its metabolite (BAY 60-8246) were calculated by non-compartmental analysis using WinNonlin (version 4.1.a).

Pharmacodynamic Analysis

Urine samples and 20 ml blood samples for pharmacodynamic (PD) analysis were collected at baseline, pre-dose and 8 hours post-dose on days 1 and 14 of cycles 1 and 2 and on day 1 of cycle 3, and pre-dose on day 1 of each subsequent cycle. The following parameters were measured: plasma soluble VEGFR-2 (sVEGFR-2), plasma VEGF, plasma basic fibroblast growth factor (bFGF), plasma IL-8, urinary VEGF. Samples were analyzed using the relevant quantitative enzyme linked immunosorbent assay (ELISA; R&D Systems Europe, Oxford, UK) according to the manufacturer's instructions.

DCE-MRI scans were performed at baseline, on day 2 of cycle 1, and on day 14 of cycles 2 and 3. We used a 1.5-T MR imaging system (Philips Medical Systems, Best, The Netherlands) using a body coil in retroperitoneal and abdominal lesions. . The tumors were localized using standard T1- and T2- fat-saturated fast spin echo sequences. Subsequently, dynamic MR imaging was performed using T1-weighted turbo field-

echo sequence with TR 5.4/TE 1.4, flip angle of 20°, nonselective inversion preparatory pulse, with delay time of 165 msec, and section thickness of 5–8 mm, with a temporal resolution, of 3 seconds during at least the first 84 seconds. Total acquisition time lasted 5 min. A power injector (Spectris; Medrad, Indianola, Pa) with injection flow rate of 2 mL/sec was used to start intravenous administration of gadopentetate dimeglumine (Magnevist, Bayer-Schering, Berlin, Germany), which was followed by a 20-mL saline flush. Bolus injection was initiated 5 seconds after the start of data acquisition.[12]

Assessed parameter was Ktrans, describing the volume transfer coefficient of contrast between blood plasma and the tumor. Empirical quantitative methods were used to quantify the signal-intensity time curve using the initial area under the contrast-agent concentration-time curve after 60 seconds ($iAUC_{60}$) and time to peak enhancement (TTPE; time period between arterial enhancement and the enhancement of the index lesions).[13,14] The second pre-contrast dynamic images were automatically subtracted from all dynamic contrast-enhanced MR images using software of the MR system.

Statistical analysis

Continuous variables are presented as mean values ± standard deviation and categorical variables as frequencies (percentages), unless otherwise stated. Comparison between variables at baseline and post-dose was performed with paired samples t-test or Wilcoxon signed rank test as appropriate. Correlations with drug exposure were assessed by Spearmans Rank correlation coefficient. All analyses were performed using SPSS version 12.01 (SPSS, Chicago, Ill, USA) and were two-sided, with a level of significance of $\alpha=0.05$.

Results

Between July 2004 and October 2006, 53 patients were enrolled. Patient characteristics are summarized in Table 1.

Safety and Tolerability

All treatment-related adverse events are summarized in Table 2. Most frequently reported treatment-related adverse events were nausea (26.4%) and hypertension (20.8%). Six episodes of grade three drug-related hypertension were observed. There was no apparent dose relationship. Grade 4 drug-related hypertension was not observed. Hypertension was easily manageable with anti-hypertensive medication in most cases.

Table 1. Baseline demographics and patient characteristics

Baseline characteristics	Patients (n (%))
Gender	
Male	29 (55)
Female	24 (45)
Age, years	
Median (range)	55 (17-76)
ECOG performance status	
0	15 (28)
1	32 (60)
2	3 (6)
Not reported	3 (6)
Prior anticancer therapies	
Surgery	51 (96)
Systemic anticancer therapy	45 (85)
Number of previous treatments (range)	2.5 (0-13)
0-1	20 (38)
2-5	29 (55)
>5	4 (8)
Radiation therapy	19 (36)
Tumor type	
Soft tissue sarcoma	11 (21)
Colorectal cancer	10 (19)
Renal cell cancer	5 (9)
Esophageal cancer	5 (9)
Other	22 (42)
Ovarian cancer	3 (6)
Osteosarcoma	3 (6)
Adrenal cancer	3 (6)
Cholangiocarcinoma	3 (6)
Melanoma	3 (6)
Pancreatic cancer	2 (4)
Bladder cancer	1 (2)
Chordoma	1 (2)
Anal cancer	1 (2)
Neuroendocrine carcinoma	1 (2)
Prostate cancer	1 (2)

ECOG: Eastern Cooperative Oncology Group

Table 2. Number of patients with treatment-related adverse events

Adverse Event	Cohort 1 20 mg od n=4		Cohort 2 75 mg od n=6		Cohort 3 150 mg od n=6		Cohort 4 300 mg od n=6		Cohort 5 600 mg od n=6		Cohort 6 900 mg once, bid later n=4		Cohort 7 900 mg od n=15		Cohort 8 1500 mg od n=6		Total incidence n=53
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Any grade n (%)
Any event	3	–	1	1	2	1	3	1	2	2	2	–	8	3	5	–	34 (64.0)
Hypertension	–	–	–	1	1	1	1	1	–	1	–	–	3	2	–	–	11 (20.8)
Hematologic toxicity																	
Anemia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
Leukopenia	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	1 (1.9)
Thrombopenia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
GI toxicity																	
Anorexia	–	–	–	–	1	–	–	–	1	–	–	–	2	–	1	–	5 (9.4)
Constipation	–	–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	2 (3.8)
Diarrhea	–	–	–	–	–	–	1	–	1	–	1	–	2	–	3	–	8 (15.1)
Nausea	1	–	–	–	2	–	1	–	3	–	2	–	4	–	1	–	14 (26.4)
Vomiting	1	–	–	–	1	–	1	–	1	–	–	–	2	–	1	–	7 (13.2)
Metabolic toxicity																	
AST/ALT	–	–	–	–	–	–	–	–	–	1	–	–	–	–	1	–	2 (3.8)
Constitutional toxicity																	
Fatigue	–	–	–	–	–	–	1	–	–	–	–	–	3	–	3	–	7 (13.2)
Dermatological toxicity																	
Dry skin	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	2 (3.8)
HFS	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1 (1.9)
Miscellaneous																	
Hemorrhage	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1 (1.9)
Headache	–	–	–	–	–	–	–	–	3	–	1	–	1	1	–	–	6 (11.3)
Hoarseness	–	–	–	–	–	–	1	–	–	–	–	–	6	–	3	–	10 (18.9)

GI: gastro-intestinal, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HFS: hand-foot syndrome

Two DLTs were observed. At 600 mg bid one episode of poorly controlled hypertension in a patient with metastatic renal cell carcinoma, prior nephrectomy and pre-existing hypertension was observed. Despite addition of a third antihypertensive agent and two dose reductions, grade 3 hypertension persisted and telatinib was permanently discontinued. At 1500 mg bid one episode of the combination of persistent grade 2 weight loss, grade 2 anorexia, and grade 2 fatigue was felt to be intolerable by the patient and therefore was considered DLT. Despite two dose reductions, this patient did not tolerate telatinib. Four additional patients experienced possible drug-related adverse events requiring dose reduction, interruption or discontinuation. One patient at 300 mg bid reported grade 2 diarrhea requiring permanent discontinuation of telatinib. One patient at 600 mg bid experienced grade 3 AST and ALT elevation, normalizing after dose reduction. One patient at 900 mg bid with well-controlled pre-existing hypertension reported grade 3 headache requiring two dose reductions of telatinib. One patient at 1500 mg bid discontinued telatinib following an episode of otherwise uncomplicated grade 3 esophageal varices bleeding. Due to the low incidence of treatment-related DLT, a formal MTD could not be defined.

Pharmacokinetics

Telatinib pharmacokinetic parameters are summarized in Table 3. Telatinib was rapidly absorbed, with t_{max} values observed less than 3 hours post-dose.

Although an overall dose proportional increase in exposure was observed in the 150-1500 mg bid dose range, high interpatient variability was observed, similar to that observed with other VEGF-R or EGF-R tyrosine kinase inhibitors.[15-20] In the intermediate dose levels (e.g. 300 mg BID and 600 mg BID) deviation from dose proportionality was observed likely due to pharmacokinetic variability. Plasma half-life of telatinib averaged 5.5 hours and is consistent with the observation that steady-state is achieved within the first 14 days of telatinib administration. A limited number of patients provided cycle 4 pharmacokinetic samples, yielding comparable results at cycle 2 day 14 and cycle 4 day 14.

There was no correlation between telatinib exposure and toxicity or time to progression. This is partly due to the low incidence of some of the toxicities and the relatively small number of patients per cohort.

Comparison of geometric mean AUC of telatinib and its metabolite BAY 60-8246 indicate that exposure to the metabolite is less than 20% of exposure to parent compound.

In a cohort of four patients in whom bioavailability of the 300 mg mesylate tablet was compared to that of the 150 mg mesylate tablet, high interpatient variability in the pharmacokinetic parameters precluded a definitive conclusion.

Table 3. Geometric mean (% coefficient of variation) of telatinib pharmacokinetic parameters, cycle 1 day, cycle 1 day 14 and cycle 2 day 14.

	Cohort						
	20 mg od (n = 4)	**75 mg od** (n = 6)	**150 mg bid** (n = 6)	**300 mg bid** (n = 6)	**600 mg bid** (n = 6)	**900 mg bid** (n = 15)	**1500 mg bid** (n = 6)
				Cycle 1 Day 1			
C_{max}, mg/L	0.106 (65%)	0.166 (85%)	0.113 (51%)	0.455 (129%)	0.597 (143%)	0.629 (81%)	1.767 (94%)
t_{max}, h [a]	2 [0.5 – 2]	2.5 [1 – 4]	3 [0.5 – 4]	3.5 [0.5 – 6]	3 [0.5 – 6.3]	2 [0.5 – 4]	1.5 [0.5 – 3]
$AUC_{(0-12)}$, [b] mg×h/L	0.596 (55%)	0.921 (102%)	0.590 (52%)	2.286 (145%)	4.592 (112%) [c]	3.735 (58%)	7.659 (79%)
Half-life, h	3.80 (12%)	4.05 (38%)	3.19 (32%)	3.96 (22%)	3.62 (23%) [c]	6.02 (87%)	3.58 (24%)
				Cycle 1 Day 14			
C_{max}, mg/L	0.135 (29%)	0.185 (58%)	0.188 (55%)	0.795 (71%)	0.822 (91%)	1.135 (60%)	1.608 (55%)
t_{max}, h [a]	2 [1 – 4]	3.5 [1 – 24]	2.5 [0.5 – 4]	2 [0.5 – 3.1]	2 [1 – 3]	1.5 [0.5 – 4]	4.5 [2 – 8]
$AUC_{(0-12)}$, [b] mg×h/L	1.082 (43%)	1.554 (30%)	1.187 (55%)	4.887 (62%)	5.060 (97%)	6.521 (49%)	12.227 (67%)
Half-life, h	5.06 (42%)	5.58 (59%)	6.91 (196%)	5.22 (66%)	5.26 (78%)	5.66 (67%)	5.42 (26%) [d]
				Cycle 2 Day 14			
C_{max}, mg/L	0.162 (25%)	0.163 (102%)	0.179 (34%)	0.482 (108%)	0.965 (86%)	0.880 (42%)	0.990 (131%)
t_{max}, h [a]	2 [0.5 – 3]	2 [1 – 10]	4.1 [1 – 8.2]	3.4 [0.5 – 5]	3 [0.6 – 3]	2 [0.5 – 4]	0.6 [0.5 – 8]
$AUC_{(0-12)}$, [b] mg×h/L	1.056 (34%)	1.117 (132%) [c]	1.101 (44%) [c]	3.203 (104%)	4.393 (115%)	5.647 (35%)	8.40 (70%) [c]
Half-life, h	4.73 (8%)	5.89 (156%) [c]	8.73 (211%) [c]	6.13 (94%)	5.45 (87%)	8.36 (72%)	4.59 (92%) [c]

a: Median [range], b: For once daily cohorts $AUC_{(0-24)}$, mg×h/L is reported, c: Sample size reduced by 1, d: Sample size reduced by 2

Pharmacodynamics

SVEGFR-2 and VEGF plasma levels

Changes in plasma levels of VEGF and sVEGFR-2 in relation to telatinib dose are summarized in figure 1A and 1B. Over the dose range studied, increasing exposure to telatinib resulted in lower plasma sVEGFR-2 levels (both pre-dose and post-dose) after 14 con-

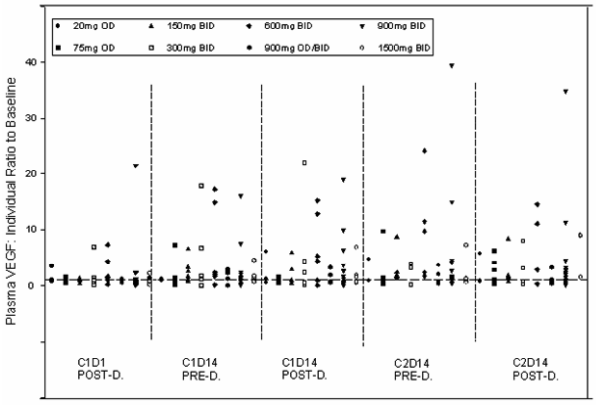
Table 4. Combined pharmacodynamic results (DCE-MRI and plasmalevels of VEGF and sVEGFR-2) : baseline and post-dose median Ktrans, $IAUC_{60}$ and TTPE values per cohort as well as plasma levels of VEGF, sVEGFR-2

		Cohort								Correlations	
		20 mg od	75 mg od	150 mg bid	300 mg bid	600 mg bid	900 mg bid	1500 mg bid	All cohorts	Telatinib $AUC_{(0\text{-}tn)}$ C1D14	Telatinib Cmax C1D14
Ktrans	N	0	0	0	0	1	13	2	16		
(min^{-1})	Pre					3.27	2.99	5.99	3.31	R = -0.285	R = -0.132
	Post					6.25	1.60	0.63	1.62	p = 0.284	p = 0.625
	% Change					+91.1	-46.7	-89.5	-51.2		
$IAUC_{60}$	N	0	0	0	0	1	13	2	16		
(mmol/ l*s)	Pre					14.14	19.26	4.10	17.07	R = -0.135	R =-0.085
	Post					14.12	17.35	2.51	14.47	p = 0.617	p = 0.753
	% Change					-0.1	-9-.9	-38.7	-15.2		
TTPE	N	1	4	3	4	4	15	1	32		
(sec)	Pre	3.00	4.55	6.00	4.95	9.80	3.20	5.80	5.25	R = 0.374	R = 0.228
	Post	3.00	4.55	6.00	5.65	9.85	5.60	11.20	5.60	p = 0.035*	p = 0.209
	% Change	0.0	0.0	0.0	+14.1	+0.6	+75.0	+93.1	+6.67		
VEGF	N	3	6	6	5	6	20	6	52		
(pg/ml,	Baseline	92.2	121.6	78.2	68.0	38.6	112.8	237.8	114.3	R = -0.222	
median)	C1D14	103.9	116.1	139.6	105.4	211.6	261.2	372.1	237.2	p = 0.140	
	Ratio mean	1.05	0.84	2.09	1.90	2.62	1.75	1.68	1.57		
SVEGFR-2	N	3	6	7	5	6	20	6	53		
(pg/ml,	Baseline	7276.1	6772.2	6500.3	7106.7	7736.9	7461.7	8000.9	7167.4	R = -0.035	
median)	C1D14	5932.1	6119.7	5866.1	5840.2	5507.3	5689.4	4919.6	5820.7	p = 0.810	
	Ratio mean	0.95	0.95	0.90	0.88	0.75	0.73	0.64	0.80		

N: number of patients, TTPE: time to peak enhancement, Ratio is mean value to baseline at cycle 1 day 14
* correlation is significant at the 0.05 level

Fig 1. Biomarker results: plasma VEGF (fig 1A) and sVEGFR-2 (fig 1B) levels; individual patient's ratios over baseline value for cycle 1 day 1 through cycle 2 day 14, pre-dose (PRE-D) and post-dose (POST-D). Plasma sVEGFR-2 ratio over baseline value versus telatinib AUC_{0-24} on cycle 1 day 14 (fig 1C) and versus telatinib C_{max} on cycle 1 day 14 (fig 1D)

1A



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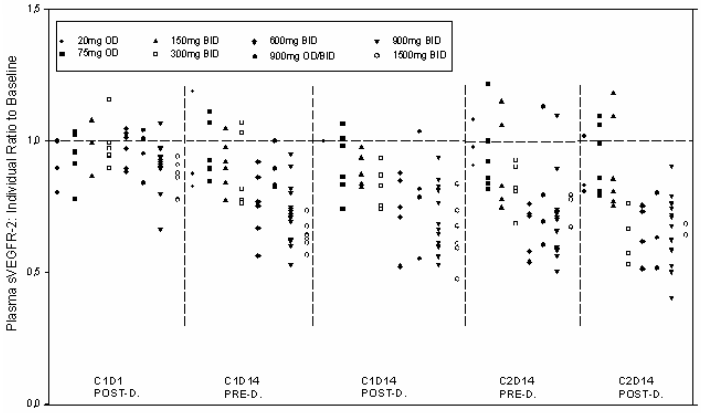
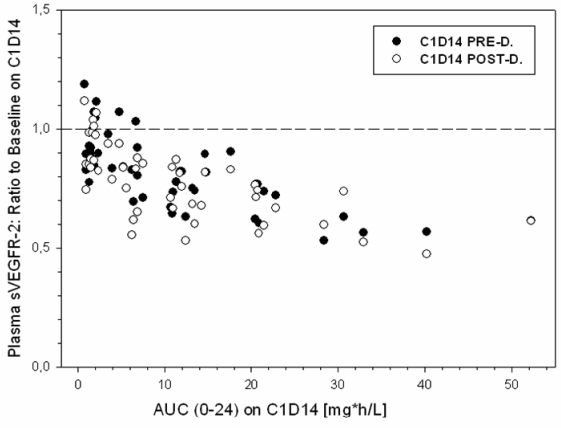


1C



1D

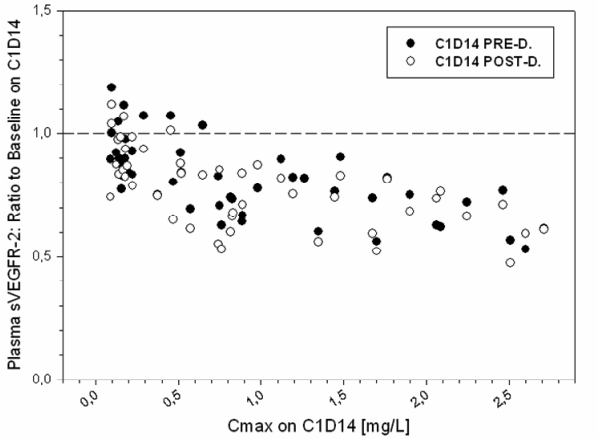


Table 5. Best Tumor Response

Cohort	N	Best Tumor Response		
		Stable disease	Progressive disease	Unknown
20 mg od	4	2	2	–
75 mg od	6	2	4	–
150 mg bid	6	2	4	–
300 mg bid	6	3	3	–
600 mg bid	6	4	1	1
900 mg once, bid later	4	2	2	–
900 mg bid	15	8	7	–
1500 mg bid	6	4	2	–

tinuous days of dosing (Figures 1C and 1D, table 4) There was no statistical correlation between dose of telatinib and plasma levels of VEGF and sVEGFR-2. Changes in plasma levels of VEGF and sVEGFR-2 plateaued at 900 mg bid, suggesting a saturable effect. There were no consistent changes in plasma levels of bFGF, and IL-8 and urinary levels of VEGF.

DCE-MRI

Reproducible DCE-MRI results for screening and at least for one post screening assessment were available from 16 subjects for evaluation of Ktrans and $iAUC_{60}$, and from 32 patients for evaluation of TTPE. DCE-MRI data for evaluation of Ktrans and $iAUC_{60}$

were missing from 37 patients for several reasons: no DCE-MRI performed (n=17), analysis unreliable due to poor quality, i.e. low signal-to-noise ratio, interference artifacts (n=14), only one scan performed (n=4), no contrast agent given (n=1), unknown (n=1). DCE-MRI data for evaluation of TTPE were missing from 21 patients for the following reasons: no DCE-MRI performed (n=17), only one scan performed (n=1), no contrast agent given (n=1), unknown (n=2).

DCE-MRI results are summarized in Table 4. For TTPE, a clear dose-response relationship was seen. TTPE changes from baseline were positively correlated to telatinib AUC.

Anti tumor activity

A disease control rate (DCR) of 50.9% was observed with 27 of 53 patients having stable disease as best tumor response (Table 5). Disease control for 6-12 months was seen in 3 patients, 12-18 months in 2, and >18 months in 4 patients. There were no complete or partial responses, however, some degree of tumor shrinkage was observed in 16 patients (30.2%).

Discussion

In this phase I dose escalation study we explored tolerability, safety and biological activity of the selective VEGFR tyrosine kinase inhibitor telatinib (BAY 57-9352).

With regard to safety, the most frequently reported treatment-related adverse events were nausea (26.4%) and hypertension (20.8%). Nausea occurred throughout all dose levels and was mild. Hypertension was easily managed with a maximum of two anti-hypertensive agents in all but one patient. Based upon previous experience and considering the potential underlying mechanisms of the observed hypertension, angiotensine converting enzyme inhibitors and calcium antagonists were most frequently prescribed. It is conceivable that hypertension should be considered an indication of biological activity of VEGF inhibitors rather than as side-effect.[1,3,15,21-25]

As only one out of 6 patients at 1500 mg bid experienced DLT (combination of grade 2 weight loss, anorexia, and fatigue), we formally could not define the MTD of telatinib based upon clinical toxicity. Eventhough grade 2 toxicity formally did not define DLT in this study, on ongoing (combination of) grade 2 toxicity induced by continuous drug administration must be considered to be cumbersome and therefore can define as intolerable.

In our study, pharmacokinetics of telatinib were dose proportional in the overall dose range studied, albeit with substantial interpatient variability and deviation from dose proportionality in the intermediate dose levels. This observation may be attributed to in-

herent variability in absorption and/or metabolism of telatinib, as well as various patient and tumor characteristics. In a parallel study with telatinib, a markedly less than dose proportional increase in exposure was observed at dose levels exceeding 900 mg bid.[26]

Telatinib induced changes in plasma levels of VEGF and sVEGFR-2 that are consistent with findings in trials with telatinib and other VEGFR inhibitors.[15,16,19,26,27] These changes plateaued at 900 mg bid suggesting a saturable effect.

Based upon the combined analysis of pharmacokinetic and pharmacodynamic results observed in the two dose escalation studies with telatinib, and based upon practical issues such as number of tablets to be taken, we defined 900 mg bid as the dose recommended for phase II studies. Based upon the mechanism of action of VEGFR-2 tyrosine kinase inhibitors, a continuous dosing schedule may prove to have optimal activity, and therefore studies exploring continuous administration of telatinib in combination with various anticancer therapies have been initiated.[28]

DCE-MRI analysis revealed changes in TTPE that are correlated to telatinib exposure. Similar studies with other angiogenesis inhibitors support our results.[29-31] A trend to a dose-effect relationship was seen, but no significant correlation could be assessed. We could not determine a statistical correlation between DCE-MRI results and clinical outcome such as disease control rate (data not shown separately). Eventhough DCE-MRI analyses should be considered a non-validated technique, results obtained in our study indicate an antiangiogenic effect of telatinib and seem to support the results of additional analyses of changes in flow mediated dilatation (FMD), nitroglycerin-mediated dilatation (NMD), and capillary density that were done in this study and are reported separately.[32]

Determining antitumor activity of telatinib was a secondary endpoint of this study. Complete or partial responses were not observed in this study, but some minor tumor regressions and prolonged periods of disease stabilization are indicative of anti-tumor activity and merit confirmation in a phase II study program. Among cases of prolonged disease stabilization is a young patient with an epitheloid hemangio-endothelioma of the scalp who is now on medication for more than three years.

Two VEGF tyrosine kinase inhibitors (sunitinib and sorafenib) have gained regulatory approval. Telatinib may have some theoretical advantages over sunitinib and sorafenib. Theoretically, side effects like thyroid dysfunction, cardiac function impairment, and reversible posterior leukoencephalopathy syndrome observed with sunitinib or sorafenib may be caused by blocking pathways not described in the pre-clinical or clinical studies or by the redirection of signals through other pathways.[33-38] These side effects can therefore by agent-specific and to date, albeit in a relatively small number of patients, telatinib has not induced any of these side effects.

Compared to telatinib, vatalanib (PTK787/ZK222584) seems to have some similarities. In our opinion, telatinib has potential benefit over vatalanib. The IC_{50} of vatalanib for VEGFR-3, c-Kit, and PDGFRβ are respectively 18, 20, and 16 times higher than the IC_{50} for VEGFR-2. For telatinib these IC_{50}'s are 0.66, 0.17 and 2.5 times higher, respectively. Activation of VEGFR-3 in lymphatic endothelial cells can facilitate lymphangiogenesis and lymphatic spread of tumor cells.[39] Therefore, theoretically, the superior potency of telatinib compared to vatalanib with regard to VEGFR-3 inhibition will hopefully translate into increased clinical efficacy. Future studies will have to prove this optimism.

In conclusion, telatinib (BAY 57-9352) administered as continuous treatment is safe and well tolerated. Based upon the combined analysis of clinical, pharmacodynamic, and pharmacokinetic endpoints, 900 mg bid is the dose recommended for future phase II studies.

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5

Pharmacogenetics of telatinib, a VEGFR-2 and VEGFR-3 tyrosine kinase inhibitor, used in patients with solid tumors

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Submitted

Abstract

Purpose

Telatinib is an orally active small-molecule tyrosine kinase inhibitor of kinase insert domain receptor (*KDR; VEGFR-2*) and fms-related tyrosine kinase 4 (*FLT4; VEGFR-3*). This study aims at the identification of relationships between single-nucleotide polymorphisms (SNPs) in genes encoding for transporter proteins and pharmacokinetic parameters in order to clarify the significant interpatient variability in drug exposure. In addition, the potential relationship between target receptor polymorphisms and toxicity of telatinib is explored.

Methods

Blood samples from 33 patients enrolled in a phase I dose-escalation study of telatinib were analyzed. For correlation with dose normalized $AUC_{(0\text{-}12)}$, ATP-binding cassette (ABC) B1 *(ABCB1)*, *ABCC1*, and *ABCG2* were the genes selected. For correlation with telatinib toxicity, selected genes were the drug target genes *KDR* and *FLT4*.

Results

No association between dose normalized $AUC_{(0\text{-}12)}$ and drug transporter protein polymorphisms was observed. In addition, no association between toxicity and *KDR* or *FLT4* genotype or haplotype was seen.

Conclusions

Our pharmacogenetic analysis could not reveal a correlation between relevant gene polymorphisms and clinical and pharmacokinetic observations of telatinib.

Introduction

Single nucleotide polymorphisms (SNPs) in genes encoding for drug transporters and drug targets contribute to interindividual heterogeneity of drug efficacy and toxicity in cancer therapy.[1,18] This type of research is referred to as pharmacogenetics. In our current study we analyze pharmacogenetic factors likely to be involved in telatinib disposition and mechanism of action.

Telatinib (BAY 57-9352) is an orally active, small-molecule tyrosine kinase inhibitor of kinase insert domain receptor (*KDR;* vascular endothelial growth factor receptor *(VEGFR)-2*) and fms-related tyrosine kinase 4 (*FLT4; VEGFR-3*). Telatinib is metabolized by various cytochrome P450 (CYP) isoforms including *CYP3A4*/*3A5*, *CYP2C8, CYP2C9*, and *CYP2C19* as well as by uridine diphosphate glucuronosyltransferase 1A4 (*UGT1A4*), with the formation of the N-glucuronides of telatinib as the major biotransformation pathway in man. In vitro studies showed telatinib to be a weak substrate of the adenosine triphosphate binding cassette (ABC) B1 (*ABCB1*) transporter.

In a phase I and pharmacological study we showed that pharmacokinetics (PK) of telatinib were dose proportional (manuscript accepted by JCO, see Chapter 4). However, substantial interpatient variability was observed (C_{max} and $AUC_{(0\text{-}12)}$ % coefficient of variation 20-150%) and no clear association between telatinib exposure and toxicity could be established. However, in this class of agents an increase in toxicity is generally observed with increasing dose.[3,17] Although in general limited information on drug metabolism and toxicity is available in early stages of drug development, pharmacogenetic research may be valuable. For example, if significant side effects could be linked to a certain drug transporter polymorphism, this could influence further drug development or could become an important issue in patient selection.

The current study examines the potential relationships between SNPs in genes coding for transporter proteins and pharmacokinetic parameters of telatinib in order to identify factors contributing to the significant interpatient variability in drug exposure. In addition, this study explores the potential relationship between target receptor polymorphisms and toxicity of telatinib.

Methods

This study was conducted in a subset of patients enrolled into a two-centre, phase I dose-escalating study of telatinib (manuscript accepted by JCO, see Chapter 4). The aim of this exploratory pharmacogenetic study was to identify possible relationships between SNPs in genes coding for drug transporters and PK parameters; and drug target

related SNPs and side effects of telatinib. From 33 of the 53 patients treated in the phase I study residual blood samples were available for pharmacogenetic analyses. Demographic, toxicity and pharmacokinetic characteristics were comparable for included and excluded patients (data not shown). Four of these 33 patients were treated with telatinib oral solution or 25 mg tablets, the remaining patients with 150 mg tablets.

Since bioavailability of the telatinib formulations differ, a decision was made to restrict the current analysis to one telatinib formulation. Therefore, in the association analysis with PK, only the 29 patients treated with the 150 mg tablets were included.

Patients and samples

Eligibility criteria, drug administration procedures and clinical and pharmacokinetic results are described in detail elsewhere (manuscript accepted by JCO, see Chapter 4). Briefly, patients with histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 were eligible. Telatinib was administered orally, once daily (od) or twice daily (bid), on a continuous basis. The clinical trial had a standard 3+3 phase I dose escalation study design. Because of significant interpatient variability in pharmacokinetics the decision was made to expand all cohorts to a minimum of six patients from the second cohort onwards. Response evaluation was performed every 2 cycles and was assessed according to RECIST.[19]

Residual blood samples taken for the routine patient care were stored at -20ºC at the local hospital laboratories. One frozen blood sample for each patient was collected from the two participating hospitals (Leiden University Medical Center, Leiden and Erasmus Medical Center, Rotterdam). All samples were anonymized by a third party, according to the instructions given in the "Code of Conduct for the use of data in Health Research" and "Code for Proper Secondary Use of Human Tissue" (www.federa.org). Approval from the institutional medical ethical review boards was obtained.

Pharmacokinetic and toxicity parameters

PK evaluation was performed by collecting blood samples on days 1 and 14 of cycle 1, and day 14 of cycles 2 and 4. Pharmacokinetic parameters were calculated by non-compartmental analysis using WinNonlin (version 4.1.a).

In this study cycle 1 day 14 (representing steady-state) dose normalized $AUC_{(0-12)}$, calculated as $AUC_{(0-12)}$/actual dose administered, was selected as the PK parameter to associate with transporter genetic polymorphisms.

Patients were evaluated for adverse events and toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0. In general, the NCI-CTC toxicity score distinguishes between mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening or disabling toxicity (grade 4) and death related to adverse events (grade 5). Telatinib administration resulted in limited toxicity. Grade 3-4 toxicity was only seen in 3 patients. Therefore, despite the fact that grade 3-4 toxicity is more clinically relevant, the occurrence of any grade 1-4 toxicity was considered to be the best candidate parameter for association analyses with drug target receptor genetic polymorphisms. Since toxicity observed in the first cycle was limited we decided to use overall toxicity observed in all treatment cycles for statistical association studies. In addition, hypertension is considered to be one of the more serious telatinib side effects, and grade 1-4 hypertension was also selected for association analyses.

Selection of candidate genes

Candidate genes were selected based on the information of preclinical pharmacology studies as reported in the Investigator's brochure (Bayer Pharmaceutical Corporation, data on file). For association with PK parameters *ABCB1*, *ABCC1*, and *ABCG2* were the genes selected. For correlation with telatinib toxicity selected genes were the drug target genes encoding *KDR* and *FLT4*. For the major biotransformation pathway in man, the formation of the N-glucuronides through *UGT1A4*, no SNP met the criteria for selection described below.

The SNPs were selected, taking into consideration one or more of the following criteria: validated SNP assay, SNP causes preferably non-synonymous amino acid change, indications for clinical relevance from previous publications, and a preferred minor genotype frequency of ~10%.

DNA extraction and SNP analysis methods

DNA was isolated from whole blood samples with MagNA Pure DNA Isolation kit (Roche Diagnostics, Almere, The Netherlands). DNA concentrations were quantified using a NanoDrop spectrophotometer (Isogen, IJsselstein, The Netherlands). Taqman assays were obtained from Applied Biosystems (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). As a quality control, 4 samples were genotyped in duplicate for all assays and 2 assays were tested in duplicate on all samples. As negative controls water was used. Overall, no inconsistencies were observed in the results.

The following SNPs were analyzed: *ABCB1* 3435C>T (rs1045642), *ABCB1* 1236C>T (rs1128503), *ABCB1* 2677G>A/T (rs2032582), *ABCB1* -129T>C, *ABCC1* C>G (rs129081),

ABCC1 825T>C (rs246221), *ABCC1* 1062T>C (rs35587), *ABCC1* 2012G>T (rs45511401), *ABCG2* 346G>A (rs2231137), *ABCG2* 421C>A (rs2231142), *FLT4* 1480A>G (rs307826), *FLT4* 2670C>G (rs448012), *KDR* 1719A>T (rs1870377), *KDR* -604T>C (rs2071559), and *KDR* 1192G>A (rs2305948).

SNP genotyping was performed with BIOMARK 48.48 dynamic array (Fluidigm Corporation, South San Francisco, CA, USA). All assays were performed according to protocols provided by the manufacturer.

Statistical analysis

Differences in pharmacokinetic and toxicity parameters among genotypes were analyzed by Student's t-test, ANOVA or Kruskal-Wallis test for continuous variables or chi-square test for dichotomous variables where appropriate. For toxicity, differences in genotype distribution were tested by 3 x 2 cross-tabulations for each genotype, and by 2 x 2 cross-tabulations for carriers versus noncarriers, with analysis by 2-sided chi-square test.

Polymorphisms within a gene were tested with the chi-square test (P-value < 0.05) to detect linkage disequilibrium (LD). If LD between SNPs was detected, haplotypes were determined for each individual with gPLINK (http://pngu.mgh.harvard.edu/purcell/plink/).[11] No phase uncertainty in the defined haploblocks and haplotypes (Rh ^ 2> 0.98) was seen.

Associations between the number of copies of a haplotype and clinical parameters were performed using a chi-square test for dichotomous variables and Student's t-test, ANOVA or Kruskal-Wallis test for continuous variables.

All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL) and were two-sided, with a level of significance of $\alpha=0.05$.

Results

Baseline patient characteristics, observed treatment-related toxicities, pharmacokinetics and treatment duration are presented in Table 1. Telatinib doses used were 20 mg od (n=2), 75 mg od (n=2), 150 mg bid (n=4), 300 mg bid (n=4), 600 mg bid (n=3), 900 mg bid (n=16), and 1500 mg bid (n=2). Our population comprised 100% Caucasians with 45% males and 55% females. Most frequent tumor types were soft tissue sarcomas (27%) next to colorectal cancer (15%) together with a high number of other tumor types (58%) consistent with the phase I nature of the clinical study. Median number of treatment courses was 5.5, ranging from 1 to 30, with one course being 3 weeks of telatinib administration.

Table 1. Patient characteristics, overall telatinib-induced toxicity, pharmacokinetic results and outcome data of telatinib treated patients.

Characteristics	Patients (n (%))
Gender	
Male	15 (45)
Female	18 (55)
Age, years	
Mean (range)	53 (22-77)
Tumor type	
Soft tissue sarcoma	9 (27)
Colorectal cancer	5 (15)
Adrenal cancer	3 (9)
Ovarian cancer	3 (9)
Cholangiocarcinoma	2 (6)
Esophageal cancer	2 (6)
Melanoma	2 (6)
Miscellaneous	7 (21)
ECOG performance score	
0	9 (27)
1	24 (73)
Nr of previous treatment lines	
Mean (range)	3 (0-13)
Toxicity	
Any toxicity grade 1-4	23 (70)
Any toxicity grade 3 or 4	3 (9)
Hypertension grade 1-4	7 (21)
Hypertension grade 3 or 4	2 (6)
Pharmacokinetic parameters	
Mean (range)	
Dose normalized AUC(0-12)	
(μg.hr/L per mg of dose)	9.26 (0.98-34.60)
Number of treatment courses	
Mean (range)	5.5 (1-30)

ECOG: Eastern Cooperative Oncology Group
Dose normalized AUC: area under the curve/dose

Telatinib toxicity was generally mild, with any grade 1-4 toxicity during all treatment cycles occurring in 23 out of 33 patients (70%). Grade 3-4 toxicity was only observed in 3 patients. Hypertension was the most frequently observed side-effect (n=7) and was unrelated to dose.[16]

The success rates for all genotyping assays were 100%. Genotype frequencies for 13 of 15 SNPs were in Hardy-Weinberg equilibrium (P >0.05). *ABCB1* -129T>C and *ABCC1* 2012G>T did not adhere Hardy-Weinberg equilibrium, which was most likely caused by the limited population size. Genotype frequencies for both SNPs were in line with previous publications and frequencies reported in the NCBI database (www.ncbi.nlm.nih.gov).

There was no association between telatinib dose normalized $AUC_{(0-12)}$ and genetic polymorphisms in *ABCB1*, *ABCC1*, or *ABCG2* (Table 2). Haploblock for *ABCB1* included 3435C>T, 1236C>T, and 2677G>A/T; haploblock for *ABCC1* included 825T>C, and 1062T>C. Haplotype frequencies for *ABCB1* were TTT 0.392, CTT 0.017, TCG 0.093, and CCG 0.498, and for *ABCC1* CC 0.197, TC 0.061, and TT 0.724. Also *ABCB1* and *ABCC1* haplotypes did not show an association with telatinib dose normalized $AUC_{(0-12)}$.

The number of telatinib treatment courses was not related to any of the genetic polymorphisms and haplotypes analyzed. Since this was an exploratory study with a relatively small number of patients, different dose levels, different tumor types, and variable previous treatment lines association analyses between polymorphisms and treatment outcome were not performed.

No association between any grade 1-4 toxicity and *KDR* or *FLT4* genotype or haplotype was observed (Table 3).

Discussion

The development of tailor-made pharmaceutics is especially useful in the field of oncology, as most standard anticancer agents have a very narrow therapeutic index, leading to nonspecific anti-tumor response in combination with a high level of side effects. For example, in 3-5% of patients with severe 5-FU-related toxicity. dihydropyrimidine dehydrogenase (DPD) deficiencies are described.[2,13] In addition, the genetic variant of the gene encoding UDP glucuronosyltransferase (UGT) 1A1 polymorphism, *UGT1A1*28*, is associated with a higher incidence of toxicity, mostly hematological toxicity, in irinotecan treatment.[4,5,7]

Most research to improve cancer treatment through genetics has focused on polymorphisms in genes encoding the drug transporters and drug metabolizing enzymes but less is known about genetic variation in drug targets. Directing treatment on the vascular endothelial growth factor (VEGF) pathway, one of the key players in angiogenesis, is a focus of more recent research. VEGF inhibitors have only become available for clinical use in the last few years and consequently, very little is known regarding the influence of polymorphisms in VEGF or its receptor, VEGFR.[8,10] One CA repeat polymorphism in the *KDR* (*VEGFR2*)) gene is described previously, with a higher promoter activity in the

Table 2. Association between genetic polymorphisms and telatinib pharmacokinetic data.

				Dose normalized AUC	
Gene	**Polymorphism**	**Genotype**	**No.**	**Mean**	**SD**
ABCB1	3435C>T	CC	7	7.30	3.35
		CT	16	11.10	9.10
		TT	6	6.65	4.76
		P-value		0.343	
	1236C>T	CC	9	7.84	3.26
		CT	15	11.60	9.44
		TT	5	4.82	1.81
		P-value		0.167	
	2677G>A/T	GG	9	7.84	3.26
		TG	15	11.60	9.44
		TT	5	4.82	1.81
		P-value		0.167	
	-129T>C	TT	26	8.67	7.51
		TC	2	15.65	6.06
		CC	1	11.79	n.a.
		P-value		0.430	
ABCC1	# (rs129081)	CC	5	10.22	7.57
		GC	15	10.44	9.07
		GG	9	6.77	3.16
		P-value		0.494	
	825T>C	TT	18	8.26	6.54
		TC	11	10.90	8.76
		P-value		0.362	
	1062T>C	TT	17	8.36	6.73
		TC	10	9.95	9.15
		CC	2	13.45	3.30
		P-value		0.631	
	2012G>T	GG	24	8.80	5.89
		GT	4	13.46	14.57
		TT	1	3.63	n.a.
		P-value		0.391	
ABCG2	346G>A	GG	25	9.78	7.83
		AG	4	6.00	2.69
		P-value		0.353	
	421C>A	CC	23	8.95	7.96
		CA	6	10.48	5.26
		P-value		0.661	

#=*801 number from termination codon TGA (5397). 3UTR

Table 3. Association between genetic polymorphisms and telatinib-induced toxicity.

			Toxicity: any toxicity grade 1-4 all cycles		
Gene	**Polymorphism**	**Genotype**	**No**	**Yes**	**P-value**
FLT4	1480A>G	AA	7	20	0.336
		AG	3	3	
	2670C>G	CC	6	11	0.813
		CG	3	9	
		GG	1	3	
KDR	1719A>T	AA	1	1	0.809
		TA	4	9	
		TT	5	13	
	-604T>C	CC	3	7	0.870
		CT	5	13	
		TT	2	3	
	1192G>A	CC	5	19	0.091
		TC	4	4	
		TT	1	0	

11-repeat polymorphism compared to the 12-repeat polymorphism.[6] Four SNPs in the *KDR* gene were identified by Park et al (-92G>A, 54A>G, 889G>A, and 1416T>A) and associated with atopy.[9] Recently, Schneider et al reported that *KDR* genotypes were not associated with toxicity or efficacy of paclitaxel with or without bevacizumab treatment in advanced breast cancer patients.[15]

VEGF inhibitors can induce very specific side effects which are hard to predict. This is even more relevant while in future use these angiogenesis inhibitors most likely will be combined with various chemotherapeutic agents. Pharmacogenetic research might help to identify the patients at risk for specific side effects and select patients or doses needed for optimal treatment without adding potentially harmful side effects.

In this exploratory study we could not find an association between polymorphisms in genes encoding transporter proteins and telatinib pharmacokinetics or between drug target gene polymorphisms and telatinib induced toxicity. This lack of association might be explained by, for example, the limited number of patients, the relatively limited toxicity, and the variability in tumor types, number of previous treatment lines, and performance scores. Since toxicity was limited we used toxicity reported over all treatment cycles. This may have caused bias, and therefore number of treatment cycles was used as a covariate in the multivariate analysis. Since different telatinib doses were used, we corrected by associating polymorphisms with dose normalized $AUC_{(0\text{-}12)}$.

Pharmacogenetic testing is important for all new drug applications. Knowledge on pharmacokinetics and pharmacodynamics of both registered and new developing drugs is increasing far more rapidly than the knowledge on genetic variants in metabolizing enzymes, transporters and drug target genes.[1,12,14] Therefore, DNA collection for future genetic studies, retrospective and prospective, is required and all patients in clinical trials should be asked to consent for DNA collection for future studies. Often side effects are based on single gene polymorphisms affecting drug metabolism, interaction with cellular targets or transport. Therefore, hypothesis based pharmacogenetic research of candidate genes is important in phase I and II studies to limit the number of patients unnecessarily exposed to a toxic dose or drug. This information may reduce the size, costs and duration of subsequent phase III studies.

In general, in the preclinical and phase I setting little is known about drug pharmacokinetics and pharmacodynamics. With this exploratory study we tried to increase that knowledge, because, despite of the rapidly increasing use of VEGF inhibitors, the knowledge of determinants that predict response and toxicity in the individual patient is still lacking. Therefore, it remains highly important to conduct pharmacogenetic association studies in early drug development in order to increase knowledge on interpatient variability of drug response.

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6

Hypertension and rarefaction during treatment with telatinib, a small molecule angiogenesis inhibitor

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Abstract

Purpose

Hypertension is a commonly reported side effect in antiangiogenic therapy. We investigated the hypothesis that telatinib, a small molecule angiogenesis inhibitor, impairs vascular function, induces rarefaction, and causes hypertension.

Experimental Design

A side-study was done in a phase I trial of telatinib, a small molecule tyrosine kinase inhibitor of vascular endothelial growth factor receptors 2 and 3, platelet-derived growth factor receptor, and c-KIT in patientswith advanced solid tumors. Measurements of blood pressure, flow-mediated dilation, nitroglycerin-mediated dilation, aortic pulse wave velocity, skin blood flux with laser Doppler flow, and capillary density with sidestream dark field imaging were done at baseline and after 5 weeks of treatment. Blood pressure and proteinuria were measured weekly.

Results

Mean systolic and diastolic blood pressure values increased significantly at +6.6 mm Hg ($P = 0.009$) and +4.7 mm Hg ($P = 0.016$), respectively. Mean flow-mediated dilation and mean nitroglycerin-mediated dilation values significantly decreased by -2.1% ($P = 0.003$) and -5.1% ($P = 0.001$), respectively. After 5 weeks of treatment, mean pulse wave velocity significantly increased by 1.2 m/s ($P = 0.001$). A statistically significant reduction of mean skin blood flux of 532.8% arbitrary units was seen ($P = 0.015$). Capillary density statistically significantly decreased from 20.8 to 16.7 capillary loops ($P = 0.015$). Proteinuria developed or increased in six patients during telatinib treatment.

Conclusion

The increase in blood pressure observed in the treatment with telatinib, an angiogenesis inhibitor, may be caused by functional or structural rarefaction.

Introduction

Dysregulated signaling through the vascular endothelial growth factor (VEGF)/VEGF receptor-2 (VEGFR-2) pathway mediates neoangiogenesis and thereby promotes tumor development and metastasis.[1,2] Overexpression of VEGF is common in solid tumors and has been associated with poor prognosis[3]. Furthermore, the overexpression or increased activation of VEGFR-2 has been associated with a poor prognosis in solid tumors.[4,5] In preclinical models, inhibition of the tyrosine kinase activity of the VEGFR-2 blocks angiogenesis and inhibits the growth of tumors.[6]

Hypertension is a commonly reported side effect in trials with inhibitors of VEGF/VEGFR-2 signaling, like bevacizumab and sunitinib.[7–12] The mechanisms leading to this increase in blood pressure during antiangiogenic therapy have not been elucidated. Proposed mechanisms include reduced formation of nitric oxide (NO) by endothelial cells, a reduced responsiveness of vascular smooth muscle cells to NO, an increased production of or reaction to vasoconstricting stimuli, a reduced compliance and distensibility of the vascular wall, and microvascular rarefaction.[13–15] Because microvessels (arterioles and capillaries) are a major contributor to total peripheral vascular resistance, functional rarefaction (a decrease in perfused microvessels) or anatomic rarefaction (a reduction in capillary density) may play an important role in the development of hypertension.

We hypothesized that systemic inhibition of VEGF impairs vascular function and causes rarefaction, which then leads to the development of hypertension in patients treated with antiangiogenic agents.

Materials and methods

This study was conducted on a subset of patients enrolled into an open-label, nonrandomized, two-center, phase I dose-escalating study of oral telatinib (Bay 57-9352).[16] The purpose of this study was to search for possible mechanisms that cause hypertension in patients treated with antiangiogenic therapy and to confirm our hypothesis that systemic inhibition of VEGF inhibits vascular function and causes rarefaction.

Patients

Patients with advanced solid tumors with no standard treatment available were eligible for study participation. Inclusion criteria were age of 18 y or older; WHO performance status of 0 to 2; life expectancy of at least 12 wk; and adequate bone marrow, liver, and renal function. Exclusion criteria were history of cardiac disease; history of HIV,

hepatitis B, or hepatitis C infection; active clinically serious infection; serious nonhealing wound, ulcer, or bone fracture; symptomatic metastatic brain or meningeal tumors; pregnancy or breast feeding; treatment with any anticancer agent or investigational drug 4 wk before the first dose; antiangiogenic therapies/VEGFR-2 inhibitors before enrollment.

The side-study was conducted on patients that were treated in the Leiden University Medical Center. The study protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center. All patients gave written informed consent.

Intervention

Telatinib (Bay 57-9352) is an orally active, small molecule inhibitor of the VEGFR-2 (IC50 in biochemical assay, 6 nmol/L), VEGFR-3 (IC50, 4 nmol/L) tyrosine kinases, and the growth factors receptors platelet-derived growth factor receptor-α (IC50, 15 nmol/L) and c-Kit (IC50, 1 nmol/L). Telatinib was continuously given once daily or twice daily in an oral formulation as solution or tablet. Patients were divided into cohorts with escalating doses. Therapy continued until progressive disease, unacceptable toxicity, death, consent withdrawal, or withdrawal from study at the discretion of the investigator. Telatinib was provided by Bayer Pharmaceuticals Corporation.

We assessed blood pressure, vascular function, and structure variables at baseline, and after 5 wk of treatment, in addition to regular evaluation of variables for safety, pharmacokinetics, and efficacy.

Hemodynamic, vascular function, and vascular structure variables and proteinuria

Blood pressure, flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), aortic pulse wave velocity (PWV), skin blood flux with laser doppler flow, and capillary density with sidestream dark field (SDF) imaging were assessed at baseline and after 5 wk of treatment with telatinib. All measurements were done by the same experienced investigator, in the morning, in a quiet, temperature-controlled room.

Peripheral blood pressure measurements were also done at every weekly visit to the outpatient clinic.

Peripheral blood pressure

Peripheral blood pressure measurements at baseline and at the 5-wk visit were done after 15 min rest, measuring thrice in a supine position with 5-min intervals, using an automatic device (Datex-Ohmeda S/5 Light Monitor, Datex-Ohmeda, Inc.) with the cuff

placed at the brachial artery. For statistical analysis, we used the mean of three consecutive measurements. Peripheral blood pressure measurements at the weekly visit to the outpatient clinic were done by the treating physician, using an aneroid sphygmomanometer (Maxi-Stabil 3, Speidel & Keller, Welch Allyn) with the auscultatory method.

Central blood pressure
Application tonometry of the brachial and external carotid artery (SphygmoCor SCOR-PVx device, AtCor) was done. The mean of the three peripheral blood pressure measurements was used to calculate central aortic pressure.[17]

Aortic pulse wave velocity
Measurements were done at the right carotid and femoral arteries using standard blood pressure transducers (SphygmoCor SCOR-PVx device, AtCor) with simultaneous electrographic gating. This enabled the base of the pressure wave to be recorded and the time delay between the carotid and femoral waves to be calculated. The distance between the two sites was measured. PWV was defined as the distance traveled by the pressure waves divided by the time delay.

Flow mediated dilation
The FMD measurements were done in a quiet, temperature-controlled room. Postischemic vasodilator responses in the brachial artery were measured using a Wall Track System (WTS 2, Pie Medical). This system consists of a standard 7.5-MHz linear array ultrasound transducer connected to a PC equipped with a data acquisition board and software. Subjects were investigated in a supine position, and three ECG leads were attached. Ischemia was induced in the forearm by inflation of a blood pressure cuff just below the elbow of the right arm for 5 min. After deflation of the cuff, changes in brachial artery wall diameter were measured every 20 s for 4 min. WTS measurements were stored and analyzed off line using WTS software. FMD was expressed as percentage change in brachial artery diameter after ischemia.

NMD
NMD was assessed in the same way as FMD, with the exception that 0.4 mg of nitroglycerin were given sublingually, instead of cuff inflation and deflation, before measurements were started.

Laser Doppler flowmetry
Forearm skin blood flux was measured using laser Doppler flowmetry (Periflux PF4001, Perimed; wavelength, 782 nm) before and during forearm postischemic hyperemia.[18]

Flows were recorded by the Perisoft program, with the time constant set at 3 s downstream from a broadband filter (12 MHz). Results were reported as arbitrary flow units (10 mV). The percentage of change in arbitrary units from baseline (before ischemia) to maximal flow in the postischemic hyperemic phase was reported.

Capillary density measurements with SDF imaging

Patients were situated in a supine position with the investigator at the head side of the bed. An SDF hand-held device (MicroScan Video Microscope System, MicroVision Medical) was introduced into the open mouth and gently pushed to the mucosal surface of the inner lip. SDF imaging consists of a light guide surrounded by light-emitting diodes that emit green light (540 F 50 nm) which penetrates the tissue and directly illuminates the tissue microcirculation. The SDF technique and the technique of its precessor orthogonal spectral polarization imaging are described in detail in previous publications.[19,20]

Images of the mucosal microcirculation were projected on a computer screen. The final on-screen magnification of the images obtained with the SDF imaging device was 325 times original. When images of satisfying quality were seen, video images of at least 30 s were obtained. Images were obtained from four different lip quadrants (mucosal readings of the left and right upper inner lip quadrant and the left and right lower inner lip quadrant) using the SDF probe. From every quadrant, at least three 30-s video images were obtained. Video images were stored on digital videotape in .avi format.

Off line, at least five still frames of each quadrant were captured from these video images. The number of capillary loops per frame was counted. Capillary density for each frame was expressed as the mean number of capillary loops per mm^2. The mean capillary density per lip quadrant and total lip was calculated.

All measurements were done by one technician, not blinded to the time point in treatment of the patients. Off-line analysis (counting of the number of capillary loops) was done by two observers, who were blinded to the time point in treatment of the patients.

Whereas the technique has not been used very frequently in the measurement of microcirculation of the mucosal surface of the inner lip, additional quality measurements were done. In 10 healthy volunteers, no difference in capillary density was observed between the different lip quadrants. The reproducibility of the SDF technique to determine capillary density was moderate to high, showing a coefficient of variation of 4.6%.

Proteinuria

Urinalysis, measured by dipstick, was done weekly in all patients to monitor proteinuria. Proteinuria was recorded according to the National Cancer Institute Common Toxicity Criteria version 3.0. Grade 1 is defined as 1+ by dipstick, grade 2 as 2+ or 3+ by dipstick,

grade 3 as 4+ by dipstick, and grade 4 as nephrotic syndrome. We report the development of proteinuria (grade 0 before treatment increasing to grade >0 during treatment) and the worsening of proteinuria (increase of proteinuria by z1 grade compared with baseline).

Pharmacokinetic analysis

Serial blood samples were collected for pharmacokinetic analysis on days 1 and 14 of cycle 1. Telatinib plasma concentrations were analyzed by a noncompartmental method using the KINCALC software package, Bayer AG, version 2.33 or higher. Peak plasma level (C_{max}), area under the concentration-time curve [$AUC_{(0\text{-}tn)}$], were calculated.

Statistical analysis

Continuous variables are presented as mean values ± SD and categorical variables as frequencies (percentages), unless otherwise stated. Comparisons between variables at baseline and after 5 wk were done with paired Student's *t*-tests and were two-sided, with a level of significance of $\alpha = 0.05$. For skin blood flux and capillary density, the Wilcoxon signed-rank test was used. The relationship between blood pressure, vascular function and structure variables, and telatinib daily dose and telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$] was investigated by correlation analysis. Correlation analysis was done using Pearson's and Spearman's correlation coefficients where appropriate. Correlations with proteinuria were done using an armitage test for trend. For correlation purposes proteinuria was reported as presence of new proteinuria or increase in existing proteinuria (yes or no). All analyses were done using SPSS version 12.01 (SPSS).

Results

Eighteen of 33 patients treated in our hospital were included in this side study. Reasons for exclusion were vaso-active hormone producing adrenal carcinoma ($n = 3$), absence of measurements for logistics reasons between June and December 2005 ($n = 7$), absence of measurements at 5 weeks due to early drop out for early progressive disease ($n = 2$), anatomic anomaly of the arm ($n = 1$), absence of appropriate drug compliance (supported by pharmacokinetic data; $n = 1$), and failure to upheld appointment baseline visit ($n = 1$). NMD measurements were not done in two patients; both had a preexisting headache and refused sublingual nitroglycerin administration.

Baseline demographics and patient characteristics of the 18 patients included in this study are listed in Table 1. Patients received the following starting doses of Bay 57-9352:

Table 1. Baseline demographics and patient characteristics

Baseline characteristics	
N	18
Male gender	9 (50)
Age, y (range)	55 (22-76)
Additional cardiovascular risk factors	
Body mass index, kg/m2 (range)	24.7 (20.5-29.7)
Nicotine abuse, in past or present	5 (28)
History of cardiovascular disease	0 (0)
History of hypertension	1 (6)
Renal impairment (creatinine > ULN)	5 (28)
WHO performance scale	
0	4 (22)
1	14 (78)
Prior treatment	
Surgery	13 (72)
Chemotherapy	17 (94)
Radiotherapy	8 (44)
Blood pressure lowering drugs at entry	2 (11)
Tumor type	
Anal carcinoma	1 (6)
Carcinoid tumor	1 (6)
Cholangiocarcinoma	1 (6)
Colorectal carcinoma	3 (17)
Esophageal carcinoma	1 (6)
Ovarian carcinoma	3 (17)
Prostatic carcinoma	1 (6)
Renal cell carcinoma	1 (6)
Soft tissue sarcoma	5 (28)
Urothelial cell carcinoma	1 (6)

NOTE: Data are presented as n (%) unless otherwise specified. Abbreviation: ULN, upper limit of normal.

patient 1, 20 mg solution once daily; patients 2 to 3, 75 mg (25 mg tablets) once daily; patients 4 to 5, 150 mg (150 mg tablets) twice daily; patients 6 to 9, 300 mg (150 mg tablets) twice daily; patient 10, 600 mg (150 mg tablets) twice daily; and patients 11 to 18, 900 mg (150 mg tablets) twice daily.

Blood pressure results

Both peripheral systolic blood pressure and peripheral diastolic blood pressure increased in 14 of 18 patients (78%) after 5 weeks treatment with telatinib compared with baseline

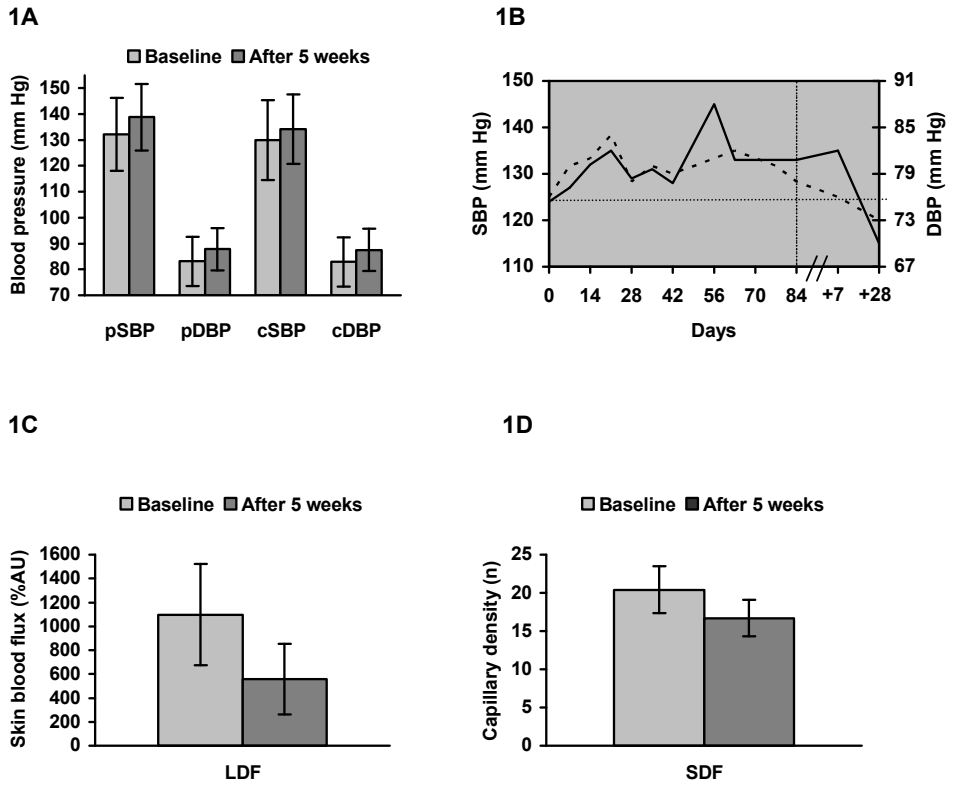

Fig. 1. Blood pressure (A), skin blood flux (C), and capillary density (D) results at baseline and after 5 wk of treatment with telatinib. B, mean systolic blood pressure (*continuous line*) and mean diastolic blood pressure (*dashed line*) before treatment, weekly during treatment, and after discontinuation of telatinib treatment. A horizontal dashed line was added at baseline systolic blood pressure and baseline diastolic blood pressure for facilitation of reading. Left from the vertical line blood pressures measured in the first 84 d of treatment. Right from the vertical line blood pressures measured 7 and 28 d after discontinuation of treatment. pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; LDF, laser doppler flow; %AU, percentage of change from baseline in arbitrary units; *n*, number.

values. The mean peripheral systolic blood pressure significantly increased from 132.2 to 138.8 mm Hg ($P = 0.009$), and the mean peripheral diastolic blood pressure values increased from 83.1 to 87.8 mm Hg ($P = 0.016$; Table 2; Fig. 1A). The increase in central systolic blood pressure (4.3 mm Hg) was not statistically significant ($P = 0.106$). Both peripheral and central pulse pressure showed no change after 5 weeks of treatment.

Mean peripheral blood pressures measured at the weekly visits showed a similar increase in both systolic and diastolic blood pressure (Fig. 1B). Blood pressure results for

Table 2.

A. Hemodynamic and vascular function/structure variables at baseline and after 5 wk of treatment with telatinib

	Baseline values	After 5 wk treatment	Change ± SD	95% Confidence interval	*P*
pSBP (mm Hg)	132.2	138.8	+6.6 ± 9.5	(1.9 to 11.3)	0.009*
pDBP (mm Hg)	83.1	87.8	+4.7 ± 7.4	(1.0 to 8.4)	0.016*
cSBP (mm Hg)	129.9	134.2	+4.3 ± 10.9	(-1.0 to 9.8)	0.106
cDBP (mm Hg)	82.9	87.5	+4.6 ± 7.8	(0.7 to 8.4)	0.024*
MAP (mm Hg)	102.5	107.6	+5.1 ± 7.2	(1.5 to 8.7)	0.008*
pPP (mm Hg)	54.3	57.1	+2.8 ± 12.9	(-3.6 to 9.2)	0.369
cPP (mm Hg)	47.0	46.8	–0.2 ± 10.2	(-5.2 to 4.9)	0.946
FMD (%)	6.0	3.9	–2.1 ± 2.6	(0.8 to 3.5)	0.003*
NMD (%)	17.0	11.9	–5.1 ± 4.1	(2.9 to 7.3)	0.001*
PWV (m/s)	8.5	9.7	+1.2 ± 0.8	(0.8 to 1.7)	0.001*
Skin blood flow (%AU)	1091.5	558.7	–532.8 ± 362.0	(-912.7 to -152.9)	0.015*
Capillary density (*n*)	20.8	16.7	–4.1 ± 3.3	(-7.2 to -1.1)	0.015*

B. Individual blood pressure data before treatment, during treatment, and after discontinuation of telatinib treatment

Pt	Systolic blood pressure, d														
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**+7**	**+28**
1	110	105	110	115	115	110	130		120	115		120	115		110
2	115	115	120	120	125	120	115							130	120
3	130	160[a]	135	140	150	128	150		180[a]	145		145		135	
4	130	125	120	138	120	125	125								
5	115	120	125	120	120	125	130								110
6	145	150	145	155[a]	140		140		135	130		160[a]	150		120
7	110	100	120	125	110		110			120		125	105		110
8	130	140	150	150	*160*	160	130			130		125	140		110
9	105	130	130	140	125	130	110								
10	130	120	126	140	135	120	140			130		135	130	140	100
11	120	120	130	130	135		120								
12	140	130	135	130	125	170[a]	130			*160*		149	163	150	110
13	120	125	150	140		156[a]	140		150	130		130			130
14	125		143	130	130	112									120
15	120	125	110	120	120	135	120								120
16	135	130	*150*	145	130	110								118	
17	130	125	130	135	120	128	125								
18	125	140	145	*150*	125	130	125		140	135		105	130	ongoing	

B. Individual blood pressure data before treatment, during treatment, and after discontinuation of telatinib treatment

Pt	Diastolic blood pressure, d														
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**+7**	**+28**
1	60	60	55	70	55	70	65		60	60		70	55		70
2	70	80	80	80	80	80	80							80	80
3	75	80	75	80	70	72	70		80	80		70		75	
4	85	90	90	85	65	60	70								
5	75	80	75	85	80	75	80								70
6	85	85	85	85	80		90		85	85		90	90		80
7	70	70	75	85	85		80			80		80	70		70
8	75	70	80	90	90	89	85			75		80	80		60
9	70	90	85	90	80	80	70								
10	75	80	81	85	80	85	70			85		80	75	65	70
11	75	75	80	85	85		80								
12	80	80	85	80	80	102[a]	90			*100*		100	98	85	75
13	70	80	89	85		97	90		90	80		80			75
14	80		79	85	80	78									75
15	75	80	70	70	80	80	80								80
16	85	90	*110*	90	75	70								77	
17	85	80	85	90	80	74	80								
18	80	85	85	90	85	85	80		90	90		70	80	ongoing	

NOTE: Data in italics indicate antihypertensive medication started.
Abbreviations: pSBP, peripheral systolic blood pressure; pDBP, peripheral diastolic blood pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; MAP, mean arterial pressure; pPP, peripheral pulse pressure; cPP, carotid pulse pressure; %AU, percentage of change from baseline in arbitrary units; *n*, number.
*$P < 0.05$.
[a]No antihypertensive treatment started, regardless of protocol.

the individual patients are reported in Table 2B. Results for the first 84 days on treatment are reported. The number of patients on telatinib treatment after 84 days was too small for reliable results to be reported ($n = 7$). None of the seven patients remaining on study medication after 84 days developed a new increase in blood pressure. In all patients, the blood pressure values returned to baseline within 4 weeks after the discontinuation of the telatinib.

One patient received antihypertensive medication before start of treatment (thiazide diuretic). Four additional patients were started on antihypertensive treatment: one patient receiving 600 mg telatinib daily and three patients receiving 1800 mg daily. Antihypertensive medication consisted of a thiazide diuretic in one patient, a calcium antagonist in one patient, and an ACE inhibitor in two patients.

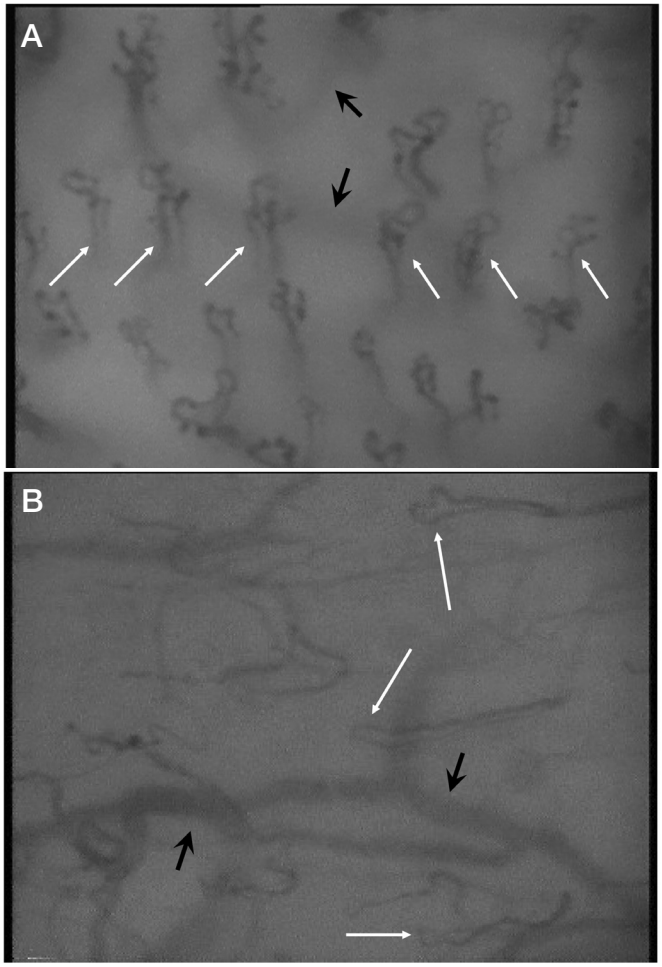

Fig. 2. SDF images demonstrating visible capillary loops of a representative patient. A, at baseline. B, after 5wk of telatinib treatment. Black arrows, larger venules; white arrows, individual superficial capillary loops.

Vascular function and vascular structure assessments

FMD decreased from baseline in 15 of 18 patients (83%) after 5 weeks treatment with telatinib. At 5 weeks, the mean decrease in FMD, compared with baseline, was statistically significant, from 6.0% to 3.9% ($P = 0.003$; Table 2). After 5 weeks of treatment, NMD decreased in 94% of patients. The mean change in NMD from 17.0% at baseline to 11.9% after 5 weeks was statistically significant ($P = 0.001$; Table 2). An increase in PWV was seen in 17 of 18 patients (94%). Mean PWV significantly increased from 8.5 m/s at baseline to 9.7 m/s after 5 weeks treatment ($P = 0.001$; Table 2). Mean forearm skin blood

Table 3. Telatinib daily dose and pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$]

Patient	Daily dose (mg)	Cycle 1, day 1		Cycle 1, day 14	
		C_{max}, mg/L	$AUC_{(0\text{-}tn)}$, mg h/L	C_{max}, mg/L	$AUC_{(0\text{-}tn)}$, mg h/L
1	20	0.2061	0.5284	0.1658	0.9628
2	75	0.2927	1.7464	0.1888	1.8779
3	75	0.3734	1.4246	0.4526	1.6941
4	300	0.0675	0.4141	0.1822	1.7680
5	300	0.0935	0.7527	0.1355	1.0164
6	600	1.2678	3.4588	1.1915	5.9823
7	600	0.1250	0.4504	0.6493	3.3407
8	600	0.1888	1.1649	0.2897	2.3776
9	600	1.2809	8.7848	1.4486	10.3795
10	1200	2.1691	16.7094	1.6995	16.4708
11	1800	0.4298	2.4610	1.7637	5.2292
12	1800	1.0484	7.0520	1.1216	7.3369
13	1800	0.2856	3.0094	0.2229	1.9701
14	1800	0.0552	0.8416	0.0969	0.8832
15	1800	0.2918	2.7700	0.8145	6.7397
16	1800	1.2599	3.4356	0.5728	3.1803
17	1800	1.6730	9.7474	2.6011	12.2049
18	1800	0.4515	2.9511	0.7626	6.2329

flux decreased significantly (-532.8 %AU, $P = 0.015$; Table 2). SDF imaging was done in seven patients. In all of the patients, the number of capillary loops markedly decreased after 5 weeks of treatment (Figs. 1 and 2; Table 2). Capillary density, the mean number of capillary loops per image, decreased from 20.8 at baseline to 16.7 after 5 weeks treatment with telatinib ($P = 0.015$).

Proteinuria

In four patients, proteinuria was reported at baseline, grade 1 proteinuria in one patient, and grade proteinuria in three patients. Proteinuria increased in one of those patients from grade 1 to grade 2. Five patients developed new onset proteinuria during telatinib treatment: grade 1 in three patients and grade 2 in two patients. Five of these six patients with new onset or increasing proteinuria were receiving the highest dose of telatinib at 1,800 mg daily. After discontinuation of treatment in three of six patients, the proteinuria returned to normal. For the other three patients, no data for proteinuria after discontinuation of telatinib were available. In two of the six patients with new

or increasing proteinuria, an increase in blood pressure above 150 mm Hg systolic or above 100 mm Hg diastolic was reported. These two patients were treated with an ACE inhibitor, resulting in disappearance of the proteinuria. The other four patients were not treated for the proteinuria.

Pharmacokinetic analysis and correlations

Telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$] are shown in Table 3. There was no correlation between either blood pressures or vascular function/structure variables and daily dose of telatinib or telatinib pharmacokinetic variables [C_{max} and $AUC_{(0\text{-}tn)}$]. No correlation between development or increase of proteinuria and blood pressure measurements or any of the other variables was seen. However, there was positive correlation between daily dose of telatinib and proteinuria (linear-by-linear association, 5.0; $P = 0.025$). All patients with SDF measurements done received 1,800 mg of telatinib a day. No correlation between SDF results and daily dose could therefore be calculated.

Discussion

We studied the effects of telatinib, a tyrosine kinase inhibitor and potent inhibitor of angiogenesis, on the vasculature to determine a mechanism by which small molecule angiogenesis inhibitors cause an increase in blood pressure. The rarefaction (reduction in capillary density) and change in microvascular characteristics observed in this study provide a plausible mechanism for the increase in systolic and diastolic blood pressure. Telatinib caused a significant decrease in endothelium-dependent and endothelium-independent vasodilation. VEGF inhibition by itself decreases NO synthesis, which promotes vasoconstriction, increases peripheral resistance, and therefore can induce an increase in blood pressure.[21–24] It remains unclear whether the key problem is impaired NO synthesis, the change in capillary structure leading to impaired NO vascular smooth muscle cell responsiveness, or a combination of both. Aortic pulse wave velocity is a variable for vascular stiffness, a which is known to increase with age, and is an independent predictor of cardiovascular risk and all-cause mortality in renal disease, hypertensive patients, and patients with diabetes mellitus.[25–27] We observed a significant increase in PWV, which correlated with the increase in mean arterial pressure. Although blood pressure is a known independent determinant of pulse wave velocity, it cannot be excluded that inhibition of angiogenesis has a direct effect on stiffness of the arterial tree.[28] In a subgroup of patients, we did SDF imaging to visualize a the microvessels in the buccal mucosa. All patients showed a reduction in the number of mucosal capillaries (rarefaction) during antiangiogenic treatment. Vessels smaller than 150 μm in diameter are the

most important segment of the vascular bed to regulate blood flow and blood pressure.[29,30] A reduction in the number of (functional) arterioles and capillaries leads to increased peripheral vascular resistance and blood pressure. Rarefaction is a consistent finding in patients with hypertension,[30–32] and it is also reported in normotensive young adults with a genetic predisposition to high blood pressure.[33] Blocking the growth of capillaries by VEGFR inhibitors and other angiogenesis inhibitors might lead to the same results even in subjects that are not predisposed to the development of hypertension. Whether the observed rarefaction is structural (disappearance of capillaries) or functional (i.e., nonperfused existing capillaries) is unclear, as visualization of microvessels based upon the SDF technique depends on perfusion of these vessels. Although the rapid normalization of blood pressure within weeks and reversal in proteinuria in some patients after discontinuation of telatinib may indicate improvement in functional rarefaction, this is more likely in functional then structural rarefaction. It remains uncertain whether the changes in microvessel architecture are reversible upon discontinuation of the treatment. While capillary density measurements were done in only seven patients, one should be careful with the interpretation of these results. These results have to be confirmed in a larger patient sample.

The exact mechanism by which telatinib leads to rarefaction and hypertension is unclear. Telatinib is a small molecule tyrosine kinase inhibitor, blocking the ATP-binding site of the VEGFR-2, VEGFR-3, platelet-derived growth factor receptor-α, and c-Kit receptors. Platelet-derived growth factor and c-Kit receptor activation result in activation of pathways that, for a large part, are also activated by VEGFR-2. However, hypertension is rarely seen in the treatment with platelet-derived growth factor and c-Kit inhibitors, such as imatinib and nilotinib.[34,35] In contrast, selective inhibitors of VEGF/VEGFR-2 signaling, such as sunitinib or bevacizumab, frequently cause hypertension.[7–10] The increase in blood pressure is therefore most likely caused by the inhibition of the VEGFR signaling. However, we cannot rule out that c-KIT or platelet-derived growth factor inhibition has a role in mediating the blood pressure changes or changes in any of the other measured variables. A recently published preclinical observation suggests that VEGF signaling is required for vascular homeostasis.[36] Our findings could be the clinical proof of that concept.

Our study has several limitations. First, the study was set up as a side-study of a phase I dose-finding study. Therefore, different dosages of telatinib were used by our patients. However, there was no correlation between changes on blood pressure, vascular structure/function variables, capillary density, and daily dose of telatinib or telatinib exposure. Even in the patients with lower doses of telatinib, significant changes in all measured variables were seen. Second, due to the small number of patients it was not possible to reliably quantitate capillary characteristics, such as length, diameter size, and

tortuosity. Third, no control group was measured and distinction between treatment and placebo effects is therefore not clear. Fourth, no vascular measurements were done after discontinuation of treatment. Whereas all patients had advanced tumors with a low life expectancy, we chose not to burden these patients with additional measurements after cessation of the study drug. Finally, the temporal relationship between rarefaction and hypertension is unclear. Therefore, future studies, in larger patient samples, with measurements before, during, and after treatment are necessary.

In the most extensively studied VEGF inhibitor bevacizumab, the increase in blood pressure is dose dependent.[13] We did not observe this in our study. This could have been due to the small study size. In addition, the start of antihypertensive medication may have masked a correlation between blood pressure and daily dose of telatinib. However, the development or increase of proteinuria was dose dependent. Another explanation for the sole dose dependency for proteinuria is that telatinib may have an effect on glomerular endothelial cells, which is independent of blood pressure and independently caused by the VEGF blockade.[37–40]

In conclusion, we report that 5 weeks of treatment with a small molecule tyrosine kinase inhibitor, blocking VEGFR-2 and VEGFR-3, results in a significant increase in both systolic and diastolic blood pressure. The reduction in capillary density and microvascular flow, associated with a reduced vasodilatory capacity, may suggest that rarefaction is a mechanism that underlies the increase in blood pressure induced by telatinib and possibly other antiangiogenic agents. Further research in larger patient samples is needed to confirm this hypothesis.

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7

Reversibility of capillary density after discontinuation of bevacizumab treatment

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Abstract

Background

VEGF inhibition is known to decrease capillary density. Decreased capillary density may be the basis for VEGF inhibitor related side effects. We investigated whether the effects of bevacizumab on capillary density are reversible.

Patients and methods

Capillary density, assessed by sidestream dark field imaging of the mucosal surface of the lip, was measured at baseline, after 6 weeks of bevacizumab treatment and >3 months after discontinuation. Additional measurements included blood pressure (BP) measurements, flow-mediated dilation (FMD), nitroglycerin-mediated dilation (NMD), and aortic pulse wave velocity (PWV).

Results

Fourteen patients were included. Seven patients completed measurements at all 3 predefined timepoints. Capillary density significantly decreased after 6 weeks of bevacizumab treatment and was reversible after discontinuation of the bevacizumab ($p=0.00001$ using a general linear model repeated measures test). Blood pressure, FMD and NMD remained unchanged. Mean PWV increased after 6 weeks treatment ($p=0.027$) and decreased after bevacizumab discontinuation. Amongst the 6 patients with the best response were the 3 patients showing the clearest decrease in capillary density after 6 weeks of bevacizumab treatment.

Conclusions

Bevacizumab induced decrease in capillary density is reversible. Non-invasive assessment of capillary density during treatment with anti-angiogenic drugs may be useful as a marker of treatment efficacy.

Introduction

Bevacizumab, a monoclonal antibody against the vascular endothelial growth factor (VEGF), is currently being used in the treatment of various types of cancer, including colorectal, breast, renal cell and non-small-cell lung cancer.[1-6]

A clear decrease in capillary density has been reported in the treatment with two VEGF inhibitors, telatinib and bevacizumab.[7,8] Decreased vasculary density, or rarefaction, may be a possible mechanism for various side effects seen in the treatment with VEGF inhibitors. One of these side effects is hypertension, with grade 3-4 hypertension, using the common toxicity criteria, developing in 9-16% of bevacizumab-treated patients develops.[9,10] Research in rats also showed that vascular rarefaction may be the cause of sunitinib-induced hypothyroidism and bevacizumab-induced enteric perforations.[11] Since bevacizumab-treated patients may have a prognosis of several years in palliative setting and many years when adjuvant schedules will prove to be beneficial, the patient group is relatively large. Increased knowledge on bevacizumab-induced vascular changes may help to identify patients at risk for complications, stimulate the sense of urgency to monitor patients and to start early treatment in case of hypertension, hypothyroidism, encephalopathy or other vascular-related complications.[12]

The aim of this study is to investigate whether the bevacizumab-associated vascular and blood pressure effects are reversible after discontinuation of bevacizumab treatment. An additional goal is to provide more information on the mechanisms in the development of bevacizumab-associated side effects.

Patients and Methods

Eligibility Criteria and study outline

Patients receiving bevacizumab monotherapy or combination therapy for breast or colorectal cancer were eligible for participation. Patients with previous bevacizumab treatment, major surgery within the last 4 weeks, or clinical significant cardiovascular disease in the previous year were excluded. Written informed consent was obtained from all patients before any study related procedure was performed, and approval from the institutional medical ethical review boards was obtained before study initiation.

Blood pressure measurements and vascular assessments were performed at baseline (<7 days before first bevacizumab administration), after 6 weeks (before third 3-weekly bevacizumab administration) and >3 months after last bevacizumab admin-

istration. All measurements were performed by the same experienced investigator, in a quiet, temperature-controlled room.

Assessments

Capillary density measurements with SDF imaging

Patients were situated in a supine position with the investigator at the head side of the bed. An SDF hand-held device (MicroScan Video Microscope System, MicroVision Medical) was introduced into the open mouth and gently pushed to the mucosal surface of the inner lip. SDF imaging consists of a light guide surrounded by light-emitting diodes that emit green light (540 F 50 nm) which penetrates the tissue and directly illuminates the tissue microcirculation. The SDF technique is described in detail in previous publications.[7,13,14] Images of the mucosal microcirculation were projected on a computer screen. The final on-screen magnification of the images obtained with the SDF imaging device was 325 times original. When images of satisfying quality were seen, video images of at least 30 s were obtained. Images were obtained from four different lip quadrants (mucosal readings of the left and right upper inner lip quadrant and the left and right lower inner lip quadrant) using the SDF probe. From every quadrant, at least three 30-s video images were obtained. Video images were stored on digital videotape in avi format. Off line, at least five still frames of each quadrant were captured from these video images. The number of capillary loops per frame was counted. Capillary density for each frame was expressed as the mean number of capillary loops per mm^2. The mean capillary density per lip quadrant and total lip was calculated. Using offline computer analysis, capillary diameter was measured. The mean diameter of at least 80 capillaries per visit of each patient was calculated. All measurements were done by one technician, not blinded to the time point in treatment of the patients. Off-line analysis (counting of the number of capillary loops and measurement of capillary diameter) was done by two observers (JR and NS), who were blinded to the time point in treatment of the patients. The reproducibility of the SDF technique to determine capillary density in healthy volunteers was moderate to high, showing a coefficient of variation of 4.6%.[7]

Peripheral blood pressure measurements

Brachial blood pressure was measured at home, 4 times a day for at least three consecutive days, after 15 min rest, measuring thrice in a supine position with 5-min intervals, using an automatic device (Datex-Ohmeda S/5 Light Monitor, Datex-Ohmeda, Inc.) with appropriate cuff size. For statistical analysis, we used the mean of the measurements.

Central blood pressure measurements

Application tonometry of the radial and external carotid artery (SphygmoCor SCOR-PVx device, AtCor) was done. The mean of the three peripheral blood pressure measurements was used to calculate central aortic pressure.[15]

Flow mediated dilation

The FMD measurements were done in a quiet, temperature-controlled room. Postischemic vasodilator responses in the brachial artery were measured using a Wall Track System (WTS 2, Pie Medical). This system consists of a standard 7.5-MHz linear array ultrasound transducer connected to a PC equipped with a data acquisition board and software. Subjects were investigated in a supine position, and three ECG leads were attached. Ischemia was induced in the forearm by inflation of a blood pressure cuff just below the elbow of the right arm for 5 min. After deflation of the cuff, changes in brachial artery wall diameter were measured every 20 s for 4 min. WTS measurements were stored and analyzed off line using WTS software. FMD was expressed as percentage change in brachial artery diameter after ischemia.

Nitroglycerin-mediated dilation

NMD was assessed in the same way as FMD, with the exception that 0.4 mg of nitroglycerin was given sublingually, instead of cuff inflation and deflation, before measurements were started.

Aortic pulse wave velocity

Measurements were done at the right carotid and femoral arteries using standard blood pressure transducers (SphygmoCor SCOR-PVx device, AtCor) with simultaneous electrographic gating. This enabled the base of the pressure wave to be recorded and the time delay between the carotid and femoral waves to be calculated. The distance between the two sites was measured. PWV was defined as the distance traveled by the pressure waves divided by the time delay.

Statistical analysis

Continuous variables are presented as mean values and categorical variables as frequencies (percentages), unless otherwise stated. Comparisons between variables at baseline, after 6 weeks, and after discontinuation of bevacizumab treatment, were made with a general linear model repeated measures test or with a paired Student's t-test were appropriate, and were two-sided, with a level of significance of $\alpha = 0.05$. All calculations were made by computer-assisted analyses using SPSS 16.0 (Chicago, IL) statistical package for Windows.

Results

Between March 2007 and July 2008, a total of 14 patients were enrolled in the study. In 8 patients measurements could be performed after discontinuation of bevacizumab treatment. Baseline patient and treatment characteristics of both groups are listed in Table 1.

Patients received bevacizumab for colorectal cancer (12 patients) or breast cancer (2 patients). All patients had metastasized disease. Bevacizumab dose was 7.5 mg/kg/3weeks for the patients with colorectal cancer and 10 mg/kg/2weeks for the breast cancer patients. Bevacizumab was combined with various chemotherapy schedules; capecitabine plus oxaliplatin (7 patients), capecitabine (3 patients), irinotecan (2 patients), paclitaxel and capecitabine (1 patient), paclitaxel (1 patient).

Reversibility of vascular changes

Measurements after discontinuation of bevacizumab treatment could be performed in 8 patients. In one of these patients SDF data were not available due to technical reasons.

Capillary density measurements with SDF imaging

Using a general linear model repeated measures test on the 7 patients with 3 evaluable SDF measurements, before, during and after bevacizumab treatment, the change in capillary density due to bevacizumab treatment was significant, with a p-value of 0.00001 (Fig 1). More importantly, after discontinuation of the bevacizumab treatment the changes in capillary density showed to be reversible, with a p-value of 0.001 (Fig 1).

Figure 2 shows the SDF images of a representative patient, demonstrating visible capillary loops at baseline, decreased capillary density after 6 weeks of bevacizumab treatment, and normalization of capillary density >3 months after bevacizumab discontinuation.

Of the 7 patients, 6 patients were without any systemic anticancer therapy in the three months before the third visit. One patient was still treated with capecitabine plus oxaliplatin, however without bevacizumab.

At baseline, 2 patients received a single blood pressure lowering agent (thiazide diuretic or angiotensin-II receptor antagonist), 3 patients received two agents (β-blocker plus thiazide diuretic, β-blocker plus angiotensin converting enzyme (ACE) inhibitor, and β-blocker plus angiotensin-II receptor antagonist, all in 1 patient respectively), and 1 patient received 3 blood pressure lowering agents (β-blocker plus loop diuretic plus angiotensin-II receptor antagonist. These blood pressure lowering medication schedules remained unchanged for all 6 of the above described patients for the duration of the

Table 1. Baseline demographics and patient characteristics

Patient characteristics	Pts with data at 3 visits, including after bevacizumab discontinuation	Pts with data before and during bevacizumab, without data after discontinuation
N	8	14
Male gender	4 (50)	6 (43)
Age (years, range)	59 (49-71)	61 (45-74)
Center		
Leiden University Medical Center	6 (75)	8 (57)
Rijnland Hospital Leiderdorp	1 (13)	4 (29)
Deaconess Hospital Leiden	1 (13)	2 (14)
Additional cardiovascular risk factors		
BMI (kg/m2, range)	27.3 (22.8-33.7)	26.2 (20.7-33.7)
Nicotine abuse; in past or present	5 (63)	6 (43)
History of CVD	1 (13)	2 (14)
History of hypertension	2 (25)	4 (29)
Renal impairment (creatinine > ULN)	0 (0)	0 (0)
WHO performance scale		
0	5 (63)	7 (50)
1	3 (38)	6 (43)
unknown	0 (0)	1 (7)
Prior treatment		
Surgery	5 (63)	7 (50)
Chemotherapy	1 (13)	5 (36)
Radiotherapy	4 (50)	5 (36)
Blood pressure lowering drugs at entry	3 (38)	6 (43)
Tumor type		
Colorectal cancer	7 (88)	12 (86)
Breast cancer	1 (13)	2 (14)

BMI: body mass index; CVD: cardiovascular disease; ULN: upper limit of normal; WHO: world health organization
Data are presented as n (%) unless otherwise specified

entire study. One additional patient, patient 14, was started on antihypertensive treatment with a calcium antagonist during bevacizumab treatment.

Blood pressure, vascular function and vascular structure assessments

With a general linear model repeated measures test there were no significant changes in blood pressure or vascular function and vascular structure parameters, besides FMD (Table 2). Comparing results of visit 3 with visit 2, FMD remained unchanged after discontinuation of bevacizumab treatment.

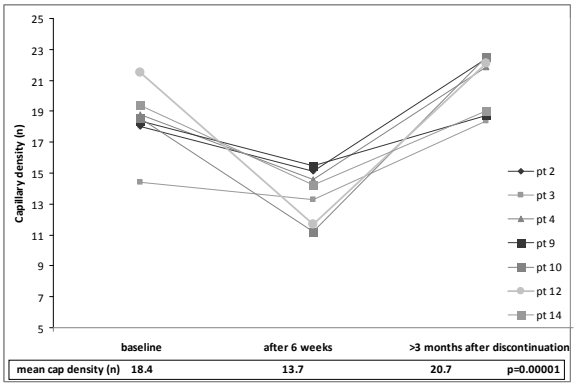

Fig. 1. Capillary density of the 7 patients with 3 evaluable SDF measurements. Visit 1: baseline; visit 2: after 6 weeks of bevacizumab treatment; visit 3: >3 months after discontinuation of bevacizumab treatment.P-value generated by general linear model repeated measures test

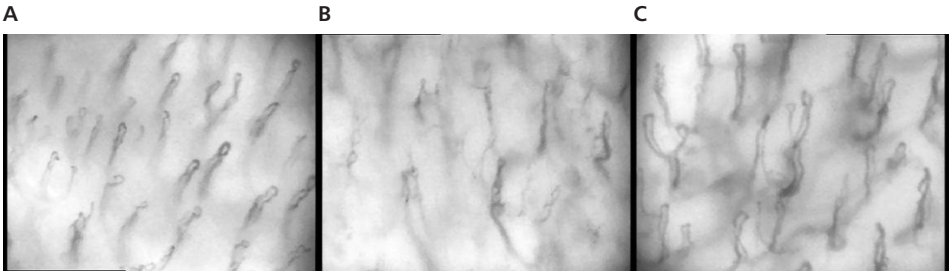

Fig. 2. SDF images demonstrating visible capillary loops of a representative patient. A: at baseline; B: after 6 weeks of bevacizumab treatment; C: >3 months after bevacizumab discontinuation.

Vascular changes during bevacizumab treatment

In a total of 14 patients measurements were performed before and during treatment with bevacizumab.

In 6 patients additional measurements after discontinuation of bevacizumab treatment could not be performed due to various reasons; 2 patients died, 2 patients were in poor clinical condition due to tumor progression, 1 moved out of the region and 1 was still receiving bevacizumab treatment.

Capillary density measurements with SDF imaging

In all 14 patients, the number of capillary loops markedly decreased from 18.2 at baseline to 13.3 ($p=0.00002$) after 6 weeks treatment with bevacizumab (Table 2).

Table 2. Hemodynamic and vascular function/structure variables; mean values of all patients.

2A: 8 patients with measurements available at all 3 predefined timepoints; baseline, after 6 weeks of bevacizumab treatment and >3 months after discontinuation. 2B: 14 patients with data available at baseline and after 6 weeks of bevacizumab treatment, but not after bevacizumab discontinuation.

2A

n=8	Visit 1 baseline values	Visit 2 after 6 weeks treatment	Visit 3 >3 months after discontinuation	Repeated measures overall p-value ‡	Visit 1 vs. 2 p-value †	Visit 2 vs. 3 p-value †
Capillary density (n) **	18.4	13.7	20.7	0.00001 *	0.002 *	0.001 *
Capillary diameter (mm)	6.9	5.6	5.7	0.094	0.051	0.816
pSBP (mm Hg)	128.4	134.1	132.6	0.536	0.286	0.768
pDBP (mm Hg)	83.9	90.0	85.3	0.203	0.042 *	0.166
cSBP (mm Hg)	134.4	135.5	132.3	0.877	0.891	0.570
cDBP (mm Hg)	83.8	86.4	86.3	0.634	0.484	0.963
FMD (%)	7.0	3.5	3.8	0.031 *	0.039 *	0.699
NMD (%)	15.5	16.1	9.7	0.186	0.302	0.060
PWV (m/s)	8.9	9.7	9.4	0.169	0.064	0.558

* $p < 0.05$

** patient 6 no SDF data available at visit 3

‡ general linear model repeated measures test

† paired Student's t-test

n: number; pSBP: peripheral systolic blood pressure; pDBP: peripheral diastolic blood pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; FMD: flow mediated dilatation; NMD: nitroglycerin mediated dilatation; PWV: aortic pulse wave velocity.

2B

n=14	Visit 1 baseline values	Visit 2 after 6 weeks treatment	Visit 1 vs. 2 p-value †
Capillary density (n) **	18.2	13.3	0.00002*
Capillary diameter (mm)	6.9	5.6	0.002 *
pSBP (mm Hg)	129.3	133.1	0.368
pDBP (mm Hg)	82.2	87.7	0.033 *
cSBP (mm Hg)	134.8	139.6	0.322
cDBP (mm Hg)	83.4	87.9	0.081
FMD (%)	6.3	3.2	0.006 *
NMD (%)	12.5	13.7	0.521
PWV (m/s)	8.9	9.6	0.027 *

* $p < 0.05$

† paired Student's t-test

n: number; pSBP: peripheral systolic blood pressure; pDBP: peripheral diastolic blood pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; FMD: flow mediated dilatation; NMD: nitroglycerin mediated dilatation; PWV: aortic pulse wave velocity.

Blood pressure, vascular function and vascular structure assessments

There was no significant change in most of the blood pressure parameters after 6 weeks treatment with bevacizumab (Table 2). There was a significant increase in peripheral diastolic blood pressure. The decrease in FMD from 6.3% to 3.2% after 6 weeks treatment was statistically significant (p=0.006) when compared with baseline (Table 2). There was no change in NMD. Mean PWV significantly increased from 8.9 m/s at baseline to 9.6 m/s after 6 weeks treatment (p= 0.027; Table 2).

Association of capillary density with bevacizumab efficacy

Figure 3 shows the relationship between capillary density changes and bevacizumab efficacy. Amongst the 6 patients that were alive and showed no tumor progression within 6 months of start of bevacizumab treatment were the 3 patients showing the clearest decrease in capillary density after 6 weeks of bevacizumab treatment.

Discussion

We conducted this study to investigate whether bevacizumab-associated vascular and blood pressure effects are reversible after discontinuation of bevacizumab treatment. We additionally aimed at providing more information on the mechanisms in the development of bevacizumab-associated side effects.

To our knowledge, this is the first study describing effects on blood pressure and the vascular system after discontinuation of VEGF inhibitory treatment. Until now, the reversibility of vascular changes was never documented in humans. We now report that the bevacizumab induced decrease in capillary density is reversible. Although this study was performed in a limited number of patients, our results are consistent and in line with previous preclinical data. Preclinical data show rapid reversibility of capillary regression after cessation of VEGF inhibition in normal organs and tumors in mice.[16-18] Strikingly, most capillaries grew back within 2 weeks after cessation of treatment.

In this journal, Mourad et al reported endothelial dysfunction and capillary rarefaction in 18 bevacizumab treated patients.[8] We previously reported a reduction in capillary density (rarefaction) and microvascular flow, associated with a reduced vasodilatory capacity induced by telatinib, an orally active, small molecule inhibitor of VEGFR-2, VEGFR-3, platelet-derived growth factor receptor-a, and c-Kit.[7] With the current study, we confirm that treatment with bevacizumab results in a clear decrease in capillary density. And, after studying both a small molecule tyrosine kinase inhibitor of the VEGF receptor and a monoclonal antibody against VEGF, we conclude that the induced decrease in

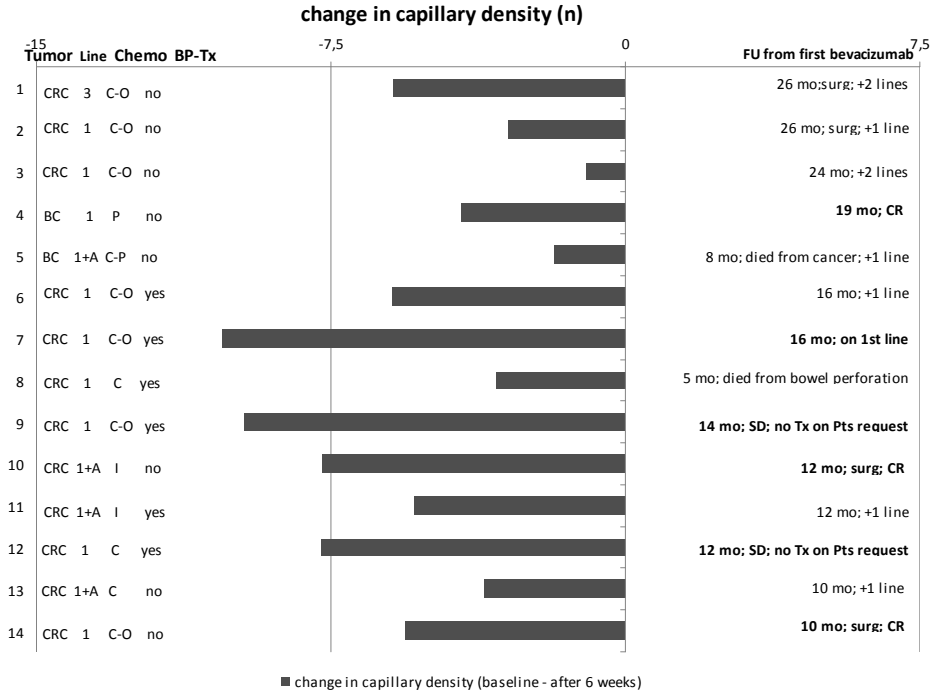

Fig. 3. Change in capillary density after 6 weeks of bevacizumab treatment, compared to baseline. Individuals clinical and efficacy parameters are added. In bold patients that were alive and showed no tumor progression within 6 months of start of bevacizumab treatment.
BP-Tx: blood pressure lowering treatment before start of bevacizumab treatment; CRC: colorectal cancer; BC: breast cancer; A: adjuvant chemotherapy; C: capecitabine; O: oxaliplatin; P: paclitaxel; I: irinotecan; mo: months; surg: surgery; CR: complete response; Tx: therapy; Pt: patient

In this study there was a significant decrease in capillary density after 6 weeks of bevacizumab treatment, however, there was no significant increase in blood pressure. This might be explained by the timing of the measurements, i.e. after six weeks or 2 half-lives of bevacizumab. The decrease in capillary density may be induced before an increase in blood pressure develops. Other explanations may be the limited number of patients, or the use of blood pressure lowering agents in almost half of the patients.

We designed our study, such that our data can be extrapolated to the general patient population treated with bevacizumab containing anti cancer treatment. To maximally imitate daily life, we decided not to exclude patients using blood pressure lowering agents at baseline. While multiple measurements were performed in the same patients and patients therefore were their own internal control, this resulted in accurate statisti-

cal data. We also decided to include patients receiving bevacizumab in combination with various cytotoxic agents. Since vascular changes were seen in all patients, we can conclude that the changes in vascular parameters are linked to the bevacizumab treatment, and are irrespective of the cytotoxic agents used in the bevacizumab combination.

The sidestream dark field (SDF) method is a very elegant method for measuring capillary density. It is a non-invasive, not painful method, with no side effects for the test subject. Measurements can be performed in almost all subjects while the mucosal surface of the inner lip is intact and easy to reach in most patients. Moreover, the measurement time for the patient is limited, while large parts of the data analysis can be performed off line. The technique is fairly easy to learn, and reproducibility of data is generally high.[7,13,14]

Our results may have several implications in the treatment of cancer patients with VEGF inhibitors. Recently, increased blood pressure during treatment with VEGF inhibitors has been associated with a longer time to tumor progression.[19-22] Possibly, capillary density measured by the noninvasive SDF method can be used as an even earlier marker for response. In our study we could show that decreased capillary density during bevacizumab treatment may be associated with a better prognosis. However, this is exploratory and confirmation with adequate sample size is needed. Also, theoretically, combining capillary density measurements with blood pressure changes can results in a better predictive marker than blood pressure alone. Moreover, we would like to stimulate the sense of urgency to monitor patients, since the reversibility of vascular changes after discontinuation of VEGF-inhibiting therapy might suggest that timely intervention may reduce or prevent certain side effects with sometimes high morbidity and even mortality. This is the first study reporting that the bevacizumab-induced capillary density decrease is reversible after discontinuation of bevacizumab treatment. In combination with earlier results in VEGF tyrosine kinase inhibitor treatment, we also report that VEGF-associated rarefaction is a class-effect generated by all VEGF-inhibitors.

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A phase I study of the combination of daily oral sunitinib with intravenous ifosfamide in patients with advanced solid malignancies

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Abstract

Introduction
Sunitinib is an orally available inhibitor of the vascular endothelial growth factor (VEGF), platelet-derived growth factor, kit oncogene, and fms-related tyrosine kinase 3 receptors. As combinations of VEGF-inhibitors with cytotoxic therapy are promising, this phase I study aimed to determine the recommended phase II dose (RP2D) of sunitinib in combination with 2 different ifosfamide schedules.
Methods
Patients with progressive solid tumors, good performance score, organ function, and no standard therapy available, were eligible. Continuous once daily sunitinib, in escalating doses per cohort, was combined with one of two ifosfamide schedules, 3g/m^2/days1-3 and 1.2g/m^2/days1-5, both given intravenously every 3 weeks. At RP2D, additional patients were enrolled to assess pharmacokinetics. Circulating endothelial cells (CECs) were measured prior to the 1st, 3rd and 6th cycle.
Results
The results of the first 26 patients accrued in this phase I study are reported. Combining 12.5 mg sunitinib with ifosfamide 3g/m^2/days1-3 was not feasible due to neutropenia >7 days in 2 out of 6 patients. However, when using G-CSF, the RP2D was ifosfamide 3g/m^2/days1-3 plus 12.5 mg sunitinib. Ifosfamide 3g/m^2/days1-3 combined with 25 mg sunitinib and G-CSF (n=5) was not feasible due to febrile neutropenia in 2 patients and hypertension with cardiac chest pain in 1 patient. Sunitinib at 12.5 mg in combination with ifosfamide 1.2g/m^2/days1-5 was also feasible with 1 out of 6 patients developing encephalopathy as dose limiting toxicity.

Sunitinib co-administration did not affect the pharmacokinetics of ifosfamide or one of its metabolites. No consistent change in the number of CECs during treatment was observed. Of 25 evaluable patients, 4 showed a partial response (16%) and 12 patients had stable disease (48%) as best tumor response.
Conclusions
Sunitinib at 12.5 mg/day with ifosfamide 3g/m^2/days1-3, and sunitinib at 12.5 mg/day with ifosfamide 1.2g/m^2/days1-5 every 3 weeks is tolerable if supported by G-CSF.

Introduction

The use of the so-called targeted drugs including monoclonal antibodies and tyrosine kinase inhibitors (TKIs) is rapidly increasing in oncology.[1] The anti-tumor effects of these targeted drugs applied as single agent, however, is modest in most tumors. Therefore, combined therapy of targeted drugs and standard cytotoxic agents has become a treatment and research strategy of interest. Early reports on combining sunitinib with various standard chemotherapeutical agents show promising results.[2-12]

Sunitinib is an orally available inhibitor of the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), kit oncogene (C-KIT), and fms-related tyrosine kinase 3 (FLT3) receptors. Sunitinib is effective as single agent in several solid tumor types and is registered for use in advanced renal cell cancer, and imatinib-resistant or -intolerant gastrointestinal stromal tumors (GISTs).[13-17] The most common adverse events reported in single agent trials are fatigue, diarrhea, nausea, sore mouth, skin discoloration, and hypertension. Hematological adverse events are manageable with grade 3/4 neutropenia in 13% of patients, anemia in 7% and thrombocytopenia in 3%. Infectious complications of neutropenia are very rare.

Ifosfamide is one of the oldest chemotherapeutic agents and induces anti-tumor activity through DNA alkylation. It is used in the treatment of several tumor types including advanced breast cancer, testicular cancer, small cell lung cancer, non-small cell lung cancer, soft tissue sarcomas, bone sarcomas, and central nerve system (CNS) tumors such as medulloblastomas.[18-24] Grade 3/4 toxicities occurring in more than 5% of the patients during treatment with ifosfamide comprise neutropenia (56%), neurotoxicity (11%), nausea/vomiting (10%), and infection (10%).[23]

Combining VEGF-pathway inhibitors with cytotoxic agents has several potential advantages. VEGF produced by tumor cells results in the formation of new vasculature which is abnormal in structure and function. These new vessels are leaky and, therefore, result in a higher interstitial pressure within the tumor. Inhibition of VEGF-mediated activities by sunitinib results in a decrease of this interstitial pressure and enhanced delivery of the concomitantly administered cytotoxic drug.[25,26] Therefore, the possibility of decreasing ifosfamide dose in order to decrease side effects, without decreasing ifosfamide exposure to the tumor, may be advocated. Other mechanisms that may account for synergistic interaction between VEGF-pathway inhibitors and conventional cytotoxic drugs include prevention of endothelial progenitor cell mobilization from the bone marrow induced by chemotherapy and decreased expression of tumor factors conferring resistance against chemotherapy.[27-31]

In addition to potential synergistic interaction, several issues are important when selecting a combination of a targeted drug and a standard chemotherapeutical agent,

including single agent activity of both agents, different mechanisms of action, and a non-overlapping toxicity profile. In theory, all of these are met by the combination of sunitinib and ifosfamide. In this study, two different ifosfamide regimens, which are both widely used, are explored for their feasibility to be combined with sunitinib.

Patients and Methods

Eligibility criteria

Patients with histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 were eligible. Other inclusion criteria were: evaluable or measurable disease by RECIST version 1; age ≥18 years; life expectancy ≥12 weeks; adequate bone marrow, liver, and renal function (hemoglobin ≥6.0 mmol/l; absolute neutrophil count ≥1.5 x 10^9/L; platelet count ≥100 x 10^9/L; total bilirubin ≤1.5x the upper limit of normal (ULN); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤2.5x ULN, (liver metastases AST/ALT <5x ULN); serum creatinine ≤1.5x ULN, creatinine clearance ≥ 60 ml/min and 2 functioning kidneys); systolic blood pressure <150 mmHg and diastolic blood pressure <90 mmHg (treatment with 2 antihypertensive drugs is allowed). Exclusion criteria were: history of cardiovascular disease; known HIV seropositivity; signs or symptoms of central nervous system metastases; pregnancy or breast-feeding; history of any condition that could endanger the safety of the patient; anticancer treatment <4 weeks before the first dose.

The study was designed and conducted under the appropriate institutional review boards' approvals and in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from each participant.

Dose-levels and Dose Escalation Procedure

Daily oral sunitinib was planned to be evaluated in three escalating dose cohorts, 12.5 mg, 25 mg, and 37.5 mg, in combination with a fixed dose of ifosfamide 3 g/m^2/day for three days intravenously administered at 3-weekly intervals. After establishing the recommended phase II dose (RP2D) of sunitinib with ifosfamide at 3 g/m^2/day for three days, this sunitinib dose was also evaluated with ifosfamide iv at 1.2 g/m^2/day for 5 days. This second ifosfamide schedule was additionally assessed for its feasibility to be combined with sunitinib as both ifosfamide schedules are frequently used.

Using the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0, dose-limiting toxicity (DLT) was defined as the following toxicity during the first treat-

ment cycle: grade 4 neutropenia ≥7 days, febrile neutropenia, grade 4 thrombocytopenia, creatinine ≥2x ULN and any drug-related grade 3 or 4 non-hematological toxicity excluding, nausea and vomiting not refractory to anti-emetics, grade 3 fatigue <7 days, and hypertension not refractory to anti-hypertensive medication. If a DLT was observed in one patient, three additional patients were recruited at that dose level, with dose escalation proceeding if in <2 of 6 patients a DLT occurred. If a DLT was observed in ≥2 patients in a cohort, RP2D had been exceeded. The RP2D of sunitinib was defined as the highest dose level which resulted in pre-defined dose limiting toxicity encountered during the first cycle in less than 33% of the patients.

At the beginning of each cycle with ifosfamide, patients had to have neutrophils ≥1.5 x 10^9/L and platelets ≥100 x 10^9/L. Treatment could be delayed for a maximum period of 2 weeks for hematological recovery. Dose reduction of more than 50% of the initial ifosfamide dose was not allowed. If patients developed a systolic blood pressure >160 mmHg, a diastolic blood pressure >100 mmHg or an increase of diastolic blood pressure >20 mmHg, which despite antihypertensive medication with an ACE-inhibitor and a calcium-channel blocker was not adequately controlled within 2 weeks, treatment with sunitinib was stopped. In case of grade 4 hypertension sunitinib was also stopped.

If a patient experienced an ifosfamide related DLT the dose of ifosfamide was reduced with 25% at every occurrence. Dose reduction of more than 50% of the initial ifosfamide dose was not allowed. Patients who experienced a DLT that had not resolved to ≤grade 1 within 5 weeks after day 1 of the previous ifosfamide administration (a maximum of two weeks delay for the next cycle was allowed) were withdrawn from the study. In those patients experiencing a DLT related to sunitinib, sunitinib was withheld for a maximum of 2 weeks. If toxicity resolved to ≤grade 1 continuation at the next lower dose cohort level was allowed for the subsequent courses.

Patients were treated for a maximum of 6 ifosfamide cycles. Those patients who experienced a benefit from the combination of sunitinib and ifosfamide were allowed to continue treatment with sunitinib alone. Treatment was continued until disease progression or unacceptable toxicity.

Pre-treatment Evaluation and Safety Assessment

Pre-treatment evaluation consisted of a complete medical history, physical examination, WHO performance status assessment, vital signs, 12 lead ECG, blood sample for complete blood count (CBC), biochemistry analysis, serum pregnancy test for women with child-bearing potential, and baseline tumor measurements.

Weekly evaluation consisted of a brief history and physical examination, concomitant medication, vital signs, blood samples for CBC (twice weekly in the first cycle), and bio-

chemistry. Response evaluation was performed every 2 cycles and was assessed according to RECIST, version 1.[32] Patients were evaluated weekly for adverse events and toxicity according to the NCI-CTC, version 3.0.

Pharmacokinetic Evaluation

In order to determine whether co-administration of sunitinib affects ifosfamide pharmacokinetics (PKs), plasma concentrations of ifosfamide and its most important metabolites, 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide, and 4-hydroxy-ifosfamide, were monitored during the first two cycles. This was performed in the additional patients who were treated at the RP2D of sunitinib in combination with the ifosfamide 3g/m^2/days 1-3 schedule. In these patients sunitinib treatment started on day 8.

Blood sample collection

Blood samples for PK evaluation were collected during cycles 1 and 2 via an indwelling intravenous catheter. A 7 mL blood sample was collected in the presence of lithium heparin as anticoagulant pre-dose, 3, 6, 10, 24 hours after the start of the ifosfamide infusion, and thereafter every 12 hours until the end of infusion, prior to the end of infusion and 1, 3, 6, 12, and 24 hours after the end of infusion. Blood samples were centrifuged within 15 minutes after collection for 10 minutes at 3000 x *g* at 4°C. Subsequently, an aliquot of exactly 1 mL of the plasma supernatant was transferred into a vial containing 100 μL of a 2M semicarbazide solution and was stored at <-70°C until analysis of 4-hyroxy-ifosfamide. The remaining plasma was stored at <-70°C, without any additive, until the simultaneous analysis of ifosfamide its 2- and 3-dechloroethyl metabolites.

Analysis of ifosfamide and its metabolites

Ifosfamide and the 2- and 3-dechloroethyl metabolites were simultaneously quantitated by a validated liquid-chromatography-tandem triple quadrupole mass spectrometry (LC-MS/MS) assay. The analytes were extracted from 10 μL aliquots of plasma with 1.5 mL of ethyl acetate after the addition of 10 μL of a 1 μg/mL cyclofosfamide solution in methanol (internal standard). Following vigorous vortex mixing for 5 min and centrifugation for 10 min at 18,000 x *g*, an aliquot of the clear supernatant was evaporated to dryness under a gently stream of nitrogen at 70°C. Subsequently the residue was dissolved in an aliquot of 100 μL of a 20% methanol solution in water, from which an aliquot of 5 μL was injected onto the LC-MS/MS system. The analytes were separated by high-performance liquid chromatography (Model 2795 XC, Waters, Mildford, MA)

on a Nucleosil C18-AB (150x4.6mm, 5mm) analytical column (Macherey-Nagel, Duren, Germany). The mobile phase was composed of methanol and water containing ammonium formate (2mM) at a flow rate of 0.5 mL/min. The first 9 minutes the mobile phase consisted of 20% methanol which was linearly increased in 0.5 min to 45% methanol. Subsequently the percentage methanol was held at 45% until 20 min after which it was linearly decreased to 20% in 0.5 min. The retention times of 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide, ifosfamide and the internal standard cyclofosfamide were 4.7, 5.9, 13.8 and 14.7 min, respectively, with an overall run time of 25 min. Detection was performed with a MicroMass Quatro Micro triple-quadropole mass spectrometer (Cary, NC) in the positive ion mode. The electrospray ionization operated at 3.0 kV and at a cone voltage of 35 V. The detector was programmed to allow the [MH]+ ions of 2-dechloroethyl-ifosfamide (m/z 199), 3-dechloroethyl-ifosfamide (m/z 199), ifosfamide (m/z 261) and cyclofosfamide (m/z 261) to pass through the first quadropole and into the collision cell. The collision energy for collision-induced dissociation of 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide, ifosfamide and cyclofosfamide was set at 22 eV, 20 eV, 22 eV and 20 eV, respectively, with argon used as collision gas at a pressure of 0.005 mbar. The daughter ions of 2-dechloroethyl-ifosfamide (m/z 92), 3-dechloroethyl-ifosfamide (m/z 78), ifosfamide (m/z 92) and cyclofosfamide (m/z 140) were monitored through the third quadropole. The dwell time per channel for data collection was 0.100 seconds. Weighted (1/concentration^2) linear regression analysis of peak area ratios of analytes and internal standard, versus concentration of analytes were used for the quantitation. Peak area ratios were a function of the concentration from 50.0 to 5,000 ng/mL for ifosfamide and its 2- and 3-dechloroethyl metabolites. The method was validated in accordance with the Guidance for Industry, Bioanalytical Method Validation, as specified by the Food and Drug Administration.[33] For ifosfamide, the within and between-run precisions at five tested concentrations, including the lower limit of quantitation (LLQ), were ≤3.7 and ≤3.6%, respectively, while the average accuracy ranged from 92.3 to 104.7%. For 2-dechloroethyl-ifosfamide, the within and between-run precisions were ≤4.8 and ≤3.1%, respectively, with the accuracy ranging from 90.0 to 103.1%. And for 3-dechloroethyl-ifosfamide, the within and between-run precisions were ≤4.9 and ≤4.1%, respectively, while the average accuracy ranged from 97.8 to 105.4%.

4-Hydroxy-ifosfamide was analyzed by a separate validated LC-MS/MS method, based on the method describe above. 4-Hydroxy-ifosfamide was extracted from 50 μL aliquots of plasma with 1.5 mL of ethyl acetate after the addition of 10 μL of a 1 μg/mL cyclofosfamide solution in methanol (internal standard). Samples were further processed as described above and injected onto the same system and analytical column. The first 2 minutes the mobile phase, delivered at a flow rate of 0.5 ml/min, consisted of 20% methanol in water which was linearly increased in 0.5 min to 45% methanol. Subsequently the

percentage methanol was hold at 45% until 10 min after which it was linearly decreased to 20% in 5 min. The retention times of 4-hydroxy-ifosfamide and cyclofosfamide were 5.8 and 8.5 min, respectively, with an overall run time of 20 min. The electrospray ionization operated at 3.0 kV and at a cone voltage of 20 V for 4-hydroxy-ifosfamide and of 35 V for cyclofosfamide. The daughter ions of 4-hydroxy-ifosfamide (m/z 334>80; collision energy 27 V) and cyclofosfamide (m/z 261>140; collision energy 20 V) were monitored, with argon at a pressure of 0.005 mbar. The dwell time per channel for data collection was 0.150 seconds. Weighted (1/concentration^2) linear regression analyses of peak area ratios of 4-hydroxy-ifosfamide and internal standard, versus concentration of 4-hydroxy-ifosfamde was used for the quantitation. Calibration curves for were linear from 50.0 to 5,000 ng/mL. The accuracy ranged from 94.0% to 105.4%, the within-run precisions were ≤4.7% and the between-run precisions were ≤5.2% at five tested concentrations, including the lower limit of quantitation of 50.0 ng/mL.

Pharmacokinetic Data Analysis

Individual pharmacokinetic parameters for ifosfamide, 2- and 3-dechloroethyl-ifosfamide and 4-hydroy-ifosfamide were estimated using noncompartmental analysis (1/y weighting factor) using the software program WinNonLin 5.0 (Pharsight, CA, USA).

Circulating Endothelial Cells

Two 10 ml blood samples for analysis of circulating endothelial cells (CECs) were collected at baseline, on day 0 of cycles 3 and 6, and 6 weeks after discontinuation of ifosfamide administrations. Enumeration of CECs was performed using cellsearch analysis as previously described.[34]

Results

This report describes the results of the first 26 of the total number of 32 patients enrolled in the recently closed study. Patient characteristics are summarized in Table 1.

Safety and Tolerability

All treatment-related adverse events during combined treatment with sunitinib and ifosfamide are summarized in Table 2. Using the combination of sunitinib at 12.5 mg with ifosfamide 3g/m^2/days1-3 all patients developed hematological toxicity. Two out

Table 1. Baseline demographics and patient characteristics.

Baseline characteristics	Patients (n (%))
Gender	
Male	15 (58)
Female	11 (42)
Age, years	
Median (range)	51 (36-69)
WHO performance status	
0	7 (27)
1	19 (73)
Prior anticancer therapies	
Surgery	20 (77)
Systemic anticancer therapy	22 (85)
Number of previous treatments (range)	1 (0-4)
0	4 (15)
1	15 (58)
≥2	7 (27)
Radiation therapy	12 (46)
Tumor type	
Sarcoma	12 (46)
Chondrosarcoma	2 (8)
Ewing sarcoma	2 (8)
Leiomyosarcoma	3 (12)
Liposarcoma	2 (8)
Osteosarcoma	1 (4)
Sarcoma NOS	1 (4)
Soft tissue sarcoma	1 (4)
Carcinoma of unknown primary	3 (12)
Neuroendocrine carcinoma	2 (8)
Miscellaneous	9 (35)

WHO: World Health Organization

of 6 patients had grade 4 neutropenia >7 days (DLT) and therefore this combination exceeded the recommended phase II dose (RP2D) and was considered not feasible. As neutropenia was the sole DLT, an amendment was made to continue the study with the addition of granulocyte-colony stimulating factor (G-CSF; pegfilgrastim 6 mg once per cycle) in all subsequent patients.

In none of the initial three patients at the dose level of sunitinib at 12.5 mg in combination with ifosfamide 3g/m^2/days1-3 a DLT occurred. In the subsequent cohort evaluating sunitinib 25 mg plus ifosfamide 3g/m^2/days1-3 and G-CSF group, three out of

Table 2. Treatment-related adverse events during combination therapy with sunitinib (S) and ifosfamide (I).

Adverse Event	Cohort 1 n=6		Cohort 2 n=3		Cohort 3 n=5		Cohort 4 n=6		Total incidence n=20		Cohort 5 n=6*	
	I 3 g/m^2 3 days								I 3 g/m^2 3 days		I 1.2 g/m^2 5 days	
	S 12.5 mg		S 12.5 mg		S 25 mg		S 12.5 mg		S 12.5-25 mg		S 12.5 mg	
	No G-CSF		Plus G-CSF		Plus G-CSF		Plus G-CSF				Plus G-CSF	
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2 n (%)	Grade 3-4 n (%)	Grade 1-2	Grade 3-4
Hematologic toxicity												
Anemia	5	1	3	–	5	–	5	1	18 (90)	2 (10)	6	–
Leucopenia	1	5	1	2	2	3	–	6	4 (20)	16 (80)	1	–
Neutropenia	1	5	1	2	2	3	–	6	4 (20)	16 (80)	–	–
Thrombocytopenia	5	1	3	–	3	2	2	4	13 (65)	7 (35)	3	1
Febrile neutropenia		–		–		2		1		3 (15)		–
GI toxicity												
Anorexia	–	1	1	–	2	–	3	–	6 (30)	1 (5)	1	–
Nausea	4	–	2	–	5	–	4	–	15 (75)	–	5	–
Vomiting	3	–	1	–	5	–	3	–	12 (60)	–	3	–
Metabolic toxicity												
AST/ALT	6	–	1	–	4	–	4	–	15 (75)	–	2	–
Constitutional toxicity												
Fatigue	4	1	3	–	4	1	5	1	16 (80)	3 (15)	3	1
Dermatologic toxicity												
HFS	2	–	1	–	1	–	1	–	5 (25)	–	–	–
Miscellaneous												
Alopecia	5	–	3	–	4	–	5	–	17 (85)	–	3	–
Hypertension	–	–	–	–	–	1	1	–	1 (5)	1 (5)	–	–

GI: gastro-intestinal, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HFS: hand-foot syndrome
* Ongoing patients received 1 cycle, 2 cycles and 4 cycles respectively, results include toxicity developed in these cycles.

5 patients developed a DLT and thus the RP2D was exceeded. DLTs consisted of 2 cases of febrile neutropenia and 1 case of hypertension and cardiac chest pain. One out of 9 patients treated with sunitinib 12.5 mg in combination with ifosfamide 3g/m^2/days1-3 and G-CSF developed febrile neutropenia. Therefore, the RP2D was established at once daily, continuously dosed sunitinib 12.5 mg in combination with ifosfamide 3g/m^2/days1-3 and G-CSF. The median number of ifosfamide cycles at the RP2D was 3.7, with a median dose of 2.9 g/m^2 ifosfamide per cycle over all cycles. The median dose of sunitinib at the RP2D was 246 mg per course, i.e.11.7 mg of sunitinib per day during the cycles administered in combination with ifosfamide. The total given number of sunitinib cycles ranged from 1 to >21 (patient still on treatment).Across the dose levels of sunitinib, for all patients treated with ifosfamide 3g/m^2/days1-3 combination, grade 3-4 hematological toxicity developing during ifosfamide and sunitinib combination treatment cycles, consisted of anemia in 10%, leucopenia in 80%, neutropenia in 80% and thrombocytopenia in 35% of patients. Febrile neutropenia was only seen in 3 patients, once in the combination with sunitinib 12.5 mg plus G-CSF and twice in the combination with sunitinib 25 mg plus G-CSF. Grade 3-4 non-hematological toxicity consisted of fatigue (15%), anorexia (5%), and hypertension (5%).

When ifosfamide 1.2g/m^2/days1-5 was combined with sunitinib 12.mg and G-CSF 1 out of 6 patients developed a DLT, ifosfamide induced encephalopathy. Therefore, this combination was considered feasible as well and was expanded with 6 patients for PK analysis. Results of the patients in this additional cohort are not yet available. The most frequently reported treatment-related grade 3-4 adverse events in the first 6 patients treated with ifosfamide 1.2g/m^2/days1-5 were thrombocytopenia (17%), and fatigue (17%).

Pharmacokinetics

Ifosfamide pharmacokinetic parameters derived from patients at the RP2D level (sunitinib 12.5 mg in combination with ifosfamide 3g/m^2/days1-3 and G-CSF) are summarized in Table 3. Ifosfamide pharmacokinetics were similar to those reported in the literature.[35,36] Sunitinib co-administration did not affect the pharmacokinetics of ifosfamide or one of its metabolites. Figure 1 shows the mean concentrations of ifosfamide and its metabolites of the patients treated in the sunitinib 12.5 mg and ifosfamide 3g/m^2/days1-3 combination.

As treatment in the sunitinib and ifosfamide 1.2g/m^2/days1-5 combination was still ongoing, no pharmacokinetic data for these patients can be reported. Also, data on sunitinib pharmacokinetics are not available, yet.

Table 3. Plasma pharmacokinetic parameters of ifosfamide and ifosfamide-metabolites during cycle 1 (without sunitinib) and cycle 2 (with sunitinib) in RP2D level patients treated with sunitinib 12.5 mg in combination with ifosfamide 3g/m^2/days1-3 and G-CSF.

		Plasma pharmacokinetic parameters of ifosfamide				
Patient	**Cycle**	**Dose (mg)**	**Cmax (μg/mL)**	**t1/2 (hour)**	**AUC0-∞ (μg*h/mL)**	**CL (L/hour)**
008	1	6200	358	3.79	2210	2.81
	2	6200	354	3.71	1954	3.17
009	1	4400	434	3.32	2415	1.82
	2	4400	401	3.49	2270	1.94
010	1	4900	429	2.06	1707	2.87
	2	4900	344	2.26	1622	3.02
108	1	7200	395	3.79	2104	3.42
	2	7200	416	3.82	2287	3.15
110	1	6600	322	3.71	1834	3.60
	2	6600	425	3.38	2039	3.24
111	1	6900	452	3.42	2403	2.87
	2	5100	315	4.01	1750	2.91

	AUC Ratio C1/C2[1]			
	Ifosfamide	**N2-DCE-Ifosfamide**	**N3-DCE-Ifosfamide**	**4OH-Ifosfamide**
008	1.13	1.17	1.21	0.93
009	1.06	0.89	0.92	0.72
010	1.05	1.00	0.93	0.72
108	0.92	1.35	1.36	n.a.
110	0.90	1.04	0.94	n.a.
111	1.02	1.06	1.15	0.89

Cmax: maximal concentration; t1/2,z: terminal half-life; AUC0-¥: areas under the curve up to infinite time, CL: systemic clearance, N2-DCE-Ifosfamide: 2-Dechloroethyl-ifosfamide, N3-DCE-Ifosfamide: 3-Dechloroethyl-ifosfamide, 4OH-Ifosfamide: 4-hydroxy-ifosfamide, n.a.: not available, [1]corrected for dose.

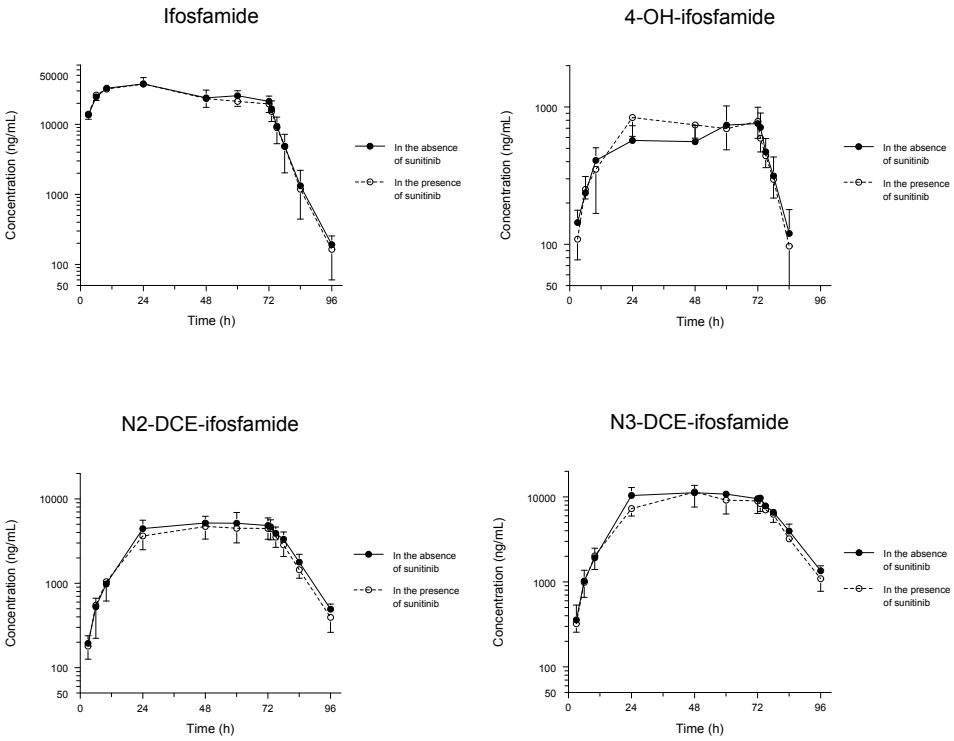

Fig. 1. Mean concentrations of ifosfamide and its metabolites of patients treated with the sunitinib 12.5 mg and ifosfamide 3g/m2/days1-3 combination.

Circulating Endothelial Cells

In 13 patients data for CECs were available at baseline and after 6 weeks of sunitinib and ifosfamide treatment (Figure 2). No consistent change in the number of CECs during treatment was observed.

Anti tumor activity

Twenty-five patients were evaluable for anti tumor activity. Best tumor response was a partial response seen in 4 patients (16%) and stable disease in12 patients (48%; Table 4). Two patients receiving sunitinib and ifosfamide 3g/m^2/days1-3 combination treatment have long-lasting responses with stable disease for 42 and 63 weeks, respectively. In these patients, with mesenchymal chondrosarcoma and chordoma, respectively, treatment with sunitinib is still ongoing.

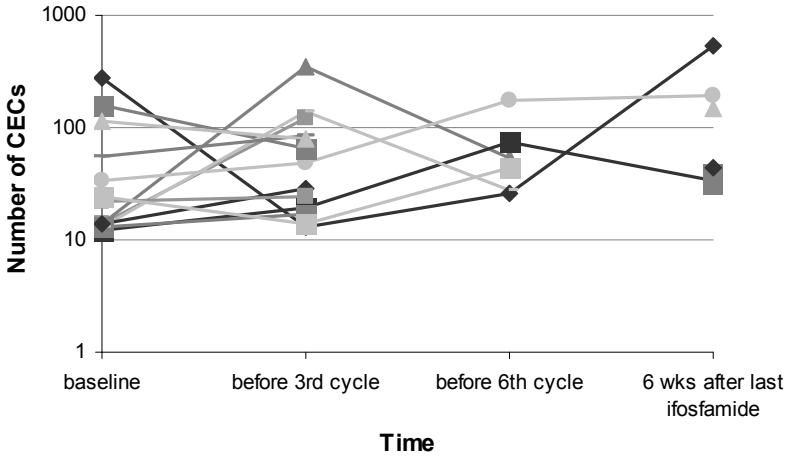

Fig. 2. Circulating endothelial cells during treatment with sunitinib in combination with ifosfamide 3g/m2/days1-3.

Table 4. Best tumor response of evaluable patients receiving sunitinib in combination with ifosfamide 3g/m^2/days1-3 (cohorts 1-4) and sunitinib in combination with ifosfamide ifosfamide 1.2g/m^2/days1-5 (cohort 5).

Cohort	N	Ifosfamide	Sunitinib	G-CSF	Best Tumor Response		
					Partial response	Stable disease	Progressive disease
1	6	3g/m^2/d1-3	12.5 mg/day	no	1	3	2
2	3	3g/m^2/d1-3	12.5 mg/day	yes	0	1	2
3	5	3g/m^2/d1-3	25 mg/day	yes	2	2	1
4	6	3g/m^2/d1-3	12.5 mg/day	yes	1	4	1
5	5*	1.2g/m^2/d1-5	12.5 mg/day	yes	0	2	3

* 1 patient ongoing, no evaluation performed yet.

Discussion

This study shows that combining sunitinib administered at 12.5 mg daily with either ifosfamide 3g/m^2/days1-3, or with ifosfamide 1.2g/m^2/days1-5 is feasible, when supported with G-CSF.

Ifosfamide monotherapy is known for substantial grade 3-4 side effects, including clinically relevant hematological toxicity.[23] In our study, the rate of febrile neutropenia in-

creased when the dose of sunitinib was increased to 25 mg, suggesting that the addition of sunitinib to ifosfamide increases hematological toxicity. Whether this is mainly the result of addition or synergism of the two agents on the bone marrow is unclear. Preliminary results show no influence of sunitinib on ifosfamide PK parameters. The effects of ifosfamide on sunitinib PK are unknown and results will follow. Concerning the relatively high frequency of neutropenia, one should also bear in mind that this study enrolled a pretreated group of patients (27% ≥2 previous systemic treatment lines) unlike most patients treated with ifosfamide. Previously, grade 3-4 neutropenia was reported in 20% of all ifosfamide courses in the first-line and in 31% in the second-line with a 5 g/m^2/1 day schedule, while for the 3 g/m^2/3 days schedule the rates were 56 and 77%, respectively.[23] In this latter study patients with ≥2 previous systemic anticancer treatment lines were excluded.

Recently, various combinations of sunitinib with chemotherapeutical agents have been studied, including combinations with capecitabine, carboplatin plus paclitaxel, gemcitabine, irinotecan, gemcitabine plus cisplatin, and 5-fluorouracil plus irinotecan.[2-12] In these phase I and II studies most combinations appeared to be feasible, however at the expense of increased hematological toxicity. The rate of neutropenia might be related to the dose and schedule of sunitinib and on the cytotoxic agent or agents in the combination. For example, when sunitinib is combined with capecitabine, grade 4 neutropenia was reported in <10% of patients.[5,10] Sunitinib in combination with irinotecan or carboplatin/paclitaxel resulted in grade 3/4 neutropenia in 30-60% of patients.[6,11,12] At this moment, these combination studies are only reported in abstract form, and therefore data, and interpretation of data, is limited.

Sunitinib RP2D was established at 12.5 mg, continuously dosed, when combined with ifosfamide. Given as monotherapy, the recommended sunitinib dose is 50 mg given daily for 28 days every 6 weeks.[15] Another widely used schedule is 37.5 mg sunitinib, once daily, administered continuously. Though data from randomized studies are lacking, theoretically, continuous dosing of sunitinib is likely to be more effective, as continuous inhibition of angiogenesis pathways probably resorts in higher anti-tumor effects than intermittent inhibition. Recently, George et al reported that continuous daily sunitinib dosing of 37.5 mg achieved and sustained effective drug concentrations without additional accumulation across cycles.[37]

The recommended sunitinib dose of 12.5 mg, when combined with ifosfamide, is considerably lower that the recommended doses of single agent sunitinib. However, theoretically low doses of VEGF-pathway inhibitors may even be more beneficial in combination therapy. As previously mentioned, VEGF inhibition, in general, results in a decrease in interstitial fluid pressure, normalization of tumor vasculature, and increased delivery of the chemotherapeutical agent to the tumor site. The optimal dosing and scheduling of VEGF inhibitors may be critical. Excessive suppression of the tumor vasculature with

complete vasoconstriction or vessel disappearance may result in decreased delivery of the chemotherapeutical agent and decreased anti-tumor activity. Therefore, lower doses of sunitinib might even result in better anti-tumor efficacy then higher doses. Indeed, this was previously reported for sunitinib in a study where interstitial fluid concentrations of the cancer chemotherapeutic drug temozolomide were increased when tumors were pretreated with sunitinib at 10 mg/kg but not at 40 mg/kg.[4] In addition, a phase I study of sunitinib monotherapy showed therapeutic sunitinib plasma concentrations and tumor responders even in the lower dose sunitinib group.[38] To optimize and study these effects of sunitinib on ifosfamide delivery it might be beneficial to evaluate tumor blood flow using noninvasive imaging techniques.

In our study, we investigated the effects on circulating endothelial cells in order to establish whether this is a prognostic factor and reflects treatment-induced antitumor activity in patients treated with the combination of sunitnib and ifosfamide. We did not observe consistent changes in the number of CECs, suggesting no relevance of CEC level as biomarker in the sunitinib and ifosfamide combination. However, patient numbers are limited.

One of the tumor types for which the combination of sunitinib and ifosfamide is interesting is soft tissue sarcoma. In patients with advanced soft tissue sarcoma, it was recently revealed that the combination of a VEGF-inhibitor with doxorubicin, a frequently applied drug in soft tissue sarcomas, is not feasible because of an unacceptable high incidence of doxorubicin-mediated cardiotoxicity.[39] Ifosfamide is the only drug, besides doxorubicin, with consistent efficacy against soft tissue sarcomas and is therefore frequently used as first-line treatment against this tumor entity.[40] As ifosfamide is not featured by the occurrence of cardiotoxicity, the combination of sunitinib and ifosfamide is attractive to explore in soft tissue sarcomas. In addition, this combination can be explored in other tumor types, including relapsed testicular cancer, advanced breast cancer, lung cancer, small blue round cell tumors and certain central nervous system tumors. Today, to our knowledge, no reports on the use of ifosfamide in combination with other VEGF inhibitors are published.

In conclusion, sunitinib at 12.5 mg/day with ifosfamide 3g/m^2/days1-3, and sunitinib at 12.5 mg/day with ifosfamide 1.2g/m^2/days1-5 every 3 weeks supported by G-CSF is tolerable in patients with advanced solid tumors. Future studies should aim at evaluating efficacy in specific tumor types.

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9

A phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib (PHA-739358) in patients with advanced or metastatic solid tumors

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Abstract

Purpose

Danusertib (PHA-739358) is a small-molecule pan-aurora kinase inhibitor. This phase I dose escalation study was conducted to evaluate safety and tolerability of danusertib with additional pharmacokinetics, biomarker and efficacy assessments.

Patients and methods

Patients with solid tumors refractory to standard therapies or with no standard therapy available were enrolled. Danusertib was administered intravenously on days 1,8,15 every 28 days in 6-hour or 3-hour infusion schedules (6h-ivS, 3h-ivS). Dose levels from 45 mg/m^2 in the 6h-ivS, and from 250 mg/m^2 in the 3h-ivS were studied.

Results

Fifty patients were treated. For the 6h-ivS, the most frequently reported side effects were neutropenia (55%), nausea (25%), anorexia (23%), fatigue (20%), and diarrhea (18%). In the 3h-ivS, fatigue (70%), neutropenia (60%), diarrhea (50%), and nausea (30%) were seen. Non-hematological toxicity was mild to moderate. Neutropenia was dose limiting. The maximum tolerated dose was 330 mg/m^2 for the 6h-ivS and not identified for the 3h-ivS. The systemic exposure to danusertib increased linear with dose. The infusion rate did not appear to influence remarkably the pharmacokinetics of danusertib. Biomarker analysis showed inhibition of histone H3 phosphorylation, indicative of Aurora B inhibition, at doses $\geq$190 mg/m^2. Stable disease was observed in 23.7% of evaluable patients with disease stabilization $\geq$6 months in 5 patients.

Conclusions

Dose limiting toxicity of danusertib is neutropenia which was short lasting and generally uncomplicated, with limited non-hematological toxicity. The recommended dose of danusertib for phase II studies is 330 mg/m^2 infused over 6 hours on days 1, 8, 15 every 28 days.

Introduction

Aurora kinases are serine/threonine kinases with a key role in mitosis. The aurora kinase family consists of 3 members, aurora-A, B, and C. Aurora-A is localized to the centrosomes of interphase cells and to the mitotic spindle of cells from prophase throughout telophase, and is required for proper spindle maturation and assembly.[1-3] Aurora-B is critical for chromosomal condensation, the attachment of the microtubules to the kinetochore of chromosomes and for proper execution of cytokinesis.[4,5] Aurora-C is found in the testes where it has a role in spermatogenesis. In addition aurora-C might act as a chromosomal passenger protein that can complement aurora-B kinase function in mitotic cells.[6,7]

Since aurora kinases are largely involved in cell cycle progression and mitosis, which is disturbed in cancer cells, their inhibition is considered to have potential as anti cancer treatment. In vitro, inhibition of aurora-A or aurora-B activity in tumor cells results in impaired chromosome alignment, weakening of the mitotic checkpoint, polyploidy, and subsequent cell death.[8,9]

Danusertib is a potent small-molecule inhibitor of the ATP site of the aurora-A (IC50: 13 nM), aurora-B (IC50: 79 nM) and aurora-C (IC50: 61 nM) serine/threonine kinases.[10,11] The chemical structure of danusertib is demonstrated in Figure 1. Danusertib is active in a wide range of cancer cell lines and xenografts models.[10] In mice, danusertib inhibits phosphorylation of histone H3, a protein implicated in chromosome condensation that is phosphorylated by aurora-B. This effect is observed in skin, bone marrow and xenograft tumors.[12] Therefore, inhibition of histone H3 phosphorylation has been identified as marker of danusertib biological activity. Preclinical pharmacokinetics of danusertib were dose-proportional and time-independent. The major route of metabolism involved the formation of the N-oxide derivative. The N-oxide metabolite was deter-

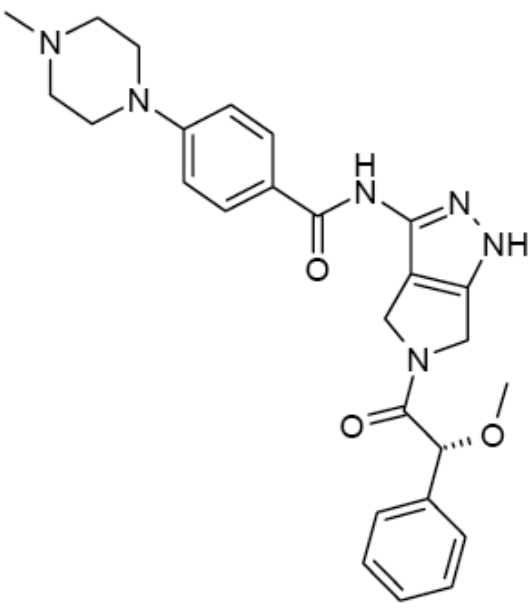


Fig. 1. Chemical structure of PHA-739358

mined to have less than 1% of the activity of the parent compound. Danusertib did not inhibit any cytochrome P450 isoenzymes and was not a potent inhibitor of P-glycoprotein.[11] We performed a phase I pharmacological and biomarker study of danusertib in patients with solid tumors. Objectives of this study were to (1) determine the maximum tolerated dose (MTD) and define dose-limiting toxicities (DLT), (2) characterize safety, (3) characterize pharmacokinetics, (4) analyze biomarkers of biological activity, including histone H3 phosphorylation in skin biopsies, and (5) evaluate preliminary antitumor activity.

Patients and Methods

Eligibility Criteria

Patients with histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤1 were eligible. Other inclusion criteria were: evaluable or measurable disease by RECIST[13]; age ≥18 years; life expectancy ≥12 weeks; tumor progression prior to study entry, adequate bone marrow, liver, and renal function (hemoglobin ≥10.0 g/dl; absolute neutrophil count ≥1,500/mm^3; platelet count ≥100,000/mm^3; total bilirubin ≤1.5x the upper limit of normal (ULN); ALT and AST ≤2.5x ULN, (<5x ULN in case of liver metastases); serum albumin ≥3.0 g/dL; serum creatinine ≤1.5 mg/dL); blood pressure ≤140/90 mm Hg. Exclusion criteria were: previous high-dose chemotherapy requiring bone marrow rescue; known brain or leptomeningeal disease; pregnancy or breast-feeding; active inflammatory bowel disease, bowel obstruction or chronic diarrhea; abnormal left ventricular ejection fraction, thromboembolic events in the year prior to enrollment; ongoing cardiac dysrhythmias grade ≥2; known active infections; any condition that could endanger the safety of the patient.

Written informed consent was obtained from all patients before any study related procedure was performed, and approval from the institutional medical ethical review boards was obtained.

Drug Administration and Dose Escalation Procedure

Danusertib was administered intravenously for 3 consecutive weeks in 4-week cycles. Patients were divided into cohorts with escalating doses, starting with 6h-ivS. After MTD definition with the 6h-ivS, in the attempt of shortening the in hospital-time, two additional cohorts of patients were included to study the 3h-ivS. Based on animal

toxicology and pharmacokinetic data, the starting dose for the 6h-ivS of danusertib was 45 mg/m^2 (target exposure 1/10th of the AUC at MTD in dogs, most sensitive species in toxicology studies). The starting dose for the 3h-ivS was 250 mg/m^2 based on toxicity and pharmacokinetic results of the 6h-ivS. Dosing schedules were based on preclinical animal toxicity studies, with higher doses and/or shorter infusion times resulting in increased bone marrow, gastrointestinal, cardiovascular and renal toxicity.

A two-stage accelerated titration design was adopted. During the initial phase a rapid dose escalation scheme was used with 100% dose increments until occurrence of drug-related first cycle DLT in 1 patient or grade ≥2 drug-related toxicity in ≥2 patients during any treatment cycle. For subsequent dose escalation steps a modified Fibonacci scheme was foreseen with 50, 40 and 33% dose increments in subsequent dose levels.

DLT was defined as grade 4 neutropenia ≥7 days, febrile neutropenia, neutropenic infection, grade 4 thrombocytopenia, grade 3 thrombocytopenic bleeding, and any drug-related grade 3 or 4 non-hematological toxicity (excluding nausea, vomiting or diarrhea not refractory to adequate treatment), decrease in LVEF to ≤40% or a decrease of ≥20% compared to baseline, interruption of infusion due to a diastolic blood pressure increase of >20 mm Hg or to >150/100 mm Hg during drug administration, next cycle delayed by ≥2 weeks, and omission of day 8 and/or 15 dose due to danusertib-related toxicity (after the 250 mg/m^2 6-hour cohort protocol amendment allowed dosing on day 8 and/or 15 in the event of grade 3 uncomplicated neutropenia). If DLT was observed in one patient, three additional patients were recruited at that dose level, with dose escalation proceeding if <2 of 6 patients exhibited DLT. If DLT was observed in ≥2 of 3 or ≥2 of 6 patients, the MTD had been exceeded, and additional patients were recruited at the previous lower dose level.

The MTD was defined as the highest dose level that could be given to 6 patients with no more than 1 patient experiencing DLT. If a patient experienced a drug related DLT, further danusertib administration was withheld in that cycle. If the toxicity resolved to ≤grade 1, the dose was reduced to the previous lower dose level. Otherwise, the patient was withdrawn from the study.

Therapy continued until disease progression or unacceptable toxicity

Pre-treatment Evaluation and Safety Assessment

Pretreatment evaluation consisted of a complete medical history, physical examination, ECOG performance status assessment, vital signs, ECG, blood sample for complete blood count (CBC; hemoglobin, white blood cell count with differential, platelet count) and biochemistry analysis (BUN or blood urea, creatinine, albumin, aspartate aminotransfer-

ase, alanine amintotransferase, bilirubin, alkaline phosphatase, lactate dehydrogenase, sodium, potassium), sample for urinalysis, serum pregnancy test, multigated acquisition (MUGA) scan, chest X-ray and baseline tumor measurements.

On days 1, 8, 15 and 22 of each cycle evaluation consisted of a brief history and physical examination, vital signs, blood samples for CBC and biochemistry, urinalysis, ECG. MUGA scans were repeated after cycle 1, and every even cycle. Response evaluation was performed every 2 cycles according to RECIST[13]. Patients were evaluated weekly for adverse events and toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 3.0.

Pharmacokinetic Evaluation

Pharmacokinetic (PK) evaluation was performed by collecting blood samples via an indwelling intravenous catheter in the opposite arm of the infusion. In cycle 1, on days 1 and 15 a 5 mL sample was collected pre-dose and at 0.5, 1, 3 and 6 (5 min before end infusion) h after start of the infusion, and 5, 15 and 30 min, 1, 2, 4 and 6 h after the end of the infusion. On days 2-4 and 15-18 blood samples were taken corresponding to 24, 48 and 72 h after the start of infusion. On day 8, blood samples were taken predose and 5 min before the end of infusion. On day 22, one blood sample was taken. In subsequent cycles an abbreviated sampling schedule was used. Urine samples were collected predose and up to 72 h after the first dose of cycle 1.

Pharmacokinetic evaluation was carried out using a non-compartmental approach with the aid of WinNonlin software (version 3.1, Pharsight Inc., Mountain View, CA, USA). Plasma and urine concentrations of danusertib and of its N-oxide metabolite were measured by validated liquid chromatography-tandem mass spectrometry techniques. Detailed methods are described in the online only appendix.

Biomarker Analysis

Skin biopsies for biomarker analysis were performed on day 1 of the first cycle, before start and 10 minutes before end of the infusion. Biopsies were processed for immunohistochemistry (IHC), using an anti-phospho histone H3 antibody, as a measure of aurora-B inhibition.[10,14,15] Detailed methods are described in the online only appendix.
Blood samples for blood pressure biomarker analysis (norepinephrine, epinephrine, endothelin A and B, vascular endothelial growth factor, and angiotensin II) were scheduled to be taken pre-dose and every hour during infusion in cycle 1 and in case of a hypertensive event.

Table 1. Baseline demographics and patient characteristics.

Baseline characteristics	PHA-739358 6-h Infusion N=40	PHA-739358 3-h Infusion N= 10
Gender, n (%)		
Male	29 (73)	8 (80)
Female	11 (28)	2 (20)
Age, years		
Median (range)	54 (22-75)	61 (46-74)
ECOG performance scale, n (%)		
0	6 (15)	3 (30)
1	34 (85)	7 (70)
Previous lines of systemic therapies		
Median (range)	4 (0*-12)	3 (1-6)
Tumor type, n (%)		
Colorectal cancer	13	6
Sarcoma	6	1
Esophageal cancer	4	–
Pancreatic cancer	3	–
Cholangiocarcinoma	2	–
Ovarian cancer	2	–
Prostate cancer	2	–
Renal cancer	2	–
Other		
ACUP	1	1
Adrenal cancer	1	–
Bladder cancer	1	1
Breast cancer	1	–
Mesothelioma	–	1
NSCLC	1	–
Thyroid cancer	1	–

ECOG: Eastern Cooperative Oncology Group,
ACUP: Adenocarcinoma of unknown primary, NSCLC: non small cell lung cancer
* 3 pancreatic cancer, 1 cholangiocarcinoma had only previous surgery

Results

Between June 2004 and September 2007, 52 patients were enrolled. Two patients never started treatment because of clinical deterioration due to rapid tumor progression. Patient characteristics are summarized in Table 1.

The percentage of evaluable patients was 94% for PK analyses, 60% for Histone H3 analyses, 100% for toxicity, and 78% for efficacy. A total of 148 cycles were administered. The median number of cycles per patient was 2 (range 1-28). Dose reductions were required in 12% of patients. Reasons for study discontinuation were lack of efficacy (69%) and adverse events (20%). Two patients withdrew consent, and one patient is still on treatment.

Safety and Tolerability

Dose levels for the 6h-ivS were 45 mg/m^2 (n=3), 90 mg/m^2 (n=7), 135 mg/m^2 (n=4), 190 mg/m^2 (n=4), 250 mg/m^2 (n=10), 330 mg/m^2 (n=8), and 400 mg/m^2 (n=4), and 250 mg/m^2 (n=3), and 330 mg/m^2 (n=7) for the 3h-ivS.

In the 6h-ivS, DLT consisted of grade 2 hypertension leading to interruption of infusion in one patient (90 mg/m^2); febrile neutropenia and grade 3 fatigue in one patient (330 mg/m^2); dose omission due to grade 4 neutropenia in 2 patients (400 mg/m^2). Using the 3h-ivS DLT consisted of dose omissions due to grade 4 neutropenia and grade 3 fatigue (330 mg/m^2).

All treatment-related hematological and non-hematological adverse events are summarized in Table 2.

For the 6h-ivS (total of 120 cycles), most frequently observed drug-related side effects were neutropenia, nausea, anorexia, and fatigue. For the 3h-ivS (total of 28 cycles), most frequently observed drug-related side effects were fatigue, neutropenia, diarrhea, and nausea. Grade 3-4 drug-related events were neutropenia, febrile neutropenia, leucopenia, and fatigue reported at doses of 250 mg/m^2 and higher for the 6h-ivS and fatigue, diarrhea, neutropenia, leucopenia, and dehydration at 330 mg/m^2 of the 3h-ivS. Injection site reactions were reported in 3 patients each with both infusion schedules.

For the 6h-ivS drug-related adverse events requiring dose reduction or omission were mainly due to hematological toxicity and started at 250 mg/m^2 (5 cases). In the 3h-ivS dose reduction was pursued in 1 patient for hematological toxicity (330 mg/m^2). Permanent treatment discontinuation for drug-related toxicity was required in 3 patients, for grade 2 anemia associated with fatigue, pain and nausea (190 mg/m^2, 6h-ivS), grade 1 hypertension (330 mg/m^2, 6h-ivS), and grade 3 fatigue (330 mg/m^2, 3h-ivS).

Neutropenia was uncomplicated except for one case of febrile neutropenia (330 mg/m^2, 6h-ivS). Median time to neutropenia nadir was 15 days and median time to recovery 7 days.

The MTD was 330 mg/m^2 for the 6h-ivS, and not defined for the 3h-ivS. The 250 mg/m^2 dose level was not further expanded to confirm it as the MTD for the 3h-ivS, as available data supported the feasibility of a safe administration of 330 mg/m^2 using the 6h-ivS.

Table 2. Number of patients with treatment-emergent hematological adverse events and/or treatment-related non-hematological adverse events per patient during all cycles. Every * represents 1 patient with dose limiting toxicity (DLT). 2A: 6-hour infusion schedule. 2B 3-hour infusion schedule.

2A:

Adverse Event	Cohort 1 6-h Infusion 45 mg/m² n=3		Cohort 2 6-h Infusion 90 mg/m² n= 7		Cohort 3 6-h Infusion 135 mg/m² n=4		Cohort 4 6-h Infusion 190 mg/m² n=4		Cohort 5 6-h Infusion 250 mg/m² n=10		Cohort 6 6-h Infusion 330 mg/m² n=8		Cohort 7 6-h Infusion 400 mg/m² n=4		Total incidence 6-h Infusion n=40
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Any grade n (%)
	Treatment-Emergent Hematological Adverse Events														
Anemia	1	–	4	1	1	–	2	–	6	–	2	1	4	–	22 (55.0)
Leukopenia	1	–	3	–	1	–	3	–	6	3	5	1	1	3	27 (67.5)
Neutropenia	1	–	2	–	1	–	3	–	3	3	2	4	1	2**	22 (55.0)
Febrile neutropenia	–	–	–	–	–	–	–	–	–	–	–	1*	–	–	1 (2.5)
Thrombopenia	2	–	2	–	–	–	1	–	1	2	–	–	1	–	9 (22.5)
	Treatment–Related Non–Hematological Adverse Events														
Any event	2	–	4	–	1	–	3	–	4	3	5	1	1	3	27 (67.5)
GI toxicity															
Anorexia	–	–	1	–	–	–	2	–	2	–	2	–	2	–	9 (22.5)
Constipation	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1 (2.5)
Dehydration	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
Diarrhea	1	–	1	–	–	–	–	–	1	–	2	–	2	–	7 (17.5)
Dyspepsia	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1 (2.5)
Nausea	–	–	1	–	1	–	–	–	4	–	2	–	2	–	10 (25.0)
Vomiting	–	–	–	–	–	–	–	–	2	–	1	–	–	–	3 (7.5)
Constitutional toxicity															
Fatigue	–	–	2	–	–	–	–	–	3	–	2	1*	–	–	8 (20.0)
Miscelaneous															
Abdominal pain	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1 (2.5)
Alopecia	–	–	–	–	–	–	–	–	1	–	1	–	–	–	2 (5.0)
Dizziness	–	–	–	–	–	–	–	–	1	–	1	–	–	–	2 (5.0)
Headache	–	–	1	–	–	–	–	–	–	–	2	–	–	–	3 (7.5)
Hypertension	–	–	2*	–	–	–	–	–	–	–	1	–	–	–	3 (7.5)
Influenza like	–	–	1	–	–	–	–	–	1	–	–	–	–	–	2 (5.0)
Myalgia	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)
Phlebitis	–	–	–	–	–	–	–	–	–	–	1	–	1	–	2 (5.0)
Somnolence	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0 (0.0)

GI: gastro intestinal

2B:

Adverse Event	Cohort 8 3-h Infusion 250 mg/m^2 n=3		Cohort 9 3-h Infusion 330 mg/m^2 n=7		Total incidence 3-h Infusion n=10
	Grade 1-2	Grade 3-4	Grade 1-2	Grade 3-4	Any grade n (%)
	Treatment-Emergent Hematological Adverse Events				
Anemia	2	–	4	–	6 (60.0)
Leukopenia	2	–	2	2	6 (60.0)
Neutropenia	1	–	1	4*	6 (60.0)
Febrile neutropenia	–	–	–	–	0 (0.0)
Thrombopenia	–	–	1	–	1 (10.0)
	Treatment–Related Non–Hematological Adverse Events				
Any event	3	–	2	4	9 (90.0)
GI toxicity					
Anorexia	–	–	2	–	2 (20.0)
Constipation	–	–	2	–	2 (20.0)
Dehydration	–	–	–	1	1 (10.0)
Diarrhea	2	–	1	2	5 (50.0)
Dyspepsia	1	–	1	–	2 (20.0)
Nausea	1	–	2	–	3 (30.0)
Vomiting	1	–	1	–	2 (20.0)
Constitutional toxicity					
Fatigue	2	–	3	2*	7 (70.0)
Miscelaneous					
Abdominal pain	–	–	2	–	2 (20.0)
Alopecia	–	–	1	–	1 (10.0)
Dizziness	–	–	–	–	0 (0.0)
Headache	–	–	1	–	1 (10.0)
Hypertension	–	–	–	–	0 (0.0)
Influenza like	–	–	–	–	0 (0.0)
Myalgia	–	–	2	–	2 (20.0)
Phlebitis	–	–	2	–	2 (20.0)
Somnolence	–	–	2	–	2 (20.0)

Pharmacokinetics

Danusertib pharmacokinetic parameters are summarized in Table 3. Day 1 danusertib plasma concentrations after 6-hour infusion dose of danusertib of a representative patient at each dose level are plot in Figure 2. The pharmacokinetics of danusertib were characterized by high volume of distribution and low to moderate plasma clearance

Table 3. Plasma pharmacokinetic parameters (mean ± SD) of PHA-739358 during cycle 1 (6h and 3h infusion schedules). Percentage coefficient of variation (%CV) and range for the recommended phase 2 dose (RP2D, 330 mg/m^2) in italics.

6-hour infusion schedule						
Day 1						
Dose mg/m^2	Cmax μM	t1/2,z hour	AUC0-∞ μM·hour	CL L/hour	Vz L	CLR L/hour
45 (n=3)	0.83±0.3	17.6±0.8	5.9±2.2	33.4±11.0	857±312	4.46±2.21
90 (n=7)	2.25±0.6	27.2±22	14.0±3.0	27.4±6.8	1010±725	3.50±1.56
135 (n=4)	2.56±1.4	19.5±3.9	13.9±3.6	38.3±10.5	1041±198	5.73±2.42
190 (n=4)	3.86±1.1	24.4±7.8	27.5±6.2	30.0±7.6	1085±565	3.06±1.1
250 (n=10)	4.75±1.6	25.1±13	30.8±9.2	35.1±11.8	1272±645	5.37±2.64
330 (n=7)	5.62±2.5	33.3±17	38.5±11	38.3±12.5	1832±933	5.66±1.84 (n=5)
RP2D %CV	*44.9*	*50.8*	*28.6*	*32.6*	*50.9*	*32.6*
RP2D range	*3.3 - 10.3*	*16.0 - 69.0*	*20.7 - 53.0*	*27.6 - 59.0*	*698 - 3095*	*3.7 - 8.4*
400 (n=4)	6.31±2.3	37.7±22	49.3±11	35.5±9.8	1872±1030	4.65±2.43
45-400 (n=39)	4.00±2.3	27.0±15.4	27.1±15.4	34.0±10.4	1312±752	4.69±2.20
Day 15-Day 1 ratio	0.98		0.94			
3-hour infusion schedule						
Day 1						
Dose mg/m^2	Cmax μM	t1/2,z hour	AUC0-∞ μM·hour	CL L/hour	Vz L	CLR L/hour
250 (n=2)	7.10±0.9	32.3±1.5	28.7±2.4	38.7±10.4	1787±400	3.18±0.59
330 (n=6)	10.10±1.7	28.5±9.8	52.7±30	32.8±14.8	1386±762	1.48±0.53 (n=2)
250-330 (n=8)	9.34±2.0	29.4±8.5	46.7±28	34.3±13.4	1487±687	2.33±1.09
Day 15-Day 1 ratio	0.95		0.75			

Cmax: maximal concentration; t1/2,z: terminal half-life; AUC0-∞ : areas under the curve up to infinite time, CL: systemic clearance, Vz: volume of distribution, CLR: renal clearance, %CV: percentage coefficient of variation.

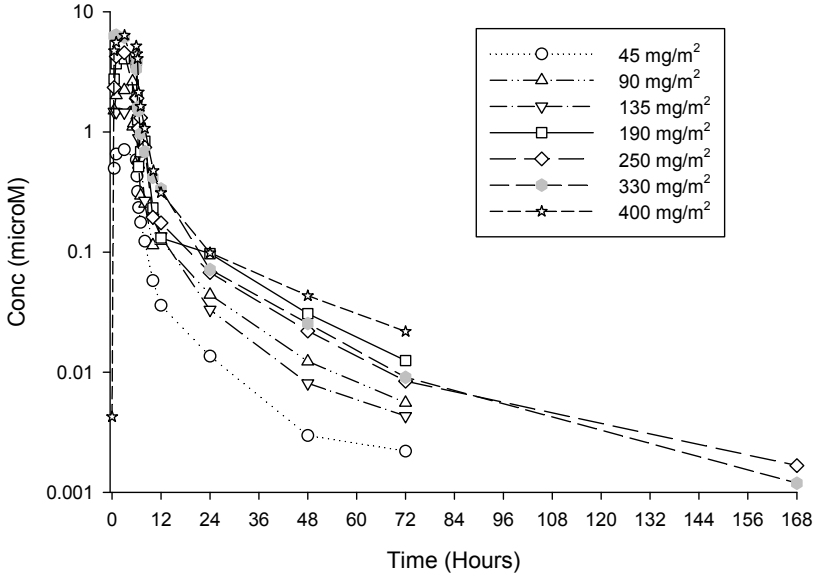


Fig. 2 Representative day 1 individual plasma concentrations (μM) of PHA-739358 after 6 hour infusion of PHA-739358 at each dose level

(range 10-59 L/hour). The half-life was about 30 hours. Accumulation was negligible. Renal clearance accounted for a small proportion of plasma clearance. The metabolite to parent AUC ratio was similar across doses and approximately equal to 1. Metabolite concentrations declined in parallel with those of the parent compound. The systemic exposure to danusertib increased linear with dose (Figure 3A). Pharmacokinetics of danusertib were not influenced by infusion rates (p-values >0.1; independent samples Student's t-test). However, patient numbers were limited. PK data on days 1 and 15 were comparable (p-values >0.1; paired samples t-test; Figure 3B).

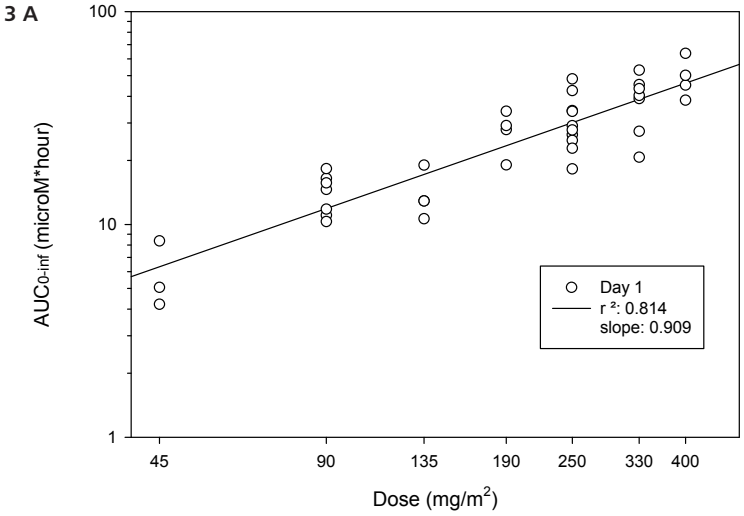
Correlation between Toxicity and Exposure

Figure 4 shows a positive correlation between the percentage decrease in neutrophil counts in cycle 1 in function of the AUC, thus demonstrating that a higher AUC is related to a greater decrease in neutrophil counts during danusertib treatment.[16]

Biomarker Analysis

Histone H3 phosphorylation in skin

Pre-and on-treatment skin biopsies were obtained from 35 patients in the danusertib 6-hour infusion schedule and in 8 patients in the 3-hour schedule. Samples from pa-



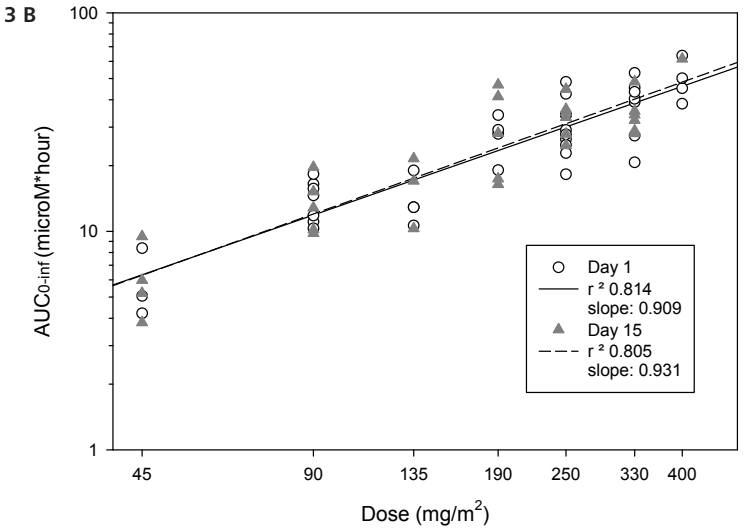


Fig. 3 A Day 1 individual AUC0-∞ of PHA-739358 vs. dose after 6-hour infusion of PHA-739358. Slope t-test: Day 1: t = 1.277, NS (df = 37)

Fig. 3 B Day 1 and day 15 individual AUC0-∞ of PHA-739358 vs. dose after 6-hour infusion of PHA-739358. Slope t-test: Day 1: t = 1.277, NS (df = 37)

tients at the 90 and 135 mg/m^2 dose levels (6h-ivS) were not evaluated because no phosphorylated histone H3 (pH3) was appreciated by Western blot (WB). In total, 30 patients had both pre and post treatment evaluable samples by IHC. By both WB (data not shown) and IHC (Figure 5) more than 80% pH3 inhibition was observed starting from the 190 mg/m^2 dose level (6h-ivS). These results are in agreement with the literature. Ex-

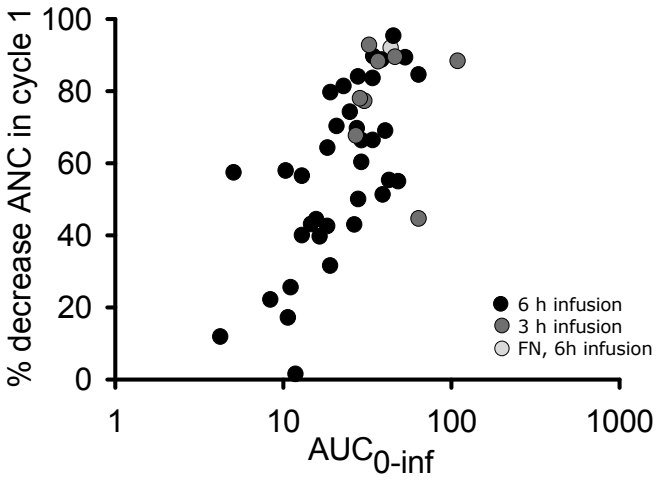


Fig. 4 Correlation between the percentage of decrease in neutrophil count, nadir vs baseline, during cycle 1 and the plasma AUC of PHA-739358.

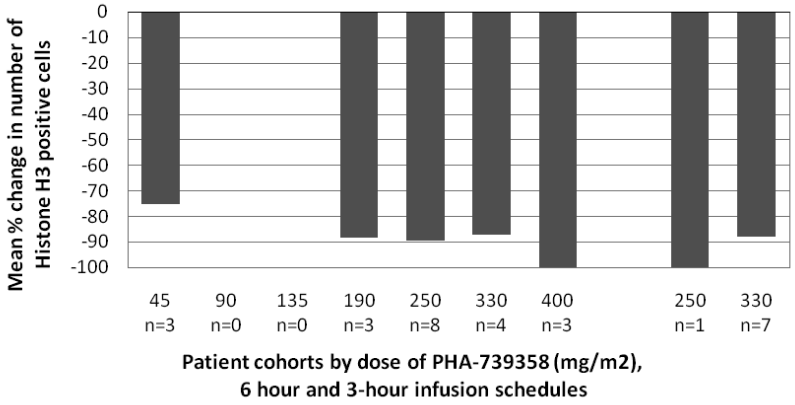


Fig. 5 Mean % change in number of Histone H3 positive cells by immunohisto-chemistry in skin biopsies; PHA-739358 on-treatment compared to pre-treatment.

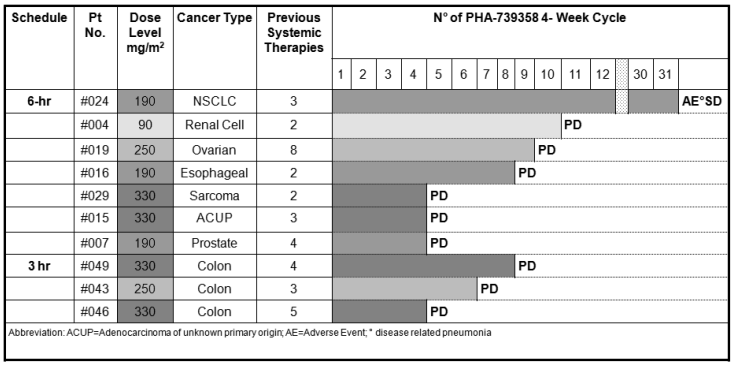


Fig. 6 Characteristics of patients with stable disease.

ploratory analysis of correlation between pH3 and clinical outcome was not conducted due to limited patient numbers.

Blood Pressure Mediators in Plasma

In the absence of a clear modulation of blood pressure mediator levels and blood pressure increase in two patients (one with hypertension during infusion and one without) (data not shown), these markers were not further explored and the blood sampling for this purpose was stopped.

Anti tumor activity

There were no complete or partial responses. An overall disease control rate (DCR) of 20.0% (6/30 patients) was observed in the 6h-ivS. DCR was 37.5% in the 3h-ivS (3/8 patients). Disease stabilization lasting >6 months was seen in 4 patients in the 6h-ivS, and in 1 patient in the 3h-ivS. One patient with progressive non small cell lung cancer prior to study entry, showed disease stabilization for over 2 years on the 6h schedule (Figure 6).

Discussion

In this study we demonstrate that treatment with the pan-aurora (A, B, and C) kinase inhibitor danusertib is well tolerated.

As aurora kinases are key regulators of mitosis, inhibition of their activity is likely to result in effects on bone marrow and other organ systems. Indeed neutropenia is dose

limiting in this and other studies with aurora kinase inhibitors.[17-27] Neutropenia is generally uncomplicated and of short duration. Limited non-hematological toxicity, such as mucositis, nausea, vomiting, diarrhea or alopecia is seen.

Recently aurora-A knockout mice were generated.[28,29] The aurora-A null mice died early during embryonic development, supporting the fact that aurora-A has a critical role in normal mitosis. Disturbingly, aurora-A heterozygote mice showed an increased incidence of malignancy.[28] The long-term effects of aurora kinase inhibition in man remain unknown.

Inhibition of pH3 more than 80%, indicating adequate aurora-B inhibition, was observed at dose levels ≥190 mg/m^2. This is in line with other publications.[10,12,14,21,25] However, since pH3 was inhibited in almost all patients, even in patients with clear tumor progression, the usefulness of this biomarker should be subject to exploration in future phase II and III studies. Other biomarkers like the number of mitotic cells in basal epithelium, FDG-PET, and dynamic-contrast enhanced magnetic resonance imaging are also being evaluated.[17,22,26,27,30]

Determining antitumor activity of danusertib was a secondary endpoint. Complete or partial responses were not observed. However, the overall disease control rate of 23.7% and long lasting disease stabilization (≥6 months) in some patients are indicative of antitumor activity and merit confirmation in a phase II study program.

Due to the limited patient numbers superiority or equivalence of the 3h or 6h schedule could not be concluded based on the PK results. The decision to recommend 330 mg/m^2 danusertib infused over 6 hours using the days 1, 8, 15 in a 28-day cycle schedule as the dose regimen for phase II investigations in solid tumors of is based on two observations. First, by shortening the infusion time to 3 hours, the dose intensity would have been lower that with the 6h-ivS (250 vs. 330 mg/m^2). Second, incidence and severity of toxicities was higher at the 330 mg/m^2 dose level when infusion time was shortened. Phase I studies investigating 24-hour infusion of danusertib are ongoing. danusertib also inhibits wild-type and mutated form of Abl, including the T315I mutant. A pilot phase II clinical study with the 6-h-IV schedule every 28 days is ongoing in patients with chronic myeloid leukemia (CML) relapsing on imatinib or other c-ABL therapy.[31,32] Preliminary results showed objective responses in 2 out of 7 CML patients with T315I mutations with an acceptable tolerability and safety profile.[33] Other cross-reactivities, including FGFRs, Ret and TrkA have been identified and could open additional venues for clinical development of danusertib.[10,11]

Currently many aurora-selective small-molecule inhibitors are undergoing preclinical and clinical studies. All have their individual advantages and disadvantages. MLN8054 was the first aurora kinase inhibitor with the advantage of oral administration. However, in phase I studies grade 3 somnolence was the main dose limiting toxicity, resulting from

binding of MLN8054 to the γ-aminobutyric acid α 1 benzodiazepine receptor.[17,26] MK-0457 is an intravenously administered aurora kinase inhibitor with positive off-target effects blocking the T315I-mutant BCR-ABL leading to clinical responses in 3 BCR-ABL dependent leukemia patients.[34] Danusertib, that inhibits all three aurora kinases, is also able to inhibit wild-type Abl as well as the most clinically frequent imatinib-resistant Abl mutants.[31]

The aurora kinase inhibitors have the advantage of not inducing alopecia and neurotoxicity related to other microtubular inhibitory agents. This can be taken into account when combining aurora kinase inhibitors with standard chemotherapy or targeted agents which will likely be part of future investigations.

In conclusion, danusertib administered in a 6h-ivS and 3h-ivS on days 1, 8, 15 of a 28 day cycle is safe and well tolerated. Based upon clinical endpoints, 330 mg/m^2 as 6h-ivS is the recommended dose for phase II studies.

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Appendix:

Pharmaceutical preparation of danusertib

Danusertib was supplied as a 10 mg/mL concentrate for solution for infusion, dosed at 15 mL/vial. One vial contained 150 mg of Danusertib in 5% dextrose solution adjusted to pH 5 with hydrochloric acid or sodium hydroxide (the hydrochloride salt of Danusertib is formed in situ during sterile aqueous solution manufacture)..

Pharmacokinetic Evaluation

Concentrations of danusertib were determined in human plasma by liquid chromatography-tandem mass spectrometry techniques (LC-MS-MS) following plasma protein precipitation in the 96-well plate format..

Briefly, plasma samples were extracted with acetonitrile containing a stable labeled internal standard. After centrifugation, the organic phase was transferred into a fresh 96-well and dried under nitrogen gas at 37°C. The residue was re-constituted with 15 mM ammonium formate buffer solution pH 3.0 and then aliquots injected into the LC-MS-MS system. Detection was by positive ion electrospray tandem mass spectrometry using Multiple Reaction Monitoring (MRM) following reversed phase chromatography on a Bonus RP column. The method was fully validated within the calibration range of 0.5-500 ng/ml.

Phospho histone H3 analysis in skin

Skin biopsies for biomarker analysis were performed on day 1 of the first cycle, before start and 10 minutes before end of the infusion. Skin samples were fixed in formalin, paraffin embedded and then analyzed for the phospho histone H3 staining by immunohistochemistry (IHC). The sections were stained with an anti-phosphorylated histone H3 (pH3) Ser10 polyclonal antibody (Upstate Biotechnology, NY, USA) and then counterstained with hematoxylin. A median number of linear dermis evaluated was 16 mm/sample. For every patient, the number of pH3 positive cells every 2 mm of dermis at pretreatment and at the end of treatment was defined as well as the % of change versus pretreatment. Biopsies were processed for immunohistochemistry (IHC), using an anti-phospho histone H3 antibody.

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Influence of pharmacogenetic variability on the pharmacokinetics and toxicity of the aurora kinase inhibitor danusertib

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Abstract

Purpose
Danusertib is a serine/threonine kinase inhibitor of multiple kinases, including aurora-A, B, and C. This explorative study aims to identify possible relationships between single nucleotide polymorphisms in genes coding for drug metabolizing enzymes and transporter proteins and clearance of danusertib, to clarify the interpatient variability in exposure. In addition, this study explores the relationship between target receptor polymorphisms and toxicity of danusertib.
Methods
For associations with clearance cancer patients treated in a phase I study were analyzed for *ABCB1*, *ABCG2* and *FMO3* polymorphisms. Association analyses between neutropenia and drug target receptors, including *KDR, RET, FLT3, FLT4, AURKB* and *AURKA,* were performed in patients treated at recommended phase II dose-levels in three danusertib phase I or phase II trials.
Results
For the *FMO3* 18281AA polymorphism, a significantly higher clearance was noticed, compared to patients carrying at least 1 wild type allele. For the other enzymes and transporters, no relationships between danusertib clearance and drug metabolizing enzymes and transporter protein polymorphisms were found. No effect of target receptor genotypes or haplotypes on neutropenia was observed.
Conclusions
The relationship between *FMO3* polymorphisms and clearance of danusertib warrants further research, as we could study only a relatively small and heterogeneous group of patients. However, as we did not find any major correlations between pharmacogenetic variability in the studied enzymes and transporters and pharmacokinetics nor toxicity, it is unlikely that danusertib is highly susceptible for pharmacogenetic variation. Therefore, no dosing alterations of danusertib are expected in the future, based on the polymorphisms studied here.

Introduction

Aurora kinases are serine/threonine kinases with a key role in mitosis.[1-9] Danusertib (PHA-739358) is a new active moiety in cancer treatment which selectively inhibits the ATP site of aurora-A (AURKA, IC_{50} = 13 nM), aurora-B (AURKB, IC_{50} = 79 nM) and aurora-C (AURKC, IC_{50} = 61 nM) kinases.[10,11] Inhibition of aurora-A or aurora-B activity in tumor cells results in impaired chromosome alignment, weakening of the mitotic checkpoint, polyploidy, and subsequent cell death.[12,13] Danusertib shows anti-tumor activity in a wide range of cancer cell lines and xenograft tumor models.[10] Tested in a panel of 32 kinases, danusertib also showed increased affinity for multiple other kinases (IC_{50} <0.50 μM), including ret proto-oncogene (RET), vascular endothelial growth factor receptor 3 (FLT4, VEGFR3), and fms-related tyrosine kinase 3 (FLT3). Therefore, inhibition of these kinases may influence danusertib efficacy or toxicity in cancer patients.

The major route of metabolism of danusertib involves the formation of the N-oxide metabolite, mainly through the enzyme flavin containing monooxygenase 3 (FMO3), forming an inactive metabolite. Furthermore, danusertib was found to be a substrate for efflux proteins ATP-binding cassette B1 (ABCB1/MDR1) and G2 (ABCG2/BCRP in *in vitro* studies (unpublished data). In addition, it has been shown that histone H3 is phosphorylated by aurora-B and phosphorylation of histone H3 is inhibited in skin, bone marrow and xenograft tumors after treatment with danusertib.[10] As a consequence, the extent of histone H3 phosphorylation is studied as a pharmacodynamic biomarker of danusertib effectiveness.

Recently, this new compound has been introduced in human research. In a phase I study, performed at the Leiden University Medical Center, Leiden and the Erasmus University Medical Center, Rotterdam (The Netherlands), the pharmacokinetics of danusertib were characterized by relatively low to moderate plasma clearances (range 10-59 L/hour) and an elimination half-life of about 30 hours.[14] Danusertib showed linear pharmacokinetics over the dose-range studied. At all dose levels, the inter-patient variability of the primary pharmacokinetic parameters of danusertib was remarkably high, with a coefficient of variation of 40-50%, which is in line with other anti-cancer drugs. Toxicity increased with danusertib dose. However, currently it is unclear whether pharmacogenetic variability in drug metabolizing or transporting proteins can explain a large part of the inter-individual variability in pharmacokinetics and/or toxicity-profile. Therefore, the current explorative study aims to identify possible (and clinically relevant) relationships between single-nucleotide polymorphisms (SNPs) in genes coding for drug metabolizing enzymes and for transporter proteins and pharmacokinetic parameters of danusertib. In this study we also explore the possible relationship between polymorphisms in genes encoding the drug target receptors and toxicity of danusertib.

Methods

This study was conducted on three different groups of patients. Group A consisted of patients enrolled into a phase I dose-escalating study of danusertib in patients with advanced or metastatic solid tumors. Group B and group C consisted both of subsets of patients enrolled into phase II studies of danusertib in patients with various tumor types, including breast cancer, pancreatic cancer, colorectal cancer, ovarian cancer, or hormone refractory prostate cancer.

From patients treated in the phase I study (Group A) residual blood samples were available for pharmacogenetic analyses to compare with pharmacokinetics (all patients) and toxicity (patients at the recommended phase II dose (RP2D)). Groups B and C consisted of patients treated in two ongoing phase II studies. They were all treated at the RP2D and had blood samples available for pharmacogenetic analysis to compare with toxicity.

Patients and samples

Eligibility criteria, drug administration procedures, safety, pharmacokinetic and efficacy methods as used in the phase I trial are described in detail elsewhere.[14]

Briefly, Group A patients had histologically or cytologically confirmed advanced or metastatic solid tumors for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status ≤ 1. Danusertib was administered intravenously on days 1, 8, 15 every 28 days. Doses were escalated from 45 mg/m^2 to 400 mg/m^2 in the 6-hour infusion schedule, and from 250 mg/m^2 to 330 mg/m^2 in the subsequent 3-hour infusion schedule. The trial had a standard 3+3 phase I dose escalation study design. In the phase II study from which group B patients were entered, men with metastatic hormone refractory prostate cancer, progressive after docetaxel treatment were eligible. Patients of group B were randomized between treatment with 330 mg/m^2 of danusertib on days 1, 8, 15 every 28 days in a 6-hour infusion schedule or with 500 mg/m^2 of danusertib on days 1 and 15 every 28 days in 24-hour infusion schedule according to the phase II study protocol. The total exposure in both groups is expected to be identical, and in line with the RP2D as determined in phase I studies.[8,14] For group C, patients with several tumor types (see table 1), progressive after 1 or 2 lines of chemotherapy depending on tumor type were eligible. Treatment consisted of 500 mg/m^2 of danusertib on days 1 and 15 every 28 days in 24-hour infusion schedule. No pharmacokinetic analyses were performed in the phase II trials.

For all groups, patients were evaluated for adverse events and toxicity according to the National Cancer Institute Common Terminology Criteria (NCI-CTC), version 3.0. Response evaluation was performed every 2 cycles and was assessed according to RECIST 1.0.[15]

Table 1. Patient characteristics, danusertib induced toxicity in the first cycle, and pharmacokinetic/pharmacodynamic results.

Baseline characteristics	All patients n (%) N=63	Phase I patients n (%) N=48*	RP2D patients n (%) N=30**
Gender, male	43 (68)	35 (73)	20 (67)
Race, caucasian	62 (98)	47 (98)	29 (97)
Age, years			
Median (range)	58 (22-75)	58 (22-75)	60 (38-74)
Patient Group			
A (phase I)	48 (76)	48 (100)	15 (50)
B (phase II, prostate cancer)	7 (11)	–	7 (23)
C (phase II, various tumor types)	8 (13)	–	8 (27)
RP2D Group			
330 mg/m2 d1,8,15 every 4 weeks	20 (31)	15 (31)	20 (67)
500 mg/m2 d 1 and 15 every 4 weeks	10 (16)	–	10 (33)
Tumor type			
Colorectal cancer	19 (30)	18 (38)	10 (33)
Breast cancer	6 (10)	1 (2)	5 (17)
Esophageal cancer	4 (6)	4 (8)	2 (7)
Ovarian cancer	3 (5)	2 (4)	1 (3)
Pancreatic cancer	4 (6)	3 (6)	1 (3)
Prostate cancer	8 (13)	1 (2)	7 (23)
Miscellaneous	19 (30)	19 (39)	4 (13)
ECOG performance score			
0	15 (24)	8 (17)	11 (37)
1	48 (76)	40 (83)	19 (63)
Nr of previous treatment lines			
Median (range)	3 (0-12)	3 (0-12)	3 (1-7)
Toxicity during cycle 1			
Any toxicity grade 1-4	51 (81)	37 (77)	28 (93)
Any toxicity grade 3 or 4	22 (35)	15 (31)	15 (50)
Neutropenia grade 1-4	34 (54)	25 (52)	19 (63)
Neutropenia grade 3 or 4	18 (29)	12 (25)	13 (43)
Febrile neutropenia	1 (2)	1 (2)	1 (3)
Clearance day 1 cycle 1 (L/hour/m^2), n=47			
Median ± SD	n.a.	17.8 ± 5.8	n.a.
Histone H3 phosphorylation, n=28, Δ%			
Median ± SD	n.a.	-92.3 ± 13.1	n.a.
Number of treatment courses			
Median (range)	2 (1-31)	2 (1-31)	2 (1-15)

RP2D: Recommended phase 2 dose; ECOG: Eastern Cooperative Oncology Group; SD: standard deviation; Histone H3 phosphorylation:% change in number of positive cells by immunohistochemistry for Histone H3 phosphorylation, * One patient included in toxicity analyses, but no PK data available, **Fifteen patients of the phase I trial were treated at the RP2D level.

Residual blood samples taken for routine patient care were stored at -20°C at the local hospital laboratories. Of each patient, one frozen whole blood sample was collected from the two participating hospitals. All samples were anonimyzed by a third party, according to the instructions stated in the Codes for Proper Use and Proper Conduct (www.federa.org). Approval from the institutional medical ethical review boards was obtained prior to analysis.

Pharmacokinetic, toxicity and biomarker parameters

Pharmacokinetic (PK) evaluation was performed by collecting blood samples on days 1 to 4, day 8, days 15 to18 and day 22 of cycle 1, and days 1 and 15 of cycles 2 and 4. Pharmacokinetic evaluation was carried out using a non-compartmental approach with the aid of WinNonlin software (version 3.1, Pharsight Inc., Mountain View, CA, USA). In this study, danusertib clearance (L/hour/m2) was selected as the pharmacokinetic parameter to associate with enzyme and transporter genetic polymorphisms. As mentioned in the phase I report, clearance was not influenced by duration of infusion and was comparable in both 3-hour and 6-hour infusion schedules; 16.2 and 18.0 L/hour/m^2 respectively.[14] Clearances in our study were also comparable to mean danusertib clearance reported in another phase I study using even a 24-hour infusion schedule.[8] Therefore, patients treated at both 3-hour and 6-hour infusion schedules were included in the pharmacokinetic association analyses.

The most frequent and clinically relevant danusertib induced side effects, known from phase I trials, are grade 3 and 4 neutropenia, defined as neutrophil counts 0.5-1.0*10^9/L and <0.5*10^9/L, respectively, and febrile neutropenia. These side-effects were therefore considered to be the best candidate toxicity parameters for the association analyses with drug target receptor genetic polymorphisms.

For the association analysis with neutropenia, we included patients treated at the RP2D (thus, 330 mg/m^2 days 1, 8, 15 q4w or 500 mg/m^2 days 1 and 15 q4w equivalent). Since grade 3-4 neutropenia was associated with danusertib dose, association analyses were performed with neutropenia developing in the first cycle only, excluding the effects of cumulative danusertib dose and dose reductions in subsequent cycles.[14] The probability of grade 3 or 4 neutropenia in the first danusertib cycle was not influenced by infusion duration and this toxicity was also comparable in both used schedules.[8,14] Therefore, for the purpose of analyzing associations between drug target receptor polymorphisms and neutropenia all patients treated at the RP2D were combined. Only one case of febrile neutropenia was observed, and as a result association analyses with febrile neutropenia could not be performed.

Skin biopsies for biomarker analysis (Group A) were performed at baseline, and

10 minutes before the end of the first infusion in the first cycle. As a biomarker for aurora-B inhibition, the inhibition of histone H3 phosphorylation in the skin was measured by immunohistochemistry.[10,16,17] Change in histone H3 phosphorylation was used as parameter for association analyses with polymorphisms in the aurora-B receptor.

Selection of candidate genes

Candidate genes were selected based on the information of preclinical pharmacology studies as reported in the Investigator's brochure (Nerviano Medical Sciences on file). For association with clearance *ABCB1*, *ABCG2,* and *FMO3* were the genes selected. For correlation with danusertib toxicity selected genes were the drug target genes encoding *AURKA, AURKB, AURKC, C-ABL, NTRK1*, *RET*, *FGFR1, LCK, FLT4, C-KIT, KDR, CDK2A, STLK1*, and *FLT3*.

The single-nucleotide polymorphisms (SNPs) were selected, taking into consideration one or more of the following criteria: a validated SNP assay, SNP should preferably cause non-synonymous amino acid changes, an indication for clinical relevance should be available from previous publications, and the preferred minority genotype allele-frequency should be at least ~10% in Caucasians. For *ABCB1*, *ABCG2, FMO3, AURKA, AURKB, RET*, *FLT4, KDR,* and *FLT3*, one or multiple SNPs could be selected according to these criteria.

DNA extraction, SNP analysis, and haploblock selection

DNA was isolated from EDTA-blood samples with MagNA Pure Compact DNA Isolation kit (Roche Diagnostics, Almere, The Netherlands). DNA concentrations were quantified on the nanodrop (Isogen, IJsselstein, The Netherlands). From the patients of whom whole blood samples were unavailable, DNA was isolated from blood-serum with MagNA Pure Compact DNA Isolation kit. Taqman assays were obtained from Applied Biosystems (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). SNP genotyping was performed with the BIOMARK 48.[48] dynamic array (Fluidigm Corporation, South San Francisco, CA, USA). All assays were performed according to protocols provided by the manufacturer. As a quality control, 4 samples were genotyped in duplicate for all assays and 2 assays were tested in duplicate on all samples. As negative controls water was used. Overall, no inconsistencies in genotypes were observed. To genotype DNA extracted from blood serum on the Biomark, a pre-amplification step was necessary. Briefly, to 1.25 μl of serum-DNA a dilution of all taqman assays in a total volume of 1.25 μl and 2.5 μl of pre-amplification mastermix (Applied Biosystems) was added, and amplified on a conventional PCR machine. This mixture was 20x diluted and 2.5 μl was used in the Biomark conform their protocol.

Genotype distributions are presented in Table 2. The success rates for all genotyping analyses were 100%, except for RET 135G>A with 22% invalid results, despite repeated analyses. Genotype frequencies for 21 of 22 SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$). KDR 1719A>T (rs1870377) was not in Hardy-Weinberg equilibrium which was most likely due to the limited population size. Our genotype frequencies were in line with previously reports and frequencies for Caucasians, as reported in the NCBI database (www.ncbi.nlm.nih.gov).

If linkage disequilibrium between SNPs was detected, haplotypes were set with gPLINK (http://pngu.mgh.harvard.edu/purcell/plink/).[18] No phase uncertainty in the defined haploblocks and haplotypes (Rh^2> 0.98) was seen. The haploblock for *ABCB1* included 1236C>T, 2677G>A/T, and 3435C>T; the haploblock for *ABCG2* included 15994G>A, and 1143C>T; and the haploblock for *FMO3* included 15167G>A, 21443A>G, and 18281G>A (Table 4).

Statistical analysis

Differences in pharmacokinetic and pharmacodynamic parameters among genotypes were analyzed by the Student's *t*-test, or analysis of variance (ANOVA) for continuous variables or chi-square test for dichotomous variables, where appropriate. For toxicity, differences in genotype distribution were tested by 3×2 cross-tabulations for each genotype, and by 2×2 cross-tabulations for carriers versus noncarriers, with analysis by a two-sided chi-square test. Polymorphisms within a gene were tested with the chi-square test (P-value < 0.05) to detect linkage disequilibrium. Associations between the number of copies of a haplotype and clinical parameters were performed using a chi-square test for dichotomous variables and Student's *t*-test, ANOVA for continuous variables.

All statistical analyses were performed using SPSS 16.0 software (SPSS, Chicago, IL) and were two-sided, with a level of significance of $\alpha=0.05$. Because of the explorative nature of this study, we did not perform a correction for multiple comparisons.

Results

Baseline patient characteristics, observed treatment-related toxicities, pharmacokinetics and treatment duration are presented in Table 1. Our population comprised 98% Caucasians with 68% males and 32% females. Most frequent tumor types were colorectal cancer (30%), prostate cancer (13%) and breast cancer (10%). Danusertib doses used ranged from 45 mg/m^2 to 500 mg/m^2, with infusion times of 3 (14%), 6 (70%) and 24 hours (16%), according to the designs of the mentioned phase I and phase II trials.

Table 2. Genotype frequency results.

Gene and Variant	No. Patients	p*	q**
ABCB1 1236C>T	63	0.556	0.444
ABCB1 2677G>A/T	63	0.563	0.437
ABCB1 3435C>T	63	0.484	0.516
ABCG2 421C>A	63	0.889	0.111
ABCG2 346G>A	63	0.952	0.048
ABCG2 1143C>T	63	0.770	0.230
ABCG2 15994G>A	63	0.690	0.310
FMO3 15167G>A	63	0.563	0.437
FMO3 21443A>G	63	0.794	0.206
FMO3 18281G>A	63	0.873	0.127
KDR -604T>C	63	0.508	0.492
KDR 1192G>A	63	0.921	0.079
KDR 1719A>T	63	0.254	0.746
KDR 54G>A	63	0.563	0.437
KDR -92G>A	63	0.762	0.238
RET 37412G>A	63	0.810	0.190
RET 135G>A	49	0.776	0.224
FLT3 738C>T	63	0.397	0.603
FLT4 1480A>G	63	0.881	0.119
AURKB 893G>A	63	0.889	0.111
AURKA 169G>A	63	0.778	0.222
AURKA 91A>T	63	0.778	0.222

*p: frequency of wild-type allele; **q: frequency of variant allele

Haplotype frequencies for *ABCB1* were GCC 0.443, TTT 0.412, GTC 0.096, and GCT 0.024, for *ABCG2* CC 0.691, TT 0.230, and TC 0.079, and for *FMO3* AGA 0.437, GGG 0.437, and GAG 0.127. Haploblock for *KDR* included -604 T>C, 1192G>A, and 1719A>T, for *RET* 37412G>A, and 135G>A, and for *AURKA* 169G>A, and 91A>T. Haplotype frequencies for *KDR* were TCT 0.410, CCT 0.316, CCA 0.109, TCA 0.087, CTA 0.047, CTT 0.021, and TTA 0.0114, for RET GG 0.602, and GA 0.938, and for AURKA GA 0.556, AA 0.222, and GT 0.222.

There was no apparent association between cycle 1 day 1 danusertib clearance

Table 4. Association between genetic polymorphisms and grade 3-4 neutropenia in cycle 1 in all patients at RP2D levels.

Gene	Polymorphism	Genotype	Total No. Patients	Neutropenia grade 3-4		p-value
				No	Yes	wt/wt vs wt/m vs m/m
KDR	-604T>C	TT	6	3	3	0.308
		TC	15	7	8	
		CC	9	7	2	
	1192G>A	GG	27	15	12	1.000
		GA	3	2	1	
	1719A>T	AA	1	0	1	0.426
		AT	15	8	7	
		TT	14	9	5	
	54G>A	GG	9	5	4	0.673
		GA	16	10	6	
		AA	5	2	3	
	-92G>A	AA	3	3	0	0.265
		AG	7	4	3	
		GG	20	10	10	
RET	37412G>A	GG	24	14	10	0.507
		GA	5	2	3	
		AA	1	1	0	
	135G>A	GG	16	9	7	0.489
	3 missing	GA	8	6	2	
		AA	3	1	2	
FLT3	738C>T	CC	5	4	1	0.414
		CT	15	7	8	
		TT	10	6	4	
FLT4	1480A>G	AA	23	12	11	0.427
		AG	7	5	2	
AURKB	893G>A	GG	22	11	11	0.407
		GA	8	6	2	
AURKA	169G>A	GG	18	10	8	0.672
		GA	11	6	5	
		AA	1	1	0	
	91A>T	AA	20	11	9	0.110
		AT	6	2	4	
		TT	4	4	0	

(L/hour/m^2) and genetic polymorphisms in *ABCB1 or ABCG2* (Table 3). However, for *FMO3*, patients carrying at least one G allele had a significantly slower clearance compared to the 18281 AA patients (P = 0.017).

No relationship between observed grade 3-4 neutropenia in the first treatment cycle and *KDR, RET, FLT3, FLT4, AURKB* or *AURKA* genotype was observed (Table 4). Also the *ABCB1*, *ABCG2*, and *FMO3* haplotypes did not show an association with danusertib clearance, nor did *KDR, RET, AURKA* haplotypes relate to danusertib induced grade 3-4 neutropenia (Table 5).

Also, no association was observed between the studied AURKB polymorphism and change in level of histone H3 phosphorylation induced by danusertib. The decrease in histone H3 phosphorylation for AURKB homozygous wild type genotypes (GG) was 91% (SD 13.3%), while the heterozygous genotype (GA) had a decrease of 84% (SD 12.2%, P=0.223).

Discussion

Aurora kinase inhibitors are relatively new and promising agents in development for anticancer treatment.[1-9] The current knowledge on treatment actions, toxicity, biomarkers and efficacy is still very limited. danusertib is the first aurora kinase inhibitor in which a pharmacogenetic pathway analysis has been performed to clarify pharmacokinetic and pharmacodynamic features of the drug.

In the last decade, well known examples of anti-cancer drugs can be given, for which initial recommended dose-levels had to be changed based on toxicity in subgroups of patients.[19-22] These subgroups of patients, with in general decreased enzymatic function based on genetic polymorphisms, could have been identified earlier if pharmacogenetic knowledge was available at an earlier stage. Therefore, it is recognized more and more that pharmacogenetic research in the earliest stages of development of new anti-cancer agents is highly relevant. While the basic characteristics of the new agent have to become more clear, also selection of patients with potential increased toxicity, or decreased efficacy, should be performed as early as possible. Therefore, the decision was made not to delay the pharmacogenetic analyses till after registration of the compound, but to explore potentially clinical relevant pharmacogenetic variation at this stage of development.

Our study was conducted in patients of a recent phase I trial and subsets of two phase II trials of danusertib, and therefore patient numbers for both pharmacokinetic and pharmacodynamic association analyses are relatively limited. However, in the phase I trial DNA-data were available for almost the entire patient population, making selec-

Table 3. Association between genetic polymorphisms and danusertib clearance.

Gene	Polymorphism	Genotype	No. Patients	Clearance L/hour/m^2 d1c1	
				Mean	SD
ABCB1	1236C>T	CC	16	17.8	6.5
		CT	21	18.3	5.9
		TT	10	18.6	4.9
		P-value		0.930	
	2677G>A/T	GG	17	18.2	6.6
		GT	20	18.5	5.8
		TT	10	17.7	4.9
		P-value		0.948	
	3435C>T	CC	13	19.5	6.1
		CT	21	17.7	5.9
		TT	13	17.8	5.6
		P-value		0.638	
ABCG2	421C>A	CC	36	18.1	6.1
		CA	11	18.8	4.8
		P-value		0.621	
	346G>A	GG	45	18.2	5.9
		GA	2	18.6	5.2
		P-value		0.755	
	143C>T	CC	29	18.9	6.1
		CT	15	17.3	5.5
		TT	3	16.5	3.6
		P-value		0.537	
	15994G>A	GG	23	18.5	5.0
		GA	21	18.2	6.2
		AA	3	16.5	3.6
		P-value		0.859	
FMO3	15167G>A	GG	15	19.5	6.6
		GA	20	18.1	6.0
		AA	12	16.9	4.2
		P-value		0.537	
	21443A>G	AA	27	19.2	6.3
		AG	18	16.9	5.1
		GG	2	16.4	1.3
		P-value		0.382	
	18281G>A	GG	35	18.1	5.2
		GA	11	17.2	6.1
		AA	1	34.0	n.a.
		P-value		0.017	

n.a: not applicable

Table 5. Haplotype analysis: uncorrected P values using Pearson X^2 analysis, independent samples Student's t-test or one-way ANOVA where appropriate

Gene	SNPS	Haplotype	Neutropenia grade 3-4 p-value
KDR	-604T>C	TCT	0.146
	1192G>A	CCT	0.460
	1719A>T	CCA	1.000
RET	37412G>A	GG	0.773
	135G>A	GA	0.773
AURKA	169G>A	GA	0.205
	91A>T	AA	0.672
		GT	0.110
Gene	**SNPS**	**Haplotype**	**clearance L/hour/m^2 d1c1 p-value**
ABCB1	1236C>T	GCC	0.953
	2677G>A/T	TTT	0.935
	3435C>T		
ABCG2	15994G>A	CC	0.859
	1143C>T	TT	0.588
FMO3	15167G>A	AGA	0.537
	21443A>G	GGG	0.603
	18281G>A	GAC	0.017

tion bias less likely. The two phase II trials are still ongoing and blood for DNA analysis was available for all patients included in the trials at the Erasmus University Medical Center at the moment of pharmacogenetic analyses.

A correlation between danusertib pharmacokinetics and pharmacogenetic variation is only seen for the FMO3 18281G>A homozygous variant and clearance. We can not exclude that this is the result of chance, but also for the other 2 SNPs (15167 G>A and 21443G>A) in this gene, a pattern to altered clearance, based on genotype, is suggested. As FMO3 is responsible for the main route of metabolism of danusertib, this warrants further research.

Currently, the mechanism causing neutropenia after danusertib treatment is unclear and could be associated with peak values (Cmax) or threshold values. Based on the new pharmacogenetic data from our current analysis no predisposition for the severity of hematological toxicity could be identified.

Clearly, this study has its limitations. As described earlier, due to the phase I and II nature of the studies patient numbers were limited and the group is quite heterogeneous. The power to find statistical significant differences in genotype of haplotype analyses was limited.

That correlations between the main enzymes and transporters involved in danusertib metabolism, and pharmacokinetics and toxicity are absent, does not mean that these results are unimportant. This study outcome makes the chance that danusertib is highly susceptible to pharmacogenetic variation less probable. More discrete differences, based on pharmacogenetic variability, should be explored further in additional (population based) pharmacogenetic studies for this compound.[23-25] The relatively high inter-individual variation observed could not be explained through pharmacogenetics and, for instance, the role of environmental factors might be important.

The reason for a lack of association between Histone H3 phosphorylation and the studied AURKB polymorphism is unclear. Whether the 893G>A mutation results in altered gene function is unknown.

Since danusertib is currently used in two treatment schedules, 330 mg/m^2 on days 1, 8 and 15 every 4 weeks and 500 mg/m^2 on days 1 and 15 every 4 weeks, a population based pharmacokinetic-pharmacogenetic model might help in selecting the optimal treatment schedule.[23-25] A second advantage of a population based pharmacokinetic-pharmacogenetic model is that the relative impact of all individual SNPs as covariates can be explored.

In conclusion, in this explorative study no highly significant associations between polymorphisms in genes coding for drug metabolizing enzyme, for transporter proteins and clearance of danusertib, between target receptor polymorphisms and toxicity of danusertib and between polymorphisms in the aurora kinase B receptor and the extent of histone H3 phosphorylation were seen. Future studies, including analyses of more patients on danusertib treatment and the use of population based pharmacokinetic-pharmacogenetic models to select the optimal danusertib treatment schedule are planned.

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11

General discussion

Targeted therapy

Conventional chemotherapeutical agents act by creating toxic effects on all dividing cells, frequently resulting in severe damage of normal tissues leading to side effects like myelosuppression, alopecia, or gastrointestinal problems. The optimum goal is to find a treatment modality that specifically kills malignant cells and causes little or no side effects. Targeted therapies were developed to target key elements that play a role in tumor development and tumor growth.

There is not one unanimous definition for the term 'targeted therapies'. In theory all therapies are 'targeted', so the term 'targeted therapies' is artificial when not used by a certain definition.[1] One common definition, and used in this thesis, is 'Targeted therapy is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with rapidly dividing cells (e.g. with traditional chemotherapy)'. There are various ways to categorize targeted agents, including categorizing by mode of inhibition of a signaling pathway, e.g. small molecule vs. antibody or by type of receptor that is blocked, e.g. EGFR, VEGFR, c-KIT etc. Another way of categorizing targeted agents is by effects in the tumor development, e.g. angiogenesis inhibitor, apoptosis inducer etc.

Trastuzumab (Herceptin®) is an excellent example of a targeted agent in the category of the monoclonal antibodies and is directed against the Her2 (EGFR2) receptor. In the category of targeted small molecules, imatinib (Gleevec®/Glivec®) was the first agent that was successfully developed. The results of imatinib in GIST, a tumor that is poorly affected by chemotherapy and radiotherapy, were astonishing and lead to a boost in research of small molecule tyrosine kinase inhibitors in solid tumors.[2,3] Also, the prognosis of Her2 positive breast cancer patients has improved significantly since the development of trastuzumab.[4,5]

On the contrary, most targeted agents do not meet up to the high expectations. The hope was that when crucial receptor and downstream signaling could be inhibited, proliferation of cancer cells was blocked and cancer could subsequently become a chronic illness. However, it becomes more and more clear that proliferation of cancer cells, in general, cannot be stopped by blocking only one or two signaling pathways.

Drug-development and patient selection in the treatment with targeted agents

Alterations should be made to the conventional phases of drug-development in oncology. In my opinion, maximum tolerated dose (MTD) can no longer be the only end-point

in oncological phase I studies, since targeted agents have limited side effects and MTD might never be reached. Instead, phase I studies should aim at identifying the maximum biological active dose, i.e. the dose that creates the maximum target inhibition. In our phase I study with telatinib, a small molecule angiogenesis inhibitor, we performed additional measurements with DCE-MRI, and plasma VEGF and s-VEGFR levels. Biomarker analyses showed dose-dependent increase in VEGF levels and decrease in sVEGFR-2 levels, with a plateau at 900 mg bid. A decrease in tumor blood flow (K_{trans} and $IAUC_{60}$) was observed with DCE-MRI. These results, together with the PK results were the main factors in determining the recommended phase II dose of a drug with limited side effects.

In phase II/III studies, instead of response rate, other endpoints should be chosen, like time to progression, while with targeted agents it might take some time before stabilization of the disease occurs. When agents like telatinib or danusertib are evaluated in phase II trials these changes to, in my opinion, 'old' trial designs should be made.

The observation that the efficacy of most targeted agents is limited in unselected patients warrants studies aiming at selecting subgroups of patients for which these drugs are beneficial. Exploration of predictive receptor polymorphisms or specific tumor subtypes with specific overactivity of certain signaling pathways may help to achieve this goal. In phase III studies, selection of the study population should be made based on biogenetics of the tumor, and investigations should also include pharmacodynamic analysis of target inhibition. In previous large phase III trials in unselected patients, targeted agents were incorrectly judged to be ineffective, and research of an effective drug has incorrectly been stopped.

In the studies reported in this thesis we aimed at selection of the best patient groups. In sarcomas we searched for EGFR and Her2 expression to select those patients who could benefit from EGFR/Her2 directed therapies. With pharmacogenetic studies we aimed to select those patients treated with the aurora kinase inhibitor danusertib or the small molecule VEGF inhibitor telatinib who would benefit most from those targeted agents without experiencing unnecessary side effects.

Toxicity of targeted agents

Unexpectedly, the toxicity of these new and specific agents is sometimes severe and may be unrecognized by the treating physician due to lack of experience. One example is the difference in monitoring of cardiovascular toxicity of trastuzumab and sunitinib, both known for their cardiotoxic side-effects. Trastuzumab has been part of the therapeutic arsenal far longer then sunitinib. In the five major randomized adjuvant trials, the use

of trastuzumab combination therapy resulted in severe congestive heart failure (New York Heart Association class III or IV) in 0-3.9% of patients treated in the trastuzumab arms versus 0-1.3% in the control arms. Ejection fraction decline of ≥10- 15% was reported in 3-34% of trastuzumab treated patients in these trials.[6] For sunitinib, data on cardiovascular toxicity are more recent, however in some reports the rate of congestive heart failure and reductions in ejection fraction are even higher then with trastuzumab treatment.[7,8]

As new therapies become available, new side effects emerge. Physicians have to be aware of those new side effects and monitoring for newly emerging side effects should be optimalized. Ongoing research should include studies on side effects of new and older agents, and studies to prevent or limit these toxicities. Clinical studies with translational research on mechanisms of toxicity and pharmacogenetic investigations have expanded the insight of and experience with selected targeted anti-cancer drugs.

This thesis focusses on hypertension, a specific side effect that is frequently seen in the treatment with VEGF inhibitors. The relevance of hypertension monitoring and treatment during anti-angiogenic treatment in cancer is often underestimated by clinicians. Acute rises in blood pressure caused by VEGF inhibitors may cause posterior leukoencefalopathy syndrome, with high morbidity and even mortality, which may be prevented by in time regulation of blood pressure.[9] Even more, multiple trials have shown that poorly controlled hypertension can lead to serious cardiovascular problems.[10,11] A long-term rise in diastolic blood pressure of 5-6 mm Hg is associated with 35-40% more stroke and 20-25% more coronary heart disease within 5 years.[12] We have shown that treatment with bevacizumab and telatinib, a monoclonal antibody against VEGF and a small molecule VEGF tyrosine kinase inhibitor, both result in decreased capillary density. This decreased capillary density is probably the basis for the VEGF-inhibitor induced hypertension. We could also show that the changes in capillary density are reversible after discontinuation of the VEGF-inhibitor. These studies increase the knowledge on the mechanisms of action of angiogenesis inhibitors and on angiogenesis induced side effects.

Future directions

In the coming years the use of targeted agents will probably expand even more rapidly. The indications might expand to almost all tumor types, to advanced and adjuvant therapy settings, and to combinations of multiple targeted agents or combinations of a targeted agent with conventional chemotherapy. Many clinical trials are already initi-

ated to explore which (combination of) agents in specific patient groups have the most potential and should be further investigated. The final position of certain targeted drugs in anti-cancer treatment will become more clear in the next decades. Agents will be registered for use when a clear survival benefit is observed for a certain indication, first in advanced stages of disease and then in the adjuvant setting. Moreover, new targeted agents directed at new pathways important in angiogenesis, apoptosis, cell division, and many others will hopefully be found.

This thesis reports a few of many trials that will increase the knowledge on targeted agents, on how to use them, whom to give them and hopefully increase the life expectancy and quality of life of future cancer patients.

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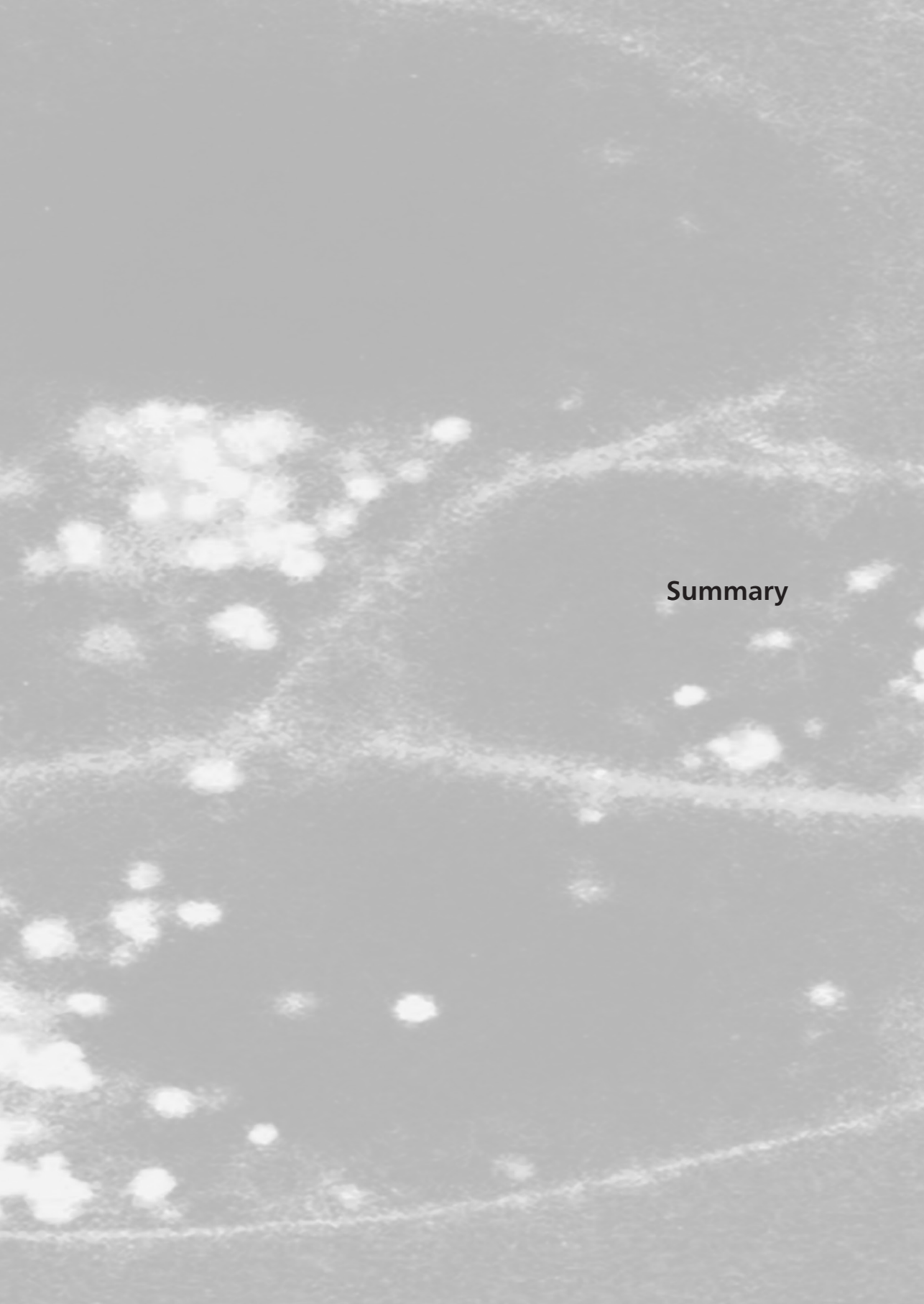


Summary

In this chapter the reported studies presented in this thesis are summarized.
This thesis focuses on targeted anti cancer agents in solid tumors. Pharmacokinetics, safety, pharmacodynamics, and pharmacogenetics of selected targeted therapies, alone or in combination with conventional chemotherapy, are studied. Moreover, new targeted treatment options for sarcomas are explored. **Chapter 1** gives a general introduction of this thesis.

In **Chapter 2**, a review of small molecule tyrosine kinase inhibitors in the treatment of solid tumors is presented. It updates the information on the small molecule tyrosine kinase inhibitors (TKIs) that are already registered for use or those who are in an advanced stage of development. Furthermore, the future role of tyrosine kinase inhibitors in the treatment of solid tumors is discussed.

Targeted agents may have potential in a wide range of tumor types, including sarcomas for which only limited treatment options are available. **Chapter 3** describes the construction and analysis of a tissue micro-array with 18 different types of soft tissue tumors. Positive membranous staining for EGFR (Her1) was observed in various sarcoma subtypes, including liposarcomas, leiomyosarcomas, synovial sarcomas, malignant peripheral nerve sheath tumors, rhabdomyosarcomas, solitary fibrous tumors, and angiosarcomas. Immunohistochemical staining for ERBB2 (Her2Neu) was negative in all subtypes. However, the immunohistochemical presence of growth factor receptors does not necessarily implicate that the subsequent pathway is activated, or is a potential target for therapy. These results, however, open the possibility to study the effect of EGFR blocking therapies, and give insight into previous study results showing that ERBB2 is not a potential treatment target in sarcomas.

A phase I dose escalation study of telatinib (BAY 57-9352), an orally available tyrosine kinase inhibitor, in patients with advanced or metastatic solid tumors is reported in **Chapter 4**. This phase I dose escalation study was conducted to evaluate the safety and tolerability of telatinib, with additional pharmacokinetics, pharmacodynamics and efficacy assessments. Telatinib was safe and well tolerated up to 1500 mg bid. Based upon pharmacodynamic and pharmacokinetic endpoints, telatinib 900 mg bid is the recommended dose for subsequent phase II studies.

Pharmacogenetics of telatinib are described in **Chapter 5.** This study was an exploratory side study conducted on a subset of patients enrolled into the phase I dose-escalating study of oral telatinib. Our pharmacogenetic analysis could not reveal an association between relevant genetic polymorphisms and clinical and pharmacokinetic observations of telatinib.

Chapter 6 focuses on hypertension and rarefaction during treatment with telatinib. Hypertension is a side-effect in anti-angiogenic therapy. We performed measurements of blood pressure, flow-mediated dilatation (FMD), nitroglycerin-mediated dilatation (NMD), aortic pulse wave velocity (PWV), skin blood flux, and capillary density during treatment with telatinib. A significant increase in blood pressure and PWV, combined with a significant reduction in NMD, FMD, skin blood flux and capillary density are reported. This study shows that the increase in blood pressure observed in the treatment with angiogenesis inhibitors may be caused by rarefaction, a functional or structural decrease in perfused microvessels.

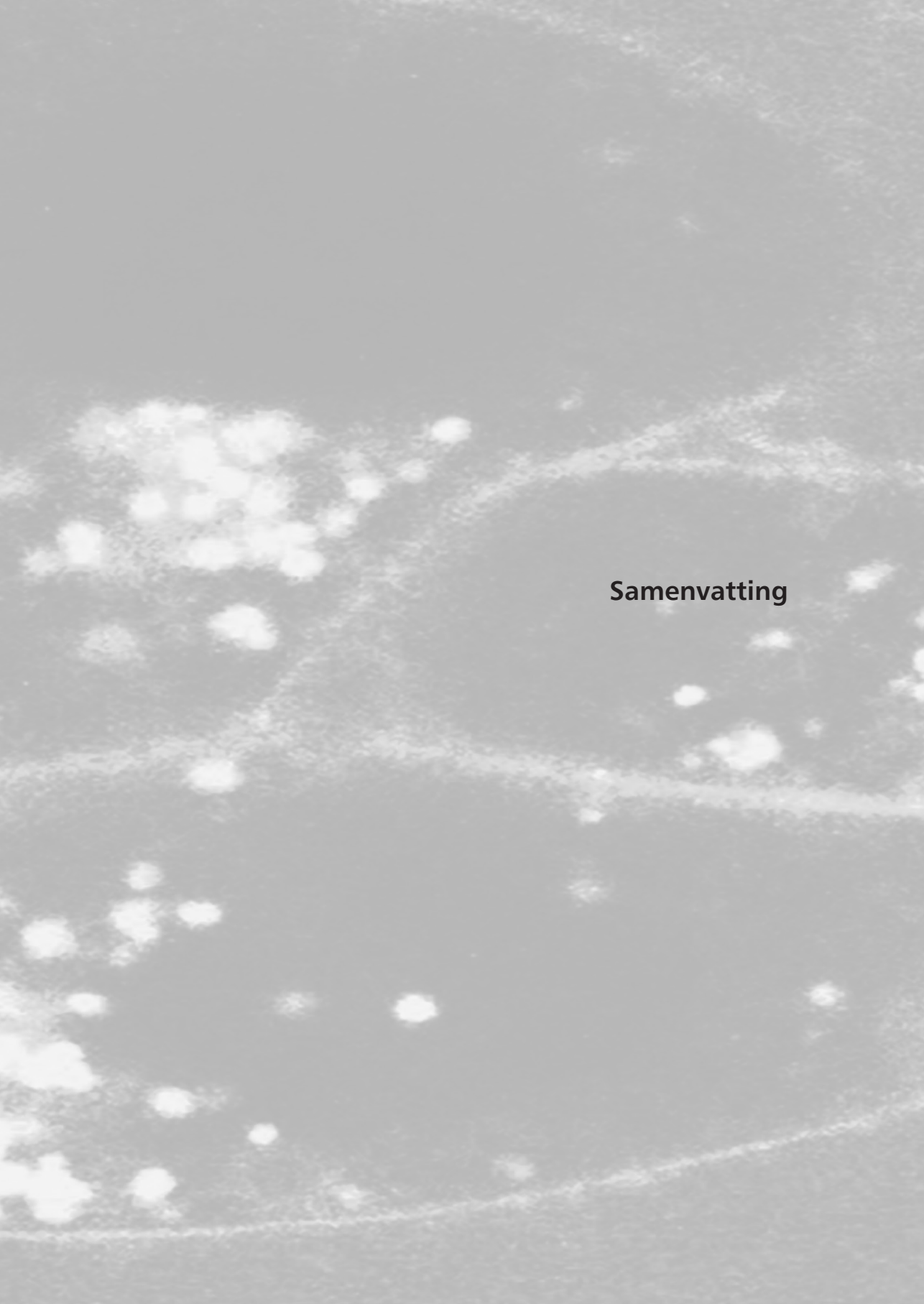
The underlying mechanisms of bevacizumab (Avastin®) related hypertension are reported in **Chapter 7**. Hypertension is a common side effect of bevacizumab, a monoclonal antibody directed at VEGF, and can lead to severe complications. We demonstrated that the decreased capillary density induced by bevacizumab treatment is reversible after discontinuation of the bevacizumab treatment. In combination with earlier results in VEGF tyrosine kinase inhibitor treatment, we also conclude that VEGF-associated rarefaction is a class-effect generated by all VEGF-inhibitors. These results implicate rarefaction, a decreased capillary density, as the most probable cause for hypertension in VEGF inhibition.

In **Chapter 8**, a phase I dose escalation study of sunitinib in combination with ifosfamide is reported. Patients with progressive solid tumors, good performance score, adequate organ function, and no standard therapy available, were eligible. Continuous once daily sunitinib, in escalating doses per cohort, was combined with one of two ifosfamide schedules, 3g/m^2/days1-3 and 1.2g/m^2/days1-5, both given intravenously every 3 weeks. Sunitinib co-administration did not affect the pharmacokinetics of ifosfamide or one of its metabolites. No consistent change in the number of circulating endothelial cells during treatment was observed. Sunitinib at 12.5 mg/day with ifosfamide 3g/m^2/days1-3, and sunitinib at 12.5 mg/day with ifosfamide 1.2g/m^2/days1-5 every 3 weeks supported by G-CSF are tolerable in patients with advanced solid tumors. Grade 3/4 neutropenia was the most reported side effect, seen in 89% of patients (8/9) treated at the recommended phase II dose. Neutropenia was uncomplicated in all but one patient (1/9).

A second phase I study of a new targeted agent is reported in **Chapter 9**. It involves a phase I dose-escalation study of the small-molecule pan-aurora kinase inhibitor danusertib. This dose escalation study was conducted to evaluate the safety and tolerability of danusertib, with additional pharmacokinetics, biomarker and efficacy assessments. Dose limiting toxicity of danusertib is neutropenia (short lasting and generally

uncomplicated), with limited non-hematological toxicity. The recommended dose for subsequent phase II studies is 330 mg/m^2 infused over 6 hours.

Chapter 10 reports the pharmacogenetics of danusertib. The aim of this exploratory side study was to identify possible associations between single-nucleotide polymorphisms (SNPs) in candidate genes with pharmacokinetic and pharmacodynamic parameters of danusertib. In patients with the *FMO3* 18281AA polymorphism, a significantly higher clearance was noticed, compared to patients carrying at least 1 wild type allele. For the variants in the genes encoding the transporters ABCB1, ABCG2, no relationships with danusertib clearance were found. Also for variants in the genes encoding the drug targets AURKA, AURKB, RET, FLT4, KDR and FLT3, no associations with neutropenia were observed. These findings make it unlikely that danusertib's pharmacokinetics and pharmacodynamics are highly susceptible for pharmacogenetic variation.



Samenvatting

In dit hoofdstuk worden de studies die in dit proefschrift worden beschreven samengevat en bediscussieerd. Dit proefschrift richt zich op kankercel specifieke middelen, ofwel 'targeted anti cancer agents', in solide tumoren. Farmacokinetiek, veiligheid, faramcodynamiek en farmacogenetica van kankercel specifieke middelen, alleen of in combinatie met conventionele chemotherapie, wordt onderzocht. Daarnaast worden nieuwe therapie mogelijkheden voor sarcomen bekeken. **Hoofdstuk 1** geeft een algemene inleiding op het onderzoek beschreven in dit proefschrift.

Hoofdstuk 2 bespreekt de intracellulair werkende tyrosine kinase remmers, ofwel 'small molecule tyrosine kinase inhibitors', in de behandeling van solide tumoren. De nieuwste informatie over de intracellulair werkende tyrosine kinase remmers die al geregistreerd zijn voor behandeling of in een ver stadium van ontwikkeling zijn, wordt besproken. Daarnaast wordt de toekomstige rol van de intracellulair werkende tyrosine kinase remmers in de behandeling van solide tumoren bediscussieerd.

Kankercel specifieke middelen zouden effectief kunnen zijn in veel verschillende soorten tumoren. Dit geldt mogelijk ook voor sarcomen, waarvoor nu slechts beperkte behandelmogelijkheden zijn. In **Hoofdstuk 3** wordt het maken en analyseren van een weefsel micro-array bestaande uit 18 verschillende typen weke delen tumoren beschreven. Membraan aankleuring voor epidermale groeifactor 1 (EGFR; Her1) werd gezien in meerdere sarcoom subtypen, o.a. in liposarcomen, leiomyosarcomen, synoviosarcomen, maligne perifere zenuwschede tumoren, rhabdomyosarcomen, fibrosarcomen en angiosarcomen. Immunohistochemische aankleuring voor epidermale groeifactor 2 (ERBB2; Her2Neu) was afwezig in alle sarcoom subtypen. Ondanks het gegeven dat een bepaalde groei factor receptor bij immuunhistochemisch onderzoek aanwezig is op de celmembraan, hoeft dit niet altijd te betekenen dat de betreffende signaal transductie route geactiveerd is, of überhaupt een potentieel doel is voor behandeling. Onze resultaten bieden wel de mogelijkheid om het potentiële effect van EGFR remmers in sarcomen te gaan bestuderen. Tevens ondersteunt dit de resultaten van eerdere studies waarbij duidelijk is geworden dat Her2 geen potentieel doel is om behandeling in sarcomen tegen te richten.

Een fase I dosis escalatie studie naar de behandeling met telatinib wordt beschreven in **Hoofdstuk 4**. Telatinib (BAY 57-9352) is een orale tyrosine kinase remmer van VEGFR-2, VEGFR-3, PDGFR- β en c-KIT. Deze fase I dosis escalatie studie werd uitgevoerd om de veiligheid en verdraagzaamheid van telatinib te onderzoeken. Daarbij werd additioneel gekeken naar farmacokinetiek, farmacodynamiek en effectiviteit. Telatinib kon veilig worden gegeven zonder veel bijwerkingen tot een dosis van 1500 mg twee keer per dag.

Gebaseerd op farmakokinetiek en farmacodynamiek uitkomsten, is telatinib in een dosis van 900 mg twee keer per dag de aanbevolen dosis voor toekomstige fase II studies.

Farmacogenetisch onderzoek van telatinib wordt beschreven in **Hoofdstuk 5**. Dit is een onderzoek verricht in een deel van de patiënten die behandeld werden in de fase I studie met oraal telatinib. Onze farmacogenetische analyses konden geen associatie aantonen tussen relevante genetische polymorfismen en klinische uitkomsten of farmacokinetiek kenmerken van telatinib.

Hoofdstuk 6 richt zich op hypertensie en rarefactie, een functionele of structurele vermindering van het aantal kleine capillairen en venulen, tijdens behandeling met telatinib. Hypertensie is een bijwerking in anti-angiogenese therapie. Wij verrichtten metingen gericht op bloeddruk, endotheel-afhankelijke vasodilatatie (flow-mediated dilatation; FMD), endotheel-onafhankelijke vasodilatatie (nitroglycerin-mediated dilatation; NMD), aorta pols golf snelheid (aortic pulse wave velocity; PWV), bloed flux in de huid en dichtheid van de capillairen tijdens behandeling met telatinib. Wij zagen een significante stijging in bloeddruk en PWV, gecombineerd met een significante daling in NMD, FMD, blood flux van de huid en dichtheid van de capillairen. Deze studie toont dat de stijging van bloeddruk tijdens de behandeling met angiogenese remmers veroorzaakt zou kunnen worden door rarefactie, een functionele of structurele vermindering van het aantal kleine capillairen en venulen.

Het onderzoek naar onderliggende mechanismen verantwoordelijk voor de hypertensie die vaak wordt gezien bij behandeling met bevacizumab (Avastin®), wordt beschreven in **Hoofdstuk 7**. Hypertensie is een vaak voorkomende bijwerking van bevacizumab, een monoklonaal antilichaam tegen VEGF, wat kan leiden tot ernstige complicaties. Wij zagen een daling in de dichtheid van de capillairen veroorzaakt door behandeling met bevacizumab die reversibel is na het stoppen van de bevacizumab therapie. In combinatie met de resultaten uit onze eerdere studie met een telatinib bevestigt dit het vermoeden dat de geïnduceerde verlaging van de dichtheid van de capillairen een klasse-effect is, veroorzaakt door alle VEGF remmers. Onze resultaten impliceren dat rarefactie, een vermindering van het aantal kleine capillairen en venulen, het belangrijkste proces is dat ten grondslag ligt aan de hypertensie die wordt gezien bij het gebruik van angiogenese remmers.

In **Hoofdstuk 8** wordt een fase I dosis-escalatie studie van sunitinib in combinatie met ifosfamide beschreven. Patiënten met vergevorderde of uitgezaaide solide tumoren, goede conditie, goede orgaan functie, en geen beschikbare standaard behandeling kon-

den worden geincludeerd. Continu 1 keer per dag gegeven sunitinib, in oplopende doseringen per cohort, werd gecombineerd met 1 van 2 ifosfamide schema's, namelijk 3 g/m^2/dag1-3 of 1,2 g/m^2/dag1-5, intraveneus toegediend, elke 3 weken. Het bijgeven van sunitinib beïnvloedde de farmacokinetiek van ifosfamide of van diens metabolieten niet. Er werd geen consistente verandering gezien in het aantal circulerende endotheelcellen tijdens de behandeling. Sunitinib in een dosis van 12,5 mg per dag gecombineerd met ifosfamide 3 g/m^2/dag1-3 en sunitinib 12,5 mg/dag in combinatie met ifosfamide 1,2 g/m^2/dag1-5 elke 3 weken ondersteund met granylocyten-groeifactoren (G-CSF) wordt goed verdragen door patiënten met vergevorderde solide tumoren en is de aanbevolen dosis voor toekomstige fase II studies. Graad 3-4 neutropenie was de meest voorkomende bijwerking gezien in 89% van de patiënten (8 van de 9) die werden behandeld met de aanbevolen dosis voor toekomstige fase II studies. Neutropenie verliep in bijna alle patiënten ongecompliceerd (8 van de 9 patiënten).

Een tweede fase I studie betreffende een nieuw kankercel specifiek middel wordt beschreven in **Hoofdstuk 9**. Het betreft een fase I dosis-escalatie studie met de aurora kinase remmer danusertib (PHA-739358) in patiënten met vergevorderde of uitgezaaide solide tumoren. Danusertib is een intracellulair werkende pan-aurora kinase remmer. Deze dosis-escalatie studie werd uitgevoerd om de veiligheid en verdraagzaamheid van danusertib te onderzoeken, en daarnaast te kijken naar farmacokinetiek, biomarkers en effectiviteit. De dosis limiterende bijwerking van danusertib is neutropenie, welke kortdurend is en meestal ongecompliceerd verloopt, met verder nauwelijks niet-hematologische bijwerkingen. De aanbevolen dosis voor verdere fase II studies was 330 mg/m^2 geïnfundeerd in 6 uur.

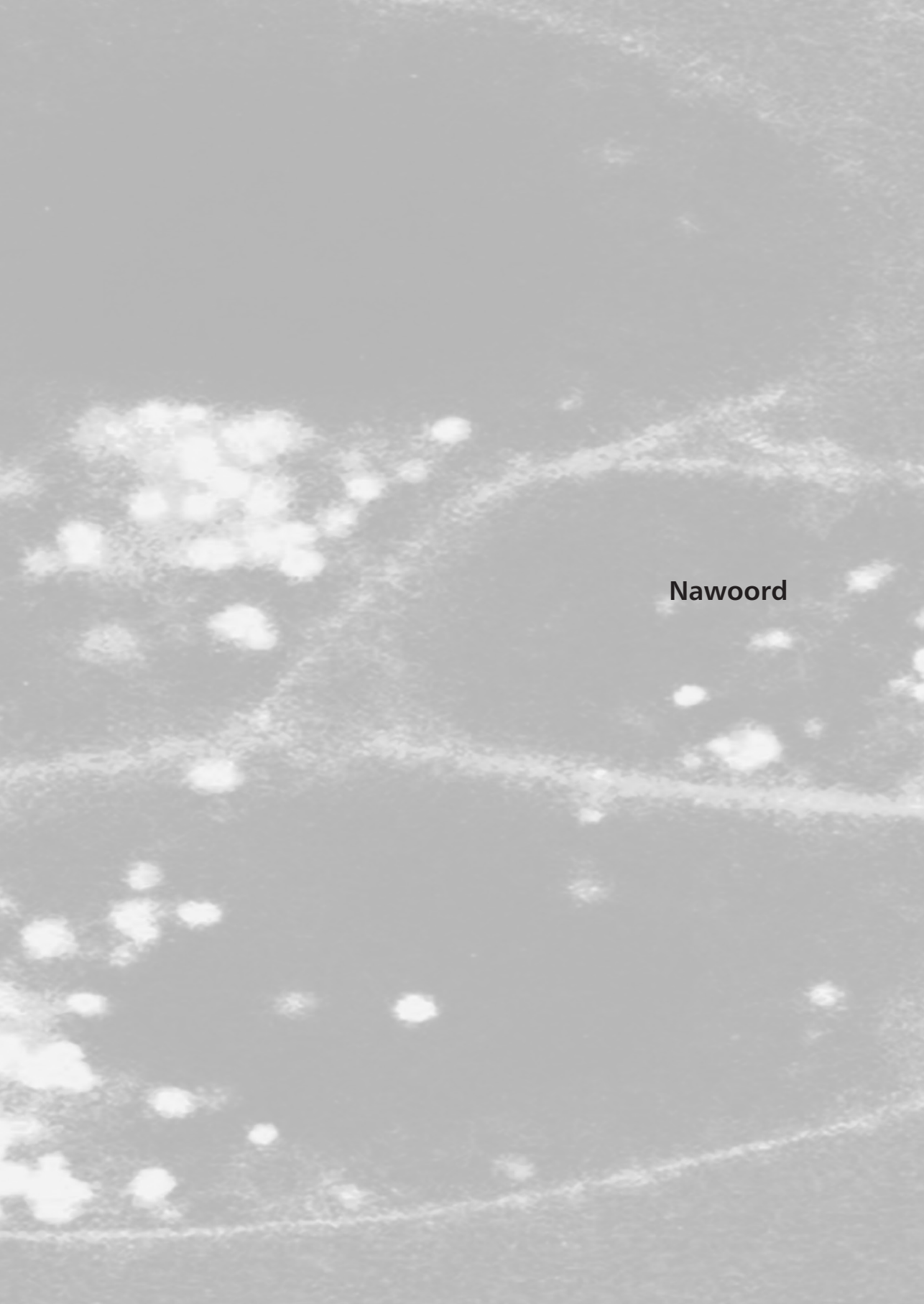
Hoofdstuk 10 beschrijft de farmacogenetica van danusertib. Het doel van deze studie was mogelijke associaties tussen geselecteerde genetische polymorfismen en farmacokinetische of farmacodynamische kenmerken van danusertib te onderzoeken. In patiënten met het *FMO3* 18281AA polymorfisme werd een significant hogere klaring van danusertib gezien, in vergelijking met patiënten die tenminste 1 wildtype allel dragen. Voor de varianten in de genen coderende voor de transporters ABCB1 en ABCG2, werd geen associatie met de klaring van danusertib gezien. Voor varianten in de genen coderend voor AURKA, AURKB, RET, FLT4, KDR en FLT3 werd geen associatie met neutropenie gevonden. Deze uitkomsten maken het erg onwaarschijnlijk dat de farmacokinetiek en farmacodynamiek van danusertib beïnvloed wordt door farmacogenetische variaties.

Hoofdstuk 11 geeft de algemene discussie weer en de toekomstperspectieven van de kankercel specifieke middelen. De ontwikkeling en registratie van kankercel specifieke

middelen gaat opmerkelijk snel. Helaas lijken de meeste kankercel specifieke middelen niet zo'n goed effect te hebben als men verwachtte. De hoop was dat wanneer cruciale receptoren en signaaltransductie routes konden worden geremd, proliferatie van kankercellen kan worden geblokkeerd en kanker daarmee een chronisch verlopende ziekte zou worden. Het feit dat de effectiviteit van de meeste kankercel specifieke middelen beperkt is in ongeselecteerde patiënten benadrukt dat verder onderzoek zich o.a. moet richten op het selecteren van die subgroepen patiënten die het meeste baat hebben van deze middelen. Het onderzoeken van voorspellende receptor polymorfismen of tumor subtypen met specifieke overactiviteit van bepaalde signaaltransductie routes kunnen helpen om dit doel te bereiken.

De bijwerkingen van deze nieuwe en specifieke middelen kunnen soms zeer ernstig zijn en verlopen vaak heel anders dan bij de bekende conventionele chemotherapeutische behandeling. Daarom is aanhoudend onderzoek naar nieuwe effectieve anti-kanker middelen, naar bijwerkingen van bekende en nieuwe middelen, en naar manieren om bijwerkingen te voorkomen of te beperken noodzakelijk. Vele reeds afgeronde klinische studies met translationeel onderzoek hebben intussen het inzicht en de ervaring met kankercel specifieke middelen vergroot.

In de komende jaren zal het gebruik van kankercel specifieke middelen ongetwijfeld enorm gaan toenemen. De indicaties zullen mogelijk uitbreiden tot bijna elke vorm van kanker, in gemetastaseerde en adjuvante situaties, en in combinatie met andere kankercel specifieke middelen of in combinatie met chemotherapie. Vele klinische onderzoeken zijn al opgestart met het doel uit te zoeken welke (combinaties van) middelen in specifieke patiëntengroepen het grootste te verwachten effect hebben en dus verder onderzocht zouden moeten worden. Dit proefschrift beschrijft een klein aantal van vele studies die de kennis over kankercel specifieke middelen zal vergroten, over hoe deze middelen te gebruiken en aan wie ze voor te schrijven, in de hoop de levensverwachting van toekomstige kankerpatiënten te vergroten.



Nawoord

Dit proefschrift is het resultaat van de inspanning en hulp van vele mensen. Als eerste wil ik de patiënten bedanken die meegedaan hebben aan deze studies. Zonder hun hulp had dit onderzoek nooit verricht kunnen worden.

Ik wil alle mensen van de afdeling oncologie bedanken die mij hebben bijgestaan en geholpen. Vydia, Ariënne, Ellie, Marjolein jullie hebben mij in de zoek naar statussen en afspraken altijd weer geholpen. Jan, Margret zonder jullie hulp bij de instructie en begeleiding van patiënten, het verzamelen van alle monsters en data en de hulp met de altijd weer opdoemende SAE's had ik het nooit gered. Judith, Ellen, Saskia jullie hebben me geholpen om de combinatie van dit onderzoek en mijn opleiding tot internist-oncoloog te kunnen combineren. Vincent, Peter-Paul zonder de steun (en de drankjes op vrijdag) had ik een hoop vreugde moeten missen de laatste jaren. Hans, je bent fantastisch geweest. Je hebt me daar laten gaan waar ik wilde gaan en me altijd geholpen. Dank je! Ook mijn beide promotores dank ik hartelijk voor de fantastische begeleiding.

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Daphne, Elise, Jacq, Hanneke, Ruth, Valeska, de etentjes, high tea, het beachvolleybal, het heeft me op de been gehouden. Bianca, Nicole, Yvette zonder die uren praten op onze weekenden en gewoon tussendoor was ik allang gestrand. Cindy en Martha, mijn paranimfen. Jullie zijn geweldig. Cin, ik hoop dat wel altijd onze verhalen kunnen blijven delen en we ooit nog een keer naar de Boney M gaan. Martha, ook jij kent al mijn geheimen en hopelijk blijft dit voor altijd. Joost, ik hoop niet dat we nog vaak zullen zeggen: 'Het is al weer veel te lang geleden'. Ik mis je.

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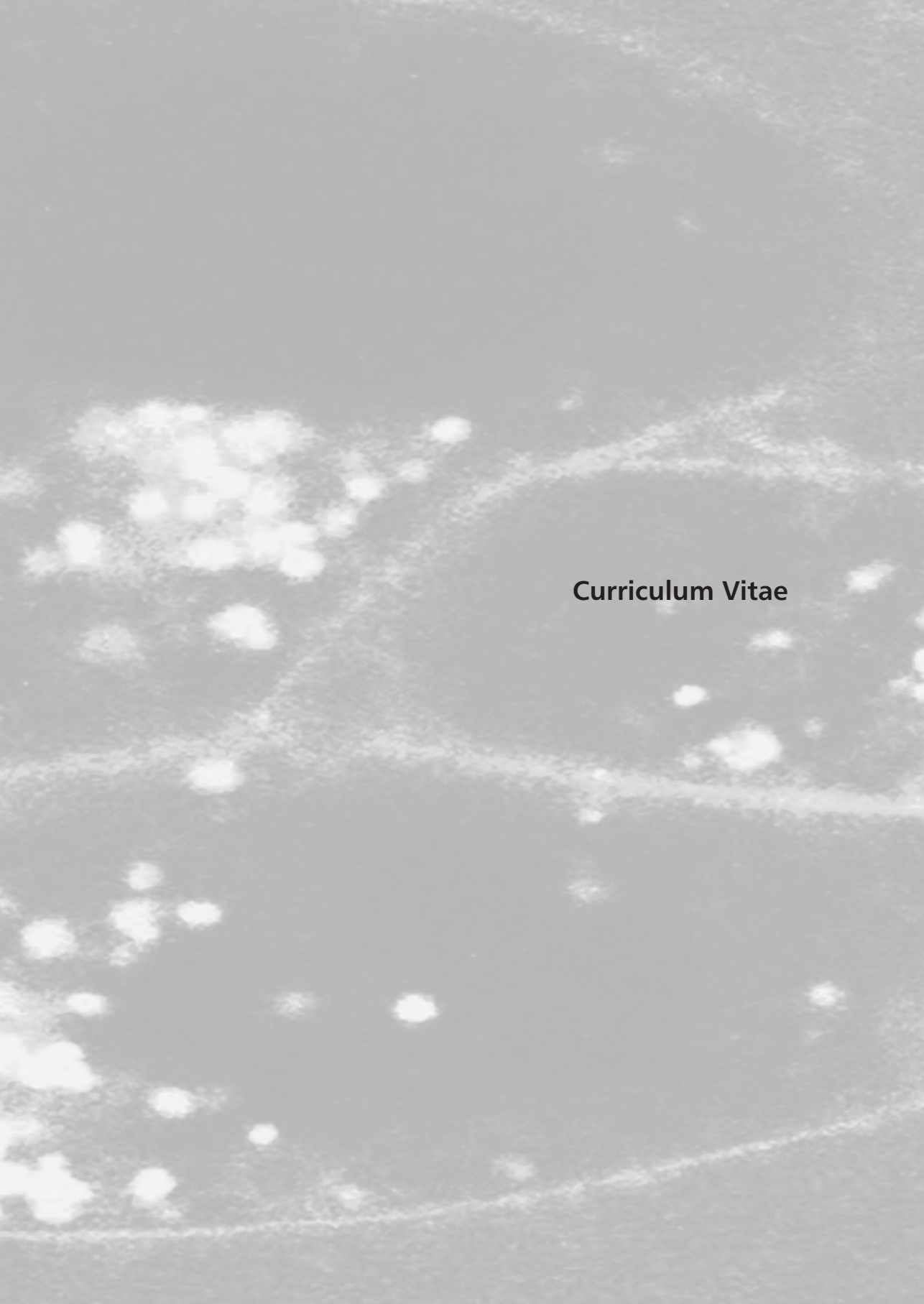
Jasper, Thomas, Koen, mijn mannetjes, jullie maken de wereld zo mooi.

Ton, jou wil ik het allermeest bedanken, voor je liefde, je motivatie, je hulp om altijd weer een oplossing te zoeken. Dank je voor de momenten waarop je zorgde dat ik gewoon eens even niets deed. Ik hou van je.



Curriculum Vitae

Neeltje Steeghs werd op 6 oktober 1977 geboren te Breda. In 1995 behaalde zij haar VWO diploma aan het St. Stanislascollege te Delft en startte met de studie biomedische wetenschappen aan de Rijksuniversiteit Leiden. In 1996 behaalde zij haar propedeuse biomedische wetenschappen en startte hierna met haar opleiding geneeskunde in Leiden. In december 2001 behaalde zij haar artsexamen, waarna zij in januari 2002 startte met de opleiding interne geneeskunde. De eerste 2 jaar van haar opleiding was ze werkzaam in het Rijnland Ziekenhuis te Leiderdorp (opleider: Dr. F.H.M. Cluitmans) waarna zij haar opleiding vervolgde in het Leids Universitair Medisch Centrum (opleiders: Prof. dr. A.E. Meinders en Prof. dr. J.A. Romijn). De laatste 2 jaar van de opleiding interne geneeskunde werden gecombineerd met het aandachtsgebied oncologie (opleider Prof. dr. J.W.R. Nortier). Dit resulteerde in de registratie tot internist-oncoloog in maart 2009. Onder leiding van Dr. A.J. Gelderblom, Prof. dr. J.W.R. Nortier en Prof. dr. H-J. Guchelaar verrichtte zij gedurende haar opleiding tot internist-oncoloog onderzoek op de afdeling klinische oncologie van het Leids Universitair Medisch Centrum. Dit onderzoek resulteerde in dit proefschrift. Vanaf 1 mei 2009 is zij werkzaam als internist-oncoloog in het Antoni van Leeuwenhoek Ziekenhuis te Amsterdam. De auteur van dit proefschrift is in 2002 getrouwd met Ton van der Zwet. Samen hebben zij drie kinderen, Jasper, Thomas en Koen.

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