EARLY CLINICAL STUDIES ON TARGET SPECIFIC ANTICANCER AGENTS

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Ter nagedachtenis
aan mijn vader

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CONTENTS

CHAPTER 1

INTRODUCTION TO THE THESIS

INTRODUCTION

Traditionally, chemotherapeutic treatment of advanced cancer is characterized by the use of cytotoxic agents that inhibit one or more important steps in the nuclear DNA replication process. By inhibiting these processes cancer cell death is promoted and tumors are likely to shrink in size. While being effective on the one hand, the side-effects induced by these agents often limit their clinical application. Cytotoxic anticancer agents generally are not specific for cancer cells, but also target various other cells and organ systems such as bone-marrow, mucous membranes, hair-follicles and nervous system leading to a range of dose-limiting and sometimes even life-threatening side-effects. In addition, the activity of cytotoxic anticancer agents is limited by the occurrence of tumor resistance, caused by a variety of mechanisms such as inactivation of the p53 suppressor gene, overexpression of Bcl-2 proteins and overexpression of the MDR-1 gene enhancing the production of P-glycoprotein. The occurrence of dose-limiting toxicity, the development of drug resistance and the non-specific mechanisms of action therefore all contribute to the fact that currently advanced cancer can only rarely be cured with chemotherapy.

In the last decades basic and molecular research has unraveled various specific intra- and extracellular mechanisms that are crucial for malignant transformation of cells, cancer cell proliferation and formation of metastases. As a result a large number of new anticancer agents targeting specifically one or more of these extracellular, transmembrane or intracellular (but extranuclear) processes have been developed. By targeting these processes, anticancer treatment is directed more specifically against the cancer cells and therefore is more likely to leave normal cells and tissues unaffected. Although these new so-called target specific anticancer agents can be toxic to cancer cells, frequently their activity results in growth inhibition rather than cell death and therefore these agents are often being referred to as cytostatic agents. Because of their specific mechanism of action, not interfering with the cellular reproduction cycle, these cytostatic agents potentially are less toxic compared to the classic cytotoxic agents. In addition, since several of these new agents are not targeting the cancer cell, but rather the surrounding matrix, harbouring non-mutated cells, drug resistance is less likely to occur.

The clinical application of these new target specific anticancer agents has had great consequences for the design of early clinical phase I and II studies. Traditionally phase I studies with cytotoxic agents focus on defining dose-limiting toxicities and the maximum tolerated dose in order to determine the recommended dose for further studies, whereas subsequent phase II studies are performed to gain insight in the antitumor activity, using tumor regression as surrogate endpoint. As mentioned, target specific anticancer agents have a completely different mechanism of action and toxicity profile, and therefore the design of phase I and II studies has to be adapted accordingly.

This thesis describes some of the issues encountered when designing clinical trails for these target specific anticancer agents and describes the results of several phase I studies that have been performed with these new agents, when used as single agent or in combination with commonly used cytotoxic anticancer regimens.

ABSTRACT

Recently a large number of new anticancer agents targeting specifically one or more of the extracellular, transmembrane or intracellular (but extranuclear) processes involved in malignant transformation of cells or carcinogenesis have been developed. These agents show target specificity, predominantly resulting in growth inhibition in tumor models and less frequently in tumor regression, acting in a cytostatic rather than a cytotoxic way. In addition, based on their specific mechanism of action, these target specific agents are expected to have a more favorable toxicity profile. In exploring new anticancer agents, phase I studies generally focus on toxicity and primarily are designed to describe dose-limiting toxicity and to determine the maximum tolerated dose and the dose recommended for phase II studies. These phase II studies are subsequently performed in small groups of patients using the percentage tumor regression to screen for anticancer efficacy. Due to the anticipated low toxicity profile and the mainly growth inhibiting activity of target specific agents, the design of phase I and II studies involving these agents will have to be adapted in several ways. It is emphasized that, although it is helpful to distinguish cytotoxic from cytostatic anticancer agents, this dichotomy can be a simplification. In this paper we will discuss important issues that will have to be faced when developing clinical trials with these agents and we will specifically translate this into the already known concepts of trial design exploring cytotoxic and cytostatic agents.

CHAPTER 2

CLINICAL TRIAL DESIGN FOR TARGET SPECIFIC ANTICANCER AGENTS

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INTRODUCTION

Traditionally the systemic treatment of advanced cancer encompasses the use of cytotoxic drugs, targeting nuclear processes. Recently the development of new anticancer agents has been accelerated by the unraveling of various processes involved in malignant transformation of cells and carcinogenesis, yielding a large number of new anticancer agents targeting specifically one or more of these extracellular, transmembrane or intracellular (but extranuclear) processes. Examples of these new agents are receptor tyrosine kinase inhibitors, farnesyl transferase inhibitors (FTIs), matrix metalloproteinase inhibitors (MMPIs) and angiogenesis inhibitors.
In general these agents show target specificity, predominantly resulting in growth inhibition in tumor models and less frequently in tumor regression. These agents usually require prolonged administration to optimally inhibit tumor growth. Target specific agents are considered to act in a cytostatic (growth inhibitory) way rather than a cytotoxic one. Based on their specific mechanism of action, target specific agents are expected to have a more favorable toxicity profile. The specific mechanism of action and anticipated low toxicity profile make these agents attractive anticancer drugs and therefore exploring them in clinical trials seems warranted. Many of these new agents indeed have already entered such a clinical trial program.
In exploring new anticancer agents, phase I studies generally focus on toxicity and primarily are designed to describe dose-limiting toxicity (DLT), and to determine maximum tolerated dose (MTD) and the dose recommended for phase II studies. These phase II studies are subsequently performed in small groups of patients using the percentage tumor regression to screen for antitumor efficacy. Finally, in randomized phase III trials the antitumor efficacy of new anticancer agents is assessed using endpoints like time to progression (TTP), disease free survival, and overall survival. Increasingly these studies include quality of life analyses, in order to assess whether the possible improvement in TTP or survival of the new treatment outweighs the disadvantages of this treatment e.g. in terms of inconvenience and toxicity.
This traditional trial design has been used for several decades and has resulted in the registration of various new and effective anticancer agents. The trial design, however, is based on the assumptions that (a) anticancer agents are almost by definition non-specific and therefore toxic for the body, (b) antitumor activity and toxicity are dose dependent with a steep dose-response curve, (c) tumor regressions are necessary to predict benefit for patients and (d) the recommended phase II dose is near to the MTD. Although this may hold for cytotoxic anticancer agents, it could be anticipated that for cytostatic agents (a) by targeting specific processes either inside or outside the tumor cell, side effects are less frequently expected and defining DLT and MTD could be difficult, if at all possible, (b) the dose-response curves with respect to antitumor activity and toxicity are frequently more shallow, (c) it is debatable whether tumor regressions are to be expected when in preclinical models only growth inhibition is observed and (d) the recommended dose with optimal biological activity (OBD) can be far below the MTD. Therefore, in particular, the design of phase I and II studies involving such agents will

have to be adapted in several ways. Several authors have addressed these issues and made recommendations for drug design studies exploring cytostatic agents [1-5].
What have we learned from clinical studies performed with cytostatic agents thus far? For several of the MMPIs tested an MTD could be defined based on musculoskeletal or skin toxicity [6]. The studies published thus far concerning FTIs also suggest that toxicity is more frequently observed than was expected based upon preclinical data and that toxicity differs from agent to agent [7]. Also in phase I studies with the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors ZD1839 and OSI-774 DLTs were observed, an MTD could be defined and tumor regressions were observed [8]. In contrast, for most angiogenesis inhibitors studied no or only minimal toxicity was observed with most of the agents tested. And although the majority of these agents can indeed be considered to act in a cytostatic way, however, some of them must be considered cytotoxic by their ability to cause rapid destruction of blood vessels resulting in objective tumor regressions [9]. Another remarkable example is imatinib mesylate (STI571) which was designed to specifically inhibit the BCR-ABL associated tyrosine kinase and was shown to cause apoptosis in BCR-ABL expressing cells. Apart from the ability to induce hematological and cytogenetic responses in patients with Philadelphia chromosome positive acute and chronic myeloid leukemia [10,11], impressive tumor regressions were noted in patients with gastrointestinal stromal tumors related to the inhibition of the tyrosine kinase activity of c-kit [12].
Therefore, although it is helpful to distinguish cytotoxic from cytostatic anticancer agents, this dichotomy can be a simplification. In this paper we will discuss important issues that will have to be faced when developing clinical trials with these agents and we will specifically translate this into the already known concepts of trial design exploring cytotoxic and cytostatic agents.

PHASE I STUDIES

The principal goal of phase I studies is to describe safety and toxicity, describe pharmacological behavior and to define an optimal and safe dose and schedule for subsequent efficacy studies. The way this goal is reached depends heavily on the characteristics of the agent tested. For cytotoxic agents, usually a steep dose-response relationship is observed, and therefore the recommended dose for efficacy studies in general is near the MTD. Toxicity is a major endpoint in these studies, and defining the MTD is usually easy to accomplish. Cytostatic agents, however, are expected to cause less acute toxicity based on their target specificity. Therefore, describing the MTD can be difficult or even impossible (Figures 1 and 2). Furthermore, since it is anticipated that for optimal antitumor activity prolonged administration is necessary, it is better to define a dose that permits such a prolonged administration. The assumption that cytostatic agents are essentially non-toxic and that MTD is difficult to define, however, is not always correct. As mentioned before, in several phase I studies with various cytostatic agents,

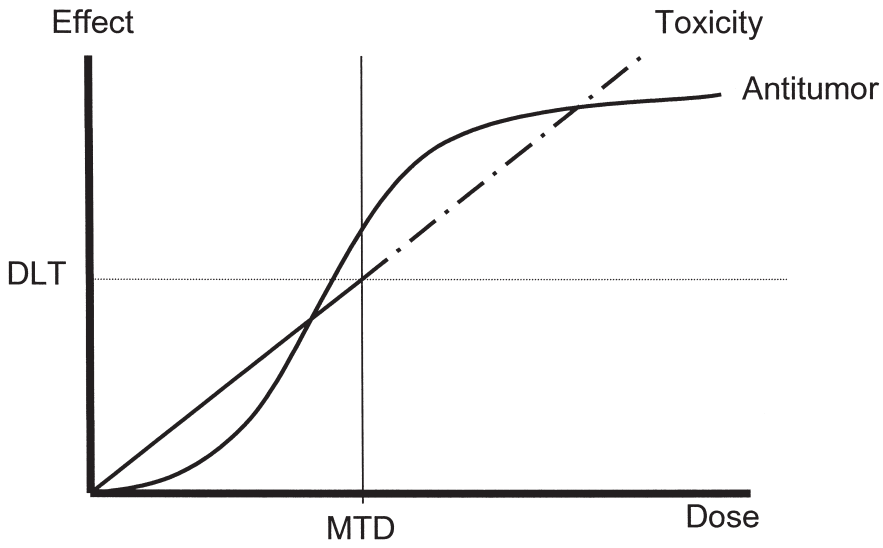

Figure1. Hypothetical Dose-Response and Dose-Toxicity Curve for Cytotoxic Agent.

Abbreviations: DLT, dose limiting toxicity; MTD, maximum tolerated dose.

dose-escalation was hampered by the occurrence of DLT. Therefore the dichotomy cytotoxic versus cytostatic is not synonymous with the presence or absence of DLT.

In the absence of DLT it is important to try to define other endpoints. Pharmacokinetic endpoints can be used to make decisions with regard to dose escalation. This pharmacologically guided dose escalation (PGDE) is based on the concept that when comparing animal to human doses, one can expect equal toxicity with equal drug exposure. In other words, although there is a large variability in the ratio of human to animal tolerable doses, at approximately equitoxic doses there is much less variability with respect to the drug exposure ratio [13]. This knowledge can be used in a PGDE and can result in more rapid dose escalation when compared to the more conservative dose escalation schemes such as the modified Fibonacci scheme, thereby minimizing the number of patients receiving low (non-therapeutic) doses. This principle holds true for both cytotoxic and cytostatic drugs. For cytostatic drugs the toxic dose can be much higher than the OBD and therefore the plasma concentration area under the curve and trough ratio correlating with antitumor activity in animal tumor models can be used to make either dose escalation decisions and to define alternative study endpoints. A good example of PGDE is the dose escalating study of STI571 in patients with chronic myeloid leukemia. While no formal MTD was defined, 400 mg daily was set to be the recommended dose for further studies, based upon the clinical response rate which was maximal from 300 mg onwards, pharmacokinetic analysis showing drug trough levels at doses from 400 mg well above

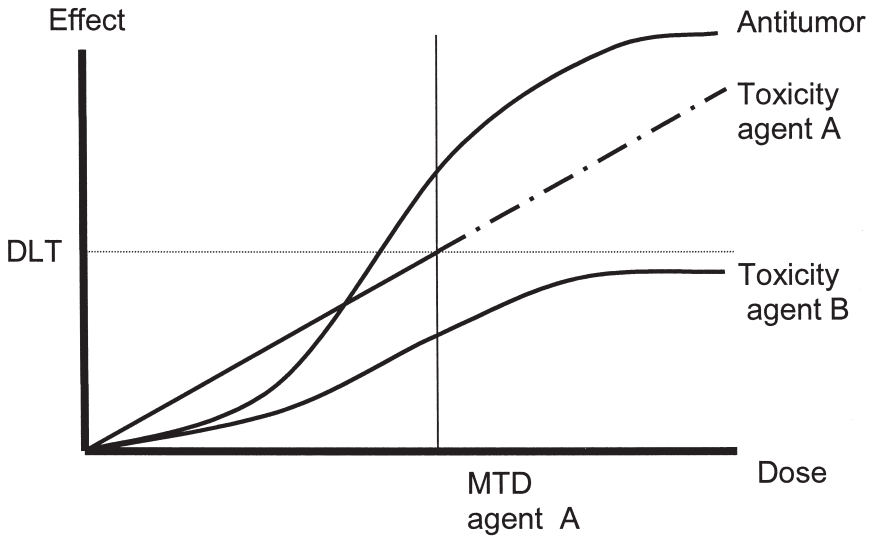

Figure 2. Hypothetical Dose-Response and Dose-Toxicity Curve for Cytostatic Agent With (A) and Without (B) DLT.

Abbreviations: DLT, dose limiting toxicity; MTD, maximum tolerated dose.

levels resulting in maximal cell kill in preclinical studies, and pharmacodynamic analysis showing inhibition of phosphorylation of a BCR-ABL substrate protein in peripheral leucocytes reaching a plateau between 250 and 750 mg [10]. Another example of PGDE is the halt of further dose escalation of BAY 12-9566, an orally available MMPI. As plasma concentration curves showed a plateau suggestive of saturable drug absorption in this essentially non toxic compound, further dose escalation was considered to be useless [14].

Pharmacodynamic endpoints such as the measurement of target (enzyme) inhibition within the tumor can also be used to guide dose escalation. Optimally, attempts should be made to define the OBD. However measurements of such target inhibition within the tumor must be validated and correlated with anticancer response. Although taking repeated tumor biopsies has been shown to be feasible by some [15], most clinicians will feel uncomfortable doing so. It has been suggested to limit the number of biopsies by randomizing the patients either to undergo a biopsy before start of the study or to undergo such a biopsy after a predefined period on therapy and to compare these two groups [4]. It is doubtful whether in this concept enough tumor biopsies can be taken and whether enough reproducible and correlative information can be obtained and therefore more convenient ways are being looked for to measure target inhibition. Measuring farnesyl transferase activity in leucocytes and buccal scrapings, determination of EGFR phosphorylation status in skin biopsies and measurement of proagioagenic factors like vascular endothelial growth factor (VEGF) and fibroblast growth factor in plasma in

studies with angiogenesis inhibitors are examples of alternatives for target inhibition in tumor tissue [16-19]. While analyzing such an alternative target inhibition, it is important that a correlation exists between this inhibition and the inhibition of the target within the tumor itself. In addition there has to be a thorough knowledge about the relation between target inhibition and tumor progression. This can be illustrated with an example of FTIs: Since the activity of farnesylated Ras oncoproteins was found to be essential in tumor progression, specific inhibitors of the key enzyme farnesyl transferase have been developed. These FTIs indeed demonstrated antitumor activity in animal tumor models, but remarkably enough this growth inhibitory activity was largely independent of the presence of oncogenic Ras protein, and it has been suggested that inhibiting farnesylation of other proteins such as Rho B is perhaps more important for antitumor activity [20]. Therefore determining the presence of mutated Ras proteins in a tumor can be misleading. In addition it might show that measurement of the enzyme activity is inferior to measurement of downstream effector molecules since in cells these downstream effector molecules can be influenced by various signaling pathways. Obviously, implementing such measurements in a phase I trial requires optimal laboratory backup and it should be kept in mind that the number of patients needed to define the OBD generally exceeds the number of patients needed in a classical MTD based trial [5].

Consecutive measurements of serum tumor marker levels such as prostate specific antigen (PSA), carcinoembryonic antigen, CA 15.3, CA 19.9, and CA 125 can be used to assess drug-target interactions and serve as a surrogate endpoint. In a number of phase I/II studies with the MMPI marimastat, changes in tumor marker levels were used to assess tumor growth inhibition in relation to dose, since it was anticipated that marimastat would not cause tumor regressions [21]. The authors defined a dose for subsequent efficacy studies based upon the combination of tumor marker level change, toxicity data and pharmacokinetic analysis. However, it should be kept in mind that using the analysis of tumor marker levels to predict antitumor efficacy is not a validated method. Additionally, in the marimastat study mentioned the decision was also largely based upon a change in the slope of increase of the tumor marker, and such a change can also be observed in the natural history of the disease.

Advanced imaging techniques like dynamic enhanced Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) scanning are increasingly being used in oncology research. Apart from their use as imaging techniques assessing tumor mass, they can be used to study more functional aspects such as changes of tumor blood flow and tumor viability. In a study with the anti-VEGF antibody HuMV833 a clear reduction of tumor microvascular permeability was shown following treatment using contrast enhanced MRI techniques. No correlations with tumor response were given however [22]. In a phase II study using PET scanning in patients with gastrointestinal stromal tumors treated with STI571, a decrease of the standardized uptake value to < 2.0 between day 21 and 40 after start of treatment correlated with a tumor regression as confirmed with CT scans, while these objective tumor regression frequently

occurred after day 40 [23]. In a phase I study with OSI-774, a decreased fluorine-18 fluorodeoxyglucose (FDG) uptake was observed in a few patients. These patients however, were subsequently found to have tumor regressions [24]. Therefore both for dynamic MRI and PET it remains to be seen whether following treatment with a cytostatic agent decreases in viability and blood flow can be found in the absence of tumor regressions. Perhaps the observed changes only reflect tumor regression or have to be considered as early predictors of tumor regression.
The optimal use of cytostatic agents in oncology practice is expected to be in situations with low tumor volumes, e.g. in the adjuvant setting or in the setting of minimal residual disease following cytoreductive treatment in advanced disease. In both situations it is anticipated that these agents will have to be used for a prolonged period of time and therefore in the early clinical testing program special emphasis should be given to the description of chronic toxicity. This means that in future phase I trials with these agents preferably patients in good clinical condition or patients with tumors known to progress slowly should be enrolled in order to optimally assess chronic toxicity.

PHASE II/III STUDIES

Randomized phase III trials are necessary to investigate the efficacy of a new treatment in terms of improvement of cure, survival or quality of life or a combination of these. Since the improvements generally are small, large numbers of patients are needed to meet the statistic criteria of significance and likewise these trials are time, money and patient consuming. The main goal of phase II studies is to screen a new agent or a combination of agents for potential antitumor activity, with the purpose of selecting the most promising agents to enter the pivotal phase III trials. For phase II studies it is generally agreed upon to use the percentage of tumor regression as an endpoint, making the assumption that the occurrence of tumor regression correlates with patient benefit. Although defining a threshold percentage above which it is decided to enter a drug in phase III trials is somewhat arbitrary, depending amongst others on historical response rates with known regimens, this criterion has been shown valid by the fact that in recent decades numerous new agents have been registered following this strategy. But what to do with agents that are not expected to cause tumor regressions? Selecting these cytostatic agents to enter phase III trials is difficult, if possible at all. Although, theoretically, one can choose to directly jump from phase I to large randomized phase III studies, the large number of new agents, the limited number of patients and ethical considerations will definitely limit this practice. For the majority of agents therefore some kind of selection in smaller phase II trials is necessary. When tumor regression is not expected to occur, other endpoints are needed to establish clinical activity. Numerous alternative clinical endpoints have therefore been defined: TTP, number of patients progressing within a certain period of time, change in tumor marker levels, specific target inhibition, and the use of modern imaging techniques such as PET and dynamic enhanced MRI. None of these endpoints however, have been validated thus far.

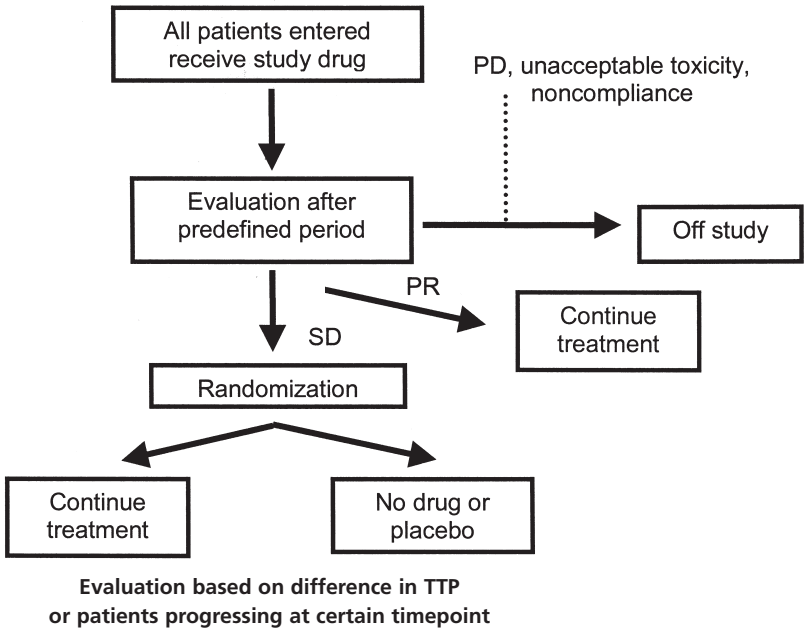

Figure 3. Randomized Discontinuation Design, Schematic Representation.

Abbreviations: SD, stable disease; PR, partial remission; PD, progressive disease; TTP, time to progression.

Using TTP as an endpoint is hampered by the fact that there is a lack of reliable historical data concerning the TTP, due to variability in patient characteristics, disease status, prior treatment and evaluation technique. In addition, determining TTP in a prospective study population will be influenced by the same confounding factors. A way to circumvent this problem is to use the patient as his own control by using a 'growth modulation index' [1,25]. Such an index is defined as the ratio of a patient's TTP in a current treatment program (TTP2) and the TTP in the previous treatment period (TTP1). The index can be considered as indicative of antitumor activity, if the value is above a certain threshold. While some authors suggest a threshold of 1.33 (a 33% increase of TTP), others suggest that even a value of 1.0 could be useful, since the natural history of the disease would normally result in a TTP2 being shorter than the TTP1. Although this growth modulation index seems attractive, many objections exist. Often it will be difficult to determine TTP in previous treatment periods, whereas the determination of the threshold value indicating antitumor activity is also highly debatable, as mentioned above.

Another approach to assess antitumor activity consists of introducing early progression as an endpoint in phase II studies [2,26]. Using the combination of tumor regression and early progression

facilitated early termination of trials with ineffective agents, as compared with conventional models using tumor regression only. It was suggested that by adaptation of the parameter with emphasis on the early progressions, this approach can be used in phase II studies with tumor types such as gliomas that are difficult to evaluate or in studies with cytostatic agents. This approach seems very interesting, but until now no studies with cytostatic agents based on this concept have been published.

To circumvent the problem of patient selection, defining the required TTP or acceptable percentage of early progressions, performing randomized phase II trials is an interesting alternative. By using an alpha error of 0.20, the required number of patients entering such trials can be kept relatively low [5]. Although in this situation 20% of agents tested could go on in large phase III trials while being inactive, the testing of large numbers of ineffective agents in phase III trials can be prevented. When performing randomized phase II studies, a new cytostatic agent can be compared to placebo or a cytostatic agent combined with a known chemotherapy regimen can be compared to chemotherapy alone. An example of this approach are the trials performed with the small molecule inhibitor of the VEGF receptor pathway SU5416 combined with 5-fluorouracil/leucovorin and cisplatin/gemcitabine as first line treatment in colon cancer and non-small cell lung cancer respectively. In both trials an interim analysis was performed after 80 patients had been followed for 12 weeks, after which the decision whether or not to proceed to a registrational phase III study was taken [27].

Another alternative is to perform a phase II trial according to the so-called randomized discontinuation design (Figure 3) [4]. In this design, all patients receive study drug for a predefined period of time, after which patients without progressive disease, unacceptable toxicity or noncompliance are randomized to either continue treatment or to receive no drug or placebo. Time to disease progression or a decrease in percentage of patients progressing at a certain time point is then used as an endpoint. Advantages of this design are the ease to accrue as all patients will receive active medication and increased efficiency since generally fewer patients will have to be randomized [28]. Major disadvantages, however, are differences in TTP observed that often cannot be generalized, the inability to quantify toxicity because of the selection and the impossibility to test potential curative treatments. In addition, although the number of randomized patients is relatively small, the total number of patients needed to really establish relevant clinical differences of activity frequently approaches that of a classic randomized phase III trial design.

From the above it is obvious that thus far no good alternatives for tumor regressions have been defined. The use of tumor marker levels has been mentioned before, but it has to be remembered that the use of tumor marker levels to define antitumor activity is not validated. For example, in the phase I-II studies with marimastat a biological effective dose was defined in various tumor types based upon changes in tumor marker levels. Subsequent phase III studies in gastric, pancreatic, non-small cell lung cancer and glioblastoma multiforme, however, failed

to show a substantial benefit, thereby indeed questioning this concept [29-32]. In some situations, however, observing changes in tumor marker levels can be acceptable. In hormone refractory prostate cancer phase II studies are difficult to carry out since most patients present with non-measurable disease. Since in these patients it was shown that the rate of change in PSA was a strong predictor of prostate cancer specific death, it is suggested that changes in PSA levels can be considered a valuable endpoint in trials [33]. Such studies should not be considered conclusive with respect to efficacy of a new drug, but they can be helpful in deciding whether a new drug is active enough to be tested in a large phase III trials. A placebo controlled randomized phase II study with an EGFR tyrosine kinase inhibitor using this surrogate endpoint of antitumor efficacy is ongoing in our center.

Measuring target inhibition will not be very helpful in phase II trials with cytostatic agents [5]. First, because measuring target inhibition is limited by the accessibility of the target as such, due to the requirement of additional patient selection criteria with respect to the presence of the target and due to the impossibility to perform repeated biopsies. Secondly, although inhibition of the biological target might be demonstrated in a certain number of patients, it is still not clear whether this will correspond with real clinical benefit and is enough evidence to enter the new agent into large randomized phase III trials. However, examining the presence of a target in pretreatment tumor biopsies qualitatively and quantitatively can be of use to correlate tumor response or TTP with the presence of that specific target. Knowledge of this correlation is of vital importance in the design of subsequent phase III studies and the future clinical use of the new agent. An example of this correlation is the use of the monoclonal antibody trastuzumab, which was found to be effective only in patients with HER-2/neu overexpressing breast cancer [34].

PET scanning has been found to be able to predict tumor regression. In a study with neoadjuvant chemotherapy in patients with operable esophageal cancer it was possible to predict a good pathological response with PET scanning using FDG uptake at baseline and 14 days after initiation of chemotherapy at the primary tumor side [35]. Also for a number of other tumor types small studies have shown that quantification of the change in FDG uptake may be an early marker of the tumoricidal effect of anticancer drugs [36]. All these studies however concern cytotoxic drugs. It is not yet clear what the additional value of PET scanning might be in early clinical studies with cytostatic agents.

RECOMMENDATIONS

In the recent years many new anticancer agents targeting various extranuclear processes involved in malignant transformation of cells and carcinogenesis have been developed. The majority of these agents is target specific and as such these agents are supposed to cause only minor toxicity while their effect is growth inhibitory. The relative paucity of adverse events and the lack tumor regressions seen in models has led to recommendations with regard to clinical

Table 1. Questions Important to Answer Before Trial Design

1. Serious toxicity expected based on preclinical data?
2. Tumor regressions expected based on preclinical data?
3. Is the target clear?
4. Does target inhibition correlate with tumor response?
5. Is it possible to measure the target inhibition in tumor samples or surrogate tissues?
6. Are there possible surrogate measurements correlating with target inhibition?

trial design with more emphasis being put on defining the OBD instead of the MTD and with alternative endpoints to replace tumor regression. This dichotomy is an oversimplification, since numerous of these so called cytostatic agents do cause tumor regression and/or cause serious side effects. Therefore a more tailor made approach is suggested, based on the answers to questions raised and summarized in Table 1.

For target specific anticancer agents showing tumor regression in preclinical models, the traditional phase I and II design could be followed. However, since toxicity is not necessarily dose limiting, it is important to include pharmacokinetic and pharmacodynamic endpoints in such a phase I study to allow for an optimal determination of the recommended dose for subsequent phase II studies. If however, serious toxicity is observed, the DLT and MTD should obviously be defined. The recommended dose based on either pharmacokinetic, pharmacodynamic or toxicity data can be subsequently used in phase II studies, which can be non-randomized if tumor regression is the major endpoint.

For essentially non-toxic target specific agents showing growth inhibitory activity in preclinial models, performing a conclusive phase I study strongly depends upon the ability to define the OBD. For this, pharmacokinetic and pharmacodynamic measurements demonstrating unequivocal target inhibition are a prerequisite. For target specific growth inhibitory agents with serious toxicity observed in preclinical models, describing DLT and defining MTD will remain important. In this situation PGDE is recommended to accelerate the phase I process and limit the number of patients treated at sub-therapeutic doses. In either case further phase II trials will have to be performed which by the lack of tumor regression and validated surrogate endpoints should preferentially be randomized. Finally, phase III trials will remain pivotal for the determination of clinical relevant patient benefit, determined by increased cure rates and /or survival.

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ABSTRACT

Purpose: *This phase I study was conducted to assess the tolerability, pharmacokinetics and antitumor activity of the oral, selective epidermal growth factor receptor tyrosine kinase inhibitor PKI166 in patients with advanced solid malignancies.*

Patients and methods: *PKI166 was first administered once daily continuously and in the second part of the study once daily for 2 weeks every 4 weeks to establish the maximum tolerated dose (MTD). Ten additional patients were studied at MTD to acquire additional safety information and characterize the effect of food intake on PKI166 pharmacokinetics. Pharmacokinetics of PKI166 were characterized after single and multiple doses at all dose levels.*

Results: *Fifty-four patients received a total of 116 28-day cycles of PKI166. Dose-limiting transaminase elevations were observed in two of seven and two of eight patients using 50 and 100 mg PKI166 continuously. In the second part with PKI166 once daily for 2 weeks every 4 weeks MTD was set at 750 mg. Dose-limiting toxicity consisted of diarrhea, skin rash and transaminase elevations. Pharmacokinetic analysis revealed fast absorption, a linear dose-response relationship without drug accumulation after multiple doses. At MTD, no significant influence of food intake on PKI166 pharmacokinetics was observed. Stable disease for more than two cycles was observed in 11 patients.*

Conclusion: *PKI166 given once daily for 2 weeks every 4 weeks is well tolerated with linear pharmacokinetics, compatible with once daily dosing, and without significant effect of food intake on absorption. The recommended dose for further studies is 750 mg once daily for 2 weeks every 4 weeks.*

CHAPTER 3

PHASE I AND PHARMACOLOGICAL STUDY OF PKI166, AN EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITOR, IN PATIENTS WITH ADVANCED SOLID MALIGNANCIES

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INTRODUCTION

Amplified epidermal growth factor receptor (EGFR) signaling is frequently found in human tumors and can be caused by various mechanisms [1,2]. As amplified EGFR signaling plays a role in carcinogenesis, inhibiting this process is a rational target for anticancer drug development. Inhibition of EGFR activity can be achieved by monoclonal antibodies that bind to the extracellular domain, antisense oligonucleotides interacting with mRNA to inhibit expression of EGRF, the use of ligands as carriers of cytotoxins and the use of small molecule inhibitors of tyrosine kinase activity [3]. A large number of these small molecule inhibitors have been synthesized and several have shown encouraging anticancer activity in both preclinical models and clinical studies [4-7]. Erlotinib, one of these small molecule tyrosine kinase inhibitors, has recently been approved by the Food and Drug Administration, for treatment of patients with locally advanced or metastasized non-small cell lung cancer after failure of at least one prior chemotherapy regimen.

PKI166 belongs to the pyrrolo-pyrimidine class of EGFR tyrosine kinase inhibitors and is active in the low nanomolar range, showing high selectivity against serine/threonine kinases and moderate selectivity against other tyrosine kinases (Figure 1). PKI166 shows potent antiproliferative effects in various EGFR-dependent and/or overexpressing cell lines, while inhibition of EGFR- independent cell lines is achieved at significantly higher concentrations. Potent growth inhibition is seen in several EGFR-dependent nude mouse tumor models following daily oral administration of doses between 10 and 100 mg/kg [8]. In the 253J B-V bladder tumor model producing abundant levels of basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and interleukine-8 it was shown that PKI166 was able to decrease the levels of bFGF and VEGF in the tumor microenvironment with 48 and 64% respectively [8]. This finding correlated with a significant decrease of blood vessel density within the tumor, suggesting that the activity of PKI166 may be mediated in part by inhibition of tumor angiogenesis. Following a single 100 mg/kg oral dose to A-431 human epidermoid carcinoma-bearing nude mice, it was shown that PKI166 was rapidly absorbed and produced complete inhibition of EGFR autophosphorylation in the tumor up to 8 hours post treatment, with > 50% inhibition being present after 24 hours [8]. Toxicology studies in mice and rats revealed that the maximum non-lethal single oral doses of PKI166 were > 2000 and 700 mg/kg, respectively [8]. In rats and dogs the no-adverse-effect-level following oral treatment for 4 consecutive weeks was 7.5 and 8.0 mg/kg/day, respectively [8]. PKI166 was shown to be extensively bound to plasma proteins and was metabolized by glucuronidation at the phenolic moiety, without signs of extensive oxidative metabolism. Excretion occurred mainly in the bile as a glucuronide [8].

We did a phase I and pharmacological study of orally given PKI166 in patients with advanced solid malignancies. The principal objectives of this study were to (a) determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of PKI166 given once daily, (b) charac-

terize safety and tolerability of PKI166 including acute and chronic toxicities, (c) characterize single and multiple dose pharmacokinetics of PKI166, (d) characterize the effect of food intake on PKI166 pharmacokinetics and (e) seek preliminary evidence of antitumor activity.

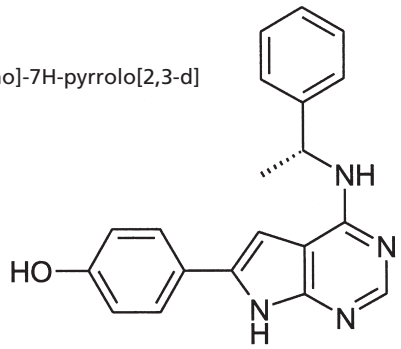
PATIENTS AND METHODS

Eligibility criteria Patients with a histologically confirmed diagnosis of an advanced solid malignancy for whom standard therapy options did not exist were eligible. Additional eligibility criteria included: age ≥18 years; World Health Organization performance status ≤ 2; an estimated life expectancy of ≥3 months; adequate bone marrow function (hemoglobin ≥ 6.2 mmol/L, granulocyte count › 1.5 x 10^9/L, platelet count › 100 x 10^9/L), hepatic function [bilirubin within normal limits, serum transaminases (alanine aminotransferase and aspartate aminotransferase) ‹ 2.5 times upper level of normal or ‹ 5 times in case of liver metastases] and renal function (creatinine within normal limits); no previous chemotherapy within 30 days (6 weeks for nitrosureas or mitomycin C); and no surgery within 2 weeks or radiotherapy within 3 weeks. Specific exclusion criteria included: impairment of gastrointestinal function that could significantly alter the absorption of PKI166; and the use of medication altering gastric pH (mild antacids were permitted if taken either 2 hours before or after drug administration). This study was approved by local ethics committees and all patients gave written informed consent.

Study design PKI166 was supplied by Novartis Pharma AG (Basel, Switzerland) as hard gelatin capsules, containing either 50 or 100 mg of the active study drug. The capsules were taken once daily in the morning at least 1 hour before breakfast without interruption. A treatment cycle was defined as 28 days of treatment. At days of pharmacokinetic evaluation

Figure 1. Chemical Structure of PKI166.

Product code:	PKI166 (CGP 75166)
Chemical name:	(R)-4-[4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d] pyrimidin-6-yl]-phenol
Molecular formula:	$C_{20}H_{18}N_4O$
Molecular weight:	330.39
Chemical structure:	



PKI166 was taken 2 hours before breakfast. The starting dose was 50 mg once daily, which corresponded with one-fifth of the toxic dose low in the rat, the most sensitive preclinical species. Cohorts of three patients were studied, with dose-doubling between cohorts until toxicity as defined by two episodes of National Cancer Institute Common Toxicity Criteria version 2.0 grade 2 toxicity or a single episode of DLT during the first cycle was observed. A Modified Continuous Reassessment Method was used thereafter for dose escalation decisions [9,10]. The MTD was defined as the highest dose level with no more than 25% of patients experiencing DLTs during the first cycle. DLT was defined as National Cancer Institute Common Toxicity Criteria ≥ grade 3 neutropenia, thrombocytopenia or anemia, and/or ≥ grade 3 non-hematological toxicity (excluding nausea responsive to anti-emetic treatment and alkaline phosphatase elevation), and/or certain grade 2 toxicities (i.e., neurotoxicity, cardiac or renal toxicity). Intrapatient dose escalation was not allowed. Due to the onset of unexpected grade 3 transaminase elevations, as well as indications of unexpected drug accumulation following continuous treatment, an alternative dosing regimen with PKI166 once daily for 2 weeks every 4 weeks was studied. For practical reasons related to pharmacokinetic evaluation patients used the study drug 15 days instead of 14 days during the first cycle only. In addition inclusion criteria for serum transaminases were changed to < 2.5 times upper limit of normal irrespective of the presence of liver metastases.

Pretreatment and follow-up studies Before therapy, a complete medical history was taken and a physical examination was done. A complete blood cell count, including WBC differential and serum biochemistry, which included sodium, potassium, chloride, bicarbonate, creatinine, albumin, total protein, serum transaminases, total bilirubin, calcium, phosphorus, glucose, and alkaline phosphatase were done, as were urinalysis, electrocardiogram and chest X-ray. Weekly evaluations during the first two cycles and every other week thereafter included history, physical examination, toxicity assessment, complete blood count including white blood cell differential and serum biochemistry. Tumor measurements were done every two 2 cycles. Response was assessed using the World Health Organization criteria [11]. Patients were allowed to continue treatment in the absence of progressive disease or unacceptable toxicity.

Pharmacokinetic sampling and data analysis For pharmacokinetic analyses, 5-mL blood samples were collected from an indwelling i.v. canula before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12 and 24 hours after administration of the drug on days 1 and 15 of the first cycle. Blood samples were collected in precooled lithium-heparin containing tubes and plasma was separated by centrifugation. Plasma samples were stored at –80 ^{0}C until analysis using a method involving liquid-liquid extraction and high-performance liquid chromatography with ultraviolet absorbance detection (320 nm). The lower limit of quantification is 10 ng/mL, when using 0.2 mL of plasma [12].

Noncompartmental analysis of the data was conducted using WINNONLIN (version 3.2). The pharmacokinetic variables area under the plasma concentration time curve (AUC) from 0 to 24 hours and 0 to infinity, peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}) of PKI166 and its major metabolite PKI166-glucuronide were reported as mean values ± SD. Apparent total body clearance (CL/F) was calculated by dividing the dose by the $AUC_{0\text{-}inf}$. The terminal elimination rate constant ß was estimated by linear regression from the terminal concentration-time data. The elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 by ß. The apparent volume of distribution (Vz/F) associated with the terminal phase was calculated by dividing CL/F by ß.

Pharmacodynamic sampling and assays For analysis of VEGF 5-mL blood samples were collected into precooled EDTA-containing tubes at baseline, immediately before dosing on days 1, 8, 15, 22 of cycle 1, days 1 and 15 of cycle 2, day 1 of further cycles and 10 hours after dosing on days 1 and 15 of cycle 1. After collection, samples were immediately cooled in an ice bath and plasma was separated by centrifugation within 30 minutes. Plasma samples were kept frozen at –80 °C until analysis. VEGF analysis was done in duplicate according to previously described methods [13].

To determine inhibition of EGFR tyrosine kinase activity in the dermis, punch biopsies were taken from the forearm skin at baseline and at days 14 and 28 of cycle 1 in the second part of the study with PKI166 once daily for 2 weeks every 4 weeks. Immediately after removal samples were placed in Tissue-Tek® (O.C.T. Compound, Sakura Finetek, Torrance, CA, USA) and stored at –80 °C. For determination of EGFR tyrosine kinase activity, frozen tissue sections were fixed in cold acetone (–20 °C) for 10 minutes, rinsed with PBS and incubated with a protein block consisting of 4% fish gelatin in PBS (Aurion, Electron Microscopy Sciences, Fort Washington, PA, USA) for 10 minutes. Excess solution was removed and the samples were incubated with mouse anti-phosphoEGFR (Chemicon, International, Inc.,Temecula, CA, USA; 1:100 dilution in 4% fish gelatin) and incubated overnight in a humidity chamber at 4 °C. The samples were washed with PBS 3x3 minutes and incubated with protein block for 10 minutes followed by a 1:400 dilution of Alexa 594 conjugated goat anti-mouse IgG (Molecular Probes, Inc., Eugene, Oregon, USA) for 60 minutes at room temperature. The samples were rinsed with PBS and coverslipped using a Vectashield fluorescence mounting media containing 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA) and examined in a Zeiss Axioplan microscope (Carl Zeiss, Inc., Thornwood, NY, USA), equipped with a 100-W lamp and a Hamamatsu 5810 CCD camera (Hamamatsu, Hamamatsu City, Japan). Fluorescence images were captured at the same settings to allow for comparison of fluorescence intensities. 4',6-Diamidino-2-phenylindole staining of nuclei was helpful in the identification of tissue architecture. Results are expressed in a qualitative way (negative, weakly positive, positive and strong positive for activated EFGR).

Hair follicle samples obtained at baseline, days 14 and 28 of cycle 1 to determine EGFR tyrosine kinase activity were collected from patients enrolled in the 600- and 750-mg cohorts. At each time point five hair follicles were obtained, placed in a 15-mL polyprophylene tube containing cold acetone and stored on ice for 30 minutes. Thereafter acetone was removed and 15 mL of cold Calcium and Magnesium free Dulbecco's PBS was added. After gently inverting the tube several times the Dulbecco's PBS was removed and new Dulbecco's PBS was added and this procedure was repeated. The hair follicles were stored in Dulbecco's PBS at 4 °C until shipment. To analyze quantitative changes in EGFR tyrosine kinase activity, automated fluorescence measurements using a Laser Scanning Cytometer (LSC, Compucyte, Cambridge, MA, USA) were done on hair follicle samples. Hair follicle samples were prepared as described above. Immediately following the second protein block, tissue samples were incubated with a 1:100 dilution of Cy5-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA) for 60 minutes at room temperature. The samples were rinsed with PBS and analyzed using a LSC equipped with an air-cooled 20 mW, 488-nm argon ion laser and a 5 mW 632-nm HENe laser. Cy5 was detected through a 650-longpass filter. The LSC was directed via a computer program to sample 800 regions (otherwise known as phantom contours) within one hair follicle sample. Integrated fluorescence (Cy5 Integral) as well as fluorescence data for individual pixels within the phantom contour were collected by the LSC and expressed as fluorescence signal (Max Pixel). Data were acquired and analyzed with Wincyte acquisition software (Compucyte) and expressed quantitatively.

Food effect evaluation The effect of food intake on $AUC_{0\text{-}24h}$, C_{max} and T_{max} was analyzed in 10 additional patients at MTD. For the fasted/fed analysis, five patients swallowed PKI166 in a fasted state on day 1, and were not allowed to eat or drink for 2 hours after ingestion. On the second day of treatment, patients swallowed PKI166 within 30 minutes of a standardized breakfast. The other five patients had the reverse sequence. The standardized breakfast contained 550 calories and approximately 30 g of fat. The sequence of intake fasted or fed was determined by a predefined randomization schedule. For pharmacokinetic analysis, 5-mL blood samples were collected in precooled lithium-heparin-containing tubes from an indwelling i.v. canula, on days 1 and 2 at the same time points as described in the section pharmacokinetic sampling and data analysis.

RESULTS

Fifty-four patients, whose characteristics are summarized in Table 1, received a total of 116 cycles of PKI166. The number of patients and cycles given as a function of schedule and dose are listed in Table 2.

With PKI166 given once daily continuously, dose-limiting grade 3 transaminase elevations were observed in two of seven and two of eight patients in the 50- and 100-mg cohort, respectively. In

Table 1. Patient Characteristics

Patient Characteristic	**No. of Patients**	
	PKI166 Administration Regimen	
	Continuous	**2 Weeks Every 4 Weeks**
Male / Female	9/6	26/13
Age (years)		
Median	52	55
Range	40-67	28-79
Prior chemo/immuno/hormonal therapy		
1-3 prior regimens	8	28
> 4 prior regimens	6	8
Prior radiotherapy	8	17
Prior radiotherapy and chemotherapy	7	14
Tumor type		
Esophageal carcinoma	1	4
Colorectal carcinoma	2	7
Ovarian carcinoma	0	3
Non-small cell lung cancer	2	2
Breast carcinoma	4	3
Melanoma	2	0
Sarcoma	0	6
Renal cell carcinoma	0	6
Adenoid cystic carcinoma	2	1
Hepatocellular carcinoma	0	2
Mesothelioma	1	2
Miscellaneous	1	3

Table 2. Dose Escalation Scheme

Administration Regimen	**Dose Level (mg)**	**No. of Patients**	**Total no. of Cycles**	**Median no. Cycles (Range)**	**No. of Patients With DLT in Cycle 1 or 2**
PKI166 Once Daily Continuously					
	50	7	10	1 (1-3)	2
	100	8	16	2 (1-7)	2
PKI166 Once Daily for 2 Weeks Every 4 Weeks					
	50	9	20	2 (1-5)	2
	200	3	4	2 (1-2)	1
	400	3	4	1 (1-2)	0
	600	6	16	2 (2-5)	0
	750	13	38	2 (1-8)	1
	900	5	8	2 (1-2)	3

Table 3. Principal PKI166-Related Side Effects

Side Effect	PKI166 Administration Regimen, Dose (mg/day), No. of Patients															
	Continuously				2 Weeks Every 4 Weeks											
	50 (n=7)#		100 (n=8)		50 (n=9)		200 (n=3)		400 (n=3)		600 (n=6)		750 (n=13)		900 (n=5)	
Grade	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4
Anorexia	2	0	1	0	0	0	0	0	1	0	1	0	1	0	2	0
Nausea	5	0	4	0	3	0	2	1	2	0	2	1	7	0	4	0
Vomiting	2	0	3	0	0	0	2	0	1	0	2	1	6	0	3	0
Diarrhea	3	0	2	0	1	0	0	0	1	0	5	0	11	0	3	1
Abd pain	1	0	0	0	2	0	0	0	0	0	0	0	3	0	0	0
Rash	0	0	1	0	1	0	1	0	2	0	3	0	5	0	1	1
ALT	0	1	1	2	1	2	0	0	0	0	0	1	3	1	0	1
AST	0	2	3	0	3	0	0	0	0	0	1	0	4	0	1	0
Fatigue	1	1	3	0	4	0	0	0	0	0	1	0	1	0	2	1
Dry mouth	0	0	0	0	0	0	0	0	0	0	1	0	2	0	2	0
Myalgia	0	0	0	0	1	0	1	0	0	0	1	0	3	1	0	0

Abbreviations: n, number; Abd pain, abdominal pain; ALT, alanine aminotransferase; AST aspartate aminotransferase.

the second part of the study with PKI166 given once daily for 2 weeks every 4 weeks dose levels studied were 50, 200, 400, 600, 750 and 900 mg. In the 900-mg cohort three of five patients experienced DLT during the first cycle, consisting of grade 3 skin rash (one patient), grade 3 transaminase elevation (one patient) and grade 3 diarrhea (one patient). Three additional patients were treated at the next lower dose level of 600 mg without experiencing DLT and therefore, an intermediate dose level of 750 mg was explored. Only 1 of 13 patients treated at this dose level experienced DLT during the first two cycles, and therefore 750 mg was set to be the MTD.

Toxicity Occurrence of side effects, as a function of the schedule and dose is listed in Table 3. Transaminase elevations, diarrhea, skin rash, nausea and vomiting were the principal toxicities of PKI166. No hematologic toxicity was observed.

Dose-limiting transaminase elevations were observed in both the 50- and 100-mg dose cohorts of the first part of the study with the continuous use of PKI166. The transaminase elevations rapidly normalized after discontinuation of the study drug in three of the four patients. In the fourth patient progression of liver metastases contributed to the lack of normalization. In the second part of the study with the use of PKI166 for 2 weeks every 4 weeks, two of nine patients in the first cohort of 50 mg had reversible grade 3 transaminase elevations, with maximum values occurring in week 3 or 4. One of these patients continued PKI166 in the same dose and developed grade 2 transaminase elevations in the second cycle, while in the third and fourth cycle no transaminase elevations were recorded. In one patient in the 600-mg cohort, grade 3 transaminase elevations occurred in the fourth cycle. After returning to grade 1, PKI166 dose was reduced to 400 mg, but again transaminase elevations occurred. In the 900-mg cohort one patient had grade 3 transaminase elevation in the first cycle. Apart from the grade 3 transaminase elevations, mild transaminase elevations were seen in several other patients. In the dosing cohorts with PKI166 for 2 weeks every 4 weeks no relationship was found between the dose of PKI166 and the occurrence of transaminase elevations. Transaminase elevations sometimes occurred in the 2 weeks period without PKI166 and frequently did not worsen in time with ongoing treatment and sometimes even improved with ongoing treatment. No bilirubin elevations were observed.

Diarrhea was frequently observed in both the continuous and 2 weeks every 4 weeks regimen. The diarrhea was generally mild and easily manageable with loperamide. In addition, in patients with PKI166 for 2 weeks every 4 weeks the diarrhea was often self-limiting, with spontaneous recovery in the obligatory period of 2 weeks without study drug. Grade 3 diarrhea was only seen in one patient in the 900-mg cohort and diminished with adequate loperamide use in subsequent cycles.

Mild and transient cutaneous toxicity manifested as either dry skin, folliculitis or skin rash was frequently observed. Skin rash was the most common manifestation and was usually located in the face and the trunk. One patient in the 900-mg cohort experienced a reversible grade 3 skin rash which was painful and itching, covering neck, chest, back, abdomen and buttocks and appeared from day 5 onwards. A skin biopsy revealed the classical picture of a toxic dermatitis with a perivascular lymphocytic infiltrate with several eosinophilic and polymorph nuclear cells. The rash almost completely disappeared in the 2 weeks off study drug. In the second cycle using 600 mg PKI166, the skin rash reappeared, but did not exceed grade 2.

Other frequently observed toxicities were mild nausea and vomiting. These toxicities were not related to schedule or dose and could be treated effectively with antiemetics such as metoclopramide or domperidone. In those patients who used the PKI166 for 2 weeks every 4 weeks nausea and vomiting decreased in severity or disappeared rapidly in the period without study drug. One patient had grade 3 nausea and vomiting in the third cycle which was the reason

Table 4. Pharmacokinetic Parameters of PKI166 (Mean ± SD)

Dose (mg)		Day 1						
	No. of Patients	AUC_{0-24}# (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h)	Vz/F (L)	
Continuously								
50	7	304 ± 121	59 ± 27	1.2 ± 0.9	5.9 ± 2.3	180 ± 88	1468 ± 855	
100	8	1126 ± 697	170 ± 116	1.6 ± 0.7	8.9 ± 1.3	104 ± 61	1287 ± 676	
2 Weeks Every 4 Weeks								
50	8	443 ± 216	72 ± 41	1.6 ± 0.8	6.9 ± 3.1	131 ± 65	1222 ± 743	
200	3	2628 ± 180	443 ± 220	1.7 ± 0.8	9.1 ± 1.2	64 ± 7	841 ± 45	
400	3	3662 ± 3128	430 ± 249	5.0 ± 2.7	6.6 ± 2.8	206 ± 210	2448 ± 3245	
600	5	13356 ± 9686	1446 ± 876	2.2 ± 0.6	11.8 ± 3.1	54 ± 43	1025 ± 1050	
750	7	12023 ± 6763	1396 ± 908	3.5 ± 2.5	10.9 ± 3.3	70 ± 53	1328 ± 1418	
900	4	20238 ± 3956	2354 ± 251	3.0 ± 0.8	10 ± 1.4	37 ± 9	522 ± 104	
		Day15						
	No. of Patients	AUC_{0-24} (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h)	Vz/F (L)	R§
Continuously								
50	7	613 ± 244	92 ± 54	2.2 ± 1.8	12.1 ± 6.7	96 ± 45	NA	1.6
100	6	2853 ± 1392	324 ± 210	2.2 ± 1.1	11.4 ± 2.7	42 ± 19	NA	1.9
2 Weeks Every 4 Weeks								
50	3	560 ± 331	62 ± 40	1.0 ± 0.0	19.3 ± 17.8	128 ± 101	NA	0.9
200	2	3334 ± 331	273 ± 116	4.6 ± 5.1	NA	60 ± 6	NA	0.6
400	3	3555 ± 2433	403 ± 281	4.1 ± 1.8	7.2 ± 5.2	197 ± 194	NA	0.9
600	5	11028 ± 4103	1128 ± 405	2.6 ± 1.0	60.2 ± 20	60 ± 20	NA	0.8
750	7	13727 ± 7725	1319 ± 399	1.9 ± 1.2	16.1 ± 6.7	58 ± 14	NA	0.9
900	4	18853 ± 8688	2159 ± 1149	2.4 ± 1.2	16.2 ± 9.4	58 ± 32	NA	0.9

Abbreviations: AUC_{0-24}, area under plasma concentration-time curve from time 0 to 24 hours; C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; $t_{1/2}$, elimination half-life; CL/F, apparent clearance; Vz/F, apparent volume of distribution; NA, not available.
§ R; C_{max} day 15/C_{max} day 1.

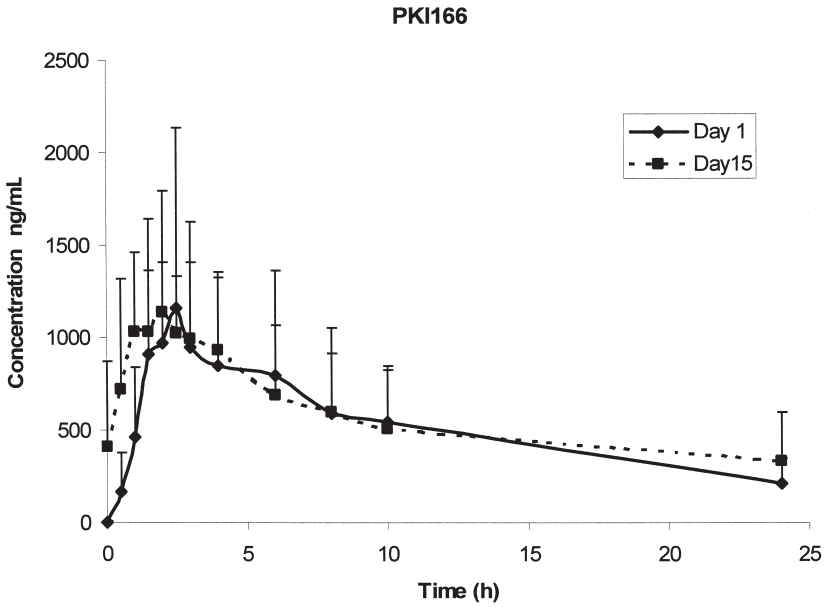


Figure 2. Plasma Concentration-Time Profiles (Mean ± SD) of PKI166 After Administration of 750 mg on Days 1 and 15 of Cycle 1 (Seven Patients).

to discontinue the PKI166 on day 12 of cycle 4. The nausea and vomiting coincided with and were probably caused by concomitant tramadol use.

Pharmacokinetics The pharmacokinetic variables are summarized in Table 4 (data of metabolite PKI166-glucuronide not shown). Representative plasma concentration-time profiles of PKI166 at the MTD of 750 mg for 2 weeks every 4 weeks on days 1 and 15 are shown in Figure 2. The plasma concentration-time profiles suggest a multiphasic decline consistent with preclinical findings. The T_{max} after oral administration was approximately 2 hours indicating fast absorption. PKI166 undergoes metabolism by direct glucuronidation by UDP-glucuronosyl transferase at the phenolic moiety to form the PKI166-glucuronide metabolite. Drugs that undergo metabolism by glucuronosyl transferase have been shown to undergo enterohepatic recirculation. This process was also observed for PKI166 as evidenced by the appearance of a secondary peak in plasma concentration in a number of patients. Both AUC and C_{max} showed a dose-dependent increase on days 1 and 15. For PKI166, with a $t_{1/2}$ of approximately 12 hours, the variables on day 15 would be indicative of steady-state pharmacokinetics. The $AUC_{0\text{-}inf}$ following the first dose was similar to or slightly higher than the $AUC_{0\text{-}24h}$ on day 15, indeed showing that steady state was achieved. A similar pattern was observed for C_{max}. There was a high degree of variability in individual pharmacokinetic parameters with a coefficient of variation in AUC and C_{max} of 50-60%. The C_{max} at steady state is a good indicator of accumulation. If the

C_{max} at steady state is comparable to that of the first dose, there is no drug accumulation, but if C_{max} at steady state is much higher than that of the first dose there is significant accumulation. As shown in Table 4, there is no significant accumulation following multiple doses of PKI166 (R value of approximately 1-2). The pharmacokinetic behaviour of the glucuronide metabolite of PKI166 was very similar to the parent molecule.

Food effect evaluation For the food effect evaluation, pharmacokinetic data were available from nine patients. In four patients pharmacokinetic data were obtained on day 1 (fasted) and on day 2 (fed). In the other five patients the reverse sequence was followed. Since pharmacokinetic data showed that no drug accumulation occurred at day 15, the pharmacokinetic data of the fasted and fed state of both groups were analyzed together. The results for both fasted and fed state are for T_{max} = 2.22 ± 0.62 and 3.30 ± 0.94 hours (P = 0.02), for C_{max} = 1,419 ± 800 and 1,303 ± 837 ng/mL (P = 0.64), and for $AUC_{0\text{-}24h}$ = 11,433 ± 6,530 and 11,135 ± 7,659 ng h/mL (P = 0.84, two-sided paired Student's t tests), respectively. These results suggest that the intake of food only has a significant (but clinically not likely relevant) effect on the absorption rate of PKI166 without significantly influencing peak plasma concentrations or total drug exposure.

Pharmacodynamics There were no significant changes in serum VEGF levels in response to PKI166 treatment (data not shown). Paired skin biopsy samples of days 1 and 14 or from days 1, 14 and 28 were available for 30 patients. In nine of these patients no significant changes in EGFR phosphorylation status were observed. In the other 21 patients the changes observed were relatively minor and appeared random in nature without clear correlation with the dose. In nine patients a decrease of EGFR phosphorylation was observed on day 14. Of these nine patients with a decrease of activity on day 14, an increase was observed in five patients and a further decrease in two patients on day 28. In six patients a decrease of EGFR phosphorylation from baseline was observed on day 28. In the remaining six patients an increase of EGFR phosphorylation was observed either after 14 days or 28 days. Paired hair follicle samples for analysis by LSC-automated fluorescence were collected from two patients in the 600-mg dose cohort and eight patients in the 750-mg dose cohort once daily for 2 weeks every 4 weeks. Paired samples of days 1 and 14 and days 1, 14 and 28 were available for 10 and six patients respectively. The median change of fluorescence intensity from baseline to day 14 was –29.3% (range, –58.9 to 25.4%). On day 28 the median change from baseline was –28.7% (range, –73.1 to 7.95%). In general, patients who experienced a decrease in EGFR phosphorylation maintained the decrease through day 28. Exploratory analysis revealed no relationship between pharmacodynamic markers and one of the pharmacokinetic parameters AUC, C_{max} or $t_{1/2}$.

Antitumor activity There were no tumor responses observed. Stable disease lasting more than two cycles was seen in eleven 11 patients (two in the continuous dosing regimen and nine in the intermittent dosing regimen). The median number of cycles in these patients was 4 (range 3-8). There was no apparent relationship between the occurrences of stable diseases lasting more than two cycles and the dose, although five out of thirteen patients from the 750-mg cohort for 2 weeks every 4 weeks were on study for more than two cycles (range 3–8).

DISCUSSION

This phase I study was initially designed to evaluate the feasibility of oral administration of PKI166 in a continuous daily regimen. However, due to the occurrence of grade 3 transaminase elevations in a significant number of patients in the first two dosing cohorts, it was decided to study an alternative dosing regimen with PKI166 given once daily for 2 weeks every 4 weeks. This change of regimen was also supported by preliminary pharmacokinetic data suggesting drug accumulation. With this regimen of PKI166 for 2 weeks every 4 weeks, at the recommended phase II dose of 750 mg the major toxicities were generally mild and consisted of transaminase elevations, diarrhea, cutaneous toxicity, nausea and vomiting. The diarrhea and cutaneous toxicity are also frequently observed in other early clinical studies with small molecule inhibitors and monoclonal antibodies of EGFR [4-6]. It is suggested that these toxicities have a common underlying mechanism of action, related to EGFR inhibition. The exact pathogenesis of these toxicities is still largely unknown. The cutaneous toxicity is characterized by a rash, predominantly on the face and trunk, while with other small molecule inhibitors of the EGFR frequently an acneiform drug eruption is described. Histopathological examination in one patient showed an allergic skin reaction with eosinophylic and polymorph nuclear cell infiltrations of the dermis. The rash frequently is only mild or modest in severity and in the intermittent dosing regimen often self-limiting. There is no clear relationship with dose however, which suggests a possible allergic nature of the skin reaction. The diarrhea observed was mild and easily managed with loperamide, on an as-needed basis. After discontinuation of the study drug a quick recovery of diarrhea was observed. It is suggested that epithelial cells of the gastrointestinal tract contain large number of EGFRs important for maintaining integrity of the intestinal mucosa. Inhibition of these EGFRs could result in damaging of the mucosa resulting in diarrhea. In addition PKI166-glucuronide the major metabolite of PKI166 is secreted mainly through the bile. Although the glucuronide is not active in inhibiting the EGFR, deconjugation of PKI166-glucuronide could result in free PKI166 causing EGFR inhibition of the intestinal mucosa. The fact that the diarrhea is dose independent suggests that the PKI166-glucuronide is not responsible for the diarrhea, but individual differences in the deconjugation could be. This of course is hypothetical and would require additional investigations (e.g. measurements of fecal excretion of the study drug and metabolites). The other major toxicity occurring with PKI166 is the transaminase elevation. Although in general the elevations were quickly reversible with discontinuation of the study drug, in a number of patients the transaminase elevations

peaked in the 2-week period without study drug. Compared to other EGFR tyrosine kinase inhibitors the hepatotoxicity is not unique, but the frequency of the hepatotoxicity seems higher. Remarkably enough in a phase I study with OSI-774, mild to moderate hyperbilirubinemia was frequently observed without accompanying transaminase elevations [6].

Two other studies are evaluating PKI166 given once daily continuously and thrice weekly (Monday, Wednesday and Friday) [14,15]. Preliminary results from these studies support the results from this study, with PKI166 being well tolerated with the majority of the adverse events being mild. In the once-daily regimen, the most frequently observed toxicities included rash, diarrhea and reversible transaminase elevations [14]. MTD has been defined at 450 mg, with two of four patients experiencing DLTs during the first cycle at the 600-mg cohort, including acute renal failure, fatigue, anorexia and decrease in performance status. In the Monday, Wednesday, Friday regimen, grade 3 transaminase elevations were observed in 2 out of 10 patients at the 400-mg dose level [15].

The pharmacokinetic profile revealed that PKI166 is orally bioavailable, quickly absorbed and suitable for once daily dosing. Although in the first two dose levels of the continuous dosing regimen drug accumulation was suggested, in the 2-weeks-every-4-weeks regimen this could not be demonstrated. A dose proportional increase of drug exposure was observed and at the recommended phase II dose of 750 mg daily for 2 weeks every 4 weeks, peak plasma levels were achieved that were thousand fold higher than the concentrations required to inhibit both in vitro EGFR phosphorylation and cellular proliferation assays. In addition, our food effect study clearly showed no influence of food intake on drug exposure.

Information obtained from the skin biopsy was not conclusive with regard to the inhibition of activated EGFR by PKI166. Although a decrease of activated EGFR was observed in a significant number of patients, in 6 of these 15 patients the decrease was only observed at day 28 after a 2-week period without PKI166. In addition in six patients an increase of activated EGFR was observed while using PKI166. The question is whether these inconsistent results reflects the inability of PKI166 to inhibit EGFR activity at the dose levels studied or whether the skin biopsy assay we used is not sensitive enough to detect such inhibition. Since the peak PKI166 plasma concentrations reached at MTD are at least thousand fold higher than the concentration IC50 of the EGFR tyrosine kinase activity as determined in preclinical models, the former is not very likely. In addition, the results of the quantitative hair follicle analysis performed at the higher dose levels suggest that PKI166 at MTD is actually able to inhibit EGFR phosphorylation at least to some extent. Although inhibition of EGFR phosphorylation has been studied in punch biopsies of the skin and hair follicles, other, at present time, more consistent parameters such as p44/42 mitogen-activated protein kinase (MAPK) activation, Ki67 proliferation index or induction of cyclin-dependent kinase 2 inhibitor p27KIP1 have not been evaluated, since the information now available was not available at the time of the study [16,17]. Thus, whereas we can state that administering a maximum orally daily dose of 750 mg for 2 weeks

every 4 weeks is feasible, we do not know if this dose corresponds to an optimal biological dose. Further studies are warranted to determine whether significant EGFR pathway inhibition can be achieved with PKI166, and whether this reflects clinical response or disease stabilization.
In our study no formal tumor responses were observed among the 54 patients included. Eleven patients, however, had stable disease for more than two cycles during PKI166 therapy at doses ranging from 50 to 750 mg.
In conclusion PKI166 is a novel agent belonging to the pyrrolo-pyrimidine class of EGFR tyrosine kinase inhibitors. PKI166 given once daily for 2 weeks every 4 weeks is well tolerated, with linear pharmacokinetics and without significant effect of food intake on absorption and at the MTD of 750 mg achieves biologically relevant plasma concentrations. The recommended dose of this schedule is set at 750 mg once daily.

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ABSTRACT

Purpose: *BMS-214662 is a potent and selective inhibitor of the farnesyl transferase enzyme with in vitro and in vivo antitumor activity. The aims of this study were to characterize the toxicities and to determine the pharmacokinetic profiles of BMS-214662 when administered in combination with cisplatin, and to determine the constitutive farnesyltransferase activity as a surrogate pharmacodynamic endpoint.*

Patients and methods: *Twenty-nine patients with advanced solid malignancy, refractory to conventional therapy, and with adequate hematologic, renal and hepatic function were treated with escalating doses of BMS-214662 administered as a 1-hour infusion, followed after an interval of 30 minutes by 75 mg/m^2 cisplatin administered as a 4-hour infusion and repeated every 21 days. Blood and urine samples for pharmacokinetic and pharmacodynamic analyses were collected during the first cycle of treatment only.*

Results: *Dose-limiting toxicities occurred in 4 of 9 patients enrolled at the 225 mg/m^2 BMS-214662 dose cohort, and included elevation of hepatic transaminases, nausea, vomiting, diarrhea, and renal failure. There was no apparent pharmacokinetic interaction between the two drugs at the recommended dose levels, and a dose-dependent inhibition of farnesyltransferase activity was observed, which returned to control levels within 24 hours of drug administration. There were no objective responses, but disease stabilization was observed in 15 patients, including 4 patients with stable disease after 6 cycles of treatment.*

Conclusion: *A dose of 200 mg/m^2 of BMS-214662 administered as a 1-hour infusion with 75 mg/m^2 cisplatin over 4 hours is the recommended dose for additional studies.*

CHAPTER 4

A PHASE I PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF THE FARNESYL TRANSFERASE INHIBITOR BMS-214662 IN COMBINATION WITH CISPLATIN IN PATIENTS WITH ADVANCED SOLID TUMORS

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INTRODUCTION

Proto-oncogenes, which result in overexpression or aberrant function of their encoded proteins, represent an attractive molecular target for the development of novel anticancer therapies. Activating mutations of the ras genes are among the most common genetic aberrations known in human cancers, particularly in pancreatic and colon carcinomas [1]. The proteins encoded by the ras genes localize to the inner plasma membrane and play a critical role in intracellular signaling by functioning as a molecular switch, alternating between inactive guanosine 5/-diphosphate (Ras.GDP) and active guanosine 5/-triphosphate (Ras.GTP) bound forms in a highly regulated manner [2,3]. Ras.GTP activates several downstream effector pathways mediating cellular proliferation, cellular adhesion and apoptosis transmitting a variety of extracellular signals from the cell surface, including growth factors and cytokines [4]. Farnesylation of Ras proteins is required for their membrane association which, in turn, is critical for their biological functions [5]. Inhibition of this step alone may be sufficient to abrogate the cell signaling and transforming function of constitutively activated Ras in tumor cells. Therefore, farnesyltransferase (FT), the enzyme that catalyzes this reaction [6], has become an interesting target for the design of novel anticancer agents.

Inhibitors of FT were originally regarded as specific and sensitive inhibitors of Ras-mediated cellular proliferation [5,7]. However it has become apparent that the critical target of FT inhibitors (FTIs) may not be Ras proteins or may include other polypeptides in addition to Ras [8-10]. More than 100 proteins have been identified that possess a "CAAX" sequence that can potentially be farnesylated [10], and up to 20 of these have been shown to undergo farnesylation including rho B, lamins A and B, transducin and CENP-E and CENP-F. Currently, at least 3 proteins have been identified, inhibition of which may be implicated in the cytotoxic actions of FTIs, and these include rho B, which regulates cytoskeleton organization [11], the centromeric proteins CENP-E and CENP-F, which interact with microtubules [12], and proteins associated with the phosphoinositide 3-OH kinase AKT pathway [13]. Thus, the molecular targets of FTIs remain unclear, but are likely to include several key proteins and possibly include some or all of the Ras isoforms.

BMS-214662 is an imidazole-containing tetrahydrobenzodiazepine [14]. It is a potent and selective FTI, active in the low nanomolar range in vitro and with good cytotoxic potency and selectivity against a number of human tumor cell lines including colon, breast, ovarian, prostate and squamous cell carcinomas [15]. Furthermore BMS- 214662 can inhibit FT and H-Ras processing and induce tumor responses in in vivo mouse models [15]. However, no clear correlation was observed between ras mutation status and sensitivity of the tumors to BMS-214662.

The potentially wide therapeutic index of FTIs with low toxicity and relatively low risk of myelosuppression raises the possibility that these agents can be safely combined with conventional cytotoxic agents [16,17]. Cisplatin has a broad spectrum of anti-tumor activity in a variety of solid tumors including testicular, lung and ovarian cancer [18]. Cisplatin-induced

cell death is primarily due to apoptosis [19] and cisplatin resistance is associated with defects in the induction of apoptosis [20]. Cells transfected with mutant ras genes, in particular RV-H ras, exhibit increased resistance to cisplatin [21-23] potentially as a result of ras-induced activator protein transcription factor leading to elevated expression of genes known to be involved in conferring cisplatin resistance [24,25]. Furthermore, there is some evidence that transfection with H-ras confers increased resistance to cisplatin whereas transfection with K-ras does not [26]. In vitro evidence suggests that the combination of an FTI with cisplatin is synergistic [27]. FTIs have been associated with increased apoptosis, and decreased DNA synthesis in animal tumors, leading to enhanced in vivo efficacy when combined with various cytotoxic agents including cyclophosphamide, 5-fluorouracil and vincristine [28]. Therefore the combination of BMS-214662 and cisplatin could potentially be more active than either agent alone.
Consequently, a phase I study was initiated with BMS-214662 administered i.v. over 1 hour in combination with 75 mg/m^2 cisplatin administered as a 4-hour infusion once every three weeks. The dose and schedule of cisplatin is that which is most frequently used in Europe either as a single agent or in combination chemotherapy regimens. The aims of this study were to characterize the toxicities of BMS-214662 when administered as a 1-hour i.v. infusion in combination with 75 mg/m^2 cisplatin administered as a 4-hour infusion both given once every three weeks and to determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT) and recommended dose for subsequent phase II studies. In addition, the pharmacokinetic (PK) profiles of BMS-214662 and cisplatin were determined and constitutive FT activity in peripheral blood mononuclear cells (PBMNs) was assessed as a surrogate pharmacodynamic (PD) end point.

PATIENTS AND METHODS

Patients and eligibility criteria This was a non-randomized, open label, phase I, dose escalating study performed at the Beatson Oncology Centre (Glasgow, United Kingdom) and at the Erasmus MC, University Medical Center (Rotterdam, The Netherlands). The study was approved by the Local Research Ethics Committee at each of the participating institutions, and all of the patients gave written, informed consent.
All patients entered into this study had an advanced solid malignancy, verified by histology or cytology, refractory to conventional therapy or for which there was no effective treatment other than single agent cisplatin. Eligible patients were those with a life expectancy of at least 3 months; age $>$ 18 years; WHO performance status 0 - 2; no chemotherapy, immunotherapy, or radiotherapy (involving $\geq$ 25% of hematopoietic reserves) within 4 weeks of entering the study (6 weeks for nitrosoureas and mitomycin C); no drugs known to be substrates of cytochrome P450-3A4 for 7 days prior to protocol therapy; adequate hematological (absolute neutrophil count $\geq$ 1.5 x 10^9/L, platelets $\geq$ 100 x 10^9/L), hepatic (total bilirubin $\leq$ 1.5 mg/dl, alanine aminotransferase and aspartate aminotransferase $\leq$ 2.5 x upper limit of normal) and renal function (creatinine clearance $\geq$ 60 ml/min as calculated by the Cockcroft and Gault formula). Patients with calculated creatinine clearance $<$ 60 ml/min were included if on undergoing an

EDTA Cr^{51} assessment their creatinine clearance was ≥ 60 mL/min. Patients with intolerable toxicity to prior cisplatin therapy, > 3 prior chemotherapy regimens for metastatic disease, peripheral neuropathy ≥ grade 2 (National Cancer Institute Common Toxicity Criteria, version 2.0), symptomatic pulmonary or cardiac disease, recent history of myocardial infarction, cardiac arrhythmia, second or third degree heart block, QT_c interval > 450 ms on electrocardiogram or inability to discontinue therapy with drugs known to prolong the QT interval, were excluded. Patients with cerebral metastases or uncontrolled infection were also excluded.

Treatment administration Pretreatment evaluation included a complete history and clinical examination, full blood count, biochemical profile, creatinine clearance (Cockcroft Gault or EDTA Cr^{51} determination), urinalysis, chest X-ray, electrocardiogram and pregnancy test (either serum or urine). Relevant radiological studies to evaluate sites of disease were performed up to 4 weeks before starting chemotherapy.

BMS-214662 was supplied in glass vials as the methanesulfonic acid salt (20 mg/mL as the Free base) and was diluted for use with 5% dextrose for injection, United States Pharmacopeia, to concentrations between 0.2 mg/mL and 2.5 mg/mL. The dilutions were stable when stored in polyvinylchloride i.v. bags, at room temperature, for up to 24 hours. BMS-214662 was administered through a PVC giving set as a 1-hour i.v. infusion followed after an interval of 30 minutes by cisplatin administered as a 4-hour i.v. infusion, both given every 21 days. Cisplatin was administered in 1000 mL of 0.9% saline or 250 mL of 3.0% saline depending on local use. Prehydration (1000-1500mL of 0.9% saline or 5% dextrose over 3 hours) was administered before the BMS-214662 infusion and patients received posthydration after the cisplatin infusion (1500-3000ml of 0.9% saline or 5% dextrose over 3-13 hours) with appropriate electrolyte supplementation. All patients received antiemetic premedication with either granisetron (3 mg) or ondansetron (8 mg) given as a 15-minute i.v. infusion in 100 mL of 0.9% saline 30 minutes before administration of BMS-214662 and also received dexamethasone 8 mg administered as a 15-minute i.v. infusion in 100 mL of 0.9% saline after completion of the BMS-214662 infusion and before cisplatin administration. Preclinical studies suggest that BMS-214662 may prolong the QT interval. Consequently, 12-lead electrocardiograms were obtained predose, between 5 and 30 minutes (to correspond with the time of expected maximum plasma drug concentration), and 24 hours after completion of the BMS-214662 infusion.

On the basis of previous studies of single-agent BMS-214662 administered as a 1-hour infusion on a 3 weekly schedule, a starting dose 126 mg/m^2 of BMS-214662 was chosen combined with a fixed dose of cisplatin 75 mg/m^2 [29]. The dose of BMS-214662 was escalated in subsequent cohorts according to a modified Fibonacci schema (Table 1), with the dose of cisplatin remaining at 75 mg/m^2 in all of the dose cohorts. Patients were able to continue treatment with cisplatin to a maximum of six courses of chemotherapy (or a cumulative dose of 450 mg/m^2) and BMS-214662 to a maximum of eight administrations provided there was no evidence of disease progression or unacceptable toxicity.

Evaluation of toxicity and dose escalation Chemotherapy toxicity was graded using National Cancer Institute Common Toxicity Criteria version 2.0. Toxicity assessment, full blood count and a biochemical profile were performed weekly during the study. Full physical examination and WHO performance status were recorded before each cycle (or more frequently if clinically indicated). Calculation and/or measurement of creatinine clearance was also repeated before each cycle of treatment. Dose escalation decisions, description of DLTs, and determination of MTD were based on toxicity occurring during the first cycle of chemotherapy, but cumulative toxicity was also recorded.

DLT was defined as grade 4 neutropenia ≥ 5 days or febrile neutropenia (fever ≥ 38.5 ^{o}C with an absolute neutrophil count < 1.0 x 10^9/L); grade 4 thrombocytopenia (platelets < 10 x 10^9/L) or a bleeding episode requiring platelet transfusion; ≥ grade 3 nausea or emesis despite maximal antiemetics; ≥ grade 3 nonhematological toxicity with the exception of grade 3 alanine aminotransferase and aspartate aminotransferase elevations which resolved to baseline within 2 weeks; and a treatment delay of 2 consecutive weeks due to failure of toxicity to resolve to baseline or grade 1.

The MTD was defined as the dose level below that at which > 1 of 3 or ≥ 2 of 6 patients experienced a DLT. Once the MTD had been defined a further 6 patients were recruited at the MTD to gain further experience with this regimen. Dose escalation occurred after all 3 of the patients (or 6 patients in any expanded cohort) had completed at least 1 cycle of treatment. No intrapatient dose escalation was performed.

Dose delays and modifications Dose delays and modifications were performed on the basis of toxicity. Administration of both agents was delayed for 1 week if drug related toxicity from the previous chemotherapy cycle had not resolved to pretreatment levels or ≤ grade 1; if toxicity did not resolve after a delay of 2 weeks study treatment was discontinued. In the event of grade 3-4 non-hematological toxicity patients could be retreated after reducing the BMS-214662 dose to the dose of the previous cohort. Cisplatin was omitted if an EDTA Cr^{51} determined creatinine clearance was < 60 mL/min, in which case BMS-214662 could be administered as a single agent at the investigators discretion. Similarly, cisplatin administration was discontinued in the event of ≥ grade 3 peripheral neuropathy or clinically significant ototoxicity. In addition, patients who developed a hematological DLT could be retreated after reduction of the dose of BMS-214662 to the previous dose level, with no modification of the cisplatin dose. After a dose reduction of either agent, for whatever reason, no dose escalation for subsequent chemotherapy cycles in that patient was allowed, with the exception of recovery of creatinine clearance to ≥ 60 mL/min.

Disease evaluation and response assessment Tumor assessments were performed by radiological evaluations (computed tomography scan of disease sites) and

clinical assessments before starting chemotherapy, and these assessments were repeated after every 2 cycles of treatment. Patients who received at least 2 cycles of treatment were evaluable for response. In addition, patients who developed rapid tumor progression, or died of progressive disease prior to response evaluation were considered evaluable for response. Responses to treatment were defined using the WHO criteria [30] and all analyses were carried out on an intention-to-treat basis.

Pharmacokinetics Blood and urine samples were collected for pharmacokinetic analysis during the first treatment cycle only. Blood was sampled from a site contralateral to the peripheral vein used for treatment during the first cycle of treatment only. Blood samples for BMS-214662 pharmacokinetics were collected in EDTA Vacutainer tubes before treatment with BMS-214662, 30 minutes after the start of the infusion, immediately before the end of the 1-hour infusion and at 10, 20 and 30 minutes and 1, 2, 3, 5, 7, 9 and 23 hours after the end of the infusion. Plasma was separated by centrifugation, transferred to appropriately labeled tubes and transported to Bristol-Myers Squibb (New Brunswick, NJ, USA) for analysis. For measurement of BMS-214662 in human plasma, 0.5-mL aliquots of plasma were transferred to screw-capped vials, and followed by addition of 0.5-mL of 1 M phosphate buffer and 5 mL of 1-chlorobutane. The tubes were capped and mixed thoroughly. After centrifugation, the upper organic phases were transferred to clean tubes and their contents evaporated under nitrogen. The dried residues were reconstituted in 300 μL of acetonitrile/50-mM ammonium acetate (pH 4.7) and a 100-μL aliquot injected onto the high-performance liquid chromatography. BMS-214662 was separated from endogenous plasma interference using a 4.6 x 150 mm C-18 column with a mobile phase containing acetonitrile/50 mM ammonium acetate (pH 4.7) and BMS-214662 was detected by its UV absorbance at a wavelength of 305 nm.
Blood samples for measurement of cisplatin concentrations were obtained in 4.5 mL heparinized glass tubes taken before infusion; at 2 hours after the start of the infusion; at the end of the 4-hour infusion; and 0.5, 1, 2, 4 and 20 hours after the end of the infusion. Immediately after sampling plasma was separated by centrifugation at 3000 x *g* for 10 minutes. Next, 500-μL aliquots of the plasma supernatant were added to 1-mL of ice-cold (–20 °C) ethanol. After mixing on a vortex-mixer for 10 seconds, the ethanolic samples were stored until the day of analysis of unbound cisplatin. The remaining plasma was stored for determination of total cisplatin. Urine samples for cisplatin PK analysis were collected before the cisplatin infusion and 0-4, 4-8, 8-12 and 12-24 hours after the start of the cisplatin infusion.
For measurement of unbound cisplatin, the ethanolic supernatant was collected by centrifugation of the samples at 23,000 x *g* for 5 minutes, which was subsequently transferred to a clean vial. A volume of 600 μL was evaporated to dryness under nitrogen at 60 °C, and the residue reconstituted in 200 μL (or 600 μL in the case the concentrations were higher than the highest standard of the calibration curve) water containing 0.2% (v/v) Triton X-100 and

0.06% (w/v) cesium chloride by vigorous mixing. A volume of 20 µL, in duplicate, was eventually injected onto the graphite furnace of the atomic absorption spectrophotometer. For determination of total platinum concentrations, a 100-µL volume of plasma was added to 900 µL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride. Of this solution, a volume of 20 µL, in duplicate, was injected into the atomic absorption spectrophotometer. Samples were analyzed on a Perkin-Elmer Model 4110 ZL spectrometer with Zeeman-background correction using peak area signal measurements at a wavelength of 265.9 nm and a slid width of 0.7 nm. The area under the plasma concentration time curve (AUC) of total cisplatin was calculated to the last sampling time point (i.e., 20 hours after the end of infusion), by the linear trapezoid method, using the software package Siphar v4.0 (SIMED, Creteil, France). The AUC of unbound platinum was calculated to the last sampling time point with detectable drug levels by the linear trapezoid method and extended to infinity. The clearances of total and unbound cisplatin were calculated by dividing the dose administered (expressed in mg/m^2 cisplatin) by the observed AUCs. The terminal disposition half-life ($t_{1/2}$) of unbound cisplatin was calculated as $\ln 2/k$, where k is the terminal elimination rate constant (expressed in h^{-1}). Cisplatin pharmacokinetic parameters are reported as mean values ± S.D. Differences in pharmacokinetic parameters of cisplatin between the different dosing groups of BMS-214662 were evaluated statistically using one way of analysis (ANOVA), while differences between the two sites were evaluated by two-tailed Student's *t* tests, using the software package SPSS for Windows (version 9.0). Probability values of <0.05 were regarded as statistically significant.
The pharmacokinetic parameters AUC, clearance, apparent volume of distribution at steady state and the $t_{1/2}$ of total BMS-214662 were calculated using noncompartmental methods by the PK-MENU application using the Statistical Analysis System (version 6.12; SAS, Cary, NC, USA).

Pharmacodynamics As a surrogate pharmacodynamic end point of activity of BMS-214662, the inhibition of constitutive FT activity in PBMCs was determined in 22 of the patients during the first cycle of treatment only. Blood samples were collected into a Benton-Dixon

Table 1. Number of Patients and Cycles of Chemotherapy per Dose Level

BMS-214662 Dose (mg/m^2)	No. of Patients	No. of Cycles, Total (Range)
126	3	14 (1-6)
168	6	19 (1-6)
200	11	25 (1-6)
225	9	29 (1-6)

Table 2. Patient Characteristics

Patient Characteristic	No. of Patients
Male / Female	16/13
Age (years)	
Median	57
Range	35-76
Performance status (WHO)	
0	8
1	21
No. of prior chemotherapy regimens	
0	14
1	9
2	4
3	1
4	1
Prior radiotherapy	11
Prior hormonal/immunotherapy	2

CPT Vacutainer tubes before treatment with BMS-214662, at the end of the infusion and at 5 and 23 hours following completion of the infusion. The tubes were centrifuged for 30 minutes at 1700 x *g* and 20 °C within 15 minutes of collection. The PBMCs were transferred to separate polypropylene tubes and washed twice with 10 mL of ice-cold PBS. After each washing, cells were separated by centrifugation for 10 minutes. After the second washing, the PBMC pellets were stored at –70 to –80 °C. PBMC preparations were lysed, and the FT activity in the cellular extracts determined using exogenous [3H]farnesylpyrophosphate and H-Ras substrates. FT activity was normalized by cellular protein concentrations. FT activity was plotted as a function of time. In addition, FT activity (measured as percentage FT activity from baseline) was assessed as a function of BMS-214662 plasma concentration using an inhibitory Sigmoid Emax Model (Pharsight Inc.) according to the following relationship: $E = Emax [1-(C^n)/C^n + EC_{50}{}^n)]$, where E is the effect, measured as the percentage reduction in FT activity from the predose level, Emax is the maximal effect at a concentration (C) of BMS-214662, EC_{50} is the plasma concentration of BMS-214662 needed to reduce the maximal effect by 50% and n is the Hill coefficient of the curve.

RESULTS

Twenty-nine patients were recruited into the study. They received a total of 87 cycles of treatment (Table 1). Patient characteristics at baseline are summarized in Table 2. Tumor types included cancer of the pancreas (6 patients), of unknown primary origin (4), the head and neck (4),

Table 3. Cumulative Toxicity (All Cycles): Worst Grade per Patient by Dose Cohort, Number of Patients (%)

Toxicity (Grade#)	BMS-214662 Dose Level				
	126 mg/m^2	168 mg/m^2	200 mg/m^2	225 mg/m^2	All
	(n = 3)	(n = 6)	(n = 11)	(n = 9)	(n = 29)
Fatigue					
1	1 (33)	1 (17)	1 (9)	2 (22)	5 (17)
2	1 (33)	4 (67)	4 (36)	4 (44)	13 (45)
3	-	-	-	-	1 (3)
Nausea					
1	-	3 (50)	3 (27)	2 (22)	8 (28)
2	2 (67)	1 (17)	2 (18)	4 (44)	8 (28)
3	-	-	-	-	1 (1)
Vomiting					
1	-	2 (33)	3 (27)	5 (56)	10 (34)
2	1 (33)	2 (33)	2 (18)	3 (33)	8 (28)
3	1 (33)	-	-	1 (11)	2 (7)
Diarrhea					
1	-	2 (33)	3 (27)	3 (33)	8 (28)
2	1 (33)	1 (17)	-	3 (33)	5 (17)
3	-	-	-	2 (22)	2 (7)
Edema					
1	-	1 (17)	1 (9)	-	2 (7)
Infection					
2	-	-	-	1 (11)	1 (3)
4	-	-	-	1 (11)	1 (3)
Neuropathy					
1	2 (67)	1 (17)	2 (18)	3 (33)	8 (28)
2	-	1 (17)	-	1 (11)	2 (7)

Grade according to National Cancer Institute Common Toxicity Criteria, version 2.0

colon (3), ampulla (2), stomach (2), and miscellaneous (8). Fifteen patients had received at least one previous chemotherapy regimen for advanced disease including 6 patients who had previously been treated with cisplatin or carboplatin. The median number of cycles administered of both BMS-214662 and cisplatin in combination was 2. The dose of BMS-214662 was escalated from 126 mg/m2 to 225 mg/m^2 in successive cohorts. Following on from this an additional 3 patients were entered at the 168 mg/m^2 dose level. Finally 11 patients were treated at the intermediate dose of 200 mg/m^2.

Table 4. Cumulative Hematological Toxicity (All Cycles): Worst Grade per Patient by Dose Cohort, Number of Patients (%)

Toxicity (Grade#)	BMS-214662 Dose Level				
	126 mg/m^2	168 mg/m^2	200 mg/m^2	225 mg/m^2	All
	(n = 3)	(n = 6)	(n = 11)	(n = 9)	(n = 29)
Leukopenia					
1	-	-	2 (18)	4 (44)	6 (21)
2	2 (67)	2 (33)	3 (27)	1 (11)	8 (28)
3	-	1 (17)	2 (18)	3 (33)	6 (21)
Neutropenia					
1	2 (67)	-	1 (9)	2 (22)	5 (17)
2	-	3 (50)	1 (9)	2(22)	6 (21)
3	1 (33)	-	3 (27)	2 (22)	6 (21)
Thrombocytopenia					
1	2 (67)	2 (33)	4 (36)	5 (56)	13 (45)
2	-	1 (17)	-	1 (11)	2 (7)
3	-	-	1 (9)	-	1 (3)
Anemia					
1	1 (33)	2 (33)	6 (54)	1 (11)	10 (35)
2	2 (67)	3 (50)	3 (27)	8 (89)	16 (55)
3	-	-	1 (9)	-	1 (3)

Grade according to National Cancer Institute Common Toxicity Criteria, version 2.0

Toxicity All of the 29 patients were evaluable for toxicity. Treatment toxicities are listed in Tables 3-5. DLTs were observed in 4 of 9 patients enrolled at the 225 mg/m^2 BMS-214662 dose level. This dose cohort was expanded after the treatment of the first cohort of 3 patients rather than escalating the dose of BMS-214662 further, based on preliminary information that was available at that time from studies of BMS-214662 as a single agent. DLTs consisted of transient grade 3 elevation of hepatic transaminases (1 patient), grade 3 nausea (1), grade 3 diarrhea with grade 4 hepatic transaminase elevation (1) with recovery to baseline occurring after 6 days, and grade 3 diarrhea, vomiting and renal failure (1). A dose of 225 mg/m^2 BMS-214662 in combination with 75 mg/m^2 cisplatin over 4 hours was, therefore, the maximum administered dose. Consequently, an additional 3 patients were entered at the next lower dose level of 168 mg/m^2 (for a total of 6 patients at this dose level) with no DLTs being observed. The dose of BMS 214662 was escalated subsequently to an intermediate dose of 200 mg/m^2. Two of 11 patients treated at this dose level experienced DLTs with one transient grade 4 hepatic transaminase elevation (resolving to baseline after 4 days) and one transient grade 3 elevation of creatinine (resolving to grade 2 after 24 hours). A dose of 200 mg/m^2 BMS-214662

Table 5. Cumulative Biochemical Toxicity (All Cycles): Worst Grade per Patient by Dose Cohort, Number of Patients (%)

Toxicity (Grade#)	BMS-214662 Dose Level				
	126 mg/m^2	168 mg/m^2	200 mg/m^2	225 mg/m^2	All
	(n = 3)	(n = 6)	(n =11)	(n = 9)	(n = 29)
Total bilirubin					
1	-	-	2 (18)	1 (11)	3 (10)
2	-	1 (17)	4 (36)	1 (11)	6 (21)
3	-	-	-	3 (33)	3 (10)
AST§					
1	-	3 (50)	4 (36)	4 (44)	11 (38)
2	-	1 (17)	2 (18)	3 (33)	6 (21)
3	-	1 (17)	1 (9)	1 (11)	3 (10)
4	-	-	1 (9)	1(11)	2 (7)
ALT					
1	-	2 (34)	3 (27)	3 (33)	8 (28)
2	-	2 (34)	2 (18)	4 (44)	8 (28)
3	-	1 (17)	3 (27)	2 (22)	6 (21)
Creatinine					
1	2 (67)	2 (34)	3 (27)	2 (22)	9 (31)
2	-	-	1 (9)	3 (33)	4 (14)
3	-	-	1 (9)	1 (11)	2 (7)

Grade according to National Cancer Institute Common Toxicity Criteria, version 2.0
§ Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase

administered 3-weekly as a 1-hour infusion in combination with 75 mg/m2 cisplatin as a 4-hour infusion 3-weekly was defined as the MTD and the recommended dose for subsequent phase II studies.

No episodes of febrile neutropenia, defined as grade ≥ 3 neutropenia with temperature ≥ 38.5 °C occurred. There was no apparent cumulative toxicity, and there were no deaths due to drug-related toxicity.

Dose modifications and delay Chemotherapy was delayed for > 3 days in 19 cycles in 12 patients due to inadequate recovery of the neutrophil count in 10 cycles, inadequate recovery of platelet count in 1 cycle, and for other reasons in 8 cycles (patient request, 3 cycles; delayed recovery of nonhematological toxicities, 3 cycles; and administrative reasons, 2 cycles). Chemotherapy was discontinued due to toxicity in five patients. For three patients, this was due to a single toxicity: renal failure (1 patient), electrocardiogram change (T-wave inversion; 1), and persistent leukopenia and neutropenia for at least 2 weeks (1). For the other

Table 6. Pharmacokinetics Parameters (Mean ± SD) of BMS-214662

Parameter	BMS-214662 Dose Level			
	126 mg/m^2	168 mg/m^2	200 mg/m^2	225 mg/m^2
No. of Patients	3	6	8	10
C_{max} (ng/mL)#	3625 ± 669	5084 ± 867	6388 ± 1829	8589 ± 3556
AUC (ng mL/h)	4825 ± 1120	7481± 1759	9982 ± 3425	17844 ± 8042
CL (mL/min/m^2)	449 ± 96	573 ± 227	689 ± 301	285 ± 148
Vss (L/m^2)	29 ± 6	37 ± 16	60 ± 18	32 ± 23
$t_{1/2}$ (h)	1.8 ± 0.4	1.9 ± 0.9	1.8 ± 2.5	2.5 ± 0.9

Abbreviations: Cmax, peak plasma concentration; AUC, area under the plasma concentration-time curve; CL, clearance; Vss, apparent volume of distribution at steady state; $t_{1/2}$, elimination half-life.

two patients, chemotherapy was discontinued due to multiple toxicities: diarrhea, vomiting, raised creatinine and infection in one patient; and sensory neuropathy, nausea and vomiting in the other. The dose of BMS-214662 was reduced by one dose level from 225 mg/m^2 to 168 mg/m^2 due to DLT in two patients after 1 cycle of treatment. The dose of BMS-214662 was reduced from 200 mg/m^2 to 168 mg/m^2 in one patient after 1 cycle. Cisplatin was discontinued in two patients due to decreased creatinine clearance.

Response and survival Twenty-three patients received at least 2 cycles of treatment and were evaluable for assessment of antitumor activity, of which all had measurable or evaluable disease at baseline. Six patients received only 1 cycle of chemotherapy and were withdrawn due to toxicity (2 patients), rapid clinical deterioration in keeping with disease progression (1), withdrawal of consent (1), physician decision due to inadequate venous access and recurrent infection (2). There were no objective responses. Disease stabilization for at least 2 cycles was observed in 15 patients. Disease stabilization was observed for at least 4 cycles in seven patients with cancer of the bladder, head and neck, ampulla, esophagus, stomach, mesothelioma and bronchoalveolar cancer. Four patients with cancer of the bladder, stomach, mesothelioma and broncheoalveolar cancer had stable disease on completion of 6 cycles of treatment.

Pharmacokinetic analysis Blood samples for pharmacokinetic analysis of BMS-214662 were available for 27 of the patients including eight of the patients at the recommended dose level of 200 mg/m^2 (Table 6). Of the 27 patients from whom samples were obtained for pharmacokinetic analysis of cisplatin in plasma and urine, all but one were evaluable for

Table 7. Pharmacokinetics Parameters (Mean ± SD) of Cisplatin

Parameter	BMS-214662 Dose Level			
	126 mg/m^2	168 mg/m^2	200 mg/m^2	225 mg/m^2
	Site 1, Glasgow			
No. of Patients	2	0	3	5
CL_{total} (L/h/m^2)#	1.40, 1.00	-	1.11 ± 0.145	1.06 ± 0.089
Cl_{unb} (L/h/m^2)	NA	-	NA	NA
$t_{1/2(unb)}$ (h)	NA	-	NA	NA
Urine Excr (%)	14.3, 15.7	-	24.2, 33.3	14.6 ± 4.13
	Site 2, Rotterdam			
No. of Patients	0	6	5	5
CL_{total} (L/h/m^2)	-	1.21 ± 0.107	1.32 ± 0.293	1.16 ± 0.225
Cl_{unb} (L/h/m^2)α	-	17.7 ± 3.53	22.0 ± 4.06	30.6 ± 7.61
$t_{1/2(unb)}$ (h)	-	0.71 ± 0.22	0.56 ± 0.13	0.73 ± 0.25
Urine Excr (%)ß	-	34.9 ± 5.15	33.1 ± 4.57	26.3 ± 4.40

Abbriviations: CLtotal, clearance of total cisplatin derived platinum from plasma; CLunb, clearance of unbound cisplatin derived platinum from plasma; Exrc, percentage of dose of platinum excreted in urine; $t_{1/2}$, elimination half-life; NA, not available.

α P = 0.006 (ANOVA).
ß P = 0.028 (ANOVA).

total cisplatin pharmacokinetic analysis in plasma, and 23 were evaluable for urine analysis. A total of 16 patients also had samples collected for the analysis of unbound cisplatin, of whom 15 were evaluable. The pharmacokinetic analysis of cisplatin measurements are summarized in Table 7.

Pharmacodynamic analysis There was a precipitous drop in FT activity (65-80%) in the PBMCs following BMS-214662 infusion that appeared to be dose dependent (Figure 1). The level of activity generally returned to control levels within 24 hours. The percentage of FT activity with increasing BMS-214662 concentration is shown in Figure 2. On the basis of fitting the results to the inverse sigmoid model, the estimated Emax value was 89.4%, the EC_{50} was 127 ng/mL, and the n was 0.24.

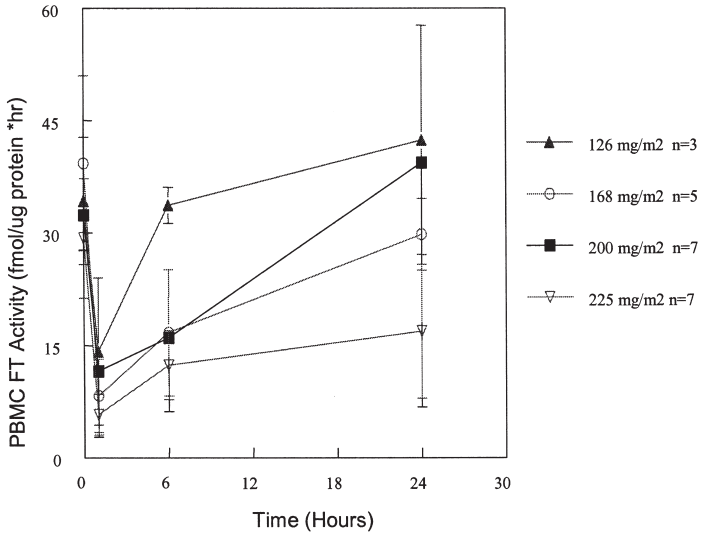

Figure1. Figure 1. Demonstrates the Inhibition of Farnesyltransferase (FT) Activity (Mean ± SD) in Peripheral Blood Mononuclear Cells (PBMC) with Increasing Doses of BMS-214662 (with Fixed Dose of Cisplatin 75 mg/m^2), Over the First 24 Hours After Administration of BMS-214662.

DISCUSSION

The combination of cisplatin and BMS-214662 was well tolerated at doses up to 200 mg/m^2 of BMS-214662 in combination with cisplatin 75 mg/m^2, and this was the MTD of BMS-214662 in this combination. The most common adverse events were uncomplicated neutropenia, reversible elevation of hepatic transaminases, nausea and vomiting and diarrhea. Toxicities were consistent with those predicted from single agent studies [18,31].

The pharmacokinetic studies showed that the clearance of unbound cisplatin seems dependent on the dose of BMS-214662 (P = 0.006, ANOVA), with higher clearance of unbound cisplatin at higher doses of BMS-214662. The mechanism behind this phenomenon might be related to higher protein binding of cisplatin with higher doses of BMS-214662. At the highest dose of BMS-214662, no pharmacokinetic interaction with cisplatin is observed. However, this study was not designed to evaluate the potential interaction of BMS-214662 on the clearance of cisplatin. Nevertheless, the urine excretion of cisplatin is dependent on the dose of BMS-214662 (P = 0.028, ANOVA), with lower excretion at higher doses of BMS-214662, and this would be in agreement with the above hypothesis that there may be an interaction at the level of protein binding, because only the unbound fraction of cisplatin can be excreted by the kidneys (i.e., lower unbound fractions results in lower urinary excretion).

The AUC of BMS-214662 increased in a ratio of 1:1.5:2.1:3.7 in a dose ratio of 1:1.3:1.6:1.8. The

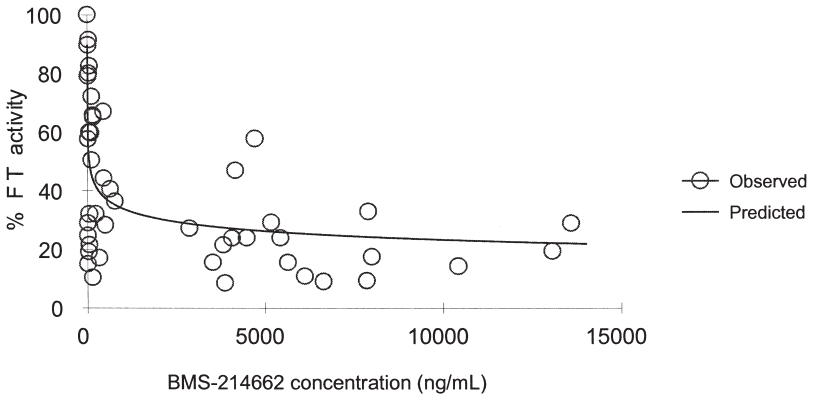

Figure 2. Demonstrates the Percentage of Farnesyltransefrase (FT) Activity in Peripheral Blood Mononuclear Cells With Increasing Plasma Concentration of BMS-214662.

greater than proportional increase in the exposure at the highest dose studied (225 mg/m^2) may in part be due to the wide variability in the AUC values (45% coefficient of variation). If this study is compared with single agent BMS-214662 pharmacokinetic studies, cisplatin does not appear to have an effect on the disposition of BMS-214662 [31]. In summary, at the recommended dose of BMS-214662, a pharmacokinetic interaction between BMS-214662 and cisplatin is not apparent. However, because the number of patients studied per dose cohort is small, and different hydration schedules were used for cisplatin administration, a relevant interaction can not be completely excluded.

Pharmacodynamic studies demonstrate a precipitous drop in FT activity in the PBMCs after BMS-214662 infusion that appears to be dose dependent, with FT activity returning to control levels within 24 hours. The Emax inverse sigmoid model gave an EC_{50} of 127 ng/mL. However, with a plasma protein binding of drug of approximately 99%, this value is similar to the IC_{50} value for FT in in vitro assays, suggesting that there is a close correlation between the in vitro and in vivo values for FT IC_{50} with BMS-214662. These pharmacodynamic studies, taken with the short elimination half-life of BMS-214662, would suggest that a single i.v. infusion of this agent may not be the optimal schedule to produce a sustained pharmacodynamic effect. However, it is not clear what is the duration of the biological effect of FT inhibition within tumors, nor what duration of biological effect is required for optimal potential synergy with

cisplatin. Studies of single-agent BMS-214662 administered weekly as a 24-hour infusion have suggested that schedules of administration of BMS-214662 that provide sustained plasma exposures and FT inhibition may be required for optimal pharmacological activity [32,33].
An important initial observation with the FTIs was that many of their cellular effects appeared to be cytostatic rather than cytotoxic [34], and thus potentially antagonizing the effects of classical cytotoxic drugs. In vitro studies with BMS-214662, however, demonstrated that it is one of the most potent of the FTIs in inducing apoptosis, suggesting that it could potentially be combined with classical cytotoxic agents to give an additive or synergistic effect. There were no objective responses observed in this study. However, disease stabilization was observed in 15 patients with four patients having disease stabilization on completion of 6 cycles of treatment.
This study defined a dose of 200 mg/m^2 BMS-214662 administered as a 1-hour infusion in combination with 75 mg/m^2 cisplatin over 4 hours as the recommended dose. However, the optimal dose and schedule for FTIs in patients with advanced cancer may not be the MTD as determined in phase I studies using standard clinical toxicity criteria. It is likely that a biological threshold exists such that additionally increasing the dose of drug does not lead to further gain. Consequently, future studies should incorporate metabolic tumor imaging and the measurement of robust surrogate markers of intratumoral FT activity to determine the optimal biologically effective dose and schedule.

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ABSTRACT

Purpose: *ABT-510 is an angiogenesis inhibitor derived from thrombospondin-1, a naturally occurring inhibitor of angiogenesis. We investigated ABT-510, administered subcutaneously in patients with advanced solid malignancies, to assess safety, pharmacokinetics and serum markers of angiogenesis.*

Patients and Methods: *ABT-510 was administered subcutaneously as continuous infusion (100 mg/24 hours) and bolus injections (100, 200 and 260 mg once daily; 50 and 100 mg twice daily) in 28-day cycles.*

Results: *Thirty-nine patients received a total of 144 treatment cycles. Administration by continuous infusion was hampered by the onset of painful skin infiltrates at the injection site. In the bolus injection regimens, the most common toxicities observed were mild injection-site reactions and fatigue. Maximum tolerated dose was not defined, but 260 mg was defined as the maximum clinically practical dose. ABT-510 pharmacokinetics were linear across the dosage ranges tested and the potential therapeutic threshold (plasma concentrations above 100 ng/mL > 3 hours/day) was achieved with all dose regimens. Median (range) serum basic fibroblast growth factor levels decreased from 14.1 (0.5-77.7) pg/mL at baseline to 3.2 (0.2-29.4) pg/mL after 56 days of treatment (P = 0.003). No correlations with time on study, ABT-510 dose or exposure were observed for individual changes in bFGF. Stable disease lasting ≥ 6 cycles was seen in 6 patients.*

Conclusion: *ABT-510 demonstrated a favorable toxicity profile and linear and time-independent pharmacokinetics with biologically relevant plasma concentrations. The significant number of patients with prolonged stable disease and the convenient method of dosing merit further studies with this angiogenesis inhibitor.*

CHAPTER 5

A PHASE I SAFETY, PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF THE THROMBOSPONDIN-1-MIMETIC ANGIOGENESIS INHIBITOR ABT-510 IN PATIENTS WITH ADVANCED CANCER

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INTRODUCTION

Cancer progression is characterized by cell growth, tissue invasion and metastasis. Angiogenesis is essential for these processes. Thrombospondin-1 (TSP-1) is a large adhesive glycoprotein that is activated by the tumor suppressor gene p53 and has an inhibitory effect on angiogenesis [1,2]. It inhibits the activity of multiple pro-angiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and interleukin-8 (IL-8) [3]. The anti-angiogenic activity of TSP-1 depends on activation of $p59^{fyn}$ and p38MAPK through interaction with the CD36 receptor or related proteins. Endothelial cell apoptosis induced by TSP-1 is mediated by the Fas/Fas ligand interaction and is accompanied by activation of caspase-3 and FAK fragmentation [4-6].

The angiogenesis-inhibiting activity of TSP-1 has been mapped to the 50,000-dalton N-terminal third of the molecule and, more specifically, to the properdin Type-1 repeats in this region. A single D-amino acid replacement in one properdin-region heptapeptide leads to a 1000-fold increase of angiogenesis inhibitory activity in preclinical models [3]. ABT-510 is a nonapeptide analog of this active heptapeptide that mimics the natural angiogenesis inhibiting activity of TSP-1 (Figure 1). ABT-510 competes with TSP-1 for binding to the endothelial cells, induces Fas ligand expression in endothelial cells and inhibits VEGF- and bFGF-stimulated migration of human microvascular endothelial cells.

In vivo, ABT-510 inhibits VEGF-induced corneal neovascularization in mice and inhibits

Figure 1. Chemical Structure of ABT-510 and Metabolites.

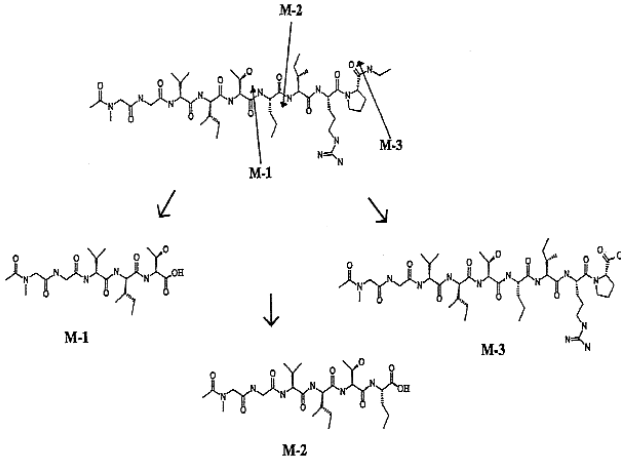
Product code: ABT-510

Chemical name: N-acetylsarcosyl-glycyl-L-valyl-D-allo-isoleucyl-L-threonyl-L-norvalyl-L-isoleucyl-L-arginyl-L-propyl-N-ethylamide

Molecular formula: $C_{46}H_{83}N_{13}O_{11}$

Molecular weight: 994.23 g/mole

Chemical structure:



tumor growth in several mouse xenograft models, in a dose-dependent manner. ABT-510 also inhibits B16 F10 melanoma lung metastases formation [7]. Studies in rats with ABT-510 administered intravenously daily for 1 week revealed dose-limiting renal toxicity at a dose of 75 mg/kg/day and a no adverse effect dose of 25 mg/kg/day. In monkeys, doses up to 75 mg/kg/day intravenously for 1 month were tolerated without dose-limiting toxicities [7]. Preclinical studies indicate that cytochrome P-450 enzymes are not involved in metabolism of ABT-510 and that ABT-510 has no inhibitory effects on cytochrome P-450 enzymes. ABT-510 is metabolized primarily by cleavage of peptide bonds to form M-1, M-2 and M-3 peptides, which are mainly excreted in bile and urine (Figure 1). In vivo, M-1, a five-amino acid peptide formed by peptide bond hydrolysis between threonine-5 and norvaline-6, predominates. ABT-510 is not extensively bound to plasma proteins.

Modeling of pharmacokinetic and pharmacodynamic data from preclinical experiments in 11 different murine models was performed to identify a pharmacokinetic target for clinical studies. Efficacy measures varied by model and included tumor volume, number of metastases and VEGF- or bFGF-stimulated new vessel density. As a result the clinical pharmacokinetic target was aimed at reaching plasma concentrations greater than 100 ng/mL for at least 3 hours per day, which, on average, achieved 75% of maximum efficacy in these models [8].

We performed a phase I safety, pharmacokinetic and pharmacodynamic study with ABT-510 administered subcutaneously to patients with advanced solid malignancies. The principal objectives of this study were to determine single and multiple dose plasma pharmacokinetics, with special emphasis on correlation with preclinical pharmacological data and to establish the safety profile and determine the maximum tolerated dose (MTD) of ABT-510 when administered by subcutaneous continuous infusion and by subcutaneous injection once daily or twice daily for 28 consecutive days.

PATIENTS AND METHODS

Eligibility criteria Patients with a histologically confirmed diagnosis of an advanced solid malignancy refractory for standard therapy were eligible. Additional eligibility criteria included: age ≥ 18 years; World Health Organization (WHO) performance status < 3; an estimated life expectancy of ≥ 3 months; no radiotherapy, chemotherapy or hormonal therapy within 4 weeks before study start, with the exception of small field radiation. Specific exclusion criteria included: a known human immunodeficiency virus positive status, a diagnosis of primary brain tumor or known central nervous system metastases; and evidence of uncontrolled clinically significant disease unrelated to the primary malignancy. The study was approved by the local ethics boards of the two participating centers and all patients gave written informed consent.

Drug administration ABT-510 (Abbott Laboratories, Chicago, IL, USA) was supplied in vials containing 1.1 mL ABT-510, 100 mg/mL dissolved in dextrose 5%. The vials were stored

at 2-8 ºC and brought to room temperature 1 hour before dosing. Three methods of drug administration were studied: subcutaneous continuous infusion, and once daily and twice daily subcutaneous bolus injection. For continuous infusion, a MiniMed® 470C micro-infusion pump (MiniMed®, Sylmar, CA, USA) was used. This pump is specifically developed for the subcutaneous delivery of liquid medications. It uses syringes with a maximal capacity of 3 mL and can deliver volumes from 0 to 0.350 mL/hour with 0.001 mL increments and an accuracy of ± 2%. Patients used the abdominal wall as the injection site and changed the injection site every 3 days. The reservoir was changed once daily, preferably in the morning. Patients in the subcutaneous bolus regimen cohorts injected themselves in the abdominal wall. The maximum volume of 1 injection was set at 1.3 mL, with a maximum of 2 injections a day; this accounted for a maximum clinically practical daily dose of 260 mg. Patients in the group administering bolus injections once daily were instructed to inject themselves in the morning, preferably at the same time each day, and patients in the group administering bolus injections twice daily were instructed to inject themselves in the morning and evening, with an interval of 12 hours in between doses. Times of injection were recorded in a diary. The starting doses were 100 mg/24 hours continuous infusion, and 100 mg once daily and 50 mg twice daily bolus injections, based on safety and pharmacokinetic data obtained in the phase I healthy volunteer's study [7]. No adjustments for body surface area or weight were made. Medication was administered daily without interruption; 28 days of treatment defined a treatment cycle. Cohorts of 3 to 6 patients were studied. Dose escalation decisions were made after review of observed toxicities and pharmacokinetic data and discussion between investigators and sponsor. Toxicity was assessed according to National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. Escalations were pursued until either MTD was identified or the maximum clinically practical daily dose of 260 mg, as defined above, was reached. The MTD was defined as the highest dose of ABT-510 given for at least 1 treatment cycle at which not more than 1 of 6 patients would experience dose-limiting toxicity (DLT). A DLT was defined as grade 3 or 4 adverse event (except inadequately treated nausea or vomiting) or any grade 2 adverse event requiring dose modification or treatment delay occurring in the first treatment cycle. Once the MTD was determined or the maximum clinically practical dose of 260 mg daily was reached for each schedule, cohort size would be expanded up to a total of 10 patients.

Pretreatment and follow-up studies Before therapy, a complete medical history was taken and a physical examination, electrocardiogram and chest X-ray were performed. The following were performed at baseline and at each scheduled visit: complete blood cell count (CBC), including white blood cell differential; serum biochemistry, including sodium, potassium, chloride, magnesium, bicarbonate, creatinine, blood urea nitrogen, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, total bilirubin, calcium, phosphate, glucose, alkaline phosphatase and amylase;

prothrombine time (PT); activated partial tromboplastin time (aPTT); and urinalysis. Plasminogen, fibrinogen and factor VIII were collected at baseline and thereafter as clinically indicated. Urine was collected over 24 hours at baseline and on day 22 for albumin excretion. Weekly evaluations during the first treatment cycle included history, physical examination, toxicity assessment, CBC, PT, aPTT and serum biochemistry. The same evaluations were also performed after 2 months and every 3 months thereafter. Additional visits were allowed at the discretion of the responsible physicians. Tumor measurements were performed after 2 cycles and every 3 cycles thereafter. Tumor response was assessed using WHO criteria [9]. Patients were allowed to continue treatment in the absence of progressive disease or unacceptable toxicity.

Pharmacokinetic sampling and assays Blood samples (5 mL) for pharmacokinetic analysis were collected from the continuous infusion patients using an indwelling intravenous canula before switching the reservoir and 0.5, 1, 2, 4, 6, 8 and 10 hours following the reservoir changes on days 1 and 22 of the first cycle. For the bolus injection patients, blood samples were collected before dosing and 5, 15 and 30 minutes and 1, 2, 4, 6, 8 and 10 hours following the morning dose on days 1 and 22 of the first cycle. Samples were collected in EDTA-containing tubes, then placed on ice and centrifuged at 2,000 x *g* for 10 minutes within 1 hour using a refrigerated (4 ºC) centrifuge. Plasma was stored in polypropylene tubes at -20 ºC until analysis. Plasma concentrations of ABT-510 and its major metabolite M-1 were determined using a validated liquid chromatography with mass spectrometry (LC/MS/MS) method. Sample preparation involved a plasma aliquot supplemented with internal standard (ABT-818), and solid phase extraction with SPEC® C18AR discs in a 96-well plate format (Varian, Lake Forest, CA, USA). Extracts were injected via autosampler into a BHK ODS-W/S C18 5µm 100 x 3.0 mm analytical column (BHK Laboratories, Inc., Naperville, IL, USA), with mobile phase of acetonitrile/0.1% formic acid in water, pH 4.0 at a flow rate of 0.3 mL/min. Analysis was performed on an API III+® mass spectrometer (Perkin Elmer Sciex, Concord, ON, USA), with positive ionization using the Turbo IonSpray source. Multiple reaction monitoring (MRM) with the following nominal transitions, in chromatographic elution order, was m/z 502 → 72 for ABT-388 (M-1 metabolite), m/z 1210 → 249 for internal standard and m/z 995 → 270 for ABT-510. Peak areas were integrated using MacQuan™ v1.6 (Perkin Elmer Sciex). Watson v6.2.0.02 LIMS (PSS, Inc., Wayne, PA, USA) was used for regression (weighted $1/x^2$) and quantitation. The lower limits of quantification in plasma were approximately 0.5 ng/mL for ABT-510 and 3 ng/mL for the M-1 metabolite.

Urine was collected on days 1 and 22 of the first cycle in the bolus injection cohorts only. Two samples of at least 15 mL were collected immediately before dosing on day 1 for the baseline drug assay. Urine was collected over 24 hours following dosing on days 1 and 22 for the patients dosed once daily and from 0 to 12 and 12 to 24 hours after dosing on days 1 and 22 for the patients dosed twice daily. The urine samples were refrigerated during the collection

period and total volumes were measured. Two 15 mL samples from each sampling period were stored at -20 ºC until analysis. For measurement of urine concentrations of ABT-510 and major metabolite M-1, 200 µL of urine was combined with internal standard and diluted. In addition, a trapping column (20 x 2.0 mm, 5 µm, ODS-W/S C18, BHK Laboratories, Inc.) was used on line for cleanup of the diluted urine samples. The analytes were eluted from the trapping column and onto the analytical column for chromatographic separation and introduction to the source of the mass spectrometer. The mass spectrometer detection and quantification were the same as for the plasma assay. The lower limits of quantification were approximately 6 ng/mL for ABT-510 and 99 ng/mL for the M-1 metabolite.

Pharmacodynamic sampling and assays Blood samples (5 mL) for determination of VEGF, bFGF and IL-8 levels were drawn at baseline, day 22, end of cycle 2, every 3 cycles thereafter and at the final study visit. Samples were preferably drawn in the morning before the injection of ABT-510 or change of the reservoir. Samples were collected in Becton Dickinson Vacutainer serum separation tubes. After collection, samples were allowed to clot and were centrifuged within 30 minutes at 800 x *g* for 15 minutes. Serum was stored in polypropylene tubes at -20 ºC until analysis. At the same collection times, urine samples of at least 10 mL were collected. After collection, urine was stored in the refrigerator and, within 4 hours, the following protease inhibitors were added: 80 µg aminoethyl benzenesulfonic acid (AEBSA, Pefabloc SC, Pentafarm, AG, Basel, Switzerland), 200 µg EDTA-sodium, 0.2 µg leupeptin (Roche, Molecular Biochemicals, Basel, Switzerland) and 0.2 µg pepstatin (Roche). Thereafter the urine specimen was centrifuged at 4 ºC, 3,000 x *g* for 10 minutes. The supernatant was stored in polypropylene tubes at -20 ºC until analysis. Serum and urine VEGF, bFGF and IL-8 were measured using commercially available kits (Human VEGF Quantikine Immunoassay Kit, Human FGF basic Quantikine Immunoassay Kit, Human IL-8/CXLX8 Quantikine ELISA Kit, R&D System Inc., Minneapolis, MN, USA). The lower limits of quantification in serum were 0.9, 0.22 and 0.5 pg/mL for VEGF, bFGF and IL-8, respectively. The lower limits of quantification in urine were 1.0 and 0.1 ng/g creatinine for VEFG and bFGF, respectively.

Pharmacokinetic analysis Noncompartmental methods were used to determine values of pharmacokinetic variables of ABT-510 after administration by continuous infusion or subcutaneous bolus injection using WinNonlin-Pro, version 4.1 (Pharsight Corporation, Cary, NC, USA). Maximum measured concentration (C_{max}), minimum measured concentration (C_{min}) and time of maximum observed concentration (T_{max}) were calculated from the concentration-time curves. Additional parameters estimated were: terminal elimination rate constant (ß), the corresponding half-life ($t_{1/2}$), apparent clearance (CL/F) and apparent volume of distribution (Vz/F). The trapezoidal rule was used to calculate area under the blood concentration-time curve to infinity ($AUC_{0-\infty}$) on day 1 and area under concentration-time curve for dosing

interval for day 22 (AUC_{0-T}). The percent of dose recovered in urine as ABT-510 and M-1 was calculated as the amount recovered in urine divided by the dose multiplied by 100. The amount of M-1 recovered in urine was converted to equivalent ABT-510 amount by multiplying by the ratio of molecular weights (994/501).

Statistics The sample size was based on clinical justification and patient numbers historically used for testing of new anti-neoplastic compounds. In models for the analysis of safety data, dose was treated as a factor with discrete levels or as a continuous variable. The nonparametric Friedman's test was performed to compare pharmacodynamic data on days 1, 22 and

Table 1. Patient Characteristics

Patient Characteristic	No. of Patients
Male / Female	30/9
Age (years)	
Median	57
Range	19-83
Performance status (WHO)	
0	16
1	20
2	3
Prior chemotherapy/immunotherapy	
0 prior regimens	6
1-3 prior regimens	30
> 4 prior regimens	3
Prior radiotherapy	14
Prior radiotherapy and chemotherapy	14
Tumor type	
Colorectal cancer	7
Non-small cell lung cancer	7
Renal cell cancer	7
Sarcoma	6
Esophageal cancer	4
Neuroendocrine carcinoma	2
Testicular germ cell tumor	1
Cervix cancer	1
Pancreatic cancer	1
Cholangiocarcinoma	1
Adamantinoma	1
Squamous cell carcinoma skin	1

Table 2. Dose Escalation Scheme

Treatment Schedule Dose (mg)	No. of Patients	Total no. of Cycles[#]	Median no. of Cycles[#] (Range)
Continuous infusion			
100 mg/24 hours	4	8	2 (2)
Bolus injection			
100 mg once daily	6	15	2 (1-5)
200 mg once daily	7	29	2 (1-17)
260 mg once daily	6	21	2 (1-10)
50 mg twice daily	6	26	4 (2-8)
100 mg twice daily	10	45	2 (1-19)
All	39	144	2 (1-19)

A cycle consisted of 28 days of treatment. ABT-510 was administered subcutaneously.

Table 3. Side Effects Possibly or Probably Related to ABT-510 (Occurring in > 5% of Patients)

	ABT-510 Administration (No. of Patients)					
Side Effect	**100 mg CI[#] (n=4)**	**100 mg QD (n=6)**	**200 mg QD (n=7)**	**260 mg QD (n=6)**	**50 mg BID (n=6)**	**100 mg BID (n=10)**
Fatigue	3	1	0	1	0	4
Headache	2	0	0	1	0	2
Dizziness	0	0	0	0	0	2
Insomnia	2	0	0	0	1	0
Taste perversion	0	0	0	0	0	2
Injection site reaction	4	1	5	3	0	7
Injection site pain	0	0	0	2	0	0
Rash	0	0	3	0	0	0
Ecchymosis	0	0	1	0	2	0
Anorexia	3	0	0	0	0	0
Nausea	2	0	0	1	0	2
Vomiting	1	0	0	0	0	2
Dyspnea	1	0	0	1	0	0
Hyperglycemia	0	0	0	0	0	2

Abbreviations: CI, continuous infusion; QD, once daily injection; BID, twice daily injection.

ABT-510 was administered subcutaneously. All toxicities were grade 1 or 2 according to NCI-CTC version 2.0 criteria except 2 patients with hyperglycemia (grade 3 and 4) in the 100 mg twice daily cohort.

56. Two-sided P-values < 0.05 were considered significant. For the pharmacokinetic analysis, descriptive statistics of parameters were determined with a breakdown by regimen and dose level on days 1 and 22.

RESULTS

A total of 39 patients were enrolled into 6 dosing cohorts. The patient characteristics are listed in Table 1. Four patients received ABT-510 by continuous infusion at a dose of 100 mg/24 hours. The remaining 35 patients received ABT-510 by bolus injection (50 and 100 mg twice daily and 100, 200 and 260 mg once daily). A total of 144 cycles of ABT-510 were administered, with a median of 2 (range, 1-19 cycles) (Table 2).

Toxicity The incidence of the observed side effects, possibly or probably related to ABT-510, as a function of the dose and schedule are listed in Table 3. All patients in the continuous infusion cohort developed grade 2 skin infiltrates at the site of the ABT-510 infusion after 48 hours. These infiltrates consisted of erythema and edema of the skin with a maximum diameter of 5 cm. The infiltrates were sometimes painful and persisted for 7-21 days after discontinuation of therapy. A skin biopsy of one of these infiltrates revealed an influx of neutrophils around the smooth vascular endothelium without any signs of vasculitis. These skin infiltrates did not reoccur when the infusion-site was changed daily instead of once every 3 days. Based on the observed skin reactions and the inconvenience of daily changing of infusion site, dosing by continuous infusion was discontinued. In the bolus injection cohorts, only mild to moderate (grade 1-2) skin reactions were observed consisting of redness, slight edema and sporadic pain at the injection site. These symptoms were of short duration and disappeared within a few minutes to 1 hour after the injection. Mild to moderate skin reactions and fatigue were the most common side effects observed with ABT-510 administration. There was no correlation between side effects observed and ABT-510 dose. MTD could not be defined in the bolus injection schedules. A total of 17 severe adverse events (SAE) were reported in 10 different patients. Only 3 SAEs were considered to be possibly related to ABT-510: a fatal intracranial hemorrhage, a transient ischemic attack and new-onset diabetes mellitus.

The intracranial hemorrhage occurred in a 57-year-old male patient with non-small cell lung cancer. He was hospitalized on day 4 of cycle 2 of treatment with ABT-510 100 mg once daily, due to progressive headache, accompanied by dizziness and nausea. Neurologic examination revealed cerebellar ataxia and MRI evaluation showed cerebellar bleeding in a previously undetected cerebellar metastasis. His medical history was positive for hypertension but negative for cardiovascular events, diabetes mellitus, hyperlipidemia, hemorrhagic diatheses or anticoagulant medication. Laboratory evaluation did not indicate a coagulopathy. This patient died the next day. Although a causal relationship with ABT-510 cannot be ruled out, hemorrhage in brain metastases is considered not uncommon in non-small cell lung cancer [10,11]. The

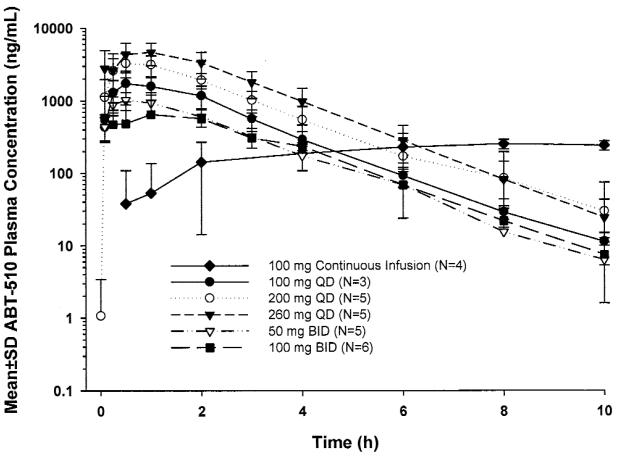
Table 4. Pharmacokinetic Parameters (Mean ± SD) of ABT-510

Parameters	CI#	Bolus Injection				
	100 mg/24h	100 mg QD	200 mg QD	260 mg QD	50 mg BID·	100 mg BIDα
			Day 1			
No. of Patients	**4**	**3**	**5**	**5**	**5**	**6**
T_{max} (h)	8.0 ± 2.8	0.7 ± 0.3	0.6 ± 0.3	0.6 ± 0.4	0.6 ± 0.3	0.7 ± 0.3
C_{max} (ng/mL)	271 ± 20	1757 ± 822	3293 ± 1105	4942 ± 1761	1060 ± 265	1958 ± 624
$AUC_{0-\infty}$ (ng h/mL)	NC	4584 ± 1656	8568 ± 1321	13302 ± 3622	2710 ± 722	4872 ± 1897
$t_{1/2}$ (h)ß	NC	1.2 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	1.2 ± 0.2	1.1 ± 0.1
CL/F (L/h)	NC	24.0 ± 9.2	23.8 ± 3.6	20.8 ± 6.0	19.4 ± 4.6	23.3 ± 9.3
Vz/F (L)	NC	43.6 ± 19.6	42.3 ± 17.2	31.8 ± 8.5	34.2 ± 8.3	36.4 ± 11.0
Time > 100 ng/mL (h/day)	21.8 ± 1.8	5.9 ± 0.5	7.0 ± 1.9	7.5 ± 1.5	10.6 ± 2.2	11.2 ± 2.4
			Day 22			
No. of Patients	**4**	**2**	**4**	**4**	**6**	**7**
T_{max} (h)	4.5 ± 1.0	0.5	0.8 ± 0.3	0.8 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
C_{max} (ng/mL)	319 ± 41	1702	3432 ± 1169	5279 ± 1850	921 ± 251	1677 ± 465
C_{min} (ng/mL)	133 ± 30	0	0 ± 0	1 ± 2	2 ± 1	3 ± 3
AUC_{0-T} (ng h/mL)	5811 ± 461	3807	8934 ± 680	14539 ± 7033	2575 ± 234	4214 ± 1311
$t_{1/2}$ (h)ß	NC	1.1	1.1 ± 0.1	1.0 ± 0.2	1.2 ± 0.3	1.1 ± 0.1
CL/F (L/h)	17.3 ± 1.4	27.0	22.5 ± 1.8	20.8 ± 8.5	19.5 ± 1.8	26.0 ± 9.4
Vz/F (L)	NC	41.2	35.4 ± 3.9	28.9 ± 7.1	36.8 ± 10.7	39.7 ± 10.7
Time >100 ng/mL (h/day)	24.0 ± 0.1	5.4	6.8 ± 0.6	7.6 ± 1.8	12.4 ± 3.0	11.4 ± 2.0

Abbreviations: CI, continuous infusion; QD, once daily; BID, twice daily; NC, not calculated; T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; C_{min}, minimum concentration; AUC, area under the plasma concentration-time curve from time 0 to infinity (∞) or dosing interval (τ); $t_{1/2}$, elimination half-life; CL/F, apparent clearance; Vz/F, apparent volume of distribution.
α Parameter estimates are for the morning dose.
ß Harmonic mean ± pseudo-standard deviation.
ABT-510 was administered subcutaneously.

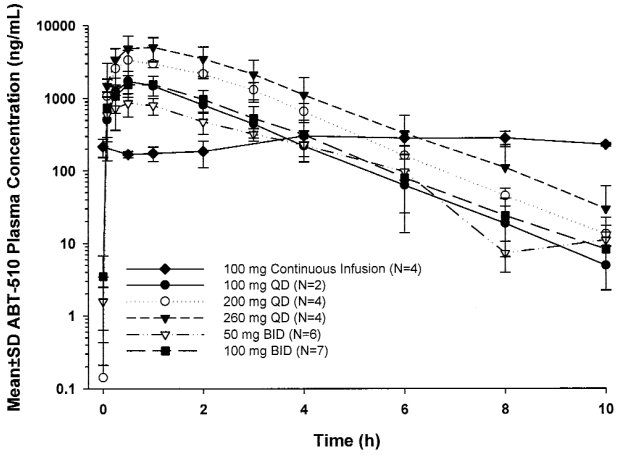
transient ischemic attack occurred in a 53-year-old woman with an advanced leiomyosarcoma receiving ABT-510 260 mg once daily. The patients had received extensive prior therapy. She experienced motor aphasia and facial nerve palsy at the clinic, prior to dosing for pharmacokinetic sampling on day 22 of cycle 1. She had no history of cardiovascular events, hypertension, diabetes mellitus or hyperlipidemia, but she used oral anticonceptives. The brain MRI and electrocardiogram were normal. Additional laboratory investigations revealed a platelet count of $709x10^9$/L, slightly elevated D-dimers and normal antithrombin III and aPTT. The

Figure 2. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 at Day 1 After Subcutaneous Continuous Infusion and Bolus Injection Doses. For the Twice-daily Curves, Data Shown are the Data Following the Morning Dose of ABT-510.



Abbreviations: QD, once daily; BID, twice daily.

Figure 3. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 at Day 22 After Subcutaneous Continuous Infusion and Bolus Injection Doses. For the Twice-Daily Curves, Data Shown are the Data Following the Morning Dose of ABT-510.



Abbreviations: QD, once daily; BID, twice daily.

Table 5. Serum bFGF, VEGF and IL-8 Concentrations Before and on Days 22 and 56 of ABT-510 Treatment (values, pg/mL).

Total daily dose of ABT-510		Day 1		Day 22		Day 56	
	No. of Patients	Median	Range	Median	Range	Median	Range
bFGF#							
100 mg	11	10.9	0.5-77.7	6.7	0.2-31.6	1.3	0.2-6.2
200 mg	14	9.4	2.2-59.6	9.7	4.6-16.6	4.0	0.2-29.4
260 mg	5	22.8	5.4-27.3	10.0	6.3-52.7	6.9	1.0-12.2
All	30	14.1	0.5-77.7	9.7	0.2-52.7$^{\alpha}$	3.2	0.2-29.4^{ß}
VEGF							
100 mg	11	384.4	128.5-894.7	583.4	135.4-1851	443.6	172.5-1063.6
200 mg	10	371.8	92.5-694.6	455.5	138-905.8	569.6	241.8-2478
260 mg	4	377.2	153.3-1882.8	1033.2	340.3-1363.4	687.7	475.9-899.5
All	25	377.9	92.5-1882.8	583.4	135.4-1851	497.1	172.5-2478
IL-8							
100 mg	9	19.4	8.8-49	42.2	5.2-135	26.4	10.1-118
200 mg	8	20.4	5.7-105	24.9	12.2-218	23.5	0.5-176
260 mg	6	21	5.4-41.1	13.6	0.5-55.5	27.7	24.5-59.4
All	23	19.4	5.4-105	32.8	0.5-218	26.4	0.5-176

Abbreviations: bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; IL-8, interleukin-8.
α *P* vs day 1 = 0.07.
ß *P* vs day 1 = 0.003.

elevated platelet count was already present at baseline. She was treated with an oral platelet aggregation inhibiting agent and subcutaneous low molecular weight heparin. As a relation with ABT-510 could not be ruled out, this drug was discontinued. In the following months no new cardiovascular events were observed. Both platelets and D-dimers remained elevated after discontinuation of ABT-510 and were considered related to the advanced malignancy.

A 54-year-old male patient with an advanced liposarcoma receiving ABT-510 100 mg twice daily was diagnosed with diabetes mellitus and grade 4 hyperglycemia on day 7 of cycle 1. The patient was known to have lung and bone metastases and an infiltrating mass in the pancreatic region. No other risk factors for diabetes mellitus were present. The patient was treated with subcutaneous insulin twice daily and continued with ABT-510 injections for 2 cycles. The diabetes was most likely caused by an infiltrating mass in the pancreatic region, although a relationship with ABT-510 could not be ruled out.

Figure 4. Individual Patient Serum Basic Fibroblast Growth Factor (bFGF) Concentrations (pg/mL). Solid Lines, Subjects with Long-Term Stable Disease Defined as ≥ 6 Cycles; Dotted Lines, Subjects without Long-Term Stable Disease.

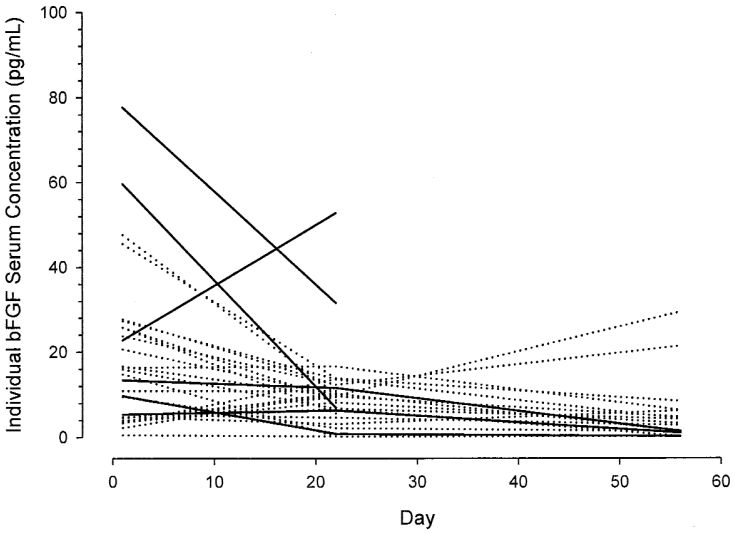


Figure 5. Individual Patient Serum Vascular Endothelial Growth Factor (VEFG) Concentrations (pg/mL). Solid lines, Subjects with Long-Term Stable Disease Defined as ≥ 6 Cycles; Dotted Lines, Subjects without Long-Term Stable Disease.

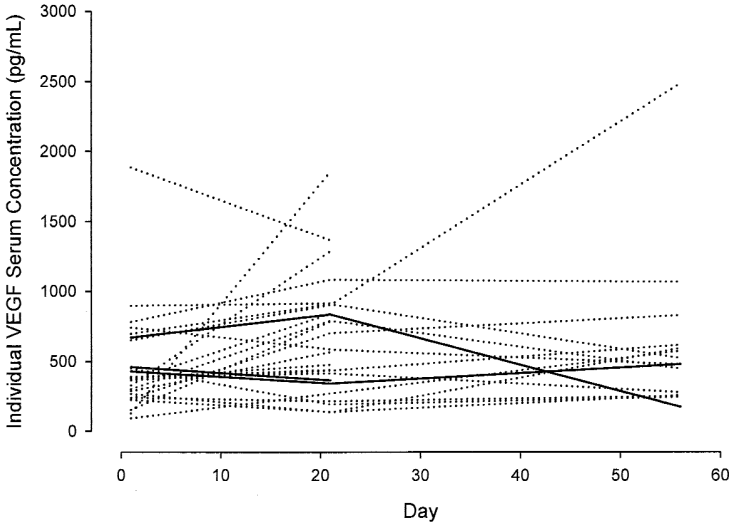
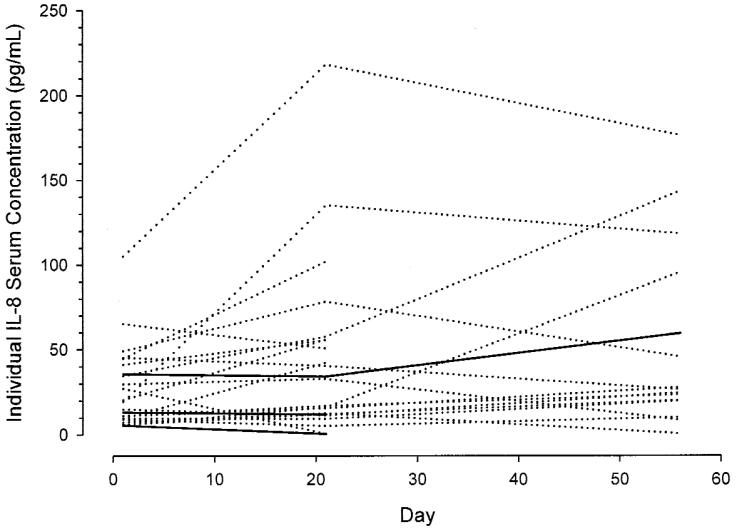


Figure 6. Individual Patient Serum Interleukin-8 (IL-8) Concentrations (pg/mL). Solid Lines, Subjects with Long-Term Stable Disease Defined as ≥ 6 Cycles; Dotted Lines, Subjects without Long-Term Stable Disease.



Pharmacokinetics Plasma sampling for pharmacokinetic studies was performed on day 1 of the first cycle in 38 patients and on day 22 in 36 patients. As plasma concentration data were not available at all time points from some subjects, a complete pharmacokinetic analysis could be performed using only 28 subjects on day 1 and 27 subjects on day 22. The values for the pharmacokinetic parameters after dosing as continuous infusion or subcutaneous bolus injections are summarized in Table 4. The ABT-510 plasma concentration-time curves on days 1 and 22 for all dose levels studied are shown in Figures 2 and 3, respectively.

The continuous infusion cohort achieved a steady-state ABT-510 concentration of 242 ± 19 ng/mL on day 22 (N=4). Following subcutaneous bolus injection, ABT-510 was rapidly absorbed and eliminated with overall (N=47) values of 0.7 ± 0.3 hour for T_{max}, 22.5 ± 6.5 L/hour for clearance (CL/F), 36.8 ± 10.8 L for apparent volume of distribution (Vz/F) and 1.1 ± 0.2 hour for $t_{1/2}$; these appeared to be similar across all the bolus injection regimens and across days 1 and 22. ABT-510 plasma concentrations did not accumulate with the once-daily or twice-daily dosing regimens.

Pharmacokinetic evaluation of the major metabolite M-1 showed that the continuous infusion regimen produced a steady-state concentration of 273 ± 66 ng/mL on day 22 (N=4). Following subcutaneous bolus injections, the overall mean $t_{1/2}$ of M-1 was 2.9 hours; this was similar across all dosing groups for both days 1 and 22. On average, 58 ± 19% (N=10) of the ABT-510 dose was recovered in the urine as M-1 and approximately 1% was recovered as unchanged drug. The percent of dose excreted in the urine as M-1 was similar following the

morning and evening doses in the groups dosed twice daily. There was no consistent trend across doses or days in the percent of dose excreted as M-1.

Pharmacodynamics Serum bFGF, VEGF and IL-8 results are presented in Table 5 and Figures 4-6. Median serum bFGF concentrations decreased between days 1 and 56 ($P = 0.003$), while median serum VEGF and IL-8 concentrations did not change significantly. The duration of treatment was not correlated with the change in serum concentrations of bFGF. Changes in serum concentrations of bFGF did not correlate with changes in serum concentrations of VEGF and IL-8 and the changes in serum concentrations of bFGF, VEGF or IL-8 over time were not correlated across individuals with ABT-510 dose, C_{max} or AUC values.
Urine concentrations of bFGF and VEGF did not change significantly (N=23), nor did they correlate with changes in serum concentrations of bFGF. Median urine concentrations of bFGF on days 1 and 56 were 0.1 (range, 0.1-0.7) and 0.2 (range, 0.1-0.8) ng/g creatinine ($P = 0.99$), respectively, and urine concentrations of VEGF on days 1 and 56 were 41 (range, 4-412) and 48 (range, 1-222) ng/g creatinine ($P = 0.32$), respectively.

Antitumor activity There were no partial or complete responses observed. Stable disease lasting more than 2 cycles was seen in 13 patients, with 6 of them experiencing stable disease lasting ≥ 6 cycles. One female patient with a recurrent angiosarcoma on the upper left leg, who developed new metastatic skin lesions every week prior to the start of ABT-510, had a period of stable disease without development of new lesions for 10 cycles. One male patient with renal cell cancer, who had bone metastases and three new primary tumors and/or metastases in the other kidney, experienced stable disease for 17 cycles. One male patient with a mixoid chondrosarcoma and multiple lymph node and pulmonary metastases has been treated for 19 cycles without clinical and radiological signs of progression. There was no apparent relationship between the occurrence of prolonged stable disease and the ABT-510 dose or treatment schedule.

DISCUSSION

This phase I, two-center, open-label, multiple dose-escalation study of ABT-510 is the first clinical study in cancer patients with an agent that mimics the naturally occurring angiogenesis inhibitor TSP-1. This study demonstrates that ABT-510 has linear, time-independent pharmacokinetics and a favorable toxicity profile.
In preclinical mouse tumor models, ABT-510 inhibits tumor growth and metastasis formation, but does not induce tumor regression. Efficacious doses range from 0.1 mg/kg/day to 200 mg/kg/ day depending on tumor model and mode of administration (intraperitoneal, subcutaneous bolus or continuous infusion). A limited number of tumor regressions, along with prolonged disease stabilization, were observed in a study involving tumor bearing companion dogs evaluating ABT510 at doses of 0.5 mg/kg/day twice daily. Regressions were observed in dogs

with soft tissue sarcomas, epithelial tumors and lymphomas [12]. These composite preclinical data, suggest that ABT-510 would primarily exhibit cytostatic activity with occasional tumor responses. In addition, ABT-510 exhibited a favorable safety profile in preclinical efficacy and toxicology studies, with dose limiting renal toxicity being observed at a dose of 75 mg/kg/day in rats. Based upon the low likelihood of observing tumor responses with ABT-510, particularly in a phase I study population, and the potential that an MTD would not be defined or that the efficacious dose would be significant lower than the MTD, potential biomarkers for antitumor activity were being looked for in order to assist in the selection of an optimal biological active phase II dose.

A potential pharmacokinetic endpoint was defined as a plasma concentration exceeding 100 ng/mL for at least 3 hours per day, as preclinical models had shown that this "time over threshold" produced 75% of maximally observed efficacy in 11 different models [8]. This finding is consistent with in vitro studies that demonstrate an exposure > 20 nM (~20 ng/mL) for approximately 4 hours is required to induce Fas ligand expression in endothelial cells (unpublished data). Increases in time over threshold beyond 3 hours, as well as further increases in overall exposure (AUC) correlated with increased efficacy in some models. With all doses of ABT-510 administered by bolus subcutaneous injection, the pharmacokinetic target of 100 ng/mL for at least 3 hours per day was achieved (Table 4). Based on the concept of time over threshold, it is obvious that increasing the frequency of injections from once daily to twice daily results in more prolonged time above threshold than doubling the dose. The 100 mg twice daily dose regimen, for example, results in approximately 11 hours above the threshold of 100 ng/mL compared to approximately 7 hours for the 200 mg once daily dose regimen. For this reason twice daily administration is being recommended for evaluation in phase II studies. It remains to be seen, however, whether time above threshold will correlate with clinical outcome in humans and can be used as a surrogate endpoint.

Dose escalation was halted at 260 mg/day once daily because this involved 2 injections of 1.3 mL, the predefined maximum volume and number of injections per day. Also, the incremental exposure over the target threshold projected for increased doses was determined to be of modest relative value. This defined maximum dose is relative since, even at this volume of injection, the toxicities generally were mild and the local skin reactions were acceptable. Based on this study, the recommended phase II dose of ABT-510 is 100 mg twice daily subcutaneously, although additional evaluation of the dose-response effect (dose-ranging) with ABT-510 might be of value.

Dose escalation in the 24-hour continuous subcutaneous infusion arm was discontinued at 100 mg/day due to the skin toxicity observed at the injection site in the 4 subjects treated with this regimen. Although the skin infiltrates were not dose limiting according to the defined criteria, further dose escalation was not considered feasible. The pharmacokinetic data from this dosing cohort also predicted drug exposure < 100 ng/mL with a 50% dose reduction, therefore, no further administration by this dosing method was pursued.

The evaluation of ABT-510 effects on serum markers of angiogenesis such as VEGF, bFGF and IL-8 was performed in this phase I study strictly as an exploratory exercise to investigate their value as potential biomarkers. The evaluation of serum angiogenesis markers identified a significant decrease in median serum bFGF levels comparing day 1 with day 56. VEGF and IL-8 levels did not show significant changes. The cause of the observed bFGF decrease remains speculative. It could be related to direct effects of ABT-510 and/or changes in tumor status. However, the clinical significance of circulating bFGF in relation to tumor status is still controversial [13]. The fact that no changes in VEGF levels were observed during treatment with ABT-510 is consistent with preclinical studies showing that inhibition of carcinogenesis and angiogenesis by TSP-1 was not due to changes in VEGF expression, receptor binding or receptor activation [14]. Associations between bFGF, VEGF, IL-8 and the angiogenic state in cancer patients are complex, as this state is controlled by the angiogenic switch in such a way that predominance of inducers results in angiogenesis and predominance of inhibitors results in vascular quiescence [15]. It is yet far from clear whether serial measurements of these proangiogenic factors during treatment with an angiogenesis inhibitor will be useful as a marker of their activity. In previous studies with other angiogenesis inhibitors, monitoring of urinary or plasma VEGF and bFGF provided no significant information [16-19].

No tumor regressions were observed among the patients treated with ABT-510 in this study; this was not unexpected based on the preclinical efficacy profile. However, in a number of patients prolonged stable disease was observed, of which six patients had stable disease for more than 6 cycles; tumor types included sarcoma (N=2), renal cell carcinoma, cervix carcinoma, colorectal carcinoma and germ cell tumor. Although from this uncontrolled trial setting it cannot be concluded whether this is a drug effect or due to indolent growth patterns, these data potentially add to the fact that relevant plasma concentrations were achieved.

Currently, ABT-510 is being tested in phase II studies, either as single agent or in combination with cytotoxic chemotherapy, in patients with soft tissue sarcoma, renal cell cancer, lymphoma and non-small cell lung cancer. Several of these trials are randomized, comparing different dosages of ABT-510. The absence of cytochrome P-450 interactions and the favorable safety profile make ABT-510 well-suited for combination therapy, both with chemotherapeutic agents and with other anti-angiogenic agents. Several pre-clinical models have demonstrated that both elevated VEGF expression and down-regulation of TSP-1 are necessary for tumor angiogenesis [20,21]. Blockade of pro-angiogenic signaling accompanied by simultaneous augmentation of a suppressed inhibitory signal may be an attractive approach to inhibit tumor angiogenesis. Pre-clinical studies evaluating this approach have been initiated. Phase I studies combining ABT-510 with several standard chemotherapy regimens (5-fluorouracil/ leucovorin and cisplatin/gemcitabine) have recently been completed. The combinations appeared feasible without pharmacokinetic interactions and without additional toxicity [22].

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ABSTRACT

Purpose: *We performed a phase I study with the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 combined with 5-fluorouracil and leucovorin (FU/LV) to determine safety profile and assess pharmacokinetic interactions.*

Patients and Methods: *Patients with advanced solid malignancies received LV 20 mg/m² followed by FU 425 mg/m² both administered intravenously in 15 minutes daily for 5 days every 4 weeks. ABT-510 was administered subcutaneously twice daily continuously from day 2 onwards. Blood and urine samples for pharmacokinetic analyses were collected at days 1, 5 and 22.*

Results: *Twelve patients received a total of 45 cycles of FU/LV combined with ABT-510. ABT-510 dose levels studied were 50 and 100 mg. The combination was well tolerated, with a toxicity profile comparable to that of FU/LV alone. At the dose levels studied no significant pharmacokinetic interactions were observed.*

Conclusion: *These data indicate that ABT-510 administered twice daily subcutaneously can be safely combined with FU/LV administered daily for 5 days, every 4 weeks.*

CHAPTER 6

PHASE I STUDY OF THE THROMBOSPONDIN-1-MIMETIC ANGIOGENESIS INHIBITOR ABT-510 WITH 5-FLUOROURACIL AND LEUCOVORIN: A SAFE COMBINATION

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INTRODUCTION

ABT-510 is a new angiogenesis inhibitor derived from the naturally occurring angiogenesis inhibitor thrombospondin-1. It is a parenterally available nonapeptide with potent in vitro and in vivo antitumor activity [1,2]. In two phase I studies exploring prolonged continuous administration of ABT-510 in patients with advanced solid malignancies, either in a once- or twice-daily subcutaneous administration schedule, ABT-510 was devoid of dose-limiting toxicities. Only mild toxicities mainly consisting of injection site reactions and fatigue were observed [3,4]. In one patient with a leiomyosarcoma a partial remission was observed and in a significant number of patients prolonged disease stabilization was observed. Plasma pharmacokinetics were linear across the dose ranges tested, without signs of drug accumulation following prolonged administration. Daily doses of twice daily 10 mg or above yielded plasma concentrations exceeding concentrations active in preclinical models and were maintained for several hours per day [2-4]. Currently, single agent phase II studies are being performed with ABT-510 in patients with renal cell carcinoma, soft tissue sarcoma and lymphoma.

Generally, angiogenesis inhibitors are expected to yield tumor growth inhibition rather than tumor shrinkage. Theoretically, the best antitumor yield is expected to occur in case of minimal tumor load, e.g. in the adjuvant setting following surgical and or radiotherapeutic treatment or in a situation of minimal residual disease following optimal cytoreductive treatment. In addition, combining cytotoxic agents with angiogenesis inhibitors has meanwhile proven to be an attractive approach [5-7].

We choose to explore a combination of ABT-510 administered subcutaneously and 5-fluorouracil and leucovorin (FU/LV). The combination of FU/LV has been used frequently for adjuvant treatment of node positive colorectal cancer and treatment of advanced colorectal cancer. Toxicity is usually mild with stomatitis, diarrhea and leukopenia being the most frequent reported adverse events [8,9]. In the present study we explored a combination of twice daily subcutaneous administration of ABT-510 continuously with short intravenous infusions of FU/LV administered daily for 5 days every 4 weeks (Mayo Clinics Regimen [8]), to establish the safety profile of this combination and exclude clinically relevant pharmacokinetic interactions.

PATIENTS AND METHODS

Eligibility criteria Patients with a histologically confirmed diagnosis of an advanced solid malignancy for whom standard therapy options did not exist or for whom the combination FU/LV was considered an appropriate treatment were eligible. Additional eligibility criteria included: age ≥ 18 years; World Health Organization (WHO) performance status ≤ 2; an estimated life expectancy of ≥ 3 months; no radiotherapy, chemotherapy or hormonal therapy within 4 weeks before study start with the exception of small field radiation; ability to receive subcutaneous injections of the study drug. Specific exclusion criteria included: a known

human immunodeficiency virus positive status, a diagnosis of primary brain tumor or known central nervous system metastases; evidence of uncontrolled clinically significant disease unrelated to the primary malignancy. The study was approved by local ethics boards of the two participating centers and all patients gave written informed consent.

Drug administration The FU/LV was administered intravenously as short infusions daily for 5 days, every 4 weeks. LV was administered over 15 minutes at a dose of 20 mg/m^2 dissolved in 100 mL 0.9% saline, followed by FU over 15 minutes at a dose of 425 mg/m^2 dissolved in 100 mL 0.9% saline. ABT-510 (Abbott Laboratories, Chicago, IL, USA) administered twice daily subcutaneously was given from day 2 onwards, continuously. ABT-510 was supplied in vials containing 1.1 mL ABT-510 (100 mg/mL) or 0.75 mL ABT-510 (80 mg/mL) dissolved in dextrose 5%. The vials were stored at 2-8 ^{0}C and brought to room temperature 1 hour before dosing. Patients injected themselves subcutaneously preferably at the same time in the morning and evening with an interval of approximately 12 hours. The starting dose of ABT-510 was 50 mg twice daily, based upon safety and pharmacokinetic data obtained in single agent phase I studies with ABT-510 [3,4]. No adjustments for body surface area or weight were made. Cohorts of six patients were studied.
Dose-limiting toxicity (DLT) was defined as any grade 3 or 4 adverse event (except inadequately treated nausea or vomiting) or grade 2 adverse event requiring dose modification or treatment delay possibly or probably related to ABT-510 and occurring in the first treatment cycle (i.e. 4 weeks). Escalation of ABT-510 dose was pursued until either maximum tolerated dose (MTD) was identified or the highest recommended dose from the single agent study was reached (twice daily 100 mg). There was no dose escalation within an individual patient. The MTD was defined as the highest dose of ABT-510 given for at least one treatment cycle during which no more than one of six patients would experience DLT.

Pretreatment and follow-up studies Before therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell count, including white blood cell differential, reticulocyte count, mean corpuscular volume, mean corpuscular hemoglobin, and serum biochemistry which involved sodium, potassium, chloride, magnesium, bicarbonate, creatinine, blood urea nitrogen, albumin, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase, total bilirubin, calcium, phosphate, glucose, alkaline phosphatase and amylase were performed, as were prothrombine time, activated partial tromboplastin time, urinalysis, electrocardiogram and chest X-ray. Plasminogen, fibrinogen and factor VIII were collected at screening and thereafter only when clinically indicated. In addition 24 hours urine for albumin excretion was collected at screening and at day 22. Weekly evaluations during the first treatment cycle included history, physical examination, toxicity assessment according to National Cancer Institute Common

Toxicity Criteria (NCI-CTC) version 2.0, complete blood count, prothrombin time, activated partial thromboplastin time and serum biochemistry. The same evaluations were also performed after 2 cycles and every 2 cycles thereafter. Additional visits were allowed at the discretion of the responsible physicians. Tumor measurements were performed every 2 cycles. Response was assessed using the World Health Organization (WHO) criteria for response [10]. Patients were allowed to continue treatment of both FU/LV and ABT-510 in the absenceof progressive disease or unacceptable toxicity. In case of discontinuation of FU/LV due to toxicity, patients were allowed to continue treatment with ABT-510, provided there were no signs of disease progression.

Pharmacokinetic sampling and assays For pharmacokinetic analysis of FU 4.5-mL blood samples were collected in EDTA containing tubes from an indwelling intravenous canula contralateral to the site of the FU infusion. Samples were collected before infusion of FU, at the end of the FU infusion and 5, 15, 30 minutes and 1, 2 and 4 hours following the infusion of FU on days 1 and 5 of cycle 1. Blood samples were immediately placed in an ice bath and plasma was separated by centrifugation. Plasma samples were stored in polypropylene tubes at -20 ^{0}C until analysis. The analyzing method of FU and its metabolite 5,6-dihydrofluorouracil (FUH_2) has recently been described [11]. The lower limits of quantification in plasma for FU and FUH_2 were 0.040 μg/mL and 0.075 μg/mL, respectively. Blood samples (4.5 mL) for pharmacokinetic analysis of ABT-510 were collected in EDTA containing tubes before dosing of ABT-510 and 5, 15, 30 minutes and 1, 2, 4, and 8 hours following the morning dose of ABT-510 on days 5 and 22 of cycle 1. On day 5 the morning dose of ABT-510 was administered about 30 to 60 minutes before the start of the infusion of LV. After collection blood samples were placed on ice and plasma was separated by centrifugation after which plasma samples were stored in polypropylene tubes at -20 ^{0}C until analysis. Plasma concentrations of ABT-510 and its major metabolite M-1 were determined using a validated liquid chromatography with mass spectrometry (LC/MS/MS) method described previously [3]. The lower limits of quantification in plasma for ABT-510 and major metabolite M-1 were 0.5 ng/mL and 3 ng/mL, respectively.
Urine for pharmacokinetic analysis of ABT-510 was collected on days 1, 5 and 22 of treatment cycle 1. Two samples of 15 mL were collected immediately before dosing of FU/LV on day 1 for the base-line drug assay. Urine was sampled from 0 to 12 and 12 to 24 hours after dosing of ABT-510 on days 5 and 22. The urine samples were refrigerated during the collection period and total volumes were measured. Two 15-mL samples from each sampling period were stored at -20 ^{0}C until analysis. For measurement of urine concentrations of ABT-510 and metabolite M-1, 200 μL of urine was processed according to previously described methods [3]. The lower limits of quantification in urine for ABT-510 and metabolite M-1 were 6 ng/mL and 99 ng/mL, respectively.

Pharmacokinetic analysis Noncompartmental methods were used to determine values of pharmacokinetic variables of FU, FUH_2, ABT-510 and metabolite M-1 using WinNonlin-Pro, version 4.1 (Pharsight Corporation, Cary, NC, USA). The peak plasma concentration and time of peak plasma concentration were reported as C_{max} and T_{max}, respectively. The value of the terminal elimination rate constant (ß) was obtained from the slope of the linear regression of the logarithms of the plasma concentration versus time data from the terminal log-linear phase of the profile. The terminal log-linear phase was identified using WinNonlin-Pro and visual inspection. The terminal phase elimination half-life ($t_{1/2}$) was calculated as (ln2/ß). The area under the plasma concentration-time curve over an ABT-510 dosing interval ($AUC_{0\text{-}12h}$) was calculated by the linear trapezoidal rule, while the concentration at 12h was set equal to the predose concentration. The AUC from zero to infinite time ($AUC_{0\text{-}\infty}$) was calculated by adding AUC from time zero to the time of the last measurable concentration to AUC_{ext}, where AUC_{ext} was calculated by dividing the last measurable concentration by ß. Clearance (CL) or apparent clearance (CL/F) was calculated by dividing the dose by the AUC, and the volume of distribution (Vz) or apparent volume of distribution (Vz/F) was calculated by dividing CL or CL/F by ß. The fraction of the dose recovered in urine as ABT-510 and M-1 was calculated as the amount recovered in urine over the dosing interval divided by the dose. The amount of M-1 recovered in urine was converted to equivalent ABT-510 amount by multiplying by the ratio of molecular weights (994/501).

Statistics The sample size was based on clinical justification and patient numbers historically used for testing of new anti-neoplastic compounds. In models for the analysis of safety data, dose was treated as a factor with discrete levels or as a continuous variable. For the pharmacokinetic analysis, descriptive statistics of parameters were determined with a breakdown by regimen and dose level on days 1, 5 and 22.

RESULTS

Twelve patients, whose characteristics are listed in Table 1, received a total of 45 cycles (median 4, range 1-7) of FU/LV in combination with ABT-510. All patients were evaluable. ABT-510 dose levels studied were twice daily 50 mg (6 patients) and twice daily 100 mg (6 patients).

Toxicity The frequency of grade 3 and 4 adverse events is listed in Table 2. The frequency of grade 3 and 4 adverse event is considered as normal for administration of FU/LV at this dose in a daily-times-five schedule. None of these adverse events were considered to be possibly or probably related to ABT-510, with the exception of one episode of atrial fibrillation in a patient with rectal carcinoma. The patient was hospitalized on day 11 of the first cycle with febrile neutropenia and grade 2 mucositis and treated with broad spectrum antibiotics. The

Table 1. Patient Characteristics

Patient Characteristic	No. of Patients
Male / Female	9/3
Age (years)	
Median	54
Range	47-78
WHO Performance status	
0	5
1	7
Prior chemo/immuno/hormonal therapy	
0-3 prior regimens	10
> 4 prior regimens	2
Prior radiotherapy	7
Tumor type	
Colorectal carcinoma	2
Head and neck carcinoma	2
Renal cell carcinoma	2
Non-small cell lung carcinoma	1
Esophageal carcinoma	1
Carcinoma unknown primary	1
Colorectal and ampulla of Vater carcinoma	1
Synovial sarcoma	1
Thymus carcinoma	1

Table 2. Grade 3 and 4 Adverse Events Observed During the Study, Number of Patients (%)

Adverse Event	ABT-510 Dose (Twice Daily)		
	50 mg (n=6)	**100 mg (n=6)**	**All Doses (n = 12)**
Diarrhea	1 (17%)	1 (17%)	2 (17%)
Fatigue	0	1 (17%)	1 (8%)
Atrial Fibrillation	1 (17%)	0	1 (8%)
Dyspnea	0	1 (17%)	1 (8%)
Bilirubinemia	1 (17)	0	1(8%)
Transaminase	1 (17%)	1 (17%)	2 (17%)
Hyperglycemia	0	1 (17%)	1 (8%)
Neutropenia	4 (67%)	4 (67%)	8 (67%)

Table 3. Pharmacokinetic Parameters (Mean ± SD) of FU and FUH2 Without (Day 1) and With (Day 5) Co-administration of ABT-510 Following Administration of FU Over 15 Minutes at a Dose of 425 mg/m^2

Parameters	**FU**		**FUH2**	
	Day 1	**Day 5**	**Day 1**	**Day 5**
No of patients	**12**	**12**	**12**	**12**
T_{max} (h)#	0.25 ± 0.04	0.28 ± 0.05α	0.61 ± 0.15	0.86 ± 0.52
C_{max} (μg/mL)	22.2 ± 3.5	22.2 ± 6.6	3.7 ± 0.9	2.9 ± 0.6,
$AUC_{0-\infty}$ (μg h/mL)	7.9 ± 1.6	9.2 ± 2.7	6.0 ± 1.8	5.6 ± 1.6
$t_{1/2}$ (h)§	0.1 ± 0.0	0.2 ± 0.1ß	0.8 ± 0.1	0.8 ± 0.1
CL (L/h/m^2)	56.2 ± 11.9	50.2 ± 16.0	NC	NC
Vz (L/m^2)	11.6 ± 2.7	14.3 ± 5.7	NC	NC

Abbreviations: T_{max}, time to peak plasma concentration; C_{max}, peak plasma concentration; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; $t_{1/2}$, elimination half-life; CL, clearance; Vz , volume of distribution; NC, not calculated.
§ Harmonic mean ± pseudostandard deviation.
α $P < 0.05$.
ß $P < 0.01$.

Table 4. Pharmacokinetic Parameters (Mean ± SD) of ABT-510 With (Day 5) and Without (Day 22) Co-administration of FU/LV

Parameter	**ABT-510 Dose**			
	50 mg BID		**100 mg BID**	
	Day 5	**Day 22**	**Day 5**	**Day 22**
No. of Patients	**6**	**6**	**6**	**6**
T_{max} (h)#	0.67 ± 0.26	0.67± 0.38	0.50 ± 0.27	0.75 ± 0.27
C_{max} (ng/mL)	997 ± 493	1181 ± 366	1877 ± 513	1789 ± 708
AUC_{0-12} (ng h/mL)	2204 ± 885	2445 ± 705	5137 ± 1332	5338 ± 2077
$t_{1/2}$ (h)§	1.0 ± 0.1	1.0 ± 0.2	1.1 ± 0.3	1.3 ± 0.4
CL/F (L/h)	25.8 ± 10.3	21.9 ± 6.3	20.8 ± 6.4	20.8 ± 6.7
Vz/F (L)	36.1 ± 12.0	33.1 ± 6.0	33.5 ± 12.0	42.8 ± 22.0

Abbreviations: Tmax, time to peak plasma concentration; Cmax, peak plasma concentration; AUC0-12, area under the plasma concentration-time curve over a dosing interval (from time 0 to 12 hours); $t_{1/2}$, elimination half-life; CL/F, apparent clearance; Vz/F, apparent volume of distribution.
§ Harmonic mean ± pseudostandard deviation.

Figure 1. Plasma Concentration-Time Profiles (Mean ± SD) of FU and Metabolite FUH2 Without (Day 1) and With (Day 5) Co-administration of ABT-510 (Either ABT-510 Dose Levels, 12 Patients).

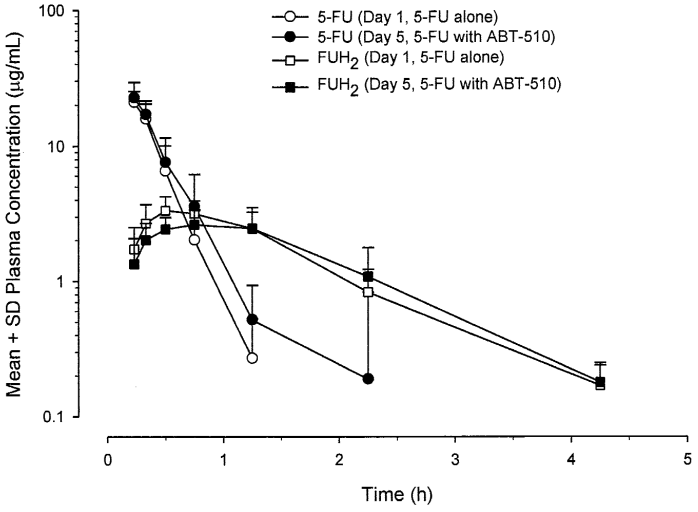


Figure 2. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 and Metabolite M-1 With (Day 5) and Without (Day 22) Co-administration of FU/LV Following the Morning Dose of ABT-510 50 mg BID (6 Patients).

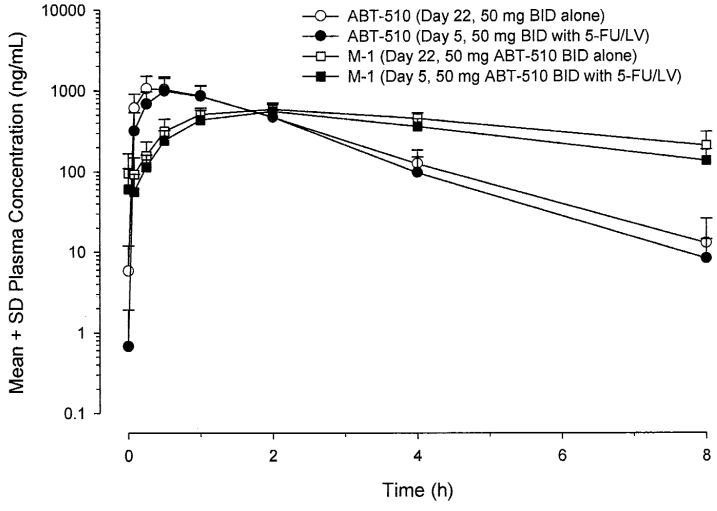
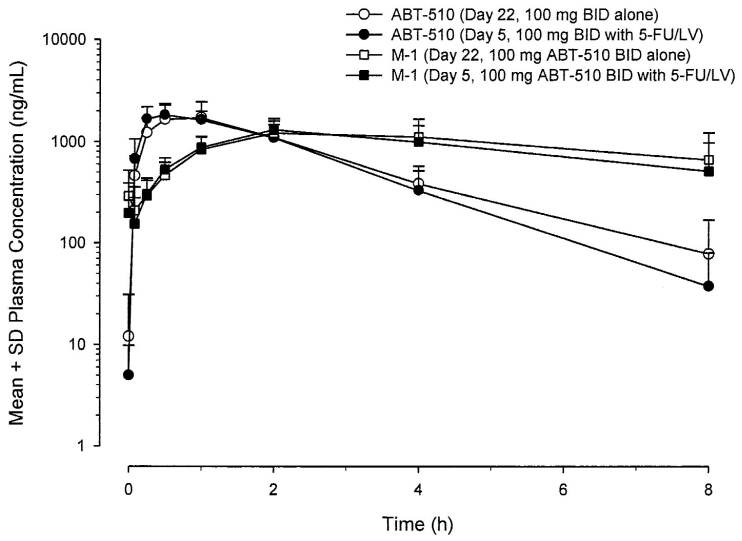


Figure 3. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 and Metabolite M-1 With (Day 5) and Without (Day 22) Co-administration of FU/LV Following the Morning Dose of ABT-510 100 mg BID (6 Patients).



atrial fibrillation occurred on day 14 and was successfully treated with medication. Grade 1 or 2 adverse events possibly or probably related to ABT-510 and occurring in more than one patient were fatigue (25%), injection site reactions (33%) and dizziness (17%).

Pharmacokinetics Plasma samples for pharmacokinetic analysis of FU (days 1 and 5) and ABT-510 (days 5 and 22) were available from all patients, and results are summarized in Table 3 and 4. The pharmacokinetics of FU were largely unaffected by co-administration with ABT-510, including no significant effect on FU C_{max} or AUC (Table 3 and Figure 1). Similarly, ABT-510 pharmacokinetics were not affected by co-administration with FU (Table 4 and Figures 2 and 3), including the amount of ABT-510 and M-1 recovered in urine (data not shown). Together, these results indicate no pharmacokinetic interaction when ABT-510 is co-administered with FU at the doses studied.

Antitumor activity No tumor regressions were observed. Stable disease for more than 4 cycles was found in four patients (33%) diagnosed with non-small cell lung cancer, colorectal cancer, renal cancer and head and neck cancer. One patient with colorectal cancer withdrew consent after cycle 7 (28 weeks) and was shown to have stable disease at 30 weeks.

DISCUSSION

In this phase I study we explored the feasibility of combining the new angiogenesis inhibitor ABT-510 with FU/LV administered as a short intravenous infusion daily for 5 days every 4 weeks. It was shown that ABT-510 administered at doses of 50 and 100 mg twice daily subcutaneously (the recommended single agent doses) could be safely combined with this FU/LV regimen. The observed toxicity in this study was comparable to that following treatment with FU/LV alone [8,9]. Given the small number of patients in this study, we can obviously not exclude the possibility of rare side effects occurring due to this combination. Therefore further studies on the combination should still include close monitoring of toxicity. In addition to the clinical safety profile, there were no relevant pharmacokinetic interactions. Although it cannot be excluded that differences in T_{max} and $t_{1/2}$ of FU on day 5 compared to day 1 could have been due to an interaction with ABT-510, this observed differences are only very small, whereas overall drug exposures is even unchanged. The lack of pharmacokinetic interaction observed in this study is consistent with the different disposition pathways involved in the elimination of ABT-510 and FU/LV.

While we believe that FU/LV can be safely combined with the recommended single dose of ABT-510 given twice daily subcutaneously, a discussion could evolve as to which dose of ABT-510 to use in further clinical efficacy studies. Defining the optimal dose for angiogenesis inhibiting agents is challenging since the MTD frequently can not be assessed, due to a lack of severe toxicity. Surrogate endpoints such as circulating levels of pro-angiogenic factors (e.g. vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF)) and/or imaging assessments on changes in tumor blood flow could be helpful in determining the optimal biological dose. Unfortunately, neither of these methods has been validated so far. In our phase I study with single agent ABT-510 we observed a significant decrease of bFGF but no relevant changes in VEGF and interleukine-8 [3]. However, the changes of bFGF observed were independent of the ABT-510 dose and did not correlate with duration of treatment. For several other naturally occurring angiogenesis inhibitors animal tumor models suggested that antitumor and angiogenesis inhibiting activity may improve with continuous infusion compared with bolus administration [12,13]. For ABT-510, the preclinical models suggest that the efficacy following continuous subcutaneous infusions is similar compared to subcutaneous bolus doses. In addition, studies in 11 different murine antitumor models showed that 75% of maximal activity was reached when plasma concentrations exceeded 100 ng/mL for at least 3 hours a day [2]. Therefore, time over this 100 ng/mL threshold per day is the major element for selecting the optimal dose of ABT-510. This exposure could be reached with a dose of 10 mg twice daily or higher [3,4].

It is important to explore the various possible ways of using angiogenesis inhibitors. One way might be to use them in the adjuvant setting, after primary curative surgical resection. However, this will require long lasting large studies as well as more convincing data on activity

and more information on long term safety of ABT-510 than currently is available. In view of the currently obtained data it is also conceivable to explore the use in metastatic or advanced cancer to yield long lasting absence of progression. Yet another way would be using angiogenesis inhibitors after primary cytoreductive chemotherapy for advanced disease, to prolong the time to progression. Finally it is potentially worthwhile to combine angiogenesis inhibitors with cytotoxic treatment, the first evidence of which has lead to registration of bevacizumab, a recombinant humanized monoclonal antibody to VEGF, for treatment of metastatic colorectal cancer in combination with a FU containing regimen [5,6]. Combining FU/LV with ABT-510 is attractive because of different mechanisms of action, lack of overlapping toxicity and the practical feasibility of this combination. However, the use of such combinations would require special attention since recent experiences with several other angiogenesis inhibiting agents tested in combination with FU/LV revealed unexpected major toxicities. For example, SU5416, a VEGF receptor tyrosine kinase inhibitor, which was studied in a phase I/II setting combined with FU/LV [14]. While this study did not reveal any dose-limiting toxicity, a subsequent randomized phase III study of FU/LV with or without SU5416 in patients with metastatic colorectal cancer had to be terminated prematurely due to an unexpected high incidence of thromboembolic events in the SU5416 arm. A similar, albeit less pronounced observation was reported for bevacizumab combined with FU/LV [15]. Similar problems have not been encountered in our current small study. Importantly the rate of thromboembolic events with ABT-510 is minimal [3,4], contrasting the above experience with other angiogenesis inhibitors. Whether this relates to differences in mechanism of action is currently unknown.

In conclusion we found that ABT-510 administered twice daily subcutaneously continuously could be combined safely with FU/LV administered daily intravenously for 5 days every 4 weeks. Clinically relevant pharmacokinetic interactions were not observed. In view of the lack of additional toxicity, the ease of administration and the interesting clinical activity observed in single agent studies with ABT-510, additional efficacy studies with this combination seem warranted.

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ABSTRACT

Introduction: *To determine the safety profile, pharmacokinetics, and potential drug interactions of the angiogenesis inhibitor ABT-510 combined with gemcitabine-cisplatin chemotherapy in patients with solid tumors.*

Patients and Methods: *Patients with advanced solid tumors received gemcitabine 1250 mg/m^2 intravenously on days 1 and 8 and cisplatin 80 mg/m^2 intravenously on day 1 of a 3-week cycle in combination with ABT-510. ABT-510 was administered subcutaneously twice daily at doses of 50 mg or 100 mg starting on day 2 of cycle 1 and continuing until patient discontinuation. Plasma samples for pharmacokinetics were obtained on days 1 (gemcitabine, cisplatin as single agents), 15 (ABT 510 as single agent), and 22 (gemcitabine, cisplatin, ABT-510 as combination). Toxicity was scored according to NCI-CTC version 2.0 criteria. Antitumor activity was evaluated after every 2 cycles.*

Results: *Thirteen patients received ABT-510 as either 50 mg twice daily (7 patients) or 100 mg twice daily (6 patients) subcutaneous injections in combination with gemcitabine-cisplatin. The most common reported adverse events reflected the known toxicity profile induced by gemcitabine-cisplatin without ABT-510. One episode of hemoptysis occurred in a patient with non-small cell lung cancer (NSCLC) after 13 days of treatment. Complete pharmacokinetics data were available for 11 patients. No clinically significant pharmacokinetic interactions between ABT-510, gemcitabine, its metabolite 2-difluoro-2-deoxyuridine (dFdU), and total and unbound platinum were observed. Three partial responses were observed in 12 evaluable patients (1 head and neck cancer, 1 melanoma, and 1 NSCLC).*

Conclusion: *Combining ABT-510 with gemcitabine-cisplatin is feasible. Pharmacokinetic interactions were not observed, and adding ABT-510 does not appear to increase toxicity.*

CHAPTER 7

A PHASE I STUDY ASSESSING THE SAFETY AND PHARMACOKINETICS OF THE THROMBOSPONDIN-1- MIMETIC ANGIOGENESIS INHIBITOR ABT-510 WITH GEMCITABINE AND CISPLATIN IN PATIENTS WITH SOLID TUMORS

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Submitted

INTRODUCTION

Endothelial cells are an attractive target for anticancer therapy because of increasing evidence that the tumor vasculature is important for sustaining tumor growth and metastasis [1]. Inhibition of angiogenesis as anticancer therapy has been pursued for several years. The naturally occurring angiogenesis inhibitor thrombospondin-1 (TSP-1) is of particular interest, as it blocks multiple pro-angiogenenic factors [2,3], while it promotes endothelial cell apoptosis [4]. Normal tissue expression of TSP-1 inhibits neo-vascularization. Decreased expression of TSP-1, resulting from mutation or altered expression of tumor suppressors and oncogenes, is associated with increased angiogenesis. A downregulation of TSP-1 transcription in areas of tumorigenesis has been observed in many solid tumors [5].

ABT-510, a TSP-1-mimetic peptide, is a parenterally available nonapeptide analog of the anti-angiogenic properdin-repeat-region heptapeptide in which isoleucine has been replaced by a D-amino acid [6]. This substitution increases the in vitro anti-angiogenic activity of ABT-510 by 1,000 fold [3]. ABT-510 competes with TSP-1 for binding to endothelial cells. Subcutaneous (SC) administration of ABT-510 in murine models of different tumor types is effective in slowing tumor growth at doses as low as 3 mg/kg/day (Data on file Abbott Laboratories).

In 2 phase I studies conducted in cancer patients, ABT-510 administered as daily SC single agent injections showed no major toxicity [7,8]. The most common reported adverse events were fatigue, headache, insomnia, anorexia, nausea, rash, and injection site reactions. No maximum tolerated dose (MTD) was identified. The pharmacokinetic target of ABT-510 plasma concentrations of 100 ng/mL for more than 3 hours per day as determined from the murine efficacy models was achieved at a dose of 10 mg twice daily. By increasing the frequency of injections from a once- to twice-daily (BID) regimen, a longer time above threshold was achieved in comparison to doubling the dose. In the 2 phase I studies, ABT-510 administered as SC bolus injections induced long term stable disease in several patients and a partial response in a patient with sarcoma [7,8].

Advanced solid malignancies are commonly treated with cytotoxic drugs. However, drug resistance often leads to treatment failure. While monotherapy with angiogenesis inhibitors has shown a limited response rate [9-11] the combination of angiogenesis inhibitors with cytotoxic therapy remains an attractive strategy due to a potential increase in antitumor activity through synergy between the two treatment modalities [12]. For example bevacizumab, an anti-VEGF antibody, showed modest antitumor activity in patients with advanced renal cell cancer. However, in a phase III trial in metastatic colorectal cancer patients, the addition of bevacizumab to treatment with 5 fluorouracil, leucovorin, and irinotecan resulted in increased response rates and prolonged progression-free and overall survival [12,13].

This combination approach was investigated in the current phase I study in which ABT-510 treatment was combined with gemcitabine and cisplatin. The combination of gemcitabine and cisplatin is often used in the treatment of solid tumors, including non-small cell lung

cancer (NSCLC) and bladder cancer [14,15]. It is known from preclinical metabolism studies that ABT-510 is metabolized by hydrolase enzymes to form a weakly active M-1 metabolite [7]. Gemcitabine, a nucleoside analogue is primarily metabolized to its inactive form 2-difluoro-2-deoxyuridine (dFdU) by cytidine deaminase and is eliminated in urine. Cisplatin, a heavy metal complex, is largely eliminated in the urine. Based on the disposition pathways, we anticipated no drug interaction when ABT-510 is combined with gemcitabine and cisplatin therapy. The objectives of this study were to determine the safety profile and pharmacokinetics of ABT-510 and to exclude clinically relevant drug interactions with gemcitabine and cisplatin.

PATIENTS AND METHODS

Eligibility criteria Patients with a histologically confirmed diagnosis of an advanced solid malignancy for whom no standard therapy options were available or for whom the combination of gemcitabine and cisplatin chemotherapy was considered an appropriate treatment were eligible. Additional eligibility criteria included: age ≥ 18 years; World Health Organization (WHO) performance status < 3; an estimated life expectancy of ≥ 3 months; no radiotherapy, chemotherapy, or hormonal therapy within 4 weeks before start of the study with the exception of small field radiation therapy; and the ability to receive SC injections of ABT-510. Specific exclusion criteria included: a known positive human immunodeficiency virus status; clinical signs of brain tumor or known central nervous system metastases; and evidence of uncontrolled clinically significant disease unrelated to the primary malignancy. The study was approved by the local ethics boards of the two participating university medical centers. All patients gave written informed consent.

Drug administration On days 1 and 8 of each repeating 21-day cycle, gemcitabine (Gemzar®, Eli Lilly, Indianapolis, IN, USA) 1,250 mg/m^2 was administered intravenously (IV) in 250 mL 0.9% NaCl solution over 30 minutes. Cisplatin (Platinol®, Faulding, Warwickshire, UK) 80 mg/m^2 dissolved in 1 L 0.9% NaCl solution was administered IV over 3 hours after completion of the gemcitabine infusion on day 1.

ABT-510 (Abbott Laboratories, Chicago, IL, USA) was supplied in vials containing 1.1 mL ABT-510 (100 mg/mL) or 0.75 mL ABT-510 (80 mg/mL) dissolved in 5% dextrose. The vials were stored at 2-8 ºC and brought to room temperature one hour prior to dosing. ABT-510 was administered SC twice daily every day from day 2 of cycle 1 and continued until the patient went off study. The morning dose of ABT-510 on days when combined with chemotherapy was administered 30 to 60 minutes before gemcitabine infusion. Patients self-administered ABT-510 in the abdominal wall in the morning and evening, preferably at the same time each day, with an interval of approximately 12 hours in between doses. Times of these twice-daily injections were recorded in a diary. The starting dose of ABT-510 was 50 mg twice daily, based on safety and pharmacokinetic data obtained in a phase I study of single-agent ABT-510 [7].

Study design The ABT-510 dose levels studied were 50 and 100 mg BID, with 6 to 12 patients enrolled at each dose level. For each dose level, at least one cycle was to be completed between the entry of the first 3 and the next 3 patients. A cycle was defined as 21 days (3 weeks). Every patient who completed one full cycle was considered evaluable for toxicity. ABT-510 dose escalation to 100 mg BID was based on the safety assessments of all patients in the first cohort using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0. There was no dose escalation within a cohort. Before the dose could be escalated, at least 3 patients having received one full cycle were evaluated at a given dose level. An additional 3 patients at the same dose were treated if one of the first 3 patients exhibited a dose-limiting toxicity (DLT). If 2 patients exhibited a DLT, the same dose level was considered to be the MTD. DLT was defined as any grade 3 or 4 adverse event, (except inadequately treated nausea or vomiting or adverse event deemed treatable by the investigators) or any unexpected grade 2 adverse event possibly or probably related to treatment requiring dose modification or treatment delay and occurring in the first treatment cycle. A maximum of 6 chemotherapy cycles were to be administered. In case of a tumor response or stable disease after 6 chemotherapy cycles patients could continue treatment with single agent ABT-510. Patients were discontinued from the study in cases of progression of disease (PD), unacceptable toxicity, or refusal of treatment.

Treatment modifications In case of inadequate bone marrow function on day 8 of a cycle, the gemcitabine dose was to be reduced to 1,000 mg/m^2. Gemcitabine was to be withheld if the WBC was $< 1.0 \times 10^9$/L or platelets were $< 50 \times 10^9$/L. If at 2 consecutive counts in one week, the neutrophil count was $< 0.5 \times 10^9$/L, platelet count was $< 50 \times 10^9$/L, or in the event of febrile neutropenia, the gemcitabine dose was to be reduced to 1,000 mg/m^2 in all subsequent cycles. If the bone marrow continued to recover poorly after a gemcitabine dose reduction, a second dose reduction to 750 mg/m^2 was to be followed. On day 22, the gemcitabine administration was to be delayed up to 2 weeks if the neutrophil count was $< 1.5 \times 10^9$/L or platelet count was $< 100 \times 10^9$/L. Cisplatin administration was to be discontinued when serum creatinine reached > 1.5 times the upper limit of normal or if the creatinine clearance declined to < 50 mL/min. No ABT-510 dose reduction was to be performed. If gemcitabine and cisplatin were discontinued due to toxicity, patients were allowed to continue treatment with ABT-510 provided there were no signs of disease progression.

Pretreatment and follow-up studies Before therapy, a complete medical history was taken and a physical examination, an electrocardiogram, and a chest X-ray were performed. Patients were monitored with weekly complete blood counts. Biochemistry measurements, prothrombin time (PT), activated partial thromboplastin time (aPTT), and urinalysis were performed at baseline and each scheduled evaluation. Plasminogen, fibrinogen, and factor

VIII were collected at baseline and thereafter as clinically indicated. Toxicity was assessed after each cycle. Tumor measurements were performed after every 2 cycles using the WHO criteria for response [16].

Blood sampling and assay for pharmacokinetics All blood samples for pharmacokinetic analysis were collected from an indwelling IV canula into EDTA-containing tubes. The samples for pharmacokinetics analysis of ABT-510 were drawn before ABT-510 dosing and at 5, 15, and 30 minutes, and at 1, 2, 4, and 8 hours following the morning dose on days 15 and 22. Samples for gemcitabine pharmacokinetics were collected before the gemcitabine infusion, and at 15 and 30 minutes after the start of the gemcitabine infusion, and at 15, 30, and 45 minutes and 1, 2, 4 and 6 hours following completion of the infusion on days 1 and 22. Samples for cisplatin pharmacokinetics were collected before the cisplatin infusion, and at 1, 2, and 3 hours after the start of the cisplatin infusion, and 30 minutes and 1, 2, 3, and 5 hours following completion of the infusion on days 1 and 22. After collection of blood samples for ABT-510 and gemcitabine, the samples were immediately placed on ice and centrifuged at 2,000 x *g* at 4 °C for 10 minutes within one hour after collection, after which plasma was stored at -20 °C until analysis. Plasma samples for cisplatin were separated by centrifugation at 3,000 x *g* for 10 minutes, after which 500-µL aliquots of the plasma supernatant were added to 1.0 mL of ice-cold (-20 °C) ethanol, and the remaining plasma was stored frozen until analysis. The ethanol treated samples were mixed on a vortex-mixer for 10 seconds, and stored at -20 °C or colder for at least 5 hours, after which they were centrifuged at 23,000 x *g* for 5 minutes. The ethanol treated samples supernatant fractions were subsequently stored at -70 °C or colder until analysis.
Plasma concentrations of ABT-510 and its major metabolite M-1 were determined using a validated liquid chromatography with tandem mass spectrometric (LC-MS/MS) method described previously [7]. The lower limits of quantification (LLOQ) in plasma for ABT-510 and major metabolite M-1 were 0.5 ng/mL and 3 ng/mL, respectively.
Plasma concentrations of gemcitabine and its metabolite dFdU were determined using a Merck-Hitachi autosampler L7200 with a Merck-Hitachi pump L7110 and a Merck-Hitachi Diode array detector L7450 (VWR, Amsterdam, The Netherlands). The integration software used was Merck-Hitachi Model D7000 'HPLC System Manager' version 3.1.1 (1994-1999) (VWR, Amsterdam, The Netherlands). Sample preparation included combining 1 mL of isopropanol, 50 µL of the internal standard, and 200 µL of plasma in a glass centrifuge tube. After mixing on a vortex mixer, 2.5 mL ethyl acetate was added and mixed on the vortex mixer. The sample was centrifuged at 2,500 x *g* for 10 minutes. The supernatant was transferred into a nipple-shaped glass tube and evaporated to dryness under a gentle stream of nitrogen at ambient temperature. The residue was reconstituted in 100 µL of cyclohexane and then transferred into a limited volume insert of an autosampler vial. A 50-µL sample was injected into the HPLC system. An Alltech Econosphere NH_2 5 µm 250 x 4.6 mm column performed

the chromatographic separations. The components were detected at different wavelengths; with the detector set at 265 nm, dFdU was eluted at a retention time of 7.2 minutes. At 280 nm, the internal standard was eluted at a retention time of 11.7 minutes. The LLOQ in plasma was 0.125 μg/mL for gemcitabine and dFdU.

For measurement of unbound platinum, aliquots of 1000 μL of the ethanolic supernatant was evaporated to dryness under nitrogen at approximately 80 °C, and the residue was reconstituted in 200 μL water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride (diluent). A volume of 20 μL, in duplicate, was eventually injected into the graphite furnace of the atomic absorption spectrophotometer (AAS, Perkin Elmer Model 4110 ZL spectrometer with Zeeman-background) [17]. Platinum peak areas were measured at 265.9 nm and a slit width of 0.7 nm. The LLOQ of this assay was established at 0.0300 μg/mL platinum in plasma. For the determination of total platinum concentrations in plasma, a 100 μL volume of plasma was added to 500 μL diluent. A volume of 20 μL, in duplicate, was injected into the AAS. The LLOQ of this assay was established at 0.200 μg/mL.

Urine sampling and assay for pharmacokinetics Urine for pharmacokinetic analysis was collected from 0 to 12 and 12 to 24 hours after dosing of ABT-510 on days 15 and 22. Two samples of at least 15 mL were collected immediately before dosing on day 1 for the baseline drug assay. Urine samples were refrigerated during the collection period. Two 15 mL aliquots from each sampling period were stored at -20 °C until analysis. For measurement of urine concentrations of ABT-510 and metabolite M-1, urine was processed according to previously described methods [7]. The LLOQ in urine for ABT-510 and metabolite M-1 were 6 ng/mL and 99 ng/mL, respectively [7]. The assay for the determination of total platinum in urine was similar to that in plasma; aliquots of 100 μL of urine were added to 1,000 μL of diluent, from which aliquots of 20 μL were injected into the AAS. The LLOQ was 0.01 μg/mL.

Circulating endothelial cells (CEC) collection and analysis

Blood samples (approximately 5 mL) were collected by venipuncture in EDTA containing vacutainer tubes at baseline (day 1), every 2 cycles thereafter, and at the final visit. Samples were immediately sent to Esoterix (a laboratory contracted by ChromaVision Laboratories; San Juan Capistrano, CA, USA) for slide preparation, within 24 hours, followed by CEC measurement. The red blood cells in the blood samples were lysed with a RBC lysis buffer and the resulting white blood cell population was placed on slides at a density of $1x10^6$ cells/slide. Slides were stained using an endothelial specific monoclonal antibody and a secondary antibody containing red chromogen with alkaline phosphatase for visualization by indirect immunocytochemistry. Slides were counter-stained with hematoxylin and CECs were counted by cytomorphologic review following ACIS analysis. Samples were acceptable for CEC analysis if the viability of the cells was more than 80%.

Pharmacokinetic analysis Non-compartmental methods were used to determine values of plasma pharmacokinetic parameters of ABT-510, M-1, gemcitabine and its major metabolite dFdU, and total and unbound platinum (from cisplatin) using WinNonlin-Pro™, version 4.1 (Pharsight Corporation, Cary, NC, USA). For ABT-510 and M-1, maximum observed concentration and time of maximum observed concentration from the plasma concentration-time curves were reported as C_{max} and T_{max}, respectively. The area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration (C_{last}, $AUC_{0\text{-}last}$) was determined using the linear trapezoidal rule. To facilitate determination of AUC over the 12-hour ABT-510 dosing interval ($AUC_{0\text{-}T}$), 12 hour concentrations were estimated by linear regression of the logarithms of the plasma concentration-time data from the terminal log-linear phase of the profile, based on visual inspection. The value of the terminal elimination rate constant (ß) was obtained from the slope of the linear regression of the logarithms of the plasma concentration vs time data from the terminal log-linear phase of the profile. The terminal log-linear phase was identified using WinNonlin-Pro and visual inspection. The terminal phase elimination half-life ($t_{1/2}$) was calculated as (ln2)/ß. The apparent clearance (CL/F) was calculated by dividing the dose by $AUC_{0\text{-}T}$, and the volume of distribution (Vz/F) was calculated by dividing CL/F by ß. The time over which ABT-510 concentrations were above 100 ng/mL per day was determined from the observed and estimated concentrations.
For gemcitabine and unbound platinum, the parameters estimated were: C_{max}, T_{max}, ß, $t_{1/2}$, clearance (CL) and volume of distribution (Vz). The AUC from time 0 to infinite time ($AUC_{0\text{-}\infty}$) was calculated by adding $AUC_{0\text{-}last}$ and AUC_{ext}, where AUC_{ext} was calculated by dividing C_{last} by ß. The unbound platinum fraction was calculated by dividing the $AUC_{0\text{-}8h}$ for unbound platinum by the $AUC_{0\text{-}8h}$ for total platinum. The fraction of the dose excreted in urine for total platinum, ABT-510 and M-1 was calculated as the amount recovered in the urine over the dosing interval divided by the dose. The amount of M-1 recovered in urine was converted to equivalent ABT-510 amount by multiplying the M-1 amount by a ratio of the molecular weights of ABT-510 over M-1 (994/501).

Statistical analysis The sample size was based on clinical justification and patient numbers historically used for testing of new anti-neoplastic compounds. In models for the analysis of safety data, dose was treated as a factor with discrete levels or as a continuous variable. For the pharmacokinetic analysis, descriptive statistics of parameters were determined with a breakdown by regimen and dose level on days 1, 5 and 22. An analysis of variance (ANOVA) was performed on the parameters of drugs when administered as single agents and with combination therapy by including patient and day as classification variables. Two sided *P*-values < 0.05 were considered significant.

RESULTS

A total of 13 patients were enrolled into 2 dosing cohorts in the study from December 2002 to July 2003. Patient characteristics are listed in Table 1. Seven patients received 50 mg ABT-510 BID, and 6 patients received 100 mg ABT-510 BID. Forty-four cycles of ABT-510 combined with gemcitabine and cisplatin were administered. The median number of combined cycles was 3 (range, 1 - 6). Gemcitabine reduction was performed in 3 cycles in 3 patients (1 patient in the second cycle and 2 patients in the fourth cycle) due to grade 3 thrombocytopenia or grade 4 leucocytopenia. Gemcitabine and cisplatin were prematurely discontinued in 5 patients due to prolonged myelosuppression in 3 patients, a liver abscess in one patient with gallbladder cancer, and pain of bone metastasis in one patient. After discontinuation of gemcitabine and cisplatin in these patients, ABT-510 was continued at the same dose level for 1 to 3 cycles (13 cycles total).

Toxicity The incidence of grade 3 or 4 toxicities reported during combined treatment are listed in Table 2. The frequency of grade 3 and 4 adverse events is considered normal for the administration of the gemcitabine-cisplatin combination. None of these adverse events were

Table 1. Patient Characteristics

Characteristic	No. of Patients
Male / Female	6/7
Age (years)	
Median	50
Range	35-72
Performance status (WHO)	
0	4
1	7
2	2
Prior chemo/immuno/hormonal therapy	
1-3 prior regimens	8
> 4 prior regimens	2
Prior radiotherapy	2
Prior radiotherapy and chemotherapy	2
Tumor type	
Non-small cell lung cancer	4
Esophageal cancer	3
Melanoma	3
Sarcoma	1
Head and neck cancer	1
Gallbladder cancer	1

Table 2. Grade 3 or 4 Adverse Events Observed During the Study, Number of Patients

Adverse Event	ABT-510 Dose (Twice Daily)	
	50 mg (n = 7)	100 mg (n = 6)
Nausea	0	1
Vomiting	1	1
Neutropenia	3	2
Thrombocytopenia	3	2
Anemia	0	3
Dyspnea	1	0
Bone pain	3	0

considered to be possibly or probably related to ABT-510 except for an episode of hemoptysis in a 72-year-old male patient with NSCLC occurring on day 13 of 50 mg ABT-510 BID of treatment. He had received no prior chemotherapy and was known to have a history of tumor-related hemoptysis without using anticoagulant medication. The administration of ABT-510 was discontinued and a causal relationship with ABT-510 could not be ruled out. The patient died 2 months after the event due to disease progression. As the patient did not complete the first cycle of chemotherapy, an additional patient was enrolled in the first cohort. No DLT was observed, and the ABT-510 dose was escalated to 100 mg BID. Local skin reactions at the injection site, with elevated redness, sometimes painful, but no inflammation or hemorrhage, were mild and common side effects possibly or probably related to ABT-510. Both dose levels of ABT-510 appeared feasible and exhibited acceptable tolerance profiles.

Pharmacokinetics Plasma sampling for pharmacokinetics analysis of ABT-510 and cisplatin were available from 11 patients and for gemcitabine from 13 patients. Urine samples for calculating percent recovery were available from 10 patients for ABT-510 and M-1 and 11 patients for total platinum.

The pharmacokinetic parameters of ABT-510 (parent and M-1) as single agent for the 50 and 100 mg BID groups were similar to those reported earlier in a similar cohort of patients [6] (Table 3, Figures 1 and 2). Following SC bolus injections, ABT-510 was rapidly absorbed, with a T_{max} of approximately 0.5 hours. The C_{max} and AUC increased dose-proportionally. The percent extrapolated AUC in the calculation of $AUC_{0-\infty}$ was less than 6% for ABT-510. CL/F, Vz/F and $t_{1/2}$ were similar for the two dose levels. Both doses achieved the pharmacokinetic target of plasma ABT-510 concentrations over 100 ng/mL for more than 3 hours per day. This target was determined from preclinical studies. The pharmacokinetic parameters of ABT-510 (parent and M-1) when administered as a single agent (day 15) and in combination with

Figure 1. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 and M-1 on Day 15 (Alone) and Day 22 (With Gemcitabine/Cisplatin) Following the Morning Dose of 50 mg ABT-510.

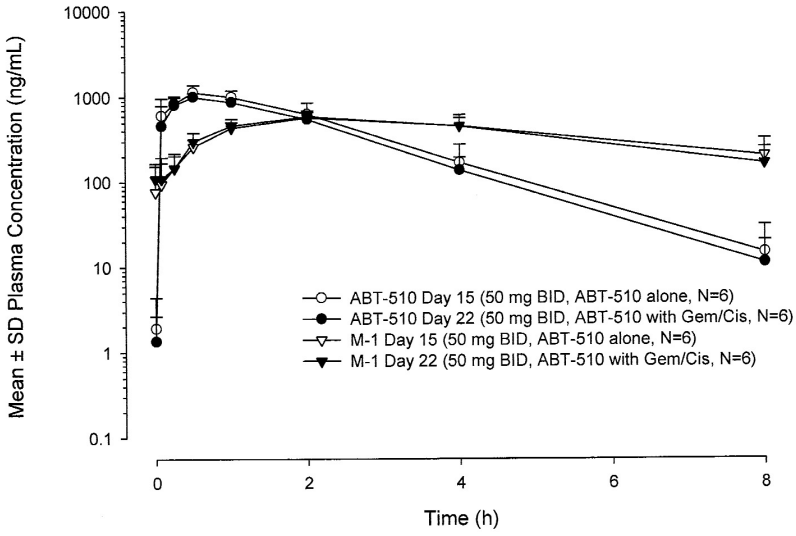


Figure 2. Plasma Concentration-Time Profiles (Mean ± SD) of ABT-510 and M-1 on Day 15 (Alone) and Day 22 (With Gemcitabine/Cisplatin) Following the Morning Dose of 100 mg ABT-510.

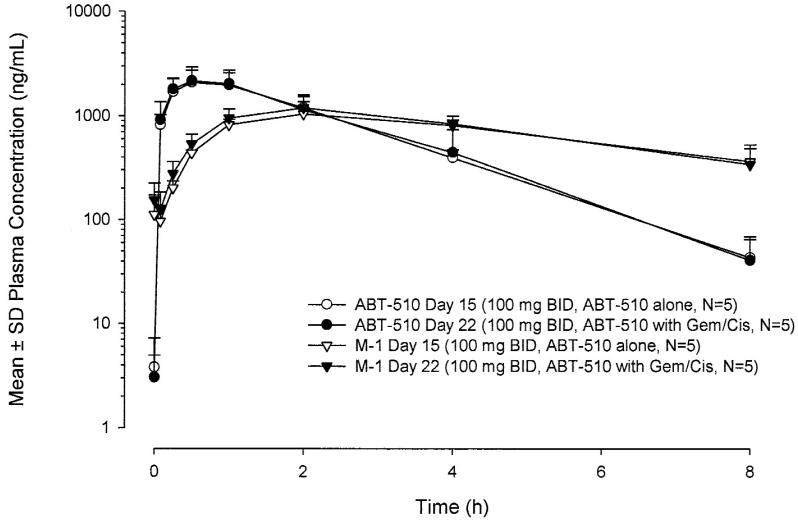


Table 3. Pharmacokinetic Parameters (Mean ± SD) of ABT-510 and M-1 Without (Day 15) or With (Day 22) Gemcitabine/Cisplatin Co-administration

Parameter	ABT-510+			
	50 mg BID#		100 mg BID	
	Day 15	Day 22	Day 15	Day 22
No. of patients	6	6	5	5
T_{max} (h)	0.5 ± 0.3	0.5 ± 0.3	0.7 ± 0.3	0.6 ± 0.2
C_{max} (ng/mL)	1143 ± 242	1032 ± 114	2123 ± 832	2171 ± 574
AUC_{0-T} (ng h/mL)B	2925 ± 887	2512 ± 393	5825 ± 1636	6009 ± 1757
$t_{1/2}$ (h)§	1.0 ± 0.1	1.0 ± 0.3	1.2 ± 0.2	1.1 ± 0.4
CL/F (L/h)	18.3 ± 5.0	20.3 ± 3.1	18.4 ± 5.5	17.8 ± 4.8
Vz/F (L)	27.0 ± 5.5	29.8 ± 6.9	36.2 ± 22.2	31.1 ± 14.
Time (h) > 100 ng/mL/24h	10.4 ± 2.6	9.8 ± 2.4	14.6 ± 0.6	14.0 ± 2.0
	M-1+			
No. of patients	6	6	5	5
T_{max} (h)	2.2 ± 1.0	2.3± 0.8	2.0 ± 0.0	2.0 ± 0.0
C_{max} (ng/mL)	572 ± 95	611 ± 76	1039 ± 153	1190 ± 177
AUC_{0-T} (ng h/mL)	3615 ± 1201	3467 ± 706	6572 ± 1033	6867 ± 1311

Abbreviations: BID, twice daily; T_{max}, time to maximum plasma concentration, C_{max}, maximum plasma concentration; AUC_{0-T}, area under plasma concentration-time curve from time 0 to dosing interval; $t_{1/2}$, elimination half-life; CL/F, apparent clearance; Vz/F, apparent volume of distribution.
§, Harmonic mean ± pseudostandard deviation;
+, Parameters estimated for the morning dose.

Gemcitabine-Cisplatin (day 22) were similar. There were no statistical significant differences in C_{max} and AUC for ABT-510 and M-1 between days 15 and 22. On days 15 and 22, the majority of the ABT-510 was recovered in the urine as M-1. Little parent drug (< 1% of the dose on average) was recovered in the urine. There was no difference in the recovery of M-1 between days 15 and 22 (data not shown). The percent of the dose recovered in the urine was similar for the 0 to 12 and 12 to 24 hour collection intervals on days 15 and 22.

Gemcitabine, dFdU, unbound and total platinum pharmacokinetics were similar with or without ABT-510 co-administration (Table 4, Figures 3 and 4). There were no statistically significant differences in the pharmacokinetic parameters, including C_{max} and AUC, with and without ABT-510 co-administration. The unbound platinum fraction on days 1 and 22 were also similar 21.7 vs 20.3%, respectively, as was total platinum excreted in the urine. Therefore, no pharmacokinetic interaction was observed between ABT-510 and gemcitabine, dFdU, and platinum.

Figure 3. Plasma Concentration-Time Profiles (Mean ± SD) of Gemcitabine and dFdU on Days 1 and 22 Relative to the Start of a 0.5-hour Infusion of Gemcitabine (1250 mg/m^2).

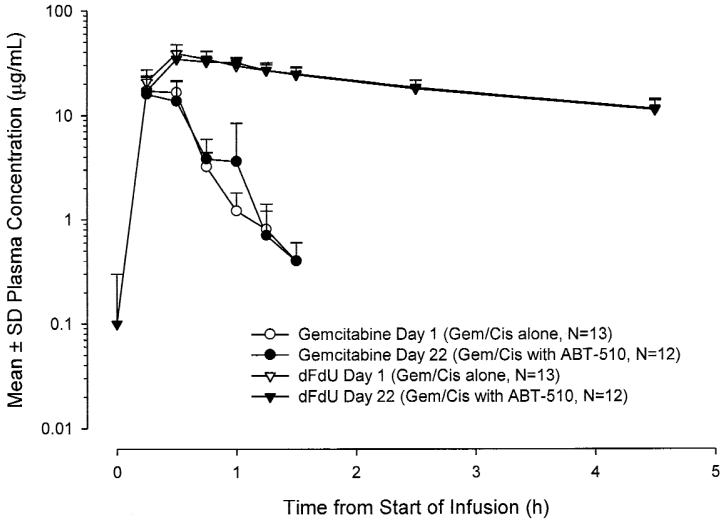


Figure 4. Platinum Concentration-Time Profiles (Mean ± SD) of Total and Unbound Platinum on Days 1 and 22 Relative to the Start of a 3-hour Infusion of Cisplatin (80 mg/m^2).

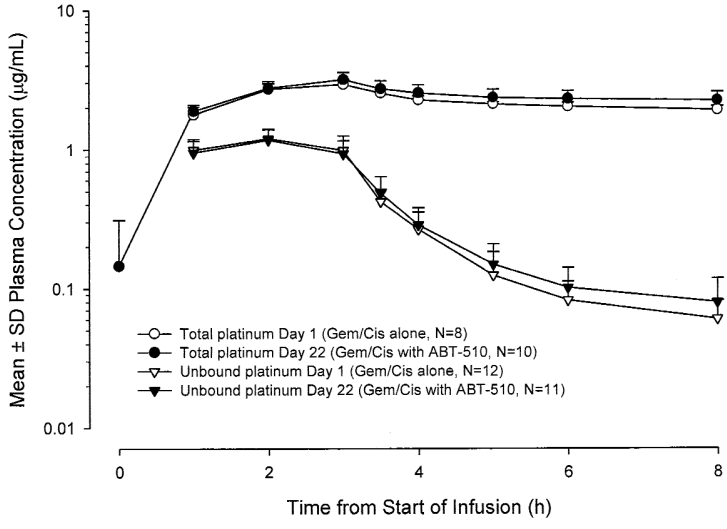


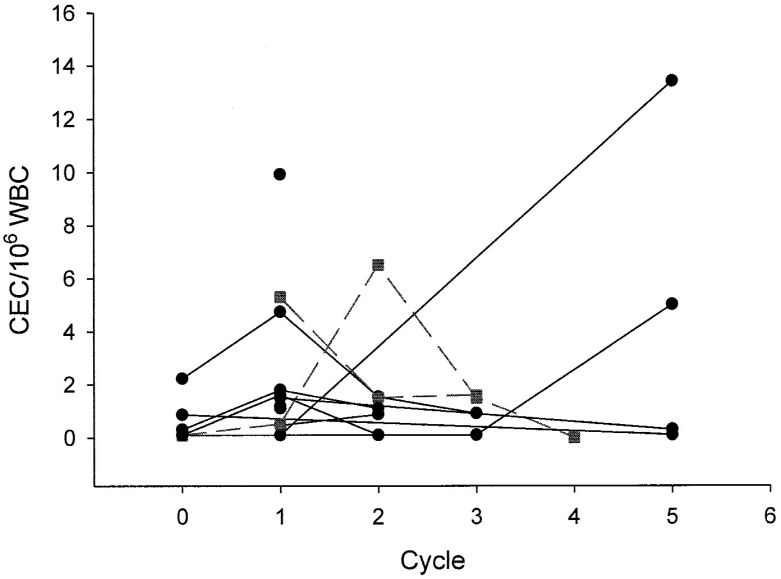
Table 4. Pharmacokinetic Parameters (Mean ± SD) of Gemcitabine, dFdU, and Unbound and Total Platinum, Administered Without (Day 1) or With (Day 22) ABT-510 Co-administration

Parameter	Gemcitabine		Unbound Platinum	
	Day 1	Day 22	Day 1	Day 22
No. of patients	13	12	12	11
T_{max} (h)#	0.3 ± 0.1	0.4 ± 0.1	2.3 ± 0.8	2.0 ± 0.5
C_{max} (μg/mL)	19.2 ± 4.8	18.6 ± 4.7	1.2 ± 0.2	1.2 ± 0.2
$AUC_{0\text{-}8h}$ (μg h/mL)	NC	NC	3.6 ± 0.5	3.7 ± 0.7
$AUC_{0\text{-}\infty}$ (μg/mL)	10.1 ± 2.5	9.6 ± 2.6	3.9 ± 0.6	4.1 ± 0.9
$t_{1/2}$ (h)§	0.2 ± 0.1	0.2 ± 0.1	3.4 ± 0.9	3.3 ± 1.5
Vz (L/m^2)	45.7 ± 19.7	49.8 ± 34.1	102.0 ± 24.5	88.9 ± 32.0
CL ($L/h/m^2$)	132.5 ± 39.4	141.2 ± 48.2	20.8 ± 3.6	19.6 ± 5.6
	dFdU		Total Platinum	
	Day 1	Day 22	Day 1	Day 22
No. of patients	13	12	8	10
T_{max} (h)	0.6 ± 0.1	0.6 ± 0.2	2.8 ± 0.4	3.0 ± 0.1
C_{max} (μg/mL)	39.1 ± 8.0	36.7 ± 5.0	3.0 ± 0.3	3.1 ± 0.4
$AUC_{0\text{-}8h}$ (μg h/mL)+	90.6 ± 15.8	89.8 ± 11.2	16.3 ± 0.9	18.3 ± 2.3
C_{last} (μg/mL)^	11.1 ± 2.7	11.4 ± 2.9	1.9 ± 0.1	2.2 ± 0.3

Abbreviations: T_{max}, time to maximum plasma concentration, C_{max}, maximum plasma concentration; $AUC_{0\text{-}8h}$, area under plasma concentration-time curve from time 0 to 8 hours or infinity; $t_{1/2}$, elimination half-life; Vz, volume of distribution; CL, clearance; C_{last}, last measurable concentration; NC, not calculated.
§, Harmonic mean ± pseudostandard deviation.
+, AUC for dFdU is over 4.5 h from start of infusion.
^, Clast for dFdU at 4.5 hours and for total platinum at 8 hours after the start of infusion.

Circulating endothelial cell analysis The individual CEC/10^6 WBC over time is presented in Figure 5. CEC data were available from 13 patients. One patient that had long term stable disease defined as progression free survival of more than 6 cycles had a decrease in CEC numbers at the last measurement as compared to measurements in the previous cycles. Another patient with long term stable disease had low CEC numbers at baseline and had an increase in the numbers at cycle 2, but the numbers returned to baseline by cycle 4. Both these patients received ABT-510 at the dose of 100 mg BID. Two patients (one in 50 mg BID and other in 100 mg BID group) had low CEC number at baseline, but they increased just before their disease was determined to be progressive. There was no trend in correlation of ABT-510 doses, length of ABT-510 treatment and change in CEC over time. Overall, from the available data there is no clear trend in changes in CEC numbers following ABT-510 treatment.

Figure 5. Individual Patient Circulating Endothelial Cell (CEC) Levels/10^6 WBC over Treatment Cycles.



Tumor response Three patients (one head and neck cancer, one melanoma, and NSCLC) experienced a PR after two cycles of treatment. The durations of these PRs were 4 cycles, 4 cycles and 2 cycles (the patient with NSCLC discontinued study drug due to adverse event). Stable disease lasting more than 2 cycles was observed in eight patients; three of these patients (one with NSCLC, one with head and neck cancer, and one with melanoma) had SD lasting more than 4 cycles.

DISCUSSION

The present phase I study shows that the combination of ABT-510, a new TSP-1-mimetic angiogenesis inhibitor, with gemcitabine and cisplatin is feasible. ABT-510 administered subcutaneously twice daily, in combination with gemcitabine and cisplatin had a similar toxicity profile as the chemotherapy alone and seemed to be well tolerated. The toxicity of the combination of ABT-510 with gemcitabine and cisplatin mainly consisted of myelosuppression, nausea, and vomiting. One patient with NSCLC experienced hemoptysis, which was considered to be possibly related to the combination of ABT-510 with chemotherapy, although it may be related to NSCLC disease process. No thromboembolic complications were observed. The scheduled dosages of gemcitabine and cisplatin were comparable to those commonly used in

regimens without an angiogenesis inhibitor [14,15]. The dosages of ABT-510 were comparable to those used in a previous phase I single-agent study [7]. No new toxicity of ABT-510 was observed and there were no clinically significant pharmacokinetic interactions during the combination treatment. In this phase I clinical combination trial, antitumor activity was observed with three patients with a PR.

Several studies have assessed the effectiveness of angiogenesis inhibitors in combination with a variety of chemotherapy regimens. In one phase I study, the experimental angiogenesis inhibitor SU5416, a VEGF-2 tyrosine kinase inhibitor, was combined with gemcitabine 1,250 mg/m^2 and cisplatin 80 mg/m^2 [18]. This combination caused a higher than reported incidence of thromboembolic events for SU5416 as a single agent (2.2%), ranging from deep venous thrombosis to cerebrovascular accident in 8 out of 19 patients (42%) and the study was prematurely terminated [19]. The increase in thromboembolic events could not be explained by a direct pharmacokinetic interaction among the drugs. A proposed mechanism behind these events is possibly related to a treatment, cycle-dependent increase of thrombin [18]. In another combination study in which SU5416 was combined with paclitaxel, 3 of the 12 patients (25%) experienced a thromboembolic event [20]. It was speculated that the combination of SU5416 with chemotherapeutic drugs activates both the coagulation cascade and endothelial cells, producing a net effect of shifting the coagulation cascade towards a prothrombotic state.

In several other studies, the VEGF pathway was targeted by bevacizumab, a monoclonal antibody to VEGF. In a randomized phase II study, previously untreated metastatic colorectal cancer patients received 500 mg/m^2 5-fluorouracil and 500 mg/m^2 leucovorin weekly every 6 weeks followed by 2 weeks of rest or treatment with 5 or 10 mg/kg bevacizumab [21]. In a recently reported phase III trial in metastatic colorectal cancer, the addition of bevacizumab (5 mg/kg every 2 weeks) to weekly 5 fluorouracil (500 mg/m^2), leucovorin (20 mg/m^2), and irinotecan (IFL) (125 mg/m^2) resulted in a significant increase in survival from 15.6 months to 20.3 months [12]. There was no difference in the incidence of venous and arterial thrombotic events reported in both arms (19.4% for IFL+ bevacizumab vs. 16.2% for IFL; $P = 0.26$). The frequency of grade 3 or 4 bleeding complications was similar in both arms, as was the incidence of proteinuria. However, the incidence of hypertension was higher in the IFL+bevacizumab arm (22.4% vs. 8.3%; $P < 0.01$) [12]. Overall, this is the first combination study in which the targeted VEGF pathway combined with a chemotherapy combination results in a survival benefit. Although minor mucosal bleeding (eg, epistaxis), hypertension, and proteinuria are adverse events that have been observed in other clinical trials of bevacizumab, of concern is a recently reported trial in patients with NSCLC in which carboplatin and paclitaxel were combined with bevacizumab (7.5-15 mg/kg). In this trial, bleeding complications, including life threatening pulmonary hemorrhages, were more frequent in the bevacizumab arm [22]. Especially patients with squamous type carcinoma of the lung were more at risk for these complications resulting in excluding those patients in current lung cancer studies [23]. So, while SU5416

and bevacizumab both interact with the VEGF pathway to inhibit angiogenesis, combining either of these with chemotherapy regimens may increase the vascular toxicity profile.
The TSP-1-mimetic ABT-510 is an endogenous angiogenesis inhibitor that, when combined with a chemotherapeutic schedule, does not appear to increase vascular and thromboembolic toxicity. Of interest is the question of whether endogenous angiogenesis inhibitors such as TSP-1 and angiostatin added to combination chemotherapy result in less frequent adverse vascular and thromboembolic events when compared to combinations in which the VEGF pathway is inhibited. There are preliminary data from a small phase II study in patients with NSCLC in which recombinant human angiostatin was added to carboplatin and paclitaxel [24]. This combination was active in NSCLC patients and was well tolerated; only 3 of the 24 patients enrolled developed thromboembolic complications (2 pulmonary embolisms and 1 transient ischemic attack). More combination studies with endogenous angiogenesis inhibitors are needed to investigate their anti-tumor potential and define the toxicity profile.
In the present study, pharmacokinetics of ABT-510, gemcitabine and cisplatin as single agents were similar to those reported in previous studies at their respective doses. In addition, the pharmacokinetic parameters of ABT-510, gemcitabine, and cisplatin when administered in the combination appeared to be similar to those found when the same doses were given as single agents. Therefore, the combination of ABT-510 with gemcitabine and cisplatin does not produce pharmacokinetic interactions. The lack of pharmacokinetic interaction observed in this study is consistent with the different disposition pathways involved in the elimination of ABT-510, gemcitabine and cisplatin.
Three of 12 assessable patients with various tumor types experienced PRs. Based on previous phase I data, several phase II trials have already been initiated in NSCLC, lymphoma, sarcoma, and renal cell cancer patients with single-agent ABT-510 regimens or ABT 510 in combination with a cytotoxic regimen. Any antitumor activity of combination treatment with ABT-510 needs to be explored in randomized phase II and III studies.
In conclusion, a regimen of SC twice daily doses of 50 or 100 mg ABT-510 in combination with 1,250 mg/m^2 gemcitabine on days 1 and 8 and 80 mg/m^2 cisplatin on day 1 IV in a 3-week cycle can be administered with acceptable toxicity. No significant pharmacokinetic interactions were observed. Further phase II and III studies are warranted to evaluate the safety and efficacy of this combination in the treatment of advanced solid tumors.

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ABSTRACT

Malignant tumors are characterized by invasive growth and metastasis. To facilitate this invasive behavior, the enzymatic breakdown of the extracellular matrix is a prerequisite. Many human tumors are characterized by locally increased concentrations of matrix metalloproteinases, enzymes that are able to degrade this ECM. A vast number of matrix metalloproteinase inhibitors have been developed in recent years and after extensive preclinical testing, the results of the first clinical studies with several of these compounds have recently been presented. In this review we will describe some of the basic concepts of the degradation of the extracellular matrix, with special emphasis on the role of matrix metalloproteinases in the progression of cancer. Furthermore we will review the results of preclinical and clinical studies with matrix metalloproteinase inhibitors and discuss their future perspective.

CHAPTER 8

MATRIX METALLOPROTEINASE INHIBITORS: CURRENT DEVELOPMENTS AND FUTURE PERSPECTIVES

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INTRODUCTION

The importance of proteinases in tumor invasion was first recognized in 1925 when Fischer found that a lytic substance from sarcoma cells could degrade the fibrin stroma. Later it was found that the serine proteinase plasminogen activator (PA) played an important role in activating plasminogen to plasmin. Apart from PAs, proteinases such as serine-, cysteine- and metalloproteinases have been associated with cancer [1]. It is important to realize that high levels of extracellular proteolytic activity are not restricted to the malignant phenotype, but are also seen in a number of physiological processes such as embryo implantation, wound healing and angiogenesis. A common feature in these processes is the breaching of histological barriers with the degradation of the extracellular matrix (ECM) composed of basement membrane and extracellular stroma.

Matrix metalloproteinases (MMPs) are enzymes able to degrade most components of the ECM such as collagens, laminins, fibronectins, elastins and the protein core of proteoglycans.

Table 1. The Matrix Metalloproteinase Family

Group	Descriptive name	Number	Principal substrate
Collagenases	Interstitial collagenase	MMP-1	Fibrillar collagen types I, II, III
	Neutrophil collagenase	MMP-8	Fibrillar collagen types I, II, III
	Collagenase-3	MMP-13	Fibrillar collagen types I, II, III
	Collagenase-4	MMP-18	
Stromelysins	Stromelysin-1	MMP-3	Proteoglycans, laminin, fibronectin, non fibrillar collagen
	Stromelysin-2	MMP-10	Proteoglycans, laminin, fibronectin, non fibrillar collagen
	Matrilysin	MMP-7	Proteoglycans, laminin, fibronectin, non fibrillar collagen
Gelatinases	Gelatinase A (72 kDa)	MMP-2	Gelatins, non fibrillar collagen types IV, V
	Gelatinase B (92 kDa)	MMP-9	Gelatins, non fibrillar collagen types IV, V
Membrane type	MT1-MMP	MMP-14	Progelatinase A, procollagenase-3
	MT2-MMP	MMP-15	Progelatinase A
	MT3-MMP	MMP-16	Progelatinase A
	MT4-MMP	MMP-17	
	MT5-MMP	MMP-21	
Others	Stromelysin-3	MMP-11	Serine protease inhibitor
	Metalloelastase	MMP-12	Elastin, non fibrillar collagen
	Enamelysin	MMP-20	
		MMP-19	
		MMP-23	
		MMP-24	

At this moment more than 20 different MMPs have been identified and classified. They show a consistent sequence homology and in general share a pre-domain which is a signal peptide for secretion, a pro-domain important for maintaining latency, a catalytic domain with a highly conserved zinc-binding site and a haemopexin-like domain. Most MMPs are secreted in the latent form, a few MMPs however, have a transmembrane domain and remain attached to the cell membrane (membrane-type MMPs or MT-MMPs). Based on sequence homology and substrate specificity, MMPs can be classified in 5 subgroups (Table 1). The classification is somewhat arbitrary, since the true physiological substrates are a matter of debate. In situ hybridization techniques showed that most MMPs are not produced by tumor cells but by adjacent stromal cells. It is suggested that tumor cells produce a stimulatory factor (Extracellular Matrix Metalloproteinase Inducer, EMMPRIN), that induces stromal fibroblasts to produce MMPs [2,3]. In addition various growth factors, hormones, oncogenes and tumor promoters are thought to play an important role in the regulation of MMP gene transcription. After translation, the MMPs are secreted in a latent form. Following proteolytic cleavage of the NH_2 terminal pro-domain by other MMPs or other proteases, activated MMPs are being formed. Inhibition of MMPs is obtained through protease inhibitors such as $\alpha 2$-macroglobulin and by a group of specific tissue inhibitors of metalloproteinases (TIMPs). It is thought that an imbalance between the activation and inhibition of MMP activity in favor of the MMP activity plays an important role in the pathophysiology of cancer by facilitating the invasion of tumor cells through the ECM. In addition it is suggested that MMPs affect growth of primary tumors and metastases by stimulating release and activation of latent growth factors from the ECM such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and insulin-like growth factor [4]. Furthermore, degradation of the ECM is also an essential step in the process of angiogenesis, which is required for tumor growth beyond a few millimeters in size and for metastasis [5].

There is extensive literature demonstrating the association of MMP activity and tumor progression. With regard to this association, several generalizations can be made [6,7]:

- MMPs are detected in a large variety of tumors
- The number of different MMP family members detected tends to increase with progression of the tumor
- The relative levels of any individual MMP family member tend to increase with increasing tumor stage
- MMPs can be made either by tumor cells themselves or as a host response to the tumor
- The MMPs most frequently encountered are MMP-2, MMP-3, MMP-7, MMP-9, MMP-11, MMP-13 and MMP-14.

Evidence for the role of MMPs in the process of metastasis comes from several experiments using animal tumor models. Intraperitoneal injection of recombinant TIMP-1, a naturally

occurring MMP inhibitor (MMPI), reduced lung colonization of intravenously injected B16F10 melanoma cells [8]. Transfecting an expression vector encoding for MMP-9 into a tumorigenic but non-metastatic rat embryo cell line resulted in increased metastatic capacity [9]. In addition, preclinical studies using synthetic MMPIs support the important role of MMP activity in the process of tumor progression, as discussed below.

Since there is a correlation between MMP expression in the tumor and tumorstage, it is suggested that MMP expression can be used as a diagnostic or prognostic tool. Indeed it was found that serum MMP-2 levels were increased in patients with prostate cancer compared to healthy controls or patients with benign prostate hyperplasia [10]. For colon cancer it was shown that high MMP-1 expression within the tumor correlated with hematogenous metastasis independent of other established histopathological factors [11]. This inverse relationship between increased MMP expression and clinical outcome was also found for gastric cancer (MMP-2 and MMP-9), small cell lung cancer (MMP-3, MMP-11 and MMP-14), esophageal cancer (MMP-7) and breast cancer (MMP-11) [12-15].

BASIC ASPECTS

As the inverse relation between MMP expression and clinical outcome in cancer became more and more obvious, inhibiting the function of the MMP cascade became a target for the development of new anticancer drugs. In theory, therapeutic intervention in the MMP cascade is possible at the induction, production, secretion, activation or catalytic part, but thus far, most research has focused on inhibition of the MMP activity itself. TIMPs have been shown to have inhibitory activity in *in vitro* and *in vivo* tumor models, but their clinical use has been hampered by low oral bioavailability. The most interesting agents are the synthetic inhibitors of the enzyme activity. The majority of these MMPIs have been developed through the application of structure-based design rather than through conventional screening technologies [16-18]. Based on the structure of the collagen molecule at the site of the initial cleavage, peptide

Table 2. Matrix Metalloproteinase Inhibitors in Clinical Development

Agent	Class	MMP inhibition	Remarks
Batimastat	Peptido-mimetic	MMP-1, 2, 3, 7, 9	Only parenterally available
Marimastat	Peptido-mimetic	MMP-1, 2, 3, 7, 9, 12	
AG3340 (prinomastat)	Nonpeptido-mimetic	MMP-2, 3, 9, 13, 14	
BAY 12-9566	Nonpeptido-mimetic	MMP-2, 3, 9	
MMI270	Nonpeptido-mimetic	MMP-1, 2, 3, 9, 13, 14	
COL-3 (metastat)	Tetracycline derivative	MMP-2, 9	Multiple mechanisms of action
BMS-275291	Nonpeptido-mimetic	MMP-2, 9	
CP-471,358	Nonpeptido-mimetic	MMP-2, 3, 8, 9, 12, 13, 14	
AE-941(neovastat)	Shark cartilage extract	MMP-1, 2, 7, 9, 12, 13	Multiple mechanisms of action

and peptide like compounds were developed, that were able to interact with the active site of the enzyme and chelate the zinc ion at the catalytic site. The majority of these inhibitors contain a hydroxamic acid group as zinc chelator. The catalytic domains of most MMPs have a high degree of homology and therefore many of the early MMPIs exhibit a broad-spectrum inhibition profile. In order to create more specificity in binding of MMPs thought to be important in the process of tumor progression, and in order to augment oral bioavailability, research has moved to the development of peptide compounds with alternative chelators to the ubiquitous hydroxamic acid group and to nonpeptide compounds with a hydroxamate chelating group. A special group of MMPIs is formed by the tetracycline derivatives that not only inhibit MMPs by chelation of the zinc ion, but are also able to downregulate the production, inhibit the activation and increase the degradation of MMPs [19]. MMPIs investigated in clinical trials are shown in Table 2.

PRECLINICAL EXPERIENCE

MMPIs have been extensively studied in numerous tumor models. The first and most extensively studied MMPI is batimastat, a low molecular weight broad spectrum MMPI with a hydroxamate group as a zinc chelator. In *in vitro* experiments no cytotoxic activity was found [20-22], whereas in studies with various human xenograft models a significant reduction of tumor growth rate was seen when batimastat was administered shortly after tumor inoculation [20,23,24]. Administration shortly after tumor inoculation in pancreatic, orthotopic colon and orthotopic liver tumor models showed reduced growth of the primary tumor, a reduction in the onset of distant metastases and even prolongation of survival [22,25,26].

Although a significant reduction in tumor growth was seen when batimastat was administered shortly after tumor inoculation, treatment in a more advanced tumor stage did not result in a significant growth reduction in a B16 -BL6 murine melanoma tumor model [23]. The issue of the growth inhibitory effect of MMPIs in the minimal residual disease state is particularly addressed in the studies mimicking the adjuvant setting. Using orthotopic human breast cancer models (MDA-MB-435 and HOSP.1P) it was shown that administration of batimastat shortly after resection of the tumor significantly inhibited local regrowth, decreased the number and volume of pulmonary metastases and improved survival [27,28]. While treatment with batimastat for a short period of time did not result in reduction of regional lymph node metastases, prolonged treatment did. It was suggested that batimastat was not able to prevent invasion of lymphatic channels (which lack a basement membrane), but that prolonged treatment was able to inhibit subsequent growth of nodal metastases [28].

AG3340 is a selective, nonpeptide inhibitor of MMP-2, MMP-3, MMP-9, MMP-13 and MMP-14. Activity was explored in a wide range of human tumor xenograft models [29]. Oral AG3340 given twice daily, started shortly after tumor implantation, resulted in a profound delay of tumor growth in a human colon, an androgen independent human prostate, and a human

non-small cell lung cancer (NSCLC) tumor model. A similar inhibition of growth was seen when AG3340 was initiated after growth of established tumors of a human breast cancer xenograft (MDA-MB-435). AG3340 was the first MMPI tested in a human glioma tumor model (U87), where it was administered intraperitoneally starting 3 weeks after subcutaneous tumor implantation. It caused profound inhibition of tumor growth, decreasing tumor size by 78% compared to controls after 31 days, resulting in a 2,3 times increased survival [30]. Apart from the role of MMPIs as inhibitors of the remodeling of the ECM surrounding the tumor, there is also evidence that MMPIs inhibit tumor induced angiogenesis. Analyzing angiogenesis using antibodies to CD-31, an endothelial marker that is almost exclusively expressed on newly formed vessels, revealed that AG3340 decreased angiogenesis in three of the four tumor models studied [29]. Furthermore using murine endothelioma cells transformed by a polyoma middle-T oncogene which forms tumors that are constituted of recruited host cells for more than 95%, it was shown that batimastat was able induce a significant growth reduction [21].

MMPIs have been tested in combination with cytotoxic chemotherapy. In a murine Lewis lung cancer model CT1746, an inhibitor of MMP-2 and MMP-9, combined with either cisplatin or cyclophosphamide was significantly more active than single agent therapy in delaying local tumor growth and reducing number and size of pulmonary metastases [31]. The effect was most obvious when CT1746 was started shortly after tumor implantation, again suggesting that MMPIs are more active when administered under conditions of low tumor volume. AG3340 was studied in combination with carboplatin or paclitaxel using a lung colonization model after i.v. injection of B16-F10 melanoma cells in mice [32]. Neither AG3340 nor carboplatin started one day after injection of tumor cells decreased the number of lung lesions (> 1 mm^3) significantly. However the combination produced a significant decrease in the number of lung lesions. AG3340 and paclitaxel, at single agent dosages not able to reduce the number of lung lesions, in combination caused a significant decrease of the number of lung lesions. In a MV522 NSCLC model paclitaxel given at suboptimal dose was able to potentiate the activity of AG3340 resulting in enhanced tumor growth inhibition [33]. Finally in a human gastric KKLS tumor model, AG3340 not active as a single agent potentiated the activity of paclitaxel [29].

Giavazzi et al studied the effects of batimastat in combination with cisplatin in two human ovarian carcinoma xenografts (HOC22 and HOC8) inoculated in the peritoneal cavity of nude mice [34]. In the HOC22 model the early treatment with a combination of batimastat and cisplatin significantly prolonged survival compared to either single agent. In the HOC8 model, only moderately sensitive to cisplatin and not responsive to batimastat, the combination therapy resulted in a prolonged disease free survival. When treatment was started in the advanced or late stage, monotherapy with cisplatin or batimastat was not effective in the HOC22 model, but the combination resulted in an increased survival.

MMPIs IN CLINICAL TRIALS

Phase I studies. Several MMPIs have been tested in phase I/II trials. These studies are summarized Table 3.

Batimastat showed a poor oral bioavailability and also could not be given intravenously due to its limited solubility. Therefore clinical studies were performed using intraperitoneal or intrapleural administration [35-38]. Following intraperitoneal administration, rapid systemic absorption was seen with serum levels exceeding concentrations causing 50% inhibition (IC_{50}) of major MMPs for prolonged periods of time. Side effects considered to be drug related included abdominal discomfort, nausea, vomiting and fever. Although response is difficult to assess in patients with ascites, patient benefit consisting of decrease of weight, abdominal girth or frequency of drainage was observed [36,37]. In a study performed in patients with malignant pleural effusions, batimastat was administered in the pleural cavity following pleural drainage [38]. Peak plasma levels were detected between 4 hours to 1 week after administration and in patients with doses ≥ 60 mg/m^2, plasma levels were detectable for 9 to 12 weeks. Side effects were comparable to those previously mentioned, with the exception of non-symptomatic elevation of liver enzymes occurring in 44% of the patients. There was no clear relationship between the elevated liver enzymes and the batimastat dose or the peak plasma levels. Peak values of liver enzymes were generally seen in the second week and in some patients elevations persisted for up to 5 months after batimastat administration. A partial response, with a significant reduction of the need for pleural reaspiration, was achieved in 7 of 16 evaluable patients. The reason for this activity is not clear and it can not be ruled out that batimistat acted simply as a sclerosing agent, especially since no experimental data existed on MMP inhibition *in vivo*. Because batimastat could only be administered intraperitoneal or intrapleural, further clinical development was halted.

Marimastat (BB-2516) was the first oral MMPI tested in clinical trials. It is a low molecular weight peptido-mimetic agent with a hydroxamate group closely related to batimastat. Marimastat is a potent and reversible MMPI with IC_{50}s in the nanomolar range against MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 and MMP-12. The first phase I study was performed with healthy volunteers [39]. Marimastat was rapidly absorbed and well tolerated, with pharmacokinetic data indicating that a total daily dose of 50 to 100 mg can achieve trough levels exceeding 40 ng/ml which is six times the IC_{50} for the major MMPs. Since it was anticipated that no tumor regressions would be seen and that chronic administration would be necessary to exert optimal antitumor activity, a number of phase I/II studies were initiated where early information about activity was based on the rate of rise of serum tumor marker levels [40-45]. A combined analysis of these studies including 415 patients with advanced colorectal, ovarian, pancreatic and prostate cancer using the serum tumor markers CEA, CA-125, CA 19-9 and PSA,

Table 3. Clinical Phase I/II Studies of Matrix Metalloproteinase Inhibitors: Side Effects at Recommended Dose Levels

MMPI [reference]	Schedule§	Tumor type	N#	Recommended dose	Side effects at recommended dose
Batimastat [35]	Intraperitoneal	All	9	1200 mg/m^2	Abdominal pain
Batimastat [36]	Intraperitoneal	All	9	600 mg/m^2	Mild abdominal pain
Batimastat [37]	Intraperitoneal	All	23		
Batimastat [38]	Intrapleural	All	18	300 mg/m^2	Fever, elevated liver enzymes
Marimastat [40]	5 mg od – 50 mg bid	Colon	70	20 mg od – 25 mg bid	Musculoskeletal
Marimastat [41]	5 mg od – 50 mg bid	Ovary	66	10 - 25 mg bid	Musculoskeletal
Marimastat [42]	10 mg od – 75 mg bid	Pancreas	64	5 - 25 mg bid	Musculoskeletal
Marimastat [43]	5 mg od – 50 mg bid	Colon	61		
Marimastat [44]	2 mg od – 50 mg bid	Prostate	88		
Marimastat [45]	5 mg od – 75 mg bid	Ovary	66		
Marimastat [47]	25-100 mg bid	NSCLC	12		
Marimastat [48]	25 mg od – 50 mg bid	Gastric	35	25 mg od	Musculoskeletal
Marimastat [82]	25 mg	Pancreas	34		
Marimastat [83]	10 – 100 mg bid	Melanoma	26		
AG3340 [49]	2 – 100 mg bid	All	45		
BAY 12-9566 [55]	100 mg od – 800 mg bid	All	26	800 mg bid	Mild thrombocytopenia, transaminase elevation
BAY 12-9566 [56]	400 mg od – 800 mg bid	All	13	800 mg bid	
BAY 12-9566 [57]	100 mg od – 800 mg bid	All	29	800 mg bid	Mild thrombocytopenia, transaminase elevation
BAY 12-9566 [59]	100 mg od – 800 mg bid	All	21	800 mg bid	Mid thrombocytopenia, transaminase elevation, hypophosphatemia
MMI270 [62]	150 mg bid – 600 mg tds	All	92	300 mg bid	Maculopapular skin rash, musculoskeltal
COL-3 [19]	36 – 98 mg/m^2/day	All	35	36 mg/m^2	Cutaneous phototoxicity
COL-3 [63]	36 – 98 mg/m^2/day	All	26		
BMS-275291 [66]	150 – 1200 mg od	Healthy males	40		
BMS-275291 [68]	600 – 2400 mg od	All	44	1200 mg od	No DLTs
CP-471,358	Ongoing	All			

Abbreviations: N, number of patients; od, once daily; bid, twice daily, tds, three times daily.
§, All oral intake except for batimastat.

respectively, was published [46]. All patients studied had serum tumor marker levels rising by 25% or more above pre specified levels in a predefined period of 4 or 12 weeks. Marimastat doses studied varied from 2 mg once daily to 75 mg twice daily. Pharmacokinetic analysis showed that mean trough levels increased almost linear with the dose and that these levels for a given dose were substantially higher compared to healthy volunteers with mean trough levels greater than 40 ng/ml observed at total daily doses of 20 mg and above. The principal toxicity of marimastat was found to be reversible musculoskeletal events (myalgia, arthralgia and tendinitis, predominantly in the upper limbs) with frequency and severity in-creasing with higher doses. Musculoskeletal events severe enough to reduce the dose occurred mostly after the first month of treatment and particularly at doses of 25 mg twice daily or higher, resulting in dose modification or withdrawal in more than one third of the patients. Other infrequent severe side effects involved the gastrointestinal system and a few episodes of elevated liver enzymes. Evaluation of serum tumor marker levels following 4-12 weeks of treatment showed that the proportion of patients showing a rise of their tumor marker at the end of the study period of < 0% or between 0-25% increased with the dose and was significant only in patients receiving doses of 20 mg daily or higher. This single evaluation point led to major discussion and is likely to overestimate clinical potential of the agent. Because the rate of rise of tumor marker levels is not yet validated as a marker of tumor response, the authors compared the survival of the patients with a tumor marker rise of < 0% or between 0-25% ("responders") with the patients with a tumor marker rise > 25% ("non-responders"), and found that survival was significantly different in favor of the responders, thereby suggesting that these marker level changes could be a valid surrogate end point. However due to the limited number of patients and the larger number of variables, this suggestion will have to be further tested in large size trials. Based on the combined analysis of the biological activity, the pharmacokinetic data and the dose related musculoskeletal pain, the recommended dose range for further studies was 10-25 mg twice daily.

A study in patients with advanced lung cancer using marimastat at three different dose levels (25 mg, 50 mg and 100 mg all twice daily) was performed [47]. Dose-limiting toxicity consisting of inflammatory polyarthritis which occurred within 3 weeks from the start of treatment was seen in the first three patients in the 100 mg twice daily group. The next three patients at this dose level received prophylactic non steroidal anti-inflammatory drugs, but these drugs could not prevent the development of the inflammatory polyarthritis. In the 50 mg group similar though less severe toxicity was seen. Two out of three patients in this group did not complete the eight week study period because of early progression, so no reliable recommendations could be made about the optimal dose for further studies.

Trying to find evidence of biological activity based on endoscopic appearance and tumor histology, marimastat was also studied in patients with advanced gastric or gastro-esophageal cancer [48]. Initially 50 mg twice daily was used based on data of the healthy volunteers

study. After five out of six patients developed side effects (gastrointestinal or musculoskeletal), and based on pharmacokinetic data from this and other studies it was decided to continue the study using a lower dose of 25 mg once daily in 29 additional patients. Again the principal side effect was related to the musculoskeletal system. Eventually 37% of the patients experienced this reversible side effect whose frequency increased following prolonged therapy. Additionally in four patient using marimastat for more than three months, a subcutaneous skin thickening of the palmar surface of the hands resembling Dupuytren's disease developed. These side effects were also reversible to a large extent. Activity of the drug studied by endoscopic changes of the tumor with respect to hemorrhage, fibrous cover and tumor size showed a definite increase in fibrous cover in 3/6 patients in the 50 mg twice daily group and 7/29 of the patients in the 25 mg once daily group. Although in 3 patients an endoscopic reduction in tumor size was suggested, this should be interpreted with caution given the difficulties of endoscopic response assessment. Microscopic assessment of tumor tissue samples did not show major histological changes after 28 days of treatment in all but two patients where an increase in fibrous stroma was seen.

AG3340 is a selective inhibitor of MMP-2, MMP-3, MMP-9, MMP-13 and MMP-14, but not MMP-1 (collagenase-1) thought to be associated with the joint related toxicities. In a phase I study doses from 2 to 100 mg orally twice daily were studied [49]. Reversible joint related complaints typically beginning in the shoulders, knees or hands occurred in a dose and time dependent manner. Symptoms were manageable with a drug holiday of 2-4 weeks and a subsequent dose reduction. Drug holidays were necessary in a significant number of patients using doses of 25 mg twice daily and higher for more than four weeks. Preliminary data showed that AG3340 was rapidly absorbed and pharmacokinetics were linear with a plasma half-life ($t_{1/2}$) of 2-3 hours. Plasma levels reached were in the active dose range determined in preclinical tumor models [50].

BAY 12-9566 is an orally bioavailable biphenyl compound with inhibitory activity against MMP-2, MMP-3, and MMP-9. In preclinical studies growth inhibitory activity and reduction of the number of metastases was shown in various tumor models, with elevation of transaminase levels and mild depression of erythropoiesis as the principal toxic effects in animals [51-54]. Four phase I studies including a total of 89 patients have been performed [55-59]. Dose levels studied ranged from 100-1600 mg/day. The main dose related toxicities were mild anemia and thrombocytopenia, elevated transaminase levels and occasionally reversible bilirubin elevations. Other toxicities were mild nausea and vomiting, fatigue and headache. Musculoskeletal effects did not occur. Pharmacokinetic analysis showed a rapid absorption and a less than proportionate increase of plasma steady state levels (C_{ss}) with doses exceeding 100 mg/day suggesting saturable drug absorption. Since C_{ss} levels seemed to reach a plateau

at the higher dose levels (C_{ss} 122 μg/ml at doses of 1600 mg/day) which exceeded biologically active concentrations at least two or three orders of magnitude, no further dose escalation was performed and therefore the maximum tolerated dose could not be determined. Despite achieving relevant plasma concentrations, no consistent effects were found on plasma levels of MMP-2 and MMP-9. For TIMP-2 levels a small increase was found in the higher dose range [59]. Also for other surrogate markers like plasma levels of VEGF, bFGF, and urinary pyridinoline and deoxypyridoline cross links, no obvious relationship with dosing was found [56]. With regard to efficacy, no responses were reported, but about one third of the patients remained on study for more than 3 months and about 6% of patients were on study for more than one year. Based on the results of these four phase I studies, the recommended dose for further studies is 800 mg twice daily.

MMI270 (previously CGS27023A) is an orally bioavailable broad spectrum synthetic hydroxamic acid derivative able to inhibit a wide range of MMPs at nanomolar concentrations in vitro (MMP-1, MMP-2, MMP-3, MMP-9 and MMP-13). Reduced tumor growth in a breast and endometrial rat tumor model and inhibition of hematogenic metastasis of B16 melanoma cells in an experimental and spontaneous metastasis model were seen [60,61]. In a phase I study with doses ranging from 50 mg once daily to 600 mg thrice daily the main toxicities were a self limiting maculopapular rash at higher dose levels and mild to moderate myalgia and arthralgia that was not dose related [62]. The recommended phase II dose was determined to be 300 mg twice daily, as at higher dose levels a marked increase in both incidence and severity of rash was seen. Pharmacokinetic analysis showed a rapid absorption and rapid elimination from the plasma with a $t_{1/2}$ of 1.6 hours. At the recommended dose the plasma levels of MMI270 were ≥ 5 times IC_{50} of the target MMPs for more than 10 hours a day.

COL-3 is an orally available tetracycline analog. Unlike the other MMPIs, tetracycline derivatives not only inhibit collagenase activity but also downregulate its production, inhibit its activation and increase the degradation of the proenzyme. A phase I study was performed with doses escalating from 36 mg to 98 mg/m^2/day [19]. Cutaneous phototoxicity was dose limiting and occurred already at the first dose level. With sun avoidance, protective clothing and the prophylactic use of sunblock, the maximum tolerated dose (MTD) was 70 mg/m^2/day. Three out of 35 patients developed a drug induced systemic lupus erythematosus with arthralgia and fevers. In 4 patients there was unexplained anemia, while bone marrow examinations in three of these patients revealed ringed sideroblasts. Other toxicities included fatigue, anorexia, nausea, vomiting and elevated liver enzymes. Pharmacokinetic analysis revealed that peak plasma levels (C_{max}) were reached after a median of 6 hours and that in the higher dose ranges the increase in C_{max} was not dose-proportional suggesting a saturable absorption. The median single-dose $t_{1/2}$ of 56 hours could potentially lead to accumulation. No

information is given about trough levels throughout the study. Three patients, all with a nonepithelial malignancy, had stable disease for more than 6 months. The authors recommend a dose of 36mg/m^2 for further studies and higher doses when diligent sun precautions are used. In another study with COL-3 preliminary results indicate a MTD of 50 mg/m^2/day, with photosensitivity of the skin and asthenia as principal toxicities [63]. In addition, it was found that plasma MMP-2 and MMP-9 levels were considerably decreased in a number of patients, possibly reflecting a decreased production, since in peripheral blood mononuclear cells the expression of MMP-9 was also decreased. The recommended doses of both studies yielded COL-3 plasma concentrations within the dose that in preclinical models resulted in growth inhibition of primary and metastatic tumors [64]. At this moment a phase I/II study with COL-3 is ongoing in patients with high grade gliomas.

BMS-275291 is a novel orally available non-hydroxamate MMPI with potent inhibitory activity against MMP-2 and MMP-9, which in an animal model did not cause joint related toxicity [65]. In a double blind placebo controlled study with healthy volunteers using doses from 150 to 1200 mg once daily for 14 days, the agent was very well tolerated and no dose-limiting toxicity was found [66]. A phase I study was performed in 44 patients with advanced cancer with doses from 600 to 2400 mg once daily [67,68]. Again the agent was well tolerated and a maximum tolerated dose was not reached. Grade 1 and 2 arthralgia and myalgia was seen in a significant number of patients, but no frank arthritis and only one case of grade 2 tenosynovitis was observed. Based on pharmacokinetic data, showing trough levels at steady state at least 20-fold the IC_{50} values for MMP-2 and MMP-9 at a dose of 1200 mg once daily, this dose was recommended for further clinical studies.

Phase I studies of MMPIs in combination with chemotherapy As MMPIs should be regarded as cytostatic drugs that inhibit tumor growth but do not induce tumor regressions, it is theoretically attractive to combine MMPIs with cytotoxic regimen to augment their effectiveness. In preclinical models MMPIs were shown to have synergistic activity with cytotoxic regimens [27,31-34]. Several phase I studies are performed combining a wide range of commonly used cytotoxic regimens with several MMPIs (Table 4). Marimastat was tested in a number of phase I studies using doses varying from 2-20 mg twice daily, which is within the range of the recommended dose for further studies determined in single agent studies. In general the combinations were well tolerated without indication of additional toxicity. Of some concern are pharmacokinetic data from a study combining carboplatin and paclitaxel with marimastat 10 or 20 mg twice daily which show trough levels of marimastat of 19.2 and 61 ng/ml that are substantially lower than in the single agent studies and for the 10 mg twice daily group are below the target through levels of 40 ng/ml [73].
AG3340 25 mg twice daily was tested in combination with carboplatin/paclitaxel in patients

Table 4. Phase I/II Studies Combining Matrix Metalloproteinase Inhibitors with Cytotoxic Drugs.

MMPI [reference]	Schedule of MMPI[§]	Cytotoxic regimen	Tumor type	N[#]
Marimastat [69]	10 mg bid	Doxorubicin/cyclophosphamide	Breast	9
Marimastat [70]	5-10 mg bid	5-Fluorouracil continuous/bolus	All	13
Marimastat [71]	2-20 mg bid	Carboplatin	Ovarian	31
Marimastat [72]	5-20 mg bid	Gemcitabine	Pancreatic	31
Marimastat [73]	10-20 mg bid	Paclitaxel/carboplatin	NSCLC	22
Marimastat [74]	20 mg bid	Doxorubicin/docetaxel	Breast	11
AG3340 [75]	25 mg bid	Paclitaxel/carboplatin	All	15
AG3340 [76]	25 mg bid	Mitoxantrone/prednisone	Prostate	15
BAY 12-9566 [77]	800 mg bid	Paclitaxel/carboplatin		19
BAY 12-9566 [78]	400-800 mg bid	5-Fluorouracil/folinic acid		17
BAY 12-9566 [79]	400 mg od – 400 mg bid	Doxorubicin/docetaxel	All	7
BAY 12-9566 [80]	800 mg bid	Carboplatin/etoposide	All	8
MMI270 [81]	150 mg tds – 300 mg bid	5-Fluorouracil/folinic acid	Colorectal	18

Abbreviations: N, number of patients; od, once daily; bid, twice daily, tds, three times daily.
§, oral intake for all MMPIs.

with advanced tumors and with mitoxantrone/prednisone in patients with hormone refractory prostate cancer [75,76]. In both studies the combination seemed safe and well tolerated but no pharmacokinetic data were given.

BAY 12-9566 is tested in number of phase I studies in combination with carboplatin/paclitaxel, 5-fluorouracil/folinic acid, carboplatin/etoposide and doxorubicin/docetaxel [77-80]. Preliminary data suggest that in general toxicity of these combinations is acceptable and that no significant pharmacological interactions occur. In the study with 5-fluorouracil 350 mg/m^2 and folinic acid 20 mg/m^2 x5 days q 28 days with BAY 12-9566 starting on day 13, 400 mg twice daily was well tolerated, while 800 mg twice daily, the recommended dose in single agent studies, was not feasible due to occurrence of grade 2/3 thrombocytopenia.

MMI270 was also tested in combination with 5-fluorouracil/folinic acid administered according to the Gramont scheme [81]. MMI270 was added from the second cycle onward. At 300 mg twice daily, preliminary pharmacokinetic analysis did not indicate a marked effect of MMI270 on 5-FU levels. The toxicity related to MMI270 was comparable with the toxicity seen in the single agent study.

Phase II/III studies with MMPIs Two randomized phase II studies with marimastat have been performed in patients with pancreatic carcinoma and malignant melanoma, respectively, but mature results from these studies have not yet been published [82,83]. A randomized phase II study in patients with glioblastoma multiforme comparing oral temozolomide from day 1-5 every 28 days plus AG3340 or placebo daily until unacceptable toxicity or disease progression is ongoing.

Randomized phase III studies with MMPIs have been performed in a range of tumor types and a range of strategies [7]. In general, phase III study strategies include those comparing an MMPI to conventional cytotoxic chemotherapy, those comparing chemotherapy with an MMPI versus chemotherapy alone and those comparing an MMPI to placebo in patients with minimal residual disease.

In 369 patients with inoperable gastric cancer marimastat 10 mg twice daily was compared to placebo [84]. Pretreatment with chemotherapy was allowed if patients had responded or had stable disease. Progression-free survival was significantly increased in the patients using marimastat, but overall survival was not improved. In subgroups of patients with prior chemotherapy and of patients without distant metastases overall survival was significantly better in the marimastat treated group. About 10% of the patients in the marimastat group stopped their treatment due to side effects, mostly musculoskeletal complaints. This study is currently the only one suggesting a benefit of an MMPI, but one must realize that this suggestion is only based on subgroup analysis in small cohorts of patients.

In patients with advanced pancreatic cancer, marimastat (5, 10 or 25 mg twice daily) was tested as first line treatment and compared to gemcitabine 1000 mg/m^2 weekly for seven out of eight weeks [82]. Time to progression and overall survival was significantly better in the gemcitabine group, with no major differences in the marimastat subgroups. Therefore there is no reason to suggest that the difference was caused by sub therapeutic marimastat dose levels. Preliminary data of a randomized trial testing the addition of marimastat 20 mg twice daily to gemcitabine in 239 patients with advanced pancreatic cancer without prior chemotherapy did not show an advantage of the combination in terms of survival, time to progression and quality of life. A study comparing marimastat with placebo in an adjuvant setting in patients after surgery for pancreatic cancer is currently ongoing. A randomized study comparing marimastat 10mg twice daily to placebo in patients with glioblastoma multiforme or gliosarcoma following completion of surgery and radiotherapy did not show a survival benefit for the marimastat group [86]. In addition, studies with marimastat are being performed in other tumor types with minimal disease e.g. non small cell lung cancer (NSCLC) stage IIIA/IIIB with minimal disease after optimal cytoreductive treatment, small cell lung cancer in partial or complete remission after first line chemotherapy and metastatic breast cancer with stable disease or response after first line chemotherapy.

Two large phase III studies are currently ongoing in patients with NSCLC (686 patients) and hormone refractory prostate cancer (553 patients) studying the addition of AG3340 (5, 10 or 15 mg bid) or placebo to a regimen of carboplatin/paclitaxel or mitoxantrone/prednisone respectively [87,88]. Interim results of both studies, including the majority of the included patients, thus far revealed no differences in response rate, progression free survival or overall survival in the treatment arms.

BAY 12-9566 was tested in several phase III trials in which this MMPI was compared to placebo

in patients with small cell lung cancer, NSCLC and ovarian cancer with partial or complete remission after primary treatment. An interim analysis of the study in patients with small cell lung cancer showed inferior survival in the patients treated with BAY 12-9566 [89]. In another phase III trial BAY 12-9566 was compared to gemcitabine in patients with advanced pancreatic carcinoma [90]. An interim analysis, after including 277 patients, showed inferior progression free survival and overall survival in the BAY 12-9566 group, after which the accrual has been closed. Based on these negative results, clinical development of BAY 12-9566 has been suspended.
Several other compounds like BMS-275291 and AE-941 have entered phase III trials, but it is too early to report on any data.

DISCUSSION

The important role of MMPs in the process of tumor growth and metastasis has led to the development of specific inhibitors of these enzymes. Several of these inhibitors have entered clinical trials and results of these studies have recently been presented. Results from preclinical studies and the currently available data from clinical studies make clear that MMPIs will have to be regarded as antiproliferative instead of cytotoxic agents. The development of clinical trials that can optimally assess the role of these new agents forms a major challenge for oncologists, similar to the situation of angiogenesis inhibitors, farnesyl transferase inhibitors and tyrosine kinase inhibitors [91]. In contrast to cytotoxic agents, where phase I studies are being performed to define dose limiting toxicities (DLTs) and to determine the recommended dose for phase II studies, defining the recommended dose for antiproliferative or cytostatic agents is more complicated because often DLTs do not occur. As cytostatic agents will have to be administered for prolonged periods of time in order to exert optimal antitumor activity, knowledge of toxicities following this prolonged administration is important for defining an optimal dose. Furthermore, as some cytostatic agents are completely devoid of side effects, it might not even be possible at all to define one single recommended phase II dose, and instead the optimal biological effective dose must be defined based on other endpoints. Examples of these endpoints are threshold plasma levels known to inhibit tumor growth in preclinical models, threshold plasma levels exceeding IC_{50} of target MMPs, or inhibition of target enzymes within tumors. The last option is often practically impossible since this requires multiple tumor biopsies. Measurement of MMP levels in plasma and other body fluids can give insight in the activity of the MMPI, but thus far such correlative studies have been disappointing [47,59]. Perhaps this is reflecting the fact that MMPIs in general inhibit enzyme activity rather than their secretion. Measurement of surrogate markers of target inhibition can also give insight in biological activity e.g. changes in tumor marker levels (CEA, CA 15.3, CA 19.9 and PSA) or changes of blood flow assessed by PET scanning or dynamic MRI. However these methods have not yet been validated.

Classic single agent phase II studies using tumor regression as an endpoint of activity will almost certainly lead to under-estimation of potential antitumor activity of cytostatic agents. Therefore in order to select agents for further testing in large randomized phase III trials, it may make sense to perform properly designed phase II trials that should preferably be randomized [92]. In these studies tumor regression should be replaced by surrogate end points of antitumor activity e.g. time to progression, tumor marker inhibition and survival rate at a certain predefined time point. In order to avoid a bias in patient selection the study design should be randomized for example using the "randomized discontinuation" design, in which all included patients are being treated with the cytostatic agent for a predefined period of time. Patients not showing disease progression during or at the end of this period could then be randomized to either continue treatment or to receive no drug or placebo. Although these trials can never be powered to detect significant differences, performing them could prevent too early rejection of a potentially active agent or prevent performing large time consuming phase III trials with inactive agents. Until now, no results of such randomized phase II trials with MMPIs have been published.

As mentioned, the results of the few randomized phase III trials with MMPIs that have been presented so far are disappointing. However, in view of the mechanisms of action of MMPIs one can argue whether the patient population studied is the most likely to benefit from growth inhibitory and anti invasive agents such as MMPIs. Usually patients in these kinds of studies have a large tumorload, often with multiple metastases. Therefore a more realistic approach should be to perform studies with MMPIs in patients in whom the tumorload has been optimally reduced either following surgery or optimal cytoreductive, cytotoxic treatment. Such an adjuvant study using marimastat in optimally operated pancreatic carcinoma is currently ongoing.

In these situations, once again, one has to bear in mind however that toxicity occurring after prolonged periods of drug administration, becomes important and thus even toxicity regarded as mild in studies with only short lasting drug administration can turn into a serious problem following prolonged treatment.

One of the most intriguing toxicities related to treatment with MMPIs are musculoskeletal problems. The clinical spectrum varies from mild myalgia and arthralgia to frank tendinitis and arthritis. This toxicity occurs in almost all broad spectrum MMPIs especially after a prolonged period of treatment, with symptoms occurring earlier and being more severe at the higher dose ranges, although in the study with MMI270 musculoskeletal complaints were dose independent [62]. It is suggested that inhibition of MMP-1 is associated with the joint related problems and therefore MMPIs that do not inhibit MMP-1 have been developed. BAY 12-9566 is such an MMPI, and in clinical studies with this compound indeed no musculoskeletal side effects were seen, whereas AG3340, which only inhibits MMP-1 in the nanomolar range compared to inhibition of other MMPs in the picomolar range, musculoskeletal toxicity

was only seen at the higher dose levels. The exact role of MMP-1 in the pathogenesis of the musculoskeletal side effects is still a matter of debate. Another possible explanation for the differences in musculoskeletal side effects reported could be the differences in inhibition of TNF-α-converting enzyme (TACE), an enzyme belonging to the reprolysin family of Zn^{2+} metalloproteinases. TACE acts as a sheddase and is held responsible for the release of soluble TNF-α from its membrane bound precursors, while TNF-α is associated with inflammatory arthritis. [93]. In an animal tendinitis model it was found that a broad spectrum MMPI with anti-sheddase activity was active in a mouse B16 melanoma model without inducing a tendinitis, while a comparable broad spectrum MMPI but without anti-sheddase activity was also active in the cancer model but did induce development of tendinitis [94]. In the same experiments it was shown that small spectrum MMPIs did not cause development of tendinitis but were not active either in reducing tumor growth. Although these findings may not be generalized, these data show that changes in MMP specificity can influence antitumor effects and toxicity profile and that therefore further research is needed to characterize the exact role of individual MMPs in different tumor types.

CONCLUSION

The recognition of the concept of MMPs being involved in the process of tumor growth and metastasis and the subsequent development of a large number of agents able to inhibit the enzyme activity, has led to the evaluation of several of these new agents in early clinical trials and randomized clinical trials of which the first results now become available. The initial enthusiasm on the possible use of MMPIs in the treatment of cancer has clearly been damped. We seem to be in a period of considerable concern whether a balance between required activity and avoidance of toxicity, based on focused targeting of specific MMPIs can ever be achieved. We believe that the concept of MMPIs is too intriguing to completely reject their development, but that at the current stage focus should again be on preclinical research.

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CHAPTER 9

SUMMARY AND CONCLUSIONS

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This thesis presents the results of a number of phase I studies with new target specific growth inhibitory or cytostatic anticancer agents, administered as single agent or in combination with cytotoxic chemotherapy. The new target specific agents studied are an epidermal growth factor receptor tyrosine kinase inhibitor, a farnesyltransferase inhibitor and an angiogenesis inhibitor, respectively. Whereas the first two agents act on targets that are located in the cancer cell wall and within the cytoplasm, respectively the third agent is specifically developed to inhibit the formation of new blood vessels in the peritumoral stroma.

In **chapter 2** an overview is given of the challenges that are being faced when designing early clinical trials with target specific agents. In comparison to phase I studies with cytotoxic agents that primarily focus on defining the maximum tolerated dose and dose-limiting toxicities in order to determine the recommended dose for subsequent studies, phase I studies with target specific agents need a different design. As target specific agents are frequently found to have a mild or even essentially non-toxic profile, defining the optimal biological dose rather than the maximal tolerated dose is more appropriate. In order to be able to define such an optimal biological dose it is necessary to introduce new endpoints in these studies. Such endpoints encompass pharmacokinetic evaluation and comparisons as well as all sorts of measurements related to target inhibition. Although these latter measurements should ideally be done in tumor tissue, practical issues greatly hamper this methodology. For this reason alternative measurements or surrogate tissues are being used, such as the determination of EGFR phosphorylation status in skin biopsies, serial measurements of farnesyltransferase activity in peripheral blood mononuclear cells and repeated assessments of tumor blood flow using dynamic or contrast enhanced MRI techniques.
In traditional phase II studies with cytotoxic anticancer agents, the percentage of patients with tumor regression is used as a surrogate endpoint that serves to estimate the antitumor activity and to determine whether large scale phase III studies should be performed. For target specific anticancer agents this design of phase II studies is not very appropriate, as tumor regression is not likely to occur. Therefore alternative endpoints such as time to progression, changes in tumor marker levels and measuring target inhibition can be used. Phase II studies with target specific anticancer agents preferably should be randomized in order to optimally assess anticancer activity of these agents.

In **chapter 3** the results of a phase I study with PKI166, a highly specific orally available inhibitor of the epidermal growth factor receptor tyrosine kinase activity, are presented. This study describes daily oral administration of PKI166 to patients in a continuous or intermittent administration schedule. In the first part of the study PKI166 was administered daily without

interruption, but due to the onset of dose-limiting transaminase elevations a regimen of PKI166 administered daily for 2 weeks followed by 2 weeks off medication was subsequently studied. With this alternative dosing schedule, dose-limiting toxicities consisting of transaminase elevations, diarrhea and skin rash were observed and the maximum tolerated dose was set at 750 mg daily for 2 weeks every 4 weeks. Pharmacokinetic analysis revealed fast absorption and dose dependent drug exposure. At the recommended dose no significant effect of food intake on PKI166 pharmacokinetics was seen and plasma drug concentrations were reached that were thousand fold higher than those needed to inhibit EGFR activity in vitro. Results of pharmacodynamic studies determining activated EGFR tyrosine kinase activity in skin biopsies and hair follicle samples taken before and during administration of PKI166 were not consistent and therefore not conclusive. Tumor regressions were not observed in this study, but prolonged stable disease was seen in several patients suggesting antitumor activity.

In **chapter 4** the results of a phase I study combining the farnesyltransferase inhibitor BMS-214662 with cisplatin are presented. Farnesylation of Ras proteins is a prerequisite for membrane association which, in turn, is essential for their biological function, being the transduction of a variety of extracellular signals from the cell surface to several downstream intracellular pathways. Activated Ras proteins are involved in cellular proliferation, cellular adhesion and apoptosis. BMS-214662 was administered as a 1-hour infusion in escalating doses, followed by cisplatin in a 4-hour infusion at a fixed dose dose of 75 mg/m^2. It was shown that BMS-214662 could be combined safely with cisplatin, without apparent mutual pharmacokinetic interaction. The maximum tolerated dose of BMS-214662 in combination with cisplatin was set at 200 mg/m^2, and was equal to the previously determined single agent maximum tolerated dose of BMS-214662 administered once every three weeks. A dose-dependent inhibition of farnesyltransferase activity in peripheral blood mononuclear cells was observed, confirming target inhibition, in a surrogate tissue.

In **chapter 5** the results of a phase I study with the angiogenesis inhibitor ABT-510 are presented. ABT-510 is a chemical derivative of the naturally occurring angiogenesis inhibitor thrombospondin-1 and was administered subcutaneously continuously or in a once- or twice-daily bolus regimen, in patients with advanced cancer. Since administration of ABT-510 by the continuous route resulted in painful subcutaneous infiltrates, no dose escalation was performed with that type of drug administration. ABT-510 administered in a once- or twice-daily bolus regimen, however, was very well tolerated and at the dose levels studied linear dose and time independent pharmacokinetics were observed. A maximum tolerated dose could not be defined due to the fact that a predefined maximum daily injection volume of 2.6 mL was reached without dose-limiting toxicity. After it was shown that ABT-510 could be administered safely for prolonged periods of time, phase Ib studies of ABT-510 in combination with two frequently

used chemotherapy regimens were performed. The results of these studies with ABT-510 in combination with 5-fluorouracil/leucovorin and gemcitabine/cisplatin are presented in the **chapters 6 and 7**, respectively. In both studies it was shown that ABT-510 could be combined safely with these chemotherapy regimens without pharmacokinetic interaction and without additive toxicity.

In **chapter 8** the recent developments and future perspectives of matrix metalloproteinase inhibitors are described. Matrix metalloproteinases are important enzymes involved in various crucial steps in cancer cell growth and progression, and therefore inhibiting these enzymes has been considered to be a rational approach for the development of a new class of target specific anticancer agents. The various aspects of the development of the matrix metalloproteinase inhibitors and the results of a number of relevant clinical trials either as single agent or combined with commonly used cytotoxic chemotherapy regimens are reviewed. Somewhat unexpectedly, in clinical trials a recognizable pattern of debilitating musculoskeletal dose-limiting adverse event was observed, whereas on the other hand antitumor activity was disappointingly low. Currently it seems that inhibitors of matrix metalloproteinases will no longer be applied in the clinical setting of anticancer treatment, thereby damping the initial enthusiasm.

Final conclusions and future perspectives This thesis presents the results of phase I studies with some of the new target specific anticancer agents either as single agent or in combination with commonly used cytotoxic regimens. Although these target specific agents have shown promising activity in various preclinical models and settings, it is obvious that in the clinical setting results are sometimes disappointing, as was the case with matrix metalloproteinase inhibitors. Our data add to the fact that early clinical trial design for non-cytotoxic agents will need to incorporate various levels of translational research, and will be relying on endpoints other than those used for cytotoxic drugs. Yet many answers remain unanswered, partly due to the fact that the absolute functional relevance of the target for human tumor development frequently remains elucidated. For instance farnesyltransferase inhibitors such as described in chapter 4, meanwhile are known not to yield activity through Ras proteins but through other proteins that require farnesylation. The almost absent predictive value of laboratory models for the human situation remains an unsolved problem in this respect. Yet it is evident that early clinical trial design is changing, and will further have to change in order to show presence or absence of proof of concept early. These studies will allow us to assess the optimal biological dose using adequate, validated surrogate markers and will doubtlessly help us in deciding whether or not these agents can subsequently be tested in large randomized phase III studies. In the next couple of decades, it can then be expected that a number of new agents will be launched that will further improve the perspectives of cancer patients.

CHAPTER 10

SAMENVATTING EN CONCLUSIES

DANKWOORD

CURRICULUM VITAE

PUBLICATIES

SAMENVATTING EN CONCLUSIES

Bij de behandeling van kanker met chemotherapie is in de afgelopen decennia vooral gebruik gemaakt van cytotoxische middelen, die essentiële intranucleaire processen remmen die betrokken zijn bij de celdeling. Als gevolg van beschadiging van het DNA wordt celdood bevorderd en kunnen tumoren in omvang afnemen. Hoewel deze cytotoxische middelen effectief zijn, wordt de toepassing ervan in de klinische praktijk beperkt door het optreden van bijwerkingen, die vaak hinderlijk en in sommige gevallen zelfs levensbedreigend kunnen zijn. Deze bijwerkingen zijn het gevolg van de aspecifieke werking van de cytotoxische middelen, die naast tumorcellen immers ook normale cellen zoals beenmergcellen, slijmvliescellen, haarzakjes en zenuwcellen kunnen beschadigen. Naast de beperkingen die het gevolg zijn van het optreden van bijwerkingen, wordt de werkzaamheid van de cytotoxische middelen verder beperkt door het frequent optreden van resistentie tegen deze middelen. Hierbij spelen diverse verschillende mechanismen een rol, zoals inactivatie van het p53 tumorsuppressor gen, en overexpressie van genen die betrokken zijn bij het stimuleren van efflux van cytotoxische middelen uit de kankercel. Het optreden van bijwerkingen enerzijds, en de geschetste beperkingen in effectiviteit anderzijds dragen bij tot de op dit moment teleurstellende conclusie dat vooral uitgezaaide kanker momenteel nog maar zelden genezen kan worden met behulp van cytotoxische middelen.

In de laatste jaren heeft de wetenschap talrijke processen ontrafeld die specifiek zijn voor het ontstaan en overleven van kankercellen en het optreden van uitzaaiingen. Aansluitend op het ontstaan van deze inzichten zijn vele nieuwe middelen ontwikkeld die specifiek gericht zijn tegen een van deze processen, waarbij de hoop is ontstaan dat op deze wijze de kankercel meer doelgericht kan worden aangepakt. Deze nieuwe middelen kunnen zowel gericht zijn tegen processen in de kankercel zelf als tegen processen die zich in de directe nabijheid van de kankercel afspelen. Deze nieuwe generatie antikanker middelen wordt “target specific” genoemd, en omdat het effect van deze middelen vooral groeiremmend of cytostatisch is, worden ze met een enigszins verwarrende term ook wel cytostatische middelen genoemd, dit dus in tegenstelling tot de klassieke cytotoxische middelen. Als gevolg van de specifieke werking van deze nieuwe cytostatische middelen is de hoop en verwachting ontstaan dat bijwerkingen minder zullen optreden.

De klinische toepassing van deze nieuwe “target specific” antikanker middelen heeft grote invloed op het ontwerp en de uitvoering van vroeg-klinische fase I en II studies. Fase I studies met nieuwe cytotoxische middelen worden gekenmerkt door het toedienen van opklimmende doseringen aan kleine groepen patiënten, met als doel het vaststellen van de maximaal tolereerbare dosis en het beschrijven van de belangrijkste bijwerkingen om zo een aanbevolen dosis te bepalen voor vervolgstudies. Vervolgens kan in fase II studies bij patiënten met een bepaald tumortype deze aanbevolen dosering worden getest om de effectiviteit van het

betreffende middel vast te stellen. De belangrijkste maat voor deze effectiviteit is het percentage patiënten, bij wie er daadwerkelijk tumorverkleining optreedt. Omdat zoals vermeld de cytostatische of 'target specific" antikanker middelen zowel een ander werkingsmechanisme als een ander potentieel bijwerkingenprofiel zullen hebben, zal hiermee bij het ontwerp en de uitvoering van fase I en II studies terdege rekening moeten worden gehouden.

Dit proefschrift beschrijft de mogelijke aanpassingen die nodig zijn bij de opzet en uitvoering van vroeg-klinisch onderzoek van nieuwe doelgerichte c.q. "target specific" antikanker middelen. Tevens worden de resultaten beschreven van diverse fase I studies met enkele van deze nieuwe doelgerichte antikanker middelen, zowel getest als monotherapie als in combinatie met veelgebruikte cytotoxische middelen. De nieuwe doelgerichte antikanker middelen die werden bestudeerd zijn een epidermale groeifactor receptor tyrosine kinase remmer, een farnesyltransferase remmer en een angiogenese remmer.

In **hoofdstuk 2** wordt een overzicht gegeven van de aanpassingen die overwogen moeten worden bij het ontwerp en de uitvoering van fase I en II studies met doelgerichte antikanker middelen. In vergelijking met de fase I studies met cytotoxische antikanker middelen waarbij de nadruk ligt op het bepalen van de maximaal tolereerbare dosis en het beschrijven van de daarbij optredende acute bijwerkingen ter bepaling van de aanbevolen dosis voor vervolgstudies, is bij bestudering in fase I studieverband van doelgerichte antikanker middelen een andere aanpak nodig. Omdat de bijwerkingen naar verwachting geringer of mogelijk zelfs geheel afwezig zijn, is het bepalen van de maximaal tolereerbare dosis vaak onmogelijk en is het beter de dosis te bepalen waarbij het optimale biologische effect wordt bereikt. Om de dosering met het optimale biologische effect te bepalen zijn nieuwe eindpunten nodig. Voorbeelden hiervan zijn het gebruik van farmacokinetische parameters, waarbij bijvoorbeeld plasmaconcentraties die effectief zijn gebleken in diermodellen gebruikt worden als streefwaarde in studies met mensen. Verder kan onderzocht worden of het nieuwe middel ook werkelijk in staat is het beoogde doel te remmen. Dit effect zou bij voorkeur in tumorweefsel zelf bestudeerd moeten worden, maar in verband met beperkingen in de uitvoerbaarheid hiervan, wordt vaak gekeken naar het effect in surrogaat weefsels, zoals huidweefsels en witte bloedcellen.
In traditionele fase II studies met cytotoxische antikanker middelen wordt vaak het percentage patiënten waarbij een verkleining van de tumor optreedt als maat gebruikt voor de activiteit van een nieuw antikanker middel, met als doel de beste middelen te selecteren die uiteindelijk getest gaan worden in grootschalige effectiviteitsstudies. Omdat bij doelgerichte antikanker middelen er veelal geen tumorverkleining optreedt maar veeleer groeiremming ofwel stabilisatie, is deze studiemethode veelal niet geschikt en moet op andere manieren een indruk worden verkregen van de antikanker activiteit. Voorbeelden hiervan zijn het vaststellen van de tijd die het gemiddeld duurt voordat de tumor groeit en die tijd te vergelijken met historische

groeiwaarden van patiënten met soortgelijke tumoren; het gebruik van tumormerkstoffen om een indruk te krijgen van het ziekte beloop; het gebruik van metingen in tumor of surrogaat weefsel waarbij de mate en frequentie van remming van het doel wordt gemeten. Omdat het hierbij toch vaak moeilijk is om te beoordelen of een nieuw middel daadwerkelijk antikanker werking heeft, zouden de fase II studies bij voorkeur gerandomiseerd moeten zijn, zodat een betere vergelijking mogelijk is.

In **hoofdstuk 3** worden de resultaten beschreven van een fase I onderzoek met PKI166, een remmer van het epidermale groeifactor receptor tyrosine kinase. De studie beschrijft de toediening van PKI166 in tabletvorm in een eenmaal daagse dosering. In het eerste deel van de studie werd PKI166 dagelijks gegeven zonder onderbreking. In de eerste twee cohorten van 50 en 100 mg werden frequent leverfunctiestoornissen waargenomen. In verband hiermee werd in het tweede deel van de studie een alternatief schema bestudeerd met dagelijkse toediening gedurende 2 weken gevolgd door 2 weken zonder medicijnen. Met dit alternatieve schema traden de bijwerkingen, bestaande uit transaminase verhogingen, diarree en huiduitslag, pas op bij veel hogere doseringen en werd de aanbevolen dosis voor vervolgstudies bepaald op eenmaal daags 750 mg gedurende 2 weken elke 4 weken. Het farmacokinetische onderzoek toonde aan dat PKI166 snel werd opgenomen vanuit de darm, waarbij inname van voeding weinig effect had op de opname en verdeling in het lichaam en waarbij concentraties in het bloed werden bereikt die het duizendvoudige waren van actieve concentraties in diermodellen. Er werd ook gekeken naar de remming van de fosforylering in huidbiopten van de onderarm en in haarfollikels van de behaarde hoofdhuid. De resultaten van deze onderzoeken waren niet consistent. In de 54 patiënten die deelnamen aan de studie werd geen tumorregressie waargenomen, echter wel stabilisatie van de tumoren in een aanzienlijk aantal patiënten.

In **hoofdstuk 4** worden de resultaten beschreven van een fase I studie met de farnesyltransferase remmer BMS-214662 gecombineerd met cisplatin. BMS-214662 werd in opklimmende doseringen toegediend via een infuus gedurende 1 uur waarna vervolgens cisplatin intraveneus werd toegediend gedurende 4 uur in een vaste dosering van 75 mg/m2. In deze studie werd aangetoond dat BMS-214662 veilig kon worden gecombineerd met cisplatin zonder wederzijdse farmacokinetische interacties. De maximaal tolereerbare dosis van BMS-214662 werd vastgesteld op 200 mg/m2, overeenkomend met de dosering die werd toegepast als monotherapie. In deze studie kon tevens worden aangetoond dat BMS-214662 is staat was om op een dosisafhankelijke manier de farnesyltransferase activiteit in witte bloedcellen te remmen.

In **hoofdstuk 5** worden de resultaten beschreven van een fase I studie met de angiogenese remmer ABT-510. ABT-510 is afgeleid van de natuurlijk voorkomende angiogenese remmer

thrombospondin-1 en werd onderhuids toegediend via een continue lopend infuus of via een- of tweemaal daagse injecties. De toediening via een onderhuids infuus gaf al bij de laagste dosering aanleiding tot pijnlijke onderhuidse ontstekingen, zodat deze toedieningmethode niet verder werd onderzocht. Daarentegen werden de een- en tweemaal daagse injecties zeer goed verdragen. De maximaal tolereerbare dosis kon niet worden bepaald, omdat het van te voren gedefinieerde maximale injectievolume van 2.6 ml werd bereikt zonder bijwerkingen. Er was sprake van een lineaire farmacokinetiek onafhankelijk van de tijd. Nadat was aangetoond dat ABT-510 veilig voor langere tijd kon worden toegediend, werden nieuwe fase I studies uitgevoerd waarin ABT-510 werd toegediend in combinatie met twee frequent gebruikte chemotherapie regimes.

De resultaten van deze studies waarin ABT-510 werd gecombineerd met 5-fluorouracil/leucovorin en cisplatin/gemcitabine worden gepresenteerd in **hoofdstuk 6** en **7**. In deze studies werd aangetoond dat ABT-510 eenvoudig en veilig kon worden gecombineerd met deze twee regimes, zonder wederzijdse farmacokinetische interacties en zonder additionele bijwerkingen.

In **hoofdstuk 8** worden de recente ontwikkelingen en verwachtingen voor de toekomst beschreven van een andere groep doelgerichte antikanker middelen namelijk de matrix metalloproteinase remmers. Matrix metalloproteinases zijn enzymen die betrokken zijn bij de afbraak van de steunweefsels rondom tumoren en zodoende betrokken zijn bij vele essentiële stappen in de ontwikkeling van een tumor zoals tumorgroei, vorming van nieuwe bloedvaten en het uitzaaiingsproces. Remming van deze enzymen werd als een zeer rationele stap gezien in de ontwikkeling van nieuwe doelgerichte antikanker middelen, hetgeen werd ondersteund door resultaten van dierexperimentele studies. De diverse aspecten van de ontwikkeling van matrix metalloproteinase remmers en de resultaten van een aantal van de meest relevante klinische studies met matrix metalloproteinase remmers, zowel toegepast als monotherapie als in combinatie met frequent gebruikte cytotoxische chemotherapie regimes, worden beschreven. Enigszins onverwacht werden in vrijwel al deze klinische studies invaliderende spier- en gewrichtsklachten geobserveerd, terwijl anderzijds de antikanker activiteit teleurstellend laag was. Momenteel lijkt er geen indicatie meer te bestaan voor de klinische toepassing van matrix metalloproteinase remmers.

Slotconclusie en toekomst verwachting

Dit proefschrift beschrijft de resultaten van fase I studies met enkele van de nieuwe doelgerichte antikanker middelen, zowel toegepast als monotherapie als in combinatie met frequent gebruikte chemotherapie regimes. Hoewel bij toepassing van deze nieuwe middelen veelbelovende antikanker activiteit is aangetoond in verschillende dierexperimentele modellen, blijken in de klinische praktijk de resultaten nogal eens teleurstellend, zoals bijvoorbeeld

in geval van de matrix metalloproteinase remmers. Onze data bevestigen dat vroeg-klinische studies met doelgerichte antikanker middelen incorporatie vereisen van additionele translationele onderzoeken, waarbij een brug wordt geslagen tussen basale kennis en de toepassing bij de mens, en waarbij tevens gebruik wordt gemaakt van andere eindpunten dan tot nu toe het geval was bij bestudering van cytotoxische antikanker middelen. Desondanks resteren er nog vele vragen, deels omdat het werkelijke functionele belang van nieuwe "targets" bij de behandeling van kanker nog opgehelderd dient te worden. Zoals bijvoorbeeld in geval van farnesyltransferase remmers beschreven in hoofdstuk 4 waarvan ondertussen bekend is dat de antikanker activiteit niet verloopt via het Ras eiwit, maar via andere eiwitten waarbij farnesylering een belangrijke rol speelt. Het veelal ontbreken van goede laboratorium modellen die bruikbaar zijn bij de mens vormen nog steeds een onopgelost probleem. Toch is het duidelijk dat de opzet van vroeg-klinische studies aan het veranderen is en in de toekomst nog verder zal veranderen, gericht op het vroegtijdig vaststellen van de aan of afwezigheid van het "proof of concept". Deze studies zullen ons hopelijk in staat stellen de optimale dosis adequaat vast te stellen, gebruik makend van gevalideerde surrogaat markers en zullen ons ongetwijfeld helpen om te beslissen of deze nieuwe middelen vervolgens getest moeten worden in grote gerandomiseerde fase III studies. In de eerstvolgende decades, valt te verwachten dat een aantal nieuwe middelen zullen worden gelanceerd die de vooruitzichten van patiënten met kanker verder zullen verbeteren.

DANKWOORD

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CURRICULUM VITAE

Ronald Hoekstra werd op 8 januari 1965 geboren te St. Annaparochie, gemeente het Bildt (Fr). In 1983 behaalde hij het diploma gymnasium ß, aan de openbare scholengemeenschap Hendrik van der Vlist te Utrecht. In datzelfde jaar ving hij zijn studie geneeskunde aan te Rotterdam. Het artsdiploma werd behaald in mei 1990. Hierna werd tot juli 1991 de dienstplicht vervuld als arts-assistent anesthesie bij het perifere team van de Koninklijke Landmacht, gevestigd in het Franciscus Ziekenhuis te Roosendaal. Na een jaar gewerkt te hebben als arts-assistent interne geneeskunde in het St. Elisabeth Ziekenhuis te Tilburg, heeft hij van 1992 tot 1993 een jaar in het Academisch Ziekenhuis te Utrecht gewerkt als arts-onderzoeker bij de vakgroep Metabolisme en Lipiden onder leiding van prof. dr. D.W. Erkelens†. In oktober 1993 werd begonnen met de opleiding tot internist in het St. Elisabeth Ziekenhuis te Amersfoort (opleiders dr. R.A. Geerdink en later dr. O.J.J. Cluysenaer). Vanaf september 1996 werd de opleiding voortgezet in het Academisch Ziekenhuis te Utrecht (opleider prof. dr. D.W. Erkelens). Registratie tot internist vond plaats op 1 oktober 1999. In de periode van mei 1999 tot mei 2003 was hij werkzaam op de afdeling interne oncologie van het Erasmus MC, aanvankelijk locatie Daniel den Hoed, vanaf januari 2000 locatie Dijkzigt (opleider prof. dr. G. Stoter). In deze periode werden de onderzoeken verricht die werden beschreven in dit proefschrift. Registratie tot internist-oncoloog vond plaats in oktober 2001. Vanaf april 2003 is hij werkzaam als internist-oncoloog in het Twenteborg Ziekenhuis, onderdeel van de Ziekenhuisgroep Twente. Hij is getrouwd met Hendrine Verhoeven, samen hebben ze 3 kinderen, Robert-Jan, Annelot en Thomas.

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