EARLY CLINICAL STUDIES EXPLORING NEW TARGETS IN ANTICANCER TREATMENT

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CHAPTER 1

INTRODUCTION AND OUTLINE OF THE THESIS.

Ferry ALM Eskens

Chemotherapy for cancer has always greatly relied upon the use of cytotoxic agents. These agents exert their activity in the process of nuclear DNA and RNA replication, and their activity in sensitive models leads to cell death and subsequent tumor shrinkage. Although a number of human tumor types can nowadays be cured by treatment involving cytotoxic agents, the overall clinical balance of efficacy and toxicity of these agents remains disappointing.

In contrast to the situation with cytotoxic agents, where introduction as anticancer agent is usually preceded by large-scale random screening procedures, more recent research has focussed on anticancer agents for which development and design was preceded by the identification of specific tumor-related molecular targets or processes. These targets and processes are located either intracytoplasmic, in the cell membrane, or even completely outside the tumor cell itself. Examples of these molecular targets and processes are the intracytoplasmic polyamine synthesis pathway and farnesyl transferase pathway, the activity of various transmembrane signal transduction pathways, and the enzymatic breakdown of the extracellular matrix and the process of tumor-related angioneogenesis, respectively.

Numerous animal studies with these new so-called rationally designed anticancer agents, aiming at one of the above targets, yielded no or only minor toxicity. The predictive value of results of animal studies for the human situation, however, is relatively limited. Nevertheless, in theory, when performing clinical studies with such compounds, toxicity may turn out to be absent or only mild. As a resultant, defining dose limiting toxicity as an endpoint for phase I studies may not be possible, and consequently, defining a recommended dose for additional activity testing might proof to be difficult.

Many of these new anticancer agents were shown in preclinical studies to have a cytostatic rather than cytotoxic effect. Although in a limited number of these studies tumor regressions were noted, growth inhibition was the most frequently seen effect. Although, as said, such results cannot easily be extrapolated to the human situation, it may still be anticipated that these agents are not likely to induce tumor regression in clinically detectable tumor masses. Because of this, performing phase II studies might not make too much sense.

Taken these considerations together, it is obvious that the design of clinical studies with new cytostatic agents needs a thorough reappraisal.

This thesis involves clinical phase I studies performed in this shifting field of anticancer treatment. It outlines several of the problems described above and reports on efforts made to suggest alternative study endpoints.

CHAPTER 2

CLINICAL STUDIES IN THE DEVELOPMENT OF NEW ANTICANCER AGENTS EXHIBITING GROWTH INHIBITION IN MODELS: FACING THE CHALLENGE OF A PROPER STUDY DESIGN.

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ABSTRACT

Various new specifically targeted anticancer agents such as matrix metalloproteinase inhibitors, angiogenesis inhibitors, farnesyl transferase inhibitors, and tyrosine kinase inhibitors have been developed in recent years. These agents exert antitumor activity through specific target inhibition, and preclinical studies showed dose-dependent tumor growth inhibition and only sporadic tumor regressions. Toxicity of these new agents was often strikingly mild or did not occur at all. Clinical studies are now being performed.

Because these new agents might have a different toxicity profile and exert their antitumor activity in a way that is completely different from that of cytotoxic agents, the design of clinical studies will have to be adapted in several ways, and new endpoints for phase I, II, and III studies must be defined.

INTRODUCTION

Over the last few decades, cancer treatment has included hormonal therapy as well as the use of presently called "classic" or "conventional" *cytotoxic* agents. The anticancer activity of cytotoxic agents is exerted through direct interference with various steps in the process of nuclear DNA and RNA replication, resulting in dose-dependent cell kill and tumor regression in sensitive models. Unfortunately, there is a lack of selectivity due to which normal cells and tissues are also affected, resulting in a wide variety of unwanted side-effects.

More recently, basic molecular research has increasingly unraveled various processes involved in the malignant transformation of cells and in carcinogenesis. Some of these processes were identified as potential targets for specifically designed inhibitory agents. For instance, the various steps involved in the intracellular signal transduction, and the extracellular processes of matrix breakdown and angiogenesis are presently targeted by specifically and rationally designed agents. Numerous of these agents have been tested in *in vitro* studies showing target- and cell growth inhibition. Also in *in vivo* models such as human tumor xenografts, dose-dependent tumor growth inhibition was seen for most of these agents, whereas for only a few of them tumor regressions have been reported [1, 2, 3]. Agents that only show growth inhibition or retardation in models should be referred to as *cytostatic* rather than *cytotoxic*. Importantly, in contrast to the antitumor activity obtained by intermittent schedules of administration for cytotoxic agents, showing tumor regressions in models, for most of the agents inducing growth inhibition, optimal activity was obtained with prolonged or continuous administration. To enable such prolonged or continuous dosing, obviously toxicity will have to be minimal. Fortunately, and as expected based upon their mechanism of action, in animal toxicology studies the toxicity of the vast majority of cytostatic agents when administered at their respective active doses for prolonged periods of time often was strikingly mild [1].

Since pharmacokinetic studies in animals have shown that plasma concentrations could be achieved that equaled adequate inhibitory concentrations from *in vitro* studies, and *in vivo* pharmacodynamic studies were able to demonstrate

target inhibition in tumor tissue, clinical studies with these novel cytostatic agents have been initiated.

The design of clinical studies in the development of new *cytotoxic* agents has always been aimed at evaluating toxicity, safety, and efficacy respectively, and adequate endpoints to describe these characteristics have been defined over the last decades. In phase I studies, clinical, biochemical and hematological side-effects are described, and dose limiting toxicity (DLT) is defined. The maximum tolerable dose (MTD), usually the same as the recommended dose for further testing, is determined and this recommended dose is used in subsequent activity testing in phase II studies. In these phase II studies, the number and extensiveness of tumor regressions (classified according to the commonly used criteria) following treatment with a preset treatment schedule and dose is evaluated to determine hints of antitumor activity. Finally, randomized phase III studies are performed to evaluate clinical efficacy, defined by time to progression (TTP), disease free survival and/or overall survival following treatment. Clearly, these endpoints can be used for studies with novel agents, as long as these agents do show tumor regressions in studies on animal models. However, *cytostatic* agents exert their anticancer activity through inhibition of one specific target process, and most frequently, this results in dose-dependent inhibition of cell growth but not in cell death per se. Clinically, this will not translate into tumor regression. Also the toxicity profile of cytostatic agents will likely differ essentially from that of classic cytotoxic agents, and therefore some endpoints used in studies with cytotoxic agents will not be suitable for studies with agents only inducing inhibition or retardation of growth. As a consequence, different endpoints might have to be defined. In other words, clinical studies in the development of new cytostatic agents may require designs different from those used in the development of cytotoxic agents. In the rest of this paper, the term "cytostatic agent" will refer to agents only inducing growth inhibition in laboratory models, whereas the term "cytotoxic agent" will refer to agents inducing direct cell kill resulting in tumor regressions in such models. We will discuss issues in the design of phase I, II, and III studies in the development of new cytostatic anticancer agents and will highlight reflections on potential new endpoints that will have to be defined and validated for these studies. The different endpoints used in or proposed for studies in the

development of either cytotoxic or cytostatic anticancer agents are summarized in table 1.

Table 1 Endpoints of studies in the development of anticancer agents

	Cytotoxic Agents	***Cytostatic Agents***
Phase I studies	*1: Acute toxicity* *2: Maximum Tolerated Dose* Defined by: Toxicity	*1: Acute toxicity* *2: Delayed toxicity* *3: Optimal biologic effect dose* Defined by: Target AUC Inhibition of cellular target Inhibition of surrogate marker
Phase II studies	*1: Antitumor activity* Defined by: Tumor regression rate *2: Delayed toxicity*	*1: Antitumor activity* Defined by: Time to progression Surrogate marker inhibition
Phase III studies	*1: Antitumor efficacy* Defined by: Time to progression Disease free survival Overall survival Quality of life	*1: Antitumor efficacy* Defined by: Time to progression Disease free survival Overall survival Quality of life

PHASE I STUDIES

Almost without exception, *in vitro* studies with cytotoxic agents have shown a quite steep dose-response relationship. Therefore, in order to achieve maximal tumor cell kill, the administered dose should be as high as possible. However, cytotoxic agents are not cell or tissue specific and due to this, normal cells are also affected,

resulting in unwanted and often severe side-effects. To obtain the best possible balance between the targeted effect and side-effects, phase I studies are designed to describe the acute toxicity profile of drugs and to define their maximum tolerated dose (MTD), which in most terminologies is the dose that can be safely administered in subsequent studies on activity and efficacy.

For cytostatic agents, however, preclinical studies have shown that target inhibition can be achieved over a wide dosing range. On top of this, when these agents are administered at their active doses, this quite frequently results in either no or only minor acute side-effects. Therefore simply defining an MTD in phase I studies will obviously not be adequate. Defining MTD may not be possible at all if side-effects would only occur at doses that can never be administered because of factors unrelated to toxicity, such as the volume necessary to infuse the agent or the number of tablets to be taken.

In preclinical models, most of the currently studied cytostatic agents did show a sometimes dose-dependent but always completely reversible tumor growth inhibition. Optimal results were almost always achieved with prolonged schedules of administration. Translating these results to clinical studies, it is obvious that prolonged or maybe even continuous administration will have to be preferred in order to achieve optimal antitumor activity. This means that, in contrast to phase I studies on cytotoxic agents that, as said, focus on acute toxicity, the evaluation of drug-induced toxicity in phase I studies on cytostatic agents must include an assessment of both acute and delayed toxicity. However, commonly patients in phase I testing are at an end-stage of their disease, and because of tumor progression on study, many of them will not be able to continue drug administration for prolonged periods of time. Due to this, phase I studies may underestimate the occurrence of delayed or chronic toxicity. This calls for revisiting the selection of patients entering phase I studies with cytostatic agents. Not only should patients preferably be in good clinical condition, but in addition at least some of them should have tumors not expected to grow rapidly, in order to increase the likelihood of the possibility of prolonged dosing, thereby enabling assessment of both the acute and delayed toxicity profile. This is even more important since phase II studies, presently also performed to evaluate

long term tolerability, will, as we will discuss later, frequently be of no use in the development of new cytostatic agents.

Since MTD is not likely to be an appropriate endpoint, other endpoints will have to be defined. Because a data set involving results of previous studies with cytostatic agents is lacking, it is difficult at this moment to define which endpoints should be used in preference. The following possibilities are therefore only listed as suggestions. Clinically, none of them have yet been validated. In preclinical studies it is frequently possible to link antitumor activity with a certain threshold plasma concentration required for this activity, and therefore it is conceivable that pharmacokinetic parameters such as the area under the plasma concentration-time curve (or AUC) become endpoints of phase I studies, despite the fact that preclinical models are poor predictors of clinical outcome. Second, the determination of the dose inducing the optimal biologic effect could be such a pharmacologically determined endpoint, and pharmacokinetic and pharmacodynamic analyses will thus become important features of phase I studies of new cytostatic agents. With specific targets being inhibited, it should theoretically be possible to measure the target inhibition or its indirect resultants within tumors as marker for antitumor activity. However, obtaining tumor material is often practically impossible, since this approach would require that multiple tumor biopsies be taken. Therefore, only measurements of so-called surrogate markers of target inhibition may be feasible, provided that a relevant, sensitive, and specific surrogate marker of drug-target inhibition is available. If so, serial measurements of these surrogate markers should be pursued and the results of these measurements should be related to the dose of the cytostatic agent in order to indirectly measure target inhibition. Examples of surrogate markers of target inhibition currently under study are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), the levels of which can be determined in plasma, and prelamin A, that can be determined in oral mucosa scrapings and is a marker for inhibition of the enzyme farnesyl transferase [4]. In another target area one may already assume that measuring proenzymatic serum levels of matrix metalloproteinases in order to assess activity of matrix metalloproteinase inhibitors (MMPI) would not be useful, because the MMPI inhibit the activity of these

proteinases rather than their secretion [5]. Measuring target inhibition would thus require a functional test.

Another way to evaluate target inhibition directly or indirectly could possibly be obtained through minimally invasive procedures such as PET-scanning or nuclear magnetic angiography, which can yield information about tumor viability and tumor vascularisation, respectively. These techniques are interesting, but it is important to realize, as stated, that non of these suggested new endpoints have been validated yet.

Tumor markers (CEA, CA 15.3, CA 19-9, PSA) could also serve the purpose of assessing drug-target interaction, and optimal tumor marker inhibition could so become an endpoint in phase I studies [6].

As stated, to exert optimal tumor growth inhibition, cytostatic agents may have to be administered for prolonged periods of time, maybe even continuously. This means that oral administration is highly preferred for patients' convenience [7]. Determining the bioavailability of oral formulations and the potential effect of food-intake on bioavailability will become an important feature of phase I studies. This is also of vital importance since preclinical studies are well known to be unable to predict the bioavailability in humans.

In order to limit the number of patients required to achieve the aims of phase I studies and to minimize the number of patients exposed to potential subtherapeutic doses of new anticancer agents and increase the number of patients exposed to doses that are believed to be potentially effective, accelerated dose escalation schemes, such as for instance the modified continual reassessment method, are presently preferred designs for phase I studies on cytotoxic agents. However, whereas these dose escalation designs indeed have been shown to limit the number of patients exposed to potential subtherapeutic doses, for cytostatic agents it has also already been shown that they do not limit the total number of patients that needs to be included in the study, and in fact these designs frequently even lead to higher numbers of patients required as compared to studies using classical dose escalation schemes [8]. Given the to be discussed possibility that phase II studies might be omitted, this increased patient number in phase I studies, especially the increased

number of patients that will be exposed to dose levels that are believed to be potentially effective, does not necessarily have to be interpreted as disadvantageous.

PHASE II STUDIES

For cytotoxic agents, the aim of phase II single-agent studies is to look for potential antitumor activity. Phase II studies can be performed in each tumor type considered to be of interest. The number of tumor regressions qualifying for a certain response is used as endpoint. To assess tumor regressions, well-defined response criteria exist [9], and recently newly defined criteria have been agreed upon [10].

As stated, most cytostatic agents do not induce any tumor regression in preclinical models but rather delay or inhibit the growth of established tumors. For such agents single-agent phase II studies using tumor regression as endpoint of activity will almost certainly lead to under-estimation of potential antitumor activity, and thus this type of study seems senseless to perform. In a more rational scenario, and if one would not jump from phase I directly to phase III studies, a single-agent phase II study with TTP as the main endpoint could be conceivable. However, since TTP is highly variable between patients, properly designed phase II studies using this endpoint should be randomized in order to avoid a bias in patient selection, even though the design of such a randomized phase II study can never be powered to detect significant differences. The outcome of the study only prevents early rejection of a potentially active agent, and will enable a more close estimation of statistical considerations for phase III studies. A possible design to perform a single-agent randomized phase II study could be the so-called "randomized discontinuation" design in which all included patients are 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 a placebo. Time to disease progression or a decrease in percentage of patients progressing at a certain time-point, specific for the disease studied, could be used as an endpoint. This type of design enables assessment of secondary endpoints such as surrogate marker inhibition and additional pharmacokinetics and pharmacodynamics in the whole study population. Whatever the design, it is obvious

that the statistical considerations will require different assumptions as compared to the assumptions made for phase II studies with cytotoxic agents.

Antitumor activity can only be a useful parameter when preclinical studies suggest that the cytostatic agent might induce tumor regression, even if through indirect effects, and thus behaves as a cytotoxic agent. In addition, phase II studies can be considered if preclinical studies suggest synergistic activity of the combination of a known cytotoxic and the new cytostatic agent. For some cytostatic agents, this has been reported [3, 11, 12, 13]. Again, the randomized phase II design would be preferred, with the control arm involving the cytotoxic agent given as a single-agent. Once again, randomization would exclude the potential of patient selection. This study design enables assessment of toxicity of the combination of cytotoxic and cytostatic agents versus toxicity of the single-agent cytotoxic drug and could suggest, but not proof, potential differences in efficacy.

PHASE III STUDIES

Phase III studies are, and will remain the pivotal studies to show efficacy, and their endpoints TTP and/or survival will not have to be adapted. Since phase III studies by definition are randomized, the design of these studies is adequate for cytostatic agents. However, in an era where drug registration should be obtained at the earliest possible time in the development to limit the costs of drug development, handling study design in a creative way relative to the specificities of the disease remains crucial. As said, in phase III studies on cytostatic agents TTP could still be used as a parameter reflecting growth inhibitory efficacy. In preference and in view of the mechanisms of action of cytostatic agents, phase III studies should be performed in patients with limited residual disease accomplished by prior surgery or by preceding cyto-reductive chemotherapy. Ideally, the first pivotal consolidation study should be performed in patients with a tumor type with a high likelihood of rapid recurrence. In such a case, the clinical efficacy of cytostatic agents could be assessed even using different doses and the endpoints TTP, disease free survival and overall survival could be adequately analyzed in a fairly limited number of patients within a relatively short period of time.

Given the possibility that cytostatic agents at optimal doses will not induce side-effects, it becomes questionable whether an analysis of quality of life should be included. However, regulatory authorities may still insist on such an analysis to rule out a negative impact that might not be balanced against the positive gains.

Studies on adjuvant therapy with cytostatic agents will necessarily involve large numbers of patients that will have to be followed for quite prolonged periods of time to be able to properly assess the role of the agents for each specific indication, and to assess endpoints such as progression free and overall survival. Presumably, these long lasting studies will not be favored for registration purposes. However, they remain valid after registration to study potential additional indications.

Whether cytostatic agents can also be applied as chemo-preventives is yet completely unknown, although some preclinical data have shown a delay in the onset of tumors in high-risk animals [3]. Questions concerning the proper definition of a high-risk study population and dosing strategies (dose, schedule, time) are still manifold, and the design of these phase III prevention studies will thus depend on yet largely unknown factors.

CONCLUSION

Many new predominantly *cytostatic* anticancer agents have been developed in recent years, and a number of these agents have now entered clinical studies. The antitumor activity of these agents is accomplished in a way that is completely different from the one of cytotoxic agents, and the toxicity profile presumably will also be completely different. Therefore, the design of clinical phase I, II, and III studies has to be specifically adapted to the characteristics of these drugs. Assessing doses yielding optimal biologic activity rather than acute clinical toxicity should be an endpoint of phase I studies, and therefore, pharmacokinetic and pharmacodynamic analyses and analyses of surrogate markers of drug-target interactions will be of great importance. Performing non-randomized single-agent phase II studies to assess antitumor activity will not be commonly recommended, and randomized phase II and III studies should be performed to optimally assess the role of these new agents in the treatment of cancer patients.

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CHAPTER 3

PHASE I AND PHARMACOLOGICAL STUDY OF WEEKLY ADMINISTRATION OF THE POLYAMINE SYNTHESIS INHIBITOR SAM 486A (CGP 48 664) IN PATIENTS WITH SOLID TUMORS.

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ABSTRACT

Purpose: A single-agent dose-escalating phase I and pharmacological study of the polyamine synthesis inhibitor SAM 486A was performed. A dosing regimen of four weekly infusions followed by 2 weeks off therapy was studied.

Patients and methods: Fifty patients were entered into the study. Dose levels studied were 1.25, 2.5, 5, 8, 16, 32, 48, 70, 110, 170, 270, and 325 mg/m^2/week. Pharmacokinetic sampling was done on day 1, and trough samples were taken weekly during the first treatment cycle. Pharmacodynamic sampling was done on days 1 and 22.

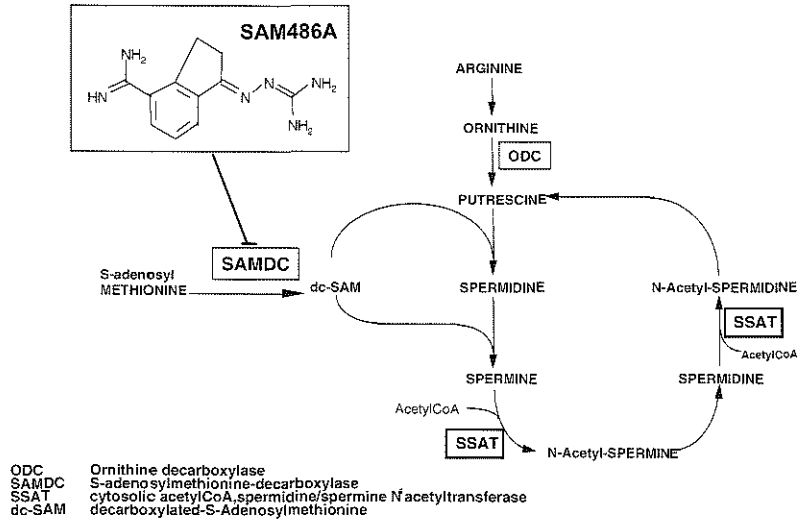
Results: At 325 mg/m^2/week, dose limiting toxicity was seen (one patient each with grade 4 febrile neutropenia, grade 3 neurotoxicity, and grade 3 hypotension with syncope and T-wave inversions on electrocardiogram). The recommended dose for further testing was set at 270 mg/m^2/week. Infusion time was increased from 10 to 180 minutes due to facial paresthesias and flushing and somnolence. Drug exposure increased linearly with dose. Mean $\pm$ SD $T_{½}$ at 70-325 mg/m^2 doses was 61.4 $\pm$ 26.2 hours with a large volume of distribution at steady state. In peripheral blood leukocytes a clear relationship between dose and inhibitory effect on S-adenosylmethionine decarboxylase or changes in intracellular polyamine pools was not recorded.

Conclusion: SAM 486A can be administered safely using a dosing regimen of four weekly infusions followed by 2 weeks off therapy. The recommended dose for phase II studies using this regimen is 270 mg/m^2/week.

INTRODUCTION

The polyamines spermine and spermidine are present in all mammalian cells, and although their exact mechanism of action remains to be elucidated, their presence is essential for maintenance of cell function, growth, and proliferation. Biosynthesis and active transport on the one hand and catabolism and efflux on the other maintain polyamine homeostasis. The biosynthesis of spermine and spermidine involves several enzymatic steps, of which those involving ornithine decarboxylase (ODC) and S-adenosyl-methionine decarboxylase (SAMDC) are rate limiting [1]. Polyamine synthesis is summarized in figure 1.

Figure 1 Polyamine synthesis



Increased intracellular concentrations of spermine and spermidine are noted in tumor cells, and aberrant polyamine metabolism is thought to play a role in carcinogenesis. Therefore SAMDC has long been considered to be a rational target

for anticancer agent development [1-5]. In the 1980's, specific and potent inhibitors of SAMDC were developed [6].

SAM 486A is a cyclic analogue of methylglyoxal-*bis* (guanylhydrazone) or MGBG, with an IC_{50} for SAMDC of 4.7 nM, being approximately 200-fold more active than the parent compound. It only impacts mitochondrial function at doses 100-fold higher than those required for cellular growth inhibition. Spermine and spermidine pools are almost totally depleted, whereas putrescine pools are increased. *In vitro* studies showed growth-inhibitory effects of SAM 486A on human melanoma, lung cancer, breast cancer, and human epidermoid carcinoma cell lines, the T24 human bladder carcinoma cell line, and the L1210 murine leukemia cell line [7-10]. *In vivo* growth-inhibitory activity showed a similar spectrum [7,9,11,12]. Preclinical data have been obtained suggesting additive and/or synergistic activity of SAM 486A in combination with currently available cytostatic agents [13].

Toxicology studies in rats and dogs revealed acute cardiovascular and respiratory symptoms with hyperemia, tachycardia, cyanosis, and reduced body temperature and dyspnea, gasping, and deep respiration, respectively. After long-term treatment, heterogeneous electrocardiogram alterations in dogs were observed together with morphological changes in liver and heart. Clearance of SAM 486A from plasma was multiexponential with extensive distribution outside the plasma compartment and a high uptake into the liver and salivary glands. SAM 486A was hardly metabolized and was excreted predominantly through renal excretion.

We have performed a phase I and pharmacological study with SAM 486A in patients with various advanced solid tumors, using a dosing regimen of four weekly infusions followed by 2 weeks off therapy. This schedule was chosen based on previous experiences with weekly administered MGBG showing a more favourable safety profile.

MATERIAL AND METHODS

Eligibility criteria

Patients with a cytologically or histologically confirmed diagnosis of a solid tumor refractory to standard treatment or for whom no standard therapy was

available were eligible for this study. Additional eligibility criteria included: (*a*) age ≥18 years; (*b*) WHO performance status ≤2; (*c*) life expectancy of ≥12 weeks; (*d*) no anticancer treatment in the previous 4 weeks (6 weeks for nitrosoureas, high-dose carboplatin/mitomycin-C, or extensive radiotherapy); (*e*) adequate bone marrow function (WBC $\geq 4.10^9$/liter, platelets $\geq 100.10^9$/liter); (*f*) normal hepatic and renal functions (bilirubin ≤25 μmol/liter, aspartate aminotransferase and alanine aminotransferase within 2.5 times the normal upper limit, serum creatinine ≤120 μmol/liter and normal age-adjusted creatinine clearance); and (*g*) a baseline left ventricular ejection fraction (LVEF) within normal limits as measured by nuclear ejection fraction determination or cardiac ultrasound. Exclusion criteria were pregnancy, active bacterial infections, fistulae, brain involvement and leptomeningeal disease, and a history of congestive heart failure or other cardiac disease with New York Heart Association classification 3 or 4. All patients gave written informed consent before the start of treatment. The study was approved by the local ethics committees.

Pretreatment and on-treatment assessments

Before therapy, a complete medical history was taken and a physical examination was performed. A complete blood count (CBC), including WBC differential and serum chemistries including sodium, potassium, calcium, phosphorus, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, and lactate dehydrogenase were performed, as were urinalysis, creatinine clearance assessment, electrocardiogram, chest X-ray and LVEF assessment. Weekly evaluations included history, physical examination, toxicity assessment according to the National Cancer Institute CTC criteria [14], complete blood count, serum chemistries, urinalysis, and electrocardiogram. Tumor measurements were performed every 6 weeks and evaluated according to the WHO criteria for response [15]. LVEF was reassessed by the same technique used before treatment every 6 weeks. In case of disease progression, patients were taken off study.

Drug and drug administration

SAM 486A is the free base of 4-(aminoiminomethyl)-2-3-dihydro-1H-inden-1-one-diaminomethylenehydrazone, and it is formulated as a salt with D,L-lactic acid for intravenous administration. Novartis AG (Basel, Switzerland) supplied SAM 486A as a freeze-dried yellow compound (10 mg of SAM 486A dry substance in 2-ml vials). The dry substance had to be protected from light and stored at temperatures <30° C. SAM 486A was reconstituted by dissolving it in 1 ml of 5% dextrose solution and then diluting it in 100 ml of 5% dextrose. The reconstituted solution had to be stored at 2-8° C and used within 8 hours after dissolving it in an infusion system completely protected from direct sunlight. Infusion time was initially 10 minutes and was increased to 20 minutes at doses of 48 and 70 mg/m^2, 1 hour at doses of 110 and 170 mg/m^2, and 3 hours at doses of 270 and 325 mg/m^2. Prophylactic antiemetics were not given routinely. A treatment cycle consisted of four weekly infusions followed by 2 weeks off treatment.

Dose and dose escalation

The starting dose was 1.25 mg/m^2/week. This dose corresponded to one-third of the human equivalent of the no adverse effect dose level with daily dosing for 3 months in the most sensitive species, the rat, being 0.06 mg/kg or 10.8 mg/m^2/month. Dose escalation was performed with decreasing rates using a Fibonacci scheme with dose doublings when no toxicities of grade >2 were seen in a previous dose level. At each dose level a minimum of three patients had to have one full course of treatment before dose escalation was allowed. When side-effects with a toxicity of grade ≥2, excluding alopecia or inadequately treated nausea or vomiting, were seen at a given dose level, at least six patients had to be treated at that dose level. The maximum tolerated dose (MTD) was the highest dose administered safely to a patient producing tolerable, manageable and reversible grade 3 toxicity in at least two out of six patients. No intrapatient dose escalation was allowed.

Pharmacological studies

For the pharmacokinetic analysis of SAM 486A, 5-ml blood samples were taken from an i.v. cannula inserted in the arm opposite the infusion arm before the

first drug administration, at the end of the infusion, and at 15, 30 and 60 minutes and 2, 4, 8, 10, and 24 hours after the end of the infusion. When infusion time was 1 hour, additional samples were taken 20 and 40 minutes after the start of the infusion. When infusion time was 3 hours, additional samples were taken 1 and 2 hours after the start of the infusion. For the second, third, and fourth administration, a blood sample was taken prior to the start of infusion. A blood sample was also taken at the 2 weekly visits after the fourth administration. The blood samples were immediately centrifuged at 3000 rpm for 5 minutes at room temperature. The separated plasma was transferred into a polyethylene tube and frozen at -18^0 C until analysis. Plasma samples were assayed by a specific and sensitive high-performance liquid chromatography (HPLC) assay [16]. The lower limit of quantitation of the assay was 5 ng/ml (variability 2.1-10.5 ng/ml). Concentration versus time data were used for calculation of the noncompartmental pharmacokinetic parameters $AUC_{0-\infty}$, peak plasma concentration C_{max}, terminal $T_{½}$, and V_{ss}, using WinNonlin Professional version 1.5 software. Excretion of SAM 486A in urine was measured for 24 hours after the first administration. Urine was collected in three 8-hour samples that were stored at 4^0 C during the collection period and subsequently frozen at -20^0 C until analysis. The volume of each 8-hour urine sample was measured. Urine samples were assayed by the same HPLC assay used for plasma analysis. The lower limit of quantitation of the urine assay was 11 ng/ml.

For pharmacodynamic studies, 10-ml blood samples were taken from an i.v. cannula inserted in the arm opposite the infusion arm before therapy and 24 hours after the end of the first and, optionally, the fourth infusion. Before the second, third and fourth administration and during the 2 weeks off treatment, trough samples were taken. Samples were immediately centrifuged at 3000 rpm for 10 minutes at 4^0 C, and then plasma was frozen at –20^0 C until analysis. Polyamines were determined in leukocytes by a capillary Gas Chromatography method using Nitrogen,Phosphorus-detection. SAMDC activity was determined in leukocytes using an assay described previously [17].

RESULTS

Fifty patients were entered into this study, all of whom were eligible and evaluable for safety. Patient characteristics are summarized in table 1.

Table 1 Patient characteristics

No of patients entered	50
No of patients evaluable	50
Male / female	30 / 20
Mean age (years)	55.8
Range	22-73
Median WHO performance status	1
Range	0-2
Prior therapy (excluding previous surgery)	
None	9
Immunotherapy only	1
Chemotherapy / hormonotherapy only	24
Radiotherapy only	3
Chemo- and radiotherapy	13
Primary tumor site	
Colorectal	16
Kidney	7
Lung	4
Unknown primary	4
Gastric	3
Head and Neck	2
Melanoma	2
Gall bladder	2
Miscellaneous	10

The total number of assessable treatment cycles was 78; most patients received 1 or 2 cycles, two patients received 4 cycles, and one patient received 5 cycles. There was no tendency towards longer treatment duration with increasing dose levels. Dose levels studied were weekly infusions of 1.25, 2.5, 5, 8, 16, 32, 48, 70, 110, 170, 270 and 325 mg/m^2. Dose escalation from 5 to 8 mg/m^2/week was performed because of the occurrence of one episode of grade 3 diarrhea at the lower dose. Infusion time was increased from 10 to 20 min at a dose of $\geq$ 48 mg/m^2/week and to 60 min at a dose of $\geq$ 110 mg/m^2/week due to acute reactions such as facial flushing and paresthesias. At 270 mg/m^2/week, infusion time was further increased to 180 min due to the additional occurrence of somnolence in three patients at this dose level.

Hematological toxicity

Hematological toxicities are summarized in table 2.

Grade >2 hematological side-effects were only recorded at the highest two dose levels. Uncomplicated grade 3 neutropenia lasting 8 days was seen in week 5 in one patient at 270 mg/m^2/week, and another patient at this dose experienced uncomplicated grade 3 neutropenia lasting 8 days in the third treatment cycle. Grade 4 neutropenia lasting 3 days complicated by fever was seen in one patient at 325 mg/m^2/week in week 6 of the first treatment cycle. No grade 3 or 4 anemia or grade 2-4 thrombocytopenia was seen. Three patients developed grade 1 thrombocytopenia (one patient each at 16, 110, and 270 mg/m^2/week). There was no treatment delay due to myelosuppression.

Nonhematological toxicity

Nonhematological toxicity was diverse, but most frequently consisted of nausea and vomiting, fatigue and/or malaise, and facial flushing and paresthesias. Fatigue, anorexia, nausea, vomiting and diarrhea occurred at all dose levels, although the incidence tended to increase at the three highest dose levels. These side-effects were usually mild and required no specific treatment. One patient at 5 mg/m^2/week had grade 3 diarrhea that subsided within 2 days without specific treatment.

Table 2 Hematological toxicity (worst per patient)

Dose level (mg/m^2/week)	Patients	Treatment cycles	Leucocytes (CTC grade)				Neutrophils (CTC grade)			
			1	2	3	4	1	2	3	4
1.25	3	4	-	-	-	-	1	-	-	-
2.5	3	5	-	-	-	-	-	-	-	-
5	4	6	1	-	-	-	-	-	-	-
8	3	6	-	-	-	-	-	-	-	-
16	4	10	1	-	-	-	1	-	-	-
32	3	5	-	-	-	-	-	-	-	-
48	3	4	-	-	-	-	-	-	-	-
70	4	5	1	-	-	-	-	-	-	-
110	4	6	1	-	-	-	-	-	-	-
170	4	5	-	-	-	-	-	-	-	-
270	8	15	-	2	1	-	1	1	2	-
325	7	7	1	-	-	1	-	-	-	1

Facial paresthesias and flushing occurred in 26 patients. One patient at 2.5 mg/m^2/week experienced grade 1 facial flushing. Twenty-two patients at $\geq$ 32 mg/m^2/week had grade 1 facial flushing and paresthesias, and two patients at the highest dose level had grade 1 flushing and grade 2 paresthesias. One patient at 32 mg/m^2/week experienced grade 3 hypersensitivity consisting of pruritis, facial flushing, dyspnea and hypertension immediately after the start of the first infusion. The infusion was stopped and antihistaminics and corticosteroids were administered. After a 30-min rest period, the infusion was restarted without sequelae apart from facial flushing. Subsequent infusions in this patient were preceded by antihistaminics and corticosteroids and were followed only by mild facial flushing.

Increasing the infusion time from 10 to 20 min at the 48 mg/m^2/week dose level and to 60 min at the 110 mg/m^2/week dose level was instrumental in the control of facial flushing and paresthesias. At 270 mg/m^2/week, due to the additional occurrence of somnolence in three patients, infusion time was further increased to 180 minutes. At 270 mg/m^2/week, grade 2 alopecia was seen in two patients, one of

whom developed sclerodermia-like skin abnormalities. Grade 1-2 local erythematous skin reactions at the infusion site were noted in four patients at doses of 170-325 mg/m^2/week.

Cardiovascular abnormalities were recorded in five patients. At 110 mg/m^2/week one patient experienced grade 3 tachyarrythmias with possible atrioventricular dissociation starting 11 days after the fourth administration in the second treatment cycle over a period of 6 days preceding death. Mild hyperkalemia (<6.1 mmol/liter) was recorded. Autopsy revealed mediastinal tumor localisation and pulmonary embolism. At 270 mg/m^2/week one patient had grade 4 cardiac ischaemia after the third drug administration. This event was considered to be possibly related to the trial drug, although the patient was known to have hypertension and hypercholesterolemia. After the occurrence of this event, continuous electrocardiographic monitoring was performed in all subsequent patients during drug administration. At 325 mg/m^2/week, on the day of the third infusion, one patient with known hypertension developed grade 3 atrial flutter and sinus tachycardia lasting 5 days. One day later the patient died due to progressive disease. At 325 mg/m^2/week, another patient developed transient grade 1 ventricular bigeminy and a first-degree atrioventricular nodal block during the first infusion of SAM 486A. This patient had a history of prior ventricular bigeminy and atrial fibrillation for which electric cardioversion had been attempted unsuccessfully. With subsequent administrations of SAM 486A, continuous electro-cardiographic monitoring revealed no arrythmias. One other patient at the 325 mg/m^2/week dose level recorded transient grade 1 sinus tachycardia (130 beats/min) only during the third administration, whereas another patient at the 325 mg/m^2/week dose level recorded transient grade 1 sinus bradycardia (45 beats/min) during the first administration only. No further administrations were given to this patient because of a rapid decline in general condition. Repeated assessments of LVEF with nuclear cardiac imaging showed no changes in cardiac contractility in any patient. Continuous electrocardiographic monitoring in subsequent patients treated at the next lower dose level of 270 mg/m^2/week revealed no arrythmias.

Renal or hepatic toxicity related to the study drug of grade 2 or greater was not recorded.

Dose limiting toxicities

At 325 mg/m^2/week, grade 4 neutropenia in week 5 of treatment lasting 3 days but complicated by fever occurred in one patient. Grade 3 hypotension with syncope and reversible T-wave inversions on electrocardiogram occurring immediately after the first infusion was seen in another patient. Grade 3 neuromotor and neurosensory toxicity of the left hand after the second administration of SAM 486A was seen in a third patient. Cerebral magnetic resonance imaging in this patient revealed no abnormalities, and the complaints subsided gradually after treatment was stopped. Accordingly, the recommended dose for further activity testing was set at the next lower dose level, *i.e.* 270 mg/m^2/week. At this dose, one episode of grade 4 cardiac ischaemia and two episodes of uncomplicated grade 3 neutropenia were seen. Other side-effects recorded at this dose level were mild facial flushing and paraesthesia, nausea, and vomiting.

Reasons for discontinuation of SAM 486A

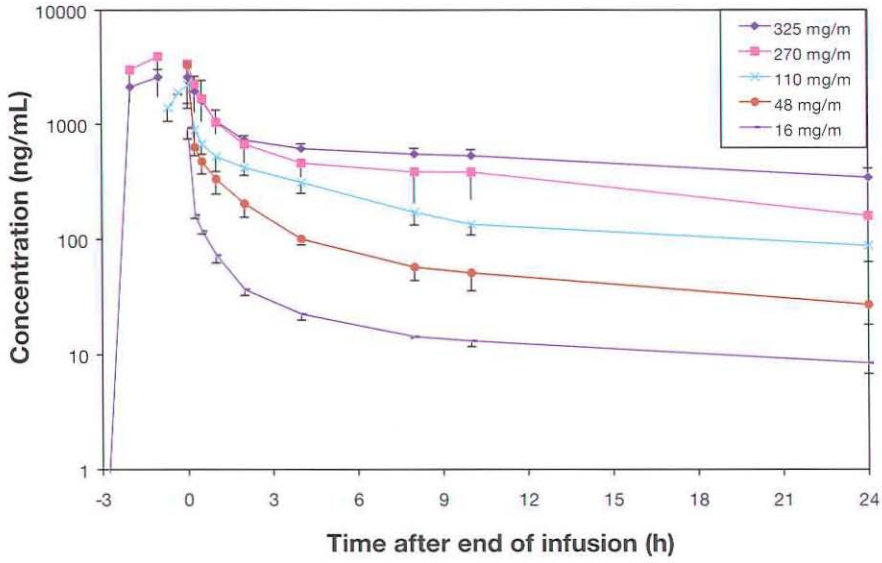
In 37 patients, progressive disease was the reason for discontinuation of SAM 486A. In 24 of these patients, SAM 486A was withheld due to progressive disease before the second treatment cycle had been completed. Two patients died before completion of two treatment cycles, three patients discontinued due to toxicity, two patients withdrew consent, and six patients discontinued treatment for various reasons (three patients discontinued treatment due to adverse events, two patients discontinued treatment due to deterioration in general condition, and one patient discontinued treatment due to increased liver enzymes suggestive of disease progression).

Pharmacokinetics and pharmacodynamics

At dose levels 1.25-8 mg/m^2/week the limits of the pharmacokinetic assay influenced calculation because plasma concentrations of SAM 486A were below the limit of quantitation for prolonged periods of time. Mean $\pm$ SD plasma concentration *versus* time profiles for the 16-325 mg/m^2/week dose levels are shown in figure 2. The relation of AUC to dose is shown in figure 3. Mean $\pm$ SD $T_{½}$ for the dose range of

70-325 mg/m^2/week was 61.4 ± 26.2 hours. Mean ± SD V_{ss} at dose levels 70-325 mg/m^2/week was 1540 ± 926 liters, indicating extensive distribution outside the plasma compartment. The interpatient variability of the parameters $T_{½}$ and V_{ss} was high, with coefficient of variation values of 43% and 60%, respectively. C_{max} was generally related to dose, but as this parameter is influenced by infusion time, no relation across all dose levels was made.

Figure 2 SAM 486A mean (SD) plasma concentration-time profiles for representative doses 16-325 mg/m^2

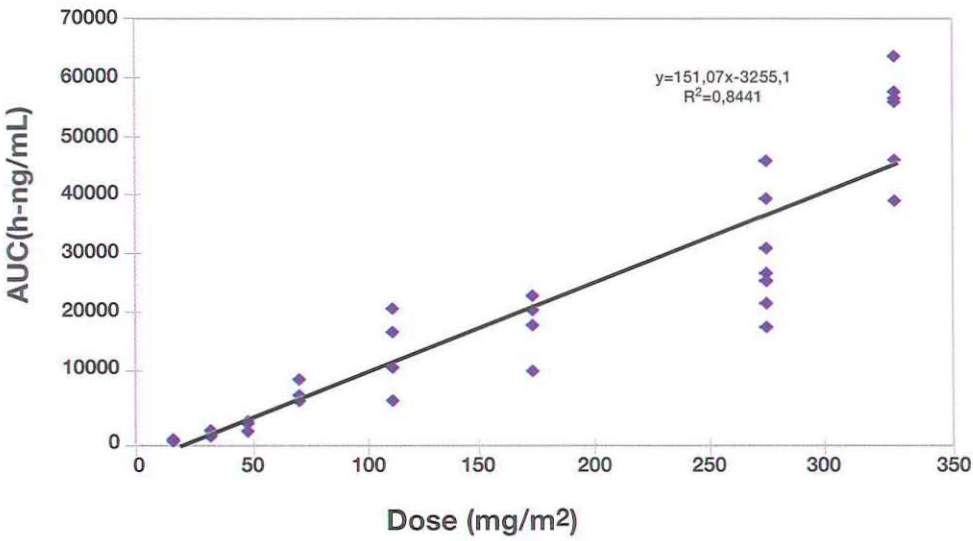


Analysis of the urine showed that 24 h excretion of SAM 486A was dependent on the dose and/or duration of infusion. Mean 24 hour urinary excretion for the 2.5-70 mg/m^2/week cohorts was 15-25% of the dose. Mean 24 hour urinary excretion for the 110-325 mg/m^2/week cohorts was 4-9% of the dose.

An exploratory analysis to investigate whether the peripheral leukocyte compartment could provide suitable material for analysis of polyamine and SAMDC activity fluctuation in response to SAM 486A administration was performed on 9

patients treated with ≥ 70 mg/m^2/week. All patients demonstrated a moderate increase in SAMDC activity after SAM 486A administration, but the results were variable and were not maintained with subsequent dosing. Analysis of polyamine pools showed high intra- and interpatient variability. Intracellular concentrations of putrescine were increased in some patients and decreased in others after administration of SAM 486A at different doses. There was no discernable relationship between polyamine or SAMDC fluctuation and the dose of SAM 486A administered.

Figure 3 AUC v Dose 16-325 mg/m^2



Responses

No partial or complete responses were seen. Stable disease was seen in seven patients. There was no tendency towards increased time to disease progression with increasing doses of SAM 486A among either the 16 patients with colorectal cancer or the 7 patients with renal cell carcinoma (data not shown separately).

DISCUSSION

We have performed a phase I and pharmacological study on the novel polyamine synthesis inhibitor SAM 486A. Hematological side-effects consisted of dose-dependent, short-term, and noncumulative neutropenia. In one patient, grade 4 febrile neutropenia caused a DLT. Myelosuppression was also recorded in two other phase I studies with SAM 486A using different treatment schedules [18,19]. Thrombocytopenia was mild, even more infrequent, and not dose dependent.

Nonhematological side-effects of SAM 486A were diverse. Gastrointestinal side-effects occurred frequently and at virtually all dose levels, with an increasing incidence of nausea and vomiting at the three highest dose levels. These side-effects were usually mild, required no specific treatment, and did not lead to interruption or withholding of treatment. Fatigue occurred in 44% of patients on study, and the incidence of this specific complaint tended to increase at the two highest dose levels. Taking into consideration the characteristics of patients entering clinical phase I studies, *i.e.* those with advanced or end-stage malignant disease, interpreting the causality of anticancer treatment with this side-effect is always somewhat hazardous. However, fatigue and weakness, on two occasions even leading to hospitalization, have also been ascribed to the parent compound MGBG when given weekly [20,21,22,23]. Considering the structural similarity between SAM 486A and MGBG, it cannot be excluded that fatigue is caused by SAM 486A. Mild (usually grade 1) facial paresthesias and flushing were recorded in 52% of patients on study, occurred at dose levels of $\geq$ 32 mg/m^2/week (except for one patient at the 2.5 mg/m^2/week dose level with grade 1 flushing), and usually occurred immediately after the start of the infusion. These symptoms were of short duration, completely reversible, and noncumulative and required no dose reduction or treatment delay. With increasing doses, concurrently increasing infusion time from 10 to 60 minutes alleviated these side-effects. Additionally, somnolence considered drug-related occurred in three patients at the dose level of 270 mg/m^2/week and onward, and because of this, infusion time was further increased to 180 minutes. Paresthesias have been described in relation to MGBG when given weekly [21,24]. The fact that higher doses of SAM 486A were better tolerated with prolonged infusion time corresponds with

results of a phase I study of MGBG [21]. The mechanism of action responsible for facial paresthesias and somnolence has not been clarified. Side-effects such as ataxia, myopathy, hypoglycemia, vasculitis-like syndromes, or skin ulcerations previously related to MGBG were not recorded in the present study, although mild mucositis and erythematous skin reactions at the infusion site were recorded. Five patients developed alopecia; in one patient this was combined with sclerodermia-like skin abnormalities.

Adverse events related to the cardiovascular system, including arrythmias and myocardial ischaemia, have been recorded in the present study. Preclinical studies with MGBG reported some cardiotoxicity in animals [3], and one single case of reproducible ventricular arrythmias after exposure to MGBG in a patient deemed susceptible for cardiac toxicity because of both disease state and previous treatment, has been published [21]. Including our study, three single-agent phase I studies with SAM 486A using different treatment schedules have been performed, including 112 patients [18,19]. In addition to the patients described in this report, so far only one patient (with a prior history of atrial fibrillation and hypertension) receiving continuous infusion of SAM 486A has developed atrial fibrillation while on treatment. Because of the diversity of cardiovascular side-effects recorded in the current study and the fact that several patients likely suffered from asymptomatic premorbid cardiac conditions, it is difficult, at this moment, to ascribe or exclude a relationship between SAM 486A and these cardiac findings. The patient with hypotensive collapse and concurrent T-wave inversions at the electrocardiogram immediately after the first infusion of the nontolerated dose (325 mg/m^2/week) of SAM 486A in this study, for example, was shown at subsequent exercise testing to develop T-wave flattening on his electrocardiogram, indicating a probable preexisting coronary atherosclerosis. Electrocardiograms after the subsequent infusions at the next lower dose level (270 mg/m^2/week) all remained normal. Because a possible relationship between the trial drug and the occurrence of cardiac arrythmias or ischemia could not be ruled out at the time of occurrence of the first cardiac event, continuous electrocardiographic monitoring during SAM 486A administration was performed for all subsequent patients. This resulted in the recording of three episodes of grade 1 cardiac arrythmias, but only in patients treated at the nontolerated dose (325 mg/m^2/week) of

SAM 486A (one episode each of transient grade 1 ventricular bigeminy, sinus bradycardia, and sinus tachycardia only during the first infusion). None of the patients receiving SAM 486A at the next lower dose level showed any cardiac arrythmia. At present, a definitive statement concerning the potential of SAM 486A to elicit cardiovascular toxicity cannot be made. In currently ongoing studies with SAM 486A, electrocardiograms are collected on a regular basis and centralized review is being performed. Thus far, additional abnormalities have not been reported.

Neurotoxicity was seen in one patient treated at the nontolerated dose of SAM 486A. Neuropathy, although infrequent, has been described in relation to MGBG [25].

Clearly, the dose limiting side-effects recorded in this study were diverse, but since DLT involved hematological, cardiovascular and neurologic toxicity, it was felt by all participants that further escalation of the dose was not warranted. At the dose recommended for further studies using this schedule of administration, organ toxicities were minor, rapidly reversible and therefore manageable.

The pharmacokinetic profile of SAM 486A shows many similarities with that of MGBG, *i.e.* a triphasic plasma elimination, a large V_{ss}, indicating tissue distribution outside the plasma compartment, and incomplete renal excretion [3,20,25,26]. The mean peak plasma concentration of the 270 mg/m^2/week patient cohort was higher than that of the 325 mg/m^2/week patient cohort until 1 h after the end of the infusion as a result of large interpatient variability, with two patients at 270 mg/m^2/week having much higher peak levels than average during this period. Mean $\pm$ SD $T_{½}$ at doses of 70-325 mg/m^2/week was 61.4 $\pm$ 26.2 h, compared with a mean $\pm$ SD $T_{½}$ of MGBG of 175 $\pm$ 84 h.[26] Mean $\pm$ SD $T_{½}$ at doses $\leq$70 mg/m^2/week could not be calculated because plasma levels at 168 hours after dose administration were below the limit of quantitation of the assay. The linear relationship between exposure to SAM 486A as represented by $AUC_{0-\infty}$, and dose administered indicates that the processes of distribution and elimination are not saturated, inhibited, or induced.

An exploratory analysis of polyamines and SAMDC activity in leukocytes after SAM 486A administration showed variable and seemingly unpredictable effects. SAMDC activity was marginally increased after the first administration of SAM 486A in all patients. This may reflect a transient stabilization of the enzyme coupled with a compensatory increase in biosynthetic activity, both known consequences of SAMDC

modulation [6]. However there was high interpatient variability and no correlation with the dose of SAM 486A administered, and this effect did not persist after multiple administrations. Intracellular concentrations of putrescine, spermine, and spermidine varied widely after the administration of SAM 486A. The likely reason for these disappointing results results may be related to the nonproliferative nature of peripheral blood leukocytes. This being so, the relative importance of SAMDC activity and polyamine synthesis in general may well be rather minimal compared to that seen in proliferating tissue. From these scant observations it must be concluded that peripheral blood leukocytes are not suitable for measuring changes in polyamine pools and activity of SAMDC in response to treatment with SAM 486A.

In conclusion, based on the results of this phase I and pharmacological study with the polyamine synthesis inhibitor SAM 486A , which was given as four weekly infusions followed by 2 weeks off treatment, the recommended dose for additional studies is 270 mg/m^2/week. At this dose, SAM 486A can be administered safely with acceptable toxicity.

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CHAPTER 4

PHASE I AND PHARMACOLOGICAL STUDY OF THE ORAL MATRIX METALLOPROTEINASE INHIBITOR MMI270B (CGS 27023A) IN PATIENTS WITH ADVANCED SOLID CANCER.

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Submitted for publication

ABSTRACT

Purpose: This phase I study of MMI270B, an orally administered matrix metalloproteinase inhibitor, assessed toxicity, pharmacokinetics, tumour response data and investigated markers of biological activity to recommend a dose for phase II studies.

Patients and methods: MMI270B was administered continuously at seven predefined dose levels (50mg od – 600mg tds). Patients were evaluated for toxicity and tumour response and blood and urine samples were taken for pharmacokinetics, bone resorption markers, direct targets of the inhibitor (MMP-2, MMP-8 and MMP-9), indirect targets (TIMP-1, TIMP-2, bFGF, VEGF, VCAM-1, suPAR, and cathepsins B and H) and for a TNF-α cytokine release assay.

Results: Ninety two patients were entered. There was no myelotoxicity. Eighteen patients developed a widespread maculopapular rash, which increased in frequency and severity at doses > 300mg bd. Thirty nine patients developed musculoskeletal side effects, which were related to duration of treatment, not to dose level. Pharmacokinetics were linear, MMI270B was rapidly absorbed and eliminated with minimal accumulation on chronic dosing. Sustained plasma concentrations in excess of 4 x mean IC_{50} for the target enzymes were observed at dose levels > 150mg bd. There were no tumour regressions, however nineteen patients had stable disease for > 90 days. There was a dose response increase of MMP-2 and TIMP-1 with MMI270B. Transient effects on the bone resorption markers were detected.

Conclusion: MMI270B was generally well tolerated, with adequate plasma levels for target enzyme inhibition. The two main toxicities were rash, resulting in a maximum tolerated dose of 300mg bd, and musculoskeletal side effects. Biological marker data indicate drug effects; the rise in TIMP-1 suggests that a reflex rise in inhibitors could modify the effects of MMI270B. The recommended phase II dose is 300mg bd.

INTRODUCTION

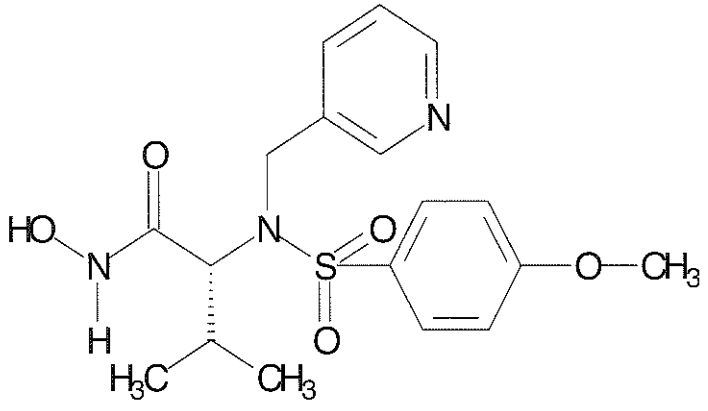
The matrix metalloproteinases (MMPs) are a family of proteinases able, between them, to break down all components of the extracellular matrix (ECM) including the basement membrane [1]. There are four subfamilies of MMPs; collagenases, gelatinases, stromelysins and membrane-type MMPs. The first three types are secreted as proenzymes, activated by cleavage of the N terminus and have highly conserved regions particularly at the catalytic site, which encloses a zinc ion. MMP activity is closely regulated by a variety of mechanisms including transcriptional control, proteolytic activation and by natural inhibitors such as α2 macroglobulin and the specific TIMP (tissue inhibitors of metalloproteinase) family of proteins [2-5]. Another important role for MMPs is increasing the bioavailability of factors bound to the cell surface and extracellular matrix, e.g. basic Fibroblast Growth Factor (bFGF) [6,7].

MMPs are important in malignant disease. Digestion of the ECM is necessary for tumour growth, invasion, metastasis and for angiogenesis. Excess MMP expression has been associated with malignancy and in several tumour types has been shown to increase along with invasive and metastatic potential [8-12]. For example, the ratio of activated to latent MMP-2 was higher in malignant than benign breast disease and increased with tumour grade [13]. Furthermore, high tumour levels of certain MMPs have been shown to correlate with poor prognosis in human cancers [14]. In view of such findings, inhibition of MMPs has become an important target for cancer therapy.

MMI270B, N – hydroxy - 2 (R) - [[4-methoxysulfonyl] (3 - picolyl) amino] – 3 - methylbutaneamide hydrochloride monohydrate (figure 1), is a novel synthetic hydroxamic acid derivative able to competitively bind the Zn^{++} ion in the active site of a wide range of MMPs, inhibiting their activity at nM concentrations in vitro (table 1). MMI270B did not show antiproliferative activity against tumour cell lines in vitro, however in rat tumour models of breast and endometrial cancer it significantly reduced the tumour burden compared to controls and enhanced the activity of cytotoxic and hormonal agents [15]. MMI270B also demonstrated anti metastatic effects in vivo and antiangiogenic effects in vitro. Very low drug doses were required

for antimetastatic effects, calculated to be equivalent to a dose of 25 mg od in humans. In a rat aorta model of antiangiogenesis, there was a dose dependent reduction in blood vessel formation, with an IC_{50} for the assay of 0.2 μM. Preclinical toxicology data demonstrated no acute toxicity in rodents, but the drug caused emesis in dogs. Chronic administration in dogs was associated with increase in bone density and minor abnormalities of serum potassium and calcium levels. Pharmacokinetic studies showed that the drug was rapidly absorbed from the gut and eliminated from plasma, suggesting that a multiple dosing might be required for inhibition of MMPs over a 24 hour period. Bioavailability after oral administration in rats was 44%. The drug appeared to be extensively metabolised prior to excretion mainly in faeces.

Figure 1 Chemical structure of MMI270B



$C_{18}H_{23}N_3O_5S \cdot HCl \cdot H_2O$ molecular weight 393.43 (free base)

Table 1 In vitro inhibition of MMPs by MMI270B

Enzyme	IC_{50} (nM)	Substrate
MMP-1, collagenase	33	synthetic
MMP-9, gelatinase B	8	synthetic
MMP-2, gelatinase A	11	synthetic
MMP-3, stromelysin	13	synthetic
MMP-13, collagenase 3	6	synthetic
MMP-1, collagenase	43	type 1 collagen
MMP-3, stromelysin	50	human proteoglycan
Angiotensin converting enzyme showed 83% inhibition at 1μM		

This paper describes the first phase I and pharmacological study of MMI270B in patients with advanced malignancy. The primary aims of the study were to evaluate toxicity and pharmacokinetics. Secondary aims were to measure tumour response data and investigate various markers of biological activity for drug related change. These included bone resorption markers, direct and indirect targets of the inhibitor and a cytokine release assay.

PATIENTS AND METHODS

Eligibility criteria

The study was open to patients with advanced solid malignancies who had failed previous therapy and/or for whom there were no conventional treatment options. Patients had to be over 21 years old, with a World Health Organisation (WHO) performance status of 0-2 and a life expectancy of greater than three months. They were required to have adequate bone marrow (Hb $\geq$9 g/dl, leukocytes $\geq 4 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l), renal and hepatic function (serum creatinine and bilirubin $\leq$1.25 times the upper limit of normal (ULN), aspartate amino transferase and alanine amino transferase $\leq$3 times ULN). All patients had recovered from the acute toxic effects of previous treatment and had not received radiotherapy within two weeks, chemotherapy within four weeks (42 days for Mitomycin C or nitrosureas) or experimental treatment within 30 days of trial entry. Pregnant women, nursing mothers and patients not using adequate contraception were excluded, as were patients with clinical evidence of cerebral metastases. Other exclusion criteria included active infection, clinically significant abnormal baseline electrocardiograms, previous exposure to MMI270B, ongoing treatment with anticancer agents, and patients with a history of non compliance to medical regimens. All patients gave written informed consent. The trial was approved by the local research ethics committees of participating centres.

Pretreatment evaluation

Pretreatment evaluation included full history, physical examination and assessment of performance status. In addition, a complete blood count, clotting screen, renal, bone and liver biochemical profiles, tumour markers, an electrocardiogram, urinalysis, radiological assessment of disease and a pregnancy test if appropriate, were performed. One cycle of treatment lasted 28 days and assessment of disease was repeated after each cycle, according to WHO criteria for response [16]. Patients were eligible to continue to further cycles provided they did not have evidence of progressive disease or unacceptable toxicity.

Dose and dose escalation

MMI270B was supplied by Novartis Pharma AG, Basel, Switzerland in size 0 capsules and swallowed with 250 ml of water. In view of the rapid elimination of the drug seen in preclinical studies, the drug was given in divided doses on a continuous daily basis The starting dose, 300mg per day, was equivalent to 0.04 times the toxic dose low in mature dogs. Dose escalation was predetermined: 150mg bd, 300mg bd, 600mg bd, 400mg tds and 600mg tds, the latter being the highest dose which in practical terms could be administered orally because of the required number of capsules.

Study design

Cohort size was planned to be 20 patients at the highest dose level and 10 patients at lower dose levels, recruited into three cancer centres. This was with the aim of maintaining at least three patients on trial for greater than eight weeks, which was achieved.

Following recruitment of the first 60 patients, the trial was expanded to include a further 32 patients at two lower doses of 50mg od and 75mg bd. Dose escalation was based on satisfactory safety data from the previous level. Toxicity was assessed using the National Cancer Institute / National Institute of Health (NCI/NIH) Common Toxicity Criteria (CTC). Dose limiting toxicity (DLT) was defined as at least 3 patients experiencing grade 3 haematological toxicity or at least 2 patients experiencing grade 4 haematological toxicity or grade 3 non-haematological toxicity (excluding nausea, vomiting and alopecia). The maximum tolerated dose (MTD) was defined as the dose level below that at which DLT was observed.

Pharmacokinetics

Patients received only the morning dose of MMI270B during the pharmacokinetic (PK) sampling on days 1 and 28 of cycle 1. Five ml blood specimens were taken from an i.v. canula inserted in the forearm before drug administration and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after receiving the dose of drug. The blood specimens were centrifuged at 2500 rpm at room temperature, and the resulting plasma samples were transferred by pipette into screw-cap plastic

tubes. Samples were immediately frozen and maintained frozen until analysis. MMI270B was determined in plasma by a validated high-performance liquid chromatography (HPLC) method. After thawing, the study and quality control (QC) samples were homogenised by shaking on a vibration shaker for a few seconds. Samples were diluted as necessary with blank human plasma. A 50-μL aliquot of the internal standard working solution (CGS 26835) was added to the study and QC samples. Aliquots of 1.0 mL were transferred into extraction tubes and analysed.

MMI270B and the internal standard (CGS 26835) were extracted from human plasma with ether:methylene chloride (2:1) after acidification of the plasma with 0.1 M potassium phosphate, monobasic. The organic layer was transferred and evaporated to dryness under nitrogen, and the residue was reconstituted in 200 μL of HPLC mobile phase. Chromatographic separation of the compounds was achieved using a 5 μm Zorbax SB-C_{18} analytical column (4.6 mm ID x 150 mm) with acetic acid (pH 3.00):acetonitrile with 9% methanol (80:20, v/v) as the mobile phase, at a flow rate of 1 ml/min. The analytes were monitored using UV detection at a wavelength of 242 nm.

Calibration curves ($y = mx + b$), represented by the plots of the peak area ratios (y) of MMI270B to the internal standard *versus* the concentrations (x) of the calibration samples, were generated using weighted ($1/x^2$) linear least-squares regression as the mathematical model. Concentrations in quality control and study samples were calculated from the resulting peak area ratios and interpolation from the regression equations of the respective calibration curves. Turbochrom II 2700 (Version 4.0 and 4.1) software from PE Nelson was used. Concentrations of MMI270B are expressed as the free base.

Specificity of the method in blank human plasma and in the predose samples on day 1 was demonstrated by the lack of interfering peaks at the retention times of MMI270B and CGS 26835. The method was linear over the concentration range of 50.8 to 5080 nM for MMI270B, with a lower limit of quantitation of 50.8 nM. Pharmacokinetic analysis was done by non-compartmental methods using WinNonlin Professional (v. 1.5) software (Scientific Consulting, Inc.). Calculated parameters included maximum concentration (C_{max}), time of maximum concentration (T_{max}),

terminal plasma elimination half-life ($T_{1/2}$) and area under the concentration-time curve (AUC).

Associated studies

In order to evaluate whether MMI270B affects collagen breakdown in bone, urine specimens were collected at baseline, 24 hours, day 14 and day 28 for the bone resorption markers pyridinoline and deoxypyridinoline. These were assayed using the Pyrilinks kit (Metra Biosystems, USA) and Pyrilinks-Dkit (Metra Biosystems, USA), respectively. Urinary creatinine was assayed with the Creatinine kit (Metra Biosystems, USA).

Heparinised or citrated plasma samples were obtained at these time points for measurement of MMP-2, MMP-8, MMP-9, TIMP-1, TIMP-2, bFGF, Vascular Endothelial Growth Factor (VEGF), Vascular Cell Adhesion Molecule-1 (VCAM-1), soluble urokinase Plasminogen Activator Receptor (suPAR), Cathepsin B (CATB) and Cathepsin H (CATH) levels. One step sandwich enzyme immunoassays using monoclonal antibodies were used to measure levels of MMP-2, MMP-8, MMP-9, TIMP-1 and TIMP-2, as published previously [17-21]. The levels of bFGF, VEGF, VCAM-1, suPAR, CATB and CATH, were measured by ELISA in citrated plasma samples. For bFGF, VEGF and VCAM-1, commercially available kits were used, (Quantikine HS (= high sensitivity) human bFGF kit, VEGF kit and Human VCAM-1 Parameter kit, all from R & D systems, Minneapolis, MN, USA). The soluble uPA-receptor assay was performed as described previously [22], but with modifications including the use of alkaline-phosphatase-conjugated monoclonal mouse anti-rabbit IgG (Sigma) and p-nitrophenylphosphate (Sigma) as substrates in the final steps to quantitate the bound fraction of rabbit-anti-human suPAR. CATB and CATH were assayed as published previously [23].

A whole blood cytokine release assay was also performed. Ten ml of heparinised blood was collected from 16 patients before drug administration and 24 hours, 7 and 28 days post treatment [24]. All procedures were carried out under sterile pyrogen-free conditions. Phytohaemoglutinine A (PHA) (2 μg/ml blood) (Murex Diagnostics Ltd.) was used as *ex vivo* mitogenic stimulant and blood samples were incubated at 37°C in 5% CO_2 saturated and humidified incubator. Twenty four hours

post stimulation samples were cool spun, separated, flash frozen and stored at -20°C prior to assay. TNF-α levels were measured using IRMA kits (Medgenix, Brussels, Belgium, range 15-500 pg/ml). The assay was calibrated with the international reference preparation (87/650) (National Institute of Biological Standards and Controls, NIBSC, Potters Bar, Hertfordshire, UK). The calibration, standardisation and the assay format were specifically developed for measuring TNF-α in plasma/serum samples.

RESULTS

Ninety two patients were entered into the study, all of whom were evaluable for toxicity. Their details are shown in table 2. Table 3 shows, for each dose level, the number of patients who failed to complete one 28 day cycle, the number of patients treated for over 90 days (all of whom had stable disease at their end of third cycle assessment) and the median and range for the number of days on treatment. Of the 17 patients who came off study before day 28, eight stopped due to toxicity and two patients withdrew consent, of whom one lost motivation to continue with treatment and the other felt her condition to be deteriorating and opted for more local palliative care. Five patients came off study due to early progressive disease, one patient had a rising activated partial thromboplastin time and was withdrawn and one patient had a pulmonary embolus, not thought to be related to the study drug. Eightyfour percent of patients, excluding those who came off study before the end of the first cycle, were compliant with their medication (measured as taking greater than 80% of their expected total dose of trial drug during the first 28 days). There was no relationship between duration of treatment and dose level.

Toxicity

There was no haematological toxicity. There were two main non haematological toxicities. The clinical details are shown in table 4. The first was rash, observed in 18 patients. Fourteen of the patients developing rash were on dose levels greater than 400mg tds.

Table 2 Patient characteristics

No of patients entered	92
No of patients evaluable	92
Median age (years)	56.5
Range	25-77
Male / female	53 / 39
WHO performance status	
0	39
1	38
2	14
Not recorded	1
Previous treatment	
Surgery	67
Radiotherapy	41
Chemotherapy	74
Tumour type	
Colorectal	26
Mesothelioma	7
Renal	6
Melanoma	6
Breast	6
Ovary	6
Lung	5
Stomach	5
Primary unknown	5
Head and neck	4
Other	16

Table 3 Duration of treatment

Dose level	No of patients treated / days			No. of days on treatment	
	total	<28	>90	median	range
50 od	22	4	5	58	7-406
75 bd	10	2	2	52	24-196
150 bd	10	1	0	40	7-133
300 bd	10	2	4	57	10-314
600 bd	10	3	2	68	10-239
400 tds	10	1	4	84	8-161
600 tds	20	4	2	44	12-328

The rash was maculopapular with a symmetrical distribution generally affecting the trunk and arms more then the neck and legs and sometimes sparing the face. A typical rash is shown in figure 2a. Two patients described associated pruritus and two felt wheezy although their chests were clear to auscultation. One patient had a skin biopsy taken of the rash (figure 2b). The histology showed a normal epidermis with a perivascular monocytic infiltrate in the dermis consistent with a toxic drug reaction. Sixteen of the 18 patients developed rash within one month of starting dosing. Four patients came off study due to the rash. Two patients were rechallenged with MMI270B and in both cases the rash recurred. In all patients the rash resolved, usually by six weeks. Fourteen patients developed rashes but were able to continue with the study drug.

The second major toxicity was musculoskeletal. Arthralgia and / or myalgia was observed in 39 patients (table 4). Typically this began following at least one month of treatment and often started with finger or shoulder stiffness, which were the most commonly affected joints. Involvement of the wrists, elbows, knees, neck and back was also observed. Symptoms worsened with ongoing treatment and were

sometimes associated with a reduction in the range of movement in the joint. One patient, who continued on the drug for over seven months, lost abduction in his shoulders above 80 degrees. This patient and two others also developed Dupuytren's contractures, after 8, 12 and 17 weeks on MMI270B. Non steroidal anti inflammatory drugs, steroids, physiotherapy and periods of time off the drug all gave symptom relief to some patients. Three patients discontinued treatment due to arthralgia, however the majority of patients' symptoms settled over time after stopping the drug. The frequency and severity of musculoskeletal toxicity did not appear to be dose related or to be reduced with once daily dosing.

Fifteen patients reported mild to moderate nausea (table 4). There was no renal or hepatic toxicity.

Table 4 Nonhematological toxicity (worst per patient)

Dose level (mg)	Rash (CTC Grade)				Musculoskeletal (CTC Grade)				Nausea (CTC Grade)		
	1	2	3	total	1	2	3	total	1	2	total
50 od	1	1	0	2	7	1	1	9	4	3	7
75 bd	1	0	0	1	3	0	2	5	2	0	2
150 bd	0	0	0	0	1	2	1	4	3	0	3
300 bd	0	1	0	1	1	1	3	5	0	0	0
600 bd	1	1	1	3	2	1	0	3	2	0	2
400 tds	2	2	2	6	0	4	0	4	0	0	0
600 tds	1	3	1	5	3	4	2	9	0	1	1
total				18				39			15

Figure 2a Typical rash observed following MMI270B

Figure 2b Histology of a skin biopsy from a patient with rash

Response

There were no partial or complete tumour responses and neither were there any significant reductions in tumour markers. Stable disease lasting $\geq$ 90 days was seen in nineteen patients (table 3). These patients had a wide variety of primary tumour types and there was no apparent relationship to dose level.

Pharmacokinetics

Blood for pharmacokinetic analysis was taken from between 5 and 10 patients at dose levels 150mg bd - 600mg tds on days 1 and 28. At the two lowest dose levels, 50 mg od and 75 mg bd, plasma concentrations of MMI270B were below the limit of quantitation of the assay used. Plasma MMI270B concentrations showed a rapid increase after dosing, followed by a rapid decrease (figure 3a) indicating that MMI270B is rapidly absorbed from the gastrointestinal tract and rapidly eliminated from plasma. On Day 28 for all patients, the median T_{max} value was 0.58 hour (range 0.3-3) and the median $T_{1/2}$ was 1.6 hour (range 0.6-7.6). Due to the relatively short $T_{1/2}$, there was little or no accumulation of the drug on bd or tds dosing.

The pharmacokinetics of MMI270B demonstrated a linear relationship between AUC values and the dose administered (correlation coefficient (r) = 0.59, $p<0.001$). There was, however marked inter-patient variability. C_{max} also increased with dose (figure 3a). Two patients in the 600 mg bd dose cohort had unusually high C_{max} values on Day 1, which did not recur on Day 28. The reason for this is unclear.

In an attempt to consider whether pharmacologically-relevant drug levels were being achieved, a plasma concentration of 200 nM was used. This level is ≥ 4 times the IC_{50} for inhibition of the MMP enzymes (table 1). The percentage of time during the course of the dosing interval that MMI270B concentrations exceeded 200 nM was determined for dose levels 150mg bd to 600mg tds (figure 3b). Pharmacologically relevant plasma levels were achieved at these dose levels. For dose levels 600mg bd to 600mg tds, MMI270B concentrations exceeded 200 nM an average of 65-75% of time during the dosing interval and for the lower two dose levels the average was about 35%. 600 mg tds produced plasma levels ≥ 4 times the IC_{50} for inhibition of the target enzymes for a mean of 18 per 24 hours.

Bone resorption markers and angiogenic factors

Percentage change from baseline of the ratios of the bone resorption markers pyridinoline and deoxypyridinoline to paired serum creatinine values were compared to the corresponding pharmacokinetic values C_{max}, AUC, and time above 670 nM.

Figure 3a Time course of mean MMI270B plasma concentration on days 1 and 28

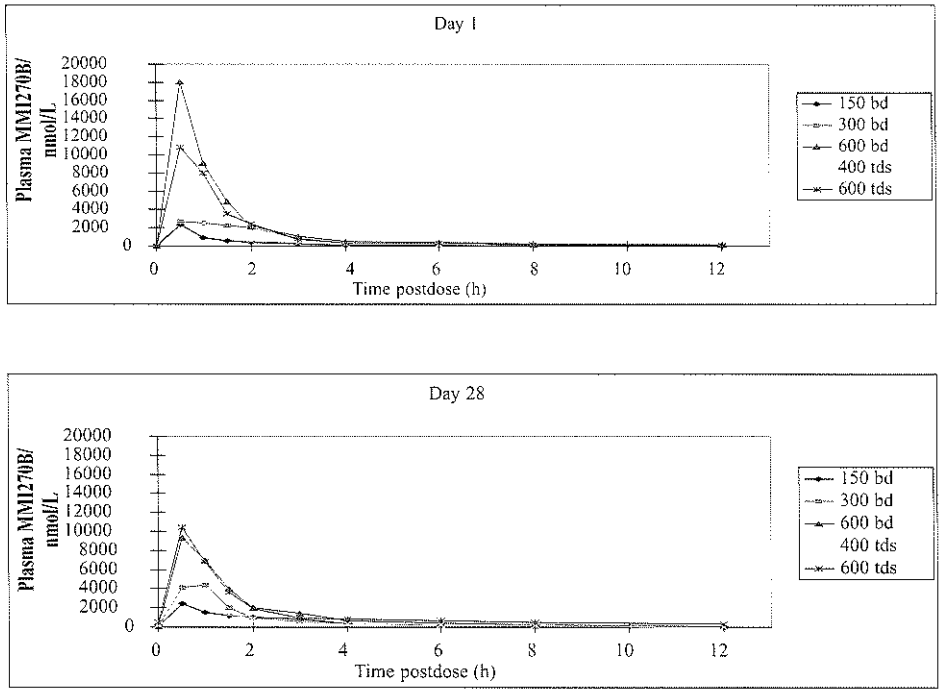
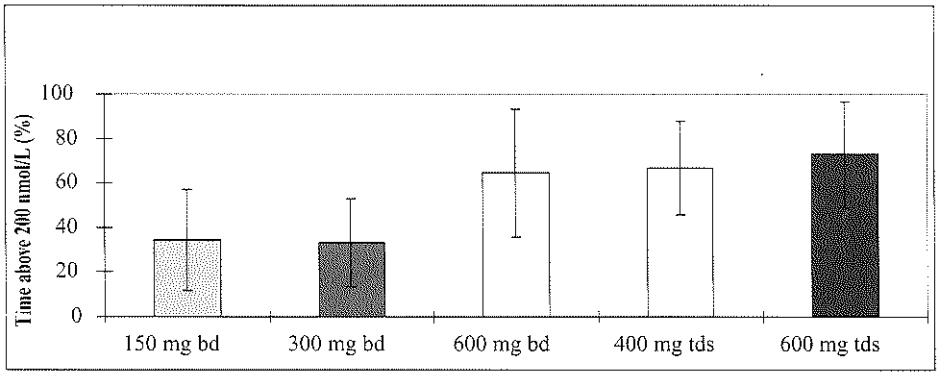


Figure 3b Percentage time MMI270B concentration is above 200 nM for dose levels150mg bd – 600mg tds



This last parameter was selected as, from animal studies, drug concentrations in an implanted tumour were approximately 30% of those in plasma, therefore a plasma concentration of 670 nM should provide complete inhibition of the target enzymes in the tumour, assuming that the animal model reflects cancer patients and that the drug is homogeneously distributed throughout the tumour. At day 14 the bone resorption marker ratios tended to fall with C_{max} and AUC but this relationship was not seen at day 28 (table 5).

There was a significant correlation for percentage increase of MMP-2 and TIMP-1 protein levels after one cycle of treatment, compared to baseline, with AUC (figures 4a and 4b), C_{max}, and $t \geq 670$ nM (table 5) and also with each other (correlation of 0.4, p=0.007) (figure 4c). These remain significant when the outlying data point is removed. Significant positive correlations were also observed between TIMP-2 with AUC and t>670, MMP-9 with C_{max} and bFGF with AUC (table 5). There were no other significant changes observed in the levels of MMP-8, MMP-9, TIMP-2, the ratio of MMP-2 to TIMP-2, bFGF, VEGF, VCAM-1, suPAR, CATB or CATH with any of the above calculated pharmacokinetic parameters.

Cytokine release assay

The cytokine release assay measured the effect of MMI270B on release of TNF-α from *ex vivo* stimulated peripheral blood cells. Pretreatment TNF-α release from stimulated whole blood cultures ranged from 50 -1250 pg/ml. TNF-α levels in the unstimulated bloods (controls) were usually below detection limits of assay *i.e.* 20 pg/ml. The mean reduction in TNF-α release was -5.9% at 4 hours, -12.9% at 24 hours, -27.3% at 7 days and -5.9% at 28 days respectively when results from all patients were combined. Although there was some inhibition of TNF-α release during treatment, the results did not reach significance, nor was there any significant difference between the results from patients receiving low (≤ 300mg bd) or high doses (> 300mg bd) of the drug.

Table 5 Significant Pearson's correlations of the biological markers to pharmacological endpoints

	PYCRE at day 14			**DEOCRE at day 14**		
	n	r	P value	n	r	P value
C_{MAX}	32	-0.48	0.01	32	-0.43	0.02
AUC	32	-0.36	0.04	32	-0.28	0.12
	MMP-2			**TIMP-1**		
	n	r	P value	n	r	P value
C_{MAX}	25	0.47	0.02	25	0.22	0.28
AUC	26	0.64	0.0004	26	0.57	0.002
$t \geq 670$	26	0.60	0.001	26	0.81	0.0001
	TIMP-2					
	n	r	P value			
AUC	26	0.46	0.02			
$t \geq 670$	26	0.57	0.002			
	MMP-9			**bFGF**		
C_{MAX}	25	0.40	0.048			
AUC				19	0.50	0.03

n = number of patients studied; r = correlation

pyrce = pyridinoline / creatinine renal excretion ratio

deocre = deoxypyridinoline / creatinine renal excretion ratio

Figure 4a Percent change from baseline at day 28 of MMP-2 levels against AUC

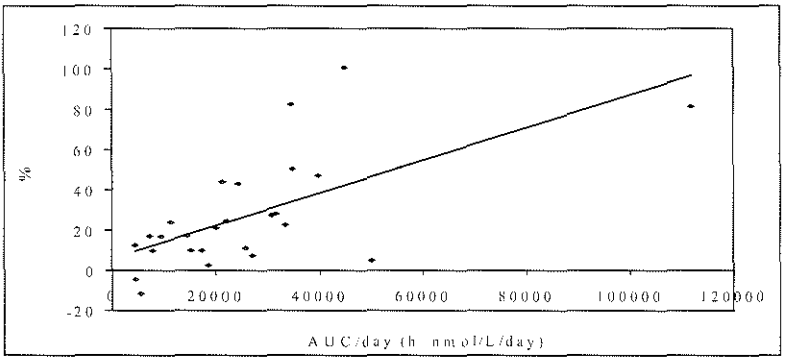


Figure 4b Percent change from baseline at day 28 of TIMP-1 levels against AUC

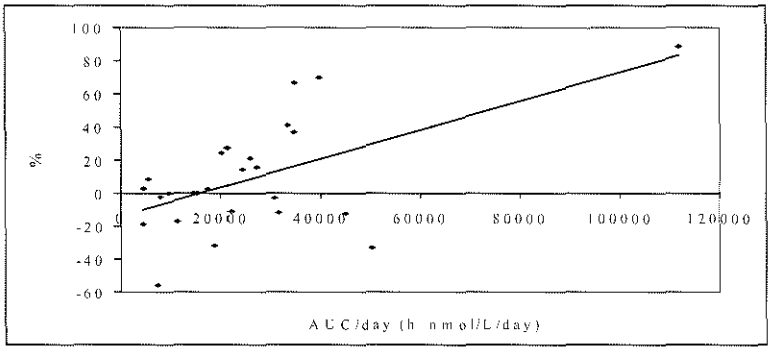
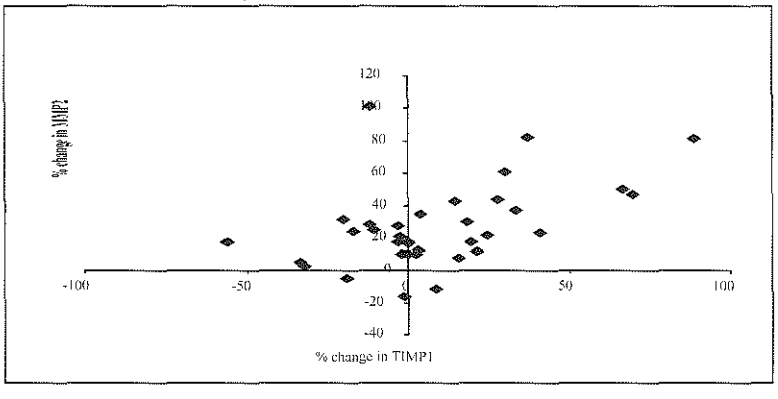


Figure 4c Percentage change from baseline at day 28 of MMP-2 against TIMP-1 after one cycle of treatment with MMI270B



The correlation coefficient between TIMP-1 and MMP-2 is r=0.4, p=0.007

DISCUSSION

Matrix metalloproteinase inhibitors (MMPIs) are among a number of antiangiogenic agents currently undergoing clinical trials. The difficulties in adapting a traditional phase I study design appropriately for investigation of such drugs has been discussed [25-27]. Biological modifiers, such as the MMPIs, may not have a serious dose limiting toxicity. Therefore the concept of the maximum tolerated dose indicating the appropriate level for phase II dosing may be inappropriate. MMPIs are likely to be most useful when prescribed over prolonged periods of time as their mode of action is prevention of invasion and metastasis. For this reason, it was desirable to collect data on toxicity associated with chronic dosing. In order to achieve this, a minimum of 10 patients were recruited at each dose level to improve the chance of some patients continuing the drug for at least eight weeks. Following completion of recruitment to the first five dose levels, the trial was further expanded with two new lower dose levels. This was as a result of further preclinical data, which indicated that MMI270B had antimetastatic effects in mouse models at markedly lower concentrations than previously determined, together with the rationale that the longterm toxicities, which emerged during the trial, might be reduced with lower doses or a once daily schedule.

In this phase I study, four separate approaches were used to assess the effects of MMI270B. Firstly patients underwent traditional assessment of toxicity. Secondly pharmacokinetic monitoring allowed dose level to be related to drug plasma levels and thereby to biological effects seen in preclinical studies. Thirdly, direct measurement of the affected target enzymes was attempted and finally indirect measures of biological activity were also studied.

There were two main toxicities associated with MMI270B, the first of which was rash. Eighteen patients developed a rash, 16 within the first month of treatment. The rash was generally mild but required cessation of treatment in four patients in the highest three dose levels. It was not a typical allergic reaction since most patients who developed rash could continue on treatment with the gradual disappearance of the skin reaction. Rash was determined to be the dose limiting toxicity as musculoskeletal side effects were related in frequency and severity to duration of

treatment rather than dose level. The maximum tolerated dose was determined to be 300mg bd, as at all dose levels higher than this, a marked increase in both the incidence and severity of rash was seen.

Musculoskeletal toxicity was the other significant side effect. Forty percent of the patients experienced symptoms ranging from general myalgia and/or arthralgia to severe tendonitis with limitation of range of movement in the affected joints. Although three patients discontinued MMI270B as a result of musculoskeletal side effects, 36 patients were able to continue on the trial. None of a variety of pharmacological and non drug therapies appeared particularly effective in reducing these symptoms, although some patients found temporary relief.

Batimastat (also known as BB-94) was the first MMPI to be assessed in clinical trials, however although it showed some activity it is not orally bioavailable and is insoluble, limiting its use [28-31]. Marimastat was the first orally bioavailable MMPI and has been extensively evaluated, currently in a series of phase III studies. In phase I studies, Marimastat showed biological activity in patients with advanced malignancy, as measured by the effects on levels of tumour markers [32]. In the phase II studies of Marimastat, the major toxicity was musculoskeletal effects, similar to those described in this trial [27]. It is probable that musculoskeletal side effects are a feature of this class of broad spectrum MMPI. Notably BAY 12-9566, an oral matrix metalloproteinase inhibitor which selectively targets MMP-2, MMP-9 and MMP-3 but not MMP-1, did not cause musculoskeletal side effects in phase I studies, however it was not clinically active either [33-36]. An explanation for the musculoskeletal side effects could be that broad spectrum inhibitors may also affect the reprolysin family of Zn^{++} metalloproteinases [37]. It is a member of this family of enzymes which has been found to hydrolyse pro TNF-α, TNF-α convertase (TACE). TNF-α release is known to be blocked by some matrix metalloproteinase inhibitors [38]. Our results on stimulated white cells analysed for TNF-α release suggest this enzyme was not inhibited by MMI270B (unlike Marimastat, unpublished data) and hence inhibition of this enzyme is not the explanation for musculoskeletal side effects.

Pharmacokinetic monitoring showed the drug to be rapidly absorbed and eliminated with a maximal plasma concentration after a median of 35 minutes and a median plasma half life of 1.6 hrs. There was minimal accumulation of drug over

time. The AUC and C_{max} of MMI270B both increased approximately proportionally with increasing dose, however marked inter-patient variability was noted. From preclinical data it was expected that plasma concentrations exceeding 200nM would result in full inhibition of the target enzymes. Thus the five higher dose levels all resulted in biologically relevant plasma levels. The 600mg tds level resulted in biologically active plasma levels for a mean of 18 per 24 hours, implying that higher doses would be unlikely to cause significant additional enzyme block. Therefore in this trial, pharmacokinetic monitoring was able to determine the maximum dose level necessary.

A concurrent study examined the effect of food intake on the pharmacokinetics of MMI270B. Although C_{max} was significantly reduced after food intake and T_{max} delayed, the mean AUC was not significantly affected and there were no recommendations that MMI270B should be taken in either the fasted or fed state [39].

MMI270B inhibits a wide range of matrix metalloproteinases at nanomolar concentrations. Serum protein levels of three of these, MMP-2, MMP-8 and MMP-9, together with their natural inhibitors TIMP-1 and TIMP-2 were measured to investigate whether a direct effect on the drug's target enzymes could be observed. It has been suggested that measuring serum MMP levels may be a method of following disease progression and response to therapy in advanced cancer patients, however other studies indicate that MMP-2 and MMP-9 are not always elevated in such patients [30,40,41]. TIMP-1 and TIMP-2 were identified in the late 1980's [3,42]. Both are effective inhibitors of a wide range of MMPs, TIMP-1 has been demonstrated to block endothelial responses to angiogenic factors such as bFGF and to inhibit angiogenesis [43]. Raised TIMP-1 mRNA levels have previously been demonstrated in tumours and this may result in the raised protein levels found in this study [44]. The ratio of MMP-2 to TIMP-2 was also calculated, as TIMPs bind stochiometrically to MMPs. In this study there was a significant trend for MMP-2 and TIMP-1 to increase at one month with increasing drug concentration and with each other, in terms of the percentage change after one cycle. The natural inhibitor for MMP-2 is TIMP-2, which also increased with AUC and $t > 670$ nM, and this may indicate a trend for MMP-2, TIMP-1 and TIMP-2 to rise in parallel. Clear interpretation of these

data is not possible, particularly as protein levels may not be an accurate surrogate for enzyme activity. However, it may be that MMP-2 is part of a negative feedback loop, such that increasing inhibition of the enzyme results in further production.

Indirect effects of matrix metalloproteinase inhibition were also sought. Pyridinoline and deoxypyridinoline are components of collagen cross links found chiefly in bone and excreted in urine. If MMPs cause breakdown of the collagen components of bone, then inhibition of these enzymes might result in a fall in urinary pyridinoline and deoxypyridinoline. With MMI270B, there was such a fall at two weeks, which was no longer observed at four weeks. It may be that these results reflect a transitory reduction in the release of ECM breakdown products at the start of MMP inhibition, which is then compensated for as time progresses by a rise in MMP-2 and TIMP-1. Clearly evaluation of intervening time points would help investigate this further.

VEGF, bFGF, VCAM-1, suPAR, CATB and CATH were also measured. Both VEGF and bFGF are important promoters of tumour angiogenesis [45,46], and raised levels have been found in cancer patients and to be associated with poorer prognosis [45-52]. Urokinase plasminogen activator (uPA) is a serine peptidase produced by many tumour cells that, when bound to its receptor, is able to cleave plasminogen to release plasmin, a known activator of MMPs [53]. Elevated plasma levels of suPAR have also been shown to correlate with poor prognosis in colorectal cancer patients [22]. Capthesins B and H are members of a family of lysozomal proteases, able to degrade various components of the ECM and to activate uPA. VCAM-1 is able to bind the integrin $\alpha_4\beta_1$ and is involved in the transmigration of leukocytes across the vascular endothelium, a process also affected by the MMPs [54]. As these molecules are all involved in alterations of the microenvironment of the ECM, it is possible that inhibition of ECM breakdown might be reflected in alterations in their levels. In a similar study, however, BAY 12-9566 did not affect VEGF or bFGF plasma levels [33].

Statistically significant correlations between the percentage changes from baseline of MMP-9 with C_{max} and bFGF with AUC were found, however, as these were related to only one of the pharmacokinetic parameters measured it may be that they represent artefacts of the statistical analysis as opposed to true biological

trends. There were no apparent correlations between other extracellular components and drug concentration, however given the wide inter-patient variability of both measured ECM components and plasma drug concentrations, it is possible that minor levels of inhibition are occurring but are not observable.

In this study there were no objective tumour responses, however prolonged disease stabilisation occurred in 19 of 92 patients. Although this will include patients with slow growing tumours, it may be indicative of drug activity. It has been suggested that a reduction in tumour markers can also be used as an indicator of disease response [32]. These were measured where appropriate in this study, however no significant reduction was observed. As discussed elsewhere, although tumour responses remain the main aim of cancer therapy, maintenance of stable disease and even delay in progression of disease would still be of clinical benefit to many patients [26]. In the future, other methods such as magnetic resonance spectroscopy, positron emission tomography, or colour doppler ultrasound, which can be used to assess tumour metabolism and blood flow, may prove useful in monitoring patient responses to anti-angiogenic agents [55].

In conclusion, MMI270B is a novel oral, broad spectrum matrix metalloproteinase inhibitor, with anti-angiogenic and anti-metastatic effects in animal models. In this large phase I study in patients with advanced malignancies, the drug was generally well tolerated with rash and musculoskeletal side effects as the main toxicities. MMI270B plasma levels were achieved at greater than four times the mean IC_{50} for the target enzymes, at the dose determined by conventional toxicity end points. Therefore, from this trial, it would be reasonable to consider MMI270B for further clinical trials at a dose level of 300 mg bd.

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CHAPTER 5

EFFECT OF FOOD ON THE PHARMACOKINETICS OF ORAL MMI270B (CGS 27023A), A NOVEL MATRIX METALLO-PROTEINASE INHIBITOR.

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ABSTRACT

Purpose: MMI270B is a matrix metalloproteinase inhibitor (MMPI) with *in vitro* and *in vivo* activity. In order to exert optimal target inhibition, MMPI must be given chronically, and therefore oral bioavailability is important. We analysed the effect of food intake on $AUC_{0\text{-}8h}$, C_{max}, and T_{max}.

Patients and methods: Seventeen patients were entered into the study. Doses of MMI270B were 150, 400 and 600 mg. The first day, patients ingested the drug in a fasted state and were not allowed to eat for 2 hours. The second day, patients ingested the drug 30 minutes after a light breakfast.

Results: Mean $AUC_{0\text{-}8h}$ was not significantly influenced by food intake. Plasma concentrations were well above the IC_{50} of several MMP's at all doses tested. Mean C_{max} was significantly decreased after food intake. Mean T_{max} was significantly delayed after food intake.

Conclusion: Food intake did not result in a significant change in exposure to MMI270B ($AUC_{0\text{-}8\ h}$), but did result in a significant, although not clinically relevant, decrease in peak plasma levels and time to reach peak plasma levels. No specific guidelines concerning the ingestion of MMI270B in either a fed or a fasted state are recommended.

INTRODUCTION

Matrix metalloproteinases (MMP) are a class of structurally related enzymes responsible for the degradation of extracellular matrix that constitutes connective tissue. Activity of MMP is controlled by naturally occurring inhibitors, but in several disease states, such as cancer, an imbalance between the activity of MMP and their inhibitors results in an increased extracellular matrix degradation. In cancer, this degradation facilitates local invasive growth and increases the potential for metastasis. Inhibiting MMP seems an attractive goal in anticancer treatment. Inhibitors of MMP should not have a direct cytotoxic effect but should control the metastatic process [1].

MMI270B (previously CGS 27023A) (N-hydroxy-2(R)-[[4-methoxysulfonyl](3-picolyl) amino]-3-methylbutaneamide hydrochloride) monohydrate) is a novel MMPI with an IC_{50} of 33 nM for recombinant human collagenase (MMP-1), 8 nM for recombinant human M_r 92,000 gelatinase (MMP-9), and 13nM for recombinant human stromylesin-1 (MMP-3). Preclinical studies with oral MMI270B *in vivo* showed growth-inhibitory effects in breast carcinoma, prostate, bladder, colon, lung adenocarcinoma, glioblastoma, and ovarian carcinoma cell lines. MMI270B is rapidly absorbed after oral administration in rats and dogs. In fasted rats, bioavailability after a single oral dose is 44%. Thus far, only one clinical study with oral MMI270B has been presented [2]. Data concerning bioavailability in humans, and the possible influence of food intake, have not been published previously. In view of the mechanism of action of MMPI's, prolonged and continuous administration will result in optimal target inhibition, and therefore, oral treatment is preferred. We performed a phase I and pharmacological study with oral MMI270B in patients with miscellaneous solid tumors [2]. As part of this study, we analysed the influence of food intake on the pharmacokinetics of MMI270B, comparing $AUC_{0\text{-}8h}$ (area under the plasma concentration *versus* time profile), C_{max} (peak plasma level), and T_{max} (time to peak plasma level) at different dose levels of MMI270B, after ingestion in both a fasted and fed state.

PATIENTS AND METHODS

Eligibility criteria

Patients with a cytologically or histologically confirmed diagnosis of a solid tumor refractory to standard treatment or for which no standard treatment was available, were eligible for the phase I and pharmacological study. Further eligibility criteria included: age ≥21 years, WHO performance state ≤2, life expectancy of ≥12 weeks, no anticancer treatment in the previous 4 weeks (6 weeks for mitomycin C or nitrosoureas), no radiotherapy in the previous 2 weeks, adequate function of bone marrow (WBC $\geq 4.10^9$/l, platelets $\geq 100.10^9$/l, haemoglobin ≥9 g/dl (5.59 mmol/l), normal hepatic and renal functions (alanine aminotransferase within three times the normal upper limit, bilirubin within 1.25 times the normal upper limit, creatinine within 1.25 times the normal upper limit). Exclusion criteria were pregnant women, the evidence of cerebral metastases, or a clinically significant abnormal electrocardiogram at baseline.

All patients gave written informed consent for the phase I and pharmacological study. Patients enrolled in the fasted/fed study gave additional and specific written informed consent.

Pretreatment assessment and follow-up studies

Prior to therapy, a complete medical history was taken and a physical examination was performed. A complete blood count, including WBC differential, and serum chemistries including sodium, potassium, calcium, phosphorus, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltranpeptidase, and lactate dehydrogenase were performed, as were urine analysis, electrocardiogram, and tumor markers if appropriate. Patients enrolled in the fasted/fed study were admitted to the hospital for 2 consecutive days for pharmacokinetic sampling.

Drug administration

MMI270B was supplied by Novartis Pharma AG, Basel, Switzerland, as a chiral hydroxamic acid derived from D-valine. It was supplied in capsules of 25, 100,

or 300 mg. Capsules had to be stored at temperatures <25^0 C and protected from light and had to be swallowed with 250 ml of water. Prophylactic antiemetics were not given routinely. For pharmacokinetic purposes, capsules were swallowed once daily on days 1 and 2. For the fasted/fed analysis, capsules were swallowed in a fasted state on day 1, and patients were not allowed to eat or drink for 2 hours after ingestion. On the second day of treatment, patients swallowed the capsules 30 minutes after they had eaten a light breakfast.

Pharmacokinetic studies

Five-ml blood samples were taken from an intravenous cannula that was inserted in the forearm. On day 1, blood samples were taken pre-dose and 30, 60, and 90 minutes, and 2, 3, 4, 6, 8, 12, and 24 hours post-dose, prior to the morning dose. On day 2, blood samples were taken pre-dose, 30, 60, and 90 minutes, and 2, 3, 4, 6, and 8 hours post-dose. Blood samples were collected in heparin-containing Vacutainer tubes that were gently inverted 8-10 times. Within 30 minutes after collection, samples were centrifuged at 2500 rpm at room temperature for 15 minutes, after which plasma was transferred into plastic tubes with a pipette, and stored at – 20^0 C until analysis. Determination of plasma concentrations of MMI270B was performed using a validated high-performance liquid chromatography method. MMI270B and the internal standard (CGS 26835) were extracted from acidified human plasma by ether:methylene chloride (2:1). The organic layer was transferred and evaporated to dryness under nitrogen, and the residue was reconstituted in high-performance liquid chromatography mobile phase for sample injection. Chromatographic separation of the compounds was achieved on a 5-µm Zorbax SB-C_{18} analytical column (4.6 mm inside diameter x 150 mm), using acetic acid (pH 3.00):acetonitrile with 9% methanol (80:20, v/v) as the mobile phase at a flow rate of 1 ml/min. The effluent from the column was monitored by UV detection at 242 nm. The lower limit of quantitation was 20 ng/ml, and the method had a linear range over the concentration range of 20 to 2000 ng/ml. The noncompartmental pharmacokinetic parameters $AUC_{0\text{-}8h}$, C_{max}, and T_{max} data were calculated using WinNonlin Professional version 1.5 software (Scientific Consulting Inc.). For $AUC_{0\text{-}8h}$ and C_{max},

the fed:fasted ratio was determined; for T_{max}, the time difference fed-fasted was determined.

Statistical considerations

The correlation between individual AUC_{0-8h} values and the administered dose was evaluated by means of Spearman's correlation coefficient (ρ) and linear regression analysis. Interpatient differences in pharmacokinetic parameters were assessed by the coefficient of variation, expressed as the ratio of the standard deviation and the observed mean. Variability in parameters between the two treatment courses and the various MMI270B dose levels was evaluated by a two-sided paired Student's *t* test plus the 95% confidence limits for the mean difference (δ) and the Kruskal-Wallis statistic, respectively. Statistical calculations were performed using Number Cruncher Statistical System (version 5.X; Jerry Hintze, East Kaysville, UT). Probability values of < 0.05 were regarded as statistically significant.

The 90% confidence intervals for the ratio of means fed versus fasted for the parameters AUC_{0-8h} and C_{max} were calculated using the ANOVA program of WinNonlin Professional version 1.5 (Scientific Consulting Inc.).

RESULTS

Seventeen patients were entered into the study. In one patient, blood sampling on day 2 was done until 6 hours post-dose. Doses studied were 150, 400, and 600 mg. None of the patients used prokinetic medication, antacids, or other concomitant medication expected to alter gastrointestinal motility.

PHARMACOKINETIC RESULTS

Mean drug exposure (AUC_{0-8h}) was related to dose (fed: Spearman's $\rho = 0.876$ and $P = 0.0007$; fasted: Spearman's $\rho = 0.869$ and $P = 0.0008$), whereas the influence of food intake on drug exposure was diverse; in 12 patients food intake resulted in a decreased drug exposure, whereas in 4 patients an opposite effect was noted (figure 1, table 1). In one patient, food intake had no effect on drug exposure.

Table 1 Summary of MMI270B pharmacokinetic data after oral administration[a]

	Dose (mg)	Fed	Fasted	95% CL[b]	*P*[c]
AUC(ng.h/ml)	150	799.3±501.9	967.0±556.8	-282<δ<-54.	0.018
	400	2422±1246	2406±1127	-319<δ<351	0.901
	600	6787±3151	7711±3592	-2859<δ<1009	0.297
C_{max} (ng/ml)	150	485.0±340.6	1091±536.4	-1080<δ<-131	0.027
	400	1754±969.8	2838±2015	-3041<δ<872	0.199
	600	4406±2264	7432±5158	-6401<δ<349	0.072
T_{max} (h)	150	1.14±0.78	0.53±0.04	-0.58<δ<1.81	0.201
	400	1.18±0.46	0.50±0.01	0.11<δ<1.26	0.030
	600	0.91±0.35	0.92±0.42	-0.17<δ<0.41	0.975

[a] Data were obtained from 17 cancer patients treated on day 1 with MMI270B at dose levels of 150 mg (n = 4), 400 mg (n = 5), or 600 mg (n = 8) after an overnight fast (Fasted) and on day 2 at 30 min after a light breakfast (Fed). Data were calculated by noncompartmental analysis and represent mean values ± SD.

[b] 95% CL, 95% confidence limits for the mean difference.

[c] Probability value from a two-sided paired Student's t test.

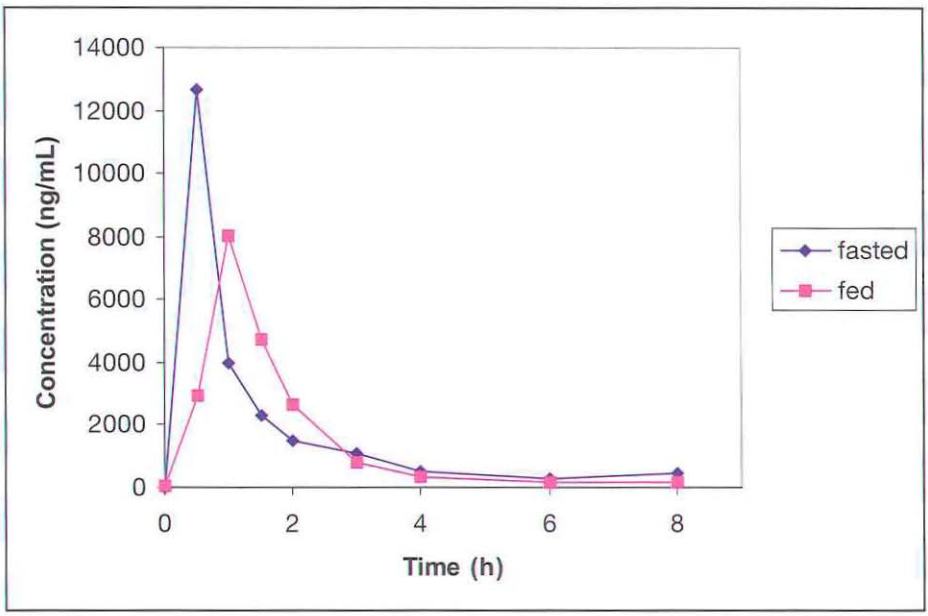
Overall, mean exposure to MMI270B was reduced by 10% after food intake. The 90% confidence interval for the ratio of means fed versus fasted (0.816-0.986) lies within the range 0.8-1.25, indicating no significant effect of food intake on $AUC_{0\text{-}8h}$. Both in the fed and fasted states, and at all doses analysed, plasma levels of MMI270B were well above the IC_{50} for the target enzymes collagenase-MMP1, gelatinese-MMP9, and stromelysin-MMP3 for considerable periods of time after administration.

Mean peak plasma levels were strongly correlated to dose (fed: Spearmans's ρ= 0.850 and P= 0.0013; fasted: Spearmans's ρ= 0.691 and P= 0.0116). In three

patients, peak plasma levels in the fed state were higher than in the fasted, whereas in 14 patients, peak plasma levels decreased after food intake. Mean C_{max} was 40% lower in the fed state. The 90% confidence interval for the ratio of means fed versus fasted (0.457-0.778) almost entirely falls outside the range (0.7-1.43), indicating a significant effect of food intake on C_{max}.

Mean time to reach peak plasma levels (1.04 $\pm$ 0.488 hr fed state, 0.704 $\pm$ 0.388 hr fasted state) was significantly increased by food intake (P = 0.042; 95% confidence limits for the mean difference: $0.04<\delta<0.65$). The absolute increase in mean time to reach peak plasma levels was 0.34 hour (or 20 minutes).

Figure 1 Concentration *versus* time profile of MMI270B in a representative patiënt (dose 600 mg)



DISCUSSION

We have performed a pharmacological study with the oral MMPI MMI270B to analyse the influence of food intake on the pharmacokinetic parameters $AUC_{0\text{-}8h}$, C_{max}, and T_{max}.

The results of this study show that exposure to MMI270B was not significantly influenced by food intake, and plasma levels of MMI270B in both the fasted and fed state, at all dose levels studied, remained well above the IC_{50} of the MMP-1, MMP-3, and MMP-9 for prolonged periods of time. Peak plasma levels of MMI270B were significantly influenced by food intake, and a correlation between change in overall drug exposure and change in peak plasma level could be determined. Although food intake significantly slowed the rate of absorption of MMI270B, the absolute change in T_{max} is not clinically relevant, especially when taking into account that MMPI's have to be administered on a continuous and prolonged basis to exert optimal target inhibition.

The results of this pharmacokinetic study indicate that although food intake slows the rate of absorption of MMI270B and significantly decreases peak plasma levels, overall drug exposure is not significantly influenced. No specific guidelines concerning the ingestion of MMI270B in either a fed or a fasted state are recommended.

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CHAPTER 6

FARNESYL TRANSFERASE INHIBITORS: CURRENT DEVELOPMENTS AND FUTURE PERSPECTIVES.

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ABSTRACT

Ras oncogenes play an important role in carcinogenesis and are frequently found in various human tumour types. Cellular activity of Ras oncoprotein, regulated through the enzyme farnesyl transferase, is crucial in the process of *ras* dependent carcinogenesis, and therefore, specific inhibition of this enzyme is an attractive goal in anticancer treatment.

Specific inhibitors of farnesyl transferase have been developed in recent years, many of them showing *in vitro* and *in vivo* growth inhibitory or cytostatic activity.

Recently, results of the first clinical studies with various farnesyl transferase inhibitors have been presented. In the design of phase I and II studies, either single-agent or combination studies, new endpoints have to be defined in order to properly assess feasibility, antitumour activity and clinical valuability.

INTRODUCTION

Mutated *ras* genes and the Ras oncoproteins they encode are found with high frequency in various human tumortypes, especially in mucinous adenocarcinomas of the digestive tract [1]. Ras oncoproteins play a causative role in the malignant transformation of cells. To exert its cellular mitogenic activity, Ras has to become localised to the inner surface of the cellular membrane following a series of posttranslational modifications, of which farnesylation, through the activity of the enzyme farnesyl transferase is the first and most important step.

A vast number of inhibitors of farnesyl transferase have been developed in recent years, and after extensive preclinical testing, the results of the first clinical studies with several compounds from this new class of potential anticancer agents have recently been presented.

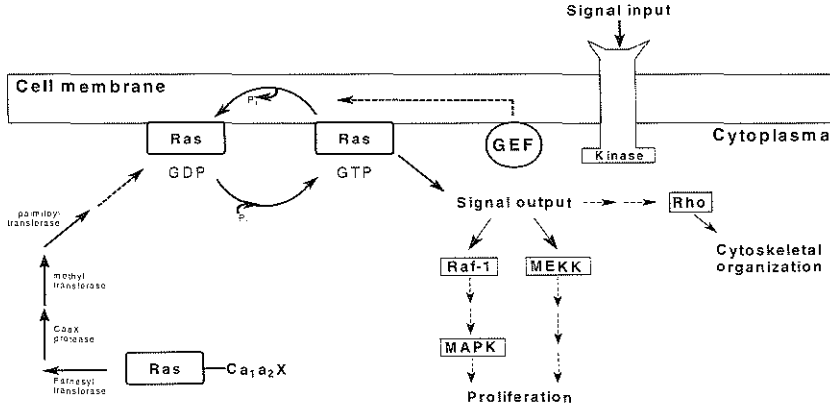
In this review we describe the process of Ras production and activation, and will review the results of preclinical and clinical studies with farnesyl transferase inhibitors that have been performed so far. The number of compounds developed so far is very large, but most of them have only undergone preclinical evaluation. For many compounds development has been halted, and despite extensive preclinical testing, the amount of available published information concerning these studies is limited. Since most of the *in vivo* studies with these compounds showed growth inhibition rather than tumour regression, at the end of this review we will discuss the consequences of these findings for the design of future clinical trials with this new class of anticancer agents.

PRINCIPLES OF CELLULAR RAS

The basis for mammalian cell functioning is located in genes stored in the nucleus. Mammalian cells contain three functional *ras* genes, H-*ras*, K-*ras* and N-*ras*, encoding for H-Ras, K-Ras and N-Ras proteins, respectively [1]. These Ras proteins play an important role in transduction of signals from receptor tyrosine kinases to several different intracellular effector pathways. Most of the signals transduced by Ras are induced by extracellular growth promoting factors like epidermal and platelet-derived growth factor, interleukin 2 and 3, and granulocyte-macrophage

colony-stimulating factor. In response to the cellular binding of these factors, membrane-bound inactive Ras.guanosine 5'-diphosphate (Ras.GDP) is converted into the activated membrane-bound Ras.guanosine 5'-triphosphate (Ras.GTP)-bound conformation. This activated conformation of Ras subsequently activates several downstream effector pathways leading to cellular proliferation on the one hand through the activation of, amongst others, the *Raf/MAPK* and *MEKK* pathways, and the induction of morphological cell changes via actin cytoskeleton activation through the *Rho* effector pathway on the other hand (figure 1). The cellular responses induced by activated Ras.GTP are counterbalanced by several mechanisms. GTP-ase activating protein (GAP) of Ras degrades activated Ras.GTP back to the inactive Ras.GDP conformation.

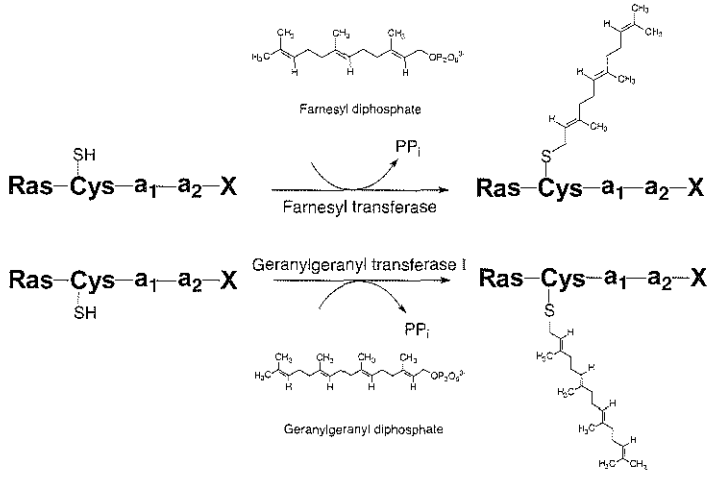
Figure 1 Function of activated Ras.GTP in signal transduction pathways



Ras.GTP activates several downstream effector pathways.
GEF denotes Ras guanine exchange factors.

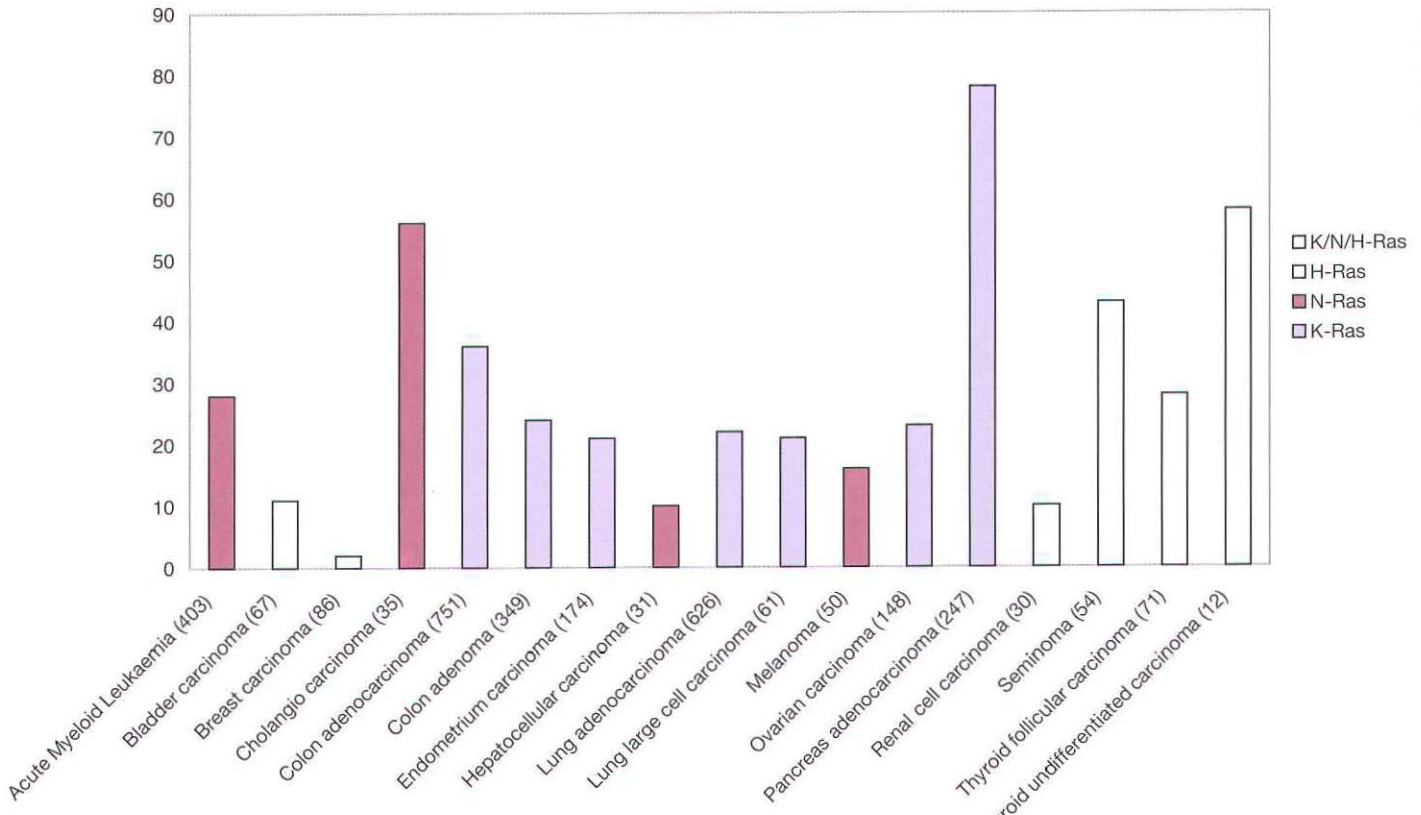
Ras is synthesised as a soluble inactive protein on free ribosomes. To become a signal transducer, a series of posttranslational modifications has to be carried out in the cytosolum prior to localisation of Ras to the inner surface of the cell membrane. The first step in this cascade of posttranslational modification or prenylation of Ras is the transfer of a C15 isoprenoid or farnesyl moiety from farnesyl diphosphate (FPP) to the sulphur atom of the cysteine locus of the carboxyterminal tetrapeptide CAAX of Ras (figure 2). The enzyme necessary for this first posttranslational enzymatic step or farnesylation is farnesyl transferase. Of this so-called CAAX box of Ras, C represents cysteine, A represents leucine (L), isoleucine (I) or valine (V), and X usually represents methionine (M) or serine (S). The CAAX box of K-Ras consists of CVIM and the CAAX box of H-Ras consists of CVLS. After the addition of the farnesyl moiety to CAAX, the three amino acids terminal to cysteine are proteolytically cleaved, and the carboxy-terminal group of cysteine is methylated, leading to a hydrophobic protein with higher affinity for the cellular membrane [2-6].

Figure 2 Farnesylation and geranylgeranylation of Ras



In various human tumour types mutated *ras* genes can be isolated (figure 3). K-*ras* mutations are most frequently found. In up to 90% of adenocarcinomas of colon, pancreas and lung these mutations can be detected. N-*ras* mutations are less

Figure 3 Frequency of Ras mutations (%)



Data adapted (1, 68, 69)

frequently found and mainly occur in acute myeloid leukaemia and myelodysplastic syndromes. H-*ras* mutations are relatively seldom found, and mainly occur in renal and bladder carcinomas [1]. All these *ras* mutations are considered to play an equally essential role in tumorigenesis. Activated Ras oncoproteins bring cells in a proliferative state in a way that is comparable to normal Ras, and farnesylation of Ras oncoprotein is equally essential for its activity. Ras.GTP oncoproteins, however, are insensitive to the hydrolytic activity of GAP, and cells harbouring these mutated Ras proteins therefore remain in a permanent proliferative state and will show autonomous growth and uncontrolled proliferation.

Farnesyl transferase specifically recognises CAAX with either methionine or serine at the X position, and the enzyme shows high affinity for this tetrapeptide. However, the activity of farnesyl transferase is not exclusively restricted to Ras. Other cellular proteins like lamin A and B, Rap 2, Rho B and E, transducin ϒ, and rhodopsin kinase also contain the CAAX tetrapeptide configuration and are farnesylated. A number of these CAAX tetrapeptide-containing substrates play a role in mitogenic signalling or are mitogenic, whereas others play a role in various physiologic processes. The nuclear lamins transducin ϒ and rhodopsin kinase, for example, are important for retinal signal transduction [5].

Recent findings have highlighted cellular Rho (B) as an important downstream effector protein for activated Ras. Rho B is a cellular protein that plays a role in the cytoskeletal stress fiber organisation. It can be either farnesylated or geranylgeranylated, and in the presence of inhibitors of farnesyl transferase in cell lines, geranylgeranylated forms of Rho B (Rho B-GG) will accumulate in these cells. The presence of increased intracellular concentrations of Rho B-GG in combination with decreased intracellular concentrations of farnesylated Rho B is sufficient for inhibition of normal cell growth and apoptosis, respectively, such as is seen in *in vitro* models of inhibitors of farnesyl transferase [7,8].

Not only farnesylation, but also geranylgeranylation, the addition of a C20 or geranyl moiety to cysteine of the carboxyterminal tetrapeptide CAAX through the activity of geranylgeranyl transferase I, is an enzymatic posttranslational modification process that leads to activation of several cellular transduction proteins (figure 2). Rap 1, Rho A, B, and G, and G-protein ϒ-subunits are proteins that are

geranylgeranylated. Geranylgeranyl transferase I preferentially recognises CAAX with leucine or phenylalanine located at the X position. Farnesyl transferase and geranylgeranyl transferase do not have absolute specificity for their substrates, and under conditions where farnesylation normally is the predominant prenylation process, in the absence of farnesyl transferase, for example due to the presence of specific inhibitors, proteins can be geranylgeranylated as a cellular 'rescue mechanism'. Because geranylgeranylation is more prevalent in normal cellular proteins than farnesylation, and because Ras oncoproteins are almost exclusively dependent upon farnesylation for their cellular activity, specifically inhibiting farnesyl transferase theoretically is the most interesting approach in the design and development of specific anticancer agents, whose activity could lead to anticancer treatment without affecting normal cellular functions.

The obvious and presumably causative role of mutated *ras* oncogenes in malignant cell proliferation, *i.e.* their role in initiating and maintaining the diverse unrestricted receptor tyrosine kinase mediated mitogenic signaling pathways that have been recognised to be essential in the development and growth of various human tumours, and the crucial role of the enzyme farnesyl transferase in the process of posttranslational activation of cellular Ras, has stimulated the development of agents aiming to inhibit this vital enzyme. However, many difficulties emerged, and underneath we will try to summarise the available preclinical information, here and there focussing on the pitfalls of bringing the concept to the clinic.

FARNESYLTRANSFERASE INHIBITORS

The substrates for farnesyl transferase are FPP and the CAAX tetrapeptides, and inhibitors of farnesyl transferase can therefore be divided into three categories;
A: compounds with structural similarity with FPP, so called FPP analogues
B: compounds with structural similarity with CAAX, so-called CAAX peptidomimetics that can be subdivided into peptide and non-peptide classes
C: bisubstrate inhibitors that combine both features

An overview of the compounds tested thus far, either preclinically and/or clinically, is given in table 1.

Table 1 Overview of farnesyl transferase inhibitors tested

	Preclinical studies	*Clinical studies (ref)*
Farnesyl diphosphate analogues	Manumycin	---
	(α-hydroxyfarnesyl) phosphonic acid	---
	J-104,871	---
	RPR 130401	---
	RPR 115135	---
	PD 169451	---
CAAX tetrapeptides	CVFM	---
	CIIM	---
	CIFM	---
CAAX peptidomimetics	BZA-5B	---
	B581	---
	B956/B1086	---
	L-731,735/L-731,734	---
	L-739,750/L-739,749	---
	L-744,832	---
	L-778,123	59
	FTI-232/FTI-244	---
	FTI-276/FTI-277	---
	A-197574	---
	FTI-2148	---
	BIM-46068	---
Non-peptide CAAX peptidomimetics	SCH 44342	---
	SCH 59228	---
	SCH 66336	60, 61, 62, 63
	R115777	64, 65, 66
	BMS 214662	---
Bisubstrate inhibitors	BMS 185878/BMS 186511	---
	BMS 184467	---

PRECLINICAL STUDIES

Farnesyl diphosphate (FPP) analogues

FPP analogues were the first reported active inhibitors of farnesyl transferase. Manumycin or UCF1-C is a natural product produced by Streptomyces that binds to farnesyl transferase, although with lower affinity than FPP itself. *In vitro*, the IC_{50} for farnesyl transferase is lower than for geranylgeranyl transferase. Manumycin inhibits the function of farnesylated proteins in yeast and has been reported to inhibit growth of K-*ras* models (such as a fibrosarcoma xenograft in syngeneic mice and the MIA PaCa-2 human pancreatic carcinoma xenograft) in a dose-dependent manner, and of an N-*ras* model (HT1080 human fibrosarcoma xenograft) [9-12].
Synthetic (α-hydroxyfarnesyl) phosphonic acid is a derivative of FPP with a tenfold higher binding affinity as compared to FPP itself. It partially inhibits H-Ras processing in cells [3,10].

Although manumycin and (α-hydroxyfarnesyl) phosphonic acid inhibit farnesyl transferase more effectively than geranylgeranyl transferase, it is yet uncertain whether this inhibition induces cellular effects, or whether these effects relate to inhibition of other enzymes such as squalene synthase and farnesyl diphosphate synthase [4].

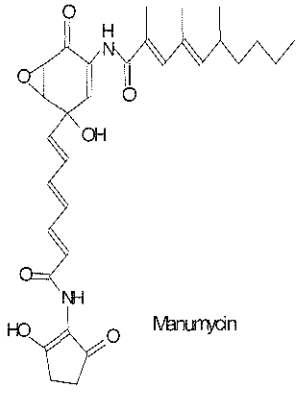
J-104,871 is a modified squalene synthetase inhibitor that inhibits farnesyl transferase in a FPP competitive manner. It very effectively inhibited rat brain farnesyl transferase, but hardly affected rat brain geranylgeranyl transferase I. It inhibits Ras processing of H-*ras* transformed NIH3T3 cells, and in *in vivo* studies it induced tumour growth inhibition in this model [13].

Benzo(f)perhydroisoindoles derivatives such as RPR 115135, RPR 130401 and PD 169451, mainly targeting K-*ras*, are selective and potent inhibitors of farnesyl transferase [14,15]. Intraperitoneally administered RPR 115135 was active against HCT 116 colon tumour xenograft, whereas oral administration of RPR 130401 induced growth arrest in this model at the highest dose tested and seemed to increase tumour free survival in mice when given after cytoreductive treatment with CPT-11 [16-18]. Interestingly, subcutaneously administered PD 169451 induced

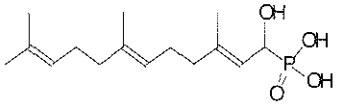
growth inhibitory effects in various human tumour xenografts, irrespective of *ras* mutational status [19].

An overview of the chemical structures of most of the thus far developed FPP analogues is given in figure 4.

Figure 4 Farnesyl diphosphate analogues



Manumycin



(a-Hydroxyfarnesyl) phosphonic acid

CAAX tetrapeptides and peptidomimetics

Tetrapeptides with inhibitory activity against farnesyl transferase contain structural modifications at the AA amino acid locations of CAAX. When this modification contains an aromatic residue at the terminal A position, the tetrapeptide is a nonsubstrate inhibitor, whereas other modifications result in CAAX tetrapeptides being alternative substrates for farnesyl transferase.

CVFM was the first CAAX tetrapeptide inhibitor. Subsequently, CIIM and CIFM were developed. Although these CAAX tetrapeptides were found to be potent inhibitors of farnesyl transferase *in vitro*, they shared a limited chemical stability and

poor cellular permeability that limited their use and halted further development. Since then various modifications of the AA aminoacid location have been studied, producing intrinsically active agents as well as their usually methyl esterified prodrugs.

BZA-5B is such a peptidomimetic methylester prodrug inhibitor of farnesyl transferase. Here the two aliphatic amino acids AA have been replaced by a benzodiazepine-based mimic of a peptide turn. *In vitro* BZA-5B is extremely potent with a higher affinity for farnesyl transferase than for geranylgeranyl transferase. Functional studies have shown that BZA-5B and other related benzodiazepine peptidomimetics, after cell entry, can block the addition of farnesyl to Ras and other proteins, resulting in normalisation of cell morphology in H-*ras* transformed Rat-1 cells, but not in untransformed Rat-1 cells [20,21].

B581 is a derivative of CVFM with increased stability and cellular penetration. It inhibits farnesylation of H-Ras and lamin A, but it does not inhibit geranylgeranylation of other cellular proteins [22]. Functional studies in H-*ras* transformed NIH3T3 models have shown growth inhibition [23].

B956 is another derivative of CVFM with structural similarity to B581 [24]. It showed broad-spectrum activity in a range of tumour cell lines expressing H-*ras*, and N-*ras* mutations, and somewhat less activity in those expressing K-*ras* mutations [25]. B1086, the methylester prodrug of B956, inhibited the growth in H-*ras* (EJ-1 human bladder carcinoma), N-*ras* (HT-1080 fibrosarcoma), and K-*ras* (HCT 116 colon carcinoma) models, respectively [24]. In addition, B1086 was found to induce a near normalisation of malignancy associated hypercalcemia and elevated PTH-related peptide (PTHrP) levels in the serum of BALB/c/nu/nu mice bearing Ras–3T3 tumours [26].

L-731,735 and its methylester prodrug L-731,734 are structural derivatives of CIIM [27-29]. Due to a masked carboxylate charge, L-731,734 showed superior cellular penetration, although its intrinsic inhibition of farnesyl transferase was less than that of L-731,735. Modest potency and limited chemical stability halted further development of both compounds. Structural modifications of L-731,735, however, resulted in more stable and active compounds, such as L-739,750 and its methylester prodrug L-739,749. These compounds are structural derivatives of the

tetrapeptide CIVM and have a more than 1000-fold higher potency for inhibiting farnesyl transferase than geranylgeranyl transferase I and II [4,28]. Interestingly, these agents equally inhibited growth of cells containing H-, N-, or K-*ras* mutations. Growth of v-*raf* or v-*mos* transformed cells, however, was not inhibited, indicating the specificity of target farnesylation being inhibited by these compounds. Growth of various *ras*-dependent tumours in nude mice was inhibited in a dose dependent way, again with similarity in activity against tumours depending on different subtypes of mutated *ras*. At active doses, there were no signs of treatment-related toxicity, and post-mortem examination of rapidly dividing tissue revealed no treatment related abnormalities [28]. Cultured *ras* transformed cells incubated with L-739,749 showed massive DNA degradation and apoptosis within 24 hours after initiation of treatment if attachment to substratum was prevented [30]. A single exposure of *ras* transformed fibroblasts to L-739,749 caused reversible morphological reversion, possibly by interference with Rho B, the protein responsible for cytoskeletal actin organisation, whereas repeated exposure caused inhibition of growth [31]. Rho B is amongst the cellular proteins which are both farnesylated and geranylgeranylated [32, 33].

L-744,832 is the isopropyl ester prodrug of L-739,750 and showed dose dependent growth inhibitory effects in a number of human tumour cell lines, irrespective of *ras* mutational status. Cell lines with wild-type *ras* and active protein tyrosine kinases, such as breast, prostate and small cell lung cancer cells, were especially sensitive [34]. In the MMTV-v-H-*ras* transgenic mouse model harbouring mutated H-*ras*, animals spontaneously develop mammary and salivary tumours. Daily subcutaneous administration of L-744,832 resulted in a dose dependent reversible regression of these tumours. When treatment was interrupted, the tumours reappeared, but again regressed when treatment was restarted [35]. This observation suggests that such an agent needs to be given for a prolonged period of time, and that treatment should be continued, even after total disappearance of the tumour. Unlike L-744,832, many inhibitors of farnesyl transferase induce growth arrest rather than tumour regression. This finding has important consequences for the development of clinical studies with these agents. In transgenic mice harbouring mammary and lymphoid tumours overexpressing N-*ras* oncogenes, treatment with L-744,832 significantly inhibited the growth rate of these tumours [36]. Taken together with the above mentioned studies

in H-*ras* models, there thus might be different sensitivity of tumours related to the different *ras* subtypes. Again, this has important consequences for potential clinical studies. L-744,832 inhibited the proliferation of astrocytoma cell lines, although these cells do not contain mutated *ras*, and in MMTV-TGFα transgenic mice and MMTV-TGFα/*neu* mice, harbouring activated receptor tyrosine kinases, mammary tumours regressed in size during treatment with subcutaneously administered L-744,832, suggesting that tumours containing activated receptor tyrosine kinase signalling pathways, irrespective of *ras* mutational status, are sensitive for farnesyl transferase inhibitors [37, 38]. Apparently, antitumour effects are possible in *ras* independent models, suggesting potentially different and yet unknown mechanisms of action.
L-778,123 is another peptidomimetic farnesyl transferase inhibitor with excellent *in vitro* target inhibition and growth inhibitory activity in either mutated H-*ras* and K-*ras* containing cell lines. In xenograft models, L-778,123 inhibited the growth of tumours containing either mutated H-*ras* or K-*ras* fibroblasts.

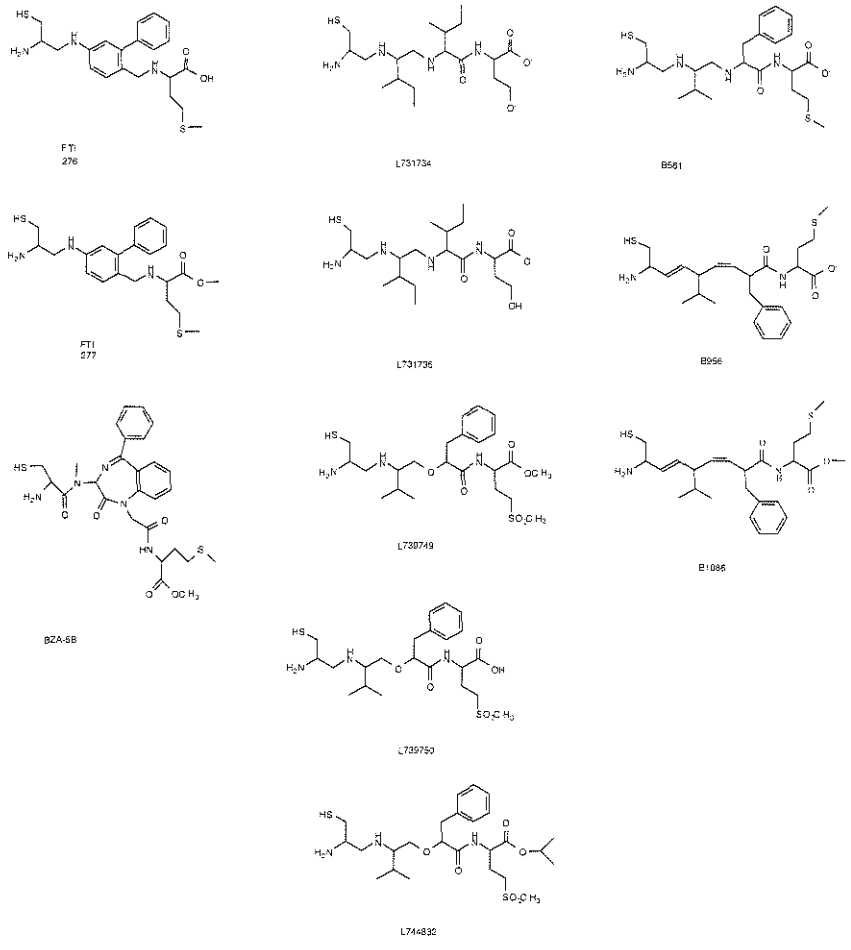
FTI-232 and its carboxyl methylated prodrug FTI-244, and FTI-276 and its carboxyl methylester prodrug FTI-277 are derivatives of CVIM [39, 40]. FTI-276 and FTI-277 have a high specificity for farnesyl transferase compared to geranylgeranyl transferase I, but FTI-276 contains a reactive thiol group that was found to non-selectively modify thiol containing proteins, which compromised its activity [41]. Although FTI-277 is a less potent inhibitor of farnesyl transferase than FTI-276 *in vitro*, increased cellular penetration resulted in better inhibition of Ras processing in whole cells. Both compounds were equally effective in blocking tumour growth in a K-*ras* mutated Calu-1 human lung carcinoma xenograft in a dose dependent way. The antitumour effect of FTI-276 was similar to that of FTI-277, indicating that the latter is converted to FTI-276 intracellularly before reaching its target, farnesyl transferase. The antitumour effect of FTI-276 was proven to be *ras* dependent and *ras* specific, whereas the compound also demonstrated a possible preventive effect on tumorigenesis [40, 42].

FTI-2148 and its prodrug FTI-2153 are non-thiol CAAX peptidominetics also derived from FTI-276. Importantly, in xenografts combining FTI-2148 with either cisplatin, paclitaxel, or gemcitabine resulted in increased growth inhibitory effects

[43]. This observation has also been made with other inhibitors of farnesyl transferase, and this is of great relevance for potential clinical development [44].

An overview of the chemical structures of most of the thus far developed peptide CAAX peptidomimetics is given in figure 5.

Figure 5 peptide CAAX peptidomimetics



Nonpeptide CAAX peptidomimetics

The 8-chlorobenzocycloheptapyridines are a group of non-peptidic non-sulphydryl tricyclic selective inhibitors that were identified as a result of a random screening of a group of antihistaminics.

SCH 44342 was the first compound of this group. It had a high specificity for farnesyl transferase over geranylgeranyl transferase I, and could block Ras-induced morphological changes of malignant cells *in vitro*. Unfortunately, *in vivo* antitumour activity was poor [45, 46].

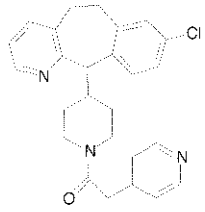
SCH 59228 and the tricyclic halogeneted SCH 66336 have shown good oral bioavailability and antitumour activity, including tumour regression achieved by SCH 66336 [47-50]. Interestingly, the growth inhibitory effects of SCH 66336 appear to be at least partly independent of *ras*-mutational status. This agent prevented the occurrence of newly formed tumours and is one of the few farnesyl transferase inhibitors inducing tumour size reduction in animal models in a dose dependent way [50]. In addition, in many models the effect of SCH 66336 was additive to the effect of cytotoxic agents such as 5-FU, vincristine, cytoxan, and paclitaxel [50, 51].

R115777 is an oral imidazole antifungal derived farnesyl transferase inhibitor with high enzyme specificity and interesting levels of growth inhibition [52, 53]. In several models, the combination of R115777 with cytotoxic agents such as cisplatin and paclitaxel induced additional antitumour effects, although the addition of R115777 to irinotecan failed to enhance the antitumour effect of this topoisomerase inhibitor [54, 55].

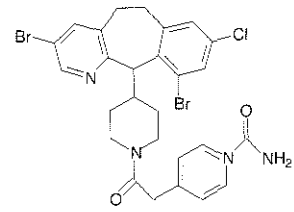
BMS 214662 is an example of a new class of non-peptide imidazol farnesyl transferase inhibitors, showing high affinity for farnesyl transferase over geranylgeranyl transferase and showing complete tumour regressions in various tumor xenograft models after both oral and intraperitoneal administration. This compound has recently entered clinical studies.

An overview of the chemical structures of most of the thus far developed non-peptide CAAX peptidomimetics is given in figure 6.

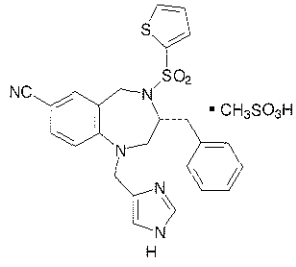
Figure 6 Non-peptide CAAX peptidomimetics



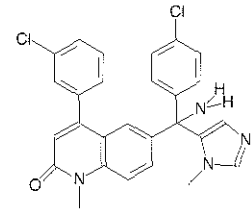
SCH 44342



SCH 66336



BMS-214662-05



R115777

Bisubstrate inhibitors

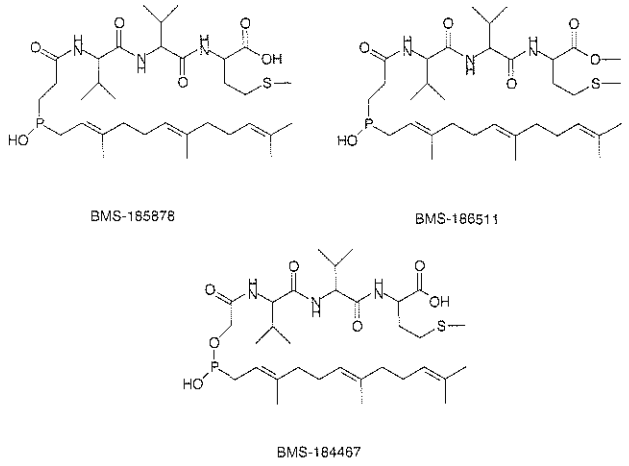
Bisubstrate inhibitors of farnesyl transferase combine the features of FPP analogues and non-peptide CAAX peptidomimetics. The chemical structure of these compounds, containing a phosphonyl or phosphinyl linkage, combines the structure of FPP with that of a non-sulfhydryl CAAX tetrapeptide. Compound 3, having a amide linker between the FPP surrogate and the farnesylgroup and its subsequently developed analogues compound -14, -15, and -16 all show selectivity for farnesyl transferase over geranygeranyl transferase, as well as growth inhibitory effects [56, 57].

BMS 185878 and BMS 184467 are phosphinate and phosphonate bisubstrate inhibitors, respectively. Although *in vitro* inhibitory effects on farnesyl transferase were seen, activity in whole cells was limited, presumably due to poor cellular permeability. BMS 186511, the methyl carboxyl prodrug of BMS 185878, however, showed good cellular activity and a 2000-fold higher affinity for farnesyl transferase over geranylgeranyl transferase I. K-*ras* transformed cells were less sensitive for growth inhibition, whereas untransformed cells were not affected. Cytotoxic effects

were not seen [58]. As yet, *in vivo* or clinical results of bisubstrate inhibitors have not been published.

An overview of the chemical structures of most of the thus far developed bisubstrate inhibitors is given in figure 7.

Figure 7 Bisubstrate inhibitors



CLINICAL STUDIES

Clinical studies with FPP analogues have not at all been performed, and clinical studies with bisubstrate inhibitors have not yet been published. The only clinical study with a peptide CAAX peptidomimetic that has yet been published is a phase I study with L-778,123 [59]. Results of this study are summarised in table 2. The drug was administered to 22 patients as a 7-days continuous infusion every three weeks. Dose levels ranged from 35-1120 mg/m^2. There was dose dependent neutropenia, and thrombocytopenia comprised dose limiting toxicity (DLT). Non-haematological toxicity consisted of fatigue and asymptomatic QTc prolongation. The recommended dose for further efficacy testing using the above mentioned dosing

regimen was set at 560 mg/m^2. At this dose, side-effects were only mild and consisted of myelosuppression, nausea and vomiting, somnolence and fatigue. At the recommended dose, steady state plasma concentrations averaged 10 μM, thereby exceeding concentrations producing antitumour activity in preclinical studies.

Several phase I studies with non-peptidomimetic inhibitors of farnesyl transferase have been performed. Four studies involved oral SCH 66336 [60-63]. Results of these studies are summarised in table 2. In these studies, different dosing regimens were used, and three studies used a BID-dosing regimen with flat doses. With 7-days dosing repeated every three weeks, DLT was seen at 400 mg BID and consisted of fatigue, nausea, vomiting and diarrhoea [60]. Extending the dosing period to 14 days repeated every 4 weeks, again DLT was seen at 400 mg BID and consisted of nausea, vomiting and fatigue [62]. Finally, when using a continuous treatment schedule, DLT was also seen at 400 mg BID with similar side-effects as reported above. After decreasing the dose in this study, DLT was again seen at 300 mg BID and consisted of myelosuppression, renal, and neurocortical toxicity consisting of rapidly reversible disorientation and confusion [61]. In the first of these studies the recommended dose for subsequent studies was determined at 350 mg BID, while in the subsequent two studies this was 200 mg BID. The study analysing the continuous BID treatment schedule was extended to study continuous once daily (OD) dosing. Again DLT, consisting of fatigue, nausea, vomiting and renal toxicity was seen at 400 mg [63]. The recommended dose for subsequent studies using the continuous OD treatment schedule was determined at 300 mg, but this total daily dose is lower than the daily dose recommended with continuous twice daily administration (200 mg BID). The four phase I studies on SCH 66336 suggest that toxicity of this compound is related to peak plasma levels rather than overall drug exposure and is independent of the dosing regimen used. Since in preclinical studies optimal antitumour efficacy was seen with continuous dosing, and taking into account the toxicity data in humans, it is obvious that the continuous daily administration schedule is recommended for further studies. Because of the total dose consideration, this should involve the BID dosing.

Table 2 Clinical studies of farnesyl transferase inhibitors: side effects at recommended dose levels

Drug (ref)	Route of admin.	Schedule	No. Pts	Recommended dose (mg)	Side effects at recommended dose
L-778,123 (59)	i.v.	d 1-7 q 3 weeks	22	560 (m^2)	ANC, plts, N/V somnolence, fatigue
SCH 66336 (60)	p.o./BID	d 1-7 q 3 weeks	14	350	N/V, diarrhea, fatigue
SCH 66336 (61)	p.o./BID	Continuous	24	200	N/V, diarrhea
SCH 66336 (62)	p.o./BID	d 1-14 q 4 weeks	21	200	N/V, diarrhea, fatigue
SCH 66336 (63)	p.o./OD	Continuous	12	300	Diarrhea, N/V, renal, fatigue
R115777 (64)	p.o./BID	d 1-5 q 2 weeks	27	?	?
R115777 (65)	p.o./BID	d 1-21 q 4 weeks	12	240 (m^2)	ANC, plts, fatigue, confusion
R115777 (66)	p.o./BID	Continuous	16	300	Skin, ANC, plts, fatigue, N/V, neuro, dizziness

Route of admin. denotes route of administration. i.v. denotes intravenously, p.o. denotes orally
BID denotes twice daily, OD denotes once daily; QTc denotes asymtomatic QTc prolongation at ECG
ANC denotes absolute neutrophile count, plts denotes platelets; N/V denotes nausea and vomiting

Pharmacokinetic analysis in studies on SCH 66336 showed slow absorption with peak plasma levels approximately 1.5-12 hours post-dose, a large volume of distribution at steady state (85-461 L), and drug exposure that increased in a more than dose proportional way. Obviously, the latter is reason for some concern, but no signs of cumulative toxicity were seen in the continuous BID dosing study where two patients were given the drug for 7 and 9 months, respectively [61]. At the recommended dose in the continuous BID dosing study, plasma levels exceeded >1.5 μM which is well above the *in vitro* inhibitory concentrations of H-*ras* (1.9 nM) and K-*ras* (5.2 nM) farnesylation. In the four phase I studies, one partial response was seen in a patient with NSCLC [60]. To some extend this confirms preclinical observations that the drug is able to induce tumour regressions.

Oral R115777 has been studied in several phase I studies. The results of these studies are also summarised in table 2. The first study used a 5-days BID flat dose dosing regimen every 2 weeks. DLT was seen at 1300 mg and consisted of neuropathy and fatigue, whereas other side-effects consisted of nausea, vomiting, headache and hypotension. Myelosuppression was mild and infrequent [64]. A second study involved a BID dosing regimen for 21 days every 28 days and yielded myelosuppression, fatigue, confusion and bilirubinemia as DLT at 420 mg/m^2. The maximum tolerable dose using this regimen was set at 240 mg/m^2 [65]. It is unclear why in this study a dosing in mg/m^2 was used, rather than the flat dosing used in the other studies with this agent. In a third study using continuous oral flat BID dosing, DLT was first seen at 500 mg BID and consisted of febrile grade 4 neutropenia with grade 3 thrombocytopenia in one patient, and grade 3 peripheral neurosensory and neuromotor toxicity in another patient. After reducing the dose to 400 mg BID, again DLT was seen consisting of grade 3 neutropenia in one patient and febrile grade 4 neutropenia in another patient. The maximum tolerable dose was set at 300 mg BID, with mild myelosuppression, nausea, vomiting, neuropathy, dizziness, fatigue and skin toxicity as side-effects [66]. R115777 pharmacokinetics showed rapid absorption (peak plasma concentrations 0.5-4 hours post dose) and a dose proportional drug exposure, albeit with significant interpatient variability and some accumulation at the higher dose levels. There were no signs of cumulative toxicity after prolonged periods of dosing. Plasma levels reached at tolerable doses were well in the range of levels

showing *in vitro* inhibitory activity. In this study one partial remission was achieved in a patient with non-small cell lung cancer treated at the highest dose, and 2 patients with colorectal cancer had stable disease with a 50% decrease in the concentration of their CEA [66].

In summary, the toxicity profiles of L-778,123, SCH 66336, and R115777 are largely comparable, although some differences exist. An overview of these side-effects is presented in table 3. At the doses recommended for subsequent activity studies, side-effects of all three compounds were mild and reversible. Whereas L-778,123 had to be administered as inconvenient continuous infusion, both SCH 66336 and R115777 could be administered orally, which might facilitate their future development. At the recommended dose, all three agents yielded plasma concentrations well above the IC_{50} of *in vitro* models.

Table 3 Clinical studies of farnesyl transferase inhibitors: Toxicity results

Drug	ANC	Plts	Fatigue	Nausea	Vomiting	Diarrhea	Neurotoxicity	Confusion
L-778,123	+	+	+	+	+	-	-	-
R115777	+	+	+	+	+	-	+	+
SCH 66336	+	+	+	+	+	+	+	+

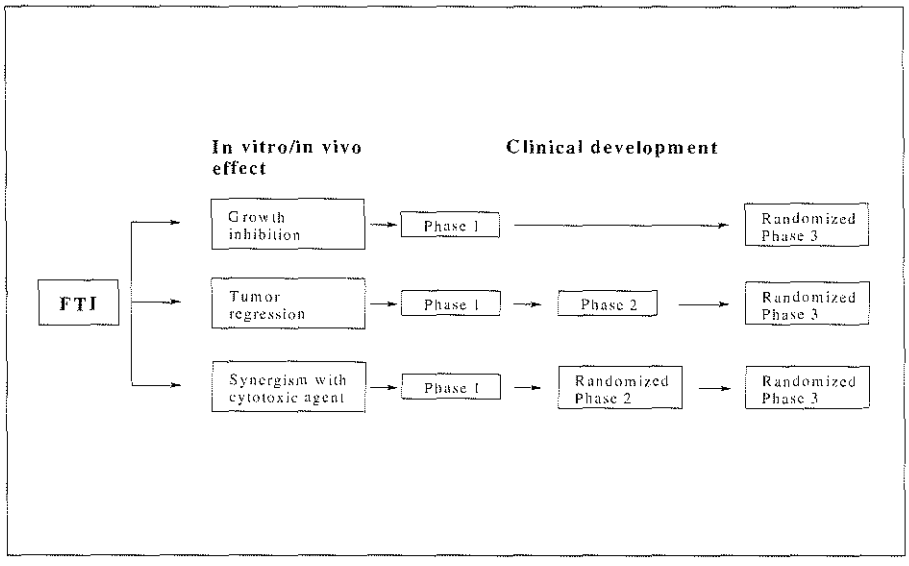
ANC denotes absolute neutrophile count
plts denotes platelets

FUTURE PERSPECTIVES

The question presently is how to proceed with the development of clinical studies with this new group of anti cancer agents. As stated previously, most farnesyl transferase inhibitors tend to be cytostatic rather than cytotoxic, and despite the fact that some preclinical studies showed tumour regressions [35, 40, 50], inhibition of tumour growth is the more rational scenario. Whereas in phase II studies of conventional cytotoxic agents the percentage or amount of tumour regressions in the

population studied can be determined, growth inhibition as endpoint of antitumour activity cannot be properly assessed in non-randomised single-agent phase II studies of cytostatic agents. Therefore, these studies seem senseless to perform as they will very likely underestimate potential antitumour activity (figure 8).

Figure 8 Clinical trial design



FTI denotes farnesyl transferase inhibitors.

When designing phase II studies for cytostatic anticancer agents, surrogate endpoints of antitumour activity should be defined to replace tumour regression. These proposed surrogate endpoints are summarised in table 4. Initiating randomised phase II studies using time to progression (TTP) or the proportion of patients surviving at a certain predefined timepoint as surrogate endpoint of antitumour activity could be conceivable. In order to obtain evaluable results within a limited timeframe, and thus to limit the number of patients to be included in these

phase II studies, they should ideally be performed in patients with tumortypes known to harbour mutated *ras* oncogenes and with a high likelihood of rapid tumour progression or disease recurrence after initial cytoreductive therapy. Pancreatic adenocarcinomas could serve this goal. For the design of randomised phase II studies for this group of patients, several alternatives exist; in the first single-agent alternative, patients could be randomised either to receive an active reference agent, for example gemcitabine, or the farnesyl transferase inhibitor. Alternatively, all patients are initially being treated with a farnesyl transferase inhibitor for a predefined period of time, and patients without disease progression after this period will subsequently be randomised either to continue treatment or to receive no further or placebo treatment. In this last situation, the study could be double-blinded. The difference between median TTP or the difference in, for example, 1-year survival rate between the two groups could be used as surrogate endpoint for the assessment of antitumour activity. These studies should be sufficiently powered to detect meaningful differences in these outcomes of activity. As yet, this design for the analysis of antitumour activity of cytostatic agents still has to be fully validated. Alternatively, one could design a randomised phase II study in which antitumour activity of the combination of a farnesyl transferase inhibitor with a cytotoxic agent is compared to that of the cytotoxic agent alone, again using the surrogate endpoints of antitumour activity mentioned above. In the case of metastatic colorectal cancer, for example, cytotoxic treatment with 5-FU/leucovorin, raltitrexed or irinotecan could be combined either with a farnesyl transferase inhibitor or a placebo to compare TTP. In this respect it is of importance that preclinical studies of SCH 66336 and R115777 have shown enhanced antitumour efficacy with various cytotoxic agents [50, 54, 55]. Preliminary results of the first clinical phase I study on the combination of continuous oral BID dosing of R115777 with bi-weekly 5-FU and leucovorin according to the so-called de Gramont regimen in patients with advanced colorectal or pancreatic carcinoma have already been presented [67].

Table 4 Surrogate endpoints in clinical studies of cytostatic anticancer agents

Phase I studies	**Phase II studies**
Optimal biologic effect dose	*Antitumour activity*
Target AUC	Time to progression
Target inhibitory concentration	(1-year) survival rate
Inhibition of cellular target activity	Tumour marker inhibition
Inhibition of tumour marker	

In addition to these clinically defined surrogate endpoints of antitumour activity, one could also analyse plasma concentrations of relevant tumour markers such as CA 19.9 for pancreatic adenocarcinoma, PSA for prostatic carcinoma, or CEA for colorectal adenocarcinoma to determine hints of antitumour activity. A decrease in plasma concentration of these markers could, although with much caution, be interpreted as indicator of antitumour activity, even in the absence of measurable or evaluable tumour regression. The analyses of these surrogate endpoints of antitumour activity are becoming increasingly important in early clinical studies of cytostatic agents that sometimes don't yield dose limiting toxicity.

Pharmacodynamic analysis by measuring inhibition of the target enzyme in readily available cells or tissues could also serve as surrogate endpoint for the assessment of antitumour activity. In the phase I study on L-778,123, this analysis revealed inhibition of prenylation of a marker protein in peripheral blood mononuclear cells [59], whereas preliminary results from pharmacodynamic analyses on SCH 66336 have shown inhibition of the farnesylation of prelamin A in oral buccal mucosa cells [60, 63]. Performing consecutive tumour biopsies will usually not be possible in clinical studies, and indirect measurements of decreased tumour proliferative activity through new methods like PET-scanning or dynamic MRI-angiography have yet to be validated.

Finally, in randomised phase III studies, the role of farnesyl transferase inhibitors administered for prolonged periods of time to larger groups of patients could be assessed with respect to TTP, disease free and overall survival. These studies in general will require a long observation time.

Whether the onset of *ras* oncogen dependent and independent tumours in high-risk patients can be effectively prevented with the prophylactic use farnesyl transferase inhibitors is unknown, although scarce preclinical data on this issue exist [50].

CONCLUSIONS

Ras oncogenes are found in many frequently occurring human tumour types. Cellular activation of the Ras oncoproteins through the process of farnesylation plays an important role in the malignant transformation of cells and tumour growth. Therefore, specific inhibition of farnesylation of Ras oncoprotein is an attractive target in anticancer treatment. Numerous specific inhibitors of farnesyl transferase showing *in vitro* and *in vivo* activity have been developed in recent years. Most of these agents are cytostatic in animal models and devoid of severe side-effects. Recently, results of clinical phase I and pharmacological studies with three different inhibitors of farnesyl transferase have been presented. DLT was recorded in all clinical studies, with a recognisable toxicity pattern. At tolerable doses, plasma concentrations able to inhibit the target enzyme were obtained, and specific target inhibitory activity was demonstrated. Taking into account the cytostatic mode of action of these compounds, specifically designed randomised phase II and III studies should be performed to more precisely determine activity and clinical valuability.

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CHAPTER 7

PHASE I AND PHARMACOKINETIC STUDY OF THE ORAL FARNESYL TRANSFERASE INHIBITOR SCH 66336 GIVEN BID TO PATIENTS WITH ADVANCED SOLID TUMORS.

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ABSTRACT

Purpose: A single-agent dose-escalating phase I and pharmacokinetic study on the farnesyl transferase inhibitor SCH 66336 was performed to determine safety profile, maximum tolerated dose, and recommended dose for phase II studies. Plasma and urine pharmacokinetics were determined.

Patients and methods: SCH 66336 was given orally BID without interruption to patients with histologically or cytologically confirmed solid tumors. Routine anti-emetics were not prescribed.

Results: 24 patients were enrolled into the study. Dose levels studied were 25, 50, 100, 200, 400, and 300 mg BID. Pharmacokinetic sampling was performed at days 1 and 15. At 400 mg BID dose limiting toxicity (DLT) consisted of grade 4 vomiting, grade 4 neutropenia and thrombocytopenia, and the combination of grade 3 anorexia and diarrhea with reversible grade 3 plasma creatinine elevation. Following dose reduction, at 300 mg BID again DLT was recorded consisting of grade 4 neutropenia, grade 3 neurocortical toxicity, and the combination of grade 3 fatigue with grade 2 nausea and diarrhea. The recommended dose for phase II studies is 200 mg BID, which was found feasible for prolonged periods of time. Pharmacokinetic analysis showed a greater than dose-proportional rise in drug exposure and peak plasma concentrations, with increased parameters at day 15 compared to day 1, indicating some accumulation upon multiple dosing. Plasma half-life ranged from 4-11 hours and appeared to increase with increasing doses. Steady state plasma concentrations were attained at day 7-14. A large volume of distribution at steady state indicated extensive distribution outside the plasma compartment.

Conclusion: SCH 66336 can be safely administered using a continuous oral BID dosing regimen. The recommended dose for phase II studies using this regimen is 200 mg BID.

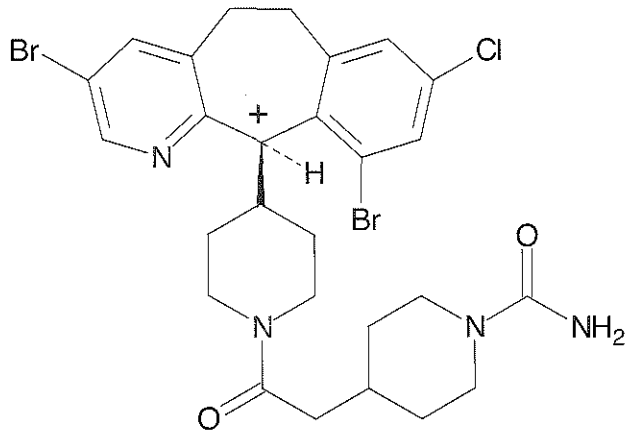
INTRODUCTION

In mammalian cells three functional *ras* genes are found. K-*ras*, N-*ras*, and H-*ras* genes encode for K-Ras, N-Ras, and H-Ras proteins, respectively. Ras is synthesized as a soluble and biologically inactive protein that undergoes several posttranslational modifications before being localized to the inner surface of the plasma membrane where it exerts its activity as transducer of various extracellular growth-promoting stimuli. An essential step in the posttranslational processing of Ras is farnesylation, the addition of a farnesyl or C_{15} isoprenoid moiety from farnesyl diphosphate to the cysteine residue at the C-terminal side of Ras. Farnesyl transferase is the crucial enzyme in this process [1-10]. Mutations in one or more *ras* genes are frequently found in various human tumor types in variable incidence [3,8,10]. Mutated *ras* oncogenes encode for oncoproteins that are synthesized in a way completely comparable to the synthesis of normal Ras. However, Ras oncoproteins are insensitive to the inhibitory activity of GTP-ase activating protein (GAP). As a result, cells harboring these Ras oncoproteins will show autonomous proliferation and malignant transformation.

As farnesylation of Ras oncoproteins is the essential enzymatic step in the process of posttranslational activation, inhibiting this step could theoretically result in the inhibition of this autonomous and malignant growth and proliferation. Thus specific inhibitors of farnesyl transferase could possibly lead the way towards a specifically targeted treatment of *ras* oncogene dependent tumors. Recently, however, evidence has emerged that the antiproliferative effects of farnesyl transferase inhibitors do not depend solely on inhibition of Ras, and that the gain of alternate prenylated (geranylgeranylated) forms of the *Rho* protein *Rho-B* mediate cell growth inhibition [11]. Besides, when inhibiting farnesylation, it has to be taken into account that this process is not restricted to Ras, as other cellular proteins also have to be farnesylated before exerting their activity [3].

Several specific inhibitors of farnesyl transferase have been developed. SCH 66336 ((11R) 4[2[4-(3,10-dibromo-8-chloro-6, 11-dihydro-5H-benzo[5,6] cyclohepta [1,2b]pyridin-11yl]-1-pyperazinyl]-2-oxoethyl]-1-piperidinecarboxamide) (figure 1) is a tricyclic nonpeptidyl, non-sulphydryl farnesyl transferase inhibitor (FTI).

Figure 1 Chemical structure of SCH 66336



In vitro it blocks farnesylation of H-Ras by purified human farnesyl protein transferase with an IC_{50} of 1.9 nM and farnesylation of K-Ras-4B with an IC_{50} of 5.2 nM. SCH 66336 blocks anchorage independent growth of K-Ras transformed rodent fibroblasts with an IC_{50} of 0.4 μM and blocks the transformed growth properties (e.g. anchorage independent growth) of rodent fibroblasts that have been transformed with mutant *ras* and human tumor cell lines containing mutated *ras* [12,13]. It does not inhibit geranylgeranyl protein transferase I in concentrations up to 50 μM. Anchorage independent growth of various mutated K-*ras* containing human tumor cell lines, like HTB 177 lung carcinoma, A549 lung carcinoma, HCT 116 colon carcinoma, and HPAF II and MiaPaCa pancreatic carcinoma is inhibited by SCH 66336 at concentrations of 0.5 μM, whereas the growth of DLD-1 colon carcinoma cell line is inhibited at 3 μM. Interestingly, several human tumor cell lines not containing *ras* mutations, like HTB 173 and HTB 175 lung carcinoma and MCF-7 breast carcinoma, are also sensitive to the growth inhibitory effects of SCH 66336. This might be explained in part by the action of oncogenes or autocrine factors that lie upstream in the Ras signal transduction pathway. In *in vivo* studies SCH 66336 showed growth inhibitory effects in human tumor xenografts, including DLD-1 and HCT 16 colon

carcinoma, A549 and HTB 177 lung carcinoma, AsPc-1, HPAF-II, HS 700T and MiaPaCa pancreas carcinoma, and DU 145 prostate carcinoma. Additionally, in a WAP-H-*ras* transgenic mouse model developing tumors of the mammary and salivary gland, dose-dependent tumor regressions have been recorded [14]. Preclinical chronic oral toxicity studies revealed dose-dependent myelosuppression, weight loss, diarrhea, and vomiting in rats and monkeys (Schering Plough Research Institute, data on file).

This phase I and pharmacokinetic study represents the first administration of SCH 66336 in patients with advanced solid tumors using a continuous twice daily oral dosing regimen.

MATERIAL AND METHODS

Eligibility criteria

Patients with a cytologically or histologically confirmed diagnosis of a solid tumor refractory to standard treatment or for whom no standard therapy was available were eligible for this study. Patients with primary central nervous system neoplasm, known brain- or leptomeningeal metastases, or known bone marrow involvement were excluded. Further eligibility criteria included: age $\geq$18 years; WHO performance status $\leq$2; life expectancy of $\geq$12 weeks; no anticancer therapy in the previous 4 weeks (6 weeks for nitrosoureas or mitomycin-C); no prior bone marrow or stem cell transplantation; no known HIV positivity or AIDS related illness; adequate function of bone marrow (hemoglobin $\geq$6.2 mmol/l, absolute neutrophil count $\geq 1.5.10^9$/l, platelets $\geq 100.10^9$/l), liver (bilirubin $\leq$25 µmol/l, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) within 2.5 times the normal upper limit), and kidney (serum creatinine $\leq$140 µmol/l); patients had to be able to take oral medication; patients were not allowed to have received more than 2 prior combination chemotherapy regimens or 1 prior combination regimen plus 2 single agent regimens.

Local ethics boards approved the protocol and informed consent brochures. All patients gave written informed consent at study entry.

Pretreatment assessment and follow-up studies

Prior to therapy, a complete medical history was taken and a physical examination was performed. A complete blood count (CBC), including white blood cell (WBC) differential, and serum chemistry including sodium, potassium, calcium, magnesium, phosphorus, urea, uric acid, creatinine, total protein, albumin, glucose, alkaline phosphatase, bilirubin, AST, ALT, γ-glutamyl transpeptidase and lactate dehydrogenase were performed, as were urinalysis, electrocardiogram (ECG) and chest X-ray. Because some visual proteins (*i.e.* rhodopsin kinase and transducin γ) are known to undergo farnesylation, patients were referred for ophthalmologic examination including retinal photography prior to treatment, after 4 and 8 weeks, and bimonthly thereafter. Weekly evaluations included history, physical examination, toxicity assessment according to the NCI-CTC, version date December 1994, CBC, serum chemistries, urinalysis and ECG. Tumor measurements were performed before treatment, at 4 and 8 weeks, and bimonthly thereafter and were evaluated according to the WHO criteria for response [15]. In case of progressive disease, patients were taken off study.

Drug and drug administration

SCH 66336 ((11R) 4[2[4-(3,10-dibromo-8-chloro-6, 11-dihydro-5H-benzo[5,6] cyclohepta[1,2b]pyridin-11yl]-1-pyperazinyl]-2-oxoethyl]-1-piperidinecarboxamide) is a crystalline solid containing one chiral center. It was supplied as 25, 100, and 200 mg blue opaque gelatin capsules by Schering-Plough Research Institute, Kenilworth, New Jersey, USA. The capsules were swallowed immediately after breakfast and after supper, with approximately 240 ml of non-carbonated water. On days of pharmacokinetic sampling, patients were administered standardized meals immediately prior to drug administration. SCH 66336 was taken for 28 consecutive days and was continued in case of stable disease or disease remission after this period for as long as no disease progression and/or no unacceptable drug-related toxicity was seen. Routine anti-emetics were not prescribed. SCH 66336 administration was immediately interrupted at the occurrence of dose limiting toxicity (DLT).

Dose and dose escalation

The starting dose of SCH 66336 was 25 mg BID. This dose was based upon the safety results of the 15-mg/kg/day dose in 3-month toxicology studies in monkeys. Although this was not a 'no effect dose', the only findings in monkeys were increased liver weight.

At the first day of treatment, patients were given a single dose for pharmacokinetic purposes.

Dose escalation was performed according to a schedule of dose doublings. At each dose level, a minimum of three patients had to have 28 days of treatment before escalation was allowed. Once DLT was seen in one patient at a given dose level, at least 6 patients had to be treated at that dose level before further dose escalation was allowed. DLT was defined as any grade $\geq$3 non-hematological toxicity, or a serum creatinine elevation of $\geq$3 times upper limit of normal. Grade 3 fever in absence of infection or grade 3 nausea or vomiting in patients not receiving adequate antiemetic treatment were not considered DLT. Neutropenia or thrombocytopenia grade $\geq$3 or grade 4 anemia constituted hematological DLT. The maximum tolerated dose (MTD) was defined as the highest dose to be administered to a group of 6 patients producing tolerable, manageable and reversible but dose limiting toxicity in at least 2 out of 6 patients. At the proposed dose for phase II studies, a maximum of 1 out of 6 patients was allowed to experience DLT. No intrapatient dose escalation was allowed.

Pharmacokinetic studies

For pharmacokinetic analysis, six-ml blood samples were taken on day 1 via an intravenous cannula prior to administration, at 30, 60 and 90 minutes, and at 2, 4, 6, 8, 12, 14 and 24 hours post-dose. On day 14, a blood sample was taken prior to the evening dose, on day 15 blood samples were taken prior to morning dosing, at 30, 60, and 90 minutes, and at 2, 4, 6, 8, 12 hours post-dose, the last sample to be taken before the evening dose. On day 16, a sample was taken prior to the morning dose. If patients were on treatment after three 28-day cycles, optional pharmacokinetic blood samples were again obtained. Blood samples were collected in sodium heparin tubes and were immediately centrifuged at 3000 rpm for 15

minutes at 10^0 C, after which plasma was divided into 2 aliquots of at least 1 ml and frozen at -70^0 C until analysis. Plasma samples were assayed by a specific and sensitive high-performance liquid chromatography (HPLC) assay [16]. The lower limit of quantitation of the assay was 1.0 ng/ml. SCH 66336 excretion in urine was measured on day 15 in urine samples collected from 0-6 and 6-12 hours post-dose. Urine samples were analyzed using the same validated HPLC assay. For urine analysis the lower limit of quantitation was 2.0 ng/ml.

For each patient, the area under the plasma concentration versus time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity by linear regression analysis. The apparent total body clearance (CL/F) (F denotes oral bioavailibility fraction) was calculated as dose/AUC. The apparent volume of distribution at steady state ($V_{d,ss}/F$) was calculated by a noncompartmental method based on the statistical moment theory [17]. The terminal disposition half-life ($T_{1/2}$) was calculated by dividing 0.693 by the fitted rate constant for drug elimination from the central compartment, estimated by linear-regression analysis of the final data points of the log-linear concentration-time plot.

Statistical analysis

Interpatient differences in pharmacokinetic parameters were assessed by the coefficient of variation, expressed as the ratio of the standard deviation and the observed mean. Pharmacokinetic parameters were analyzed as a function of the SCH 66336 dose level using the Kruskal-Wallis' one-way analysis of ranks followed by the Dunn's multiple comparison test for identifying statistically different groups. Variability in pharmacokinetics between administration days was evaluated by the paired Student's *t*-test after testing for normality and heteroscedasticity, or the Wilcoxon's test for matched pairs. Statistical calculations were performed using the Number Cruncher Statistical System 5.X series (J.L. Hintze, East Kaysville, UT, 1992). Statistical significance was considered to be reached when $P<0.05$, with a two-tailed distribution. All data are presented as mean ± standard deviation except where indicated otherwise.

RESULTS

Twenty-four patients were enrolled into the study. The patient characteristics are summarized in table 1. The median duration of treatment was 40 days (range 5-280, mean 63.4 days). Dose levels studied were 25 (n=4), 50 (n=5), 100 (n=3), 200 (n=6), 400 (n=3) and 300 (n=3) mg BID.

Table 1 Patient characteristics

No of patients entered	24
No of patients evaluable	24
Male / female	14/10
Median age (years)	56.5
Range	28-77
Median WHO performance status	1
Range	0-2
WHO 0	9
WHO 1	12
WHO 3	3
Prior therapy	
None	5
Chemotherapy	8
Radiotherapy	3
Chemo- and radiotherapy	8
Primary tumor site	
Colorectal	5
Lung	3
Breast	2
Cervix uteri	2
Unknown primary	2
Liver	2
Miscellaneous	8

Hematological toxicity

Hematological toxicities observed in this trial are summarized in table 2. Transient grade 1 neutropenia reversible without treatment interruption was seen in the fourth week and in the fourth month of treatment in one patient at 50 mg BID and in the first week and the second month of treatment in one patient at 100 mg BID. At 400 mg BID, grade 4 neutropenia lasting from day 14-28 was seen in one patient. Granulocyte-colony stimulating factor (G-CSF) was administered from day 26-29. This patient also developed transient grade 4 thrombocytopenia following withdrawal of the study drug. At 300 mg BID, grade 4 neutropenia lasting from day 17-35 was seen in one patient. No G-CSF was administered. Transient grade 1 thrombocytopenia was recorded in the third week of treatment in one patient at 25 mg and 300 mg BID, respectively. One patient at 300 mg BID developed grade 2 thrombocytopenia lasting 5 days after treatment had been stopped because of other toxicities. One patient at 400 mg BID developed grade 3 anemia six days after treatment had been stopped.

Table 2 Hematological toxicity (worst per patient)

Dose level (BID)	Patients	Neutropenia (CTC grade)				Thrombocytopenia (CTC grade)			
		1	2	3	4	1	2	3	4
25	4	-	-	-	-	1	-	-	-
50	5	1	-	-	-	-	-	-	-
100	3	1	-	-	-	-	-	-	-
200	6	-	-	-	-	-	-	-	-
400	3	-	-	-	1	-	-	-	1
300	3	-	-	-	1	1	1	-	-

Non-hematological toxicity

Major non-hematological side-effects observed in this trial are summarized in table 3. Toxicity was mainly gastrointestinal and consisted of watery diarrhea, nausea, vomiting, and anorexia. In patients with diarrhea, loperamide administered on an 'as-needed' basis resulted in prompt relief of symptoms. At lower doses vomiting was usually mild and required no specific treatment. Anorexia mainly occurred at the highest dose levels, was mild and required no specific therapy. Other toxicities consisted of grade 1-2 elevation of liver enzymes and reversible grade 1-2 elevated plasma creatinine levels recorded at all dose levels studied. In one patient at 400 mg BID, anorexia and diarrhea grade 3, together with nausea grade 2 and vomiting grade 1 resulted in creatinine grade 3 due to dehydration, defining DLT. Weight loss grade 1 was recorded in 3 patients at 200 mg BID and one patient each at 300 and 400 mg BID. Almost all patients experiencing weight loss had various concurrent gastrointestinal toxicities. Transient grade 2 fever was recorded in one patient at 300 mg BID who also developed transient grade 2 oral mucositis after SCH 66336 administration was interrupted due to other side-effects. Atrial flutter/fibrillation was recorded in the third month of therapy in a single patient at 100 mg BID. This patient had a prior history of atrial fibrillation. Asymptomatic sinus bradycardia (55 BPM) was recorded in the third week of treatment in one patient at 300 mg BID. 24-hour Holter monitoring following the day of onset revealed numerous episodes of bradycardia. Nineteen days after discontinuation of the study drug due to other toxicities, 24-hour Holter monitoring showed no further episodes of bradycardia. Serial electrocardiograms showed no relevant changes in any of the patients. Ophthalmologic examinations revealed no retinal changes.

Dose limiting toxicity

Since in the first three patients at 200 mg BID no toxicity greater than grade 1 was recorded, the dose was doubled to 400 mg BID. At this dose DLT was seen in three consecutive patients. It consisted of grade 4 vomiting in the first week of treatment in one patient, grade 4 neutropenia lasting 14 days coinciding with grade 4 thrombocytopenia lasting 5 days occurring after 2 weeks of treatment in a second patient, and the combination of grade 3 diarrhea, grade 3 anorexia, grade 2 nausea,

Table 3 Non-hematological toxicity (worst per patient)

Dose level (BID)	Patients	Nausea (CTC grade)			Anorexia (CTC grade)			Diarrhea (CTC grade)				Vomiting (CTC grade)				Fatigue (CTC grade)			Neurocortical (CTC grade)			Creatinine (CTC grade)		
		1	2	3	1	2	3	1	2	3	4	1	2	3	4	1	2	3	1	2	3	1	2	3
25	4	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
50	5	1	-	-	-	-	-	2	-	-	-	1	-	-	-	1	-	-	-	-	-	3	-	-
100	3	1	-	-	-	-	-	1	-	-	-	1	-	-	-	1	-	-	-	-	-	1	-	-
200	6	5	-	1	4	-	1	1	1	2	1	2	2	-	-	3	1	-	-	-	-	5	-	-
400	3	-	3	-	-	-	2	1	1	1	-	2	-	-	1	-	1	-	-	-	-	1	1	1
300	3	2	1	-	1	1	-	2	1	-	-	2	-	-	-	1	1	1	-	-	1	1	-	-

and grade 1 vomiting leading to reversible grade 3 elevation of plasma creatinine occurring after the first week in a third patient. Three additional patients were then treated at the next lower dose level of 200 mg BID, but as no additional dose limiting toxicities were recorded, it was decided to increase the dose to 300 mg BID. At this dose DLT was again observed in three consecutive patients, consisting of grade 4 neutropenia lasting 10 days and occurring after three weeks of treatment, reversible grade 3 neurocortical toxicity consisting of disorientation and confusion in the first week of treatment, and the combination of grade 3 fatigue with grade 2 nausea and grade 2 diarrhea occurring in the third week of treatment. No patient at 400 mg BID or 300 mg BID was able to complete 28 days of treatment. The recommended dose for phase II trials is set at 200 mg BID. The 6 patients treated at this dose level received the drug for a median of 57 days (range 52-280)

Pharmacokinetics

Pharmacokinetic studies were completed in all 24 patients. The plasma concentration versus time profiles of SCH 66336 were similar for all patients studied, with mean curves obtained at the tested SCH 66336 dose levels shown in figure 2.

Figure 2 Plasma concentration versus time profiles of SCH 66336 in patients treated at a dose level of 25 mg (o), 50 mg (•), 100 mg (◊), 200 mg (◆), 300 mg (▽) or 400 mg (▼). Mean values (symbols) and standard error (bar) are shown for all patients treated on day 1 at the indicated SCH 66336 dose level.

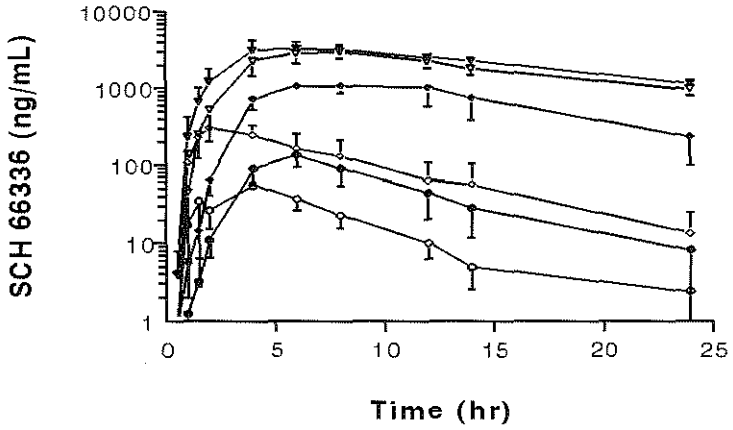


Table 4 Summary of SCH 66336 pharmacokinetic data[a]

Dose level (mg)	*n*	C_{max}[b] (ng/mL)	T_{max} (h)	AUC (μg.h/mL)	$T_{1/2}$ (h)	CL/F (mL/min)	$V_{d,ss}/F$ (liters)	$AUC_{d15}/_{d1}$[d]
25	4	63.9±2.24	3.4±1.3	0.397±0.167	3.57±1.32	1190±462	331±27.0	2.65±0.27
50	5	156±96.1	5.2±1.1	1.35±0.955	3.68±1.49	845±486	460±532	5.28±1.83
100	3	333±70.6	2.7±1.2	2.46±1.96	4.09±1.29	958±536	299±114	3.66±1.35
200	6	1380±728	6.7±2.7	17.7±14.4	5.45±1.22	253±103	114±43.8	3.29±0.56
300	3	2900±1290	7.3±1.2	56.4±20.0	10.0±0.59[c]	98.7±42.9	85.4±35.9	3.38±1.60
400	3	3610±1290	8.0±5.3	69.1±16.5	10.4±0.25[c]	101±27.3	90.4±22.4	NA

[a] Mean values ± standard deviation.

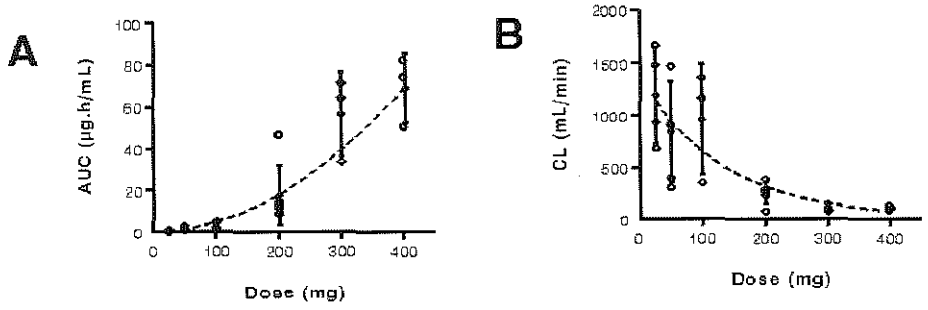
[b] C_{max}, peak plasma concentration; T_{max}, time to peak concentration; $T_{1/2}$, terminal disposition half-life; CL/F, apparent clearance; $V_{d,ss}$, apparent volume of distribution at steady-state; $AUC_{d15/d1}$, ratio of AUC_{0-12} values measured on days 15 and 1, respectively.

[c] Significantly different, $P<0.006$ (Kruskal-Wallis' test followed by Dunn's multiple comparison).

[d] Dose-independent, $P=0.103$ (Kruskal-Wallis'test) but significantly different from 1, $P=0.0016$ (Paired Student's *t*-test).

The mean single-dose non-compartmental pharmacokinetic parameters of SCH 66336 after doses ranging from 25-400 mg are summarized in table 4. Significant interpatient variability in pharmacokinetic parameters was apparent at all dose levels. The absorption of the drug was relatively slow, and peak concentrations were reached between 2.7-8.0 hours after drug intake. Peak plasma concentrations as well as AUCs increased in a greater than dose-proportional manner (figure 3a); A 16-fold increase in dose (from 25 to 400 mg) was associated with an increase in mean peak plasma concentration of approximately 56-fold and an increase in the AUC of approximately 200-fold. The apparent clearance of SCH 66336 decreased exponentially from 1190±462 mL/min at a dose of 25 mg to 101±27.3 mL/min at 400 mg (figure 3b), while the $V_{d,ss}/F$ decreased from 331±27.0 liters to 90.4±22.4 liters at the same dose levels. There was a trend to increasing plasma half-life with increasing dose which was statistically significant at the 2 highest dose levels (P<0.007; Kruskal-Wallis' test). The peak plasma concentrations (not shown) and AUC_{0-12} (table 4) increased approximately 2-5 fold upon repeated dosing in a dose independent manner (P=0.103; Kruskal-Wallis' test) which is more than expected based on accumulation effects only (P=0.0016; paired Student's t-test). In contrast, the terminal disposition half-life (data not shown) was comparable between days 1 and 15 although the mean difference reached borderline significance (P=0.04; Wilcoxon's test for matched pairs of 10 patients). This suggests that the dose-dependency in apparent clearance does not arise primarily from factors associated with saturation of excretory routes. Steady state concentrations of SCH 66336 were attained by day 7-14, with only minor intrapatient variability in trough levels (median coefficient of variation, 15.5%; range, 6-60%). The cumulative urinary excretion of unchanged SCH 66336 was dose-independent and accounted for only <0.02% of the administered dose. The mean renal clearance, i.e. the product of the dose-fraction excreted unchanged in urine and the apparent total body clearance, was estimated as 0.117±0.0105 mL/min, suggesting that SCH 66336 is not cleared by renal processes.

Figure 3 Effect of dose on the AUC (**A**) and apparent clearance of SCH 66336 (**B**) in 24 cancer patients. Closed symbols with error bars indicate mean values of the pharmacokinetic parameter at each of the tested dose levels and standard deviation, respectively.



Response

No partial or complete responses were seen. One patient with pseudomyxoma peritonei had stable disease for 9^{+} months, whereas one patient with metastatic follicular thyroid carcinoma had stable disease for 7 months with ongoing treatment.

DISCUSSION

We performed a phase I and pharmacokinetic study to explore safety, tolerability, maximum tolerated dose and pharmacokinetics of the oral farnesyl transferase inhibitor SCH 66336. In this study using continuous oral BID administration, side-effects attributable to the study drug were hematological and non-hematological, whereas DLTs included neutropenia, thrombocytopenia, various gastrointestinal side-effects and neurocortical toxicity with reversible disorientation and confusion.

Hematological toxicity of SCH 66336 in the current study consisted of dose dependent, uncomplicated and reversible neutropenia and thrombocytopenia mainly

occurring at the two highest, non-tolerable dose levels tested. At the dose level recommended for phase II studies, 200 mg BID, myelosuppression did not occur, even in the patient that was on treatment for up to 9^{+} months. This parallels the experience in three other studies using different dosing regimens of SCH 66336 where hematological toxicity was absent at these dose levels [18-20]. One of these studies also used a continuous treatment schedule [20]. This finding is in contrast to results obtained in other farnesyl transferase inhibitors. In two published reports on L-778,123, a peptidomimetic farnesyl transferase inhibitor given intravenously, myelosuppression comprised one of the DLT's and also occurred at dose levels recommended for further activity testing [21,22].

Out of three phase I studies [23-25] that have been reported on the farnesyl transferase inhibitor R115777, myelosuppression comprised DLT in two [23,24], whereas in the third study that used a 5-days on, 9 days off schedule, only minimal hematopoietic toxicity was observed [25]. In the only published phase I study with the novel farnesyl transferase inhibitor BMS-214662, exploring an intermittent treatment schedule, no myelosuppression was recorded [26]. Clearly, for farnesyl transferase inhibitors myelosuppression is a class effect, however with marked differences depending on agent and schedule of administration.

Non-hematological side-effects of SCH 66336 in our current study were predominantly gastrointestinal and consisted of mild dose dependent, non-cumulative and reversible diarrhea, vomiting, anorexia, and nausea. When diarrhea occurred at the recommended dose for phase II studies, treatment with loperamide always resulted in prompt and complete relief. Patients were advised to use loperamide on an 'on demand' basis, which always proved to be sufficient. At the recommended dose for phase II studies, vomiting was also usually mild, short lasting and required no specific treatment. Anorexia and nausea occurred at virtually all dose levels and usually were mild. Gastrointestinal side-effects were recorded in all studies of SCH 66336 and comprised DLT in all treatment schedules analyzed. This may suggest that gastrointestinal toxicity is not cumulative. Presumably partly related to these various gastrointestinal side-effects, mild weight loss was noted in almost all patients. However, patients without gastrointestinal toxicity also experienced some weight loss

that mainly occurred within the first two weeks of treatment. Remarkably, no additional weight loss was seen with ongoing treatment.

Non-gastrointestinal side-effects were diverse, infrequent, and usually mild. At the lower dose levels, non-cumulative and reversible grade 1 creatinine increases were seen, but coinciding urine analysis never revealed any abnormality, and therefore we cannot rule out mild dehydration due to various gastrointestinal side-effects as the principal cause of these creatinine increases. In the patient at the non-tolerable dose level 400 mg BID in whom grade 3 creatinine was recorded, urine analysis revealed no abnormalities and interruption of SCH 66336 dosing and intravenous rehydration resulted in a rapid and complete normalization of creatinine levels.

In the present study, two episodes of reversible atrial rhythm abnormalities (atrial fibrillation in a patient with previous cardiac history and asymptomatic sinus bradycardia) occurred, but serial electrocardiograms did not show consistent changes in all other patients. This is in sharp contrast with the data from studies with L-778,123, where prolongation of the QT-time constituted DLT [21,22]. In the current study one episode of grade 3 rapidly reversible neurocortical toxicity consisting of disorientation and confusion was recorded, but no other episodes of either neurocortical toxicity or peripheral neuropathy were recorded in any of the other studies with SCH 66336. Reversible peripheral neurosensory and –motor, as well as central neurocortical toxicity have been described with oral R115777 [23-25]. No neuropathy was recorded with BMS-214662 [26].

When considering which treatment schedule of SCH 66336 should preferrably be used in future clinical trials, it is of note that preclinical data demonstrate that SCH 66336 is a reversible competitive inhibitor of farnesyl transferase, and the biochemical effects are rapidly reversed upon withdrawl of the compound. Because the compound thus is a competitive inhibitor, the schedule most likely to result in continuous inhibition of farnesyl transferase would be the continuous schedule. This schedule achieves the highest total dose and the longest exposure time.

When summarizing the results of the recorded toxicity profiles of the farnesyl transferase inhibitors that are currently being tested in clinical studies (SCH 66336, R115777, L-778,123, and BMS-214662), it can be concluded that myelosuppression

is a common feature, whereas non-hematological toxicities differ essentially. Table 5 summarizes the results of the clinical studies with farnesyl transferase inhibitors presented to date.

This present study clearly demonstrates a dose-dependency in SCH 66336 plasma pharmacokinetics in cancer patients, which contrasts previous findings from preclinical dose-response studies. In the rat peak plasma levels reached values of 3, 10 and 30 μM at oral doses of 10, 30 and 100 mg/kg, respectively [27]. In cancer patients, both the apparent clearance and the apparent $V_{d,ss}/F$ demonstrated a >4-10 fold decrease at a dose of 400 mg, compared with 25 mg. The most likely explanation is an increase in F with multiple dose administration resulting in an apparent decrease in V_d and an apparent decrease in Cl/F. The opposing effects of these two processes on drug elimination leaves the apparent terminal disposition half-life almost dose-independent, except at the 2 highest dose levels. In addition, at repeated dosing, *i.e.* when comparing the mean drug exposure and peak plasma concentrations of the various dose levels tested at day 15 with those of day 1, substantial increases were found which were greater than predicted based on accumulation processes alone. Clearly, this may have important clinical ramifications; if clinical outcomes are related to drug exposure, then a simple percentage increase in dose will have much greater impact on total drug exposure than would be expected with a behavior based on linear pharmacokinetics. Most importantly, at the recommended dose for further clinical studies applying continuous dosing regimens with SCH 66336, trough plasma concentrations were shown to exceed 1.5 μM, which is above concentrations required *in vitro* to induce significant growth inhibition in colony assays against various primary human tumor specimens [28].

The general principles of dose-dependency in pharmacokinetics have recently been reviewed [29].The dose-dependent pharmacokinetic behavior of SCH 66336 in cancer patients most likely involves multiple nonlinear (absorption) mechanisms, including saturation of metabolic processes responsible for presystemic biotransformation (e.g. the cytochrome P450 system) or saturation of outward-directed drug-carrier systems that mediate transmembrane drug flux,

Table 5 Clinical studies (single-agent) of farnesyl transferase inhibitors; Schedule, DLT, recommended dose, toxicities

Drug (ref)	Schedule	DLT	Recommended dose (mg)	Side effects at recommended dose
SCH 66336 (18)	p.o./BID d 1-7 q 3 weeks	Diarrhea, fatigue	350 BID	ANC, platelets, N/V, diarrhea, fatigue
SCH 66336 (19)	p.o./BID d 1-14 q 4 weeks	Gastrointestinal	200 BID	N/V, diarrhea, fatigue
SCH 66336 (20)	p.o./OD continuous	Diarrhea	300 OD	Diarrhea, N/V, renal, fatigue
L-778,123 (21)	i.v. d 1-7 q 3 weeks	QTc Neutropenia	560 (m^2) OD	ANC, plts, N/V somnolence, fatigue
L-778,123 (22)	i.v. d 1-14 q 3 weeks	Neutropenia QTc	560 (m^2) OD	?
L-778,123 (22)	i.v. d 1-28 q 5 weeks	?	?	?
R115777 (23)	p.o./BID continuous	Skin, neutropenia, thrombocytopenia, neuromotor/sensory	300 BID	Skin, ANC, plts, fatigue, N/V, neuro, dizziness
R115777 (24)	p.o./BID d 1-21 q 4 weeks	Neutropenia, thrombocytopenia, confusion, fatigue, bilirubin	240 (m^2) BID	ANC, plts, fatigue, confusion
R115777 (25)	p.o./BID d 1-5 q 2 weeks	Neuropathy, fatigue	500 BID	N, fatique,neurocortical
BMS-214662 (26)	i.v. course 1 p.o course 2 d 1 q 3 weeks	Hepatotoxicity Gastrointestinal	?	Fatigue, somnolence, gastrointestinal

Route of admin. denotes route of administration
i.v. denotes intravenously, p.o. denotes orally
BID denotes twice daily, OD denotes once daily
OTc denotes asymtomatic QTc prolongation at ECG
ANC denotes absolute neutrophile count, plts denotes platelets
N/V denotes nausea and vomiting

such as *MDR1* P-glycoprotein. Saturation of presystemic metabolism or degradation in the gut lumen, the intestinal mucosae or the liver after oral administration of drugs in humans is relatively common and has been well described for the calcium antagonist verapamil[30], and also for 5-fluorouracil [31]. However, the phenomenon of a dose-dependent decrease in extravascular binding ($V_{d,ss}/F$) as seen here with SCH 66336 is highly unusual, although it has been reported to occur with 3-hour infusions of paclitaxel, presumably as a result of extensive binding to microtubules or micellar encapsulation in its formulation vehicle [32]. Further analysis of the absorption and disposition of SCH 66336 in individual cancer patients, with respect to the current findings, should be of great importance for our ability to better understand the role of the various biological factors that may influence the compound's pharmacokinetic behavior and pharmacological actions, and effects of other drug administered concomitantly.

In conclusion this phase I and pharmacological study with continuous oral BID SCH 66336 has shown that this farnesyl transferase inhibitor can be safely administered using a continuous oral BID dosing schedule. The recommended dose for phase II studies using this treatment schedule is 200 mg BID.

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SUMMARY AND CONCLUSIONS

This thesis presents results of phase I and pharmacological studies on various new, predominantly cytostatic or growth inhibitory anticancer agents.
Studies were performed on an inhibitor of the polyamine synthesis, a matrix metalloproteinase inhibitor, and an inhibitor of farnesyl transferase. Although these compounds each do inhibit different intra- or extracellular targets, their common feature is target specificity and their predominantly cell growth inhibitory activity. As these *cytostatic* anticancer agents do not induce direct cell-kill and thus exert their activity in a way completely different from that of classic *cytotoxic* agents, the design of clinical studies on these agents has to be adapted extensively in order to be able to optimally assess toxicity and potential clinical activity of these new compounds.

In **chapter 2** the background and rationale for the development of new cytostatic anticancer agents is reviewed, and some suggestions on how to change the design of clinical studies on these agents as compared to the conventional study design are presented.
Cytostatic anticancer agents are designed to inhibit one specific target or process. In vitro studies with agents inhibiting the intracellularly localized polyamine synthesis or the enzyme farnesyl transferase, transmembrane receptor tyrosine kinases, the enzymatic breakdown of extracellular matrix, or the process of tumor-related angiogenesis all showed specific target inhibition and subsequent inhibition of tumor cell growth in case of an intracellular target. In vivo studies with these agents usually showed tumor growth inhibition, although tumor regressions were recorded as well. Most agents had to be administered for prolonged periods of time, sometimes even continuously, in order to exert optimal target inhibition.
In animal studies, toxicity of these agents was often strikingly mild.
Clinical studies with various new cytostatic anticancer agents are currently ongoing, and it has been acknowledged that due to their different mode of action, endpoints used in clinical studies with cytotoxic agents can not always be used in studies with these agents. This means that new endpoints must be defined, and the design of these clinical studies thus has to be reappraised.
In phase I studies, acute dose limiting toxicity theoretically may not be reached at all, and due to the frequently required chronic drug administration, a shift towards the

assessment of chronic toxicity may become increasingly important. The specific target inhibition as noted in preclinical studies points toward biological activity, and therefore this endpoint should, if possible, also be assessed in clinical studies. However, practical limitations such as the impossibility to perform repeated invasive procedures in patients might hamper such analyses. Surrogate markers of target inhibition should thus be looked for, and for this purpose assessment of pharmacokinetic parameters, pharmacodynamic parameters in more readily available tissue, and/or the assessment of specific tumor marker inhibition could theoretically be used.

Performing single-agent phase II studies with cytostatic agents will most likely lead to underestimation of potential antitumor activity, as tumor regressions have only sporadically been described in models. Single-agent phase II studies using time to progression as endpoint, however, could be considered. Preferably, these studies should be randomized in order to prevent a bias in patient selection. For those compounds showing tumor regressions or synergistic antitumor activity when used in combination with cytotoxic agents, the classic design of phase II studies could be conceivable, although also in these cases a randomized design would be preferred.

The endpoints of phase III studies for both cytotoxic and cytostatic agent studies are the same, and therefore the design of these studies can largely remain unchanged.

In **chapter 3** the results of a phase I and pharmacological study on the polyamine synthesis inhibitor SAM 486A (formerly known as CGP 48664) administered as four weekly infusions followed by two weeks off treatment in patients with solid tumors is presented. Fifty patients were evaluable for toxicity and response. Due to the occurrence of facial paraesthesias and flushing, infusion time gradually had to be prolonged from 10 to 60 minutes, whereas at the highest two dose levels studied, additional prolongation of the infusion time to 180 minutes was necessary due to the onset of somnolence. Dose limiting toxicity was reached at a weekly dose of 325 mg/m^2 and consisted of febrile neutropenia, hypotension and collapse with synchronous abnormalities at electrocardiography, and neuromotor and neurosensory toxicity. Side effects at tolerable dose levels were mild and consisted

of myelosuppression, anorexia, nausea, vomiting, diarrhea, fatigue, and cardiovascular abnormalities, mainly dysrythmias.
Pharmacodynamic analysis in a peripheral blood mononuclear cell model failed to show a consistent inhibition of polyamine synthesis. There were no partial or complete tumor responses. The recommended dose for further activity testing using this schedule is 270 mg/m^2/wk.

Chapter 4 describes the results of a large phase I and pharmacological study of the oral matrix metalloproteinase inhibitor MMI270B (formerly known as CGS 27023A) administered one to three times daily to patients with advanced solid tumors. Ninety-two patients were evaluable for toxicity. There was no myelotoxicity. The most prominent non-hematological toxicities were cutaneous rash and arthralgia and/or myalgia. The rash was predominantly located at the trunk and arms and was only sporadically associated with pruritis. At dose levels exceeding 300 mg BID, rash was seen more frequently, was more intense, and became dose limiting. The onset of arthralgia and/or myalgia usually was insidious and most often involved the shoulder region and fingers. There was a clear relationship between the frequency and intensity of these musculoskeletal complaints and duration of treatment, but no such relationship was found with the dose of the trial drug. There were no partial or complete responses. Continuous oral treatment with 300 mg BID was determined to be the recommended dose for further activity testing.

Chapter 5 presents the results of a study on effects of food intake on overall drug exposure (as indicated by $AUC_{0\text{-}8h}$), peak plasma levels (C_{max}) and time to reach peak plasma levels (T_{max}) of MMI270B. These pharmacokinetic parameters were analyzed after the trial drug was administered either 2 hours preceding the intake of food or directly following the intake of food. Seventeen patients taking three different dose levels were enrolled into this study. At all dose levels studied, mean drug exposure was reduced by 10% when the drug was administered directly following food intake, but this was a non-significant effect. Mean peak plasma levels when the drug was administered directly following food intake were 40 % lower than in the fasted state, indicating a significant effect. Time to reach peak plasma levels was

significantly increased following drug administration in the fed state. At all dose levels studied and in both the fasted and fed situation, plasma levels exceeding target inhibitory concentrations were maintained for prolonged periods of time. These data indicate that exposure to MMI270B is not significantly influenced by food intake, and that therefore no specific guidelines concerning the ingestion of MMI270B in either a fasted or fed state have to be given.

Chapter 6 gives an overview of preclinical and clinical studies on farnesyl transferase inhibitors that have been performed so far. Three different groups of farnesyl transferase inhibitors have been developed; compounds showing structural similarity with farnesyl diphosphate or FPP, so-called FPP analogues; compounds showing structural similarity with the so-called CAAX tetrapeptide sequence of farnesyl transferase, the so-called CAAX peptidomimetics. This group can be subdivided into peptide and non-peptide classes; Bisubstrate inhibitors combine the features of the FPP analogues and CAAX peptidomimetics.

In vitro studies with farnesyl transferase inhibitors showed specific target inhibition as well as delay of cell growth, whereas in vivo studies with most of these agents showed tumor growth inhibition, although some tumor regressions in animal models were also seen.

Clinical studies with FPP analogues have not at all been performed, and clinical studies with bisubstrate inhibitors have not yet been published.

The results of phase I studies on three CAAX peptidomimetics have been presented thus far; intravenously administered L-778,123, a peptide CAAX peptidomimetic, yielded myelosuppression and prolongation of the QTc interval on electrocardiography as dose limiting toxicity. Oral R115777, an imidazole antifungal derived farnesyl transferase inhibitor, yielded myelosuppression, peripheral neuropathy, confusion, and fatigue as dose limiting toxicity, depending on the treatment schedule used. In one patient with non-small cell lung cancer a partial tumor response was noted. SCH 66336, a non-peptide CAAX peptidomimetic, yielded myelosuppression, fatigue, nausea, vomiting and diarrhea, sometimes accompanied by rapidly reversible renal toxicity assumed to be due to dehydration caused by one or more of these gastrointestinal side effects, and neurocortical

toxicity as dose limiting toxicities, also dependent on the treatment schedule used. Also here one partial tumor response was noted in a patient with non-small cell lung cancer. BMS 214662, an example of a new class of non-peptide imidazol farnesyl transferase inhibitors, is currently undergoing phase I studies. The toxicity profile of these various farnesyl transferase inhibitors seems to be highly comparable although some differences exist. Phase II studies, either single-agent or combination studies, and phase III studies, designed specifically for these cytostatic agents are either being planned or performed.

Chapter 7 presents the results of a phase I and pharmacokinetic study on the oral farnesyl transferase inhibitor SCH 66336 using a continuous twice daily treatment schedule. Twenty-four patients were enrolled into this study. At 400 mg BID dose limiting toxicity consisting of neutropenia and thrombocytopenia, vomiting, and the combination of anorexia, diarrhea and rapidly reversible creatinine elevation was seen. After reducing the dose, dose limiting toxicity was again seen at 300 mg BID, consisting of neutropenia, neurocortical toxicity, and the combination of nausea, diarrhea and fatigue. There were no partial or complete responses, two patients had stable disease for 7 and 9 months, respectively. The recommended dose for further activity testing using this treatment schedule is 200 mg BID which in this study was found feasible for prolonged periods of time.

Final conclusions and future perspectives

Due to their limited clinical success rate on the one hand, and the frequency of various, often cumbersome side effects on the other hand, the balance of effectivity and tolerance of cytotoxic anticancer agents still remains disappointing.

In recent years, many specifically designed anticancer agents inhibiting either intra- or extracellular processes that are typically involved in the process of malignant transformation of cells and tumorigenesis have been developed.

As these agents have a cytostatic rather than cytotoxic mode of action, the perception of cancer as disease and the perception on how to optimally treat cancer patients probably will change in the years to come.

It is conceivable that in the future cancer increasingly can be considered to be a chronic disease, like for example hypertension or diabetes mellitus, for which specific cytostatic treatment options exist that, devoid of cumbersome side effects, will be able to restrict new or further tumorgrowth once the tumor has been optimally reduced in size, either by surgical techniques, previous cytotoxic treatment, or a combination of these treatment modalities. As anticancer treatment in this situation probably means continuous or even chronic drug administration, oral treatment is highly preferable.

It has to realised, however, that although a large number of these new cytostatic anticancer agents have already been tested in preclinical and clinical studies, and results of numerous studies have already been presented and thoroughly discussed, at this moment the potential role of these new and theoretically promising agents in the armamentarium of the medical oncologist still remains to be fully determined.

SAMENVATTING EN CONCLUSIES

In dit proefschrift worden de resultaten gepresenteerd van fase I en farmacologische studies van enkele nieuwe, voornamelijk cytostatisch werkende antikanker middelen.

De resultaten van een studie met een polyamine synthese remmer, een remmer van matrix metalloproteinase, en een remmer van farnesyl transferase worden beschreven. Hoewel deze middelen ieder hun eigen specifieke intra- of extracellulair gelegen aangrijpingspunt hebben, is de overeenkomst tussen deze middelen gelegen in de hoge mate van specificiteit voor één aangrijpingspunt en het feit dat remming van vanuit dit aangrijpingspunt gestuurde processen wel leidt tot het stoppen van verdere tumorgroei, maar niet tot celdood met de daaruit voortvloeiende regressie van reeds aanwezige tumoren. Omdat cytostatisch werkende antikanker middelen derhalve een totaal ander werkingsmechanisme hebben als de zogenaamd klassieke cytotoxische antikanker middelen, moet bij de uitvoering van klinische studies met deze middelen rekening worden gehouden met andere effecten en eindpunten. Daarom zal, om tot een goede inschatting van potentiële werkzaamheid en toxiciteit te kunnen komen, het ontwerp van deze studies aanzienlijk moeten worden aangepast.

In **hoofdstuk 2** worden achtergrond en beweegredenen beschreven voor de ontwikkeling van nieuwe cytostatisch werkende antikanker middelen. Tevens worden voorstellen geformuleerd om te komen tot aanpassingen in het ontwerp van klinische studies met deze middelen ten opzichte van de opzet van studies met cytotoxische antikanker middelen.

Cytostatische antikanker middelen zijn zoals gezegd ontworpen om een specifiek aangrijpingspunt in of rondom de tumorcel te remmen. In vitro studies met remmers van de intracellulaire polyamine synthese of het intracellulair aanwezige enzym farnesyl transferase, maar ook studies met remmers van verschillende celwandreceptor tyrosine kinases, remmers van de afbraak van de extracellulaire matrix, of remmers van het proces van tumor gerelateerde angiogenese of bloedvatnieuwvorming lieten alle een specifieke remming van de aangrijpingspunten zien alsmede remming van celgroei in geval van een intracellulair gelegen doelwit. In vivo studies met deze middelen toonden vrijwel altijd een remmend effect op de groei

van tumor implantaten, hoewel ook een enkele maal tumor regressie werd beschreven. De meeste cytostatische middelen moesten langere tijd achtereen of soms zelfs continu worden toegediend om een optimaal antitumor effect te bereiken. Opvallend was de vrijwel volledige afwezigheid van bijwerkingen tijdens veel van deze dierproeven.

Momenteel worden talloze klinische studies met cytostatisch werkende antikanker middelen uitgevoerd. Het is gebleken dat het hanteren van eindpunten uit studies met cytotoxische antikanker middelen niet altijd mogelijk is. Dit betekent dus dat nieuwe eindpunten dienen te worden opgesteld en dat daarmee de opzet van studies met cytostatische middelen grondig dient te worden herzien.

Bij fase I studies hoeft het eindpunt van de acute dosis limiterende toxiciteit niet altijd te worden bereikt, en mede omdat langdurige en/of continue toediening van deze cytostatische middelen wenselijk of zelfs noodzakelijk is, zal de beoordeling van op langere termijn optredende toxiciteit steeds belangrijker worden.

In veel preklinische studies met cytostatische middelen is remming van een specifiek proces aangetoond, hetgeen wijst op biologische effectiviteit van het middel. Ook in klinische studies zal derhalve dit biologisch effect daar waar mogelijk moeten worden beoordeeld. Echter, dit zou betekenen dat bij patienten bij herhaling tumorbiopsieën zouden moeten worden verricht, iets dat ethisch en praktisch onmogelijk is. Daarom zal moeten worden gezocht naar andere bewijzen voor biologische effectiviteit, en hierbij valt te denken aan analyse van zogenaamde surrogaat merkers van activiteit. Analyse van farmacokinetiek gegevens, analyse van farmacodynamische gegevens in representatief materiaal dat eenvoudiger dan tumorweefsel kan worden verkregen, zoals bloed of wangslijmvlies schraapsel, en de analyse van het beloop van specifieke tumormerkstoffen tijdens de behandeling met cytostatische middelen zijn theoretisch mogelijkheden om bij patienten de biologische effectiviteit te beoordelen.

Het verrichten van fase II studies met afzonderlijke cytostatisch werkende antikanker middelen kan gemakkelijk leiden tot een onderschatting van de werkzaamheid van deze middelen, aangezien preklinische studies met deze middelen slechts bij uitzondering tumor regressie hebben aangetoond, en dit het eindpunt is bij fase II studies met cytotoxische middelen. Fase II studies met tijd tot ziekteprogressie als eindpunt zouden wel overwogen kunnen worden, maar bij voorkeur zou dit soort

studies dan gerandomiseerd van opzet moeten zijn om de kans op een vertekening in patientenselectie op voorhand zo klein mogelijk te houden. Voor cytostatische antikanker middelen waarbij in preklinische studies tumor regressie werd beschreven, en voor die middelen waarvan een synergistisch effect bij gecombineerd gebruik met cytotoxische antikanker middelen is beschreven, kan een standaard fase II studieopzet worden overwogen, maar ook hier verdient een gerandomiseerde opzet de voorkeur om redenen als boven genoemd.
De eindpunten van fase III studies met cytostatische danwel cytotoxische antikanker middelen zijn vergelijkbaar, en derhalve kan de opzet van deze studies in beide gevallen ongewijzigd blijven.

In **hoofdstuk 3** worden de resultaten gepresenteerd van een fase I en farmacologische studie met de polyamine synthese remmer SAM 486A (voorheen bekend als CGP 48 664). Vijftig patienten kregen op vier achtereenvolgende weken een intraveneuze toediening waarna twee weken zonder toediening volgden. Door het onstaan van paraesthesieën en roodheid in het gelaat moest de inloopsnelheid aanvankelijk worden verlengd van 10 tot 60 minuten, terwijl bij de twee hoogste doseringen een verdere verlenging van de inlooptijd tot 180 minuten nodig was in verband met het optreden van slaperigheid. Dosis limiterende bijwerkingen werden gezien bij een wekelijkse dosering van 325 mg/m^2, en deze bestonden uit febriele neutropenie, hypotensie, collaps met het tegelijkertijd optreden van afwijkingen op het ECG, en perifere sensorische en motorische neuropathie. De bijwerkingen op de tolerabele doses waren mild en bestonden uit myelosuppressie, anorexie, misselijkheid, braken, diarrhee, moeheid en hartritmestoornissen. Farmacodynamisch onderzoek in mononucleaire bloedcellen kon geen eenduidige remming van de polyamine synthese aantonen. Er werd geen partiele of complete tumor respons gezien. De aanbevolen dosis SAM 486A voor verdere effectiviteitsstudies met het beschreven toedieningsschema is wekelijks 270 mg/m^2.

Hoofdstuk 4 beschrijft de resultaten van een grote fase I en farmacologische studie met de oraal beschikbare matrix metalloproteinaseremmer MMI270B (voorheen bekend als CGS 27023A). Tweeënnegentig patienten kregen dagelijks een- tot

driemaal daags een of meerdere capsules. Al deze patienten konden worden geevalueerd op bijwerkingen. Er was geen beenmergremming, de meest op de voorgrond tredende bijwerkingen waren huiduitslag en spier- en/of gewrichtsklachten. De huiduitslag bevond zich voornamelijk op de romp en armen en ging slechts zelden gepaard met jeuk. Bij doseringen hoger dan 300 mg tweemaal daags werd de uitslag frequenter gezien en was deze intenser en uiteindelijk dosis limiterend. De spier- en/of gewrichtsklachten namen geleidelijk aan toe in ernst naarmate de behandeling langer kon worden voortgezet, dit effect was onafhankelijk van de dosering. Deze klachten manifesteerden zich vooral in de schouders en vingers. Er werden geen partiele of complete tumor regressies in deze studie waargenomen. De dosis MMI270B die geadviseerd wordt voor verdere effectiviteitsstudies is tweemaal daags 300 mg.

In **hoofdstuk 5** worden de resultaten van een studie naar de invloed van voedselopname op de biologisch beschikbaarheid van MMI270B gepresenteerd. De farmacokinetische parameters geneesmiddelexpositie (AUC_{0-8}), piek plasma concentratie (C_{max}) en de tijd die nodig is om de maximale plasma concentratie te bereiken (T_{max}) werden vergeleken wanneer MMI270B werd ingenomen in nuchtere toestand danwel na gebruik van een maaltijd. Er werden door zeventien patienten drie verschillende doseringen gebruikt. Bij elk doseringsniveau werd wanneer voedselinname voorafging aan de inname van MMI270B de geneesmiddelexpositie gemiddeld 10% verminderd, maar dit verschil was niet significant. De gemiddelde piek plasma concentraties bij inname van MMI270B na voedselinname was 40% lager dan in geval van inname in nuchtere conditie, dit verschil was significant. De tijd die verstreek alvorens de maximale piek plasma concentratie werd bereikt was significant langer bij inname van MMI270B na een maaltijd. In het plasma van alle patienten werden zowel in nuchtere als gevoede situatie gedurende langere tijd concentraties MMI270B gemeten die voldoende hoog zouden moeten zijn om te komen tot een adequate remming van meerdere matrix metalloproteinases.
De resultaten van deze studie betekenen dat er voor de inname van MMI270B geen specifieke adviezen hoeven te worden gegeven met betrekking tot tijdstip ten opzichte van een maaltijd.

Hoofdstuk 6 geeft een overzicht van de preklinische en klinische studies die tot op heden zijn verricht met farnesyl transferase remmers. Er bestaan drie verschillende groepen farnesyl transferase remmers; stofffen die chemisch verwant zijn aan farnesyl difosfaat, de farnesyl difosfaat analoga; stoffen die een structurele overeenkomst hebben met de zogenaamde CAAX tetrapeptide structuur van farnesyl transferase, de zogenaamde CAAX tetrapeptide analoga. Deze laatste groep kan worden onderverdeeld in een peptide en non-peptide klasse; Bisubstraat remmers tot slot combineren de eigenschappen van beide bovenbeschreven middelen.
In vitro studies met farnesyl transferase remmers tonen een duidelijke remming van het doelwit enzym alsmede een remming van celgroei, terwijl in vivo studies met de meeste middelen een rem op tumorgroei toonden, terwijl er bovendien sprake was van tumor regressies bij enkele studies.
Klinische studies met farnesyl difosfaat analoga zijn niet verricht, en resultaten van klinische studies met bisubstraat remmers zijn nog niet zijn gepubliceerd.
Tot op heden zijn de resultaten van studies met drie verschillende CAAX tetrapeptide analoga gepubliceerd; L-778,123 is een intraveneus toe te dienen farnesyl transferase remmer waarvan de dosis limiterende toxiciteit bestond uit myelosuppressie en verlenging van de QT tijd op het electrocardiogram. R115777 is een oraal beschikbare farnesyl transferase remmer met als dosis limiterende bijwerkingen myelosuppressie, perifere neuropathie, verwardheid en moeheid. Het optreden van de verschillende dosis limiterende bijwerkingen was afhankelijk van het toedieningsschema. In één van de studies met R115777 werd een partiele respons waargenomen bij een patient met niet kleincellig longcarcinoom. SCH 66336 is een eveneens oraal beschikbare farnesyl transferase remmer met myelosuppressie, moeheid, misselijkheid, braken en diarrhee, alsmede verwardheid en reversibele nierinsufficientie als dosis limiterende bijwerkingen, afhankelijk van het gebruikte schema. De nierinsufficientie wordt verklaard door milde uitdrogingsverschijnselen ten gevolge van een of meerdere gastrointestinale bijwerkingen. In één van de studies met SCH 66336 werd eveneens een partiele respons waargenomen bij een patient met niet kleincellig longcarcinoom. BMS 214662 is een voorbeeld van een nieuwe klasse non-peptide imidazol farnesyl transferase remmers. Fase I studies met dit middel worden momenteel uitgevoerd. Het patroon van bijwerkingen van de

farnesyl transferase remmers lijkt tussen de verschillende klassen goed vergelijkbaar, hoewel er ook zeker onderlinge verschillen waarneembaar zijn.
Fase II studies met uitsluitend een farnesyl transferase remmer, danwel met een combinatie van een farnesyl transferase remmer met een of meerdere cytotoxische antikanker middelen, alsmede fase III studies worden momenteel gepland of zijn reeds gestart.

In **hoofdstuk 7** worden de resultaten van een fase I en farmacokinetische studie met continu oraal tweemaal daags toegediende SCH 66336 gepresenteerd. Vierentwintig patienten namen deel aan deze studie. Op een dosisniveau van tweemaal daags 400 mg werd dosis limiterende toxiciteit gezien in de vorm van neutro- en thrombopenie, braken, en de combinatie van anorexie, diarrhee en snel reversibele nierinsufficientie. Bij een dosis van 300 mg SCH 66336 tweemaal daags werd opnieuw dosis limiterende toxiciteit waargenomen, ditmaal in de vorm van neutropenie, neurocorticale toxiciteit met verwardheid, en de combinatie van misselijkheid, diarrhee en moeheid. Er werden geen partiele of comlete responsen gezien, twee patienten hadden stabiele ziekte gedurende respectievelijk 7 en 9 maanden. De dosis die wordt geadviseerd voor verdere effectiviteitsstudies met dit schema is tweemaal daags 200 mg. In deze studie werd deze dosis goed verdragen gedurende langere tijd.

Eindconclusies en toekomstperspectieven

De vaak nog teleurstellende effectiviteit enerzijds, en het optreden van vaak hinderlijke en soms levensbedreigende bijwerkingen ten gevolge van behandeling met deze middelen anderzijds, maken dat de uiteindelijke betekenis van klassieke cytotoxische antikanker middelen voor patienten met een gemetastaseerde maligniteit helaas nog steeds beperkt is.
In de afgelopen jaren is een groot aantal nieuwe antikanker middelen ontwikkeld. Deze cytostatisch werkende middelen hebben een specifiek aangrijpingspunt in of buiten de tumorcel, waarbij dit aangrijpingspunt of het hieraan gekoppelde cellulaire proces een belangrijke rol speelt in het proces van maligne ontaarding. Deze cytostatische antikanker middelen hebben een totaal verschillend werkings-

mechanisme in vergelijking met de cytotoxische antikanker middelen, en ook het doel van behandeling van kanker met deze middelen is anders; daar waar door behandeling met cytotoxische middelen wordt gestreefd naar tumorverkleining, wordt er bij behandeling met cytostatische middelen eerder en misschien alleen gestreefd naar het tot stilstand brengen van de uitgroei van de tumor. De perceptie van de ziekte kanker zou door deze nieuwe behandelstrategie in de komende jaren wellicht kunnen gaan veranderen; kanker wordt in deze veranderde beeldvorming dan beschouwd als een chronische ziekte, net als hypertensie of diabetes mellitus, die met eenvoudige behandelingen, bij voorkeur door oraal beschikbare medicijnen die geen of slechts weinig bijwerkingen veroorzaken, onder controle kan worden gehouden. Een optimaal scenario van toekomstige kanker behandeling zou er uit kunnen bestaan dat een bestaande tumor door operatie, chemotherapie met cytotoxische middelen, of een combinatie van deze opties wordt verkleind tot minimale omvang, waarna door middel van een aanvullende, wellicht chronische behandeling hernieuwde tumorgroei achterwege blijft. Deze aanvullende behandeling zou, zeker wanneer zij chronisch dient te zijn, bij voorkeur dus met oraal beschikbare middelen dienen te worden uitgevoerd.

Echter, vooralsnog dient men zich terdege te realiseren dat, hoewel er nu reeds talloze preklinische en (vroeg)klinische studies zijn verricht met een ruim scala aan nieuwe, potentieel veelbelovende cytostatische antikanker middelen, de uiteindelijke plaats en betekenis die deze middelen eventueel krijgen in de behandeling van patienten met maligniteiten nog lang niet duidelijk is.

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Het is klaar!
Voor u ligt een proefschrift met één auteursnaam op de kaft, hoewel bij de totstandkoming ervan zijn vele mensen betrokken zijn geweest. Ik wil graag een aantal mensen met naam noemen, hoewel ik me realiseer dat zonder de grote groep van in dit proefschrift naamloze patienten het doen van klinisch wetenschappelijk onderzoek überhaupt nooit mogelijk zou zijn. De hoop, het onvoorwaardelijk vertrouwen en de getrooste inspanning die deze groep patienten de afgelopen jaren heeft getoond blijft een voortdurende stimulans om verder te gaan met dit wetenschappelijk, maar bovenal patient gebonden werk.

Mijn beide promotoren, professor Dr J Verweij en professor Dr G Stoter, zijn ieder op hun eigen manier betrokken bij dit proefschrift. Jaap, de manier waarop je mij hebt ingewerkt in het doen van wetenschappelijk onderzoek vind ik voorbeeldig; Aanvankelijk heb je me wegwijs gemaakt in deze wetenschappelijke omgeving, waarna er onder jouw toeziend oog steeds meer gelegenheid werd geboden te komen tot verdere onplooiing en zelfstandigheid. Daarnaast dwingt de snelheid en nauwgezetheid waarmee je telkens mijn manuscripten beoordeelde respect af, zeker als ik me realiseer dat je naar eigen zeggen hiervoor slechts 5 dagen van een week gebruikt. Gerrit, je bent wat meer op afstand betrokken geweest bij de ontwikkeling van dit proefschrift, maar al het werk vindt plaats binnen jouw afdeling. Hier is voor iedereen de ruimte en atmosfeer aanwezig om patientenzorg met het doen van wetenschappelijk onderzoek te combineren, hetgeen mijns inziens de beste voedingsbodem is voor verdere professionalisering van medewerkers en afdeling.

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Michiel en Lucas, er mag weer verstoppertje worden gespeeld op het kleine kamertje!

CURRICULUM VITAE

Ferry Eskens werd op 17 september 1962 in Son geboren. In 1980 behaalde hij het OVWO-diploma aan het Eckart College te Eindhoven. In datzelfde jaar begon hij de studie Geneeskunde aan de Katholieke Universiteit te Nijmegen. In de periode juli 1986-december 1986 liep hij als 'student doctor' stage in het Government Hospital, Mafeteng, Lesotho. In november 1987 behaalde hij het artsexamen.

Van april 1988 tot mei 1989 had hij twee halve banen; enerzijds was hij werkzaam als arts-onderzoeker op de ziekenhuisapotheek van het Canisius-Wilhelmina Ziekenhuis te Nijmegen (Dr EJ Vollaard), anderzijds was hij gedurende deze periode werkzaam als doseer-arts op de Trombosedienst Nijmegen eo (Dr MMJ Schuurmans). Van mei 1989 tot augustus 1990 vervulde hij zijn militaire dienstplicht als onderdeels- en kazerne arts bij respectievelijk de 43' Geneeskundige Compagnie en het Kazerne Ziekenrapport op de Johannes Post Kazerne te Havelte. Als burgerman keerde hij terug naar het Canisius-Wilhelmina Ziekenhuis te Nijmegen waar hij van september 1990 tot juli 1991 werkzaam was als AGNIO op de afdeling Interne Geneeskunde (Dr IH Go), waarna aansluitend de opleiding tot internist in hetzelfde ziekenhuis werd gestart (Dr IH Go, Dr RW de Koning). Vanaf 1 juli 1993 werd deze opleiding voortgezet in het Academisch Ziekenhuis St Radboud in Nijmegen (Prof. dr JWM van der Meer). Op dezelfde dag dat registratie als internist een feit was, 1 juli 1997, werd gestart met de vervolgopleiding Interne Oncologie in het Academisch Ziekenhuis Rotterdam, locatie Daniel (Prof. Dr G Stoter). Vanaf 1 november 1998 is hij werkzaam op de afdeling Interne Oncologie van het Academisch Ziekenhuis Rotterdam locatie Dijkzigt, aanvankelijk nog als junior internist, sinds 1 april 1999 als internist-oncoloog in vaste dienst. In de periode vanaf 1 juli 1997 werd het onderzoek verricht dat heeft geleid tot dit proefschrift. Hij is getrouwd met Antoinette Roetgerink, samen hebben ze twee zonen, Michiel en Lucas.

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