Early clinical cancer trials: Proof of concept and beyond

Inge Konings

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Early clinical cancer trials: Proof of concept and beyond

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Voor mijn ouders

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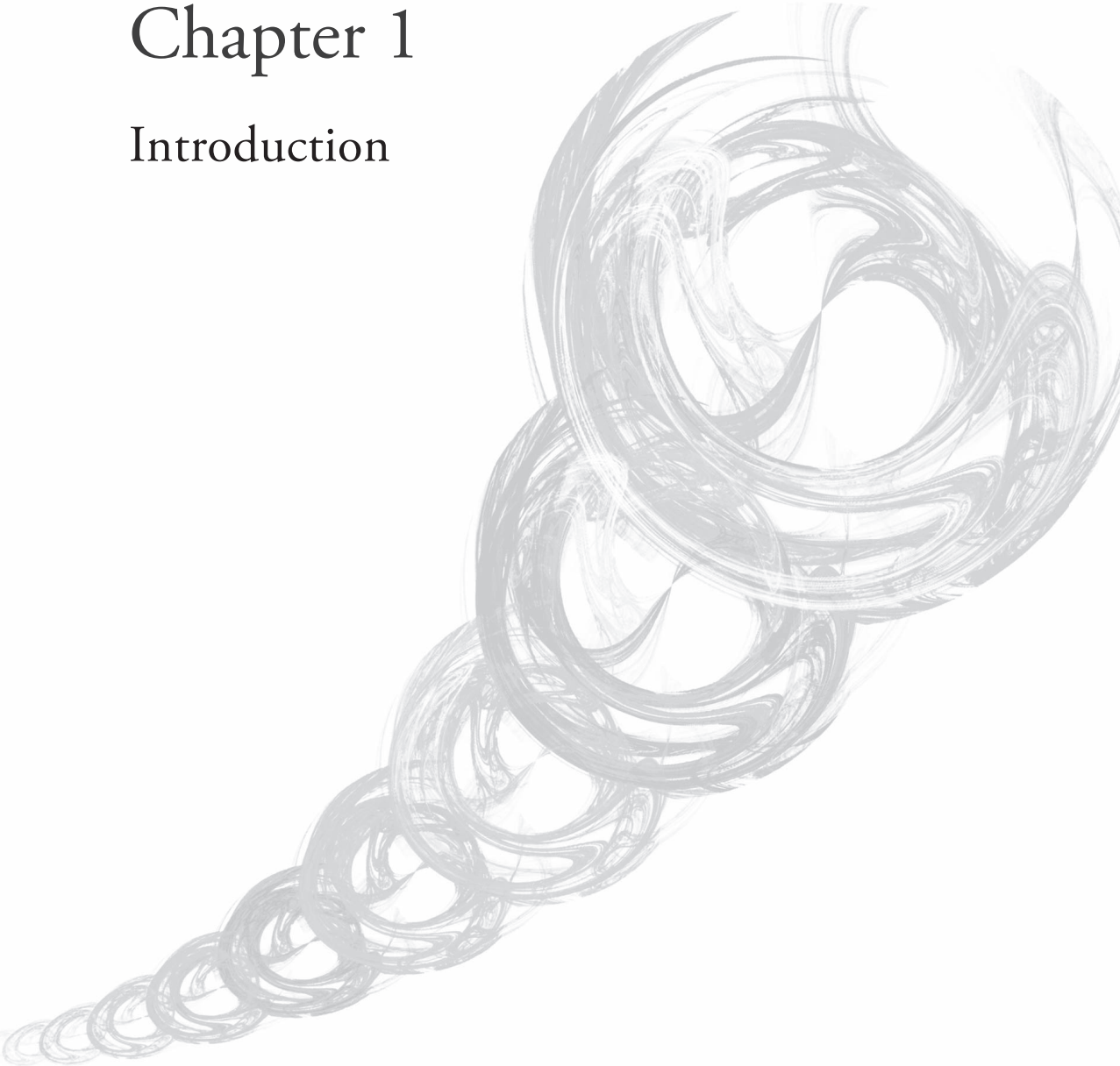
Chapter 1

Introduction



INTRODUCTION

Over the last few decades clinical cancer research has developed at accelerating speed, resulting in a tremendous increase of knowledge with regard to tumour biology, hypotheses to interfere with tumour growth and the subsequent development of anticancer therapies. Obviously, the ultimate aim of cancer research is to identify treatment approaches improving overall survival with a good quality of life. Novel anticancer therapies mostly arise from scientific insights in preclinical studies, and provided that they have looked promising *in vitro* and in animal models, at one point they have to be tested in humans. Testing new cancer therapies in patients occurs in different stages, i.e. successively in phase I, phase II and phase III trials.

Phase I trials are traditionally designed to determine the recommended dose and schedule of a new drug or combination for subsequent studies. The recommended regimen of the new drug or combination is based on the encountered toxicity together with pharmacokinetic and preferably pharmacodynamic properties.

Pharmacokinetics form a crucial part of phase I studies. Pharmacokinetic data give insight in the way the body handles the drug, i.e. its absorption, distribution, metabolism and excretion. Pharmacokinetics is thus based on changes in drug concentration as a function of time and mathematical data analysis allows for calculation of parameters such as clearance, volume of distribution, area under the curve (AUC) and half-life ($t_{1/2}$) of the studied drug. Pharmacokinetics can be used to define the most optimal schedule and to check whether drug concentrations achieved are comparable to those that yielded antitumour activity in preclinical models. Mostly, these data are based upon drug concentrations measured in blood, but it is questionable whether the obtained blood pharmacokinetic profile is directly related to tumour or tissue concentrations. For some cytotoxics a considerable difference in plasma, tissue and tumour concentrations was demonstrated in animal studies.[1-3] Also, the association with clinical parameters such as drug efficacy and toxicity is not always concordant. In view of this, it would be important to know more about pharmacokinetic processes at the tumour site itself (or at any tissue that can serve as a better surrogate for tumour than plasma) and be able to compare plasma and tumour pharmacokinetic profile, with the subsequent possibility to more accurately recommend dose and treatment schedule for further clinical testing. Next to pharmacokinetic profiling of a drug, it is just as important to understand the pharmacological effects of the administered agent on the body or target tissue. These so-called pharmacodynamic parameters may include molecular changes, antitumour effects or the occurrence of drug-specific toxicity. In particular with the development of new agents

targeting specific receptors or signalling pathways in tumour cells, pharmacodynamic data may provide evidence that the agent of interest actually reaches or modulates the putative target. Nevertheless, pharmacodynamic profiling in phase I trials is still often non-decisional and mainly exploratory, and used to create a dose – pharmacodynamic response curve at the investigated dose. This is mainly due to the fact that the best biomarkers for proof of target inhibition or activity have not been identified yet or standardized and validated assays for these biomarkers lack.[4]

Of great importance for phase I studies is to include as few patients as possible to limit the number of patients exposed to ineffective doses of the investigational agent. These experimental studies are usually offered to patients with advanced cancer for whom no standard treatment exists or is no longer effective. Several phase I study designs are being applied but most phase I trials follow a classic 3x3 design: at each dose level three patients are accrued with dose escalation to the next higher cohort if dose limiting toxicity (DLT) is < 33%. If one of the first three patients experiences a DLT, the cohort will be expanded to a total of six patients. If two or more of these six patients encounter a DLT, the maximum tolerated dose has been exceeded. Usually the recommended dose for further studies is one dose level below the maximum tolerated dose. At the recommended dose for further studies, extra patients are included at that dose level to gain more insight in the toxicity (and pharmacokinetic) profile, and provided that toxicity is not aggravated, this dose will be used in phase II studies.[5,6]

Phase I studies addressing the feasibility of the addition of a new agent to a standard treatment strategy are particularly challenging, as it is difficult to draw firm conclusions on additional toxicity caused by the new agent when the standard treatment already induces toxicity by itself. For that purpose, a subsequent randomized phase II trial would be needed to obtain more robust data. The possible interference of toxicity caused by the new agent with the background toxicity of standard treatment raises even more difficulties for combination phase I trials with curative intent: delay of standard treatment is unacceptable in that case but this may result in premature halt of dose escalation and thereby influence trial outcome and decisions regarding further drug combination development.[7]

All together, there are several aspects of phase I studies that need improvement. These include novel means to get insight into the exact pharmacokinetics of new regimens, better pharmacodynamic parameters, and novel study designs, in particular for phase I studies exploring combinations of antitumour compounds.

In phase II trials the clinical activity of the recommended dose of the drug or combination identified in the phase I trial is tested in a more defined and homogeneous patient population in order to identify as swiftly as possible whether or not a novel anticancer

approach should be investigated further. A parameter reflecting antitumour activity is the traditional primary end point for phase II trials. Commonly used end points in this regard are parameters reflecting tumour shrinkage, such as offered by the Response Evaluation Criteria in Solid Tumours (RECIST), or by parameters reflecting prolonged disease stabilisation such as progression free rate (PFR) at a specified time point.[8] However, it should be realised that these surrogate efficacy parameters in phase II trials are poor surrogates for overall survival (OS). As a result, approaches that yielded promising antitumour activities in phase II studies fail in phase III studies.

Another important point of attention in phase II studies is the wish to avoid exposing more than the minimum number of patients to a potential inactive regimen. As a result, numerous different phase II trial designs have been proposed. Increasingly applied are phase II study designs in which there are two or more stages of accrual, with a stopping rule for early discontinuation at the end of each stage once predetermined criteria with regard to insufficient activity are met. Depending on the pursued antitumour activity of a new approach and the predefined error limits with regard to the probability of accepting an inactive drug or rejecting an active drug (i.e. false positivity vs. false negativity) the sample size of the phase II trial can be computed. In case of evaluation of the addition of a new drug to an established anticancer treatment, a randomized comparative phase II trial is highly recommended in order to prevent a selection bias in the patient population that may lead to erroneous results and decision making for further investigation.[9] By including a control arm it is possible to compare the obtained results to historic data, which gives an impression whether selection bias might have occurred. A randomized phase II trial can thus provide strong evidence whether the new combination deserves further testing in a phase III trial, but is underpowered to determine true superiority.[4]

Other objectives of phase II trials include further characterization of the toxicity profile of the new drug or treatment, often accompanied by translational research to further explore the relevance of earlier assessed pharmacodynamic parameters. Pharmacodynamic end points in phase II trials include biomarkers for proof of target inhibition and an evaluation of the possible correlation between biomarker(s) and clinical outcome. The number of patients in phase II trials is often too small to reach significance in this regard, but it does allow for the identification of associations further to be analyzed in phase III studies, e.g. to identify subpopulations most likely to benefit or experience harm from therapy.[10,11]

In spite of well thought-out phase II trial designs, it has been shown that it is hard to accurately predict success of new anticancer treatments in phase III trials, underlining the importance of defining new primary endpoints with respect to antitumour activity, novel phase II designs and translational research to establish robust and validated

pharmacodynamic parameters enabling the identification of patients likely to respond to a certain therapy.[4]

In a phase III trial the new agent or combination is head-to-head compared with the "gold standard" for a certain cancer or with best supportive care in case no treatment is available. Such randomized controlled trial designs, involving many patients, are often multicenter and time consuming and therefore very expensive to conduct. The aim of a phase III trial is to show the capability of a new agent or combination to improve outcome, mostly with regard to efficacy but sometimes also to introduce an equally effective but less toxic or more convenient regimen. Preferably, phase III trials should include an interim analysis to allow for early termination in case of futility of the experimental arm or for cross over in case of distinct better outcome.[12] For long, overall survival was the primary end point used most with regard to efficacy, but as more agents became available and patients nowadays often receive multiple lines of poststudy treatments, overall survival lost some of its power. For instance, because the possibility for patients to be treated with other agents before or after a trial inherently results in a somehow biased study population and secondly because the increase in tumour treatments has also resulted in an improved survival, making efficacy trials last endlessly. Therefore, surrogate end points such as disease free survival (DFS) and progression free survival (PFS) are currently commonly used in phase III trials to describe clinical benefit.[13,14] However, as improved overall survival and/or a better quality of life are still the most important goals, it is questionable whether this is a step in the right direction.

AIMS OF THE THESIS

The road for new anticancer strategies from hypothesis to FDA and/or EMEA-approval is long and often paved with numerous obstacles and disappointments. Despite major investments in (pre)clinical cancer research only 1 out of 20 anticancer agents investigated in clinical trials will eventually receive marketing authorization. This underlines the necessity to keep focussing on the scientific reasoning for a certain strategy and taking it step-by-step without jumping to conclusions based on preclinical data. This thesis carries five early clinical cancer trials, each one addressing specific issues of early clinical trials.

As noted before, many of the currently used anticancer treatments are based on the acquired concentrations of the involved drugs measured in blood. However, antitumour response is correlated to the concentration of a drug at the target site, i.e. the tumour. Since plasma and tumour pharmacokinetics may not per definition be similar, this may

have consequences for the optimal dosing or schedule of a drug. In order to gain more insight in tumour pharmacokinetics and processes there involved we used the technique of microdialysis in patients with an easy accessible (sub)cutaneous tumour or metastasis. These proof of concept trials were performed with two different drugs and administration schemes. First, we assessed the feasibility of prolonged microdialysis sampling in cancer patients treated with an iv bolus of carboplatin, while the second trial investigated tumoural 5-fluorouracil concentrations during a 5-day continuous infusion. These trials are described in Chapter 2 and 3.

Although pharmacokinetic profiling has been firmly incorporated in phase I trials for long, the importance of pharmacodynamic profiling is increasingly being appreciated. Especially with the introduction of the new class of anticancer drugs, the targeted therapies, it may be helpful to have solid information whether they indeed influence their targeted receptor or signalling pathway. The phase I trials described in Chapter 4 and 6 each show how pharmacodynamic assessment can be part of a trial design and may be of help in making decisions with regard to the further development of a drug or combination regimen, for a multityrosine kinase inhibitor and an inhibitor of the mammalian target of rapamycin (mTOR), respectively. To support Chapter 6, Chapter 5 provides a review on the use of inhibitors of mTOR in solid tumours.

Once dose and schedule for further investigation have become clear, the next stage in drug development is a phase II trial. The clinical activity of a drug or regimen is tested in a more selected patient population with the aim to clarify as quickly as possible whether the new approach deserves further testing in a large phase III trial. Chapter 7 describes the results of a randomized phase II trial comparing the efficacy and safety of pravastatin added to epirubicin, cisplatin and capecitabine in patients with advanced gastric carcinoma. By including a control arm, efficacy results could also be compared with historic data regarding this regimen and selection bias could be excluded.

REFERENCES

1. Zamboni WC, Strychor S, Joseph E, Parise RA, Egorin MJ, Eiseman JL (2008) Tumor, tissue and plasma pharmacokinetic studies and antitumor response studies of docetaxel in combination with 9-nitrocamptothecin in mice bearing SKOV-3 human ovarian xenografts. Cancer Chemother Pharmacol 62:417-426.
2. Zamboni WC, Strychor S, Joseph E, et al (2007) Plasma, tumor, and tissue disposition disposition of STEALTH liposomal CKD-602 (S-CKD602) and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts. Clin Cancer Res 13:7217-7223.
3. Zamboni WC, Houghton PJ, Hulstein JL, et al (1999) Relationship between tumor extracellular fluid exposure to topotecan and tumor response in human neuroblastoma xenograft and cell lines. Cancer Chemother Pharmacol 43:269-276.
4. Adjei AA, Christian M, Percy Ivy S (2009) Novel designs and end points for phase II clinical trials. Clin Cancer Res 15:1866-1872.
5. Percy Ivy S, Siu LL, Garrett-Mayer E, Rubinstein L (2010) Approaches to phase I clinical trial design focused on safety, efficiency and selected patient populations: A report from the Clinical Trial Design Task Force of the National Cancer Institute Investigational Drug Steering Committee. Clin Cancer Res 16:1726-1736.
6. DeVita VT Jr, Lawrence TS, Rosenberg SA et al (2008) Cancer: principles and practice of oncology. Lippincott, Williams & Wilkins.
7. Hamberg P, Verweij J (2009) Phase I drug combination trial design: walking the tightrope. J Clin Oncol 27:4441-4443.
8. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216.
9. Van Glabbeke M, Steward W, Armand JP (2002) Non-randomised phase II trials of drug combinations: often meaningless, sometimes misleading. Are there alternative strategies? Eur J Cancer 38:635-638.
10. Booth CM, Calvert AH, Giaccone G et al (2008) Endpoints and other considerations in phase I studies of targeted anticancer therapy: recommendations from the task force on Methodology for the Development of Innovative Cancer Therapies (MDICT). Eur J Cancer 44:19-24.
11. Dancey JE, Dobbin KK, Groshen S et al (2010) Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res 16:1745-1755.
12. Parulekar WR, Eisenhauer EA (2002) Novel endpoints and design of early clinical trials. Ann Oncol 13:139-143.
13. Methy N, Bedenne L, Bonnetain F (2010) Surrogate endpoints for overall survival in digestive oncology trials: which candidates? A questionnaires survey among clinicians and methodologists. BMC Cancer 10:277.
14. Shi Q, Sargent DJ (2009) Meta-analysis for the evaluation of surrogate endpoints in cancer clinical trials. Int J Clin Oncol 14:102-111.

Chapter 2

Application of prolonged microdialysis sampling in carboplatin-treated cancer patients

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ABSTRACT

PURPOSE To better understand mechanisms underlying (in)sensitivity of tumours to anticancer drugs, assessing intratumour drug pharmacokinetics (PK) could be important. We explored the feasibility of microdialysis in tumour tissue for multiple days in a clinical setting, using carboplatin as model drug.

METHODS Plasma and microdialysate samples from tumour and adipose normal tissue were collected up to 47 hours after dosing in eight carboplatin-treated patients with an accessible (sub)cutaneous tumour.

RESULTS Pharmacokinetics were evaluable in tumour tissue in 6/8 patients and in adipose normal tissue in 3/8 patients. Concentration time-curves of unbound platinum in both tissues followed the pattern of the curves in plasma, with exposure ratios of tissue versus plasma ranging from 0.64 to 1.46.

CONCLUSIONS Microdialysis can be successfully employed in ambulant patients for multiple days, which enables one to study tissue PK of anticancer drugs in normal and malignant tissues in more detail.

INTRODUCTION

In clinical oncology many treatment regimens are in part based on acquired concentrations of the involved anticancer drugs measured in blood. This so-called pharmacokinetic (PK) profile in the peripheral circulation has been established for many of the currently used cytotoxic drugs, but varies largely between patients because of inter-individual differences in capacity to metabolize and eliminate the administered agent. Furthermore, the obtained blood PK profile may not be informative enough or may not per definition be related to tumour or tissue concentrations. This was demonstrated e.g. in mice bearing tumour xenografts treated with docetaxel, camptothecin-analogues and topotecan respectively, where plasma, tissue and tumour concentrations differed considerably.[1-3] Also, clinical parameters such as drug efficacy and toxicity are quite often not concordantly associated. Consequently, it would be interesting to gain more information about the pharmacokinetic processes at the tumour site itself.

The technique of microdialysis allows for the evaluation of tumour and tissue disposition of drugs. Microdialysis is a minimally invasive sampling method based on the diffusion of analytes from the interstitial compartment (i.e. extracellular fluid) through a semipermeable membrane. After inserting a microdialysis catheter into selected tissue followed by perfusion with an isotonic fluid, the perfusate, exchange of endogenous and/or exogenous compounds from the extracellular fluid into the perfusate takes place following their concentration gradient. The solution that exits the catheter, the microdialysate, is then collected for analysis (Figure 1A). Since there is no net fluid exchange over the membrane during microdialysis, continuous sampling for prolonged periods is possible without interfering with the pharmacokinetic behaviour of the drug.[4] The concept of microdialysis has been optimized in neurological research where microdialysis was used to monitor neurotransmitter concentrations in brain tissue. Microdialysis was shown to be applicable in oncology as well. Clinical drug disposition studies using microdialysis were performed with several anticancer drugs, such as 5-fluorouracil, capecitabine, cisplatin, carboplatin, dacarbazine and methotrexate.[5-11]

However, in these studies with anticancer drugs performed up till now, the pharmacokinetic data derived using microdialysis were collected only during a relative short period of time, i.e. up to a maximum of four hours, which might be too short to obtain informative pharmacokinetic profiles. Therefore in the present study, the feasibility of microdialysis was studied for multiple days. Carboplatin was chosen as model drug since this drug exists in two distinguishable fractions in the plasma compartment; the free fraction dissolved in plasma water (i.e. unbound fraction), which is thought to be able to penetrate tissues, and the irreversibly to plasma protein bound fraction. Given the relative

slow plasma clearance of the unbound fraction of carboplatin in comparison with for example the unbound fraction of the other widely used platinum containing anticancer agent cisplatin, it was expected that carboplatin-derived platinum concentrations could be quantitated in the dialysates for several days after the administration.[12] Investigation of normal tissue was conducted as well to compare the data obtained through microdialysis, with PK data from the tumour site.

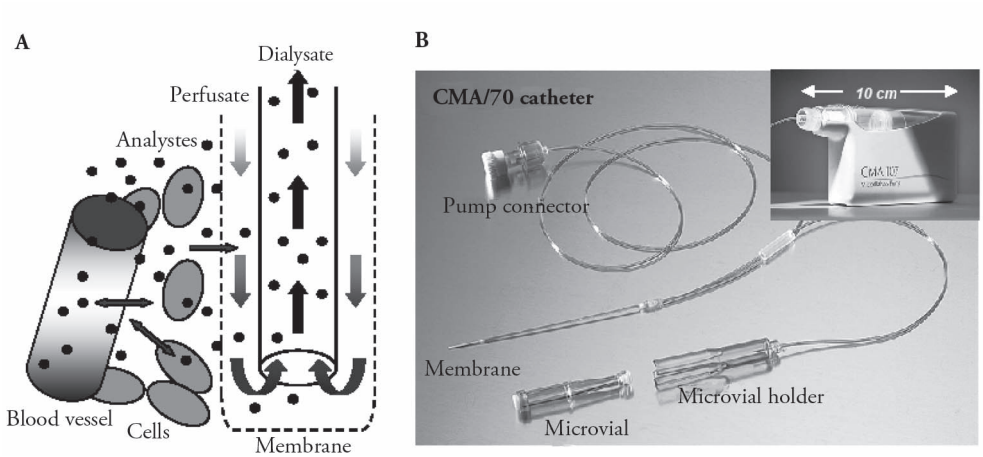

Figure 1 | Schematic representation of a microdialysis probe and the diffusion path of an analyte in tissue (A) and microdialysis instruments (B).

PATIENTS AND METHODS

Patient Selection Criteria

Patients who had a (sub)cutaneous primary tumour or metastasis and for whom carboplatin-based therapy was a viable treatment option were eligible for the study. The (sub)cutaneous lesion had to be of sufficient size (i.e. ≥ 20 mm) and readily accessible for catheter implantation. Other inclusion criteria included: no use of therapeutic doses of anticoagulans (prophylactic use was allowed); no previous serious allergic reactions to platinum compounds; WHO performance status ≤ 1; age ≥ 18 years; absolute neutrophil count ≥ 1.5 x 10^9/L; absolute platelet count ≥ 100 x 10^9/L; creatinine clearance ≥ 60 mL/min.; no other serious illness or medical unstable condition requiring treatment or history

of psychiatric disorder that would prohibit the understanding and giving of informed consent. This single-center study was approved by the ethical committee of the Erasmus Medical Center (Rotterdam, the Netherlands) and was performed in accordance with standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients gave written informed consent.

Administration of Carboplatin

Carboplatin (Carbosin, Pharmachemie, Haarlem, the Netherlands) was administered as a 1h intra-venous infusion, at an AUC of 6 mg/mL*min based on the Calvert formula.[13] To prevent allergic reactions and nausea and vomiting a premedication schedule consisting of dexamethasone, clemastine, ranitidine and granisetron was administered intravenously prior to the start of the carboplatin infusion. In case carboplatin was combined with another cytotoxic agent (e.g. paclitaxel), this second agent was administered approximately 48 hours after the administration of carboplatin.

Plasma pharmacokinetic and microdialysis sampling were performed during a single treatment course.

Microdialysis instruments and catheter insertion

The microdialysis catheters (CMA 70, brain catheters), pumps (CMA 107, flow rate adjustable) and microdialysis consumables were purchased from CMA/Microdialysis AB (Solna, Sweden; Figure 1B). The applied microdialysis instruments were, at least at start of the clinical study, the only devices CE-labeled according to the Medical Device Directive (MDD) for use in peripheral tissue and have been approved for clinical use. Prior to catheter insertion the surface of the skin was locally anaesthetized with topical lidocaine/prilocaine cream and thereafter disinfected according to local procedures. Microdialysis catheter insertion took place according to the procedure previously described by Müller.[14] Each patient had one microdialysis catheter inserted in a suitable (sub)cutaneous tumour lesion and a second one into healthy abdominal subcutaneous adipose tissue. Correct positioning of the catheter at the tumour site was determined by ultrasound.

After implantation, the microdialysis syringe was filled with 2.5 mL of Ringer's solution (147 mmol/L Na^+, 2.25 mmol/L Ca^{2+}; 4.00 mmol/L K^+, and 156 mmol/L Cl; Baxter, Utrecht, The Netherlands), connected to the microdialysis catheter and then placed in the pump. The pump automatically started with a 5-6 minute flush period (flow 15 µL/min) after which the flow automatically decreased to the preset flow rate of 0.5 µL/min.

Since implantation of the microdialysis catheter could cause adverse tissue reactions (i.e. inflammatory effects), the catheter was implanted the day before the carboplatin

infusion to allow for enough time to let these tissue reactions subside.[15] Earlier studies showed that the minimally invasive technique of microdialysis implantation has generally no effect on the performance of the implanted microdialysis catheters.[14-16] Sampling with microdialysis probes was started on the day of implantation (day 0) and up to a maximum of 48 hours after carboplatin infusion. Carboplatin infusion was started 2h after the recovery determination on day 1, during which time period carboplatin was washed out from the periprobe fluid (data not shown). Figure 2 depicts the order of procedures undertaken in this study.

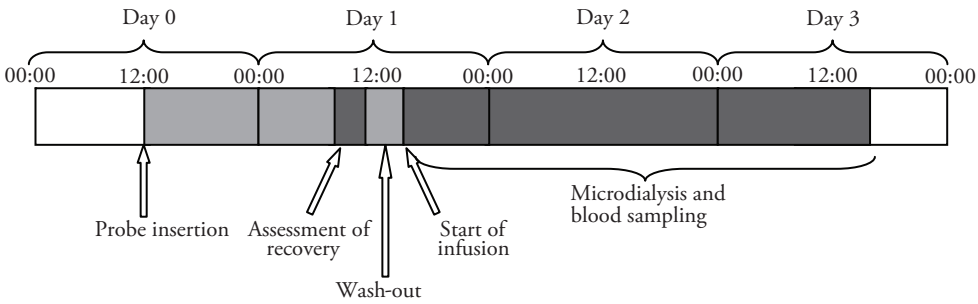

Figure 2 | Time frame describing order of procedures.

Assessment of probe recovery

As microdialysis is not performed under equilibrium conditions, the concentration of the drug in the dialysate will be different from that in the fluid around the probe (i.e. in the tissue of investigation). The relationship between the dialysate concentration and the concentration around the probe is called relative recovery, which can be depicted in a ratio.[17] As the rates of diffusion of most analytes differ between aqueous solutions and tissue, adequate validation of the *in vivo* recovery is required. Solely *in vitro* calibration of a microdialysis probe is insufficient, but is useful to investigate (i) the potential absorption of analyte to the microdialysis probe and (ii) the potential effect of the analyte concentration, at clinical relevant concentrations, on the recovery.[18]

The *in vitro* recovery of the CMA 70 catheters was assessed at ambient temperature using the extraction recovery (Equation 1) as well as the retrodialysis (Equation 2) method.

$$\text{Recovery} = \text{Conc}_{\text{dialysate}}/\text{Conc}_{\text{solution}} \times 100\% \quad \text{(Equation 1)}$$

$$\text{Recovery} = (\text{Conc}_{\text{perfusate}} - \text{Conc}_{\text{dialysate}})/\text{Conc}_{\text{perfusate}} \times 100\% \quad \text{(Equation 2)}$$

Using the extraction recovery method, the recovery was determined at a concentration of 2.0 and 20 µg/mL carboplatin (Carbosin, Pharmachemie, Haarlem, the Netherlands) in Ringer's solution at a flow rate of 0.5 µL/min, at a concentration of 20 µg/mL carboplatin at flow rates of 0.5, 1.0 and 2.0 µL/min and at a concentration of 20 µg/mL carboplatin at a flow rate of 0.5 µL/min during three days. Using the retrodialysis method, the recovery was assessed at a concentration of 20 µg/mL carboplatin at a flow rate of 0.5 µL/min. Three consecutive 30 min samples were taken for each experiment after an equilibration time of at least 30 min. Samples were stored at T< -20°C until analysis.

The *in vivo* recovery for each inserted probe was assessed on the morning of day 1, before the start of the infusion, using the retrodialysis method by perfusing a Ringer's solution containing 20 µg/mL of carboplatin through each inserted probe at a flow rate of 0.5 µl/min (patient 1 perfused at 0.5 and 1.0 µL/min). After equilibration for 15 to 30 minutes, 3 to 5 dialysate samples were collected every 30 minutes and processed as described above. The recovery (Equation 2) was calculated as the mean of the 3 to 5 observations.

Microdialysis sampling

As there is a constant flow of Ringer's solution through the membrane of the catheter, dialysate is sampled continuously. Samples are collected in microvials (Figure 1B) during sample periods varying from 1h during and shortly after the carboplatin infusion up to 3h at later time points. Samples were stored directly at the bed-side of the patient at T< -20°C. The dialysates were collected by centrifugation of the microvials upside down in microcentrifuge reaction vials for 1 min at 500 x g, after which they were stored at T< -70°C until analysis.

Blood sampling

For carboplatin plasma pharmacokinetic analysis, blood samples were collected in the presence of lithium heparin as anticoagulant at the following time points: before start of the intravenous carboplatin infusion, 30 min after start of infusion, at the end of the 1h infusion and at 1, 2, 4, 6, 8, 11, 23, 35 and 47h after the end of infusion. Within 15 minutes after collection, plasma was separated by centrifugation at 3,000xg for 10 min. Subsequently, 500-µL aliquots of the plasma supernatant were mixed with 1.0-mL

aliquots of ice-cold (-20°C) ethanol and stored at T< -20°C. Ethanolic supernatant was collected by centrifugation at 18,000 x g for 5 min after which the clear supernatant was stored at T< -70°C until analysis. The remaining plasma supernatant, for the analysis of carboplatin-derived total platinum, was stored at T< -70°C until analysis.

Carboplatin analysis

For the analysis of carboplatin-derived platinum in dialysate, the methodology was validated in the range of 0.0500 to 1.00 µg/mL (Method 1) and 0.200 to 5.00 µg/mL (Method 2) platinum in Ringer's solution. For Method 1, an aliquot of 100 µL dialysate was mixed with an aliquot of 20 µL 1.0% HNO_3 (v/v), while for Method 2 an aliquot of 20 µL dialysate was mixed with an aliquot of 100 µL 0.2% HNO_3 (v/v). A volume of 20 µL, in duplicate, was subsequently injected onto the graphite furnace of a Perkin Elmer Model 4110 ZL atomic absorption spectrophotometer (Uberlingen, Germany). Platinum peak areas were measured at 265.9 nm. The lower limit of quantitation was established at 0.0500 µg/mL carboplatin-derived platinum for Method 1 and at 0.200 µg/mL for Method 2.

Plasma concentrations of unbound carboplatin-derived platinum were determined according to a method as described for cisplatin-derived platinum.[19] This method was found to be equivalent to the ultrafiltration procedure, as described earlier by Ma and Johnsson and has been validated in our laboratory by direct comparison of ultrafiltration and ethanolic deproteinization.[20,21] On the day of analysis aliquots of 1000 µL of the ethanolic supernatant were evaporated to dryness under nitrogen at T=80°C, and the residue reconstituted in 200 µL diluent (i.e., water containing 0.2% (v/v) Triton X-100 and 0.06% (w/v) cesium chloride), from which subsequently aliquots of 20 µL were injected onto the graphite furnace as described above. The lower limit of quantitation was established at 0.0300 µg/mL.

For total platinum analysis, plasma aliquots were accurately 6-fold diluted in diluent (i.e. 100 µL plasma mixed with 500 µL diluent) from which subsequently also aliquots of 20 µL were injected onto the graphite furnace. The lower limit of quantitation for total carboplatin-derived platinum in plasma was established at 0.200 µg/mL.

Pharmacokinetic data analysis

Individual pharmacokinetic parameters for both total and unbound carboplatin-derived platinum in plasma as well as for carboplatin-derived platinum in dialysate were estimated using noncompartmental analysis using the software program WinNonLin 5.0 (Pharsight, CA, USA).

RESULTS

Patients and Microdialysis technique

Nine patients were included in the study between March and October 2007 and six patients were assessable for pharmacokinetic analysis of carboplatin in the extracellular fluid (ECF) of tumour tissue. One patient never started therapy because of rapidly progressing disease. In another patient there was leakage of the microdialysis catheter which led to inadequate sampling and therefore, catheters at both tumour and healthy tissue were removed. A third patient was considered non-evaluable because of a highly variable and decreasing recovery. The mean of the five recovery samples in that patient was 43.4% with a SD of 9.8%, with a manifest decrease in time. A summary of demographic characteristics of the six patients assessable for pharmacokinetic analysis in the ECF of tumour tissue is presented in Table 1.

Table 1 | Patient demographics of the six patients

Patient	Age	Gender	Tumour Type	Tumour probe site[1]
1	49	F	Melanoma	Thoracic wall
2	31	M	Melanoma	Leg
3	53	M	ACUP	Cervical lymph node
4	63	M	Eccrine carcinoma	Axilla
5	65	M	Esophagus carcinoma	Supraclavicular lymph node
6	57	F	ACUP	Thoracic wal

[1] Probes in subcutaneous normal adipose tissue were all placed in the abdomen.

ACUP: Adenocarcinoma of Unknown Primary.

Probe recovery

Microdialysis is not performed under equilibrium conditions, so the concentration of the drug in the dialysate will be different from that in the ECF of tumour tissue. The relative recovery refers to the relation between the drug concentration in the dialysate and the estimated ECF drug concentration and is used to calculate the actual concentration in the ECF.[17]

From initial *in vitro* recovery experiments (data not shown), it was anticipated that the microdialysis study could be performed at a flow rate of 1.0 µL/min. At this flow rate, the recovery of carboplatin-derived platinum in tumour tissue of the first studied

patient was low with a value of 26.5 ± 3.0%. In this patient the flow rate was subsequently decreased to 0.5 µL/min resulting in a higher recovery of 44.8 ± 2.5%. All subsequent patients were dialyzed at a flow rate of 0.5 µl/min and after equilibration for 15 to 30 minutes, 3 to 5 dialysate samples were collected every 30 minutes.

As shown in Table 2, the *in vitro* recoveries in the subsequent performed experiments were found to be higher compared to the observed in vivo recoveries, with values in the range of 94 to 98% observed at a flow rate of 0.5 µL/min. By increasing the flow rate to 1.0 and 2.0 µL/min, values of 81.4 ± 3.1% and 61.9 ± 1.2% were observed, respectively. The in vitro recovery was independent of the concentration tested and the method used, was found to be constant over three studied days and suggested carboplatin did not significantly bind to the microdialysis catheter.

Table 2 | In vitro recovery of carboplatin at a flow rate of 0.5 µL/min (data are presented as mean ± SD of three measurements)

	20 µg/mL carboplatin		**2.0 µg/mL carboplatin**
	Extraction Recovery(%)	**Retrodialysis (%)**	**Extraction Recovery (%)**
Day 1	95.7 ± 1.9	ND	ND
Day 2	94.1 ± 4.1	96.2 ± 0.9	ND
Day 3	95.5 ± 3.6	ND	97.9 ± 1.4

At a flow rate of 0.5 µL/min, the *in vivo* recovery in the six for tumour tissue evaluable patients varied between 44.8 to 75.8% (Table 3) and was found to be very constant in each individual patient. The recovery in adipose subcutaneous tissue in the three for normal tissue evaluable patients ranged between 25.5 and 62.9% (Table 3). In the remaining cases, one catheter was removed because of leakage, while in another patient due to incorrect flow setting of the pump, variable dialysate volumes and, as a result, highly variable recoveries were observed. In patient 6 recovery decreased in subsequent dialysates, with a extremely low recovery of 0.4% in the fifth sample taken.

Pharmacokinetics

In Table 3, a summary of the pharmacokinetic data of carboplatin-derived total and unbound platinum in plasma as well as unbound platinum in the ECF of tumour and adipose normal tissue is presented. As is also shown in Figure 3, the pharmacokinetic profiles of unbound platinum in the ECF of tumour and adipose tissue are identical to

Table 3 | Summary of pharmacokinetic data carboplatin-derived unbound platinum in plasma and tissue

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Dose Carboplatin (mg)	700	1150	850	780	700	780
Dose Platinum (mg)	368	604	447	410	368	410
Plasma						
C_{max} (µg/mL)	25.0	21.7	27.1	23.8	19.3	18.8
$AUC_{0\text{-}inf}$ (µg*h/mL)[5]	63.3	57.0	59.6	74.2	41.0	53.1
CL (L/h)	5.81	10.6	7.50	5.53	8.98	7.72
$T½_z$ (h)	4.13	4.02	4.97	5.41	4.04	4.05
Tumour Tissue						
Probe recovery (%)[1]	44.8 ± 2.5	59.9 ± 2.3	69.3 ± 1.8	75.8 ± 4.4	52.2 ± 2.0	74.4 ± 4.0
	(41.9-46.3; 3)	(54.6-60.1; 5)	(66.5-71.5; 5)	(70.1-80.5; 5)	(49.6-53.9; 5)	(68.1-77.6; 5)
C_{max} (µg/mL)	21.7	21.3	24.8	13.9	13.4	18.3
$AUC_{0\text{-}inf}$ (µg*h/mL)[5]	64.3	62.5	65.6	47.2	33.9	57.4
$T½_z$ (h)	3.64	3.85	2.70	5.68	3.71	3.08
Adipocytic Normal Tissue						
Probe recovery (%)[1]	NA[3]	25.5 ± 1.2	62.9 ± 5.4	49.6 ± 2.7	17.3 ± 9.9[4]	10.8 ± 9.8[2]
		(24.1-26.9; 4)	(53.8-68.2; 5)	(46.5-53.9; 5)	(10.1-34.5; 5)	(21.1→0.4; 5)
C_{max} (µg/mL)	NA	25.5	19.6	12.5	NA	NA
$AUC_{0\text{-}inf}$ (µg*h/mL)[5]	NA	83.5	51.5	52.9	NA	NA
$T½_z$ (h)	NA	3.50	4.26	4.37	NA	NA
Ratio's						
AUC Tumour/Plasma	1.02	1.10	1.10	0.64	0.83	1.08
AUC Adip. Tissue/Plasma	NA	1.46	0.86	0.71	NA	NA

C_{max}; maximum observed concentration, AUC; area under the concentration time curve, CL; clearance, $T½_z$; terminal disposition half-life, NA; not available; [1]mean ± SD (range; number of observations) ; [2]Highly variable recovery decreasing in subsequent samples; [3] leakage of probe observed; [4]Fluctuating and high flow of perfusate; [5]Calculated using observed data up to 24h after start of infusion

the profiles of unbound platinum in the plasma compartment. Carboplatin thus well distributed to the tumour and adipose tissue, while no sequestration of the drug in the ECF of the tissues was observed.

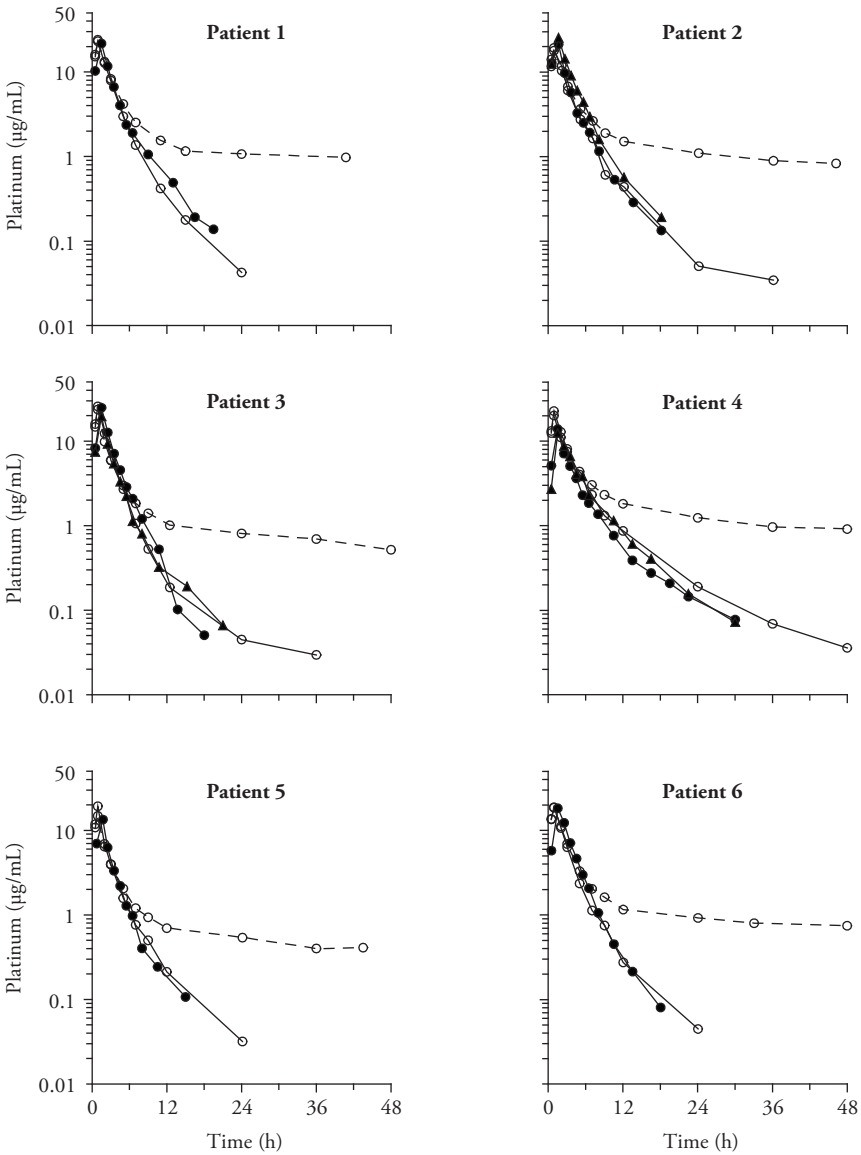

Figure 3 | Concentration time curves of carboplatin-derived total platinum in plasma (open circles, dashed line) and unbound platinum in plasma (open circles, solid line), ECF of tumour (filled circles) and ECF of normal adipose tissue (filled triangles) in the six evaluable patients.

DISCUSSION

Previously, several studies have explored microdialysis in cancer patients in order to study tissue PK of antitumour drugs. However in these studies, sampling was only conducted over a limited period of time (maximum of four hours). In this study, we have shown that microdialysis in cancer patients is also feasible for prolonged periods of time without restriction of patients' mobility. This allows examining antitumour drugs in more detail, in particular those drugs with a relative slow clearance such as carboplatin.

With regard to the obtained pharmacokinetic data, results show fairly identical PK-curves of unbound carboplatin at both the tumour site and in plasma. Although consistent with previous findings of Blochl-Daum et al. in which PK data were obtained only up to four hours after administration of carboplatin, the similar AUCs of unbound carboplatin in plasma and tumour ECF of all studied patients were unexpected.[8] For carboplatin, internalization of the compound into (the nucleus of) the cell is necessary in order to induce the formation of toxic platinum-DNA adducts, resulting in cell cycle arrest and/or cell death.[22] Due to fragile vasculature which is more permeable than normal vasculature, the interstitial fluid pressure (IFP) is known to be increased in many solid tumours compared to adjacent normal tissue. This increased IFP forms a barrier for transcapillary transport thereby impairing the uptake of cytotoxic agents.[23] A high tumour IFP is associated with a poor prognosis in breast cancer, colorectal cancer, melanomas and head and neck tumours.[24-27] A potentially increased IFP at the tumour site was thought to be reflected by differences between tumour and plasma exposures of unbound platinum in our study. A possible explanation for the unexpected similarity between plasma and tumour pharmacokinetics could be the co-administration of dexamethasone. Dexamethasone (10 mg) – given as standard anti-emetic treatment – has been shown to lower the IFP of tumours as a consequence of which carboplatin penetration into the tumour lesions could be facilitated.[28] This was demonstrated in a rat model in which the IFP of tumours was significantly lowered after administration of high dose dexamethasone resulting in an increase in the uptake of 5-fluorouracil and in a significant reduction of tumour size compared to controls.[29]

The recovery in normal subcutaneous adipose tissue was fluctuating, raising concerns about the feasibility to use microdialysates from normal adipose tissue as controls. Theoretically this could be related to a problematic catheter implantation with possible disruption of the dialysis membrane, though the implantation procedures were all carried out quickly without complications. Moreover, recovery was stable in tumour tissue, where catheter implantation took place in a similar manner. The underlying mechanisms of the relatively low, variable and over-time decreasing recoveries in adipose normal tissue

remain to be unraveled. A possible explanation could be that subcutaneous tissue is poorly vascularized compared to tumour tissue and/or the occurrence of local vasoconstriction during the course of the experiments. Further research will be necessary to evaluate the definite role of microdialysis in subcutaneous normal tissue.

As ECF sampling in tumour tissue using microdialysis is feasible for multiple days, microdialysis might be a valuable tool in clinical trials. For instance, it could be implemented to study intratumoural release of drugs from liposomes or nanosomes. The applicability has recently been shown in preclinical models for SPI-077, a liposomal formulation of cisplatin. In contrast to conventional cisplatin, platinum was not detectable in the ECF of tumour tissue after the administration of SPI-077, despite sufficient tumour penetration of the liposomes.[30] Another potential application area of microdialysis involves the study of tissue specific distribution and metabolism. Enhanced tumour concentrations of anticancer drugs like e.g. irinotecan, may be achieved by the administration of prodrugs, which have to be activated into active metabolites by enzymes in the tumour. In addition, microdialysis sampling could be helpful in further disentangling the exact sites of metabolism. Microdialysis may also be applied to unravel pharmacokinetic-response relationships. For example, in vivo experiments studying topotecan penetration in two neuroblastoma xenografts showed that topotecan exposure in the ECF of the relative sensitive xenograft was 3.5-fold greater compared to the relative resistant xenograft while plasma exposures were equivalent. This suggests that the sensitivity of the xenografts to topotecan is partly related to the extent of tumour penetration of the drug.[31]

In conclusion, we showed the feasibility of microdialysis in ambulant patients for multiple days, which enables to study tissue pharmacokinetics of anticancer drugs in normal and malignant tissues in more detail. Microdialysis could become an important method to measure drug concentrations at the site of action, as the procedure is relatively simple with no restrictions in mobilization of patients, while data can be obtained with only one catheter in situ for several days. Nevertheless, it should be mentioned that, although microdialysis is a sensitive tool and enables one to obtain unique intratumoural PK information over time, it is still subject to some technical challenge. The failed collection of microdialysis data in 2/8 patients, exemplifies the limitation of this technique. The data presented in this study are still limited given the small number of patients and the high variability in patients. Despite the high variability, an estimation of the range of drug delivery to tumour is achieved and this allows clinical determination if a “minimum” of required concentration is achievedIn the future, microdialysis could be valuable for additional PK/PD information in studies evaluating for example liposome- and immunoliposome-based cancer therapeutics or other carrier-mediated anticancer agents. The use of microdialysis may also be helpful in optimizing dosing and administration schedules of anticancer

agents, in the selection of new cytotoxic agents with the most favourable delivery profile and to gain more insight into mechanisms conferring drug resistance.

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REFERENCES

1. Zamboni WC, Strychor S, Joseph E, Parise RA, Egorin MJ, Eiseman JL (2008) Tumor, tissue and plasma pharmacokinetic studies and antitumor response studies of docetaxel in combination with 9-nitrocamptothecin in mice bearing SKOV-3 human ovarian xenografts. Cancer Chemother Pharmacol 62: 417-426.
2. Zamboni WC, Strychor S, Joseph E, et al (2007) Plasma, tumor, and tissue disposition disposition of STEALTH liposomal CKD-602 (S-CKD602) and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts. Clin Cancer Res 13:7217-7223.
3. Zamboni WC, Houghton PJ, Hulstein JL, et al (1999) Relationship between tumor extracellular fluid exposure to topotecan and tumor response in human neuroblastoma xenograft and cell lines. Cancer Chemother Pharmacol 43:269-276.
4. Müller M (2002) Science, medicine, and the future: microdialysis. Br Med J 324:588-591.
5. Müller M, Mader RM, Steiner B et al (1997) 5-Fluorouracil kinetics in the interstitial tumor space: clinical response in breast cancer patients. Cancer Res 57:2598-601.
6. Mader RM, Schrolnberger C, Rizovski B et al (2003) Penetration of capecitabine and its metabolites into malignant and healthy tissues of patients with advanced breast cancer. Br J Cancer 88:782-787.
7. Tegeder I, Brautigam L, Seegel M et al (2003) Cisplatin tumor concentrations after intra-arterial cisplatin infusion or embolization in patients with oral cancer. Clin Pharmacol Ther 73:417-426.
8. Blochl-Daum B, Müller M, Meisinger V et al (1996) Measurement of extracellular fluid carboplatin kinetics in melanoma metastases with microdialysis. Br J Cancer 73:920-924.
9. Joukhadar C, Klein N, Mader RM et al (2001) Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. Cancer 92:2190-2196.
10. Ekström PO, Andersen A, Saeter G et al (1997) Continuous intratumoral microdialysis during high-dose methotrexate therapy in a patient with malignant fibrous histiocytoma of the femur: a case report. Cancer Chemother Pharmacol 39:267-272.
11. Müller M, Brunner M, Schmid R et al (1998) Interstitial methotrexate kinetics in primary breast cancer lesions. Cancer Res 58:2982-2985.
12. Long DF, Repta AJ (1981) Cisplatin: chemistry, distribution and biotransformation. Biopharm Drug Dispos 2:1-16.
13. Calvert AH, Newell DR, Gumbrell LA et al (1989) Carboplatin dosage: prospective evaluation of a simple formula based on renal function. J Clin Oncol. 7:1748-1756.
14. Müller M, Schmid R, Georgopoulos A et al (1995) Application of microdialysis to clinical pharmacokinetics in humans. Clin Pharmacol Ther 57:371-380.
15. Chaurasia CS, Müller M, Bashaw ED et al (2007) AAPS-FDA Workshop White Paper: microdialysis principles, application and regulatory perspectives. Pharm Res 24:1014 -1025.
16. Ault JM, Riley CM, Meltzer NM et al (1994) Dermal microdialysis sampling in vivo. Pharm Res 11:1631-1639.
17. Kitzen JJ, Verweij J, Wiemer EA et al (2006) The relevance of microdialysis for clinical oncology. Curr Clin Pharmacol 1:255-263.
18. Loos WJ, Zamboni WC, Engels FK et al (2007) Pitfalls of the application of microdialysis in clinical oncology: controversial findings with docetaxel. J Pharm Biomed Anal. 45:288-294.

19. Loos WJ, de Jongh FE, Sparreboom A et al (2006) Evaluation of an alternate dosing strategy for cisplatin in patients with extreme body surface area values. J Clin Oncol 24:1499-1506.

20. Ma J, Stoter G, Verweij J, Schellens JH (1996) Comparison of ethanol plasma-protein precipitation with plasma ultrafiltration and trichloroacetic acid protein precipitation for the measurement of unbound platinum concentrations. Cancer Chemother Pharmacol 38:391-394.

21. Johnsson A, Björk H, Schütz A, Skärby T (1998) Sample handling for determination of free platinum in blood after cisplatin exposure. Cancer Chemother Pharmacol 41:248-251.

22. Wang D, Lippard SJ (2005) Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 4:307-320.

23. Heldin CH, Rubin K, Pietras K et al (2004) High interstitial fluid pressure – an obstacle in cancer therapy. Nat Rev Cancer 4:806-813.

24. Less JR, Posner MC, Boucher, Y et al (1992) Interstitial hypertension in human breast and colorectal tumors. Cancer Res 52:6371-6374.

25. Nathanson SD, Nelson L (1994) Interstitial fluid pressure in breast cancer, benign breast conditions, and breast parenchyma. Ann Surg Oncol 1:333-338.

26. Boucher Y, Kirkwood JM, Opacic D et al (1991) Interstitial hypertension in superficial metastatic melanoma in humans. Cancer Res 51:6691-6694.

27. Gutmann R, Leunig FJ, Goetz et al (1992) Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. Cancer Res 52:1993-1995.

28. Kristjansen PE, Boucher Y, Jain RK (1993) Dexamethasone reduces the interstitial fluid pressure in humon colon adenocarcinoma xenograft. Cancer Res 53:4764-4766.

29. Stuhr LEB, Salnikow AV, Iversen VV et al (2006) High-dose, short-term, anti-inflammatory treatment with dexamethasone reduces tumorgrowth and augments the effects of 5-fluorouracil on dimethyl-□-benzanthrace-induced mammary tumors in rats. Scand J Clin Lab Invest 66:477-486.

30. Zamboni WC, Gervais AC, Egorin MJ et al (2004) Systemic and tumor disposition of platinum after administration of cisplatin or STEALTH liposomal-cisplatin formulations (SPI-077 and SPI-077 B103) in a preclinical tumor model of melanoma. Cancer Chemother Pharmacol 53:329-336.

31. Zamboni WC, Stewart CF, Thompson J et al (1998) Relationship between topotecan systemic exposure and tumor response in human neuroblastoma xenografts. J Natl Cancer Inst 90:505-511.

Chapter 3

Increasing tumoural 5-fluorouracil concentrations during a 5-day continuous infusion: a microdialysis study

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ABSTRACT

PURPOSE Response to anticancer therapy is believed to be directly related to the concentration of the anticancer drug in the tumour itself. Assessment of intratumour drug pharmacokinetics can be helpful to gain more insight into mechanisms involved in the (in)sensitivity of tumours to anticancer therapy. We explored the pharmacokinetics of 5-fluorouracil in both plasma and tumour tissue during a 5-day continuous infusion of 5-fluorouracil in cancer patients. Sampling for measurement of 5-fluorouracil in tumour tissue was performed using microdialysis.

EXPERIMENTAL DESIGN In seven patients with an accessible (sub)cutaneous tumour treated with a continuous 5-fluorouracil infusion, plasma and microdialysate samples from tumour and normal adipose tissue were collected over a period of 5 days.

RESULTS For six patients drug concentrations in both tumour tissue and plasma were available. Concentration-time curves of unbound 5-fluorouracil were lower in tumour tissue compared to the curves in plasma, but exposure ratios of tumour tissue versus plasma increased during the 5-day infusion period. The presence of circadian rhythmicity of 5-fluorouracil pharmacokinetics in the tumour itself was demonstrated as 5-fluorouracil concentrations in tumour extracellular fluid were higher during the night than during daytime.

CONCLUSION Microdialysis was successfully employed in cancer patients during a continuous 5-day 5-fluorouracil infusion. Plasma and tumour pharmacokinetics of 5-fluorouracil differed substantially with increasing 5-fluorouracil concentrations in tumour over time, possibly resulting from a lowered interstitial fluid pressure by 5-fluorouracil itself. This microdialysis 5-fluorouracil model might be useful to monitor the effect of drug delivery modulating strategies in future studies.

INTRODUCTION

Traditionally, most oncological treatment regimens and doses are based on safety profiles. It is generally assumed that the obtained pharmacokinetic profile in blood is directly related to the mentioned safety and the magnitude of antitumour response. However, the efficacy of anticancer drugs will likely depend on the maintenance of adequate unbound drug concentrations in tumour tissue rather than plasma. Furthermore, the pharmacokinetic profile of a drug in blood may not per definition resemble its pharmacokinetic profile in tumour or normal tissue, as was demonstrated in a mouse model.[1-3] More detailed information about drug levels at the tumour site itself would therefore be helpful in optimizing oncological treatment.

In clinical oncology, evaluation of tumour and tissue disposition of drugs has become possible with the technique of microdialysis. This minimally invasive sampling method is based on the exchange of compounds from the extracellular fluid into perfusate, which runs through an inserted catheter, by diffusion through a semipermeable membrane following their concentration gradient. No net fluid exchange occurs during microdialysis, so prolonged continuous sampling is possible without interfering with the pharmacokinetic behaviour of the drug.[4] The exiting solution, the so-called microdialysate, can be used for both pharmacokinetic as well as pharmacodynamic analyses.

The clinical use of microdialysis to evaluate drug disposition in tumours is of considerable interest with the recognition that insufficient drug penetration into the interstitium of solid tumours may be involved in resistance to antitumour drugs.[5,6] One of the problems associated with efficient drug delivery is the presence of increased interstitial fluid pressure, a phenomenon occurring in most solid tumours.[7] To date, several studies have been performed applying the microdialysis technique in clinical oncology with several anticancer drugs.[8-14] In most of these studies the collected data covered only a relatively short period of time, i.e. up to a maximum of 4 hours (h), which might not be adequate to obtain useful pharmacokinetic profiles for many drugs. However, recently we showed the feasibility of prolonged microdialysis sampling up to 48h in ambulant cancer patients treated with intravenous (iv) bolus carboplatin.[15]

In the present study, we investigated the feasibility of microdialysis for even a longer period of time using another drug, namely 5-fluorouracil infused continuously for 5 days. The aim was to accomplish a model which could be used in further studies to monitor the influence of co-administration of other agents on tumoural 5-fluorouracil concentrations, each patient being its own control. 5-Fluorouracil belongs to the group of the antimetabolites and is a widely used agent for the treatment of colorectal, breast and head and neck cancer.[16-18] After entering the tumour cell, 5-fluorouracil is converted into

several metabolites that exert antitumour activity by interfering with DNA synthesis and DNA repair, and by incorporation in RNA. The majority of 5-fluorouracil is inactivated by the enzyme dihydropyrimidine dehydrogenase (DPD), which is mainly found in the liver but also in other tissues such as the gastrointestinal mucosa, peripheral blood cells and tumour tissue.[19] Numerous dose regimens exist, continuous administration at 1000 mg/m^2/daily for 5 days being one of the most commonly used. Given the fact that 5-fluorouracil rapidly reaches a steady state in plasma during continuous infusion, it was expected that 5-fluorouracil concentrations could be quantitated in the microdialysates during the whole 5 times 24 hours period.

The aims of the present study were to explore the feasibility of continuous microdialysis sampling over a 5-day period, to assess differences in plasma and tumour pharmacokinetics of 5-fluorouracil during a continuous 5-fluorouracil infusion and to set up a clinical model, with 5-fluorouracil as model drug, for future drug delivery modulating studies.

PATIENTS AND METHODS

Patient Selection Criteria

Patients who had a (sub)cutaneous primary tumour or metastasis and for whom 5-fluorouracil-based therapy was considered a viable treatment option were eligible for the study. The (sub)cutaneous lesion had to be of sufficient size (i.e. ≥ 20 mm) and readily accessible for catheter implantation. Other inclusion criteria included: no use of therapeutic doses of anticoagulants (prophylactic use was allowed); WHO performance status ≤ 1; age ≥ 18 years; absolute neutrophil count ≥ 1.5 x 10^9/L; absolute platelet count ≥ 100 x 10^9/L; creatinine clearance ≥ 60 mL/min.; no other serious illness or medical unstable condition requiring treatment or a history of psychiatric disorder that would prohibit the understanding and giving of informed consent. This single-center study was approved by the ethical committee of the Erasmus Medical Center (Rotterdam, the Netherlands) and was performed in accordance with standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients gave written informed consent. (EudraCT number: 2006-001070-24).

Administration of 5-fluorouracil

5-Fluorouracil (Ebefluoro, EBEWE Pharma, Belgium) was started as a 5-day continuous intravenous infusion on Day 1, at a dose of 1000 mg/m^2/day. To prevent allergic reactions, nausea and vomiting a premedication schedule consisting of dexamethasone

and granisetron was administered intravenously prior to the start of the 5-fluorouracil infusion. In case 5-fluorouracil was combined with another cytotoxic, this second agent was administered only on the last day of treatment.

Plasma pharmacokinetic and microdialysis sampling were performed during a single treatment course of five days.

Microdialysis procedures

The microdialysis catheters (CMA 63 catheters, membrane length 10 mm, molecular weight cut-off level of ~20,000 Da), pumps (CMA 107, flow rate adjustable) and microdialysis consumables were purchased from CMA/Microdialysis AB (Solna, Sweden). On Day 0, each patient had one microdialysis catheter inserted in a suitable (sub)cutaneous tumour lesion and a second one into healthy abdominal subcutaneous adipose tissue. Catheter insertion procedures have previously been described in detail.[20] The intratumoural location of the probe tip was verified by ultrasound.

As microdialysis is not performed under equilibrium conditions, the concentration of the drug in the dialysate may be different from that in the fluid surrounding the probe.[21] To correct for this difference, the relative recovery of each probe was assessed the morning after the implantation of the probes by retrodialysis with 1 µg/mL of 5-fluorouracil in Ringer's solution as described recently, at a flow rate of 0.5 µL/min (i.e., the flow rate as used during the pharmacokinetic sampling).[15]

Prior to the clinical study, *in vitro* recovery experiments (data not shown) were conducted to demonstrate that 5-fluorouracil does not irreversibly bind to the catheters, the probe recovery is independent of the concentration and is equal in both directions over the probe, similar to previous studies.[15]

During the pharmacokinetic experiment, samples were collected during the following sample periods: 0-2h, 2-5h following the start of infusion and subsequently every 5h till the end of infusion at day 5. The 5-hour interval was chosen as at a flow-rate of 0.5 µL/min the vials could not be overfilled during this period. Samples were stored directly at the bed-side of the patient at T < -20°C. The dialysates were collected by centrifugation of the microvials upside down in micro-centrifuge reaction vials for 1 min at 500 x g, after which they were stored at T < -20°C until analysis.

Blood sampling

For 5-fluorouracil plasma pharmacokinetic analysis, blood samples were collected in the presence of lithium heparin as anticoagulant at the following time points: before start of the infusion, 1, 2, 4, 7 and 12h after the start of infusion and subsequently every 12h till the end of infusion at day 5. Within 15 minutes after collection, plasma was separated by centrifugation at 3,000xg for 10 min and stored at T < -20°C until analysis.

Unbound fraction in plasma

Although the free fraction of 5-fluorouracil has been reported to be > 90%, and plasma protein binding will have a minor influence on the tissue distribution, the percentage binding may be different between patients.[22] Therefore, we estimated the free fraction of 5-fluorouracil in each individual patient. Aliquots of 250 µL of plasma samples collected each 24 hour during the infusion period, were transferred into Microcon Ultracel YM-30 Centrifugal Filter Devices (Millipore Corp., Bedford, MA, USA) with a molecular weight cut-off level of 30,000 Da. The samples were centrifuged in a fixed angle rotor at 18,000 x g for 30 min. The ultrafiltrate was stored at T < -20°C until analysis. The individual observed concentration in each sample was divided by the total concentration. The mean free fraction was subsequently used to estimate the free fraction in all samples collected

5-Fluorouracil analysis

5-Fluorouracil (molecular weight 130.1) in the dialysate (and ultrafiltrate) was quantitated using liquid chromatography (LC) with tandem mass-spectrometric detection (MS/MS). Briefly, a volume of 25 µl of the internal standard solution containing 500 ng/mL 5-chlorouracil in water was added to 25 µL of dialysate, from which aliquots of 25 µL were injected into the LC (Model 2795 XC chromatograph, Waters Alliance, Mildford, MA). Chromatographic separations were achieved on a Hypercarb column (100 x 3 mm internal diameter, 5 µm particle size, Thermo, Breda, the Netherlands), which was held at T = 40°C. The mobile phase was composed of water and acetonitrile and was delivered using a linear gradient setting. The initial flow rate was 0.75 mL/min where the composition changed from 10% to 90% acetonitrile in 1 minute. In the subsequent minute, the flow rate was linearly decreased to 0.25 mL/min and kept at this rate for 6 min. Subsequently, the flow rate was linearly increased to 1.0 mL/min in 1 min and kept at 1.0 mL/min for 2 min. Hereafter the composition changed from 90% to 10% acetonitrile in 1 minute. The overall run time was 12 min. Detection was performed with a Waters MicroMass Quatro Micro triple-quadropole mass spectrometer in the negative ion mode. The detector was programmed to allow the MH^- ions of 5-fluorouracil (m/z 129) and 5-chlorouracil (m/z 145) to pass through the first quadropole and into the collision cell. The collision energy for collision-induced dissociation of 5-fluorouracil and 5-chlorouracil was set at 20 eV and 18 eV, respectively, with argon used as collision gas at a pressure of $6e^{-3}$ mbar. The daughter ions of 5-fluorouracil (m/z 86) and 5-chlorouracil (m/z 102) were monitored through the third quadropole. The dwell time per channel for data collection was 0.150 seconds. Retention times of fluorouracil and 5-chlorouracil were 2.5 and 3.3 min respectively. Calibration curves were linear from 10.0 to 1,000 ng/mL. The accuracy ranged from 94.0% to 103.7%, the within-run precisions were ≤ 12.9% and

the between-run precisions were ≤ 2.7% at five tested concentrations, including the lower limit of quantitation of 10.0 ng/mL.

Plasma 5-fluorouracil concentrations were quantitated likewise. Aliquots of 30 µL plasma were extracted, after the addition of 10 µL of 500 ng/mL 5-chlorouracil, with 1.5 mL ethyl acetate. Following vigorous vortex mixing for 5 min and centrifugation for 5 min at 18,000 x g, the clear supernatant was evaporated to dryness at T = 70°C under nitrogen. The residue was resuspended in 100 µL aliquots of Ringer's solution, from which an aliquot of 50 µL was injected into the system as described above. LC-MS/MS system settings were as described for dialysate. Calibration curves were linear from 20.0 to 2,000 ng/mL. The accuracy ranged from 94.4% to 107.5%, the within-run precisions were ≤ 7.1% and the between-run precisions were ≤ 4.1% at five tested concentrations, including the lower limit of quantitation of 20.0 ng/mL.

During the analysis of the dialysates of the first 3 patients, a significant matrix effect was observed for 5-chlorouracil. The observed peak areas of 5-chlorouracil were significantly lower in dialysate samples compared to the Ringer's solution in which the calibration curve standard and quality control samples were prepared. This results in an overestimation of the 5-fluorouracil concentrations in the ECF. Replacing the internal standard 5-chlorouracil by stable $^{13}C/^{15}N_2$ labeled 5-fluorouracil, which elutes at the same time from the analytical column as 5-fluorouracil, this to mass spectrometry related potentially occurring issue was resolved. Multiple reaction monitoring of $^{13}C/^{15}N_2$ 5-fluorouracil was applied at m/z 132>88, with the cone voltage and collision energy set at 30 and 18 eV, respectively.

Individual pharmacokinetic parameters were estimated using non-weighted noncompartmental analysis using the software program WinNonLin 5.2 (Pharsight, CA, USA). As an increase in the ECF/plasma ratios was observed over time, partial AUCs were then calculated over 24h intervals. Although sampling time points were not based on a 24h cycle, the use of WinNonLin 5.2 allows for interpolation of AUCs.

RESULTS

Patients

Seven patients were included in the study between April 2008 and November 2009, of which six patients were assessable for pharmacokinetic analysis of 5-fluorouracil in the extracellular fluid of tumour tissue. One patient (No 2) was not evaluable as there was not enough microdialysate left for re-analysis following the switch of the internal standard in the bioanalytical methodology. Only one patient (No 4) was evaluable for analysis of

extracellular fluid of normal tissue as probe recovery in the other patients was variable. In two patients (No 1 and 6) treatment of 5-fluorouracil was combined with carboplatin, which was administered on the last day of treatment after termination of the 5-fluorouracil infusion. A summary of demographic characteristics of the six patients assessable for pharmacokinetic analysis in the extracellular fluid of tumour tissue is presented in Table 1.

Table 1 | Patient demographics of the six patients

Patient	Age	Gender	Tumour Type	Tumour probe site[1]
1	60	M	Melanoma	Leg
3	66	M	Esophageal carcinoma	Cervical lymph node
4	52	F	Non small cell lung cancer	Abdominal wall
5	59	M	Melanoma	Axilla
6	49	F	Muco-epidermoid carcinoma	Cervical tumour mass
7	59	F	Breast	Supraclavicular lymph node

[1]Probes in subcutaneous normal adipose tissue were all placed in the abdomen

Probe recovery

The *in vitro* recovery was independent of the concentrations tested and the method applied (i.e., retrodialysis or extraction recovery). Furthermore, the in vitro recovery was constant over up to 10 studied days and showed that 5-fluorouracil does not bind to the microdialysis catheter. For instance, at a flow rate of 1.0 µL/min the recovery was 91 (± 2.2), 82 (± 0.5) and 91 (± 0.2) % on days 1, 4 and 10, respectively. In addition, as increased interstitial fluid pressure is a well known characteristic of several tumours, we tested if increased pressures influenced the recovery.[7,23] Pressures up to 15 mm Hg (i.e., 20 cm of Ringer's solution above the probe) had no effect on the recovery of 5-fluorouracil.

At a flow rate of 0.5 µL/min, the in vivo recovery in the six patients evaluable for tumour tissue drug levels varied from 40-92 % (Table 2) and was constant in each individual patient.

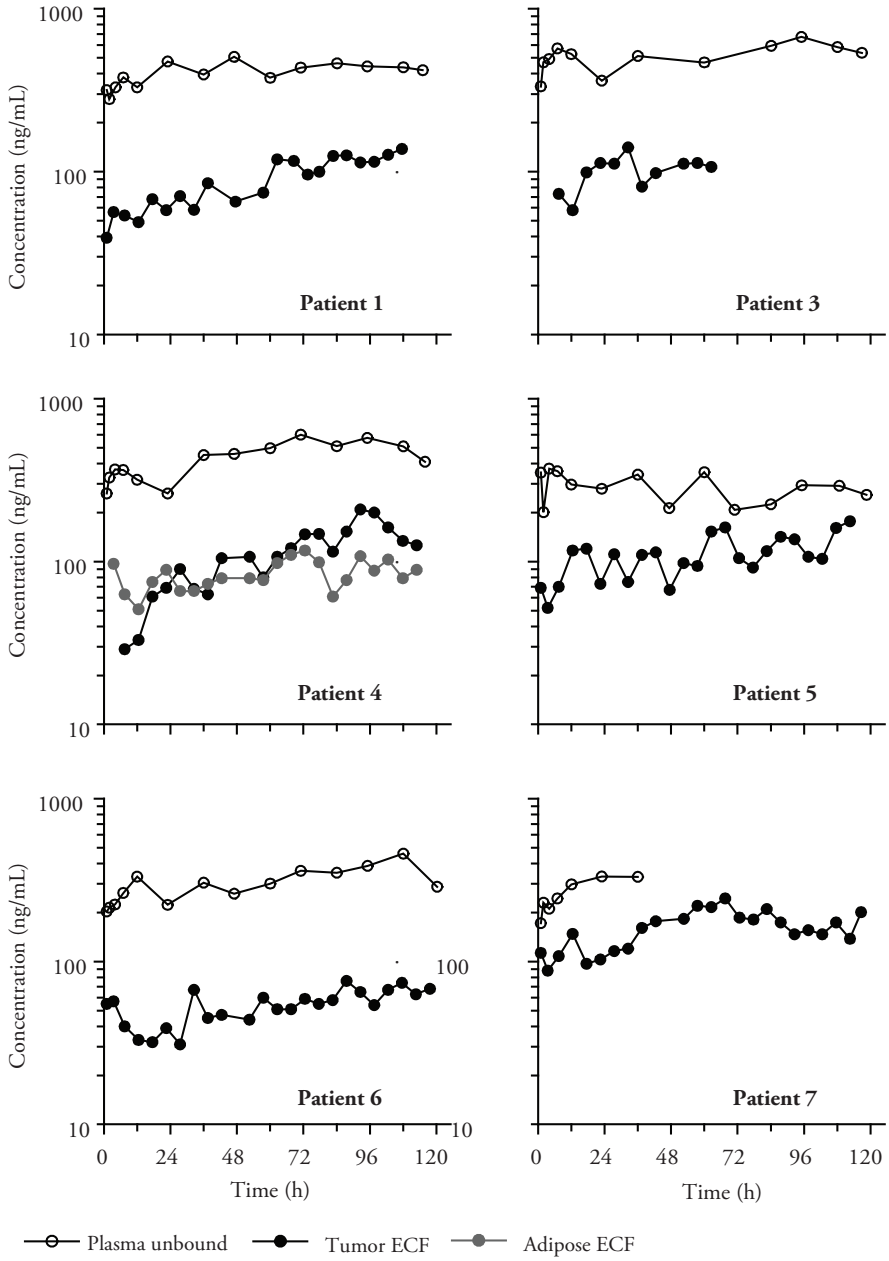

Figure 1 | Concentration time curves of the unbound fraction of 5-fluorouracil in plasma (open circles) and extracellular fluid (ECF) of tumour (filled black circles) and ECF of normal adipose tissue (filled gray circles) in the six evaluable patients.

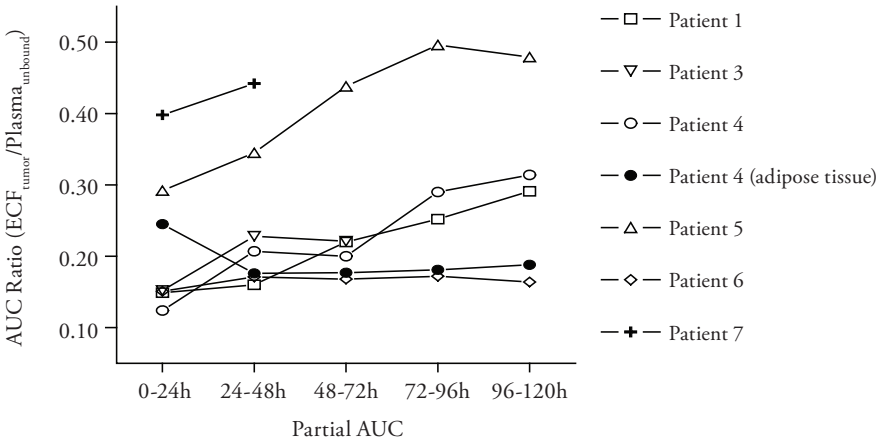

Figure 2 | Exposure ratio ECF_{tumour}/$plasma_{unbound}$ during the infusion of 5-fluorouracil. Each symbol represents a single patient. Patient 4 (i.e., the patient with evaluable normal tissue PK) is represented with circles. The closed circles represent the normal tissue versus plasma AUC ratio, while the open circles represent the tumour tissue versus plasma AUC ratio.

Pharmacokinetics

A summary of the pharmacokinetic data is presented in Table 2. As also depicted in Figure 1 and 2, the 5-fluorouracil concentrations in the extracellular fluid of tumours are lower than the concentrations of unbound 5-fluorouracil in the plasma compartment. Over time however, the difference between tumour and plasma concentrations decreased, as a result of increased tumour concentrations.

Figure 3 depicts the 5-fluorouracil concentrations in tumour extracellular fluid according to the time of sampling during the day. The tumoural 5-fluorouracil concentrations were higher during the night than during daytime. Of note, tumour ECF was not available from patient 3 at later time-points due to the significant matrix effect observed for 5-chlorouracil during the initial analysis and the inability for re-analysis of the samples (all used).

Table 2 | Summary of pharmacokinetic data of 5-fluorouracil in plasma and ECF of tumour tissue

Parameter	Patient 1	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Dose 5-fluorouracil (mg/day)	2300	1890	1700	2000	1650	1500
Plasma unbound						
Fraction unbound (%) [1]	87 ± 3.2	93 ± 11	97 ± 9.1	86 ± 8.5	83 ± 4.7	88 ± 2.7
$AUC_{0\text{-}120}$ (µg*h/mL)[1]	50.8	62.7	54.6	33.6	38.6	NA
$AUC_{0\text{-}24}$ (µg*h/mL)[2]	8.79	11.1	7.39	7.22	6.25	6.56
$AUC_{24\text{-}48}$ (µg*h/mL)[2]	10.6	11.4	9.83	7.05	6.58	7.94
$AUC_{48\text{-}72}$ (µg*h/mL)[2]	10.2	11.8	12.4	6.74	7.39	NA
$AUC_{72\text{-}96}$ (µg*h/mL)[2]	10.8	14.4	13.2	5.75	8.71	NA
$AUC_{96\text{-}120}$ (µg*h/mL)[2]	10.4	14.1	11.8	6.79	9.62	NA
ECF tumour tissue						
Probe recovery (%)[1]	40 ± 4.5	89 ± 3.4	89 ± 2.7	92 ± 2.4	63 ± 1.2	92 ± 1.2
$AUC_{0\text{-}120}$ (µg*h/mL)[2,3]	11.0 (0.22)	NA	13.0 (0.24)	13.6 (0.41)	6.38 (0.17)	19.3 (NA)
$AUC_{0\text{-}24}$ (µg*h/mL)[2,3]	1.31 (0.15)	1.69 (0.15)	0.92 (0.12)	2.09 (0.29)	0.94 (0.15)	2.61 (0.40)
$AUC_{24\text{-}48}$ (µg*h/mL)[2,3]	1.70 (0.16)	2.59 (0.23)	2.03 (0.21)	2.43 (0.35)	1.13 (0.17)	3.51 (0.44)
$AUC_{48\text{-}72}$ (µg*h/mL)[2,3]	2.23 (0.22)	2.60 (0.22)	2.49 (0.20)	2.96 (0.44)	1.24 (0.17)	5.05 (NA)
$AUC_{72\text{-}96}$ (µg*h/mL)[2,3]	2.73 (0.25)	NA	3.82 (0.29)	2.85 (0.50)	1.50 (0.17)	4.28 (NA)
$AUC_{96\text{-}120}$ (µg*h/mL)[2,3]	3.03 (0.29)	NA	3.69 (0.31)	3.26 (0.48)	1.58 (0.16)	3.87 (NA)

AUC area under the concentration time curve, ECF extracellular fluid, NA not available; [1] Mean ± SD, [2] calculated using data observed during a timeframe after start of the infusion with subscript numbers referring to start and endpoint of timeframe, [3] (AUC ratio $ECF_{tumour}/Plasma_{unbound}$)

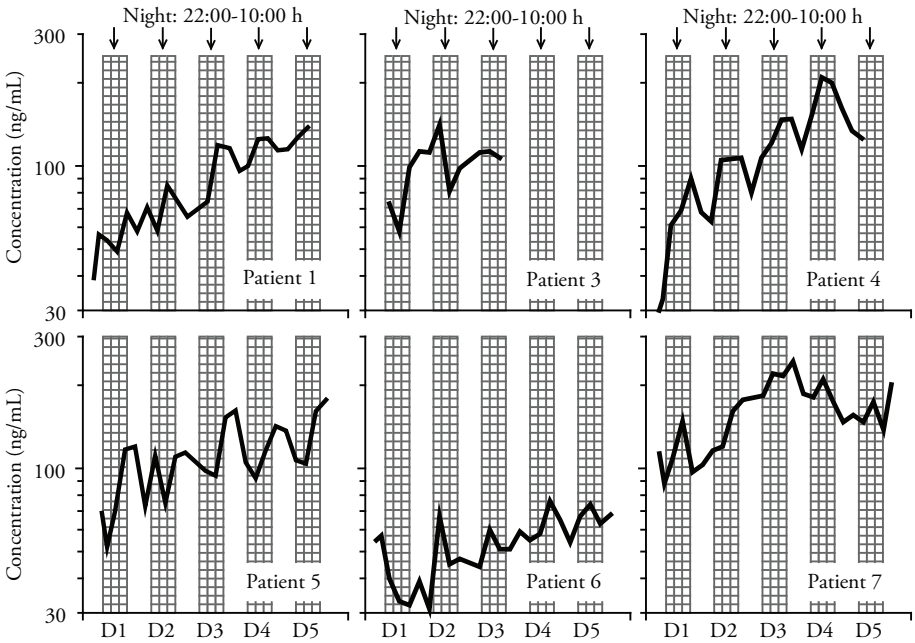

Figure 3 | Concentration time curves of the unbound fraction of 5-fluorouracil in tumour ECF during day and night.

DISCUSSION

Optimization of cancer therapy by enhancing drug delivery has become of major interest as many barriers to drug delivery are being identified, underlining the need for new therapeutic strategies increasing drug delivery to tumours. Obviously, this requires a model that can easily be applied in clinical practice and with the ability to monitor intratumoural drug concentrations. The present study addresses this need and proposes the use of microdialysis to obtain detailed information on drug concentrations at the tumour site. Earlier studies explored the applicability of microdialysis in clinical oncology with microdialysate collection with a maximum of up to 48 hours after iv bolus administration of carboplatin.[15] In this study, we showed that microdialysis is also feasible for longer periods of time in cancer patients treated with continuous 5-fluorouracil infusion during a 5x 24h period.

Our results evidently show lower concentration-time curves at the tumour site in comparison with plasma. This is in line with the results of an earlier microdialysis study by Müller et al. where the obtained area under the curve (AUC) in tumour after a single iv administration of 5-fluorouracil was 61% of the AUC in plasma.[8] A remarkable observation in our study is the fact that the difference between plasma and tumour concentrations diminished over time, as a result of increasing 5-fluorouracil concentrations in the tumour. A possible explanation for this phenomenon may be a change in interstitial fluid pressure in the tumour. Many solid malignancies display a high interstitial fluid pressure, which causes poor uptake of anticancer drugs and has been associated with poor clinical response to treatment.[5,7] Mechanisms involved yielding a high interstitial fluid pressure in tumours are a high vessel permeability, inadequate lymphatic drainage, fibrosis and contraction of the interstitial matrix and a high cell density around the blood vessels.[7] Reduction of tumoural interstitial fluid pressure is an attractive means to augment efficacy of antitumour drugs. In preclinical models, several agents have been revealed to decrease interstitial fluid pressure including dexamethasone, imatinib, and prostaglandin E1.[24-28] Also several cytotoxic agents, including 5-fluorouracil, have shown to lower tumour interstitial fluid pressure within days.[24,29,30] Although the decrease in tumour interstitial fluid pressure following 4 days of 5-fluorouracil treatment in female Sprague-Dawley rats with induced mammary tumours was not significantly different, a reduction of 35% in interstitial fluid pressure was noted on day 5 compared to day 1 (7.2 vs. 4.7 mmHg).[24] The increasing 5-fluorouracil concentration at the tumour site as observed in our study might also be due to the effect of 5-fluorouracil on the interstitial fluid pressure. Assuming that 5-fluorouracil enhanced its own tumour concentration due to its antitumour and thereby lowering effect on interstitial fluid pressure, our data would be in line with a recent study showing that modulation of convection currents by lowering interstitial fluid pressure by treatment with collagenase within tumours enhances the delivery of 5-fluorouracil approximately 1.5 fold.[31] The transport of low-molecular weight compounds, such as 5-fluorouracil, from the vascular space into the interstitial space is thus also driven by convection besides by diffusion. Though only available for one patient, 5-fluorouracil uptake in normal tissue does not seem to increase over time. In the patient with evaluable pharmacokinetic data in extracellular fluid of normal tissue, the tumour exposure increased, while normal tissue exposure was constant over time (Figure 1 and 2). This is also in agreement with the above mentioned study, that did not show a difference in 5-fluorouracil accumulation in normal tissue of mice treated with or without collagenase, i.e. with or without lowering of interstitial fluid pressure.[31]

The interstitial fluid pressure and its effect on convention currents are only two of the multiple factors influencing drug delivery at the target site. Tumour vasculature differs

significantly from that of normal tissue, with great heterogeneity in density, length and diameter, thereby impeding blood flow. Tumour pH gradients are of importance too, as membrane transport and accumulation of weakly acidic 5-fluorouracil in tumours is pH dependent.[6,32,33] DPD activity may be of influence as well, since its activity is known to be variable in tumour tissue and dysregulation of DPD is more frequent in malignant tissue.[19,34] These tumour specific characteristics may explain the differences observed in ECF_{tumour}/$plasma_{unbound}$ ratio's between the patients.

Similar to the findings of our previous microdialysis study in carboplatin-treated patients measurement of 5-fluorouracil in extracellular fluid of normal adipose tissue was troublesome because of fluctuating recovery assessments.[15] It is well confirmed now that subcutaneous adipose tissue is not the best site to use as control, most probably due to its different and poorer vascularisation. Other normal tissues should be chosen as site control in future studies, e.g. muscular tissue or preferably, tissue surrounding the tumour.

Another interesting observation was the typical pharmacokinetic pattern of 5-fluorouracil, with higher 5-fluorouracil tumour extracellular fluid concentrations observed during the night compared to daytime (Figure 3). Circadian rhythmicity of 5-fluorouracil pharmacokinetics in plasma has been an issue of investigation for decades. During continuous 5-fluorouracil infusion, 5-fluorouracil plasma concentrations turned out to be higher during the night than during daytime. Many factors such as age, diet, physical activity, high serum alkaline phosphatase and length of drug infusion were previously identified as patient variables affecting 5-fluorouracil clearance.[34,35] Also, dihydropyrimidine dehydrogenase activity has been found to change during the day, with its highest activity occurring during daytime.[34] In order to achieve the highest efficacy and least toxicity chronomodulated 5-fluorouracil therapy was developed, albeit not regarded standard of care yet.[35,36] Our study is the first to suggest the presence of a circadian rhythm of 5-fluorouracil pharmacokinetics in the tumour itself.

In summary, we showed the feasibility of microdialysis in cancer patients treated with a continuous 5-fluorouracil infusion during a 5x 24h period. Plasma levels of 5-fluorouracil were higher compared to tumour, but there was a distinct rise of 5-fluorouracil at the tumour site over time. Since 5-fluorouracil is a widely used cytotoxic agent and high concentrations at the tumour site are directly linked with a better response, evaluation of its disposition in tumour tissue is of notable importance to further optimize individual cancer patient treatment.[6,8] The technique of microdialysis can be used to improve insight into processes involved in tumour pharmacokinetics, such as e.g. interstitial fluid pressure. In a rat model the co-administration of imatinib resulted in a significant decrease in interstitial fluid pressure with as a consequence a 2-4 fold increase of the ECF_{tumour}/$plasma_{unbound}$ ratio's of ^{51}Cr-EDTA and paclitaxel respectively, and a significant tumour

volume reduction of 44% in rats treated with 5-fluorouracil and imatinib.[26,27] Clinical trials investigating the impact of co-administration of drugs thought to (in)directly effect tumour drug uptake in cancer patients are warranted and this microdialysis model, with 5-fluorouracil as representative drug for other small molecules, might be a useful tool to study pharmacokinetics and pharmacodynamics in more detail. The ongoing development of newer microdialysis probes that can be used in deeper lying (tumour) tissue, e.g. the liver, will add to the clinical use of this technique.

REFERENCES

1. Zamboni WC, Strychor S, Joseph E, Parise RA, Egorin MJ, Eiseman JL (2008) Tumor, tissue and plasma pharmacokinetic studies and antitumor response studies of docetaxel in combination with 9-nitrocamptothecin in mice bearing SKOV-3 human ovarian xenografts. Cancer Chemother Pharmacol 62:417-426.
2. Zamboni WC, Strychor S, Joseph E, et al (2007) Plasma, tumor, and tissue disposition disposition of STEALTH liposomal CKD-602 (S-CKD602) and nonliposomal CKD-602 in mice bearing A375 human melanoma xenografts. Clin Cancer Res 13:7217-7223.
3. Zamboni WC, Houghton PJ, Hulstein JL, et al (1999) Relationship between tumor extracellular fluid exposure to topotecan and tumor response in human neuroblastoma xenograft and cell lines. Cancer Chemother Pharmacol 43:269-276.
4. Müller M (2002) Science, medicine, and the future: microdialysis. Br Med J 324:588-591.
5. Trédan O, Galmarini CM, Patel K, Tannock IF (2007) Drug resistance and the solid tumor microenvironment. J Natl Cancer Inst 99:1441-1454.
6. Presant CA, Wolf W, Waluch V, et al (1994) Association of intratumor pharmacokinetics of fluorouracil with clinical response. Lancet 343:1184-1187.
7. Heldin CH, Rubin K, Pietras K, Östman A (2004) High interstitial fluid pressure - an obstacle in cancer therapy. Nat Rev Cancer 10:803-813.
8. Müller M, Mader RM, Steiner B, et al (1997) 5-Fluorouracil kinetics in the interstitial tumor space: clinical response in breast cancer patients. Cancer Res 57:2598-2601.
9. Mader RM, Schrolnberger C, Rizovski B, et al (2003) Penetration of capecitabine and its metabolites into malignant and healthy tissues of patients with advanced breast cancer. Br J Cancer 88:782-787.
10. Tegeder I, Brautigam L, Seegel M, et al (2003) Cisplatin tumor concentrations after intra-arterial cisplatin infusion or embolization in patients with oral cancer. Clin Pharmacol Ther 73:417-426.
11. Blochl-Daum B, Müller M, Meisinger V, et al (1996) Measurement of extracellular fluid carboplatin kinetics in melanoma metastases with microdialysis. Br J Cancer 73:920-924.
12. Joukhadar C, Klein N, Mader RM, et al (2001) Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. Cancer 92:2190-2196.
13. Ekström PO, Andersen A, Saeter G, et al (1997) Continuous intratumoral microdialysis during high-dose methotrexate therapy in a patient with malignant fibrous histiocytoma of the femur: a case report. Cancer Chemother Pharmacol 39:267-272.
14. Müller M, Brunner M, Schmid R, et al (1998) Interstitial methotrexate kinetics in primary breast cancer lesions. Cancer Res 58:2982-2985.
15. Konings IR, Engels FK, Sleijfer S, Verweij J, Wiemer EA, Loos WJ (2009) Application of prolonged microdialysis sampling in carboplatin-treated cancer patients. Cancer Chemother Pharmacol 64:509-516.
16. Wolpin BM, Mayer RJ (2008) Systemic treatment of colorectal cancer. Gastroenterology 134:1296-1310.
17. Seidman AD (2006) Systemic treatment of breast cancer. Two decades of progress. Oncology 20:983-990.

18. Pan Q, Gorin MA, Teknos TN (2009) Pharmacotherapy of head and neck squamous cell carcinoma. Expert Opin Pharmacother 10:2291-2302.

19. Kubota T (2003) 5-Fluorouracil and dihydropyrimidine dehydrogenase. Int J Clin Oncol 8:127-131.

20. Müller M, Schmid R, Georgopoulos A, et al (1995) Application of microdialysis to clinical pharmacokinetics in humans. Clin Pharmacol Ther 57:371-380.

21. Kitzen JJ, Verweij J, Wiemer AC, Loos WJ (2006) The relevance of microdialysis for clinical oncology. Curr Clin Pharmacol 1:255-263

22. Czejka M, Schuller J (1992) The binding of 5-fluorouracil to serum protein fractions, erythrocytes and ghosts under in vitro conditions Bindung von 5-Fluorouracil an Serumproteinfraktionen, Erythrozyten und Ghosts unter in vitro Bedingungen. Arch Pharm 325:69-71.

23. Jain RK (2001) Delivery of molecular and cellular medicine to solid tumors. Adv Drug Deliv Rev 46:149-168.

24. Stuhr LEB, Salnikov AV, Iversen VV, Salvesen G, Rubin K, Reed RK (2006) High-dose, short-term, anti-inflammatory treatment with dexamethasone reduces growth and augments the effects of 5-fluorouracil on dimethyl-□-benzanthracene-induced mammary tumors in rats. Scand J Clin Lab Invest 66:477-486.

25. Kristjansen PE, Boucher Y, Jain RK (1993) Dexamethasone reduces the interstitial fluid pressure in a human colon xenograft. Cancer Res 61:6400-6415.

26. Pietras K, Östman A, Sjöquist M, et al (2001) Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport into tumors. Cancer Res 61:2929-2934.

27. Pietras K, Rubin K, Sjöblom T, et al (2002) Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. Cancer Res 62:5476-5484.

28. Salnikov AV, Iversen VV, Koisti M, et al (2003) Lowering of tumour interstitial fluid pressure specifically augments efficacy of chemotherapy. FASEB J 17:1756-1758.

29. Feretti S, Allegrini PR, Becquet MM, McSheehy PMJ (2009) Tumor interstitial fluid pressure as an early-response marker for anticancer therapeutics. Neoplasia 11:874-881.

30. Griffon-Etienne G, Boucher Y, Brekken C, Suit HD, Jain RK (1999) Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications. Cancer Res 59:3776-3782.

31. Gade TPF, Buchanan IM, Motley MW, Mazaheri Y, Spees WM, Koutcher JA (2009) Imaging intratumoral convection: pressure-dependent enhancement in chemotherapeutic delivery to solid tumors. Clin Cancer Res 15:247-255Peters GJ, Lankelma J, Kok RM, et al (1993) Prolonged retention of high concentrations of 5-fluorouracil in human and murine tumors as compared with plasma. Cancer Chemother Pharmacol 31: 269-276.

32. Stubbs M, McSheehy PMJ, Griffiths JR, Bashford CL (2000) Causes and consequences of tumour acidity and implications for treatment. Mol Med Today 6:15-19.

33. Milano G, Chamorey AL (2002) Clinical pharmacokinetics of 5-fluorouracil with consideration of chronopharmacokinetics. Chronobiol Int 19:177-189.

34. Block KI, Block PB, Fox SR, et al (2009) Making circadian cancer therapy practical. Integr Cancer Ther 8:371-386.

35. Lévi F, Focan C, Karaboué A, et al (2007) Implications of circadian clocks for the rhythmic delivery of cancer therapeutics. Adv Drug Deliv Rev 59:1015-10135.

Chapter 4

Phase I and pharmacological study of the broad-spectrum tyrosine kinase inhibitor JNJ-26483327 in patients with advanced solid tumours

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ABSTRACT

BACKGROUND JNJ-26483327 is an oral, potent, multitargeted tyrosine kinase inhibitor, inhibiting kinases of Epidermal Growth Factor Receptor (EGFR)-1, -2 and -4, Rearranged during Transfection (RET)-receptor, Vascular Endothelial Growth Factor Receptor (VEGFR)-3, and Src family (Lyn, Fyn, Yes) at low nanomolar concentrations. This phase I, accelerated titration study assessed maximum tolerated dose, safety, pharmacokinetics and pharmacodynamic effects of JNJ-26483327.

METHODS Nineteen patients with advanced cancers received JNJ-26483327 continuous twice daily (BID) in escalating dose cohorts ranging from 100 to 2100 mg. Pharmacodynamic effects were assessed in paired skin biopsies and blood.

RESULTS JNJ-26483327 was well tolerated in doses up to 1500 mg BID, with target-inhibition related toxicity such as diarrhea and skin rash and other common reported toxicities being nausea, vomiting, anorexia and fatigue. At 2100 mg, two episodes of DLT were observed, consisting of grade 3 anorexia and a combination of grade 3 anorexia and fatigue, respectively. Pharmacokinetics were dose proportional up to 1500 mg where plasma levels were obtained showing antitumour activity in xenograft mouse models. Pharmacodynamic analysis did not show a substantial effect on expression of Ki-67, $p27^{kip1}$, pMAPK, pAkt and EGFR and serum levels of sVEGFR-2, VEGF-C and VEGF-D remained unchanged. Stable disease was noted in 6 patients (32%).

CONCLUSION JNJ-26483327 is well tolerated and shows a predictable pharmacokinetic profile; the recommended dose for further studies is 1500 mg BID.

INTRODUCTION

Protein kinases represent over 2% of all human genes and approximately 9% of known cancer genes. Overexpression or mutational and constitutive activity of these kinases plays an important role in the pathophysiology of tumours. Tyrosine kinase inhibitors (TKIs) have become an emerging new class of anticancer agents due to the importance of their targets in tumour proliferation, survival (apoptosis), tumour neoangiogenesis, invasion and metastasis.[1,2] Significant clinical successes have meanwhile been achieved with relatively selective TKIs such as imatinib mesylate (Gleevec® Glivec®), erlotinib (Tarceva®), gefitinib (Iressa®) and lapatinib (Tyverb®) as well as with the broad spectrum TKIs sunitinib (Sutent®) and sorafenib (Nexavar®).[3-9]

According to current understanding, most TKIs exert their activity in a cytostatic manner, suggesting that prolonged and maybe continuous treatment is to be recommended. An oral route of administration thus is of clear advantage and adequate oral bioavailability a prerequisite.[10] Toxicities observed with the use of TKIs appear to be linked to their primary mode of action (e.g. skin rash for EFGR-inhibitors due to EGFR-kinase inhibition in the basal layer of the skin), but other toxicities can occur for which no clear-cut pathophysiologic explanation exists as yet.[11]

JNJ-26483327 has displayed binding affinity to multiple tyrosine kinase receptors known to play a role in a wide variety of neoplasms. It demonstrated potent (IC50 < 10nM) *in vitro* inhibitory activity against EGFR kinase, several mutationally activated EGFR kinases and against RET-receptor kinase. Inhibitory activity against VEGFR-3, Her4 and Src family (Lyn, Fyn, Yes) tyrosine kinases was demonstrated over an IC50-range of 11 to 99 nM. Other tyrosine kinases inhibited by JNJ-26483327 (IC50 100-1000 nM) include c-Src, Her2, Flt3 and others. In preclinical studies anticancer activity was observed and a favourable safety and tissue distribution profile was seen, including passage of the blood brain barrier. JNJ-26483327 is administered as a twice daily oral regimen and has an oral bioavailability of ~80% (Johnson&Johnson, data on file).

This first-in-man study was designed to determine safety, maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of JNJ-26483327 in patients with refractory or advanced solid tumours. In addition, the pharmacokinetic profile, pharmacodynamic activity in various surrogate tissues and preliminary signals of antitumour activity were assessed.

PATIENTS AND METHODS

Eligibility criteria

Patients with a histologically or cytologically confirmed diagnosis of advanced solid malignancy for whom no standard options existed or who were no longer responding to established treatments were eligible. Additional eligibility criteria included: age ≥ 18 years; ECOG performance ≤ 2; life expectancy > 3 months; adequate bone marrow function, without the support of cytokines and/or erythropoietin (white blood cell count (WBC) > 3.0 x 10^9/L, absolute neutrophil count (ANC) > 1.5 x 10^9/L, platelet count > 100 x 10^9/L, hemoglobin > 10.0 g/dL), hepatic function (total bilirubin level ≤ 1,5 times institutional upper limit of normal (iULN), serum alanine transferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 times iULN or ≤ 5 times iULN in case of liver metastases) and renal function (serum creatinine < 1.5 times iULN); no chemotherapy, radiotherapy or immunotherapy within 28 days; no history of uncontrolled heart disease or arterial hypertension (systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg despite appropriate medication). Specific exclusion criteria included a history of pulmonary fibrosis, known central nervous system metastases, impairment of gastrointestinal absorption status and inability to swallow. Left ventricular ejection fraction (LVEF) based on MUGA scan was required to be > 50%. The study was approved by local Ethics Committees and all patients gave written informed consent prior to any study related procedure.

Study design

JNJ-26483327 was supplied by Johnson & Johnson Pharmaceutical Research and Development, Beerse, Belgium as 10 or 50 mg/L oral solution for doses ≤ 1200 mg, or capsules of 50, 100 or 300 mg for doses ≥ 1500 mg. Medication was taken in combination with food twice daily (BID) with 12 hour intervals. A cycle was defined as 28 days of treatment.

The starting dose was 100 mg BID, which was selected based on preclinical data in rodents (one third of the toxic dose low (TDL) and one tenth of the dose causing severe toxicity (STD) in rat) and a preceding study in healthy male volunteers. In the latter study, JNJ-26483327 was safe and well tolerated with single doses up to and including 200 mg. On the basis of the observed half life a BID dosing regimen was chosen for this study.

The study followed an accelerated escalation design. Initial cohorts consisted of 1 patient with dose doublings between cohorts in absence of grade ≥ 2 toxicity according to the National Cancer Institute Common Toxicity Criteria version 3.0 during the first

cycle (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf). Once grade ≥ 2 considered drug related toxicity occurred during the first cycle, two additional patients were enrolled at that dose level with subsequent cohorts following a conventional dose escalation (3+3) model. DLT was defined as any grade 3 or 4 non-haematological toxicity, except for nausea, vomiting or diarrhea responsive to treatment, grade 3 skin rash/acne responsive to treatment, alopecia and isolated grade 3 GGT elevations; need for loperamide for > 7 days to treat or prevent grade ≥ 2 diarrhea; need for continuous administration of 5HT-3 antagonists for > 7 days to treat or prevent grade ≥ 2 nausea or vomiting; a relative decrease of LVEF on MUGA-scan of > 20% compared to baseline; any grade 4 haematological toxicity or treatment interruptions of more than 14 days for grade ≥ 2 toxicity in cycle 1.

If during the conventional escalation stage no DLT occurred, the dose for the next cohort was incremented with 20-100%. If DLT was observed during cycle 1 in one of three patients, an additional three patients were enrolled at that dose level. If ≥ 2 out of 6 patients experienced DLT, the maximum tolerated dose (MTD) was exceeded and three more patients were enrolled at the next lower dose level, unless already six patients had been accrued at that dose. The MTD was defined as the highest dose at which less than one third of patients experienced DLT.

No intrapatient dose escalation was allowed. In the absence of DLT or disease progression, patients were allowed to continue on JNJ-26483327 at the dose level assigned.

Pretreatment and follow-up studies

Prior to therapy, a complete medical history was taken and a physical examination was done. A complete blood cell count (CBC), white blood cell (WBC) count and differential and serum biochemistry including lipid profile and coagulation tests were performed, as were urinalysis, a 12-lead electrocardiogram (ECG) and MUGA-scanning. Weekly evaluations during the first cycle, every other week during the second cycle and monthly thereafter included history, physical examination, adverse event (AE) assessment, CBC, WBC + differential, serum chemistry, ECG and urinalysis. An electrocardiogram was performed predose and 1, 2, 4, 8 and 24 hours postdose on days 1 and 28. Follow-up of LVEF by MUGA-scanning was done at the end of the first and second cycle. Tumour measurements were done pretreatment and every other cycle. Response was assessed according to RECIST.[12]

Pharmacokinetic sampling and data analysis

For pharmacokinetic analysis, blood samples (3 mL) were collected prior to dosing and 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after drug administration on days 1 and 28 and predose,

0.5, 1, 2, 4 and 6 hours postdose on day 15 of the first cycle and day 28 of the second cycle. Furthermore, trough PK samples were taken before drug administration on days 8 and 22. Blood samples were collected protected from light in EDTA-Vacutainer® tubes and plasma was separated by centrifugation and stored at – 20ºC within 2 hours of collection. Plasma concentrations of JNJ-26483327 were determined by validated, selective and sensitive liquid chromatography-mass spectrometry (LC-MS/MS) bioanalytical method. PK parameters gathered were area under the plasma concentration-time curve from time zero and extrapolated to infinity (AUC_{inf}) after the first dose and calculated over the 12h dose interval tau at steady state (AUC_{tau}), maximum observed plasma concentration (C_{max}), time to reach C_{max} (t_{max}), apparent terminal half life ($t_{1/2}$), apparent total plasma clearance (CL/F) and apparent volume of distribution (Vdz/F).

Graphical analysis was performed using dose-normalized C_{max} as well as area under the curve (AUC) of days 1 and 28, for exploration of dose proportionality across doses.

Pharmacodynamic assessments

Modulation of EGFR, phosphorylated mitogen-activated protein kinase (pMAPK), phosphorylated Akt (pAkt), Ki-67 (an indicator of cellular proliferation) and $p27^{KIP1}$ (kinase inhibitory protein 1) was assessed in skin biopsies at screening and on Day 28 of the first treatment cycle. Treatment induced changes in these cellular biomarkers were examined using immunohistochemistry (IHC) on formalin-fixed and paraffin-embedded skin tissue sections. Punch biopsies (4 mm width × 4 mm depth) were taken from the lateral aspect of the upper extremity just before the first dose and at the end of Cycle 1. Specimens were immediately fixed in 10% buffered neutral formalin for 16-24 hours, and embedded in paraffin. Tissue sections (4 µm) were mounted onto silan adhesive "Star Frost" glass slides (Waldemar Knittel Glaser, Braunschweig, Germany), dried overnight at 50 °C, deparaffinised and rehydrated.

Antibodies used were: monoclonal mouse antihuman Ki-67 antigen (clone MIB-1, DakoCytomation, Glostrup, Denmark); monoclonal mouse antihuman $p27^{KIP1}$-protein (clone 1B4, Monosan Sanbio, Uden, The Netherlands); phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (#9101, Cell Signaling Technology, Bioké, Leiden, The Netherlands); pAkt (Ser473) antibody (#3787, Cell Signaling Technology) and monoclonal mouse anti-EGFR (clone E30, DakoCytomation). A pressure cooker-enhanced procedure was used for antigen retrieval, except EGFR which required proteinase K treatment. Antigen detection and visualization (DakoEnVision) were performed according to the standard staining procedures as provided by the manufacturer's instructions. Positive and negative control specimens were used for each IHC staining batch and, if available, an appropriate blocking peptide was included as well. Treatment effects of JNJ-26483327 on

Ki-67 and $p27^{KIP1}$ were assessed by counting ≥1000 positive keratinocytes and expressing the markers as a percentage. Regarding pMAPK-, pAkt- and EGFR-positive keratinocytes, the proportion of positive cells as well as the intensity of staining were estimated using the Allred scoring system.[13]

Blood samples were taken on day 1 prior to the first dose and on days 15 and 28 in Cycle 1 and 2. Blood was collected in a 6 mL serum separator tube (Vacutainer® SST No.367784, Becton Dickinson, Franklin Lakes, USA) and allowed to clot for 30 minutes before centrifugation at 1000 x g. Serum was thereupon removed, aliquotted into three separate 3.6 mL cryotubes (Nunc, Cat. No. 366524, Thermo Fisher Scientific, Rochester, USA) and stored at ≤ -20°C until further analysis. Treatment induced changes in serum levels of sVEGFR-2, VEGF-C and VEGF-D were examined using specific Quantikine human VEGF Immunoassays (R&D systems Europe, Abingdon, UK; human sVEGFR-2 DVR200, human VEGF-C DVEC00, human VEGF-D DVED00). Absolute concentrations of serum VEGF proteins were determined according to manufacturer's protocol, normalized to baseline levels and relative changes were correlated to the administered dose of JNJ-26483327.

Statistical methods

The pharmacokinetic analysis and descriptive statistics were performed using PKAA 2.00 (developed for J&JPRD). PKAA 2.00 uses WinNonLin 5.2.1. Elimination rate constants (λ_z) were calculated as the negative of the slope of the terminal log-linear segment of the plasma concentration-time curves. The range of data used for each subject and dose were determined by visual inspection of a semilogarithmic plot of concentration versus time. At least 3 data points were used to estimate λ_z. All PK calculations and figures used validated software.

RESULTS

Nineteen patients (16 male, 3 female), with a median age of 61 years (47-74), were enrolled between October 2006 and July 2008. Patient demographics, baseline characteristics and prior anticancer treatments are listed in Table 1.

Table 1 | Patient characteristics

Total	19
Male/female	16/3
Median age, years	61
Range, years	47-74
Prior systemic therapy	18
Prior radiotherapy	5
Prior surgery	12
ECOG Performance Score*	
0	4
1	13
2	2
Tumour type	
Colorectal carcinoma	6
Oesophageal carcinoma	5
Prostate carcinoma	2
Hepatocellular carcinoma	1
Mesothelioma	1
Renal cell carcinoma	1
ACUP	1
Cholangiocarcinoma	1
Thyroid carcinoma	1

* ECOG= Eastern Cooperative Oncology Group.

A total of 44 cycles of JNJ-26483327 was administered, and the maximum number of cycles received was six (received by two subjects) with duration of treatment ranging from 11 to 168 days. Dose levels studied were 100 mg (n=1), 200 mg (n=1), 400 mg (n=1), 800 mg (n=3), 1200 mg (n=3), 1500 mg (n=6) and 2100 mg (n=4) (Table 2).

Safety

Occurrence of side effects, as a function of schedule and dose, is listed in Table 3. Nausea, diarrhea, vomiting, anorexia, fatigue and skin rash were principal toxicities of JNJ-26483327.

Diarrhea occurred in > 60% of patients at all but the lowest dose level and was mostly mild or manageable with loperamide. One patient (dose level 1500 mg) experienced grade 3 diarrhea that quickly resolved after loperamide initiation. Other common gastrointestinal side effects were nausea and, to a lesser extent, vomiting, which was usually mild and only required specific treatment in one patient. Eight patients complained of loss of appetite, with frequency increasing with dose. In two patients (dose level 2100 mg) grade 3 anorexia was observed.

Table 2 | Dose escalation scheme, treatment duration

Dose level (mg)	No. of patients	Total no. of cycles	No. of patients with DLT in cycle 1
100	1	2	-
200	1	2	-
400	1	2	-
800	3	10	-
1200	3	4	-
1500	6	17	-
2100	4	7	2

Abbreviation: DLT = dose limiting toxicity. Administration scheme: JNJ 26483327 twice daily, continuously, 28 days per cycle.

Cutaneous side effects manifested mainly as rash, occurring in 11/19 patients, with a hint of dose-dependency (6/11 received ≥ 1500 mg) and predominantly located on the chest (Figure 1). Skin toxicity predominantly occurred during the first cycle and was transient with ongoing treatment. Skin discoloration, exfoliation and dry skin were other drug-induced skin toxicities.

Another frequently observed toxicity was mild fatigue (47.4% of patients). Haematological toxicity consisted of anemia, with grade 2 anemia detected in three patients. None of the subjects with paired LVEF measurements (n=15) showed a significant decrease in LVEF and no hypertension was noted.

Dose-limiting toxicity

At 2100 mg, two episodes of DLT, consisting of grade 3 anorexia and of a combination of grade 3 anorexia and fatigue were observed in one patient each. Other coinciding toxicities observed in the latter patient were grade 2 nausea, vomiting and diarrhea, ultimately resulting in treatment interruption at day 11. All (four) patients in the 2100 mg cohort

had substantial difficulty with the capsule load (seven capsules of 300 mg BID). Based upon these combined observations the dose of 2100 mg was considered to be intolerable and therefore the MTD was set at 1500 mg BID. At this dose, three additional patients were studied, none of whom experienced DLT.

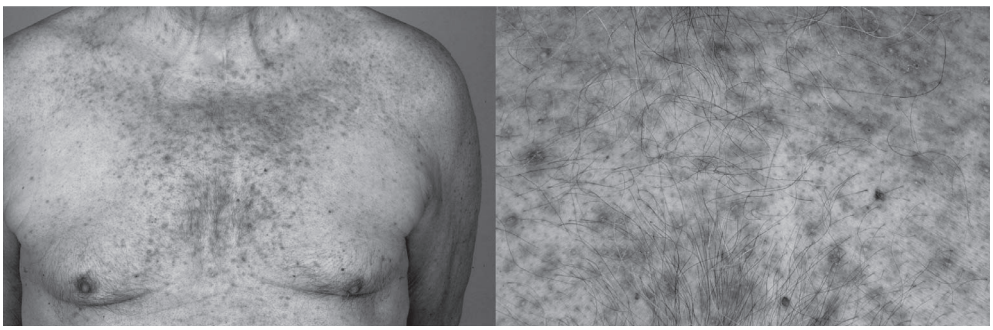

Figure 1 | Skin toxicity after administration of JNJ-26483327

Pharmacokinetics

Plasma concentrations of JNJ-26483327 rapidly increased after oral administration. Maximum plasma concentrations (C_{max}) were reached 2-3 hours (h) after the first administration of the liquid formulation (dose levels 800 and 1200 mg) and up to 4h after administration of the capsule formulation (dose levels 1500 and 2100 mg). On Day 1, C_{max} and overall drug exposure (AUC_{last}) increased with increasing doses up to 1200 mg. At this dose level, peak concentrations were on average nearly 4 µg/mL. The 1500 and 2100 mg dose groups showed lower C_{max}-values in the order of 2 µg/mL as well as a decrease in overall drug exposure. After the peak, plasma concentrations declined biphasically. For all dose levels, the terminal half life was 5 to 8h.

At steady state (as determined on days 15 and 28), C_{max} was reached 1-2h after ingestion of liquid formulation and 2-5h after administration of capsule formulation. C_{max} and AUC increased with increasing dose up to 1500 mg, but not beyond this dose. Dose-normalized C_{max} and AUC of 1200 mg liquid formulation and 1500 mg capsule formulation were comparable. Table 4 displays pharmacokinetic parameters of JNJ-26483327 for all dose levels on days 1 and 28 of Cycle 1. Mean plasma concentration versus time curves of 1500 mg BID JNJ-26483327 on days 1 and 28 of Cycle 1 are shown in Figure 2.

Table 3 | Principal JNJ-26483327-related side effects

	100 mg (n=1)		200 mg (n=1)		400 mg (n=1)		800 mg (n=3)		1200 mg (n=3)		1500 mg (n=6)		2100 mg (n= 4)		Total (n=19)	
	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	G 1/2	G 3/4	n	%
Nausea	0	0	0	0	1	0	2	0	3	0	6	0	1	0	13	68.4
Diarrhea	0	0	1	0	1	0	2	0	2	0	3	1	2	0	12	63.2
Rash	1	0	1	0	0	0	2	0	1	0	3	1	2	0	11	57.9
Vomiting	0	0	0	0	1	0	2	0	2	0	3	0	2	0	10	52.6
Fatigue	1	0	0	0	0	0	1	0	1	0	3	0	2	1	9	47.4
Anorexia	0	0	0	0	0	0	1	0	1	0	3	0	1	2	8	42.1
Anemia	0	0	1	0	1	0	0	0	1	0	0	0	0	0	3	15.8
Dysphonia	0	0	0	0	0	0	0	0	1	0	2	0	0	0	3	15.8
Pain	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	10.5
Dry skin	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	10.5
Constipation	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	10.5
Weight decreased	0	0	0	0	0	0	1	0	0	0	0	0	1	0	2	10.5
Dyspepsia	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	10.5
Dry skin	0	0	0	0	0	0	0	0	0	0	2	0	0	0	2	10.5
Skin exfoliation (palms)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	5.3
Skin discoloration	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	5.3

Table 4 | Pharmacokinetic parameters of JNJ-26483327 for Day 1 and Day 28 (steady state)

Doses (BID)	Day	Patients	AUC (ng*h/mL)	C_{max} (ng/mL)	t_{max} (h)	$t_{½}$ (h)	CL/F (L/h)	Vdz/F (L)
800 mg	D1	n=3	10621 ± 5230	2210 ± 593	2.03 (2.00-3.02)	8.4 ± 1.8	86.3 ± 33.5	1021 ± 401
	D28	n=2	10623	2390	1.52 (1.00-2.03)		76.4	
1200 mg	D1	n=3	35554 ± 22086	3707 ± 1927	3.00 (2.00-6.00)	5.2 ± 1.7	47.4 ± 35.3	342 ± 212
	D28	n=2	21230	2750	1.00 (1.00-1.00)		88.4	
1500 mg	D1	n=6	16388 ±11956	2361 ± 1765	4.00 (2.00-4.03)	4.8 ± 0.5	144 ± 104	1038 ± 799
	D28	n=6	26225 ± 20491	3375 ± 2116	3.99 (3.00-12.00)		286 ± 540	
2100 mg	D1	n=4*	20357 ± 25622	2213 ± 2983	3.54 (2.00-4.08)	6.7 ± 1.7	3055 ± 5099	36985 ± 62264
	D28	n=2	8467	1236	5.00 (4.00-6.00)		3087	

t_{max}: median (min-max); $C_{max,}$ AUC, $t_{1/2}$, CL/F, Vdz/F: mean ±SD. SD= standard deviation, given when data of >2 subjects; AUC_{inf} after the first dose and AUC_{tau} at steady state (D28); * n=3 for AUC_{inf}, $t_{1/2}$, CL/F and Vdz/F

Pharmacodynamics

Upon treatment with JNJ-26483327, no evident histopathological effects were observed in paired skin samples. Furthermore, no consistent changes in EGFR-associated cell signalling biomarkers (EGFR, pMAPK or pAKT) and indicators of cellular differentiation ($p27^{KIP1}$) and proliferation (Ki-67) were detected in keratinocytes of 16 paired skin biopsies. Baseline-normalized serum levels of sVEGFR-2, VEGF-C and VEGF-D were not affected by treatment.

Antitumour activity

Six patients had stable disease lasting more than two cycles; the median number of cycles in these patients was 4 (range 3-6). One patient with prostate cancer treated at 800 mg BID and another patient with renal cell carcinoma treated at 1500 mg BID showed stable disease at the end of the fourth cycle. Stable disease did not continue beyond 6 cycles of treatment. There was no significant relationship between the occurrence of stable disease lasting more than two cycles and dose, even though 3/6 patients received ≥ 1500 mg JNJ-26483327 BID.

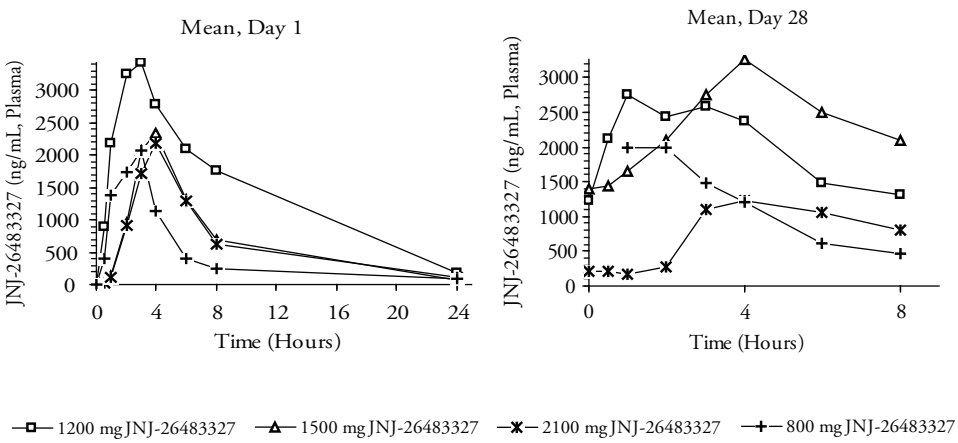

Figure 2 | Mean plasma concentration-time profile of JNJ-26483327 after administration of JNJ-26483327 orally BID, Day 1 and 28.

DISCUSSION

This study was the first-in-human study evaluating the feasibility of oral administration of JNJ-26483327 given continuously BID. Main objectives were to define its toxicity profile and maximum tolerated dose, as well as the pharmacokinetic and -dynamic properties in man.

JNJ-26483327 was well tolerated up to 1500 mg BID, with most common reported toxicities being nausea, vomiting, diarrhea, anorexia, fatigue and skin rash. Cutaneous and gastrointestinal side effects predominate in studies with EGFR tyrosine kinase inhibitors.[14,15] The exact pathogenesis is still unclear, but as functional EGFR is crucial for maintaining integrity of the gastrointestinal mucosa, for normal development and physiology of the epidermis, as well as for mucosal repair, inhibiting EGFR activity likewise can induce mucocutaneous side effects.[16-18] The occurrence of these side effects can even be considered to be indicative for true target inhibition. In this study gastrointestinal side effects showed a 95% incidence. Diarrhea, nausea, and vomiting occurred at most dose levels, but tended to be dose dependent. In those patients experiencing diarrhea, loperamide treatment was always effective. Skin toxicity consisted of usually mild rash, dry skin, skin exfoliation, erythema, skin discoloration and paronychia with an overall incidence of 68%. The frequency and/or type of skin event was not clearly dose dependent and in all circumstances local treatment with moisturizing ointments was sufficient in controlling these effects. No treatment interruption was indicated or felt necessary. Other observed side effects were also mild, never exceeding grade 2. Since JNJ-26483327 is a Her2-inhibitor potentially inducing cardiotoxicity, special attention was given towards early recognition of this phenomenon.[19] In this study, no signs of cardiac impairment or hypertension were observed.

Initially JNJ-26483327 was administered as oral solution, but as high amounts of the vehiculum Captisol® have been noted to induce soft stools or diarrhea in man, a capsule formulation was introduced for the higher dose levels (≥ 1200 mg). The maximum drug load of JNJ-26483327 was 300 mg per capsule, resulting in a substantial capsule intake at the higher dose levels. At 2100 mg BID grade 3 anorexia and a combination of grade 3 anorexia and fatigue yielded protocol-defined DLT in 2 patients, but due to substantial difficulty with capsule intake experienced by all other patients at this dose, capsule load at 2100 mg BID was considered dose limiting as well, albeit not defined by protocol. Therefore, the recommended phase 2 dose for JNJ-26483327 was set at 1500 mg BID.

Pharmacokinetic analysis demonstrated rapid absorption with C_{max} reached on average 1-2 hours earlier in case of the solution. With a half life of 5-8 hours the drug is suitable for twice daily dosing. Up to 1500 mg C_{max} and AUC were dose proportional at

steady state (Day 28) albeit with substantial inter- and intrapatient variability. This is a common phenomenon for many oral TKIs. Steady state plasma concentrations at 1500 mg were in the active range as observed in mouse xenograft models. PK data support the clinically guided conclusion that the recommended phase 2 dose should be 1500 mg BID, since the pharmacokinetic profile at 2100 mg BID showed lower overall drug exposure, probably due to a decrease in bioavailability. Very low concentrations were observed in one specific subject after 2100 mg administration which could account for the overall effect observed at this dose level; of note is that this subject had taken carbamazepine, a known inducer of CYP3A4.

Somewhat to our surprise, no clear JNJ-26483327-induced pharmacodynamic effects were observed in surrogate tissues in this study. No consistent histopathological effects were observed in skin biopsies and various biomarkers of EGFR-signaling and serum levels of regulators of (tumour)angiogenesis also remained essentially unchanged, even in patients experiencing obvious on-target side effects such as diarrhea and skin rash. As skin toxicity is a predominant side effect of many specific EGFR-TKI, the question here is whether any of the other target inhibiting effects acts as main driver of the therapeutic potential of JNJ-26483327.[16] Since JNJ-26483327 indeed targets multiple tyrosine kinases, this is a possible explanation. Another question is whether other parameters such as inflammatory cytokines (e.g. IL-1β, IL-6, IL-8), placental growth factor or circulating tumour cells should have been explored to gain better insight in pharmacodynamic alterations at the tumoural level.[20] In addition, our observations again stress the importance of performing pharmacodynamic research in the most essential tissue available, being the tumour. Although taking repeated tumour biopsies will be inconvenient or even somewhat cumbersome for patients, restricting pharmacodynamic research to surrogate tissues might lead to disappointing or even incorrect conclusions.

In our study 32% of patients had stable disease for more than 2 cycles, with a maximum of six cycles over a dose range of 800 to 2100 mg BID. Although there was no statistical correlation between dose and frequency and/or duration of disease stabilization, the observation was that at doses of and exceeding 1500 mg BID more prolonged disease stabilization was observed. Some cases of prolonged stable disease were observed, however due to the small numbers, no recommendation as to which tumour type could benefit most of JNJ-26483327 administration can be made. Most prolonged disease stabilization was seen in two patients with prostate and renal cell cancer, respectively. If in future studies with JNJ-26483327 at these dose levels a better correlation between clinical outcome and pharmacodynamic assessment could be made, this would undoubtedly help in better understanding this broad spectrum tyrosine kinase inhibitor.

In summary, JNJ-26483327 is a novel multitargeted tyrosine kinase inhibitor. It is well tolerated at the recommended dose level of 1500 BID with only mild and reversible gastrointestinal and skin toxicity. At this dose JNJ-26483327 shows a predictable pharmacokinetic profile. Further studies to establish its clinical antitumour activity are currently being considered.

REFERENCES

1. Blume-Jensen P, Hunter T (2001) Oncogenic kinase signalling. Nature 411:355-365.
2. Shawyer LK, Slamon D, Ullrich A (2002) Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 1:117-123.
3. Demetri GD, von Mehren M, Blanke CD, et al (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumours. N Engl J Med 347:472-480.
4. Perez-Soler R (2007) Erlotinib: recent clinical results and ongoing studies in non small cell lung cancer. Clin Cancer Res 13:s4589-4592.
5. Fukuoka M, Yano S, Giaccone G, et al (2003) Multi-institutional randomized phase II trial of gefinitib for previously treated patients with advanced non-small-cell lung cancer. J Clin Oncol 21:2237-2246.
6. Geyer CE, Forster, J, Lindquist D, et al (2006) Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 355:2733-2743.
7. Motzer RJ, Hutson TE, Tomczak P, et al (2009) Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. J Clin Oncol 27:3584-3590.
8. Escudier B, Eisen T, Stadler WM, et al (2009) Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 27:3312-3318.
9. Llovet JM, Ricci S, Mazzaferro V, et al; SHARP Investigators Study Group (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 24:378-390.
10. Eskens F, Verweij J (2000) Clinical studies in the development of new anticancer agents exhibiting growth inhibition in models: facing the challenge of a proper study design. Crit Rev Oncol Hematol 34:83-88.
11. Stadler WA (2006) New targets, therapies and toxicities: lessons to be learned. J Clin Oncol 24:4-5.
12. Therasse P, Arbuck SG, Eisenhauer EA, et al (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216.
13. Allred DC, Harvey JM, Berardo M, Clark GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11:155-168.
14. Hartmann JT, Haap M, Kopp HG, Lipp HP (2009) Tyrosine kinase inhibitors – a review on pharmacology, metabolism and side effects. Curr Drug Metab 10:470-481.
15. Loriot Y, Perlemuter G, Malka D, et al (2008) Drug insight: gastrointestinal and hepatic adverse effects of molecular-targeted agents in cancer therapy. Nat Clin Pract Oncol 5:268-278.
16. Li T, Perez-Soler R (2009) Skin toxicities associated with epidermal growth factor receptor inhibitors. Target Oncol 4:107-119.
17. Lacouture ME (2006) Mechanisms of cutaneous toxicities to EFGR inhibitors. Nat Rev Cancer 6:803-812.
18. Playford R, Hanby A, Gschmeissner S, Peiffer L, Wright N, McGarrity T (2006) The epidermal growth factor receptor (EGF-R) is present on the basolateral, but not the apical, surface of enterocytes in the human gastrointestinal tract. Gut 39:262-266.

19. Perez EA (2008) Cardiac toxicity of ErbB2-targeted therapies: what do we know? Clin Breast Cancer 8Suppl 3:S114-120.

20. Jain RK, Duda DG, Willet CG, Sahani DV, Zhu AX, Loeffler JS, Batchelor TT, Sorensen AG (2009) Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol 6:327-338.

Chapter 5

The applicability of mTOR inhibition in solid tumours

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ABSTRACT

The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin-pathway (PI3K/AKT/mTOR-pathway) plays a role in the regulation of cell proliferation, cell survival, angiogenesis and resistance to antitumour treatments. In many tumour types the PI3K/AKT/mTOR-pathway is found activated through several different underlying mechanisms. Since this pathway is believed to largely drive the malignant behaviour of several of these tumours, mTOR inhibition is considered an attractive means to apply as antitumour treatment. Currently, four mTOR-inhibitors are explored for clinical use: rapamycin, temsirolimus (CCI-779), everolimus (RAD001) and deforolimus (AP23573). As monotherapy, mTOR-inhibitors yield interesting antitumour activity against various tumour types at the expense of relatively mild toxicities. This recently resulted in the registration of two mTOR-inhibitors for patients with metastatic renal cell carcinoma (RCC) while randomized studies in other tumours are currently in progress. Furthermore, mTOR-inhibitors are well-suited drugs to combine with other antitumour drugs as in preclinical models mTOR-inhibition overcomes chemoresistance. Consequently, mTOR-inhibitor-containing multidrug regimens are subject to clinical studies.

As holds true for all antitumour therapies, identification of patients who are likely to respond to mTOR-inhibitor-containing therapies is of utmost importance to avoid over- or undertreatment. Preliminary results suggest that several factors reflecting activation of mTOR in tumours may be used for this purpose.

This review addresses the mechanism of action and current clinical experience with mTOR-inhibitors as well as their role in overcoming resistance to conventional therapies. Additionally, potential predictors of outcome to mTOR inhibition are discussed.

INTRODUCTION

In recent years our knowledge of the molecular mechanisms underlying malignant behaviour has expanded considerably. Signaling pathways are being unraveled and numerous genes/proteins that are crucial for tumour pathogenesis have been identified. The application of drugs that target these cancer specific factors and inhibit or impair their function, is thought to have the advantage of interacting specifically with cancer cells, leaving normal cells and tissues mostly unaffected.

One of these targets is the mammalian target of rapamycin (mTOR), a serine/threonine kinase which has become known as an important signal transducer in the PI3K/AKT/mTOR-pathway. This pathway drives several important cellular processes such as cell proliferation, cell survival and angiogenesis. In addition, it has been revealed that activation of this pathway confers resistance to apoptotic triggers such as induced by chemotherapy. The observation that in many tumour types an activated PI3K/AKT/mTOR-pathway is found, prompted the search for drugs that are able to inhibit this pathway. Over the past few years, several mTOR-inhibitors have become available yielding interesting results regarding anticancer activity in preclinical and early clinical studies. Furthermore, in preclinical models mTOR-inhibitors interact synergistically with several other antitumour agents, rendering the exploration of such combinations in humans worthwhile. This review will discuss the role of mTOR-dependent pathways in oncology and the role of these pathways in determining sensitivity and in resistance to other antitumour agents. Moreover, it will provide a summary of preclinical data obtained so far with mTOR-inhibitors, but shall mainly focus on current clinical experience with mTOR-inhibitors in solid malignancies.

IMPORTANCE OF ACTIVATED MTOR-DEPENDENT PATHWAYS IN SOLID MALIGNANCIES

Regulation of mTOR and its role in normal cells

Several mechanisms are involved in the regulation of the PI3K/AKT/mTOR-pathway in normal conditions. Activation by growth factors and cytokines after binding to the corresponding receptor is one of the most important ones. Receptors that use the PI3K/AKT/mTOR-pathway for signal transduction include the receptors for interleukin 1 (IL-1), IL-2, transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and many others (Table 1). After binding of the ligand to its cognate receptor, the intracellular tyrosine kinase domain

of the receptor becomes activated. This triggers the activation of several downstream signaling intermediates including phosphatidylinositol 3-kinase (PI3K), which catalyses the conversion of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). One of the downstream targets of PI3K is protein kinase B, also known as AKT, which is activated through phosphorylation (phospho-AKT). Once activated, phospho-AKT phosphorylates numerous other proteins, including mTOR. There are two distinct subtypes of mTOR: a rapamycin- and nutrient-sensitive multiprotein complex called mTORC1 and a second growth factor sensitive but nutrient insensitive mTOR-containing complex called mTORC2. Activated AKT has a direct effect on mTORC1 through phosphorylation, but is influenced itself by mTORC2. As such, mTOR may function both upstream and downstream of AKT. There is cross-talk between mTORC1 and mTORC2 through different (feedback) loops, but the precise regulating mechanisms are not fully understood yet.[1] The cellular effects of mTOR activation are in turn mediated by phosphorylation of several downstream messengers. Two of these downstream effectors that are well described are ribosomal p70S6 kinase (p70S6K) and eukaryotic initiation factor binding protein 1 (4E-BP1). Activated p70S6K phosphorylates the S6 protein of the small ribosomal subunit, enabling its participation in ribosome formation and consequently protein synthesis. Activation of 4E-BP1 results in its dissociation from eukaryotic initiation factor 4E (eIF-4E) and allows eIF-4E to form a new complex with eIF-4F and eIF-4G, thereby enhancing protein translation.[2] As several of these phosphorylated mTOR substrates are measurable both in tumours and in normal tissues such as peripheral blood mononuclear cells (PBMCs), they are frequently used as pharmacodynamic markers to assess the effects of mTOR-inhibitors in preclinical and clinical studies.

Table 1 | Receptors that exert their function through the PI3K/AKT/mTOR-pathway

Interleukin 1, 2, 3, 4 and 6-receptor
Insulin-like growth factor-receptor
Epidermal growth factor-receptor
Platelet derived growth factor-receptor
Insulin growth factor 1 and 2-receptor
Vascular endothelial growth factor-receptor
Transforming growth factor b-receptor
c-KIT

In addition to these downstream effectors, mTOR acts through hypoxia-inducible factor 1 alpha (HIF-1α), a transcription factor. HIF-1α stimulates the production of several oncogenic proteins including VEGF, transforming growth factor α (TGF-α), platelet derived growth factor (PDGF) and cyclin D1, ultimately resulting in malignant behaviour.

Next to activation by phospho-AKT, there are several other proteins that have an impact on mTOR activation. One of the most crucial ones is phosphatase and tensin homologue deleted on chromosome 10 (PTEN). PTEN antagonizes the actions of PI3K by dephosphorylating PIP3 back to PIP2 thereby inhibiting mTOR activation.[3] Furthermore, mTOR is regulated through a complex consisting of the tuberous sclerosis complex proteins 1 and 2 (TSC1/hamartin and TSC2/tuberin). This complex, which inhibits mTOR activation, is regulated by the presence of amino acids; in states of nutrient sufficiency the TSC1/TSC2-complex is inactivated, resulting in undisturbed mTOR kinase activity.[4] A schematic view of the PI3K/AKT/mTOR-pathway is depicted in Figure 1.

Mechanisms yielding mTOR activation in solid malignancies

Activation of the PI3K/AKT/mTOR-pathway has been found in almost all tumour types and as such, is thought to be a major driving force for the malignant behaviour of these tumours. Several underlying mechanisms accounting for activation have been elucidated (Table 2).

Table 2 | Molecular mechanisms yielding mTOR activation in solid tumours

Excess of ligand (growth factors, cytokines)
Receptor overexpression
Gain of function mutations
PI3K or AKT amplification
Mutated PTEN
TSC1/TSC2 alterations
Nutrients / MAP4K3
P53-mutations

** see text for additional details

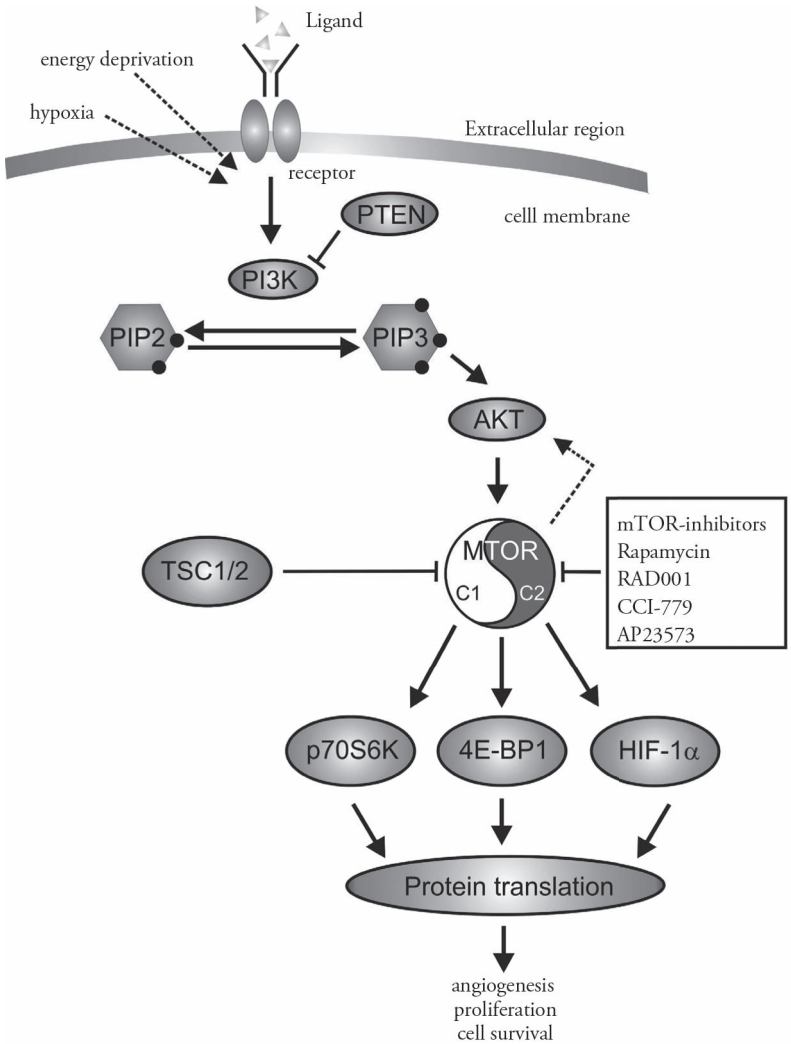

Figure 1 | Overview of the PI3K/AKT/mTOR-pathway.

One of these is an excess of ligands that exert their function through binding to receptors using the PI3K/AKT/mTOR-pathway for signal transduction. For example, overexpression of ligands such as VEGF, IGF-1, IL-4, and IL-6 has been demonstrated to occur in a wide variety of different tumour types, e.g. renal cell, small cell lung, colorectal, prostate and breast cancer, sarcomas and carcinoids.[5,6] Likewise, overexpression of

receptors at the cell surface that connect to the PI3K/AKT/mTOR-pathway to exert their function, can result in stimulation of mTOR. Examples include overexpression of the HER2/neu receptor and the IGF-1 receptor as found in various tumour entities.[7] Gain-of-function mutations in genes encoding receptors using the PI3K/AKT/mTOR-pathway can also account for increased mTOR activity. The resulting mutated receptors yield a constitutive signal transduction without the normally required ligand binding. Examples include gain-of-function mutations in c-KIT and the EGF-receptor in gastro-intestinal stromal tumours (GIST) and non-small cell lung cancer (NSCLC), respectively.[8,9]

With respect to mechanisms beyond the ligand-receptor level, gene amplifications of PI3K and/or AKT leading to mTOR activation have been found in cervical, ovarian, gastric, pancreatic, head and neck and colon cancer.[10-12] Another mechanism causing continuous mTOR activation is mutated or otherwise inactivated PTEN, which is frequently encountered in various cancer types, such as glioblastoma, prostate and endometrial cancer.[13,14] Alterations in the mTOR-regulating TSC1/TSC2-complex are specifically found in the tuberous sclerosis complex syndrome, an autosomal genetic disorder that leads to benign tumours and is associated with increased occurrence of renal cell cancer, clear cell subtype. So far, no mutations in or overexpression of mTOR itself, have been reported.[15]

In many human cancers mutations in the tumour suppressor gene p53 are described. There is evidence of intercommunication between the p53-pathway and PI3K/AKT/mTOR-pathway through PTEN. Decreased p53-function, e.g. as occurs in case of mutated p53, leads to decreased activation of PTEN as a consequence of which the mTOR-pathway is activated.[16,17]

Collectively, numerous mechanisms accounting for increased mTOR activity in malignancies have been reported, but it is unlikely that the whole spectrum of mechanisms has been revealed.

mTOR-INHIBITORS

Given the important role of the PI3K/AKT/mTOR-pathway in tumour pathogenesis, its inhibition seems an obvious means to induce antitumour activity. For this purpose, mTOR seems a logical and attractive target as it finds itself in a central position. As a consequence, since the mid-90s mTOR-inhibitors have attracted much interest, which resulted in the development of different agents. Of these, four are currently explored in the clinic, i.e. rapamycin, temsirolimus, everolimus and deforolimus.

The first one, rapamycin (sirolimus, Rapamune, Wyeth Pharmaceuticals) is a natural macrolide antibiotic, which was initially developed as an antifungal drug. Later it was studied because of its immunosuppressive activity in kidney transplant patients, resulting

in FDA-approval in 1999 for use in this field. In contrast to other immunosuppressants like e.g. cyclosporin, no increase in the occurrence of secondary malignancies was observed, but rather a decrease. This led to the initiation of studies to further explore the exact antitumour activity of rapamycin. Later, analogues of rapamycin were introduced (CCI-779, RAD001 and AP23573), mostly because of more favourable pharmaceutical properties in terms of water solubility and stability.

Rapamycin inhibits mTOR-mediated phosphorylation of downstream substrates through the formation of a cytoplasmic complex with FK506-binding protein 12 (FKBP12), which binds directly to mTOR and shuts it down.[18] Temsirolimus (CCI-779, cell-cycle inhibitor-779, Wyeth Pharmaceuticals) is a more water-soluble ester of rapamycin, available in intravenous as well as oral formulations and metabolized to rapamycin in the body. Everolimus (RAD001, Novartis) is also a rapamycin ester, available only for oral use. Their actions are similar to those of rapamycin, namely complex-formation with FKBP12 and subsequent mTOR-inhibition. Deforolimus (AP23573, Ariad Pharmaceuticals) is one of the most recently developed intravenous mTOR-inhibitors. It has been conceived using a computational modeling strategy resulting in a rapamycin-like drug with high stability in water and exhibiting high affinity binding to FKBP12 and mTOR.[19] An oral formulation of this drug is also in development.

mTOR-inhibition in preclinical models

Consistent with the presumed central role of mTOR activation in many tumour entities, rapamycin and its derivates show substantial antitumour activity against a wide variety of tumour types both in vitro and in vivo. These include RCC, NSCLC, glioblastoma, sarcoma, primitive neuroectodermal tumours, head and neck, melanoma, prostate, breast, colon, cervical, endometrial, ovary and pancreatic cancer. Inhibitors of mTOR produce tumour growth inhibition in the nanomolar and femtomolar range, concentrations that are expected to be easily achieved in humans.[20,21] From the preclinical studies, it appears that inhibition of cell proliferation, induction of apoptosis and inhibition of angiogenesis account for the antitumour activity mediated by rapamycin and its analogs.[15]

mTOR-inhibition as monotherapy in clinical trials

Rapamycin

Over the last few years several phase I/II trials were initiated with rapamycin in cancer patients. The dose of rapamycin that is used in these studies varies between 2-6 mg orally once daily (www.clinicaltrials.gov).[22] This dose was chosen because of signs of suppressed mTOR signaling in preclinical models. Also, an once weekly schedule (up to 60 mg) was well-tolerated and mainly characterized by gastro-intestinal side effects, hyperglycemia

and mild myelosuppression. Sustained p70S6K-inhibition in peripheral T-cells was observed with doses of 30 mg and upwards, suggesting adequate blocking of the PI3K/AKT/mTOR-pathway at these rapamycin levels.[23] Current phase II trials with rapamycin as single agent involve patients with pancreatic, kidney and hepatocellular carcinoma, but also malignant glioma and lymphoma.

Tem sirolimus (CCI-779)

Temsirolimus was investigated in phase I studies using various dose regimens. In a study with daily temsirolimus for 5 days every other week, the dose range varied from 0.75-24 mg/m^2/day. Administration of 15 and 19.1 mg/m^2/day turned out to be the maximum tolerated dose (MTD) for minimally and extensively pretreated patients, respectively.[24] When temsirolimus was administered once weekly as a 30-minute infusion, doses were given up to 220 mg/m^2. Although formally no MTD was reached, it was decided to stop further dose escalation because of grade 3 psychiatric toxicity in 2/9 patients: euphoria and insomnia were followed by depression in both patients, requiring hospitalization and antidepressive treatment in one patient. This toxicity was reversible after treatment discontinuation.[25] A third study was initiated to explore the feasibility of oral administration of temsirolimus daily for five days every two weeks. Pharmacokinetic data showed a moderately rapid absorption and a dose-related increase in plasma levels. Based on toxicity data the recommended oral dosage of temsirolimus was set at 75 mg/day in this schedule.[26]

In all these phase I studies the most frequently observed drug-related toxicities consisted of mucositis, dermatological reactions (acne-like, maculopapular rashes, eczematous reactions, dry skin and nail disorders), mild myelosuppression (primarily thrombocytopenia), elevated liver function tests, asymptomatic hypocalcemia, hypercholesterolemia and hypertriglyceridemia. All were reversible upon drug withdrawal. Similar to observations in animals, no relevant immunosuppression was seen with intermittent exposure to temsirolimus as opportunistic infections or significant changes in lymphocyte counts and function were not observed.[24] Overall, indications of mTOR-inhibition were observed over the entire dose range in view of decreased activation of downstream factors such as p70S6K, 4E-BP1 and HIF-1α in PBMCs. In this respect, no direct relationship with dose was seen.[20] It was decided to further explore temsirolimus at 25, 75 and 250 mg weekly given by intravenous infusion.[25] From the publications it does not become clear why exactly these regimens and not the oral ones were chosen.

Meanwhile, multiple phase II studies have been carried out in a wide variety of tumour types. Interesting results were seen in patients with advanced refractory renal cell cancer (RCC), with good tolerability and tumour responses occurring at al three

examined dose levels (25, 75, 250 mg iv). Collectively, 56 patients (51%) showed either tumour regression or stable disease. Median time to tumour progression (TTP) and overall survival were 5.8 and 15 months, respectively.[27] Compared to historical data, in particular the antitumour activity in patients belonging to the poor prognostic group according to the Motzer criteria was favourable.[28] Although this randomized study was not powered to detect differences, no evident differences between the dose cohorts were observed and a weekly dose of 25 mg temsirolimus was chosen to be further explored. In a subsequent phase III trial accruing only RCC patients with poor prognostic features, patients were randomly assigned to receive either temsirolimus monotherapy, interferon-α (IFN-α) or combination therapy of temsirolimus (15 mg) and IFN-α. Compared to IFN-α, the combination of temsirolimus and IFN-α did not improve overall survival, possibly related to the delays in treatment and dose reductions due to adverse events. In contrast, temsirolimus as single agent significantly enhanced overall survival (10.9 vs 7.3 months) at the expense of a toxicity profile judged acceptable.[29] Temsirolimus-related grade 3-4 adverse events consisted primarily of metabolic abnormalities (hyperglycemia, hyperlipidemia, hypophosphatemia and hypocalcemia), which were easily controlled medically.[30] Based on this study, FDA-approval was granted for temsirolimus as first-line treatment in patients with advanced RCC and poor prognostic features.

Encouraging results were also obtained in a phase II study with single agent temsirolimus in heavily pretreated patients with locally advanced or metastatic breast cancer.[31] However, single agent temsirolimus failed to exhibit antitumour activity in patients with extensive small cell lung cancer (SCLC), recurrent glioblastoma multiforme, metastatic melanoma and neuroendocrine tumours.[32-35] Investigators of a study involving patients with advanced soft tissue sarcoma agreed on an acceptable toxicity profile, but considered the drug ineffective for this subset of malignancies.[36] However, a close observation of the data indicates a progression free rate at 3 months in about 40% of the patients, which is deemed indicative of an active compound for soft tissue sarcomas.[37] In our opinion, this renders further research with temsirolimus in soft tissue sarcomas worthwhile.

Everolimus (RAD001)

In phase I studies, everolimus was given orally, at doses of up to 70 mg once weekly or at 2.5-10 mg daily. The most frequently observed adverse effects were only mild to moderate and included rash, stomatitis, fatigue, hyperlipidemia, myelosuppression (neutropenia), hyperglycemia and gastro-intestinal symptoms (nausea, vomiting, diarrhea and anorexia). There were no frequent or severe infections that may have indicated substantial immunosuppresion.[21] The maximal tolerated dose (MTD) was not reached in the daily

setting, but in one of two studies exploring weekly dosing, 70 mg weekly appeared to exceed the MTD.[38] Inhibition of mTOR was achieved from weekly dosages of 20 mg or daily doses from 5 mg onwards, using among others inhibited p70S6K activity in PBMCs, skin and tumour tissue as a biomarker.[38,39] The correlation with antitumour activity of everolimus was demonstrated earlier in a rat model by pharmacodynamic measurements showing reduced phosphorylation of p70S6K in PBMCs and changes of expression of p70S6K and 4E-BP1 in human prostate tissue samples.[40,41] Combination of these preclinical and clinical data led to the development of a pharmacokinetic/pharmacodynamic (PK/PD) model which can be useful in designing new studies.[81] Early hints of antitumour activity were observed in these phase I studies, especially in patients with RCC, with 5/12 RCC patients showing prolonged disease stabilization lasting longer than six months.[42] Ultimately, the proposed starting dosages for further studies when using everolimus as a single agent are 5-10 mg/day or 20-50 mg in an once weekly schedule.

Up to now, several data from phase II studies have been published. In agreement with temsirolimus, everolimus displayed antitumour activity in metastatic RCC, both as a single agent and in combination with imatinib.[43,44] Furthermore, antitumour activity was seen in patients with recurrent or metastatic breast cancer treated with single agent everolimus, but the high incidence of pneumonitis was unexpected (4/49 patients experienced a grade 2-3 pneumonitis). In melanoma, data are still inconclusive.[45] Phase II studies of everolimus in patients with advanced NSCLC and in patients with gemcitabine-refractory, metastatic pancreatic cancer failed to demonstrate any clinical activity.[46]

In a randomized phase III study in patients with metastatic clear cell RCC, treatment with everolimus (10 mg/day) was compared to placebo in conjunction with best supportive care. All patients had progressive disease after previous therapy with VEGFR-inhibitors (sunitinib, sorafenib), IFN-α, IL-2 or bevacizumab. Everolimus significantly prolonged progression free survival relative to placebo (4.0 vs 1.9 months), resulting in FDA-approval for everolimus as a second line therapy in advanced RCC.[47] Another phase III study with single agent RAD001 is conducted in patients with advanced neuroendocrine tumours where the agent is compared to best supportive care.

Deforolimus (AP23573)

Different schedules of deforolimus were evaluated in phase I studies, rendering a 30-minute intravenous infusion of 12.5 mg deforolimus once daily for 5 days (QDx5) every other week or 12.5-25 mg once weekly as the recommended dose for further studies.[48] At these doses, the drug in general was well tolerated with oral mucositis being the most frequent DLT. Other drug-related toxicities were similar to those seen with temsirolimus and everolimus such as rash, fatigue, hyperlipidemia, myelosuppression and

elevated liver function tests, but these were rarely of clinical importance. In the QDx5 schedule the MTD was determined at a dose of 18.75 mg/day.[49] At lower doses there were already signs of mTOR inhibition at a pharmacodynamic level as examplified by decreased phosphorylation of p70S6K in PBMCs and skin biopsies.[48-50] In view of the fact that the iv schedule is rather inconvenient for patients, an oral formulation is also being developed. Results of the ongoing phase I study have not yet been presented.

Until now, only a few phase II trials have been conducted with deforolimus at 12.5 mg once daily for 5 days (QDx5) every other week. Progression free periods exceeding 4 months were demonstrated in 61 patients (29%) in a trial in sarcoma patients.[51] In addition, it was demonstrated that a marked decrease of FGD-uptake on PET-imaging -suggesting antitumour activity- occurred in 18/25 patients already during the first course of treatment (two weeks) in the sarcoma trial.[52] Single agent activity of deforolimus was also reported in pretreated patients with advanced, progressive endometrial cancer.[53] In both studies most adverse events were only mild to moderate (fatigue, hyperglycemia, anemia, stomatitis and nausea/vomiting).

An overview of the recommended dosing schedules and the general toxicity profile of the current mTOR-inhibitors is depicted in Table 3.

Table 3 | Recommended dosing schedules and toxicity profile of current mTOR-inhibitors

mTOR-inhibitor	**Dosing scheme**	**Toxicity**
Rapamycin (sirolimus)	2-6 mg/day orally	– Gastro-intestinal (stomatitis, nausea, vomiting, diarrhea) – Dermatological (rash, nail disorders) – Mild myelosuppression (primarily thrombocytopenia) – Elevated liver function tests – Dyslipidemia – Fatigue – Hyperglycemia – Asthenia/psychiatric (bipolar like syndrome) – Allergic drug reaction – Pneumonitis
temsirolimus (CCI-779)	75 mg/day orally QDx5 every 2 weeks OR 25-250 mg/week IV	
everolimus (RAD001)	50-70 mg/week orally OR 10 mg/day orally	
deforolimus (AP 23573)	12.5 mg QDx5 every 2 weeks IV OR 12.5 mg/week IV	

mTOR-dependent pathways in chemoresistance and -sensitivity

As previously addressed, many processes crucial for malignant behaviour such as angiogenesis and uncontrolled proliferation can be driven by mTOR-dependent factors. In addition, there are strong indications, both preclinical and clinical, that (over)activation of the PI3K/AKT/mTOR-pathway is also involved in sensitivity and resistance to other antitumour agents. As exact measurements of mTOR and its phosphorylated form are technically challenging, activation status of mTOR is frequently determined indirectly by assessing factors up- or downstream from mTOR.

With respect to the clinical setting, data underlining that activated mTOR-signaling impairs outcome to other antitumour agents in particular exist for endocrine therapy, radiotherapy and, recently, also for trastuzumab. In several trials, endocrine-treated breast cancer patients with activated AKT, which leads to an enhanced mTOR-activity, had a poorer outcome. This was found to be the case for patients treated in the adjuvant setting as well as for patients with metastatic disease, irrespective of the endocrine agent used.[54,55] Next to this, high levels of pAKT are associated with a decreased benefit of adjuvant radiotherapy.[56]

Recently, loss-of-function mutations in PTEN and gain-of-function mutations in PIK3CA, both very likely to result in activation of mTOR, were identified as poor predictive factors for trastuzumab-containing therapy in patients with HER2/neu-overexpressing breast cancer.[57]

Particularly in preclinical studies, an activated PI3K/AKT/mTOR-pathway has been shown to impair outcome to many antitumour agents. Several studies using chemotherapy-resistant or hormone-refractory cell lines revealed high phospho-AKT-expression levels, while co-administration of an mTOR-inhibitor led to enhanced antiproliferative and apoptotic effects compared to the cell lines treated with hormonal or chemotherapy only.[58,59] Resistance to conventional drugs was also reversed when the cells were transfected with an adenovirus carrying an AKT-dominant negative mutant.[59] Furthermore, high levels of phospho-AKT in human lung tumour tissues were strongly related to diminished cisplatin chemosensitivity, cisplatin resistant cell lines had higher levels of phosphorylated p70S6K compared to controls and administration of rapamycin led to a remarkably decreased phosphorylated p70S6K and increased sensitivity to cisplatin.[59]

The effect of PTEN-status with respect to susceptibility to chemotherapy was demonstrated in a prostate cell line treated with doxorubicin. PTEN-negative cell lines -and therefore likely to exhibit an activated mTOR-pathway- were less responsive to doxorubicin than the PTEN-positive cell line. This relative resistance was reversed by transfecting the cells with PTEN, a constitutively activated AKT-dominant negative mutant or treatment with the mTOR-inhibitor rapamycin.[60,61]

In other preclinical studies, additive or even synergistic effects of mTOR-inhibitors were found with cisplatin, gemcitabine, paclitaxel, carboplatin, doxorubicin, imatinib, tamoxifen, letrozole or fulvestrant.[58,60,62-65] Investigational studies into the influence of an activated PI3K/AKT/mTOR on sensitivity to radiotherapy in a mouse model demonstrated an enhanced response after co-administration of an mTOR-inhibitor.[66]

In summary, extensive data point at an important role of activated PI3K/AKT/mTOR-pathway in resistance to many antitumour therapies and suggest that mTOR inhibition may restore responsiveness to such agents.

mTOR inhibition in multi-drug regimens in clinical trials

Given the potential of mTOR-inhibitors to augment antitumour activity of other agents, mTOR-inhibitors may be well suited to combine with other anticancer drugs. With respect to combining antitumour drugs in oncology, there are several prerequisites that have to be met: single agent activity, non-overlapping toxicity profiles, different mechanisms of antitumour action, and synergistic interaction. Currently, many studies are in progress that assess the feasibility, safety and activity of mTOR-inhibitor containing combinations (Table 4).

Rapamycin (sirolimus)

Besides its use as a single agent, rapamycin is assessed for its feasibility and activity in combination with e.g. bevacizumab, abraxane, sorafenib, erlotinib, trastuzumab and radiotherapy. To date, no clinical data have been published yet.

Temsirolimus (CCI-779)

Several phase I combination studies with conventional cytotoxic drugs have already been carried out. The combination of temsirolimus with either 5-fluorouracil/leucovorin or gemcitabine in advanced solid tumours resulted in unacceptable toxicity (mucositis) which was encountered at relatively low doses of temsirolimus.[67] A combined phase I/II trial with temsirolimus, paclitaxel and carboplatin is scheduled for patients with advanced endometrial or ovarian cancer. In a phase II study investigating temsirolimus in combination with the aromatase inhibitor letrozole, this regimen was well tolerated and led to an improvement of progression free survival compared to the letrozole alone arm.[68] Other phase I/II trials currently ongoing include concurrent therapy of temsirolimus with bevacizumab, erlotinib, sorafenib or sunitinib in renal cell cancer, melanoma or malignant glioma. Preliminary data of temsirolimus in combination with standard dose sorafenib (400mg bid) in patients with advanced solid malignancies indicate significant mucocutaneous toxicity at low dose temsirolimus (15-25 mg weekly iv), which necessitated the sorafenib dose to

be reduced.[69] Currently, investigational trials with temsirolimus with or without other targeted therapeutics are being conducted in prostate cancer (neoadjuvant), (non-)small cell lung cancer, neuro-endocrine tumours, GIST, cancer of unknown primary origin (CUP), pancreatic, endometrial, ovarian and head and neck cancer (www.clinicaltrials.gov).

Table 4 | Combination regimens with mTOR-inhibitors

mTOR-inhibitor	**Investigational combination regimens**	
Rapamycin (sirolimus)	– Bevacizumab	
	– Abraxane	
	– Sorafenib	
	– Erlotinib	
	– Trastuzumab	
	– Radiotherapy	
Temsirolimus (CCI-779))	– 5-Fluorouracil	– Sorafenib
	– Gemcitabine	– Sunitinib
	– Paclitaxel	– Interferon a
	– Carboplatin	– Letrozole
	– Bevacizumab	
	– Erlotinib	
Everolimus (RAD001)	– Paclitaxel	– AEE788
	– Gemcitabine	– Trastuzumab
	– Cisplatin	– Lapatinib
	– Capecitabine	– Bevacizumab
	– Docetaxel	– Sunitinib
	– Carboplatin	– Sorafenib
	– Etoposide	– Imatinib
	– Pemetrexed	– Letrozole
	– Erlotinib	– Octreotide
	– Gefitinib	– Radiotherapy
Deforolimus (AP 23573)	– Doxorubicin	
	– Capecitabine	
	– Paclitaxel	

Everolimus (RAD001)

In studies exploring the feasibility of everolimus in combination regimens, the concomitant use of paclitaxel and everolimus was well tolerated and showed no relevant pharmacokinetic

interactions.[70] In contrast, the combination of everolimus with gemcitabine led to an unacceptable high frequency of myelosuppression (thrombocytopenia and neutropenia) already at relatively low dosages of gemcitabine. The reason for the adverse toxicity profile of this combination remains to be defined, but pharmacokinetic interaction between gemcitabine and everolimus was not observed.[71] Trials with everolimus in combination with cisplatin, capecitabine, docetaxel, carboplatin, etoposide or pemetrexed are currently being performed and their toxicity reports are awaited. Given the synergistic interaction of everolimus with both cisplatin and radiotherapy in preclinical studies, there is a strong rationale to apply everolimus in combination with concomitant chemoradiotherapy.[17,66] A phase I trial in patients with locally advanced head and neck or cervical cancer is planned to establish the tolerability of daily everolimus in combination with chemoradiation. Furthermore, simultaneous administration of everolimus with inhibitors of c-kit (imatinib) or the aromatase receptor (letrozole) was found to be feasible and promising with regard to antitumour activity.[72,73]

A phase II trial was conducted in patients with metastatic or irresectable low- to intermediate-grade neuroendocrine tumours who were treated with everolimus (5-10 mg/d) in combination with octreotide LAR. The toxicity profile was similar to that found in everolimus single agent studies and a majority of patients had disease stabilization (70%, n=42) or a partial response (22%, n=13). Furthermore, the obtained progression free survival compared favorably with results obtained in other phase II/III studies using chemotherapy or other targeted therapies.[74] Several other combination regimens of everolimus with signal transduction inhibitors are currently under investigation in phase I/II trials (www.clinicaltrials.gov) (Table 4).

The favourable phase II outcome regarding the combination of octreotide LAR and everolimus in patients with neuroendocrine tumours has resulted in further investigation in a phase III trial. In another phase III trial co-administration of everolimus and imatinib is compared to best supportive care in patients with previously treated GIST whose disease has recurred or progressed while receiving imatinib.

Deforolimus (AP23573)

Little is known yet from combination studies with deforolimus. In a phase Ib trial, the concurrent use of deforolimus and paclitaxel proved to be safe and antitumour activity was seen even at modest doses of each drug.[75] In another study, the combination of deforolimus and capecitabine was tolerable and there were no signs of increased toxicity, although pharmacokinetic data suggested a decreased exposure to one of the catabolites, compatible with the finding of a gradually decreased dihydropyrimidinedehydrogenase activity to 60% of that before deforolimus administration.[76] Data from running studies

investigating the use of deforolimus in patients with taxane-resistant and androgen-independent prostate cancer have not been released yet.

PREDICTIVE FACTORS FOR RESPONSIVENESS TO mTOR-INHIBITORS

As for all antitumour agents, identification of patients who are likely to respond to mTOR inhibition is of key importance. Several efforts have been made to identify potential molecular predictive biomarkers for response or clinical benefit to mTOR-inhibitors.

Preclinical and in vitro studies indicated that cells deficient in PTEN or expressing high levels of phospho-AKT are more sensitive to temsirolimus.[77] In another study examining a panel of breast cancer cell lines, rapamycin sensitivity seemed associated with overexpression of phosphorylated AKT and p70S6K, but was independent of PTEN-status.[78] In a phase II trial with temsirolimus in poor prognosis RCC patients, it appeared that the poorest outcomes were seen in patients with tumours that express low levels of phospho-AKT and/or low phosphorylated p70S6K, which suggest that mTOR in the tumours of these individual patients is not a major driving force.[79] In patients with recurrent or metastatic endometrial cancer the response to temsirolimus turned out to be independent of PTEN-status, while increased tumour levels of phosphorylated p70S6K were more frequently encountered in patients with a partial response or stable disease than in patients displaying progressive disease.[80] However, these findings are derived from small patient series and independent validation is warranted.

In theory, numerous other tumour factors, that could be indicative of the dependency on mTOR for malignant behaviour, may turn out to be of predictive value as well. These include the activation status of cell surface receptors that use the PI3K/AKT/mTOR-pathway for signal transduction, p53-, PTEN- or TSC-mutational status, phosphorylated mTOR itself or the phosphorylation/activation status of downstream messengers like p70S6K, 4E-BP1 or HIF-1α. Initially, mTOR-inhibitors were thought to bind exclusively to mTORC1, but recent studies have indicated that mTORC2 is sensitive to prolonged inhibition as well, at least in a subset of cell types.[1,2] Determination of the mTORC1/mTORC2 status could therefore also be helpful in predicting which patients will benefit most from mTOR inhibition. In the earlier mentioned in vitro study in breast cancer patients by Noh et al. changes in cyclin D1 expression were suggested to be useful as a marker for response to mTOR-inhibitors. Furthermore, molecular profiling with tissue micro-array enables to evaluate the expression pattern and activation status of multiple signaling pathways in tumour specimens simultaneously and may serve as a method to develop patient selection models.

In conclusion, more detailed studies are warranted to appropriately assess predictive factors regarding responsiveness to mTOR inhibition. Information from current phase II/III trials with mTOR-inhibitors will be important to guide the way to identify reliable predictive factors for each tumour type.

BIOMARKERS

In contrast to traditional cytotoxic agents, molecular targeted drugs are currently not dosed upon the MTD but frequently upon a dose based on pharmacodynamic parameters that are supposed to reflect inhibition of a specific signal transduction pathway. These so-called biomarkers should be indicative of target inhibition and/or inhibition of downstream effects.

Regarding the PI3K/AKT/mTOR-pathway, biomarkers that have been suggested to play an important role in determining the biologically active dose are p70S6K1, eIF-4G and 4E-BP1.[38,39,81] In pharmacodynamic studies in experimental tumour bearing rat models, antitumour activity paralleled effects on p70S6K1 and 4E-BP1 phosporylation in PBMCs and tumours.[40] Possible clinical applicability of inhibited p70SK1 activity and/or diminished 4E-BP1 phosporylation was e.g. demonstrated in the studies of Tanaka and Tabernero, resulting in the earlier mentioned PK/PD model incorporating data derived in humans.[38,81]

Extensive biomarker analysis is currently included in most studies. There are several downstream effects of mTOR e.g. on HIF-1a and/or VEGF that, when inhibited, may be of similar or even greater importance for its antitumour activity than those mediated by p70S6K and 4E-BP1. Presently, the biggest challenge is to appropriately assess which biomarkers most adequately reflect the antitumour activity of mTOR inhibition. Next to that, it will be important to know in which tissues (e.g. PBMCs, skin and tumour) those biomarkers should be assessed.

CONCLUSION

Over the last decade, cumulative evidence has emerged that inhibition of signal transduction pathways is a promising attribution to current standard treatment regimens in cancer. Recently, novel agents impairing one of these pathways through inhibition of the central kinase mTOR were introduced. There are four different mTOR-inhibitors currently available (rapamycin, temsirolimus, everolimus and deforolimus), and in early

clinical trials, all show interesting antitumour activity in tumour types such as sarcoma, SCLC, endometrial and breast cancer. However, apart from a trial with temsirolimus as first-line treatment in patients with advanced poor risk RCC, which lead to registration of this drug, no randomized trials have been reported yet.

In general, mTOR-inhibitors exhibit a favourable safety profile with toxicities being relatively mild, reversible upon drug withdrawal and mainly consisting of rash, fatigue, hyperlipidemia, myelosuppression and elevated liver function tests. However, with expanding use novel untoward effects will be recognized as is examplified by publications suggesting that the frequency and severity of pneumonitis may be underreported.[30,47,82]

It has been described that mTOR activation confers resistance to a wide range of antitumour agents. Accordingly, synergistic interactions of mTOR-inhibitors with other antitumour agents in preclinical studies has been demonstrated. This, in combination with non-overlapping toxicity profiles and different mechanisms of action as well as single agent activity, warrants the clinical investigation of combined treatment with mTOR-inhibitors and other anticancer drugs. Such combinations are currently extensively explored (Table 5), but firm conclusions on their efficacy cannot be drawn since outcomes from randomized studies are not yet available.

Besides appropriately designed and performed clinical trials, there is a need for the identification and validation of predictive factors by which patients, that are likely to benefit from treatment with mTOR-inhibitors, can be selected. Preliminary results suggest that phospho-AKT and phosphorylated p70S6K levels in tumours may be particularly useful for this purpose.[79,80] Also, the tendency to rely on biomarker measurements as a potential predictor of antitumour activity and their paramount role in establishing doses for further exploration during phase I studies, stress the need for further research to determine whether doses that are biologically active according to biomarker studies, are indeed properly reflecting the optimal dose for all patient populations and subgroups. Currently there are still too many uncertainties in this respect and it seems prudent not to omit establishing the highest achievable dose of each mTOR-inhibitor as this may be of use in the future.

In conclusion, our knowledge on the use mTOR-inhibitors as antitumour agents is rapidly expanding and early clinical trials provide us with interesting and promising data. However, well-designed clinical trials with special emphasis on translational biomarker studies are necessary to establish the precise role of mTOR-inhibitors as antitumour agents in solid malignancies.

Table 5 | Summary of trials investigating the use of mTOR-inhibitors

	Rapamycin (sirolimus)	**Temsirolimus (CCI-779)**	**Everolimus (RAD001)**	**Deforolimus (AP 23573)**
Stage of development	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II
Investigated tumour types	– Breast – Hepatocellular – Lung – Malignant glioma – Pancreas – Prostate – Rectal	– Breast – Endometrial – GIST – Head and neck – Malignant glioma – Melanoma – Neuro-endocrine – (N-)SCLC – Ovarian – Prostate – Renal – Sarcoma	– Breast – Cervical – Colorectal – Endometrial – Gastric – GIST – Head and neck – Hepatocellular – Malignant glioma – Melanoma – Neuro-endocrine – (N-)SCLC – Ovarian – Pancreas – Prostate – Renal – Sarcoma	– Endometrial – Prostate – Sarcoma

ABBREVIATIONS

mTOR	mammalian target of rapamycin
PI3K	phosphatidylinositol 3-kinase
AKT	protein kinase B
RCC	renal cell carcinoma
IL-2	interleukin 2
TGF-β	transforming growth factor-ß
VEGF	vascular endothelial growth factor
IGF	insulin-like growth factor
PIP2	phosphatidylinositol-4,5-biphosphate
PIP3	phosphatidylinositol-3,4,5-triphosphate
p70S6K	ribosomal p70S6 kinase
4E-BP1	eukaryotic initiation factor binding protein
eIF-4E	eukaryotic initiation factor 4E
PBMC	peripheral blood mononuclear cell
HIF-1α	hypoxia-inducible factor 1α
PDGF	platelet derived growth factor
PTEN	phosphatase and tensin homologue deleted on chromosome 10
TSC1/2	tuberous sclerosis complex proteins ½
MTD	maximum tolerated dose
GIST	gastro-intestinal stromal tumours
NSCLC	non-small cell lung cancer
IFN-α	interferon-α
CUP	carcinoma of unknown primary
EGFR	epidermal growth factor receptor

REFERENCES

1. Abraham RT, Gibbons JJ (2007) The mammalian target of rapamycin signaling pathway: twists and turns in the road to cancer therapy. Clin Cancer Res 13:3109-3114.
2. Guertin DA, Sabatini DM (2007) Defining the role of mTOR in cancer. Cancer Cell 12:9-22.
3. Sansal I, Sellers WR (2004) The biology and clinical relevance of the PTEN tumor suppressor pathway. J Clin Oncol 22:2954-2963.
4. Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis, J (2002) Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proc Natl Acad Sci USA 99:13571-13576.
5. Samani AA, Yakar S, LeRoith D,Brodt P (2007) The Role of the IGF System in Cancer Growth and Metastasis: Overview and Recent Insights. Endocr Rev 28: 20-47.
6. Ferrara N (2004) Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 9 Suppl 1: 2-10.
7. Zhou X, Tan M, Stone Hawthorne V, et al (2004) Activation of the Akt/Mammalian Target of Rapamycin/4E-BP1 Pathway by ErbB2 Overexpression Predicts Tumor Progression in Breast Cancers. Clin Cancer Res 10: 6779-6788.
8. Corless CL, Schroeder A, Griffith D, et al (2005) PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and in vitro sensitivity to imatinib. J Clin Oncol 23:5357-5364.
9. Johnson BE, Jänne PA (2005) Epidermal Growth Factor Receptor Mutations in Patients with Non–Small Cell Lung Cancer. Cancer Res 65:7525-7529.
10. Cheng JQ, Lindsley CW, Cheng GZ, Yang H, Nicosia SV (2005) The Akt/PKB pathway: molecular target for cancer drug discovery. Oncogene 24:7482-7492.
11. Roy HK, Olusola BF, Clemens DL, Karolski WJ, et al (2002) AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. Carcinogenesis 23:201-205.
12. Cristiano BE, Chan JC, Hannan KM, et al (2006) A specific role for AKT3 in the genesis of ovarian cancer through modulation of G_2-M phase transition. Cancer Res 66:11718-11725.
13. Chow LM, Baker SJ (2006) PTEN function in normal and neoplastic growth. Cancer Lett 241:184-196.
14. Cully M, You H, Levine AJ, Mak TW (2006) Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 6:184-192.
15. Bjornsti M, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4:335-348.
16. Feng Z, Hu W, de Stanchina E, et al (2007) The regulation of AMPKb1, TSC2 and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating te IGF-1-AKT-mTOR pathways. Cancer Res 67:3043-3053.
17. Beuvink I, Boulay A, Fumagalli S, et al (2005) The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition op p21 translation. Cell 120:747-759.
18. Chan S (2004) Targeting the mammalian target of rapamycin (mTOR): a new approach to treating cancer. Br J Cancer 91:1420-1424.
19. Vignot S, Faivre S, Aguirre D, Raymond E (2005) mTOR-targeted therapy of cancer with rapamycin derivatives. Ann Oncol 16:525-537.

20. Rowinsky EK (2004) Targeting the molecular target of rapamycin (mTOR). Curr Opin Oncol 16:564-575.

21. Smolewski P (2006) Recent developments in targeting the mammalian target of rapamycin (mTOR) kinase pathway. Anti-Cancer Drugs 17:487-494.

22. Sarkaria JN, Schwingler P, Schild S, et al (2007) Phase I trial of sirolimus combined with radiation and cisplatin in non-small cell lung cancer. J Thorac Oncol 2:751-757.

23. Ratain MJ, Napoli KL, Knightley Mosier K, et al (2007) A phase Ib study of oral rapamycin (sirolimus) in patients with advanced malignancies. J Clin Oncol 25:18S (Abstract 3510).

24. Hidalgo M, Buckner JC, Erlichman C, et al (2006) A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. Clin Cancer Res 12:5755-5763.

25. Raymond E, Alexandre J, Faivre, S, et al (2004) Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. J Clin Oncol 22:2336-2347.

26. Forouzesh B, Buckner J, Adjei A, et al (2002) Phase I, bioavailability, and pharmacokinetic study of oral dosage of CCI-779 administered to patients with advanced solid malignancies. Eur J Cancer 38:s54.

27. Atkins MB, Hidalgo M, Stadler WM, et al (2004) Randomized phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. J Clin Oncol 22:909-918.

28. Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumbar B (2002) Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol 20:289-296.

29. Hudes G, Carducci M, Tomczak P, et al (2007) Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356:2271-2281.

30. Bellmunt J, Szczylik C, Feingold J, Strahs A, Berkenblit A (2008) Temsirolimus safety profile and management of toxic effects in patients with enhanced renal cell carcinoma and poor prognostic features. Ann Oncol 19:1387-1392.

31. Chan S, Scheulen ME, Johnston S, et al (2005) Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. J Clin Oncol 23:5314-5322.

32. Pandya KJ, Dahlberg S, Hidalgo M, et al (2007) A randomized, phase II trial of two dose levels of temsirolimus (CCI-779) in patients with extensive-stage small-cell lung cancer who have responding or stable disease after induction chemotherapy: a trial of the Eastern Cooperative Oncology Group (E1500). J Thorac Oncol 11:1036-1041.

33. Omuro AMP, Faivre S, Raymond E (2007) Lessons learned in the development of targeted therapy for malignant gliomas. Mol Cancer Ther 6:1909-1919.

34. Margolin K, Longmate J, Baratta T, et al (2005) CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. Cancer 104:1045-1048.

35. Duran I, Kortmansky J, Singh D, et al (2006) A phase II clinical and pharmacodynamic study of temsirolimus in advanced neuroendocrine carcinomas. Br J Cancer 95:1148-1154.

36. Okuno S (2006) Mammalian target of rapamycin inhibitors in sarcomas. Curr Opin Oncol 18:360-362.

37. van Glabbeke M, Verweij J, Judson I, Nielsen OS (2002) Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. Eur J Cancer 38:543-549.

38. Tabernero J, Rojo F, Calvo E, et al (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin pathway with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. J Clin Oncol 26:1603-1610.

39. O'Donnell A, Faivre S, Burris III HA, et al (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumours. J Clin Oncol 26:1588-1595.

40. Boulay A, Zumstein-Mecker S, Stephan C, et al (2004) Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res 64:252-261.

41. Lerut E, Roskams T, Goossens E, et al (2005) Molecular pharmacodynamic (MPD) evaluation of dose and schedule of RAD001 (everolimus) in patients with operable prostate carcinoma (PC). J Clin Oncol 23:16S (Abstract 3071).

42. Porter LL, Burris HA, Jones SF, et al (2006) Summary of results in patients with metastatic renal cell cancer (RCC) from phase I studies of RAD001 (everolimus). J Clin Oncol 24:18S (Abstract 14599).

43. Jac J, Giessinger S, Khan M, Willis J, Chiang S, Amato R (2007) A phase II study of RAD001 in metastatic renal cell cancer. J Clin Oncol 25:18S (Abstract 5107).

44. Chan JS, Vuky J, Besaw LA, Beer TM, Ryan CW (2007) A phase II study of mammalian target of rapamycin (mTOR) inhibitor RAD001 plus imatinib mesylate (IM) in patients with previously treated advanced renal carcinoma (RCC). J Clin Oncol 25:18S (Abstract 15600).

45. Rao RD, Allred JB, Windschitl HE, et al (2007) Results of NCCTG phase II trial of the mTOR inhibitor RAD-001 in metastatic melanoma. J Clin Oncol 25:18S (Abstract 8530).

46. Wolpin BM, Hezel AF, Abrahams T, et al (2008) Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. J Clin Oncol 27:193-198.

47. Motzer RJ, Escudier B, Oudard S, et al (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449-456.

48. Desai A, Janisch L, Berk LR, et al (2004) A phase I trial of a novel mTOR inhibitor AP23573 administered weekly (wkly) in patients (pts) with refractory or advanced malignancies: A pharmacokinetic (PK) and pharmacodynamic (PD) analysis. J Clin Oncol 22:14S (Abstract 3150).

49. Mita MM, Mita AC, Chu QS, et al (2008) Phase I trial of the novel mammalian target of rapamycin inhibitor deforolimus (AP23573; MK-8669) administered intravenously daily for 5 days every two weeks to patients with advanced malignancies. J Clin Oncol 26:361-367.

50. Rivera VM, Kreisberg JI, Mita MM, et al (2005) Pharmacodynamic study of skin biopsy specimens in patients (pts) with refractory or advanced malignancies following administration of AP23573, an mTOR inhibitor. J Clin Oncol 23:16S (Abstract 3033).

51. Chawla SP, Tolcher AW, Staddon AP, et al (2007) Survival results with AP23573, a novel mTOR inhibitor, in patients (pts) with advanced soft tissue or bone sarcomas: update of a phase II trial. J Clin Oncol 25:18S (Abstract 10076).

52. Sankhala KK, Chawla SP, Iagaru A, et al (2005) Early response evaluation of therapy with AP23573 (an mTOR inhibitor) in sarcoma using 18F2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) scan. J Clin Oncol 23:16S (Abstract 9028).

53. Colombo N, McMeekin S, Schwartz P, et al (2007) M. A phase II trial of the mTOR inhibitor AP23573 as a single agent in advanced endometrial cancer. J Clin Oncol 25:18S (Abstract 5516).

54. Pérez-Tenorio G, Stål O (2002) Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer 86:540-545.

55. Tokunaga E, Kataoka A, Kimura Y, et al (2006) The association between Akt activation and resistance to hormone therapy in metastatic breast cancer. Eur J Cancer 42:629-635.

56. Stål O, Pérez-Tenorio G, AkerbergL, et al (2003) Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Res 5:R37-44.

57. Berns K, Horlings HM, Hennessy BT, et al (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12:395-402.

58. Beeram M, Tan, QTN, Tekmal RR, Russell D, Middleton A, DeGraffenried LA (2007) Akt-induced endocrine therapy resistance is reversed by inhibition of mTOR signaling. Ann Oncol 18:1323-1328.

59. Liu LZ, Zhou XD, Qian G, Shi X, Fang J, Jiang B (2007) AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. Cancer Res 67: 6325-6332.

60. Grünwald V, DeGraffenried L, Russel D, Friedrichs WE, Ray RB, Hidalgo M (2002) Inhibitors of mTOR reverse doxorubicin resistance conferred by PTEN status in prostate cancer cells. Cancer Res 62:6141-6145.

61. Wendel HG, de Stanchina E, Fridman JS, et al (2004) Survival signalling by Akt en eIF4E in oncogenesis and cancer therapy. Nature 428:332-337.

62. Shi Y, Frankel A (1995) Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. Cancer Res 55:1982-1988.

63. Bruns CJ, Koehl GE, Guba M, et al (2004) Rapamycin-induced endothelial cell death and tumor vessel thrombosis potentiate cytotoxic therapy against pancreatic cancer. Clin Cancer Res 10:2109-2019.

64. Aissat N, Le Tourneau C, Ghoul A, et al (2008) Antiproliferative effects of rapamycin as a single agent and in combination with carboplatin and paclitaxel in head and neck cancer cell lines. Cancer Chemother Pharmacol 62:305-313.

65. Masiello D, Mohi MG, McKnight NC, et al (2007) Combining an mTOR antagonist and receptor tyrosine kinase inhibitors for the treatment of prostate cancer. Cancer Biol Ther 6:195-201.

66. Shinohara ET, Cao C, Niermann K, et al (2005) Enhanced radiation damage of tumor vasculature by mTOR inhibitors. Oncogene 24:5414-5422.

67. Punt CJ, Boni J, Bruntsch U, Peters M, Thielert C (2003) Phase I and pharmacokinetic study of CCI-779, a novel cytostatic cell-cycle inhibitor, in combination with 5-fluorouracil and leucovorin in patients with advanced solid tumors. Ann Oncol 14: 931-937.

68. Chollet P, Abrial C, Tacca O, et al (2006) Mammalian target of rapamycin inhibitors in combination with letrozole in breast cancer. Clin Breast Cancer 7:336-338.

69. Patnaik A, Ricart A, Cooper J, et al (2007) A phase I, pharmacokinetic and pharmacodynamic study of sorafenib (S), a multi-targeted kinase inhibitor in combination with temsirolimus (T), an mTOR inhibitor in patients with advanced solid malignancies. J Clin Oncol 25:18S (Abstract 3512).

70. Campone M, Levy V, Bourbouloux E, et al (2009) Safety and pharmacokinetics of paclitaxel and the oral mTOR inhibitor everolimus in advanced solid tumours. Br J Cancer 100:315-321.

71. Pacey S, Rea D, Steven N, et al (2004) Results of a phase 1 clinical trial investigating a combination of the oral mTOR-inhibitor Everolimus (E, RAD001) and Gemcitabine (GEM) in patients (pts) with advanced cancers. J Clin Oncol 22:14S (Abstract 3120).

72. van Oosterom A, Reichardt P, Blay J, et al (2005) A phase I/II trial of the oral mTOR-inhibitor Everolimus (E) and Imatinib Mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM: Study update. J Clin Oncol 23:16S (Abstract 9033).

73. Awada A, Cardoso F, Fontaine C, et al (2008) The oral mTOR inhibitor RAD001 (everolimus) in combination with letrozole in patients with advanced breast cancer: results of a phase I study with pharmacokinetics. Eur J Cancer 44:84-91.

74. Yao JC, Phan AT, Chang DZ, et al (2008) Efficacy of RAD001 (everolimus) and octreotide LAR in advanced low- to intermediate-grade neuroendocrine tumors: results of a phase II study. J Clin Oncol 26:4311-4318.

75. Cresta S, Tosi D, Sessa C, et al (2007) Phase Ib study defining the optimal dosing combinations of the mTOR inhibitor AP23573 and paclitaxel (PTX). J Clin Oncol 25:18S (Abstract 3509).

76. Perotti A, Maur M, Viganò L, et al (2006) Phase Ib pharmacokinetic (PK) and pharmacodynamic (PD) study to define the optimal dose for combining the mTOR inhibitor AP23573 with capecitabine (CAPE). J Clin Oncol 24:18S (Abstract 3065).

77. Shi Y, Gera J, Hu L, et al (2002) Enhanced sensitivity of multiple myeloma cells containing PTEN mutations to CCI-779. Cancer Res 62:5027-5034.

78. Noh WC, Mondesire WH, Peng J, et al (2004) Determinants of rapamycin sensitivity in breast cancer cells. Clin Cancer Res 10:1013-1023.

79. Cho D, Signoretti S, Dabora S, et al (2007) Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer 5:379-385.

80. Oza AM, Elit L, Biagi J, et al (2006) Molecular correlates associated with a phase II study of temsirolimus (CCI-779) in patients with metastatic or recurrent endometrial cancer—NCIC IND 160. J Clin Oncol 24:18S (Abstract 3003).

81. Tanaka C, O'Reilly T, Kovarik JM, et al (2008) A. Identifying optimal biologic doses of everolimus (RAD001) in patients with cancer based on the modeling of preclinical and clinical pharmacokinetic and pharmacodynamic data. J Clin Oncol 26:1596-1602.

82. Duran I, Siu LL, Oza AM, et al (2006) Characterisation of the lung toxicity of the cell cycle inhibitor temsirolimus. Eur J Cancer 42:1875-1880.

Chapter 6

Phase I study on adding everolimus to cisplatin in chemoradiation for head and neck or cervical cancer

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Preliminary report

ABSTRACT

PURPOSE To assess the feasibility of adding daily oral everolimus to cisplatin-based chemoradiation in patients with locally advanced head and neck (HNC) or cervical cancer (CC).

METHODS HNC and CC patients were to be enrolled in separate cohorts adding escalating everolimus doses (2.5, 5, 7.5 and 10 mg/daily), to standard concurrent cisplatin iv, 40 mg/m^2 weekly (HNC patients: 6 cycles; CC patients: 5 cycles), and radiotherapy (HNC patients: total dose 70 Gy; CC patients: total dose 63 Gy). Primary end point was safety and secondary end points included evaluation of pharmacokinetics (PK) and pharmacodynamics (PD). A classic 3x3 design was applied.

RESULTS Fourteen patients entered the study. In this interim analysis, two out of six patients in the HNC arm experienced dose limiting toxicity (DLT) at the lowest everolimus level explored (2.5 mg); grade 3 diarrhea in one patient and gastroparesis with omission of two cisplatin cycles in another patient. In the CC arm, no DLTs were observed at 2.5 mg and 5 mg doses (n=3 each). In both arms, most common toxicities were fatigue, haematological toxicity and diarrhea. Everolimus did not affect cisplatin PK. Inhibition of the PI3K/AKT/mTOR pathway was demonstrated at both dose levels.

CONCLUSION In HNC patients, the addition of everolimus to cisplatin-based chemoradiation turned out to be infeasible, as DLT was already encountered at the lowest dose studied. In CC patients, everolimus was well tolerated at doses up to 5 mg/d and further assessment of safety is currently ongoing at 7.5 mg.

INTRODUCTION

Over the past decades irradiation combined with cisplatin has become standard of care for a substantial part of patients with either locally advanced head and neck cancer (HNC) or locally advanced cervical carcinoma (CC). For HNC patients, this combination yields an absolute benefit in terms of overall survival of 6.5% at 5 years over radiotherapy alone.[1] Similarly, in CC patients, the risk of death is reduced by 30 to 50% as compared to radiotherapy alone.[2,3] Nevertheless, a substantial number of patients still experiences local relapse or metastatic spread of disease underlining the necessity for novel treatment approaches.

A promising strategy to improve the outcomes of concomitant cisplatin-based chemoradiation for HNC as well as cervical cancer might be inhibition of the phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway in tumour cells. This pathway, which is involved in the signal transduction of many factors responsible for cell growth and survival, has been demonstrated to be constitutively activated in many tumour types.[4-6] This leads to more aggressive malignant behaviour and resistance to both chemotherapy and radiotherapy and a consequently worse prognosis in numerous tumour types including HNC and CC.[7-14]

Currently, several mTOR-inhibitors are available, one of these being everolimus, an oral rapamycin derivative. In addition to a direct antitumour effect, rapamycin derivatives have shown to inhibit endothelial proliferation and demonstrated antiangiogenic activity in preclinical models.[9,15] As initial studies demonstrated feasibility to administer everolimus at doses leading to systemic drug levels yielding antitumour activity in vitro, everolimus was explored in various tumour types. In 2009 everolimus was FDA- and EMEA-approved as second line treatment of renal cell carcinoma and assessment of its efficacy in other tumour types is ongoing.[7,16] Besides its antitumour activity as single agent, everolimus also yields synergistic interaction in preclinical models with many other antitumour treatments, including cisplatin and radiotherapy.[7-11] Of note, the exact mechanisms underlying this synergistic interaction remain to be elucidated.

Given the importance of the PI3K/AKT/mTOR pathway in HNC and CC, the ability of everolimus to inhibit this pathway and the synergistic interaction with both cisplatin and radiotherapy in preclinical models, there is a strong rationale to explore everolimus in combination with cisplatin-based chemoradiation. This phase I study aimed to determine the recommended dose of everolimus with standard doses of cisplatin-based concomitant chemoradiation. As in HNC and CC different regimens of cisplatin and radiotherapy are applied, this phase I study was conducted in HNC and CC patients separately. In this preliminary report, results obtained up to the 5 mg dose level are presented.

PATIENTS AND METHODS

Eligibility criteria

Patients with histologically proven locally advanced HNC or CC for whom radiotherapy with concomitant cisplatin was indicated as first line treatment were eligible and included in two separate arms. The HNC patients had to be willing to undergo a standard prophylactic gastrostomy in anticipation of severe mucositis. Other eligibility criteria included: age ≥ 18 years; Eastern Cooperative Oncology Group performance 0 or 1; life-expectancy > 3 months; adequate bone marrow (absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L, platelet count ≥ 100 x 10^9/L, haemoglobin ≥ 6.0 mmol/L), hepatic (total bilirubin level ≤ 1.5 times upper limit of normal (ULN), serum alanine transferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 times ULN) and renal function (serum creatinine ≤ 1.5 times ULN, creatinine clearance ≥ 60 mL/min); no uncontrolled heart disease, myocardial infarction or cerebrovascular accident < 12 months prior to inclusion and no atrial fibrillation of any grade or ongoing cardiac dysrhythmias ≥ grade 2. Specific exclusion criteria included concurrent use of strong CYP3A4-inhibitors or -inducers and severe hyperlipidemia (cholesterol > 7.75 mmol/l or triglycerides > 5.0 mmol/l) although concomitant treatment for hyperlipidemia was allowed. The study was approved by the local medical ethical committee and was conducted in agreement with the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent prior to any study related procedure. (EudraCT number: 2007-000495-17).

Study design

A cycle was defined as 7 days comprising once daily oral everolimus, one cisplatin administration and 5-6 fractions of radiotherapy. Everolimus was to be studied in all dose levels in HNC and CC patients separately, given the difference in fields and total doses of radiation, and the difference in scheduled cycles of cisplatin. At each dose level, three patients were initially enrolled. Dose escalation decisions were made based on the safety assessments of all patients in the dose cohort using the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0. If one of three patients experienced a dose limiting toxicity (DLT), the dose level was expanded to a total of six evaluable patients. If a DLT was observed in two or more patients in a cohort, the maximum tolerated dose (MTD) was exceeded. The recommended dose (RD) for further studies was defined as the dose level directly below the MTD. After establishing the RD of everolimus combined with chemoradiation six to nine additional patients will be included to further characterize safety at that dose level and to exclude clinically relevant drug-drug interactions.

DLTs were defined as toxicities occurring during chemoradiation judged possibly, probably or definitely due to the combination of everolimus and chemoradiation and consisting of grade 4 neutropenia lasting > 7 days, febrile neutropenia, grade 4 thrombocytopenia and grade 3 or 4 non-haematological adverse events with the following qualifications: grade ≥ 3 nausea and vomiting despite optimal antiemetic treatment, grade ≥ 3 hypercholesterolemia or hypertriglyceridemia despite optimal treatment, serum creatinine ≥ 2 times ULN, grade 3 fatigue lasting longer than 7 days or grade 4 fatigue and skipping two or more administrations of cisplatin or five or more doses of radiotherapy. Since all patients in the HNC arm had a prophylactic gastrostomy, mucositis or mucositis-related symptoms were not considered a DLT.

After completion of chemoradiation and in the absence of disease progression, everolimus monotherapy could be continued for 6 months. Patients were withdrawn from the study in case of disease progression, unacceptable toxicity or refusal of treatment.

Drug administration

Everolimus was supplied by Novartis Pharma BV, Arnhem, The Netherlands, as tablets of 2.5 or 5 mg. Everolimus was given once daily, starting on day 2, i.e. the day after the first radiation and cisplatin administration, and was continued until completion of chemoradiation or if desired up to six months. Everolimus was taken orally with water on an empty stomach 30 min for breakfast. Cisplatin (Pharmachemie, Haarlem, The Netherlands), 40 mg/m^2, was administered iv over 3 hours weekly for a maximum of 5 cycles in CC patients and a maximum of 6 cycles in HNC patients. In the CC arm radiation therapy consisted of fractions of 2 Gy per day, daily for 5 days a week, to a total dose of 46 Gy in 4.5 weeks, followed by two doses of 8.5 Gy intracavitary high-dose-rate radiation one week later (total dose 63 Gy). For the HNC patients fractions of 2 Gy, six fractions per week (weekdays only), were given to a total dose of 70 Gy in six weeks. In both arms radiation therapy was started within 8 hours after the first cisplatin administration.

Prior to each administration of cisplatin, the absolute neutrophil count had to be ≥ 1.0 x 10^9/L, platelets ≥ 100 x 10^9/L and creatinine clearance ≥ 50 mL/min. Additional pre- and/or posthydration was allowed, prophylactic use of growth factors was not allowed. Treatment with cisplatin could be delayed up to 2 weeks for recovery, but if 2 or more cycles of cisplatin were missed, this was considered a DLT. Patients experiencing a DLT or other toxicity judged unacceptable went off-study and further administration of everolimus was withheld and not restarted. Their radiation therapy was continued as scheduled.

Pretreatment and follow-up studies

Prior to therapy, a complete medical history, physical examination, electrocardiography, laboratory evaluations (including blood chemistry, haematology and lipid profile), chest X-ray and CT-scan or MRI for disease assessment were performed. Vital signs were assessed weekly from start until two weeks following completion of chemoradiation. Patients were evaluated for toxicity and laboratory values before the start of every cycle during chemoradiation and every 3-4 weeks during treatment with everolimus monotherapy.

Pharmacokinetic sampling and data analysis

Serial blood samples for plasma levels of cisplatin-derived total and unbound platinum concentrations and whole blood levels of everolimus were collected before dosing and over a 24h period after administration of cisplatin and everolimus during the first two cycles (days 1-2-3 and days 8-9). Analysis of unbound platinum and total platinum were done using an atomic absorption spectrophotometer.[17] For everolimus analysis, blood samples were collected at day 2 and day 8 at pre-dose, 30 min and 1h, 1h30min, 2, 3, 4, 6, 9, 12 and 24h after administration of everolimus in standard blood collection tubes containing potassium EDTA as anticoagulant. The concentrations of everolimus were quantitated by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) following liquid-liquid extraction of 300 µl aliquots of whole blood, with the use of a stable labelled internal standard. The lower limit of everolimus quantification was 0.5 ng/mL. Individual pharmacokinetic parameters were estimated using weighted (1/y) noncompartmental analysis using the software program WinNonLin 5.2 (Pharsight, CA, USA). Differences in pharmacokinetic parameters of unbound cisplatin-derived platinum between cycle 1 (before everolimus treatment) and cycle 2 (during everolimus treatment) were assessed by a 95% confidence interval (one-sample T-test using SPSS version 15.0; SPSS Inc., Chicago, IL, USA).

Pharmacodynamic assessments

Peripheral blood mononuclear cells (PBMCs) and skin biopsies were used as surrogate tissue to evaluate pharmacodynamic effects. Skin biopsies at baseline and on day 22 were processed for immunohistochemical analysis (IHC) of phosphorylated 4E-binding protein 1 (p-4EBP1^{Thr70}), phosphorylated eukaryotic initiation factor 4G (p-eIF4G^{Ser1108}), phosphorylated p70S6Kinase (p-p70S6K^{Thr389}), phosphorylated S6 ribosomal protein (p-S6$^{Ser235/236}$) and proliferation marker Ki-67 as previously described.[18] For Ki-67, treatment effect was expressed as percentage of positive cells (≥ 1000 epidermal keratinocytes were evaluated). Regarding all other mTOR signalling markers, an alternative histoscore

based on both the proportion of positive cells and the intensity of staining estimated using the Allred scoring system was applied.[19]

Blood samples for PBMCs were taken on day 0, day 15 and day 29 (CC arm) or day 36 (HNC arm) and PBMCs were isolated according to routine standard procedures.[20] Resulting PBMC pellets were lysed in a specific cell extraction buffer (FNN0011, Invitrogen, Carlsbad, CA), containing protease inhibitor cocktail (Product No. 2714 Sigma-Aldrich, Zwijndrecht, The Netherlands) and 1 mM PMSF (Product No. 78830, Sigma-Aldrich). Treatment induced changes in protein levels of total p70S6K and phosphorylated p70S6Kinase (p-p70S6K^{Thr389}) in PBMCs were examined using specific ELISA kits (Invitrogen, Carlsbad, CA): p70S6K (Total) No. KHO0571 and p70S6K (pT389) No. KHO0581). Treatment induced effects on the phospho-/ total p70S6K ratio for the on-therapy samples were related to the baseline ratio. Relative changes were evaluated for each arm (HNC and CC arm) and correlated to the administered dose of everolimus.

RESULTS

General

For this preliminary report, data derived between January 2008 to August 2010 are presented. In this period fourteen patients received a total of 63 cycles of chemoradiation in combination with everolimus (median 5 cycles, range 1-6 and 1-5 in the HNC and CC arm, respectively). The achieved dose intensity of cisplatin over time did not differ significantly from the schedule dose intensity over time. In addition, 7 patients received a total of 149 weeks of everolimus monotherapy (median 23 weeks, range 4-28), following chemoradiation. Two patients were considered non-evaluable and therefore replaced. Both were treated at the 2.5 mg dose level and went off-study on day 8. One of those patients was included in the HNC arm and suffered from severe nausea and vomiting (grade 3) despite optimal antiemetic treatment. Everolimus was stopped at day 8 but as this had no effect on the extent of nausea and vomiting, this event was due to cisplatin and further cisplatin administration was withheld after the second cycle. In the CC arm, one patient withdrew her informed consent for psychological reasons and was regarded non-evaluable as well. Patient characteristics of the evaluable patients are presented in Table 1.

Safety

The most frequent chemoradiation-related and/or everolimus-related side effects are listed in Table 2. Haematological toxicity, including anaemia, thrombocytopenia and neutropenia, aggravated towards the end of chemoradiation and was considered to be most likely

cisplatin-related. Accordingly, it was more pronounced in HNC patients who received one extra cisplatin administration in comparison with CC patients. Grade 1 elevations of transaminases were seen in seven patients (58%), but these were transient and without further clinical implications.

Table 1 | Patient characteristics

	HNC patients[#]	**Cervical cancer patients[#]**	
	n=6	**n=3**	**n=3**
Everolimus	**2.5 mg**	**2.5 mg**	**5 mg**
Age			
Median	60	48	55
Range	44-63	25-48	42-59
Sex			
Male	6	-	-
Female	-	3	3
ECOG performance status*			
0	4	3	1
1	2	-	2
No. of cycles chemoradiation + everolimus			
Median	5	5	5
Range	4-6	5	5
Everolimus monotherapy			
No. of patients	3	3	1
Median no. of weeks	23	27	4
Range	21-26	19-28	-
Cisplatin (mg/m^2/week)			
Scheduled dose intensity	40	40	40
Achieved dose intensity	35.5	40	40
Radiotherapy dose (Gy)			
Median	70	63$^{\alpha}$	63$^{\alpha}$
Range	66-70	63$^{\alpha}$	63$^{\alpha}$
No. of patients with DLT	2	0	0

[#] Only those patients evaluable for toxicity included; [*] ECOG= Eastern Cooperative Oncology Group; [α] 46 Gy external beam radiotherapy + 2x 8.5 Gy intracavitary boost.

With respect to the HNC arm, radiation-associated pain was perceived by all patients with coinciding toxicities such as grade 1 to 3 anorexia and stomatitis, well-known to occur in this patient population. Grade 1-3 fatigue, nausea and vomiting were noted in 50-83% of patients. Both diarrhea and constipation were observed, the latter most probably due to the use of opiods for pain control. Mild dyslipidemia was detected in three patients (50%), a mild rash and dyspnea were observed in one patient each. Another patient (Body Mass Index = 43) displayed grade 3 hyperglycemia during combination treatment and started insulin s.c. therapy. During everolimus monotherapy two patients developed a one-sided peroneal neuropathy, possibly related to everolimus administration.

Table 2 | Treatment-related adverse events

	HNC n=6		**CC n=3**		**CC n=3**		**Total**			
Everolimus	**2.5 mg**		**2.5 mg**		**5 mg**		**HNC**		**CC**	
	G 1-2	**G 3**	**G 1-2**	**G 3**	**G 1-2**	**G 3**	**n**	**%**	**n**	**%**
Anemia	6	-	3	-	3	-	6	100	6	100
Thrombocytopenia	5	1	1	-	3	-	6	100	4	67
Nausea	3	-	3	-	3	-	3	50	6	100
Fatigue	4	1	3	-	3	-	5	83	6	100
Pain	6	-	2	-	2	-	6	100	4	67
Anorexia	5	1	-	-	2	-	6	100	2	33
Stomatitis	5	1	-	-	2	-	6	100	2	33
Leukopenia	3	2	2	1	2	-	5	83	5	83
Vomiting	4	-	-	-	-	-	4	67	-	-
Constipation	4	-	1	-	1	-	4	67	2	33
Hypertriglyceridemia	3	-	2	-	2	-	3	50	4	67
Diarrhea	2	1	3	-	2	1	3	50	6	100
Neutropenia	2	1	2	-	-	-	3	50	2	33
AST	3	-	2	-	-	-	3	50	2	33
ALT	2	-	1	-	1	-	2	33	2	33
Hypercholesterolemia	1	-	1	-	1	-	1	17	2	33
Peripheral neuropathy motor	1	1	-	-	-	-	2	33	-	-
Peripheral neuropathy sensory	2	-	1	-	-	-	2	33	1	17
Rash	1	-	1	-	1	-	1	17	2	33
Hyperglycemia	-	1	-	-	-	-	1	17	-	-
Dyspnea	1	-	-	-	-	-	1	17	-	-
Cognitive disturbance	-	-	-	-	1	-	0	0	1	17

HNC= head and neck cancer, CC=cervical cancer, G=grade

In CC patients, diarrhea was noted in all patients, although more severe in the 5 mg cohort. Radiation-associated pain occurred in four out of six patients, mostly of mild intensity. Dyslipidemia developed in four patients, with the necessity to start pravastatin in only one patient (2.5 mg cohort). In both cohorts a patient experienced a transient rash. Cognitive disturbance was reported by one patient in the 5 mg cohort. No treatment-related dyspnea or hyperglycemia was observed.

Dose limiting toxicity

At 2.5 mg, two DLTs were observed in two HNC patients. One patient experienced grade 3 diarrhea after five administrations of cisplatin and a cumulative radiotherapy dose of 54 Gy, and despite optimal supportive care. Diarrhea resolved during a temporary halt of everolimus of three days, recurred after restart and resolved again after definite everolimus interruption at day 32. No adjustments of cisplatin administrations or radiotherapy dose were necessary. The other patient went off-study on day 31, after four administrations of cisplatin and a cumulative radiotherapy dose of 56 Gy, due to gastroparesis, most probably caused by cisplatin but an interaction with everolimus treatment could not be excluded. Since omission of two or more cycles of cisplatin was defined a DLT, further dose escalation in HNC patients was not possible, and the addition of everolimus to cisplatin-based chemoradiation at this site and at these standard doses, infeasible.

In CC patients no DLT was encountered at the 2.5 mg nor at the 5 mg everolimus cohort. Accrual is currently ongoing at the 7.5 mg dose level.

Pharmacokinetics

For pharmacokinetic evaluation of cisplatin samples of 12 patients were available. A summary of unbound and total cisplatin-derived platinum pharmacokinetics is presented in Table 3. As illustrated in Figure 1A, the mean plasma concentration curves of the pharmacologically active unbound fraction of platinum with or without everolimus (cycle 1 vs. cycle 2) are identical. Paired individual exposures to unbound platinum in the absence or presence of everolimus are presented in Figure 1B (95% CI: 0.96-1.11; P=0.3).

Pharmacokinetic evaluation of everolimus was performed in 12 and 9 patients on day 2 and 8 respectively and is shown in Table 4. PK of everolimus is comparable to PK data from previous everolimus PK studies.[21] In combination with cisplatin dose-linearity of everolimus was unaffected and the Css (concentration at steady state) ratio$_{T=0/T=24}$ of 1.04 ± 0.34 did not hint a possible interaction.

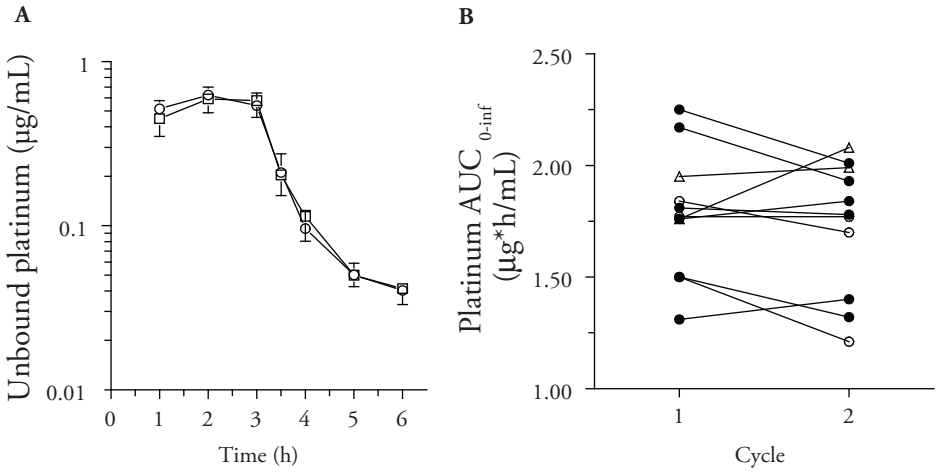

Figure 1 | (**A**) Mean ± SD (n=11) plasma concentration curves of unbound cisplatin-derived platinum given alone on Cycle 1 (circles) and in combination with everolimus on Cycle 2 (squares) and (**B**) paired individual cisplatin-derived platinum plasma exposures after treatment alone (Cycle 1) and in combination with everolimus (Cycle 2). The filled symbols in Figure B represent the individual exposures in the head and neck cancer patients in combination with 2.5 mg everolimus, while the open symbols represents the individual exposures in the cervix cancer patients (circles 2.5 mg everolimus, triangles 5.0 mg everolimus)

Pharmacodynamics

In skin tissue an increase in Ki-67 positive keratinocytes was seen in most patients, reflecting the proliferation of epidermal keratinocytes. Mean Ki-67 positivity increased from 12.1 to 17.8% ($p > 0.05$) in the HNC arm and this was even more pronounced in the CC arm (from 14.3 to 26.1% ($p > 0.05$), pooled analysis), although a clear dose effect was absent.

In the HNC arm at the 2.5 mg dose level, three out of six patients showed a decrease in p-eIF4G^{Ser1108} and p-S6$^{Ser235/236}$, while p-4EBP1^{Thr70} and p-p70S6K^{Thr389} remained largely unchanged. In CC patients similar results were obtained, with two out of three patients in the 5 mg cohort showing a decrease in p-S6$^{Ser235/236}$. Unfortunately, p-4EBP1^{Thr70} could not be evaluated in any of the 5 mg CC patients due to technical and analytical complications.

In both arms the phospho-/total p70S6K ratios in PBMCs for the on-therapy samples were mostly lower than those observed at baseline, indicating treatment induced

inhibition of the PI3K/AKT/mTOR signalling pathway. However, the relative change in ratio varied largely and was therefore not statistically significant.

Table 3 | Summary of pharmacokinetic parameters cisplatin-derived platinum[1]

Everolimus		HNC 2.5 mg n=6	CC 2.5 mg n=3	CC 5.0 mg n=3	All patients
Unbound cisplatin-derived platinum					
$AUC_{0\text{-}inf}$	Cycle 1	1.80 ± 0.366	1.70 ± 0.180	1.76; 1.95[2]	1.78 ± 0.280
(µg*h/mL)	Cycle 2	1.71 ± 0.286	1.56 ± 0.305	2.08; 1.99[2]	1.73 ± 0.295
AUC Ratio	Cycle 1/2	1.04 ± 0.083	1.11 ± 0.122	0.85; 0.97[2]	1.03 ± 0.109
CL	Cycle 1	31.8 ± 6.68	25.5 ± 0.67	29.5; 28.3[2]	29.6 ± 5.53
(L/h)	Cycle 2	32.9 ± 7.11	28.2 ± 3.50	25.0; 27.4[2]	30.4 ± 6.05
$T½_z$	Cycle 1	0.87 ± 0.41	0.55 ± 0.20	0.78; 2.10[2]	0.88 ± 0.52
(h)	Cycle 2	0.83 ± 0.41	0.56 ± 0.14	1.28; 1.16[2]	0.83 ± 0.38
Total cisplatin-derived platinum					
$AUC_{0\text{-}24h}$	Cycle 1	21.0 ± 1.20	21.2 ± 2.66	23.6 ± 2.38	21.7 ± 2.09
(µg*h/mL)	Cycle 2	24.3 ± 2.72	24.8 ± 2.47	30.5 ± 3.17	26.0 ± 3.71
$CL_{0\text{-}24h}$ (L/h)	Cycle 1	2.65 ± 0.28	2.05 ± 0.09	2.31 ± 0.24	2.41 ± 0.34

[1] Mean ± SD of patients evaluable for both courses; [2] n=2 for unbound cisplatin; individual data presented; HNC= head and neck cancer, CC=cervical cancer

Table 4 | Summary of pharmacokinetic parameters of everolimus[1]

Everolimus		HNC 2.5 mg	CC 2.5 mg	CC 5.0 mg	All patients
$AUC_{0\text{-}24h}$	Day 2	86.4 ± 52.9 (n=6)	71.2 ± 43.5 (n=3)	109; 340 (n=2)	---[2]
(ng*h/mL)	Day 8	109 ± 23.2 (n=4)	102; 115 (n=2)	217 ± 144 (n=3)	---[2]
$CL/F_{0\text{-}24h}$ (l/h)	Day 2	43.6 ± 32.3 (n=6)	43.2 ± 20.2 (n=3)	45.9; 14.7 (n=2)	41.1 ± 26.1 (n=11)
$Css_{T=0/T=24}$	Day 8	1.00 ± 0.37 (n=4)	0.81; 0.77 (n=2)	1.26 ± 0.34 (n=3)	1.04 ± 0.34 (n=9)

[1] Mean ± SD of evaluable courses; [2] No data (different doses); HNC= head and neck cancer, CC=cervical cancer

Antitumour activity

Chemoradiation was applied with curative intent. However, in the HNC arm two out of six patients had progressive disease at 6 and 8 months and died at 7 and 17 months, respectively. Both patients received full dose of standard chemoradiation. The other patients are still alive with no evidence of disease (median follow-up period of 24 months, range 20-29). In the CC arm at 2.5 mg, two out of three patients had lymphatic recurrence of disease at 13 and 18 months after chemoradiation, respectively. The other patients treated for cervical cancer show no evidence of disease at a median follow-up period of 13 months (range 1-28). Given the study design and small numbers of patients, these data need to be taken with extreme caution.

DISCUSSION

This is the first clinical trial investigating the combination of chemoradiation adding an mTOR-inhibitor. In this study, everolimus was added to cisplatin-based chemoradiation in patients with either locally advanced head and neck or cervical cancer. Though in the current report preliminary data are presented, a marked difference between both treatment groups was demonstrated with regard to tolerability of the regimen in favour of the CC arm.

The mTOR-inhibitor everolimus is known to cause mild gastro-intestinal, haematological, dermatological as well as metabolic side effects and therefore, patients were closely monitored for such adverse events.[7] In CC patients the combination of concurrent cisplatin, radiotherapy and everolimus treatment was safe and well tolerated, with fatigue, haematological toxicity and nausea being the main points of concern without undermining the standard treatment schedule. Administration of doses of 2.5 and 5 mg was possible without encountering dose limiting toxicity and accrual is currently ongoing at the 7.5 mg dose level. In contrast, in the HNC cohort, two patients experienced DLT at the lowest dose level of 2.5 mg everolimus. One DLT consisted of grade 3 diarrhea. The other DLT consisted of gastroparesis, most probably due to cisplatin treatment and resulting in pre-emptive interruption of cisplatin administration after four weeks. Since omission of two or more cycles of cisplatin was defined as DLT and a possible relationship with everolimus could not be excluded, further dose escalation in the HNC patients was not feasible. In hindsight, the early closure of this study in HNC patients might have been due to chance observation; it could be the case that by using another dose escalation study design, the combination of everolimus and chemoradiation might have shown feasibility in HNC

patients as well. For instance, the chance of falsely halting dose escalation because of the severity of background toxicity, will decrease remarkably by applying a 3+3+3 design.[22]

When comparing the observed side effects between the two cancer patient groups, side effects such as anorexia and stomatitis predominantly occurred in HNC patients (100% vs 33% in CC patients). This was anticipated based on earlier experience with chemoradiation in the head and neck area and reason for the obliged prophylactic gastrostomy before start of treatment. Notably, one has to take into account that HNC patients not only received one extra cisplatin administration but the total dose of radiation was also higher compared to that in cervical cancer patients (70 vs. 63 Gy). In general, all other toxicities were anticipated given the known toxicity profiles of the explored treatments when used as monotherapy and are seemingly not aggravated by the combination. Importantly, drawing firm conclusions on additional toxicity of a compound when added to other drugs or treatment modalities inducing toxicity by themselves, however, is not possible outside the context of a randomized trial.[22,23]

Pharmacokinetic data showed no effect of everolimus on the pharmacologically active fraction of unbound platinum plasma concentrations, as the AUC ratio of unbound platinum of cycle 1/cycle 2 was approximately 1. Although the study is not optimally designed to detect an interaction of cisplatin on everolimus PK, this seemed unlikely as the trough concentrations on day 8 (i.e., T=0) and 9 (i.e., T=24) were identical. In addition, the everolimus $AUC_{0\text{-}24h}$ at the 5 mg dose level was consistent with the observations in a previous phase I study. [21]

Inhibition of the PI3K/AKT/mTOR pathway was demonstrated in both PBMCs as well as in skin tissue by a decrease in downstream messengers. In PBMCs the phospho-/total p70S6K ratios decreased after 2.5 mg and 5 mg dosing, however this decrease was neither complete nor persistent. Accordingly, the mean phospho-/total p70S6K ratios seemed to increase again in both the CC and HNC arm at day 29 and 36, respectively. In the on-therapy skin biopsies, the extent of activation or phosphorylation of the molecular markers p-eIF4G^{Ser1108} and p-S6$^{Ser235/236}$, involved in protein translation and synthesis, was lower compared to baseline during once daily everolimus treatment. This is in accordance with findings of the phase I pharmacodynamic study with everolimus, where sustained inhibition of p-S6$^{Ser235/236}$ was demonstrated at 5 and 10 mg doses OD and complete inhibition of p-eIF4G^{Ser1108} was only seen at the 10 mg dose level.[18] Despite the ambiguity of the obtained pharmacodynamic results, it seems that the intended degree of mTOR inhibition was not reached at the dose levels investigated so far (2.5 and 5 mg).

In summary, the potential synergistic action of cisplatin-based chemoradiation together with the mTOR-inhibitor everolimus can be further explored in patients with locally advanced cervical cancer. In this patient group it has been demonstrated that the

combination is feasible at the 2.5 mg and 5 mg dose level thereby supporting the ongoing accrual in the 7.5 mg cohort. As signs of mTOR inhibition were encountered at the everolimus doses investigated, this combination deserves further study in CC patients. In HNC patients DLT was met at the lowest investigated dose level and no recommended dose was identified. The discrepancy between the feasibility results obtained in these two patient groups is most likely related to the higher irradiation and cumulative cisplatin dose in HNC patients as well as by the area of radiation.

REFERENCES

1. Pignon JP, le Maître A, Maillard E, Bourhis J (2009) Meta-analysis of chemotherapy in head and neck cancer (MACH-NC): an update on 93 randomised trials and 17,346 patients. Radiother Oncol 92:4-14.
2. Rose PG (2002) Chemoradiotherapy for cervical cancer. Eur J Cancer 38:270-278.
3. Eifel PJ (2006) Concurrent chemotherapy and radiation therapy as the standard of care for cervical cancer. Nat Clin Pract Oncol 3:248-255.
4. Amomphimoltham P, Patel V, Sodhi A, et al (2005) Mammalian target of rapamycin, a molecular target in squamous cell carcinomas of the head and neck. Cancer Res 65:9953-9961.
5. Faried LS, Faried A, Kanuma T, et al (2006) Predictive and prognostic role of activated mammalian target of rapamycin in cervical cancer treated with cisplatin-based neoadjuvant chemotherapy. Oncol Rep 16:57-63.
6. Bjornsti MA, Houghton PJ (2004) The TOR pathway: a target for cancer therapy. Nat Rev Cancer 4:335-348.
7. Konings IR, Verweij J, Wiemer EA, Sleijfer S (2009) The applicability of mTOR inhibition in solid tumors. Curr Cancer Drug Targets 9:439-450.
8. Stål O, Pérez-Tenorio G, Akerberg L, et al (2003) Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Res 5: R37-44.
9. Shinohara ET, Cao C, Niermann K, et al (2005) Enhanced radiation damage of tumor vasculature by mTOR inhibitors. Oncogene 24:5414-5422.
10. Liu LZ, Zhou XD, Qian G. Shi X, Fang J, Jiang, B (2007) AKT1 amplification regulates cisplatin resistance in human lung cancer cells through the mammalian target of rapamycin/p70S6K1 pathway. Cancer Res 67:6325-6332.
11. Shi Y, Frankel A (1995) Rapamycin enhances apoptosis and increases sensitivity to cisplatin in vitro. Cancer Res 55:1982-1988.
12. Smolewski P (2006) Recent developments in targeting the mammalian target of rapamycin (mTOR) kinase pathway. Anti-Cancer Drugs 17:487-494.
13. Faried LS, Faried A, Kanuma T, et al (2008) Expression of an activated mammalian target of rapamycin in adenocarcinoma of the cervix: A potential biomarker and molecular target therapy. Mol Carcinog 47:446-457.
14. Massarelli E, Liu DD, Lee JJ, et al (2005) Akt activation correlates with adverse outcome in tongue cancer. Cancer 104:2430-2436.
15. Guba M, von Breitenbuch P, Steinbauer M et al (2002) Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial grwoth factor. Nat Med 8:128-135.
16. Motzer, RJ, Escudier B, Oudard S, et al (2008) Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet 372:449-456.
17. Gietema JA, Hoekstra R, de Vos FY et al (2006) A phase I study assessing the safety and pharmacokinetics of the thrombospondin-1-mimetic angiogenesis inhibitor ABT-510 with gemcitabine and cisplatin in patients with solid tumors. Ann Oncol 17:1320-1327.
18. Tabernero J, Rojo F, Calvo E et al (2008) Dose- and schedule-dependent inhibition of the mammalian target of rapamycin with everolimus: a phase I tumor pharmacodynamic study in patients with advanced solid tumors. J Clin Oncol 26:1603-1610.

19. Allred DC, Harvey JM, Berardo M, Clar GM (1998) Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol 11:155-168.

20. Boulay A, Zumstein-Mecker S, Stephan C, et al (2004) Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res 54:252-261.

21. O'Donnell A, Faivre S, Burris III HA et al (2008) Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. J Clin Oncol 26:1588-1595.

22. Hamberg P, Ratain MJ, Lesaffre E et al (2010) Dose-escalation models for combination phase I trials in oncology. Eur J Cancer 46:2870-2878.

23. Hamberg P, Verweij J (2009) Phase I drug combination trial design: walking the tightrope. J Clin Oncol 27:4441-4443.

Chapter 7

The addition of pravastatin to chemotherapy in advanced gastric carcinoma: a randomized phase II trial

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ABSTRACT

PURPOSE Statins have for long been considered to play a potential role in anticancer treatment based upon their ability to inhibit the mevalonate synthesis pathway. This randomized phase II trial compared the efficacy and safety of pravastatin added to epirubicin, cisplatin and capecitabine (ECC vs. ECC+P) in patients with advanced gastric carcinoma.

METHODS Patients were randomized to receive up to six cycles of 3-weekly ECC with or without pravastatin (40 mg, once daily from day 1 of the first cycle until day 21 of the last cycle). Primary endpoint was progression free rate at 6 months ($PFR_{6months}$). Secondary endpoints were response rate (RR), progression free survival (PFS), overall survival (OS) and safety. For early termination in case of futility, a two-stage design was applied (P0=50%; P1=70%; α=0.05; β=0.10).

RESULTS Thirty patients were enrolled. $PFR_{6months}$ was 6/14 patients (42.8%) in the ECC+P arm, and 7/15 patients (46.7%) in the control arm, and therefore the study was terminated after the first stage. In the ECC and ECC+P arm, RR was 7/15 (46.7%) and 5/15 (33.3%), median PFS was 5 and 6 months and median OS was 6 and 8 months, respectively. Toxicity data showed no significant differences, although there was a trend towards more gastrointestinal side effects such as diarrhea and stomatitis in the ECC+P arm.

CONCLUSION In this randomized phase II trial the addition of pravastatin to ECC did not improve outcome in patients with advanced gastric cancer. Therefore, further testing of this combination in a randomized phase III trial cannot be recommended.

INTRODUCTION

Worldwide, gastric adenocarcinoma is the fourth most common cancer type.[1] Patients with advanced, non-resectable disease have a dismal prognosis with a median overall survival of 3-5 months. In advanced gastric carcinoma 5-fluorouracil-containing chemotherapy has shown to increase overall survival when compared to best supportive care, making chemotherapy a viable treatment option for these patients.[2-4] In addition, several randomized phase III trials have established the combination of epirubicin, cisplatin, and capecitabine (ECC) as a potential standard of care.[5] Still, prognosis of patients with advanced gastric carcinoma remains poor with a median progression free survival and overall survival of 6-7 months and 9-11 months, respectively.[6]

HMG-CoA-reductase inhibitors, frequently referred to as statins, are commonly prescribed drugs to lower serum cholesterol. Statins act by decreasing synthesis of mevalonate, the precursor of cholesterol. Mevalonate is also a precursor for isoprenoids, which play an important role in the membrane attachment of several GTP-binding proteins. These are pivotal in downstream signalling of many plasma membrane receptors involved in cellular processes such as proliferation, differentiation and apoptosis. By decreasing synthesis of isoprenoids and other yet unknown mechanisms, statins exert antiproliferative effects, attenuate metastatic potential, inhibit angiogenesis and enhance antitumour immunity in tumour cells.[7] By these mechanisms, statins exhibit antitumour activity against a wide range of tumour types including gastric carcinoma.[8,9] Moreover, in patients with advanced gastric carcinoma, high-dose statins induced disease stabilisation for 16 weeks in some patients.[9]

Statins have shown in vitro and in animal models to interact synergistically with several chemotherapeutic agents including cisplatin, 5-fluorouracil and doxorubicin, the latter being structurally almost identical to epirubicin.[10-12] The exact mechanism underlying this synergism is not fully elucidated, but might involve a statin-induced decrease in Bcl-2.[11]

Pravastatin is one of the most commonly prescribed statins and is known for its mild toxicity profile. It is more hydrophilic than other statins resulting in higher concentrations in peripheral tissues such as the stomach.[13] Clinically pravastatin demonstrated preliminary hints of efficacy in patients with hepatocellular carcinoma, where patients treated with pravastatin 40 mg once daily (OD) after chemotherapy showed increased survival compared to patients without pravastatin.[14]

Based upon its mechanism of action, non-overlapping toxicity profile, and the synergistic interaction in particular, the combination of ECC with pravastatin was chosen

in this randomized phase II study to assess its role in patients with advanced gastric carcinoma.

PATIENTS AND METHODS

Eligibility criteria

Patients with a histologically proven gastric adenocarcinoma not amenable for curative resection and with evaluable disease according to Response Evaluation Criteria in Solid Tumours (RECIST) were eligible.[15] No prior chemotherapy, radiotherapy or the use of HMG-CoA-reductase inhibitors was allowed. Other eligibility criteria included: age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) performance ≤ 2; adequate bone marrow function (white blood cell count (WBC) > 3,0 x 10^9/L, absolute neutrophil count (ANC) > 1,5 x 10^9/L, platelet count > 100 x 10^9/L, haemoglobin > 6,0 mmol/L), hepatic function (total bilirubin level ≤ 1,5 times upper limit of normal (ULN), serum alanine transferase (ALT) and aspartate aminotransferase (AST) ≤ 3 times ULN or ≤ 5 times ULN in case of liver metastases) and renal function (creatinine clearance ≥ 60 mL/min). The study was approved by the local Ethics Committee and was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent prior to any study related procedures.

Treatment

Patients were randomized to receive ECC with pravastatin (ECC+P; experimental arm) or ECC alone (control arm). ECC was given every 3 weeks for a maximum of six cycles. On day 1 of every cycle epirubicin was administered as intravenous (IV) bolus injection of 50 mg/m^2, followed by IV cisplatin at a dose of 60 mg/m^2 over three hours followed by hydration. Capecitabine 1000 mg/m^2 was taken orally twice daily on days 1-14. In the experimental arm, patients received pravastatin 40 mg OD from day 1 of the first cycle to day 21 of the last ECC cycle. Patients were not allowed to receive growth factors for myelosuppression. Dose reductions (up to 50% of starting dose of either epirubicin or capecitabine, but not cisplatin or pravastatin) and/or treatment delays (up to a maximum of 3 weeks) were allowed in case of haematological and non-haematological toxicities (e.g. renal, hepatic or skin toxicity, or hand-foot syndrome, diarrhea or mucositis).

Evaluation and outcomes

Pretreatment evaluation included a full medical history, physical examination, full blood cell count with differential, serum biochemistry including lipid profile and coagulation

tests, and computed tomography of chest and abdomen within 28 days before the start of therapy. During treatment, history taking, physical examination including toxicity assessment, haematology and serum biochemistry tests were performed before and 10-14 days after every ECC cycle. Tumour measurements were performed at baseline, every other cycle and every three months after completion of study treatment until progressive disease, as was assessed according to RECIST.[15]

Statistical methods

Primary end point was progression free rate at 6 months after randomization ($PFR_{6months}$). The expected $PFR_{6months}$ of ECC was set at 50% and it was aimed that the experimental arm would yield a $PFR_{6months}$ of 70%. Two hypotheses were tested: 1) $PFR_{6months}$ in the ECC+P arm is < 50%, which means no further testing is warranted, and 2) $PFR_{6months}$ in the ECC+P arm is > 70%, which allows for further testing. Both hypotheses were tested with type I and II errors of 0.05 (α) and 0.10 (β), respectively. According to a Simon's two-stage phase II optimal design, a sample size of 15 in each arm was required for the first stage.[16] If fewer than eight patients out of the first 15 patients in the experimental arm achieved $PFR_{6months}$, the study was to be terminated. Otherwise, another 56 patients were accrued accounting for a total of 43 patients in both arms.

Secondary endpoints included response rate (RR), progression free survival (PFS), and overall survival (OS) according to intention-to-treat analysis. Descriptive statistics were reported as estimates with accompanying 95%-confidence intervals for PFS, OS and occurrence of toxicity. PFS and OS were calculated using Kaplan-Meier methodology. PFS was calculated from the start of treatment until the date of progression or death of any cause. Comparison of adverse events was performed using the Chi-square test. Calculations were performed using SPSS v.15.

RESULTS

Patient characteristics

From February 2005 to May 2009, 30 patients were enrolled. Baseline characteristics are summarized in Table 1. The study groups were well balanced in terms of their baseline characteristics, with a median age of 58 years (range 36-74 years) and a male-female ratio of approximately 4:1. Most patients had ECOG-performance score 1 and the most frequent sites of metastatic spread were to lymph nodes (90%) and peritoneum (30%). Forty-three percent of patients had 2 or more sites of metastases.

Treatment

A total of 68 cycles in the ECC arm and 54 cycles in the ECC+P arm were administered with a mean of 4.5 and 3.6 cycles per patient (range: 1-6), respectively. The mean relative dose intensities of epirubicin, cisplatin and capecitabine were comparable in both groups as well as the mean number of days of a delay in treatment per patient (7.3 and 6.4 days, respectively). Pravastatin was taken according to predefined plan by all patients in the ECC+P arm, except for 2 patients who by mistake stopped pravastatin from day 15-21 during 2 and 4 cycles, respectively.

Table 1 | Patient characteristics

Characteristics	**ECC**	**ECC+P**
Total number of patients	15	15
Sex		
Male	13	11
Female	2	4
Age (years)		
Median	57	59
Range	42-74	36-73
ECOG performance status[∂]		
0	4	4
1	11	9
2	-	2
Prior surgery		
Yes	5	4
No	10	11
Metastatic sites[†]		
Lymph nodes	14	13
Liver	2	1
Peritoneum	4	5
Bone	-	2
Lung	2	1
Other	4	1
No. of metastatic sites		
1	8	9
2	4	4
>2	3	2

[∂] Eastern Cooperative Oncology Group; [†] Some patients had lesions at multiple sites

Study end points

One patient in the ECC+P arm was not evaluable for $PFR_{6months}$ due to stereotactic radiotherapy given after 5 months. $PFR_{6months}$ therefore was 6/14 patients (42.8%) in the ECC+P arm, and as this did not meet the predefined criteria to proceed to the second stage, the study was terminated. $PFR_{6months}$ was 7/15 patients (46.7%) in the control arm.

Responses were observed in 7/15 (46.7%) of patients in the control arm and in 5/15 (33.3%) of patients in the ECC+P arm, (p=0.473). Six patients underwent surgery or radiotherapy during follow-up. Eventually all patients progressed, except one patient who had surgery and is still alive. Median PFS was 6 (95% CI, 3.39-8.61) and 5 (95% CI, 3.83-6.17) months in the experimental and the control arm, respectively (Figure 1). Median OS was 8 (95% CI, 3.02-12.98) and 6 (95%CI, 4.93-7.08) months in the experimental and the control arm, respectively (Figure 1).

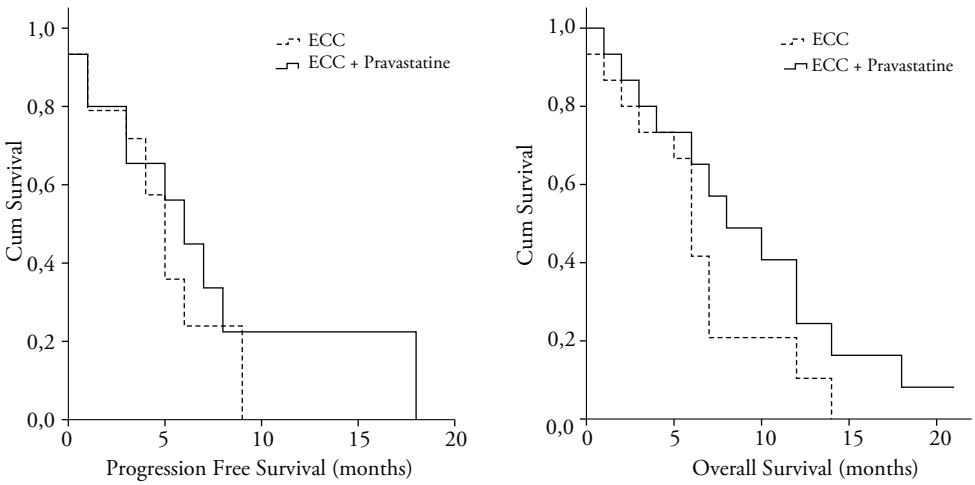


Figure 1 | Kaplan-Meier Estimates of PFS and OS.

Safety

Table 2 presents the incidence of adverse events according to the study group. As compared to ECC, haematologic toxicity and the occurrence of febrile neutropenia were similar, with the suggestion of more severe (grade 3) thrombocytopenia in the ECC+P arm. Haematological toxicity led to early termination of chemotherapy treatment in 6

patients (n=3, both arms). The occurrence of lethargy and peripheral neuropathy was similar in both arms. Diarrhea and stomatitis occurred more frequently in the ECC+P arm (26.7% vs. 66.7 %, p=0.065 and 46.7% vs. 80%, p= 0.058, respectively). At 60 days from randomization, rate of death from any cause did not differ significantly between the two arms. No patients experienced recognizable adverse events (myopathy or increased serum creatine phosphokinase concentrations) attributable to pravastin.

Tabel 2 | Toxicity profile

	ECC (n=15)		**ECC+P (n=15)**	
	NCI-CTC* grade (%)			
Adverse Event	**All Grades**	**Grade 3 or 4**	**All Grades**	**Grade 3 or 4**
Anemia	14 (93.3)	3 (20.0)	13 (86.7)	2 (13.3)
Thrombopenia	6 (40.0)	-	8 (53.3)	2 (13.3)
Neutropenia	10 (66.7)	7 (46.7)	9 (60.0)	8 (53.3)
Febrile neutropenia	-	2 (13.3)	-	3 (20.0)
Diarrhea	4 (26.7)	-	10 (66.7)	3 (20.0)
Stomatitis	7 (46.7)	-	12 (80.0)	-
Hand-foot-syndrome	1 (6.7)	-	2 (13.3)	-
Nausea and vomiting	12 (80.0)	1 (6.7)	15 (100.0)	1 (6.7)
Peripheral neuropathy	8 (53.3)	1 (6.7)	9 (60.0)	1 (6.7)
Alopecia	15 (100.0)	NA	15 (100.0)	NA
Lethargy	15 (100.0)	4 (26.7)	15 (100.0)	4 (26.7)
Thromboembolism	2 (13.3)	NA	4 (26.7)	NA
Skin rash	2 (13.3)	-	3 (20.0)	-
Myalgia	1 (6.7)	-	1 (6.7)	-
Death within 60 days after randomization	1 (6.7)	NA	1 (6.7)	NA

*NCI-CTC : National Cancer Institute Common Toxicity Criteria; NA, not applicable

DISCUSSION

In recent years, the possible beneficial effect of statins in cancer prevention and treatment has repeatedly been suggested. In particular, the role of statins in cancer prevention has been extensively investigated, but recently published large meta-analyses did not reveal such a protective effect.[17,18] The potential role of statins to potentiate antitumour activity of conventional chemotherapeutic drugs has also been explored in clinical studies; one study assessed the combination of simvastatin with first-line chemotherapy in metastatic colorectal cancer patients, while another study explored the role of fluvastatin with multi-agent chemotherapy in paediatric brain stem tumours. Both studies showed feasibility of the combinations and interesting antitumour activity when compared to historic data.[19,20] However, as both studies were not randomized, their results are somewhat difficult to interpret.

To the best of our knowledge, this study of ECC with or without pravastatin in patients with advanced gastric carcinoma is the first randomized trial assessing the effect of a statin added to standard chemotherapy in patients with a solid tumour. Consistent with observations from a large randomized trial in patients with advanced gastric carcinoma, a $PFR_{6months}$ of approximately 50% in the ECC arm was observed in our study.[6] Unfortunately enough, the addition of pravastatin to this regimen failed to increase $PFR_{6months}$ in this study, which according to predefined design was terminated after the first 30 patients were analysed. In addition to the observed lack of effect in the primary endpoint, RR, PFS and OS also did not differ between both treatment groups, further underlining a lack of benefit of pravastatin in this study. Of note here is that due to the small number of patients studied and the chosen phase II design, this study is underpowered to assess significant differences for these parameters between the two treatment arms.

With regard to safety and tolerability, the addition of pravastatin to ECC induced only mild additional toxicity, in particular diarrhea and stomatitis. Diarrhea is a well-known side effect of pravastatin occurring in 0.1-1% of patients. We consider the combination of capecitabine with pravastatin to be accountable for this side effect, which could also be the case for the observed stomatitis. Perhaps the immunomodulatory effects of pravastatin potentiate the mucocutaneous toxicity action of capecitabine given in this ECC chemotherapy.[21] The frequency of other frequently occurring side effects of ECC, such as fatigue and peripheral neuropathy, was not affected by pravastatin.

Based upon the results of this first randomized study in patients with advanced gastric cancer, we conclude that the addition of pravastatin to ECC does not improve efficacy. As advocated by the Clinical Trial Design Task Force of the National Cancer

Institute Investigational Drug Steering Committee, this phase II study had a randomized comparative design, used PFR at a given time point (i.e. six months) as primary endpoint and had sufficient power to make well balanced outcome-based decision with a sample size of 30 patients.[22] Therefore we conclude that the addition of pravastatin to ECC does not increase activity of this regimen. The conclusion from this randomized phase II trial provides strong and scientific evidence to discourage the initiation of a large and costly phase III trial to further explore the role of pravastatin in combination with ECC in patients with advanced gastric cancer.

REFERENCES

1. Kamangar F, Dores GM, Anderson WF (2006) Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 24:2137-2150.
2. Murad A, Santiago F, Petroianu A et al (1993) Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. Cancer 273:10618-10623.
3. Glimelius B, Ekström K, Hoffman K et al (1997) Randomized comparison between chemotherapy plus best supportive care with best supportive care in advanced gastric cancer. Ann Oncol 8:1-6.
4. Pyrhönen S, Kuitunen T, Nyandoto P et al (1995) Randomized comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. Br J Cancer 71:587-591.
5. Wagner AD, Unverzagt S, Grothe W et al (2010) Chemotherapy for advanced gastric cancer. Cochrane Database Syst Rev 17;3:CD004064.
6. Cunningham D, Starling N, Rao S et al (2008) Capecitabine and oxaliplatin for advanced esophagogastric cancer. N Engl J Med 358:36-46.
7. Sleijfer S, van der Gaast A, Planting AST, Stoter G, Verweij J (2005) The potential of statins as part of anti-cancer treatment. Eur J Cancer 41:516-522.
8. Chan KKW, Oza AM, Siu LL (2003) The statins as cancer agents. Clin Cancer Res 9:10-19.
9. Kim WS, Kim MM, Choi HJ et al (2001) Phase II study of high-dose lovastatin in patients with advanced gastric adenocarcinoma. Investig New Drugs 19:81-83.
10. Feleszko W, Zagozdzon R, Golab J, Jakobisiak M (1998) Potentiated antitumour effects of cisplatin and lovastatin against MmB16 melanoma in mice. Eur J Cancer 34:406-411.
11. Agarwal B, Bhendwal S, Halmos B et al (1999) Lovastatin augments apoptosis induced by chemotherapeutic agents in colon cells. Clin Cancer Res 5:2223-2229.
12. Feleszko W, Mlynarczuk I, Balkiwiec-Iskra EZ et al (2000) Lovastatin potentiates antitumor activity and attenuates cardiotoxicity of doxorubicin in three tumor models in mice. Clin Cancer Res 6:2044-2052.
13. Germershausen JI, Hunt VM, Bostedor RG et al (1997) Tissue selectivity of the cholesterol-lowering agents lovastatin, simvastatin and pravastatin in rats in vivo. Biochem Biophys Res Commun 8:1-6.
14. Kusama T, Yamasaki E, Nagase T et al (2001) Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial. Br J Cancer 84:886-891.
15. Therasse P, Arbuck SG, Eisenhauer EA et al (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216.
16. Simon R (1989) Optimal two-stage design for phase II clinical trials. Control Clin Trials 10:1-10.
17. Haukka J, Sankila R, Klaukka T et al (2010) Incidence of cancer and statin-usage linkage study. Int J Cancer 126:279-284.
18. Kuoppala J, Laminpaa A, Pukkala E (2008) Statins and cancer: a systematic review and meta-analysis. Eur J Cancer 44:2122-2132.
19. Lee J, Jung KH, Park YS et al (2009) Simvastatin plus irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) as fist-line chemotherapy in metastatic colorectal patients: a multicenter phase II study. Cancer Chemother Pharmacol 64:657-663.

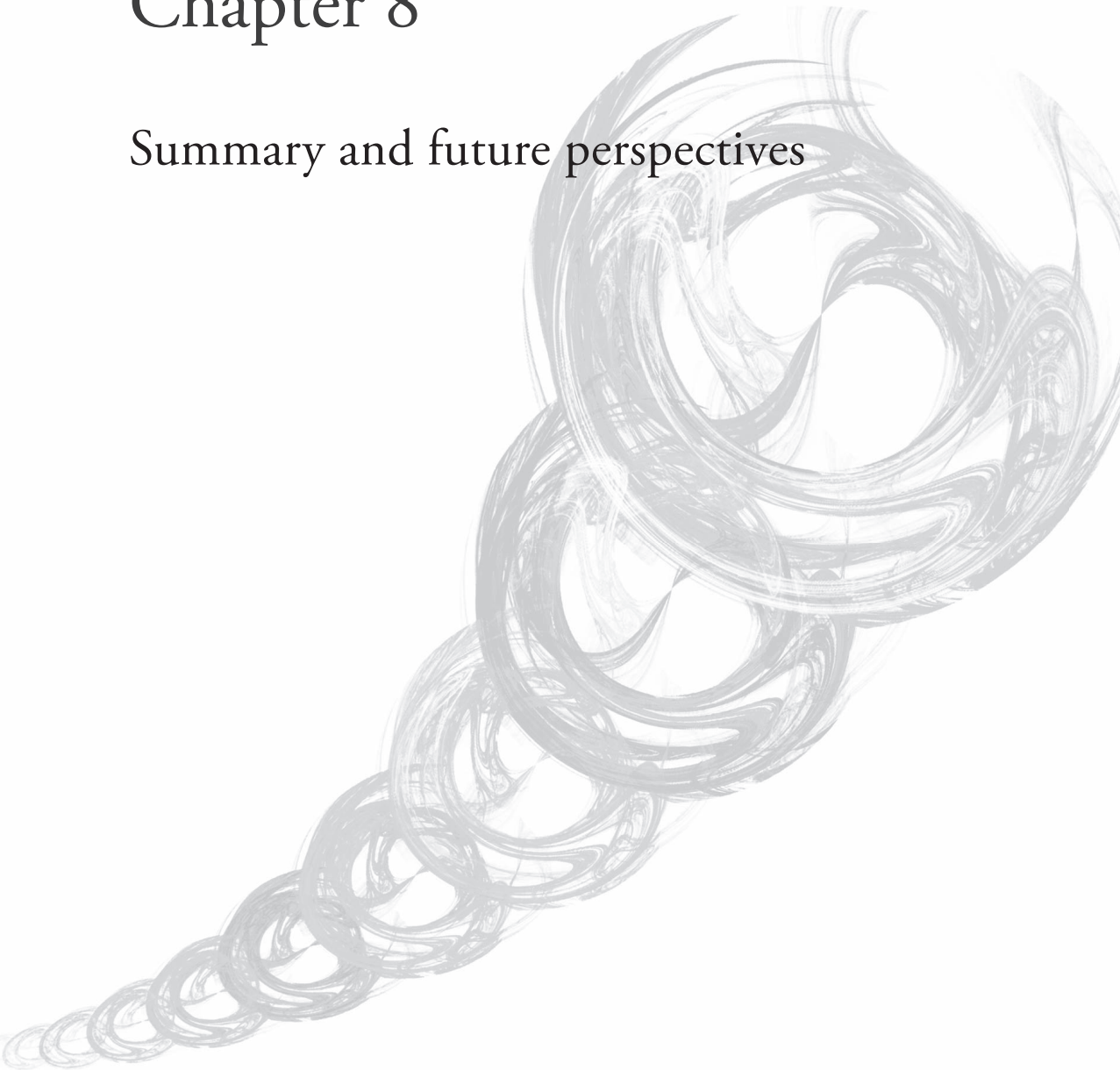
20. López-Aguilar E, Sepúlveda-Vildósola AC, Betanzos-Cabrera Y et al (2008) Phase II study of metronomic chemotherapy with thalidomide, carboplatin-vincristine-fluvastatin in the treatment of brain stem tumors in children. Arch Med Res 39:655-662.

21. Shaw SM, Fildes JE, Yonan N, Williams SG (2009) Pleiotropic effects and cholesterol-lowering therapy. Cardiology 112:4-12.

22. Seymour L, Percy Ivy S, Sargent D et al (2010) The design of phase II clinical trials testing cancer therapeutics: consensus recommendations from the Clinical Trial Design Task Force of the National Cancer Institute Investigational Drug Steering Committee. Clin Cancer Res 16:1764-1769.

Chapter 8

Summary and future perspectives



SUMMARY

In medical oncology the development of new anticancer therapies traditionally follows the track of consecutive phase I, phase II and phase III trials. However, in the era of molecularly targeted therapies with many new promising compounds requiring exploration in the clinical setting, new challenges with regard to drug development arise. Unfortunately, the abundance of novel potential agents has not resulted in a proportional increase in new, approved and registered drugs. Currently, only a small proportion (5-8%) of the investigated agents is eventually launched commercially. Furthermore, the average time from drug discovery to launch is ~13.5 years with an accompanying cost of ~1.778 billion dollar. A recent analysis of the costs of the drug development process demonstrated that clinical development (phase I - III) accounts for 63% of these costs, while 66% and 33% of phase II and III trials fail, respectively.[1] It is highly likely that a number of these agents that ultimately failed could have been identified as inactive at an earlier stage, thereby avoiding toxicity in patients exposed to these drugs at later stages of development and saving costs. On the other hand, several compounds judged inactive are probably active drugs, but could be not revealed as such since they were explored wrongly, for example in the wrong patient population. This once more stresses the importance to improve the quality of trial design in order to identify more swiftly whether or not a compound is active.

The work as described in this thesis addresses several specific aspects of early clinical cancer trials. In **Chapter 1** a general and more extensive introduction is given with regard to the different stages of clinical cancer research. It provides insight into the design of clinical cancer trials and expands on the usefulness as well as on the limitations of pharmacokinetic and pharmacodynamic parameters to guide clinical treatment development.

As noted earlier, dose and schedule of many of the currently used chemotherapeutic regimens are based on the measured drug concentrations in blood. Since blood and tumour pharmacokinetics may not per definition be the same and preliminary data suggested that concentrations of an anticancer drug in the tumour correlate with antitumour response, assessment of tumour pharmacokinetics may be more relevant in early clinical drug testing than the pharmacokinetics as measured in the peripheral circulation. We conducted two proof-of-concept studies to assess whether it is possible to measure tumour pharmacokinetics of two commonly used antitumour agents by microdialysis and to see whether these concentrations are comparable to peripheral blood concentrations. Microdialysis is a minimally invasive sampling technique based on the

diffusion of analytes from the interstitial compartment, i.e. extracellular fluid, through a semipermeable membrane. As described in **Chapter 2**, in eight patients treated with carboplatin, intratumour drug pharmacokinetics were assessed in an accessible (sub) cutaneous tumour. Plasma and microdialysis sampling of tumour and normal adipose tissue was performed up to 47 hours, whereas in previous studies this was only done up to a maximum of 4 hours. Concentration-time curves of unbound platinum in tumour tissue were practically identical to those in plasma, with exposure ratios of tumour tissue versus plasma ranging from 0.64 to 1.10. This study not only showed the feasibility of microdialysis to be employed in ambulant patients for multiple days, but also of the assessment of the pharmacokinetic profile of carboplatin in tumour after a single iv bolus. This led to the next trial, exploring the pharmacokinetics of 5-fluorouracil (5-FU) in both plasma and tumour tissue during a more prolonged period of time, namely during a 5-day continuous infusion (**Chapter 3).**

In this study, the microdialysis model was applied in seven cancer patients receiving continuous 5-FU treatment to see whether 5-FU showed the same similarity in plasma and tumour concentrations as we reported for carboplatin. However, as opposed to carboplatin, concentration-time curves of unbound 5-FU were distinctly lower in tumour tissue compared to the plasma curves. Furthermore, exposure ratios of tumour tissue versus plasma turned out to increase during the 5-day infusion period. We hypothesized that the decrease of interstitial fluid pressure caused by 5-FU itself might be a possible explanation for the substantial difference in plasma and tumour pharmacokinetics and the increase of 5-FU tumour concentrations over time. Another finding was the presence of circadian rhythmicity of 5-FU pharmacokinetics in the tumour, as 5-FU concentrations in tumour extracellular fluid were higher during the night than during daytime. For the future, this continuous 5-FU microdialysis model might be used as a proof-of-concept model to monitor the effect of drug delivery strategies.

These studies show that tumour pharmacokinetics can vary by the used agent and no general assumption can or should be made up front. Consequently, assessment of tumour pharmacokinetics should be strongly considered in early clinical testing of novel antitumour compounds. Possible explanations for the difference in pharmacokinetic behaviour between drugs could be e.g. inter-individual variability due to differences in enzymatic degradation of a drug or the influence of a high interstitial pressure, well known to occur in tumours. Microdialysis is a relatively easy means to get a better understanding in mechanisms underlying the (in)sensitivity of tumours to anticancer drugs and can be used in future trials to monitor the effect of drug delivery modulating strategies as well as to study tumour pharmacokinetics and pharmacodynamics in more detail. The ongoing

development of newer microdialysis probes that can be used for deeper lying tissue, e.g. the liver or tumour itself, will add to the clinical applicability of this technique.

Chapter 4 and 6 describe phase I trials with novel targeted therapy agents; one as single agent, the other one in combination with standard chemoradiation. In both studies extensive pharmacokinetic and pharmacodynamic analysis were performed, with interesting but also puzzling results. **Chapter 4** describes a phase I trial with the multitargeted tyrosine kinase inhibitor JNJ-26483327, which among others inhibits kinases of Epidermal Growth Factor Receptor (EGFR)-1, -2 and -4, Rearranged during Transfection (RET)-receptor, Vascular Endothelial Growth Factor Receptor (VEGFR)-3, and Src family (Lyn, Fyn, Yes). This first-in-human study followed an accelerated escalation design to assess maximum tolerated dose, safety, pharmacokinetic and pharmacodynamic effects of JNJ-26483327, which was administered orally, twice daily (BID). Dose limiting toxicity was encountered at the 2100 mg dose level, but in general it was well-tolerated. Dose proportional pharmacokinetics were observed in doses up to 1500 mg BID, with plasma levels showing antitumour activity in mouse models. Common reported toxicities were nausea, vomiting, anorexia and fatigue. Also toxicity such as diarrhea and skin rash was noted, which was thought to be due to on-target inhibition of EGFR. However, extensive pharmacodynamic analysis did not show consistent histopathological effects in skin biopsies and various biomarkers of EGFR-signalling also remained essentially unchanged, even in patients experiencing obvious on-target side effects. Although skin toxicity is a predominant side effect of many specific EGFR-tyrosine kinase inhibitors, JNJ-26483327 targets multiple tyrosine kinases and it might be that inhibition of any of the other targets acts as main driver of this specific toxicity. There were also no effects seen in serum levels of various regulators of (tumour) angiogenesis. Ultimately, based on the safety and pharmacokinetic data the recommended dose for phase II testing was set at 1500 mg and further studies to establish its clinical antitumour activity are currently being considered.

This study demonstrates one of the frequently encountered limitations of the use of pharmacodynamic data in decision making: although the target seemed to be affected clinically, this could not be confirmed by pharmacodynamic analysis of skin tissue or blood. Additionally, since both skin and blood were only used as surrogates for tumour tissue, it might be that these are not the optimal surrogate tissues to use or that other parameters such as inflammatory cytokines (e.g. IL-1β, IL-6, IL-8), placental growth factor or circulating tumour cells might have given better insight in pharmacodynamic alterations at the tumoural level. This underlines the necessity to include pharmacodynamic

research into phase I trials in order to identify the best pharmacodynamic parameters and to improve biomarker and/or biomarker assay validity.

The use of the oral mammalian target of rapamycin (mTOR)-inhibitor everolimus in combination with chemoradiation is assessed in chapter 6. Preceding that, a review is given in **Chapter 5** regarding the use of mTOR-inhibitors in solid tumours. The phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin-pathway (PI3K/AKT/mTOR-pathway) is involved in the regulation of cell proliferation, cell survival, angiogenesis and resistance to antitumour treatments. It is one of the pathways that is upregulated in many tumour types and therefore mTOR inhibition is considered an attractive means to apply as antitumour treatment. Currently, four mTOR-inhibitors are available for clinical use or research and two of these have yielded sufficient antitumour activity against various tumour types at the expense of relatively mild toxicities, to result in FDA- and EMEA-approval. Many clinical trials with mTOR-inhibitors are currently ongoing, investigating single agent therapy as well as the combination of mTOR-inhibitors with other drugs or radiotherapy. The review addresses the mechanism of action, current clinical experience with mTOR-inhibitors as well as their role in overcoming resistance to conventional therapies. Additionally, potential predictors of outcome to mTOR inhibition, such as e.g. PTEN-status or overexpression of phosphorylated AKT, are discussed.

Chapter 6 describes a phase I study in which everolimus was combined with chemoradiation, applied with curative intent. Patients with head and neck or cervical cancer (HNC and CC, respectively) were to be enrolled in separate cohorts adding escalating everolimus doses (2.5, 5, 7.5 and 10 mg/daily), to standard concurrent cisplatin iv, 40 mg/m2 weekly (HNC patients: 6 cycles; CC patients: 5 cycles), and radiotherapy (HNC patients: total dose 70 Gy; CC patients: total dose 46 Gy). We evaluated safety, pharmacokinetic and pharmacodynamic effects of this three-modality regimen and report an interim analysis. In HNC patients, the addition of everolimus to cisplatin-based chemoradiation turned out to be infeasible, as dose limiting toxicity (DLT) was already encountered at the lowest dose level studied; grade 3 diarrhea in one patient and gastroparesis with omission of two cisplatin cycles in another patient. In contrast, the combination was well tolerated in CC patients up to 5 mg/d and further assessment of safety is currently ongoing at 7.5 mg/d. In both arms, most common toxicities were fatigue, haematological toxicity and diarrhea. The addition of everolimus did not affect cisplatin pharmacokinetics. Pharmacodynamic effects of mTOR-inhibition were demonstrated in several patients from the 2.5 mg cohort onwards; in skin tissue by a decrease in downstream messengers p-eIF4G^{Ser1108} and p-S6$^{Ser235/236}$, involved in protein translation and synthesis, as well as by a decrease in the

phospho-/total p70S6K ratios in peripheral blood mononuclear cells. The discrepancy in tolerability between these two patient groups is probably caused by the higher irradiation dose, and cumulative cisplatin dose in HNC patients as well as by the area of radiation. In general, all other toxicities were anticipated given the known toxicity profiles of the explored agents when used as monotherapy and are seemingly not aggravated by the combination of the three drugs. Of note, it remains difficult to draw firm conclusions on additional toxicity caused by everolimus as it is added to other drugs with intrinsic toxicity. A randomized trial would be needed for more robust data regarding the safety profile and whether toxicity is enhanced by adding everolimus.

This study reflects the problems encountered in a combinational phase I study, since it is hard to distinguish which part of treatment is responsible for which toxicity and does not rule out the possibility that another dosage and/or administration schedule of radiotherapy, cisplatin and everolimus might have resulted in a better safety profile without compromising efficacy. Also, the variability in pharmacodynamic results once more shows that these biomarkers are not robust enough yet for guidance in drug development decisions.

Finally, in **Chapter 7** a comparative randomized two-stage phase II trial is described to show how a randomized phase II design can be of eminent value in further decision making, in particular when exploring combinations of antitumour agents. The main goal of the trial was to see whether the addition of pravastatin to epirubicin/cisplatin/capecitabine chemotherapy (ECC) would improve outcome in patients with advanced gastric cancer. The underlying hypothesis was that statins might prove useful in anticancer treatment based upon their ability to inhibit the mevalonate synthesis pathway, another pathway involved in tumorigenesis. Patients were randomized to receive either ECC with or without pravastatin and progression free rate at 6 months ($PFR_{6months}$) was used as primary efficacy end point. Response rate (RR), progression free survival (PFS), overall survival (OS) and safety were also monitored. The study was ended after the first stage because of futility as $PFR_{6months}$ was 6/14 patients (42.8%) in the ECC+pravastatin arm versus 7/15 patients (46.7%) in the control arm. Even more, with regard to toxicity a trend was noted towards more gastrointestinal side effects such as diarrhea and stomatitis in the investigational arm. By using a two-stage design it was possible to make this well balanced outcome-based decision with a sample size of only 30 patients, hereby preventing further exploration of this ECC+pravastatin regimen in patients with advanced gastric cancer in a large and costly phase III trial.

FINAL CONCLUSIONS AND FUTURE PERSPECTIVES

As detailed characterisation of erroneous signalling pathways, receptors and genetic disorders involved in tumorigenesis is increasingly possible, this may ultimately result in more tailored and individualized therapy for each cancer patient. The new class of molecularly targeted therapies will probably play an important role in this regard. In many tumours there is more than one aberrant signalling pathway or receptor involved, so it is reasonable to assume that combining agents of different groups of targeted therapy might ultimately be the best approach to improve efficacy. The currently widely used and well-established group of classic cytotoxic drugs will keep a prominent place in the treatment approaches in cancer patients, including combinations with molecularly targeted agents. The resulting enormous amount of imaginary anticancer strategies underlines the necessity to improve phase I and II trials in order to extract active regimens as quickly as possible and by this means improve the success rate of phase III trials in the end.

There are several issues that can be considered in the improvement of early clinical cancer trials. As the new molecularly targeted therapies are specifically inhibiting or blocking a certain target or activated pathway, one way to improve the velocity of clinical drug development is to include only those patients in phase I and/or phase II trials whose tumours are known to display that specific target or activated pathway. For instance, investigate a phosphatidyl-inositol 3 kinase (PI3K)-inhibitor only in tumours with proven activation of PI3K. By including only a selected patient population this will eventually contribute to patients' benefit, but to do this properly one has to be able to rely on validated and standardized assays needed to determine those biomarkers. Importantly, as for such trials the number of eligible patients is limited in comparison with phase I studies in which patients irrespective of tumour characteristics can be included, more intensive collaboration between phase I centers is indicated.

A second issue that can improve future trials is the enhanced use of pharmacodynamic analysis and parameters. In the era of targeted therapy it seems of indisputable importance to conduct pharmacokinetic and pharmacodynamic studies in conjunction. The currently used maximum tolerated dose, determined by a combination of safety and pharmacokinetic data, may in the future not be absolute anymore once valid biomarkers are available, as they may provide sufficient proof of target inhibition at doses lower than the currently used maximal tolerable dose. Since current knowledge does not enable such applications yet, incorporation of well designed pharmacodynamic research in phase I/II trials is of utmost importance to identify the best pharmacodynamic parameters and to improve biomarker validity.[2] Of note, attention should also be given to which (easily accessible) tissues can act as surrogate tissue and whether non-invasive functional imaging studies

can play a role in this regard as well. With increased scientific understanding of the target population, biomarkers may be of use in early identification of subpopulations most likely to benefit or experience harm from therapy.

Nevertheless, the incorporation of pharmacokinetic analysis in clinical cancer trials is beyond dispute. Pharmacokinetics not only give a general insight in the way the body handles the drug, but will also help to detect possible mechanisms involved in drug resistance or the extent of inter-individual variability. As was shown in the microdialysis studies described in this thesis, it is conceivable that the pharmacokinetics of anticancer drugs at the tumour level are different from those in blood. Since the achieved concentrations in the tumour are possibly better correlated with antitumour response, it may be important to evaluate tumour pharmacokinetics in a subset of patients by microdialysis for each new compound. This way it may become clear if blood and tumour pharmacokinetics overlap or if the tumour-blood ratio is constant. Furthermore, pharmacodynamic parameters may be assessed in the obtained tumour microdialysates, rendering microdialysis a useful research tool in early clinical cancer trials in general.

Another way to improve cancer drug development is by optimizing the trial design of phase I and phase II trials. For phase I trials investigating a combination of drugs, more reliable results regarding feasibility may be obtained by using a randomized design. The design should be custom-made depending on the anticipated levels of interaction of the drugs and/or treatment modalities and may eventually lead to multiple maximum tolerated doses (MTDs), with final dose determination in a subsequent randomized phase II trial.[3] To improve the efficacy of phase II trials randomisation seems to be a prerequisite. Secondly, phase II trials should be sufficiently powered to accurately capture patient benefit and predict ultimate phase III success.[4] For instance, this could be done by setting the target aims for novel therapies higher than is currently done, for example by looking for a doubling of progression free rate at a certain time point instead of a 20% increase.

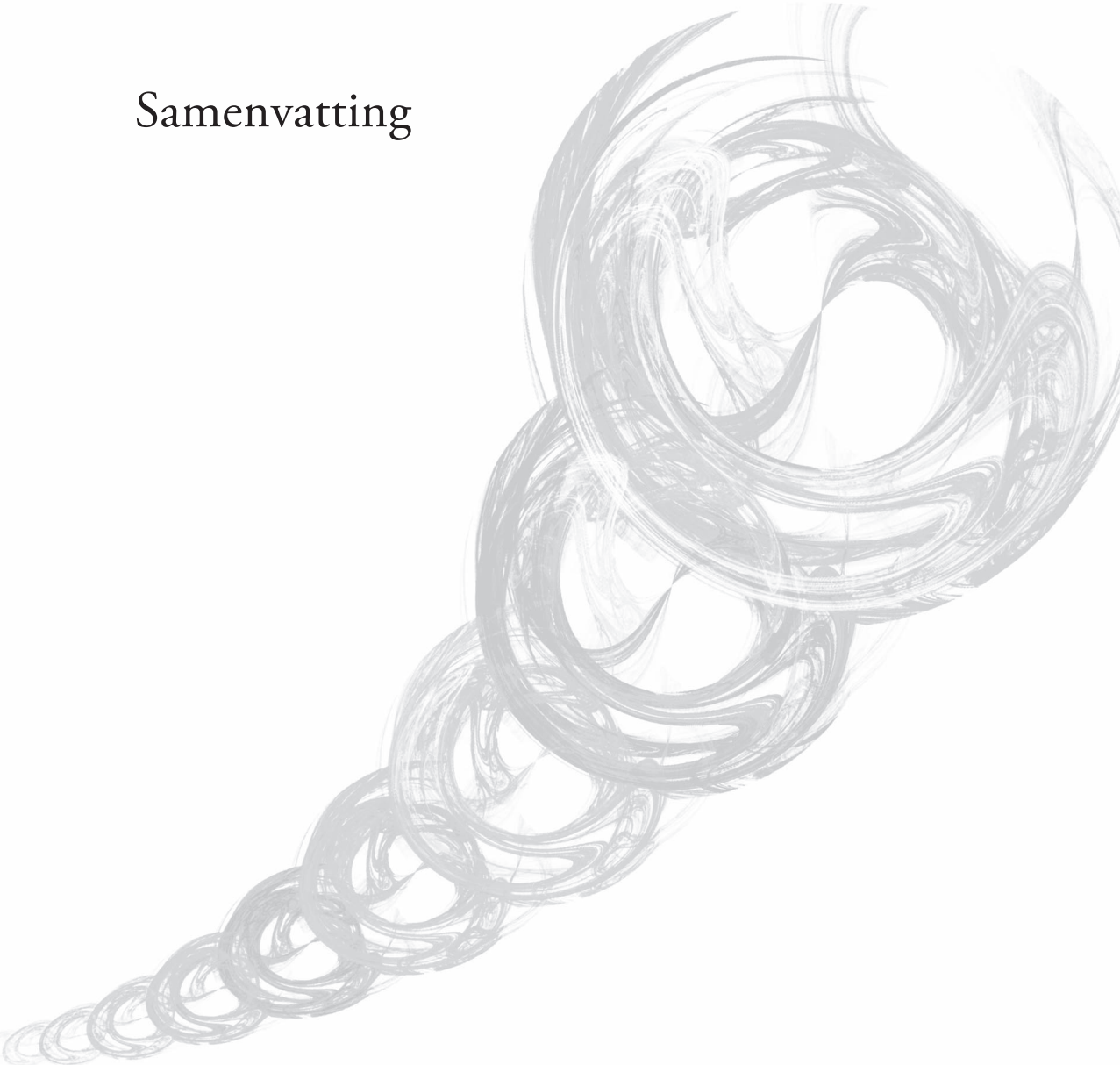
Finally, a reconsideration of the currently used end points in early clinical cancer trials seems necessary. Treatment with many of the new anticancer drugs often does not immediately result in tumour regression, but rather a stabilization of tumour growth, which can be evenly worthwhile if persisting for a long period of time. Response assessment according to the Response Evaluation Criteria in Solid Tumours may therefore well be accompanied by e.g. new functional imaging techniques such as positron emission tomography or digital contrast-enhanced magnetic resonance imaging to get a better insight in tumour response.[5] Also, since many of these drugs are administered continuously, relevant toxicity may occur much later than with the conventional cytotoxic agents making longer observation periods imperative. In the future, pharmacodynamic parameters may prove useful and robust end points but at this moment validation lacks.

In conclusion, the tremendous increase in anticancer drugs available for clinical investigation has resulted in a demand for improvement of early clinical cancer trials. Better (randomized) study designs as well as better end points, obtained best by extensive pharmacokinetic and translational research, are imperative in order to prevent that active regimens fail prematurely but also to prevent inactive regimens to enter further studies.

REFERENCES

1. Paul SM, Mytelka DS, Dunwiddie CT, et al (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nature Rev Drug Discov 9:203-214.
2. Sleijfer S, Wiemer E (2008) Dose selection in phase I studies: why we should always go for the top. J Clin Oncol 26:1576-1578.
3. Hamberg P, Ratain MJ, Lesaffre E, et al (2010) Dose-escalation models for combination phase I trials in oncology. Eur J Cancer 46:2870-2878.
4. Adjei AA, Christian M, Percy Ivy S (2009) Novel designs and end points for phase II clinical trials. Clin Cancer Res 15:1866-1872.
5. Therasse P, Arbuck SG, Eisenhauer EA, et al (2000) New guidelines to evaluate the response to treatment in solid tumours. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-216.

Samenvatting



SAMENVATTING

Het ontwikkelen van nieuwe behandelingsstrategieën voor patiënten met kanker is een essentieel onderdeel van de oncologie. Wat betreft het klinische onderzoeksdeel van nieuwe oncologische geneesmiddelen en behandelingen verloopt dit in verschillende stadia, de zogenaamde fase I, II en III studies. In fase I onderzoek wordt gekeken of een middel dat veelbelovend is gebleken in preklinisch onderzoek (d.w.z. in celkweekstudies en dierexperimenteel onderzoek) verdragen wordt door mensen en welke dosering en toedieningsvorm en -schema het meest optimaal lijkt. In deze vroegklinische onderzoeken wordt meestal nog niet geselecteerd op het soort kanker. Behalve nieuwe middelen kan het ook gaan om nieuwe combinaties van middelen of behandelingsmodaliteiten die onderzocht worden. Wanneer het optimale dosering- en toedieningschema is vastgesteld volgt een fase II onderzoek, waarin gekeken wordt naar de werkzaamheid van het nieuwe middel of de nieuwe behandeling, maar ook naar de mate waarin bijwerkingen optreden. Meestal gebeurt dit wel in patiënten met een speciaal type kanker. Zijn de resultaten hiervan nog steeds veelbelovend, dan zal een fase III onderzoek gestart worden. Het nieuwe middel of de nieuwe behandeling wordt dan in een gerandomiseerde opzet rechtstreeks vergeleken met de bestaande standaardbehandeling. Het doel is uiteindelijk met zekerheid te kunnen zeggen welke behandeling het beste is.

De ontdekking van diverse afwijkende signaaltransductie paden en receptoren die betrokken zijn bij het ontstaan van kanker heeft geleid tot een toename van nieuwe middelen die deze paden en receptoren potentieel kunnen beïnvloeden en zo een anti-tumor effect kunnen bewerkstelligen. Dit heeft helaas (nog) niet geresulteerd in een evenredige toename van nieuwe geregistreerde antitumor behandelingen, want slechts 5-8% van de onderzochte middelen wordt uiteindelijk officieel goedgekeurd en op de markt gebracht. Daarnaast neemt de totale tijd van ontdekking tot registratie van één nieuw middel gemiddeld 13,5 jaar in beslag met een bijbehorende kostenpost van 1,778 miljard dollar. Uit een recente analyse van de kosten van het totale ontwikkelingsproces van een nieuw oncologisch geneesmiddel bleek dat 63% hiervan opgaat aan het klinische onderzoeksdeel, terwijl vele nieuwe middelen juist pas falen in een relatief laat stadium; respectievelijk 66% en 33% van de fase II en fase III onderzoeken vallen negatief uit. Veel van deze laat falende middelen hadden waarschijnlijk al in een eerder stadium als onwerkzaam geïdentificeerd kunnen worden, waardoor minder patiënten blootgesteld hadden hoeven te worden aan mogelijke bijwerkingen en kosten bespaard hadden kunnen worden. Anderzijds kan het ook zo zijn dat er werkzame middelen verworpen zijn, doordat zij niet als zodanig herkend zijn door een verkeerde onderzoeksopzet, bijvoorbeeld omdat

ze onderzocht zijn in de verkeerde patiëntenpopulatie. Het is dus van groot belang om de opzet van klinisch kankeronderzoek zodanig te verbeteren dat de (in)effectiviteit van een middel al in een vroege fase blijkt.

In dit proefschrift komen verschillende aspecten van vroegklinisch kankeronderzoek aan bod. Allereerst wordt in **Hoofdstuk 1** een algemeen overzicht gegeven van de huidige opzet van klinisch kankeronderzoek, waarbij ingegaan wordt op de verschillende stadia waarin dit verloopt (de eerder genoemde fase I, II en III studies) en de opzet van deze onderzoeken. Daarnaast worden de bruikbaarheid en de beperkingen van farmacokinetische en farmacodynamische parameters met betrekking tot hun rol in de besluitvorming besproken.

De dosering en toedieningschema's van een groot aantal van de huidige chemotherapeutica is gebaseerd op de gemeten geneesmiddelconcentraties in bloed (plasma), maar deze concentraties en het resultante farmacokinetische profiel zijn mogelijk niet of onvoldoende gerelateerd aan de geneesmiddelconcentraties in tumorweefsel. Preklinisch onderzoek heeft aangetoond dat de effectiviteit van antikanker middelen gecorreleerd is met de bereikte geneesmiddelconcentratie in het doelorgaan, te weten de tumor en/of de uitzaaiingen. Het lijkt derhalve zinvol om inzicht te krijgen in de farmacokinetiek van een nieuw geneesmiddel op tumorniveau. In dit proefschrift worden twee zogenaamde "proof-of-concept" studies beschreven, waarin gekeken is of het mogelijk was om door middel van microdialyse de tumor farmacokinetiek van twee veelgebruikte cytostatica vast te stellen en deze vervolgens te vergelijken met hun farmacokinetisch profiel in plasma. Microdialyse is een relatief nieuwe en minimaal invasieve monster-afname techniek, gebaseerd op diffusie van endo- en/of exogene verbindingen vanuit het interstitium, oftewel de extracellulaire ruimte, door een semipermeabel membraan.

Hoofdstuk 2 beschrijft de toepasbaarheid van microdialyse met betrekking tot het vaststellen van een farmacokinetisch profiel in tumorweefsel van acht patiënten met een goed voor bemonstering toegankelijke tumor of metastase die werden behandeld met carboplatin. Plasma en microdialyse metingen in tumor en normaal subcutaan vetweefsel vonden plaats gedurende 47 uur, iets wat in eerdere studies slechts gedurende maximaal 4 uur was gedaan. De concentratie-tijdcurven van de vrije fractie carboplatin in tumor en in bloed waren vrijwel identiek, met blootstellingsratio's van tumor versus plasma ($AUCratio_{tumor/plasma}$) variërend van 0,64 tot 1,10. Hiermee werd niet alleen aangetoond dat het mogelijk was om microdialyse toe te passen bij ambulante kankerpatiënten gedurende meerdere dagen, maar ook werd inzicht verkregen in het tumorfarmacokinetisch profiel van carboplatin na een eenmalige intraveneuze toediening. Dit leidde tot een

vervolgonderzoek, waarbij de farmacokinetiek van 5-fluorouracil (5-FU) in zowel plasma als tumorweefsel werd bekeken gedurende een nog langere periode.

De resultaten hiervan worden beschreven in **Hoofdstuk 3.** In deze studie werd het microdialyse model toegepast bij zeven kankerpatiënten die werden behandeld met een 5-daagse continue 5-FU infusie met als onderliggende vraag of het farmacokinetisch profiel van 5-FU in plasma en tumor eveneens identiek zou zijn. In tegenstelling tot de resultaten van de carboplatin microdialyse studie bleken de concentratie-tijdcurven van het ongebonden 5-FU in tumorweefsel aanzienlijk lager te zijn dan de curven in plasma, waarbij de blootstelling aan 5-FU in de tumor wel geleidelijk toenam in de tijd. Verlaging van de interstitiële druk door de behandeling met 5-FU is hiervoor een mogelijke verklaring. Naast deze bevindingen bleek er op tumorniveau sprake te zijn van een dag-nacht ritme van de gemeten 5-FU concentraties. Er werden hogere 5-FU spiegels gemeten gedurende de nacht ten opzichte van overdag. Dit was eerder al aangetoond voor 5-FU concentraties in plasma, echter dit is de eerste studie die hiervan getuigt op tumorniveau. Uiteindelijk zou dit continue 5-FU microdialyse model goed gebruikt kunnen worden in toekomstige studies om het effect van geneesmiddelen die de tumorpenetratie beïnvloeden te monitoren.

Met deze studies is aangetoond dat het farmacokinetische profiel in tumoren per geneesmiddel kan variëren en dat er niet zonder meer vanuit gegaan kan worden dat het farmacokinetische profiel in plasma een juiste weerspiegeling daarvan is. Het is aanbevelenswaardig om meting van de intratumorale farmacokinetiek in de toekomst een standaard onderdeel te laten zijn van vroegklinisch onderzoek van nieuwe antikanker geneesmiddelen. Op deze wijze kan het op termijn duidelijk worden welke onderliggende mechanismen mogelijk verantwoordelijk zijn voor het verschil in farmacokinetiek. Hierbij kan gedacht worden aan de invloed van inter-individuele variabiliteit ten gevolge van verschillen in enzymatische afbraak van een middel of de rol van een verhoogde interstitiële druk, een fenomeen in tumoren dat veelvuldig beschreven is. De microdialyse techniek is relatief eenvoudig toepasbaar en kan gebruikt worden om mechanismen te ontrafelen die zijn betrokken bij het ontstaan van resistentie van tumoren voor bepaalde antikanker middelen. Daarnaast kan zij worden ingezet om beïnvloeding van tumorpenetratie en farmacodynamische veranderingen te monitoren. De microdialyse sondes worden continu verbeterd en mettertijd zal het mogelijk zijn deze techniek ook toe te passen op dieper gelegen weefsel, zoals bijvoorbeeld de lever of primaire tumor, waardoor de klinische toepasbaarheid zal toenemen.

In hoofdstuk 4 en 6 worden de resultaten weergegeven van twee verschillende fase I studies met nieuwe tumor(cel)specifieke geneesmiddelen. In de eerste studie werd een middel

als monotherapie onderzocht, terwijl in de tweede studie een nieuw middel toegevoegd werd aan een bestaande standaardbehandeling van chemoradiatie. Het primaire eindpunt in beide studies was verdraagbaarheid, tevens werd uitgebreid farmacokinetisch en farmacodynamisch onderzoek verricht.

Hoofdstuk 4 beschrijft de fase I studie met de multi-targeted tyrosine kinase remmer JNJ-26483327, die onder meer de kinases remt van de epidermale groeifactor receptor (EGFR), de vasculaire endotheliale groeifactor receptor en de Src familie. Dit middel werd in deze studie voor het eerst bij kankerpatiënten getest, volgens een versneld escalatie protocol. De doelstelling was de maximaal tolereerbare dosis en veiligheid te definiëren, alsmede de farmacokinetiek en farmacodynamische effecten van het middel vast te stellen. JNJ-26483327 is een oraal middel, dat tweemaal daags ingenomen dient te worden. Het middel werd in het algemeen goed verdragen, maar bij een dosering van 2100 mg traden dosislimiterende bijwerkingen op. Tot een dosering van 1500 mg was de farmacokinetiek dosis-proportioneel en daarbij werden plasma concentraties behaald waarbij in muismodellen antitumor activiteit werd waargenomen. Misselijkheid, braken, anorexie en vermoeidheid waren de meest gerapporteerde bijwerkingen, maar ook diarree en huiduitslag kwamen voor. De diarree en huiduitslag zijn hoogstwaarschijnlijk het gevolg van EGFR-remming, maar dit kon niet bevestigd worden in uitgebreide farmacodynamische analyses in huidbiopten en perifere mononucleaire bloedcellen. Huiduitslag is één van de meest voorkomende bijwerkingen van veel specifieke EGFR-remmers, maar aangezien JNJ-26483327 meerdere tyrosine kinases remt, is het mogelijk dat remming van één van deze andere kinases (in)direct verantwoordelijk is voor deze specifieke toxiciteit. Uiteindelijk werd op basis van het bijwerkingenprofiel en de farmacokinetiek de aanbevolen dosis voor fase II onderzoek vastgesteld op 1500 mg. Verdere studies naar de antitumor effectiviteit van JNJ-26483327 worden momenteel overwogen.

Deze studie toont eens te meer het manco van de bruikbaarheid van farmacodynamische data in de besluitvorming van vroegklinisch kankeronderzoek op dit moment. Hoewel er klinisch duidelijke aanwijzingen waren voor doelgerichte remming van betrokken eiwitten, kon dit niet bevestigd worden door middel van farmacodynamische analyses in huidbiopten en bloed. Aangezien huid en bloed in deze als surrogaat fungeerden voor tumorweefsel, is het mogelijk dat dit niet de optimale keuze is voor surrogaatweefsel of dat andere parameters, zoals bijvoorbeeld inflammatoire cytokines of circulerende tumorcellen, een betere weergave geven van farmacodynamische veranderingen op tumorniveau. Enkel door uitgebreid farmacodynamisch onderzoek te blijven incorporeren in fase I studies zal het mogelijk zijn om uiteindelijk de beste farmacodynamische parameters te identificeren en zal de validiteit, van zowel biomarkers als de kwaliteit van de assays om deze biomarkers aan te tonen, verbeteren.

De toevoeging van het orale middel everolimus, een remmer van mTOR (mammalian target of rapamycin), aan chemoradiatie wordt beschreven in hoofdstuk 6. Voorafgaand daaraan wordt in **Hoofdstuk 5** een overzicht gegeven van het gebruik van mTOR-remmers bij de behandeling van solide tumoren. Het mTOR-kinase is onderdeel van de fosfatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin-cascade (PI3K/AKT/mTOR-cascade). Deze cascade speelt een belangrijke rol in de regulering van cellulaire processen als proliferatie, survival, angiogenese en resistentie-ontwikkeling tegen antikanker middelen en blijkt overactief te zijn in veel tumoren. Behandeling van tumoren door remming van mTOR heeft daarom veel aandacht gekregen en op dit moment zijn er vier mTOR-remmers beschikbaar voor klinisch gebruik en wetenschappelijk onderzoek. Twee hiervan zijn reeds geregistreerd vanwege hun aangetoonde antitumor effectiviteit in verschillende tumoren en een relatief mild bijwerkingenprofiel. Momenteel lopen er diverse klinische kankeronderzoeken waarin het gebruik van mTOR-remmers wordt getest, zowel als monotherapie als in combinatie met andere middelen of radiotherapie. In dit overzichtsartikel wordt het werkingsmechanisme, de huidige ervaringen en de rol van mTOR-remmers met betrekking tot resistentie-ontwikkeling bij solide tumoren beschreven. Tevens komen mogelijke parameters aan bod die van predictieve waarde voor het resultaat van behandeling met mTOR-remmers zouden kunnen zijn, zoals bijvoorbeeld de PTEN-status of overexpressie van gefosforyleerd AKT.

In **Hoofdstuk 6** wordt een tussentijdse rapportage gedaan van de tot nu toe behaalde resultaten in de fase I studie naar de verdraagbaarheid van chemoradiatie wanneer daar de mTOR-remmer everolimus aan wordt toegevoegd. Deze combinatie wordt onderzocht in patiënten met een locaal vergevorderde tumor in het hoofdhals (HH)-gebied of van de baarmoederhals (cervixcarcinoom, CC). De standaardbehandeling, chemoradiatie, heeft een curatieve intentie en de toevoeging van everolimus mag daar geen afbreuk aan doen. De patiënten worden in afzonderlijke cohorten geïncludeerd, waarbij de dosering everolimus geleidelijk wordt opgevoerd van 2,5 naar 10 mg per dag in stappen van 2,5 mg per cohort. De chemotherapie, bestaande uit wekelijkse intraveneuze toedieningen cisplatin van 40mg/m^2 (6 cycli bij de HH-patiënten, 5 cycli bij de CC-patiënten), wordt gelijktijdig gegeven met bestraling, die op doordeweekse dagen plaatsvindt (totale dosis HH-patiënten 70 Gy, totale dosis CC-patiënten 63 Gy). Er wordt niet alleen gekeken naar het optreden van bijwerkingen, maar ook naar de farmacokinetiek en farmacodynamische effecten van deze combinatie. In deze interim-analyse bleek dat twee van de zes HH-patiënten dosisbeperkende toxiciteit ondervonden bij de laagste dosering everolimus (2,5 mg): één patiënt had last van graad 3 diarree, een andere patiënt kreeg een gastroparese met het overslaan van twee cycli cisplatin als gevolg. Het bleek derhalve niet mogelijk

om een dosering voor verder (fase II) onderzoek vast te stellen voor de HH-patiënten. Dit in tegenstelling tot de CC-patiënten die everolimus doseringen van 2,5 en 5 mg in combinatie met chemoradiatie goed verdroegen en waar momenteel de verdraagbaarheid van de combinatie met 7,5 mg everolimus wordt onderzocht. De meest voorkomende bijwerkingen in beide patiëntgroepen waren vermoeidheid, beenmergsuppressie en diarree. De farmacokinetiek van cisplatin werd niet beïnvloed door gelijktijdig everolimus gebruik. Wat betreft farmacodynamische effecten werden reeds vanaf 2,5 mg everolimus tekenen gezien van mTOR-remming, zich uitend in een verlaging van de secundaire signaaleiwitten p-eIF4G^{Ser1108} en p-S6$^{Ser235/236}$, betrokken bij cellulaire DNA-translatie en eiwitsynthese. In perifere mononucleaire bloedcellen werd daarnaast ook een verlaging van de gefosforyleerde versus totale p70S6K ratio gezien.

Het verschil in verdraagbaarheid tussen de twee patiëntengroepen kan waarschijnlijk grotendeels toegeschreven worden aan het feit dat de HH-patiënten zowel een hogere dosis bestraling als cisplatin kregen. De anatomische positie van het bestralingsgebied kan eveneens een rol gespeeld hebben. Er was vooraf geanticipeerd op de overige opgetreden bijwerkingen aangezien de afzonderlijke toxiciteit van cisplatin, bestraling en everolimus bekend is. Deze bijwerkingen lijken niet verergerd in de combinatiebehandeling. Het blijft desalniettemin lastig om harde uitspraken te doen over toegevoegde toxiciteit als gevolg van de toevoeging van everolimus aan het regime. Een gerandomiseerd onderzoek zal meer valide data genereren met betrekking tot veiligheid en verdraagbaarheid.

Uit de interim gegevens van deze studie blijkt hoe moeilijk het is om in een fase I studie waarin een combinatiebehandeling wordt gebruikt, onderscheid te maken welk deel van de behandeling verantwoordelijk is voor welke toxiciteit. Het zou kunnen zijn dat andere dosering- of toedieningschema's van bestraling, cisplatin en everolimus minder bijwerkingen tot gevolg hebben zonder afbreuk te doen aan effectiviteit. Ook in deze studie blijkt uit de grote variabiliteit in de farmacodynamische onderzoeksresultaten dat deze momenteel nog niet robuust genoeg zijn om een doorslaggevende rol te spelen in de besluitvorming van geneesmiddel- of behandelingontwikkelprocessen.

Tenslotte worden in **Hoofdstuk 7** de resultaten van een vergelijkende gerandomiseerde fase II studie beschreven om de waarde van randomisatie als onderzoeksinstrument in oncologische studies te benadrukken, vooral wanneer meerdere geneesmiddelen of behandelmodaliteiten met elkaar gecombineerd worden. De beschreven studie onderzocht de toegevoegde waarde van pravastatine aan het bekende chemotherapie-schema epirubicine/cisplatin/capecitabine (ECC) bij patiënten met een vergevorderd of gemetastaseerd maagcarcinoom. Statines zouden een potentieel antitumor effect kunnen hebben vanwege het feit dat zij een remmende werking hebben op de mevalonaatsynthese

cascade, een cascade betrokken bij het ontstaan van tumoren. De patiënten werden na inclusie gerandomiseerd voor een behandeling met ofwel de standaard (ECC) of ECC in combinatie met dagelijks pravastatine. Het primaire eindpunt was de progressievrije overleving na 6 maanden (PFR_{6mnd}). Tevens werd gekeken naar de mate van respons, de totale progressievrije overleving, overleving in het algemeen en de veiligheid. De studie was opgebouwd in twee fasen en werd na statistische analyse van de resultaten van de eerste fase gesloten vanwege onvoldoende verbetering van de werkzaamheid door toevoeging van pravastatine. In de ECC+pravastatine arm behaalden zes van de veertien patiënten (42,8%) het PFR_{6mnd} eindpunt versus zeven van de vijftien patiënten (46,7%) in de ECC arm. Observatie van de toxiciteitsdata toonde zelfs een trend aan in de richting van het vaker voorkomen van gastro-intestinale bijwerkingen, zoals diarree en stomatitis, in de experimentele arm.

Door gebruik te maken van een gerandomiseerde tweefasen studiedesign was het mogelijk om een weloverwogen en gefundeerde beslissing te maken met betrekking tot het wel of niet voortzetten van de studie op basis van de behaalde resultaten met slechts 30 patiënten. Hiermee is voorkomen dat onnodig veel patiënten bloot werden gesteld aan mogelijke extra bijwerkingen in een grote fase III studie en werd tijd en geld bespaard.

TOEKOMSTIGE ONTWIKKELINGEN

Door de toenemende inzichten in het aandeel van ontspoorde signaaltransductie cascades, receptoren en genetische afwijkingen in het ontstaan van tumoren, lijkt het mogelijk om op termijn iedere kankerpatiënt een geïndividualiseerde en op maat gemaakte behandelingsstrategie te adviseren. Het is waarschijnlijk dat de nieuwe groep van tumor(cel) specifieke moleculaire geneesmiddelen hierin een grote rol zal spelen. Aangezien er in veel tumoren vaak sprake is van meerdere ontregelde signaaltransductie cascades en receptoren, is het aannemelijk dat het combineren van verschillende tumorcelspecifieke antikanker geneesmiddelen uiteindelijk het meest optimale antitumor effect zal bewerkstelligen. Uiteraard zullen de 'klassieke' cytostatica ook gebruikt blijven worden, al dan niet gecombineerd met de nieuwe antitumor middelen. Het aantal denkbare combinaties van antikanker behandelingen wordt hiermee oneindig groot, waardoor het eens te meer wenselijk is om al in een vroeg stadium (fase I/II) duidelijkheid te krijgen over de potentie van een nieuw antitumor middel of behandelstrategie en waardoor het succespercentage van fase III studies uiteindelijk zal verbeteren.

Er zijn meerdere zaken die een rol kunnen spelen in de verbetering van de kwaliteit en snelheid van vroegklinisch kankeronderzoek. Allereerst dient overwogen te worden om

in fase I/II studies met tumor(cel)specifieke middelen alleen die patiënten te includeren bij wie is aangetoond dat hun tumor het specifieke aangrijpingspunt voor het onderzochte middel bezit of de beoogde aberrante signaaltransductie cascade aanwezig is. In dat geval zou een PI3K-remmer alleen getest worden in patiënten met tumoren met bewezen (over) activiteit van PI3K. Verfijnen van de patiëntbehandelpopulatie kan alleen plaatsvinden indien het mogelijk is om deze selectie te maken op basis van gevalideerde predictieve biomarkers. Momenteel ontbreekt het echter aan validiteit van zowel biomarkers als van de assays om deze aan te tonen. Doordat de beschikbare patiëntenaantallen voor dit soort selectieve fase I studies aanmerkelijk lager zullen zijn ten opzichte van de huidige situatie, waarbij alle patiënten in principe includeerbaar zijn, zal intensievere samenwerking tussen de fase I centra nodig zijn.

Een tweede manier om toekomstig vroegklinisch onderzoek te verbeteren is door meer gebruik te maken van farmacodynamisch onderzoek en farmacodynamische parameters. In de huidige tijd van toenemende aantallen onderzochte tumor(cel)specifieke middelen is het van groot belang om zowel farmacokinetische als farmacodynamische analyses op te nemen in de studie protocollen, zodat de gegenereerde data gelijktijdig geïnterpreteerd kunnen worden. De maximaal tolereerbare dosis, voortgekomen uit fase I studies, wordt op dit moment gebruikt als de aanbevolen dosis voor fase II studies en is gebaseerd op basis van het bijwerkingenprofiel en farmacokinetiek van het onderzochte middel. Indien er in de toekomst farmacodynamische biomarkers kunnen worden geïdentificeerd die een juiste weerspiegeling zijn van de mate van remming van de beoogde receptor of signaaltransductie cascade, zou deze aanbevolen dosering lager uit kunnen vallen dan de huidige maximaal tolereerbare dosis, zolang er maar sprake is van adequate inhibitie. Helaas reikt de wetenschap nog niet zo ver, maar hiermee wordt wel het belang van farmacodynamisch onderzoek in vroegklinische studies onderstreept. Farmacodynamisch onderzoek hoeft zich niet uitsluitend af te spelen in tumorweefsel. Het is mogelijk dat andere (makkelijker benaderbare) weefsels als surrogaat kunnen dienen of dat non-invasief afbeeldend onderzoek een rol kan spelen. Biomarkers zullen in de toekomst vermoedelijk van grote waarde worden in het identificeren van patiënten die baat zullen hebben van een bepaalde behandeling of juist at risk zijn voor bijwerkingen of zelfs een negatief behandeleffect.

Ondanks het groeiende belang van de farmacodynamiek blijft ook het uitvoeren van farmacokinetisch onderzoek uitermate belangrijk. Farmacokinetiek geeft niet alleen inzicht in de manier waarop het lichaam met het middel omgaat, zij kan ook gebruikt worden om onderliggende mechanismen die ten grondslag liggen aan resistentie-ontwikkeling op het spoor te komen of inzicht te krijgen in de mate van inter-individuele variabiliteit. De microdialyse-studies in dit proefschrift tonen aan dat de farmacokinetiek van een

middel op tumorniveau wezenlijk kan verschillen van de farmacokinetiek in bloed. Aangezien de bereikte geneesmiddelconcentraties in de tumor mogelijk beter correleren met het uiteindelijke antitumor effect, lijkt het raadzaam om voor ieder nieuw middel in een aantal patiënten het farmacokinetisch profiel in tumoren te onderzoeken door middel van microdialyse. Zo kan het duidelijk worden of er een overeenkomst is tussen tumor en bloed farmacokinetiek en of er sprake is van een constante tumor/bloedratio. Daarnaast is het ook mogelijk om farmacodynamische parameters in de microdialysaten te bepalen, wat nog meer ten gunste komt aan de waarde en inzetbaarheid van microdialyse in vroegklinisch kankeronderzoek.

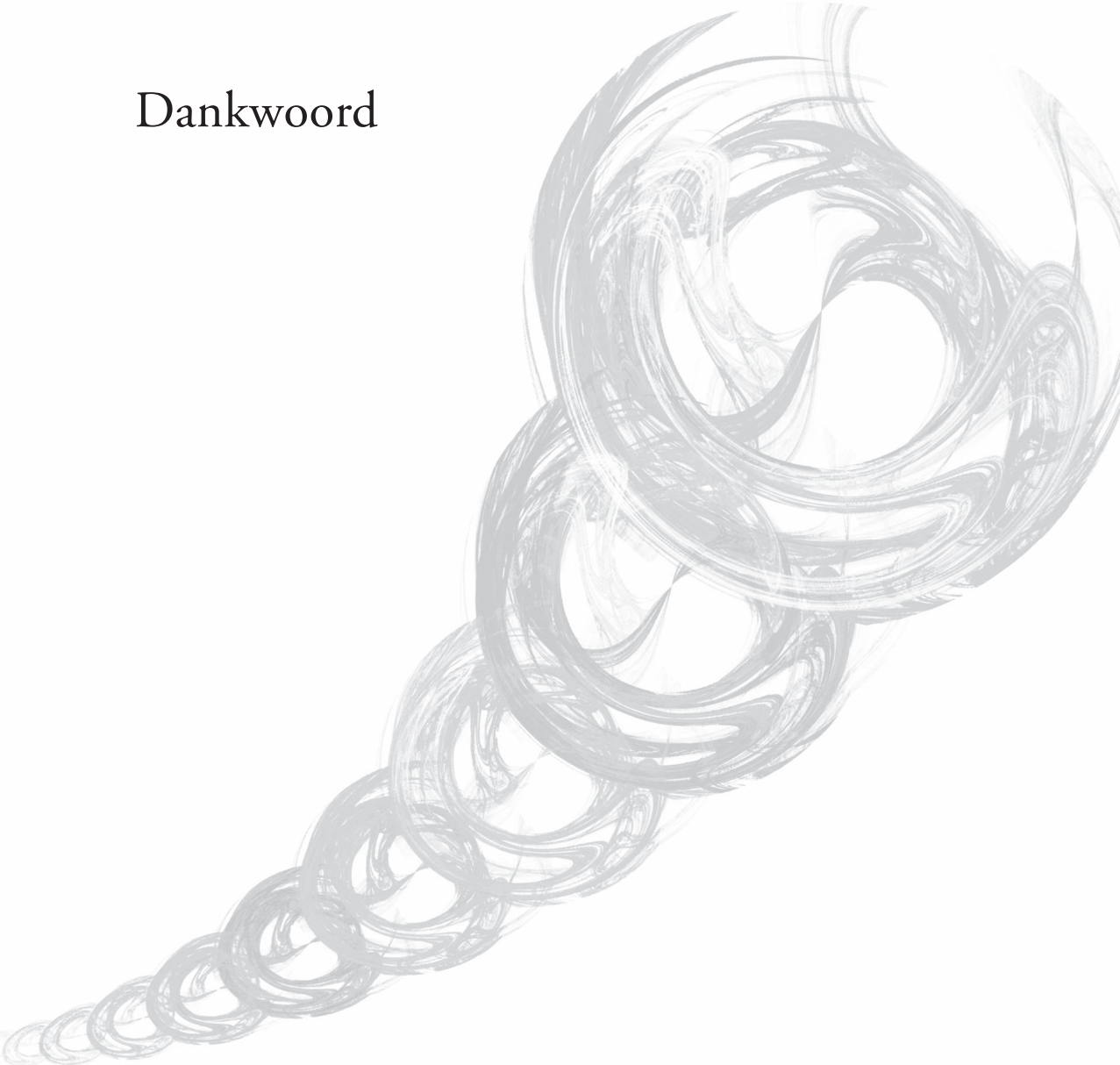
Ten derde kan optimalisatie van de opzet van fase I en II studies een belangrijke bijdrage leveren aan de verbetering en versnelling van het ontwikkelingsproces van een nieuw oncologisch geneesmiddel. Fase I studies die een combinatie van middelen onderzoeken zouden mogelijk betere, meer valide resultaten met betrekking tot verdraagbaarheid genereren wanneer er gebruik wordt gemaakt van een gerandomiseerde studieopzet. Dit studieontwerp moet dan per studie bedacht worden, rekening houdend met de te verwachten interacties tussen de middelen of behandelmodaliteiten. Het is mogelijk dat dit uiteindelijk leidt tot meerdere maximaal tolereerbare doseringen, waarna de definitieve dosering pas kan worden vastgesteld in een gerandomiseerde fase II setting. Randomisatie lijkt hoe dan ook een vereiste om de kwaliteit en snelheid van fase II studies te verbeteren. De studies dienen zodanig te worden opgezet dat zij voldoende onderscheidend vermogen hebben om nauwkeurig eventueel patiëntenvoordeel aan te tonen en daarmee het uiteindelijke succes van fase III studies correct voorspellen. Een manier om dit te bewerkstelligen is om hogere eisen te stellen aan de effectiviteit van een nieuw middel of therapie dan op dit moment gedaan wordt in gerandomiseerde fase II studies, bijvoorbeeld door te kijken naar een verdubbeling van de progressievrije overleving op een bepaald tijdstip in plaats van naar een verbetering van 20%.

Tot slot lijkt het zinvol om de huidige eindpunten in vroegklinische studies te herdefiniëren. Behandeling met veel van de nieuwe tumorcelspecifieke geneesmiddelen resulteert niet altijd direct in regressie van de tumor of metastasen, maar geeft meer stabilisatie van tumorgroei, wat eveneens zeer de moeite waard kan zijn indien dit gedurende een lange periode aanhoudt. Responsevaluatie vindt nu voornamelijk plaats volgens RECIST (Response Evaluation Criteria in Solid Tumours) en dit zou in de toekomst misschien gecombineerd kunnen worden met nieuwe functionele beeldvormende technieken zoals positron emissie tomografie (PET) of digitale contrast-enhanced magnetic resonance imaging. Aangezien de meeste nieuwe middelen een continu toedieningschema kennen, kan relevante toxiciteit pas op een veel later tijdstip optreden in vergelijking met de huidige cytostatica en het verdient daarom aanbeveling om langere observatieperiodes

in te bouwen in toekomstige studies. De rol en het belang van farmacodynamische parameters als eindpunten in vroegklinische studies in de toekomst lijkt evident, echter op dit moment nog niet opportuun.

Concluderend kan gesteld worden dat de enorme toename in potentiële antitumor middelen die klinisch onderzocht dienen te worden, vraagt om een verbetering van vroegklinisch kankeronderzoek met betrekking tot methodologie en efficiëntie. Door betere (gerandomiseerde) studiedesigns en beter gekozen eindpunten zal het mogelijk moeten worden om te voorkomen dat actieve middelen onterecht afgewezen worden, maar ook dat inactieve middelen onterecht verder gaan in het ontwikkelingsproces. Uitgebreid farmacokinetisch én translationeel onderzoek zal van toenemende importantie zijn in de besluitvorming van geneesmiddelontwikkelingsprocessen.

Dankwoord



DANKWOORD

En dan is het klaar, wat een onwerkelijk maar ook heerlijk gevoel!
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Curriculum Vitae

Inge Konings werd op 3 juni 1976 geboren te Tilburg. Zij behaalde in 1994 haar VWO Gymnasium diploma aan het Mill Hill College te Goirle, waarna zij geneeskunde studeerde aan de Universiteit van Utrecht. Ter afsluiting van de doctoraal fase deed zij gedurende een jaar preklinisch onderzoek naar de toepasbaarheid van radioactief gelabelde somatostatine-analoga bij neuroendocriene tumoren in het Cancer Research and Treatment Center van de Universiteit van Albuquerque, New Mexico in de Verenigde Staten. In april 1999 behaalde zij het doctoraal examen en begon zij aan haar co-schappen aan de Erasmus Universiteit te Rotterdam. Het artsexamen werd behaald in 2001 (cum laude), waarna zij als AGNIO (arts geneeskunde niet in opleiding) ging werken op de afdeling Interne Geneeskunde van de Reinier de Graaf Groep te Delft. Vanaf mei 2002 was zij in opleiding tot internist in het Erasmus MC (opleiders Prof.dr. H.A.P. Pols en Prof.dr. J.C.L.M. van Saase) en Medisch Centrum Rijnmond-Zuid (opleider dr. A. Berghout) te Rotterdam. Op 1 mei 2008 vond registratie plaats als internist. Zij specialiseerde zich in het aandachtsgebied Medische Oncologie in het Erasmus MC (opleider Prof.dr. J. Verweij) en startte gedurende deze opleiding met haar promotieonderzoek. Op 1 juli 2008 vond registratie plaats als internist-oncoloog, waarna zij als staflid verbonden bleef aan de afdeling Interne Oncologie van het Erasmus MC. Sinds februari 2010 is zij werkzaam als internist-oncoloog op de afdeling Medische Oncologie van het VU Medisch Centrum te Amsterdam.

Publications

Van der Bol JM, Loos WJ, de Jong FA, van Meerten E, **Konings IRHM**, Lam MH, de Bruijn P, Wiemer EA, Verweij J, Mathijssen RH. Effect of omeprazole on the pharmacokinetics and toxicities of irinotecan in cancer patients: a prospective cross-over drug-drug interaction study. Accepted Eur J Cancer.

Konings IRHM, de Jonge MJA, Burger H, van der Gaast A, van Beijsterveldt LEC, Winkler H, Verweij J, Yuan Z, Hellemans P, Eskens FALM. Phase I and pharmacological study of the broad-spectrum tyrosine kinase inhibitor JNJ-26483327 in patients with advanced solid tumours. Br J Cancer 2010; 103:987-992.

Konings IRHM, van der Gaast A, van der Wijk LJ, de Jongh FE, Eskens FA, Sleijfer S. The addition of pravastatin to chemotherapy in advanced gastric carcinoma: a randomized phase II trial. Eur J Cancer 2010; 46:3200-3204.

Konings IRHM, Sleijfer S, Mathijssen RH, de Bruijn P, Ghobadi Moghaddam-Helmantel IM, van Dam LM, Wiemer EA, Verweij J, Loos WJ. Increasing tumoral 5-fluorouracil concentrations during a 5-day continuous infusion: a microdialysis study. Cancer Chemother Pharmacol 2010, July 23, Epub ahead of print.

Konings IRHM, Verweij J, Wiemer EAC, Sleijfer S. The applicability of mTOR inhibition in solid tumors. Curr Cancer Drug Targets 2009; 9:439-450.

Konings IRHM, Engels F, Sleijfer S, Verweij J, EAC Wiemer, Loos W. Application of prolonged microdialysis sampling in carboplatin-treated cancer patients. Cancer Chemother Pharmacol 2009; 64:509-516.

Norenberg JP, Krenning BJ, **Konings IR**, Kusewitt DF, Nayak TK, Anderson TL, de Jong M, Garmestani K, Brechbiel MW, Kvols LK. ^{213}Bi-DOTA^{0},Tyr^{3})Octreotide Peptide Receptor Radionuclide Therapy of Pancreatic Tumors in a Pre-clinical Animal Model. Clin Cancer Res 2006; 12:897-903.

ABSTRACTS AND POSTER PRESENTATIONS

Konings IR, de Jonge MJ, Burger H, van der Gaast A, van Beijsterveldt LE, Winkler H, Hellemans P, Eskens FA. A phase I and pharmacological study of the broad-spectrum tyrosine kinase inhibitor JNJ-26483327, administered to subjects with advanced or refractory solid tumors. AACR-EORTC-NCI International Conference on Molecular Targets and Cancer Therapeutics Conference, November 2009, Boston, USA. Clin Cancer Res Nov 2009, Abstract A108.

Loos WJ, **Konings IR**, Engels FK, Lam MH, van Noort C, de Wit R, Sleijfer S, Wiemer EA, Verweij J. Pharmacokinetics (PK) of carboplatin in tumor tissue of humans: usefulness of microdialysis. Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, April 2008, Orlando, Florida; Clin Pharmacol Ther 83, S51(abstract PII-31), 2008.

Konings IRHM, de Man YA, van Saase JLCM, Berghout A. Metabolic consequences of bariatric surgery in patients with morbid obesity. Annual Meeting of the Netherlands Association of Internal Medicine, April 2005, Maastricht, the Netherlands. Neth J Med 2005; 63(4)suppl:7P.

Krenning BJ, **Konings IR**, Norenberg JP, de Jong M, Kvols LK, Kusewitt DF. Long-term histological organ damage in animals following peptide receptor radionuclide therapy (PRRT) with high doses of ^{90}Y- and ^{111}In-labeled DOTA^{0},TYR^{3}Octreotide (DOTATOC). 48th Annual Meeting of the Society of Nuclear Medicine, June 2001, Toronto, Canada. J Nucl Med 2001;v42(5)suppl.S, 37P-38P.

Norenberg JP, Krenning BJ, **Konings IR**, de Jong M, Garmestani K, Brechbiel MW, Atcher RW, Kusewitt DF, Kvols LK. Safety and efficacy of ^{213}Bi-DOTA^{0},TYR^{3} Octreotide (Bi-DOTATOC2001 AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics, October 2001, Miami, Florida, USA. Clin Cancer Res Nov 2001;v7(11),suppl.S, p.3732S.

Norenberg JP, Krenning BJ, **Konings IR**, de Jong, M, Srinivasan A, Garmestani K, Brechbiel MW, Kvols LK. DOTA^{0},TYR^{3} Octreotide (Bi-DOTATOC) in peptide receptor radionuclide therapy (PRRT): radiolabeling, stability and biodistribution. 44th Annual Meeting of the Southwest Chapter, Society of Nuclear Medicine, Santa Fé, New Mexico, USA; March 26-28, 1999. Clin Nucl Med 1999;24(3):212.

.Norenberg JP, Krenning BJ, **Konings IR**, de Jong, M, Srinivasan A, Garmestani K, Brechbiel MW, Kvols LK. ^{213}Bi-DOTA^{0},TYR^{3} Octreotide (Bi-DOTATOC) in peptide receptor radionuclide therapy (PRRT). 46th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, California, USA; June 7 1999. J Nucl Med 1999;40(5)suppl:103P.

Norenberg JP, Garmestani K, Krenning BJ, **Konings IR**, Atcher RW, de Jong M, Srinivasan A, Kvols LK, Brechbiel MW. Radiolabeling methods for the preparation of ^{213}Bi-DOTA^{0},TYR^{3} Octreotide (Bi-DOTATOC) for peptide receptor radionuclide therapy (PRRT). 46th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, California, USA; June 5 1999. J Nucl Med 1999;40(5)suppl:80P.