

**1 + 1 = ?**

**Combinations and combination strategies  
in early cancer-drug development**

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**Combinations and combination strategies in  
early cancer-drug development**

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**Combinaties en combinatie strategieën in vroeg klinische  
ontwikkeling van antikanker medicatie**

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# CONTENTS

<b>Chapter 1</b>	Introduction	<b>7</b>
<b>Chapter 2</b>	It takes two to tango: combinations of conventional cytotoxics with compounds targeting the vascular endothelial growth factor-vascular endothelial growth factor receptor pathway in patients with solid malignancies <i>Cancer Science 2010; 101: 7-15</i>	<b>13</b>
<b>Chapter 3</b>	Dose-escalation models for combination phase I trials in oncology <i>European Journal of Cancer 2010; 46: 2870-2878</i>	<b>33</b>
<b>Chapter 4</b>	Decreased exposure to sunitinib due to concomitant administration of ifosfamide: results of a phase I and pharmacokinetic study on the combination of sunitinib and ifosfamide in patients with advanced solid malignancies <i>British Journal of Cancer 2010; 102: 1699-1706</i>	<b>51</b>
<b>Chapter 5</b>	(Pre-)Clinical pharmacology and activity of pazopanib, a novel multikinase angiogenesis inhibitor <i>The Oncologist 2010; 15: 539-547</i>	<b>69</b>
<b>Chapter 6</b>	Pazopanib exposure decreases as a result of an ifosfamide-dependent drug-drug interaction: results of a phase I study <i>British Journal of Cancer 2014; 110: 888-893</i>	<b>87</b>
<b>Chapter 7</b>	Impact of pazopanib on docetaxel exposure: results of a phase I combination study with two different docetaxel schedules <i>Submitted</i>	<b>101</b>
<b>Chapter 8</b>	Randomized phase II study comparing efficacy and safety of combination-therapy trastuzumab and docetaxel vs sequential therapy of trastuzumab followed by docetaxel alone at progression as first-line chemotherapy in patients with HER2 <sup>+</sup> metastatic breast cancer: HERTAX trial <i>Clinical Breast Cancer 2011; 11: 103-113</i>	<b>115</b>

<b>Chapter 9</b>	Summary, discussion and future perspectives	<b>133</b>
<b>Appendix</b>		
	Samenvatting	<b>143</b>
	Dankwoord	<b>149</b>
	Curriculum vitae	<b>151</b>
	List of publications	<b>153</b>
	PhD Portfolio	<b>163</b>

Chapter

1

Introduction

The path towards an established medical anticancer treatment is a long and winding road. After thorough pre-clinical studies, novel drugs or combinations of drugs enter clinical development usually in a so-called phase I dose-finding study. These studies primarily aim to determine the dose that can safely be administered and is recommended for further studies. Subsequently, in phase II studies patients with a specific tumor entity are enrolled to screen whether in that specific population the drug or combination of interest exerts anti-tumor activity considered worthwhile enough for further studies and to gather additional information on the tolerability. Lastly, the decision whether or not the novel regimen should be implemented in clinical practice is generated from a phase III trial in which the novel regimen is compared to standard care in the population of interest.

Early cancer drug development refers to phase I studies and to a lesser extent to phase II studies. Although the development of a specific agent towards the phase III trial seems pretty straight forward, there are many important factors relevant to the dose and dosing strategy of the anticancer drug entering phase III. These include initial choices regarding dose-escalation steps as well as the determination of the maximally tolerated dose (MTD; the maximum dose that can be tolerated by patients) versus the biological optimal dose, the dose at which drug-mediated effects important for anti-tumor activity are exerted.

### **Combining agents**

The number of issues that need to be addressed in combining drugs is multiplied if compared to single agents. Most important challenges in studying combinations in early cancer-drug development, include the following:

1. Choice of the drugs
2. Determining the dose-limiting toxicity (DLT) observation time window
3. Dose escalation steps
4. Sequence versus combination

### **Choice of the drugs**

Which drugs to pair is a critical first step. In the last decade the majority of anticancer drugs gaining marketing approval are targeted agents such as monoclonal antibodies and tyrosine kinase inhibitors (TKIs). The introduction of these agents with a mechanism of action different to conventional chemotherapy also triggered trials combining these agents with conventional cytotoxic agents. In selecting two drugs, frequently encountered criteria are non-overlapping toxicity of the drugs involved, potential synergism in anti-tumor activity, a patient-population in whom the combination may be worthwhile, and an absent or limited potential for drug-drug interaction augmenting toxicity or attenuating anti-tumor effects.



### **Determining the DLT observation time window**

Early drug development studies on traditional cytotoxic agents, which in general are only applied for a limited period of time, for example for 4 or 6 cycles, hardly covered chronic toxicity. This was deemed acceptable since the majority of severe adverse events induced by traditional cytotoxic drugs determining the recommended dose for further studies are transient, do not cumulate, and resolve before the administration of the subsequent cycle. As a result, toxicity from such agents observed during the first cycle in general adequately reflects the toxicity profile of the drug during the whole, relatively short time of treatment. However, most targeted agents induce tumor growth stasis and therefore need to be administered for prolonged periods of time. Due to this longer exposure to targeted agents the margins of tolerability might be crossed in a completely different fashion and will thus have to be assessed from a different perspective. Importantly, while short lasting severe side-effects may still be considered tolerable, less severe side-effects experienced for prolonged periods may not. For example, grade 3 nausea for a few days due to a cytotoxic agent may be better tolerable than daily grade 2 nausea for a few weeks. As a consequence we may have to change our definitions of DLT for chronic dosing as well as the methodology of determining DLT in a phase I study, or may have to lean more on the safety evaluation in subsequent phase II trials.

### **Dose escalation steps**

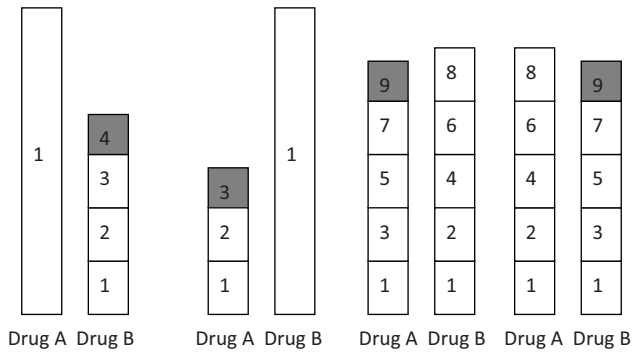
One of the other challenges in phase I studies on drug combinations is the dose-escalation schedule. In contrast to single agent studies, in which the consequence of exceeding the MTD is pretty clear, the decision rules in combination phase I studies can be applied in several ways, rendering other doses to be tested. And even in the steps towards the MTD, choices in dose escalation can greatly impact the final MTD as illustrated in figure 1. This figure illustrates the stepwise increase in dose-level, starting at dose-level 1 for both drugs. Subsequently one of the drugs is escalated at each following dose-level. The predefined dose-escalation steps in itself already determine which dose of a drug might become an MTD (Figure 1).

Usually there is a fair amount of prior data on the single drug toxicity profiles of the separate agents once explored in combination. Exploring a drug with a high incidence of severe toxicities as one of the components in a combination-study poses a challenge to the investigator. The dose-finding study should be defined in such a way that potentiating effects from the other drug on the toxicity is easily detected. In other words, the "background noise" should be identified. Additionally, recognition of a relevant drug-drug-interaction rendering a lower or higher drug-exposure should be detected at an early stage. Furthermore, during the design-process, decision rules should be considered that allow the investigator to anticipate for unexpected events. If adequately constituted, these rules ideally can result in the detection of the upper boundary of safe dosing based on safety,



pharmacokinetic and pharmacodynamics parameters. Ideally, this boundary is not just a single MTD, but multiple MTDs.

Specific challenges are posed when adding an agent with a novel mechanism to an established cytotoxic drug. Usually, the dose of the established cytotoxic therapy is defined on the basis of the occurrence of adverse events at MTD. Adding a new drug easily tilts the combination above the tolerated border rendering coincidence to have a large impact on dose-escalation possibility. For example, if an established therapy has an incidence of severe toxicity at or close to the MTD and a novel drug is added at zero milligram (placebo level) to this treatment in the first two patients and they both experience severe adverse events, than even the addition of “nothing” inadvertently creates the conclusion that this dose is above the MTD.



**Figure 1** | Four maximally tolerated doses (MTDs) determined with two drugs, solely depending on escalation steps. (Numbers are dose-levels. Grey blocks are the dose-levels exceeding the MTD). (Hamberg *et al.* Eur J Cancer 2010: 2870-2878).

### Sequence versus combination

If MTDs of the drugs in combination are defined then subsequent studies will have to evaluate the combination on hints for efficacy as well as provide more robust information on tolerance. Yet, the optimum schedule still needs refinement also in later phase studies. For example, a combination of two drugs might be tolerable, but a key-question in the end is whether the combination is better than the sequence of both single agents. Phase II trials can also be helpful in “picking a winner”, the combination or the sequence of the both drugs, that should proceed to phase III.

This thesis focuses on combining anticancer drugs in early clinical trials and addresses several of the challenges mentioned above. The combinations described in this thesis all

involve so-called targeted agents, agents designed to inhibit a specific factor or receptor, combined with conventional cytotoxic drugs.

Chapter 2 provides an overview of cytotoxic agents in combination with inhibitors of the vascular endothelial growth factor – vascular endothelial growth factor receptors (VEGFR) pathway. The third chapter introduces novel models to improve dose-finding anticancer drug combination studies in general. The fourth chapter describes a study that aims to determine the MTD of the targeted agent sunitinib, a TKI targeting, amongst others, the VEGFR, combined with two different schedules of ifosfamide. The fifth chapter reviews the properties of pazopanib, another oral TKI inhibiting VEGFRs. In the subsequent sixth and seventh chapter, pazopanib is combined with two schedules of ifosfamide and docetaxel, respectively. The eighth chapter describes a phase II study comparing a combination of two drugs to both drugs given sequentially. Finally, a summary, discussion, and future perspectives are provided in chapter 9.





Chapter

# 2

**It takes two to tango: combinations of conventional cytotoxics with compounds targeting the vascular endothelial growth factor-vascular endothelial growth factor receptor pathway in patients with solid malignancies**

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## ABSTRACT

Through advances in molecular biology, insight into the mechanisms driving malignancies has improved immensely and as a result, various factors playing an essential role in the biology of numerous tumor types have been revealed. By using compounds that specifically block the function of a single factor being crucial for tumor pathogenesis, it was hoped to exert antitumor activity while avoiding toxicities characteristic for conventional chemotherapy. One of the processes of crucial importance in the development of cancer, and consequently an attractive target, is angiogenesis. In recent years, several key factors for angiogenesis have been identified, including ligands, receptors, and transduction signaling factors. Of these, the vascular endothelial growth factor (VEGF) pathway has been found to be activated in numerous tumor types and considered one of the main drivers of angiogenesis. Roughly, VEGF-mediated angiogenesis can be inhibited by two approaches: either by monoclonal antibodies directed towards VEGF or its corresponding receptors, or by kinase inhibitors targeting the signal transduction of the VEGF receptors. As monotherapy, several kinase inhibitors exert antitumor activity in tumor types such as renal cell carcinoma. However, in most tumor types, the antitumor activity of compounds targeting the VEGF pathway is limited. In recent years, evidence is mounting that the paradigm of one single factor that drives malignant behavior applies rarely and is an oversimplification for most tumors in which there are multiple driving pathways. Consequently, multitargeting rather than single-targeting approaches are required. One of the means is by combining targeted agents with conventional cytotoxics. As the VEGF pathway also affects the sensitivity of tumor cells to chemotherapeutics, combinations of compounds targeting this pathway and conventional cytotoxics have been explored. This review addresses such combinations.

## INTRODUCTION

Recently, anticancer therapy has focused on cancer cell specific therapy, often referred to as targeted therapy. Mainly through improved molecular techniques, numerous factors involved in tumor pathogenesis have been identified. Such factors are frequently expressed both in tumor cells, and in adjacent normal cells, supporting tumor growth. Examples of tumor-driving factors include ligands (e.g. VEGF, and hepatocyte growth factor), receptors (e.g. c-KIT, VEGFR, EGFR, and human EGFR-2), and factors involved in signal transduction pathways. Initially, it was hoped that one or only a few factors would drive malignant behavior of solid tumors, and that inhibiting these factors would exert antitumor activity.

Indeed, the concept of a single pathway driving malignant behavior is illustrated by the example of gastro-intestinal stromal tumor (GIST). GIST is driven by activating mutations in the *c-KIT* gene.<sup>1</sup> The introduction of imatinib, a TKI targeting *c-KIT*, dramatically improved the out-comes of advanced GIST patients.<sup>2,3</sup> However, in contrast to GIST, in most tumors multiple pathways are active in parallel, therefore targeting one or a few pathways will frequently not yield significant antitumor activity. Multiple driving pathways require multi-targeting approaches, which can be achieved by several means; cancer cell-specific drugs are designed to have a broader range of activity, cancer cell-specific drugs can be combined, and targeted therapy may be combined with conventional chemotherapy. The present review addresses the rationale and currently available data on combinations of conventional chemotherapy and cancer cell-specific therapies directed towards the VEGF pathway.

### **Vascular endothelial growth factor-driven angiogenesis as a target for therapy in solid tumors**

Angiogenesis is crucial for tumor growth and dissemination and therefore forms an attractive pathway to target. In this process, the VEGF family plays a central role. VEGF-A is the major pro-angiogenic factor, usually referred to as VEGF. Other family members include VEGF-B, VEGF-C, VEGF-D, and placental growth factor. In addition to tumor cells, VEGF is produced by a number of cells, such as platelets, stromal, and muscle cells. Although VEGFR is sometimes expressed by tumor cells, VEGF's predominant site of action is at endothelial cells. Binding of VEGF to VEGFR-1 and VEGFR-2 initiates a cascade of downstream intracellular signal transduction pathways resulting in endothelial cell proliferation and migration, vascular permeability, and subsequently to the formation of new blood vessels.<sup>4</sup>

VEGF is overexpressed in many solid tumor types as a consequence of several underlying mechanisms. VEGF can be induced by a number of genetic and epigenetic alterations, by cytokines, growth factors, hormones, and hypoxia. One of the best examples elucidated thus far is in clear-cell renal cell carcinoma (RCC), in which the



activity of the *VHL* gene is disrupted due to mutations or methylation. Normally, *VHL* binds to and inactivates the transcription factor hypoxia inducible factor 1- $\alpha$ . Due to the disrupted *VHL* function in RCC, however, hypoxia inducible factor 1- $\alpha$  levels are elevated, inducing transcription of many factors including VEGF.<sup>5</sup> In many cancer types, increased VEGF expression is associated with poor outcome, irrespective of tumor stage or grade.<sup>6</sup> A higher potency to disseminate and chemoresistance account for this, thus rendering the VEGF pathway one of the most attractive targets for anticancer therapy.

### **Clinical studies on single agents targeting the VEGF pathway**

Currently, the VEGF pathway can be blocked by mAb or kinase inhibitors. Concerning mAb, only bevacizumab has been extensively explored in the clinic. Bevacizumab is directed towards VEGF, thereby hindering its attachment to receptors. As bevacizumab does not bind factors other than VEGF, bevacizumab is regarded as a truly single factor-targeting treatment. In contrast, kinase inhibitors targeting VEGFR often abrogate the function of other factors as well, therefore being less specific. Recently, compounds targeting the VEGF pathway have been widely explored. One of the first issues that had to be solved in the context of these studies was how to reliably assess their activity in clinical studies. Historically, the RR was used for this purpose, but data are accumulating that for many antitumor agents, in particular those targeting the VEGF pathway, antitumor activity is not adequately reflected by changes in size but more relevantly by parameters reflecting tumor stabilization, such as the ratio of tumor progression before and after starting the agent of interest, the progression free survival (PFS), and progression-free rate at a certain time point.

### ***Bevacizumab***

The first proof of the efficacy of an anti-angiogenesis treatment in human malignancy was established in advanced RCC. Monotherapy bevacizumab induced a low RR (10%), but PFS almost doubled compared to placebo.<sup>7</sup> However, apart from RCC, monotherapy bevacizumab has been explored only in a few other tumor types and only in non-randomized settings. In cervical cancer and castrate refractory prostate cancer, no antitumor activity was observed (Table 1).

In hepatocellular carcinoma (HCC), bevacizumab induced a 6-months progression-free rate of 65% compared with 40% in historical controls, although fair comparison remains difficult without a randomized control arm.<sup>8</sup> For ovarian cancer, two non-randomized phase II studies in heavily pretreated patients have been published, both showing interesting PFS and overall survival (OS)<sup>9,10</sup> compared to historical controls.<sup>11</sup> It was concluded that bevacizumab has activity against ovarian cancer, albeit this conclusion is based on non-randomized studies. Furthermore, bevacizumab has recently been Food and Drug Administration (FDA)-approved based on data of two non-randomized studies in patients with previously treated glioblastoma (AVF3708g and NCI 06-C-0064E), both not published



as full papers yet. Another non-randomized phase II study confirmed bevacizumab's activity in pretreated glioblastoma.<sup>12</sup>

**Table 1 | Trials with monotherapy bevacizumab**

Indication	Study phase	Patients (n)	Agent	End points	Reference
Metastatic clear-cell RCC 2 <sup>nd</sup> line	II	116	Bevacizumab 3 and 10 mg/kg q2w or placebo	PFS bevacizumab 10 mg/kg 4.8 m; 3 mg/kg 3.0 m; placebo 2.5 m OS ns RR 10% bevacizumab 10 mg/kg	[7]
Metastatic castrate refractory prostate carcinoma	II	15	Bevacizumab 10 mg/kg q2w non-randomized	No objective response	[73]
Platinum resistant epithelial ovarian/peritoneal serous cancer 3 <sup>rd</sup> /4 <sup>th</sup> line	II	44	Bevacizumab 15 mg/kg q3w non-randomized	PFS 4.4, OS 10.7 m, PR 15.9%, perforation 11%, 3 deaths (historical PFS 2.3–3.4 m, OS 8–10.3 m)	[10]
Epithelial ovarian/primary peritoneal cancer 2 <sup>nd</sup> /3 <sup>rd</sup> line	II	62	Bevacizumab 15 mg/kg q3w non-randomized	PFS 4.7 m, OS 17 m RR 21%	[9]
Recurrent cervical cancer 2 <sup>nd</sup> /3 <sup>rd</sup> line	II	46	Bevacizumab 15 mg/kg q3w non-randomized	PFS 3.4 m, OS 7.3 m, PR 10.9% (historically OS 4–6.6 m)	[74]
Non-metastatic unresectable HCC	II	46	Bevacizumab 5 or 10 mg/kg q2w non-randomized	PFS 6.9 m, PFS rate 65% at 6 m, OS not available	[8]
Recurrent glioblastoma	II	48	Bevacizumab 10 mg/kg q2w non-randomized	PFS 16 w, OS 31 w, RR 35% (MacDonald criteria)/ 71% (Levin criteria)	[12]

HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; RR, response rate; w, weeks; m, months.

### **Tyrosine kinase inhibitors**

The first two TKI targeting VEGFR that were widely explored in solid tumors are sunitinib and sorafenib. In addition to VEGFR-2, sunitinib also inhibits *c-KIT*, FMS-like tyrosine kinase 3, PDGF- $\alpha$ , and PDGF- $\beta$ . Sorafenib is a potent Raf kinase inhibitor that directly suppresses tumor cell proliferation, and also targets VEGFR-2, VEGFR-3, and PDGFR- $\beta$ . Sunitinib improves PFS and RR compared with IFN- $\alpha$  as first-line therapy for advanced clear-cell RCC. PFS and RR were 11 months and 47% in the sunitinib group, compared with 5 months and 12% in the IFN- $\alpha$  group respectively.<sup>13</sup> A trend to better OS in the sunitinib group was observed (26.4 vs 21.8 months [ $P=0.051$ ]). Within the IFN- $\alpha$  group, however, the majority of patients received active post-study antitumor treatment,

obscuring the true impact of sunitinib on OS. In the subgroups not receiving post-study therapy, sunitinib doubled the OS compared with IFN- $\alpha$  (28.1 vs 14.1 months, respectively),<sup>14</sup> strongly supporting sunitinib's activity in RCC. Furthermore, sunitinib is active in patients with advanced GIST failing to imatinib.<sup>15</sup>

Sorafenib improved PFS in patients with advanced clear-cell RCC pretreated with cytokine-containing therapy in a placebo-controlled phase III trial (median PFS 5.5 vs 2.8 months). Subsequently, the trial was stopped early and patients receiving placebo were allowed to cross over to sorafenib.<sup>16</sup> Although an intent-to-treat analysis demonstrated no OS benefit (17.8 vs 15.2 months, respectively), censoring placebo patients indicated a better OS for those receiving sorafenib (17.8 vs 14.3 months), suggesting an important cross-over effect.<sup>17</sup> Surprisingly, given the effects of sorafenib in second-line treatment, sorafenib and IFN- $\alpha$  had equivalent activity as first-line treatment of metastatic RCC.<sup>18</sup> In HCC, sorafenib yielded a 2% RR, but significantly improved PFS and OS over placebo. PFS was 5.5 versus 2.8 months, and OS was 10.7 versus 7.9 months, respectively.<sup>19</sup> This study clearly shows that antitumor activity of VEGF-targeting agents is frequently not appropriately reflected in RR. Furthermore, sunitinib and sorafenib have been studied in a wide range of other tumor types (Table 2).

In addition to sunitinib and sorafenib, the number of TKI targeting the VEGFR is rapidly increasing, as is the number of tumor types in which they are assessed. Recently, the results of a randomized placebo-controlled phase III trial of pazopanib as first- or second-line treatment in clear-cell RCC were presented. Compared to placebo, pazopanib improved RR and PFS.<sup>20</sup> Although the outcomes of many of the studies exploring pazopanib and other novel VEGFR TKI are promising, the lack of results from randomized studies is insufficient to give an exact definition of their role in this process. However, it is not unrealistic that besides a few exceptions, the activity of these agents as monotherapy is at best modest for most tumor types.

### ***Rationale to combine compounds inhibiting the VEGF-pathway with conventional chemotherapy***

There are several potential reasons rendering VEGF pathway-inhibiting drugs attractive to combine with conventional chemotherapeutic drugs. Besides promoting angiogenesis, VEGF expression can confer resistance against chemotherapy, potentially contributing to the generally worse outcome for patients with VEGF-overexpressing tumors. In xenografts, VEGF-producing tumor cell lines formed highly vascular tumors with accelerated growth compared to the parental cell lines, and exhibited less sensitivity to doxorubicin. Adding an anti-VEGF mAb reinforced the antitumor effects of doxorubicin.<sup>21</sup> Several mechanisms explaining how VEGF may confer chemoresistance, and why combinations of conventional chemotherapy with VEGF pathway-inhibiting agents may yield synergistic interaction have been revealed.

**Table 2** | Trials with monotherapy tyrosine kinase inhibitors

Indication	Study phase	Patients (n)	Agent	End points	Reference
Metastatic clear-cell RCC 1 <sup>st</sup> line	III	750	Sunitinib 50 mg 4 weeks q6w, or IFN $\alpha$ 9 MU 3/week	PFS sunitinib 11 m vs IFN $\alpha$ 5 m OS 26.4 vs 21.8 m, RR 42% vs 12%	[14]
Metastatic clear-cell RCC 1 <sup>st</sup> line	II	189	Sorafenib 400 mg bid or IFN $\alpha$ 9 MU 3/week. Cross-over to sorafenib 600 mg bid or sorafenib 400 mg bid	PFS sorafenib 5.7 m vs IFN $\alpha$ 5.6 m, PR 5 vs 7% Cross-over: PFS sorafenib 600 mg 3.6 m; 400 mg 5.3 m	[18]
Metastatic clear-cell RCC 2 <sup>nd</sup> line	III	903	Sorafenib 400 mg bid or placebo	Interim analysis: PFS sorafenib 5.5 m vs placebo 2.8 m. Cross-over: OS sorafenib 17.8 m vs placebo 15.2 m (ns) OS (placebo censored) sorafenib 17.8 m vs placebo 14.3 m	[6,17]
Metastatic clear-cell RCC 1 <sup>st</sup> /2 <sup>nd</sup> line	III	434	Pazopanib 800 mg or placebo	PFS pazopanib 9.2 m vs placebo 4.2 m, OS awaited, RR 30% vs 3%	[20]
Metastatic castrate refractory prostate cancer 1 <sup>st</sup> line	II	55	Sorafenib 400 mg bid non-randomized	PFS 8 w, PFS rate 1 y 13%, OS not reached	[75]
Metastatic soft tissue sarcoma 2 <sup>nd</sup> line	II	142	Pazopanib 800 mg non-randomized	PFS rate 12 w 36–49% (leiomyosarcoma, synovial); PFS rate 1 y 14%, OS rate 1 y 34%	[76]
Advanced HCC 1 <sup>st</sup> /2 <sup>nd</sup> line	II	34	Sunitinib 37.5 mg/d 4w q6w non-randomized	PFS 3.9 m, OS 9.8 m RR 2.9%, 50% stable disease	[28]
Advanced HCC 1 <sup>st</sup> line	III	271	Sorafenib 400 mg bid or placebo	PFS sorafenib 2.8 m vs placebo 1.4 m, OS 6.5 m vs 4.2 m	[77]
Advanced HCC 1 <sup>st</sup> line	III	602	Sorafenib 400 mg bid or placebo	PFS sorafenib 5.5 m vs placebo 2.8 m, OS 10.7 m vs 7.9 m	[19]
Glioblastoma 1 <sup>st</sup> /2 <sup>nd</sup> line	II	16	Cediranib 45 mg/d non-randomized	PFS 3.7 m, OS 7 m	[29]

HCC, hepatocellular carcinoma; OS, overall survival; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; RR, response rate; w, weeks; m, months; y, years; ns, non-significant.

Increased VEGF expression can protect tumor endothelial cells from apoptosis, through increased levels of Bcl-2 and surviving, two anti-apoptotic factors.<sup>22,23</sup> Furthermore, VEGF over-expression may account for chemoresistance through increased interstitial fluid pressure (IFP) in tumors. Tumor vasculature is more fragile and leaky than normal vasculature, leading to elevated IFP, which hinders the delivery of drugs from the circulation into tumors.<sup>24</sup>

Abnormal tumor vasculature also leads to reduced blood flow and perfusion, thereby further impairing delivery of anticancer drugs. Normalization of tumor vasculature by anti-angiogenic drugs can transiently reverse these abnormalities, and enhance the effects of chemotherapy (or radiotherapy), provided that it is administered during the “normalization window”.<sup>25,26</sup> In a small series of six patients with locally advanced colorectal carcinoma (CRC), bevacizumab decreased tumor perfusion, vascular volume, microvascular density, and IFP, all being signs of tumor vasculature normalization.<sup>27</sup> Furthermore, there was no change in FDG-PET uptake, despite less blood flow, indicating increased efficiency of blood vessels.<sup>27</sup> Consistently, in HCC patients treated with sunitinib, and in glioblastoma patients treated with cediranib, signs of reduced vascular permeability corresponding with vascular normalization were seen.<sup>28,29</sup> A third VEGF-mediated mechanism that may contribute to tumor growth and tumor cell repopulation after chemotherapy is the VEGF-mediated mobilization of circulating endothelial progenitor cells (EPC) after cytotoxic therapy. It is hypothesized that EPC are mobilized from the bone marrow and transported through the circulation to become incorporated into the walls of growing blood vessels,<sup>30-32</sup> thereby contributing to blood vessel formation and tumor regrowth after chemotherapy-induced cytotoxic effects. Both clinical and preclinical data showed substantial increases in viability and mobilization of EPC post-chemotherapy.<sup>33-35</sup> Notably, EPC induction by cytotoxic drugs seems to be drug-dependent. Paclitaxel, 5-fluorouracil (5-FU), and docetaxel cause acute elevations in viable EPC levels, unlike other cytotoxic agents (e.g. gemcitabine, cisplatin, and doxorubicin).<sup>34</sup> VEGF’s role has been demonstrated as the rapid induction of EPC was blocked when an anti-VEGFR mAb was added prior to paclitaxel-containing chemotherapy. Furthermore, combining the anti-VEGFR mAb with paclitaxel yielded synergistic antitumor effects that could not be observed with gemcitabine.<sup>34</sup> However, debate is still ongoing concerning the identity and relative contribution to tumor angiogenesis of EPC, as extreme variability in the contribution of EPC to tumor vasculature were reported. Altogether, several ways may yield synergy between conventional chemotherapy and VEGF pathway-targeting drugs.

### **Combinations of bevacizumab and chemotherapy**

Bevacizumab has been combined with various chemotherapeutic regimens in a wide range of tumor types. In general, combining bevacizumab with chemotherapy is safe, although exceptions exist; combining bevacizumab with doxorubicin in soft tissue sarcoma yielded a greater than expected cardiotoxicity.<sup>36</sup> Given the great number of studies on bevacizumab-containing regimens, only those combinations for which randomized data are available, and which have been published as full papers, will be addressed here.

### ***Colorectal cancer***

The first hint of bevacizumab's activity in metastatic CRC was observed in a phase III trial, comparing irinotecan, 5-FU, and leucovorin with or without bevacizumab. The addition of bevacizumab improved OS and PFS significantly. OS was 20.3 months in the combination arm, compared with 15.6 months for irinotecan, 5-FU, and leucovorin, whereas PFS was 10.6 and 6.2 months respectively. Furthermore, RR increased from 34.8% to 44.8%.<sup>37</sup> More recently, bevacizumab was explored in combination with two nowadays more widely used first-line treatment schedules for metastatic CRC; capecitabine plus oxaliplatin and FOLFOX-4. Again, PFS was improved in the combination arm, but only slightly (9.4 vs 8.0 months), while OS and RR were comparable.<sup>38</sup> In addition to combination regimens such as capecitabine plus oxaliplatin, monotherapy 5-FU or capecitabine is frequently used in patients considered unfit for combinations. The added value of bevacizumab to capecitabine, or capecitabine plus mitomycin C was investigated as first-line therapy for metastatic CRC. RR and PFS were significantly improved in the bevacizumab-containing regimens, but OS was unchanged.<sup>39</sup> Recently, the value of bevacizumab was assessed in stage II and III CRC, in which patients were treated with adjuvant FOLFOX-6 with or without bevacizumab. After a median follow up of 36 months, disease-free survival was comparable in both treatment arms.<sup>40</sup> In contrast to the findings in first-line and adjuvant settings, bevacizumab added to FOLFOX-4 significantly improved RR, PFS, and OS when given as second-line treatment for metastatic CRC.<sup>41</sup> In conclusion, bevacizumab added to conventional chemotherapy in CRC may enhance antitumor effects, but the extent to which this occurs is not fully elucidated, and seems to be dependent on regimen and setting.

### ***Breast cancer***

The first randomized study in metastatic breast cancer (MBC) compared capecitabine with capecitabine plus bevacizumab as second- and third-line treatment of MBC. The combination regimen improved RR, but there was no PFS or OS benefit.<sup>42</sup> However, bevacizumab added to paclitaxel as first-line treatment of MBC did show a benefit in PFS. In this trial, bevacizumab combined with weekly paclitaxel significantly improved the RR from 21.2% to 36.9% and PFS from 5.9 to 11.8 months.<sup>43</sup> Although less striking, preliminary data showed that bevacizumab improves RR and PFS when added to docetaxel.<sup>44</sup> So also in MBC, the effects of bevacizumab seem regimen dependent.

### ***Non-small cell lung cancer***

Two randomized phase III studies explored bevacizumab with first-line chemotherapy in NSCLC. Bevacizumab with carboplatin and paclitaxel significantly improved both OS and PFS with 8 and 6 weeks respectively; however, this was at the cost of significant side effects in terms of bleeding, hypertension, and grade 4 neutropenia. Even though patients



with squamous cell tumors were excluded, lethal pulmonary hemorrhage occurred in 1.2%.<sup>45</sup> In the second study (AVAL), two dose levels of bevacizumab were combined with gemcitabine and cisplatin in advanced NSCLC. PFS was 6.1 months in the chemotherapy alone arm, compared with 6.7 and 6.5 months for the bevacizumab low and high dose, respectively. RR was 20.1% in the chemotherapy alone group, compared with 34.1% and 30.4% in the chemotherapy plus bevacizumab low and high dose groups, respectively. The incidence of serious adverse events and pulmonary hemorrhages were comparable in all groups.<sup>46</sup> So, bevacizumab modestly enhanced the outcomes of platinum-based chemotherapy, but at the expense of increased toxicity. Particularly in older patients, toxicity seems to outbalance antitumor activity<sup>47</sup> and as most NSCLC patients are of older age with comorbidity, only a minority of patients may benefit from bevacizumab added to chemotherapy.

### ***Pancreatic cancer***

Many approaches to improve the outcomes of the current standard in advanced pancreatic cancer (gemcitabine) have failed, including the addition of bevacizumab. In a large phase III trial, adding bevacizumab to gemcitabine failed to improve RR, PFS, and OS.<sup>48</sup> Data of bevacizumab added to gemcitabine/erlotinib were recently published. Median OS was equivalent in both groups, while adding bevacizumab significantly improved PFS (4.6 vs 3.6 months).<sup>49</sup>

### **Combinations of receptor TKI and chemotherapy**

As previously mentioned, TKI harbor a broader range of activity than mAb. Consequently, TKIs may be more effective than antibodies, but at the cost of more toxic effects. Accordingly, combinations of chemotherapy with VEGFR TKI seem less feasible than combinations of chemotherapy with bevacizumab. Unfortunately, data of randomized trials on VEGFR-targeting TKI-containing regimens are currently scarce. In addition to the few randomized trials, combinations explored in phase I, including toxicity and interaction issues that arise from these studies, are discussed. As the outcomes of single-arm efficacy studies on combinations without a control arm are hard to interpret, these will not be addressed.

### **Combinations of Sorafenib and Chemotherapy**

#### ***Phase I on sorafenib-containing combinations***

Numerous chemotherapeutic drugs have been combined with sorafenib and evaluated for toxicity, and pharmacokinetic interactions (Table 3). In the majority of these trials the toxicity profiles encountered were deemed acceptable and similar to the expected toxicity from each agent when given as monotherapy. Sorafenib (from day 4 at 100, 200, or 400 mg twice a day) in combination with doxorubicin (60 mg/m<sup>2</sup>, every 3 weeks) has been explored in a dose escalation ( $n=34$ ), and an extension part ( $n=18$ ), the latter enrolling only

advanced HCC patients.<sup>50,51</sup> The most frequent grade 3–4 drug-related adverse events were neutropenia (56%), lymphopenia (18%), fatigue (12%), and hand-foot-skin reaction (HFSR) (12%). The frequency of cardiotoxicity was not higher than expected from monotherapy doxorubicin. In HCC patients, a high incidence of hepatic toxicity (change >2 grades from baseline) was observed: bilirubin (62%), albumin (24%), and alkaline phosphatase (17%). Furthermore, grade 3 diarrhea was observed (18%), and two patients withdrew from treatment due to adverse events (renal failure grade 4 and hepatic encephalopathy). DLT were experienced by eight patients (20%), mainly HFSR and diarrhea. The MTD was not reached. Sorafenib increased doxorubicin exposure, with an increase in AUC of 21% and  $C_{max}$  of 33%. The pharmacokinetics of sorafenib and one of doxorubicin's active metabolites, doxorubicinol, were not affected.<sup>51</sup>

**Table 3 |** Phase I trials combining sorafenib with chemotherapy

Study population	Patients (n)	Agent	End points	Reference
Refractory solid tumors (n=15) and metastatic melanoma (n=24)	39	Sorafenib (100, 200, 400 mg bid) Carboplatin (AUC6) and paclitaxel (225 mg/m <sup>2</sup> )	DLT: 6 rash, 1 hypertension Melanoma: 1 CR, 9 PR, PFS 10.2 m, RR 26%	[54]
Refractory solid tumors (n=27) and CRC (n=10)	37	Sorafenib (200, 400 mg bid) Oxaliplatin (130 mg/m <sup>2</sup> )	MTD not reached DLT: 2 diarrhea gr 3 2 PR (6%), SD>10 w 43% solid tumors, 78% CRC	[53]
Refractory solid tumors (n=19) and pancreatic cancer (n=23)	42	Sorafenib (100, 200, 400 mg bid) Gemcitabine (1000 mg/m <sup>2</sup> )	MTD not reached DLT: 1 fatigue gr 3 2 PR (11%), 25 SD	[52]
Refractory solid tumors (n=34) and advanced HCC (n=18)	52	Sorafenib (100, 200, 400 mg bid) Doxorubicin (60 mg/m <sup>2</sup> )	MTD not reached DLT: 7 HFSR 1 gr 3 diarrhea Solid tumor: 1 PR, 15 SD (48%) HCC: 1 PR (6%), 10 SD (63%)	[50,51]
Refractory solid tumors (n=20) and CRC (n=14)	34	Sorafenib (100, 200, 400 mg bid) Irinotecan (125 mg/m <sup>2</sup> or 140 mg)	MTD not reached DLT: 1 hemorrhage, 2 HFSR 22 SD (67%), 1 PR	[55]

CR, complete response; CRC, colorectal carcinoma; DLT, dose-limiting toxicity; HCC, hepatocellular carcinoma; HFSR, hand-foot skin reaction; MTD, maximum tolerated dose; PFS, progression-free survival; PR, partial response; RR, response rate; SD, stable disease.

Sorafenib continuously (100, 200, or 400 mg bid) has been combined with gemcitabine (1000 mg/m<sup>2</sup>; day 1, 8, 15; every 4 weeks).<sup>52</sup> The most frequent adverse events were constitutional (fatigue 78.6%), gastrointestinal symptoms, dermatological, and bone marrow toxicities. Common grade 3–4 adverse events were thrombocytopenia (28.6%), lymphopenia (21.4%), lipase elevation (19%), neutropenia (16.7%), fatigue (14.3%),

thrombosis (11.9%), and hypertension (7.1%). Grade 3–4 elevations in hepatic transaminases or bilirubin occurred in 5–10%. One DLT, grade 3 fatigue, was observed in the cohort with 400 mg bid sorafenib. Therapeutic dosages of gemcitabine and sorafenib (400 mg bid) could be administered without reaching the MTD. No clinically relevant pharmacokinetic interaction between sorafenib and gemcitabine was observed.<sup>52</sup>

Sorafenib (200 or 400 mg continuously from day 4) was combined with oxaliplatin (130 mg/m<sup>2</sup>) in 27 patients with refractory solid tumors, and 10 patients with refractory CRC in the extension part. Adverse events were generally mild to moderate. Common adverse events were diarrhea (43%), neuropathy (37%), and dermatological toxicities (51%). Two DLT were reported (grade 3 diarrhea), and the MTD was not reached. No pharmacokinetic interaction between sorafenib and oxaliplatin was found.<sup>53</sup>

The combination of sorafenib (100, 200, or 400 mg bid days 2–19) combined with paclitaxel (225 mg/m<sup>2</sup> every 3 weeks) and carboplatin (AUC6) showed promising results in 39 patients with advanced cancer, of which 24 were melanoma.<sup>54</sup> All patients experienced treatment-related adverse events, mostly hematological (95%), dermatological (85%), fatigue (59%), sensory neuropathy (59%), nausea (56%), and arthralgia (26%). Grade 4 neutropenia occurred in 62%, and HFSR grade 3 was reported in 23%. Seven patients experienced a DLT, grade 3 rash / HFSR in six patients, and hypertension in one patient. There was no clear dose-dependent relationship in treatment-related adverse events. The recommended phase II dose was sorafenib 400 mg bid, carboplatin AUC6, and paclitaxel 225 mg/m<sup>2</sup>. Although clearance of paclitaxel is dependent on the cytochrome P450 enzymes, sorafenib had no apparent effect on the pharmacokinetics of paclitaxel. One complete response and nine partial responses were observed, all among patients with melanoma.<sup>54</sup>

Sorafenib (100, 200, and 400 mg bid) was combined with irinotecan (125 mg/m<sup>2</sup> on days 1, 8, 15, and 22 of each 6-week cycle) in patients with advanced solid tumors, and in an extended cohort in CRC patients, receiving fixed-dose irinotecan (140 mg weekly). Three DLT related to sorafenib were found, with sorafenib 400 mg bid, one cerebellar hemorrhage, and two HFSR. Frequent drug-related toxicities were gastrointestinal, dermatological, constitutional, and metabolic, mostly grade 1–2. Grade 3–4 adverse events were diarrhea (40%), infection / neutropenic fever (35%), leukopenia (10%), and neutropenia (5%). The MTD was not reached. Irinotecan had no impact on sorafenib's pharmacokinetics. In contrast, sorafenib doses higher than 200 mg bid significantly increased irinotecan and SN38 exposure; however, this was not associated with increased toxicity.<sup>55</sup>

## **Randomized trials on sorafenib-containing combinations**

### ***Melanoma***

The promising results of sorafenib combined with paclitaxel and carboplatin in the abovementioned phase I trial prompted further studies in melanoma patients. Recently, a phase III study was published in which 270 patients with advanced melanoma received



second-line therapy with carboplatin (AUC6) and paclitaxel (225 mg/m<sup>2</sup>, every 3 weeks) with sorafenib (400 mg bid) or placebo. Disappointingly, no difference was observed in any of the end points of the study. Dermatological events (91% vs 73%), and fatigue (75% vs 57%) were more common in patients treated with chemotherapy plus sorafenib.<sup>56</sup> Another study with a comparable design is expected to complete accrual in 2010.

Based on a phase I study, published as an abstract, it was shown that sorafenib (400 mg bid) can be safely combined with dacarbazine (1000 mg/m<sup>2</sup>, every 3 weeks). This combination was compared with dacarbazine alone as first-line treatment in advanced melanoma patients (*n*=101). A trend for improved PFS was observed for the sorafenib group (21.1 vs 11.7 months) without any difference in OS. The combination of sorafenib with dacarbazine in therapeutic dosage was well tolerated and had a manageable toxicity profile. Grade 3–4 adverse events were reported in 50% of patients in the control arm, and in 69% of patients in the sorafenib plus dacarbazine arm, 51% of the sorafenib-treated patients had grade 3–4 hematological toxicity.<sup>57</sup>

## **Combinations of Sunitinib and Chemotherapy**

### ***Phase I on sunitinib-containing combinations***

Currently, several combinations of conventional chemotherapy and sunitinib are being studied in phase I / II settings, including combinations with ifosfamide, capecitabine, carboplatin plus paclitaxel, gemcitabine, irinotecan, gemcitabine plus cisplatin, and 5-FU plus irinotecan. Most combinations of sunitinib plus conventional chemotherapy seem feasible, but at the expense of increased hematological toxicity. The severity and frequency of neutropenia is probably determined by the dose and schedule of sunitinib, and on the toxicity profile of the cytotoxic agent used. For example, sunitinib combined with capecitabine resulted in grade 4 neutropenia in <10% of patients, whereas sunitinib in combination with irinotecan or carboplatin / paclitaxel resulted in grade 3–4 neutropenia in 30–60% of patients. Furthermore, sunitinib in combination with ifosfamide was not feasible without growth factor support.<sup>58</sup> However, none of these studies have thus far been published as full papers, and therefore will not be discussed in further detail. The same applies to randomized trials exploring sunitinib-containing combinations. Many such trials are ongoing but have not been published yet.

## **Other TKI**

### ***Phase I trials on vandetanib-containing combinations***

Vandetanib is an orally administered TKI of VEGFR2, VEGFR3, and epithelial growth factor receptor (EGFR). As monotherapy, it is well tolerated dosed at 300 mg per day.<sup>59</sup> In a phase I study, 21 patients with advanced NSCLC received vandetanib (100 or 300 mg) with pemetrexed (500 mg/m<sup>2</sup>, every 3 weeks) as second-line therapy. Both dose levels were well tolerated. Two DLT were reported, asymptomatic QT<sub>c</sub> prolongation and interstitial



lung disease, which resolved after steroid therapy. Most common adverse events were rash, anorexia, fatigue, and diarrhea (all approximately 50%), and most were grade 1–2. No pharmacokinetic interactions were found.<sup>60</sup>

The safety and tolerance of vandetanib plus FOLFOX-6 was recently investigated in patients with advanced CRC as first- or second-line chemotherapy. Seventeen patients received 14-day treatment cycles of mFOLFOX-6 plus vandetanib (100 or 300 mg). Both dose levels were tolerable, but a DLT (diarrhea) occurred in each cohort. Overall, the most common adverse events were diarrhea, nausea, lethargy (all 65%), neutropenia, and neuropathy (both 59%). There was no pharmacokinetic interaction. At steady-state exposure to vandetanib, there was an increase in exposure to oxaliplatin, but time-dependent increases have also been observed previously with oxaliplatin as monotherapy.<sup>61</sup>

### ***Phase II randomized trials on vandetanib-containing combinations***

Vandetanib has been studied with docetaxel in advanced NSCLC patients as second-line treatment in a randomized phase II study. Advanced NSCLC patients ( $n=127$ ) were treated with docetaxel ( $75 \text{ mg/m}^2$ , every 3 weeks) combined with either placebo or vandetanib (100 or 300 mg). Diarrhea and rash were most frequent and severe in patients receiving vandetanib 300 mg. Patients in both vandetanib groups showed a modest increase in blood pressure at 6 weeks. Asymptomatic  $QT_c$  prolongation was only observed in the vandetanib-treated patients. Though not adequately powered to detect small differences, RR and PFS were significantly improved in the vandetanib 100 mg group, compared with the other two groups. Combined use did not cause detectable changes in the pharmacokinetic profile of either drug.<sup>62</sup> Currently, a randomized phase III trial of docetaxel with vandetanib or placebo as second-line therapy for advanced NSCLC is ongoing.

In another randomized phase II study, the combination carboplatin / paclitaxel was compared with vandetanib monotherapy, and with carboplatin / paclitaxel combined with vandetanib in advanced NSCLC patients as first-line therapy. The vandetanib monotherapy arm was stopped early after an interim analysis. Treatment was tolerable in all three groups, but more patients receiving vandetanib plus carboplatin / paclitaxel experienced insomnia, anorexia, depression, grade 3–4 diarrhea, asymptomatic  $QT_c$  prolongation, skin disorders, and hypertension. Neutropenia was the most frequently reported grade 3 adverse event, equally distributed among the chemotherapy-containing arms. A statistically significant improvement in PFS of only 1 week was observed in the group treated with vandetanib and chemotherapy, compared with chemotherapy alone. OS and RR were not significantly different. No detectable changes in pharmacokinetic exposure to vandetanib with the addition of carboplatin/paclitaxel were observed<sup>63</sup>

### ***Phase I trial on cediranib-containing combinations***

The TKI cediranib targets VEGFR, PDGFRb, and *c-kit*. In a phase I study, cediranib (daily 30 or 45 mg) was combined with carboplatin (AUC6) and paclitaxel (200 mg/m<sup>2</sup>, every 3 weeks) in patients with advanced NSCLC as first-line therapy. Toxicity was manageable, and common side effects were fatigue, diarrhea, anorexia, and neutropenia. No DLT were reported. Steady-state levels of cediranib were comparable to those seen in single-agent therapy. Carboplatin clearance was unchanged, but paclitaxel clearance was decreased in cycle 2, which was reflected in the nadir of the platelet counts.<sup>64</sup>

In another phase I trial, cediranib (30 or 45 mg daily) was added to mFOLFOX-6 (every 14 days) in 16 metastatic CRC patients. One DLT, grade 3 diarrhea, was observed. Common grade 3 cediranib-related toxicities included hypertension, diarrhea, fatigue, and anorexia. There were no pharmacokinetic interactions between cediranib and 5-FU or free plasma oxaliplatin.<sup>65</sup> Currently, a phase III trial has been initiated, in which FOLFOX plus bevacizumab is compared with FOLFOX plus cediranib as first-line treatment of metastatic CRC.<sup>66</sup>

### **Combine targeted therapy and chemotherapy with caution, more is not always better**

To further improve the outcomes of combinations of agents targeting the VEGF pathway and chemotherapy, recently two large studies have been published in which an EGFR-targeting drug was added in patients with metastatic CRC as first-line therapy. Unexpectedly, adding panitumumab or cetuximab, resulted in worse outcome and increased toxicity.<sup>67,68</sup> Panitumumab was added to bevacizumab and oxaliplatin- and irinotecan-based chemotherapy. PFS was 10.0 and 11.4 months, and OS was 19.4 and 24.5 months for the groups with or without panitumumab respectively.<sup>67</sup> Similarly, cetuximab, added to capecitabine, oxaliplatin, and bevacizumab, resulted in significantly shorter PFS, 9.4 compared with 10.7 months for patients with or without cetuximab respectively. OS and RR were comparable.<sup>68</sup> Although a combination of agents targeting multiple signal-transduction pathways appears reasonable, the results from these studies show that theory may differ from practice. The underlying mechanisms for these results are unclear. There are no available data of a possible pharmacokinetic interaction. A possible pharmacodynamic interaction induced by EGFR inhibition could have led to diminished therapeutic effects of bevacizumab and / or chemotherapy, perhaps through EGFR-mediated alterations of downstream targets required for the activity of bevacizumab, but this is speculative.



## CONCLUSIONS AND FUTURE PERSPECTIVES

Though combining VEGF pathway inhibitors with conventional chemotherapy is theoretically attractive, this has currently only been proven for a few indications. Bevacizumab can improve the outcomes of conventional chemotherapy, but this is highly dependent on tumor type, stage, and chemotherapeutic regimen. With respect to kinase inhibitors, which in general are more difficult to combine with chemotherapy, randomized studies evaluating their added value are ongoing. Obviously, the availability of biomarkers enabling the identification of patients likely to benefit from combined regimens will augment the risk-benefit ratio of this approach. Biomarkers currently assessed include radiological tests to determine parameters such as vascular density, permeability, and volume.<sup>27,29</sup> With respect to soluble markers, baseline VEGF levels and outcome to antiangiogenic therapy as monotherapy have shown conflicting results. The predictive value in combination regimens remains to be established.<sup>69,70</sup> Increased levels of placental growth factor were associated with better outcome in CRC patients treated with bevacizumab and chemoradiation.<sup>71</sup> Furthermore, inflammatory proteins may be potential biomarkers; increased interleukin-6 levels were associated with worse outcome in patients with CRC and HCC, treated with bevacizumab and sunitinib respectively.<sup>28,71</sup> Also, circulating endothelial cells and EPC may emerge as useful biomarkers. Polymorphisms in the VEGF gene are another promising predictive factor. The VEGF-2578 AA genotype was associated with better OS in patients with MBC treated with bevacizumab and paclitaxel.<sup>72</sup> Whether or not this holds true for other combination regimens and other polymorphisms remains to be established. Clearly, the need for markers predictive for outcome to combinations of conventional chemotherapy and VEGF pathway-targeting drugs is high. In particular through the introduction of such predictive markers and thereby improved treatment individualization, combinations of conventional chemotherapy and VEGF pathway-targeting drugs are likely to redeem their great promise.

## REFERENCES

1. Hirota S, Isozaki K, Moriyama Y et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* (New York, NY) 1998; **279**:577-80.
2. Verweij J, Casali PG, Zalcberg J et al. Progression-free survival in gastrointestinal stromal tumours with high-dose imatinib: randomised trial. *Lancet* 2004; **364**:1127-34.
3. Heinrich MC, Owzar K, Corless CL et al. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol* 2008; **26**:5360-7.
4. Shibuya M, Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006; **312**:549-60.
5. Rini BI, Small EJ. Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. *J Clin Oncol* 2005; **23**:1028-43.
6. Jain RK, Duda DG, Clark JW, Loeffler JS. Lessons from phase III clinical trials on anti-VEGF therapy for cancer. *Nat Clin Pract Oncol* 2006; **3**:24-40.
7. Yang JC, Haworth L, Sherry RM et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003; **349**:427-34.
8. Siegel AB, Cohen EI, Ocean A et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008; **26**:2992-8.
9. Burger RA, Sill MW, Monk BJ, Greer BE, Sorosky JJ. Phase II trial of bevacizumab in persistent or recurrent epithelial ovarian cancer or primary peritoneal cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2007; **25**:5165-71.
10. Cannistra SA, Matulonis UA, Penson RT et al. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. *J Clin Oncol* 2007; **25**:5180-6.
11. Gordon AN, Fleagle JT, Guthrie D, Parkin DE, Gore ME, Lacave AJ. Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. *J Clin Oncol* 2001; **19**:3312-22.
12. Kreisl TN, Kim L, Moore K et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol* 2009; **27**: 740-5.
13. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**:115-24.
14. Motzer RJ, Hutson TE, Tomczak P et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 3584-90.
15. Heinrich MC, Maki RG, Corless CL et al. Primary and secondary kinase genotypes correlate with the biological and clinical activity of sunitinib in imatinib-resistant gastrointestinal stromal tumor. *J Clin Oncol* 2008; **26**:5352-9.
16. Escudier B, Eisen T, Stadler WM et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**:125-34.
17. Escudier B, Eisen T, Stadler WM et al. 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* 2009; **27**:3274-6.
18. Escudier B, Szczylik C, Hutson TE et al. Randomized phase II trial of first-line treatment with sorafenib versus interferon Alpha-2a in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**:1280-9.
19. Llovet JM, Ricci S, Mazzaferro V et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**:378-90.



20. Sternberg CN, Szczylik C, Lee E et al. A randomized, double-blind phase III study of pazopanib in treatment-naive and cytokine-pretreated patients with advanced renal cell carcinoma (RCC). *J Clin Oncol* 2009; **27**:abstract 5021.
21. Zhang L, Hannay JA, Liu J et al. Vascular endothelial growth factor overexpression by soft tissue sarcoma cells: implications for tumor growth, metastasis, and chemoresistance. *Cancer Res* 2006; **66**: 8770-8.
22. Dias S, Shmelkov SV, Lam G, Rafii S. VEGF(165) promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. *Blood* 2002; **99**:2532-40.
23. Tran J, Master Z, Yu JL, Rak J, Dumont DJ, Kerbel RS. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. *Proc Natl Acad Sci U S A* 2002; **99**:4349-54.
24. Heldin CH, Rubin K, Pietras K, Ostman A. High interstitial fluid pressure – an obstacle in cancer therapy. *Nat Rev Cancer* 2004; **4**:806-13.
25. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* (New York, NY) 2005; **307**:58-62.
26. Winkler F, Kozin SV, Tong RT et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiotensin-1, and matrix metalloproteinases. *Cancer Cell* 2004; **6**:553-63.
27. Willett CG, Boucher Y, di Tomaso E et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**:145-7.
28. Zhu AX, Sahani DV, Duda DG et al. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol* 2009; **27**:3027-35.
29. Batchelor TT, Sorensen AG, di Tomaso E et al. AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 2007; **11**:83-95.
30. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat Rev Cancer* 2002; **2**:826-35.
31. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**: 401-10.
32. Kerbel RS. Antiangiogenic therapy: a universal chemosensitization strategy for cancer? *Science* 2006; **312**:1171-5.
33. Bertolini F, Paul S, Mancuso P et al. Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. *Cancer Res* 2003; **63**:4342-6.
34. Shaked Y, Henke E, Roodhart JM et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 2008; **14**: 263-73.
35. Furstenberger G, von Moos R, Lucas R et al. Circulating endothelial cells and angiogenic serum factors during neoadjuvant chemotherapy of primary breast cancer. *Br J Cancer* 2006; **94**:524-31.
36. D'Adamo DR, Anderson SE, Albritton K et al. Phase II study of doxorubicin and bevacizumab for patients with metastatic soft-tissue sarcomas. *J Clin Oncol* 2005; **23**:7135-42.
37. Hurwitz H, Fehrenbacher L, Novotny W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**:2335-42.
38. Saltz LB, Clarke S, Diaz-Rubio E et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008; **26**: 2013-9.
39. Tebbutt NC, GebSKI V, Wilson K et al. International randomized phase III study of capecitabine (Cap), bevacizumab (Bev), and mitomycin C (MMC) in first-line metastatic colorectal cancer (mCRC): final results of the AGITG MAX trial. *J Clin Oncol* 2009; **27**: abstract 4023.
40. Wolmark N, Yothers G, O'Connell MJ et al. A phase III trial comparing mFOLFOX6 to mFOLFOX6 plus bevacizumab in stage II or III carcinoma of the colon: results of NSABP Protocol C-08. *J Clin Oncol* 2009; **27**: abstract LBA4.

41. Giantonio BJ, Catalano PJ, Meropol NJ et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* 2007; **25**:1539-44.
42. Miller KD, Chap LI, Holmes FA et al. Randomized phase III trial of capecitabine compared with bevacizumab plus capecitabine in patients with previously treated metastatic breast cancer. *J Clin Oncol* 2005; **23**:792-9.
43. Miller K, Wang M, Gralow J et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007; **357**:2666-76.
44. Miles D, Chan A, Romieu G et al. Randomized, double-blind, placebo- controlled, phase III study of bevacizumab with docetaxel or docetaxel with placebo as first-line therapy for patients with locally recurrent or metastatic breast cancer (mBC): AVADO. *J Clin Oncol* 2008; **26**: abstract LBA1011.
45. Sandler A, Gray R, Perry MC et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; **355**:2542-50.
46. Reck M, von Pawel J, Zatlouk P et al. Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAIL. *J Clin Oncol* 2009; **27**:1227-34.
47. Ramalingam SS, Dahlberg SE, Langer CJ et al. Outcomes for elderly, advanced-stage non small-cell lung cancer patients treated with bevacizumab in combination with carboplatin and paclitaxel: analysis of Eastern Cooperative Oncology Group Trial 4599. *J Clin Oncol* 2008; **26**:60-5.
48. Kindler HL, Niedzwiecki D, Hollis D et al. A double-blind, placebo- controlled, randomized phase III trial of gemcitabine (G) plus bevacizumab (B) versus gemcitabine plus placebo (P) in patients (pts) with advanced pancreatic cancer (PC): a preliminary analysis of Cancer and Leukemia Group B (CALGB). *J Clin Oncol* 2007; **25**: abstract 4508.
49. Van Cutsem E, Vervenne WL, Bennouna J et al. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**:2231-7.
50. Richly H, Henning BF, Kupsch P et al. Results of a phase I trial of sorafenib (BAY 43-9006) in combination with doxorubicin in patients with refractory solid tumors. *Ann Oncol* 2006; **17**:866-73.
51. Richly H, Schultheis B, Adamietz IA et al. Combination of sorafenib and doxorubicin in patients with advanced hepatocellular carcinoma: results from a phase I extension trial. *Eur J Cancer* 2009; **45**:579-87.
52. Siu LL, Awada A, Takimoto CH et al. Phase I trial of sorafenib and gemcitabine in advanced solid tumors with an expanded cohort in advanced pancreatic cancer. *Clin Cancer Res* 2006; **12**:144-51.
53. Kupsch P, Henning BF, Passarge K et al. Results of a phase I trial of sorafenib (BAY 43-9006) in combination with oxaliplatin in patients with refractory solid tumors, including colorectal cancer. *Clin Colorectal Cancer* 2005; **5**:188-96.
54. Flaherty KT, Schiller J, Schuchter LM et al. A phase I trial of the oral, multikinase inhibitor sorafenib in combination with carboplatin and paclitaxel. *Clin Cancer Res* 2008; **14**:4836-42.
55. Mross K, Steinbild S, Baas F et al. Results from an in vitro and a clinical/pharmacological phase I study with the combination irinotecan and sorafenib. *Eur J Cancer* 2007; **43**:55-63.
56. Hauschild A, Agarwala SS, Trefzer U et al. Results of a phase III, randomized, placebo-controlled study of sorafenib in combination with carboplatin and paclitaxel as second-line treatment in patients with unresectable stage III or stage IV melanoma. *J Clin Oncol* 2009; **27**:2823-30.
57. McDermott DF, Sosman JA, Gonzalez R et al. Double-blind randomized phase II study of the combination of sorafenib and dacarbazine in patients with advanced melanoma: a report from the 11715 Study Group. *J Clin Oncol* 2008; **26**:2178-85.
58. Hamberg AP, Steeghs N, Loos WJ et al. Phase I safety and pharmacokinetic (PK) study of sunitinib (S) in combination with ifosfamide (I) in patients (pts) with advanced solid tumors (STs). *J Clin Oncol* 2009; **27**: abstract 13520.
59. Tamura T, Minami H, Yamada Y et al. A phase I dose-escalation study of ZD6474 in Japanese patients with solid, malignant tumors. *J Thorac Oncol* 2006; **1**:1002-9.
60. de Boer R, Humblet Y, Wolf J et al. An open-label study of vandetanib with pemetrexed in patients with previously treated non-small-cell lung cancer. *Ann Oncol* 2009; **20**:486-91.



61. Michael M, Gibbs P, Smith R, Godwood A, Oliver S, Tebbutt N. Open-label phase I trial of vandetanib in combination with mFOLFOX6 in patients with advanced colorectal cancer. *Invest New Drugs* 2009; **27**:253-61.
62. Heymach JV, Johnson BE, Prager D et al. Randomized, placebo-controlled phase II study of vandetanib plus docetaxel in previously treated non-small-cell lung cancer. *J Clin Oncol* 2007; **25**: 4270-7.
63. Heymach JV, Paz-Ares L, De Braud F et al. Randomized phase II study of vandetanib alone or with paclitaxel and carboplatin as first-line treatment for advanced non-small-cell lung cancer. *J Clin Oncol* 2008; **26**:5407-15.
64. Laurie SA, Gauthier I, Arnold A et al. Phase I and pharmacokinetic study of daily oral AZD2171, an inhibitor of vascular endothelial growth factor tyrosine kinases, in combination with carboplatin and paclitaxel in patients with advanced non-small-cell lung cancer: the National Cancer Institute of Canada clinical trials group. *J Clin Oncol* 2008; **26**:1871-8.
65. Chen E, Jonker D, Gauthier I et al. Phase I study of cediranib in combination with oxaliplatin and infusional 5-Fluorouracil in patients with advanced colorectal cancer. *Clin Cancer Res* 2009; **15**:1481-6.
66. Robertson JD, Botwood NA, Rothenberg ML, Schmoll HJ. Phase III trial of FOLFOX plus bevacizumab or cediranib (AZD2171) as first-line treatment of patients with metastatic colorectal cancer: HORIZON III. *Clin Colorectal Cancer* 2009; **8**:59-60.
67. Hecht JR, Mitchell E, Chidiac T et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol* 2009; **27**:672-80.
68. Tol J, Koopman M, Cats A et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009; **360**:563-72.
69. Burstein HJ, Chen YH, Parker LM et al. VEGF as a marker for outcome among advanced breast cancer patients receiving anti-VEGF therapy with bevacizumab and vinorelbine chemotherapy. *Clin Cancer Res* 2008; **14**:7871-7.
70. Rini BI, Michaelson MD, Rosenberg JE et al. Antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J Clin Oncol* 2008; **26**:3743-8.
71. Willett CG, Duda DG, di Tomaso E et al. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. *J Clin Oncol* 2009; **27**:3020-6.
72. Schneider BP, Wang M, Radovich M et al. Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. *J Clin Oncol* 2008; **26**:4672-8.
73. Reese DM, Fratesi P, Corry M, Novotny W, Holmgren E, Small EJ. A phase II trial of humanized anti-vascular endothelial growth factor antibody for the treatment of androgen-independent prostate cancer. *Prostate J* 2001; **3**:65-70.
74. Monk BJ, Sill MW, Burger RA, Gray HJ, Buekers TE, Roman LD. Phase II trial of bevacizumab in the treatment of persistent or recurrent squamous cell carcinoma of the cervix: a gynecologic oncology group study. *J Clin Oncol* 2009; **27**:1069-74.
75. Steinbild S, Mross K, Frost A et al. A clinical phase II study with sorafenib in patients with progressive hormone-refractory prostate cancer: a study of the CESAR Central European Society for Anticancer Drug Research-EWIV. *Br J Cancer* 2007; **97**:1480-5.
76. Sleijfer S, Ray-Coquard I, Papai Z et al. Pazopanib, a multikinase angiogenesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: a phase II study from the European Organisation for Research and Treatment of Cancer-Soft Tissue and Bone Sarcoma Group (EORTC Study 62043). *J Clin Oncol* 2009; **27**:3126-32.
77. Cheng AL, Kang YK, Chen Z et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**:25-34.



Chapter

# 3

## **Dose-escalation models for combination phase I trials in oncology**

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## **ABSTRACT**

Designing combination drug phase I trials has become increasingly complex, due to the increasing diversity in classes of agents, mechanisms of action, safety profiles and drug-administration schedules. With approximately 850 agents currently in development for cancer treatment, it is evident that combination development must be prioritised, as based on a specific hypothesis, as well as a projected development path for the involved combination.

In this manuscript the most relevant issues and pitfalls for combination drug phase I trial design are discussed. Several phase I study designs that incorporate controls to circumvent bias due to imbalances in observed background toxicity are discussed.

## INTRODUCTION

Designs for phase I trials in general and combination drug phase I trials in particular are facing several challenges with the increasing diversity of classes of agents and mechanisms of action. In addition, these new classes of anticancer agents are often administered continuously rather than intermittently, and they manifest safety profiles that are completely different from those of conventional cytotoxic agents. Determining the best dose for new combination drug treatments will have to be balanced against these aspects. Longer observation periods than the usual 3–4 weeks are required, given that continuously administered drugs may show relevant toxicity only after a prolonged exposure.<sup>1</sup> There are currently approximately 850 agents in the development for cancer treatment,<sup>2</sup> which could potentially be combined into approximately 400,000 two-drug combinations, not to mention potential combinations involving already marketed agents. Computational modelling of new drug combinations may be a way forward, but as long as this has not been developed, we will have to work with realistic phase I study modelling and planning.

### Rationale

A phase I study of a new combination of drugs is only the first clinical step in the development of that specific combination and should be considered a means, not an end in itself. Before commencing a combination drug phase I trial, a plan for (tumour-type specific) subsequent developmental phase II/III studies must clearly be defined. The choice for tumour type should be based on a scientific rationale, ideally including data in an appropriate preclinical model or on the basis of clinical results for the individual agents.

### It all starts with an hypothesis

A single ideal template to perform a combination drug phase I trial will likely never exist. Every trial has to be designed based on prior knowledge of the preclinical and clinical pharmacology of the individual agents. Given the enormous number of potential combinations, in addition to a proper rationale and further development plan as outlined above, an appropriate hypothesis to guide the protocol design is also crucial. We have identified three potential general hypotheses for such studies. Whilst the hypothesis is leading for the trial design, one should obviously always keep an open eye to detect unexpected observations.

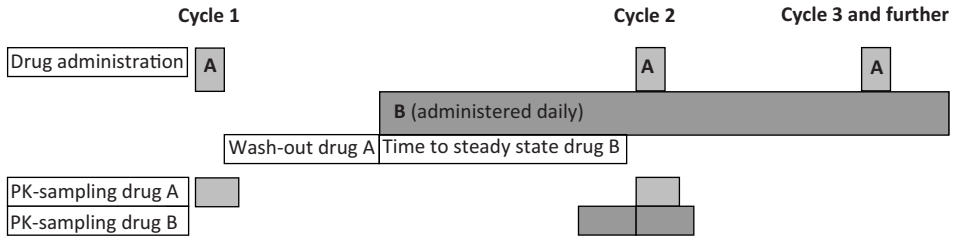
#### ***Type 1 hypothesis: interaction at the pharmacokinetic (PK) level***

Data on metabolism and pharmacokinetics of each of the drugs involved may suggest a potential interaction at the PK level, for example, if drug A is a putative CYP3A4 inhibitor while drug B is a substrate for CYP3A4. PK drug interactions are particularly plausible when



both drugs are oral, as there may be unanticipated interactions related to drug absorption and/or first-pass metabolism.

Obviously, in phase I trials based on this hypothesis extensive PK sampling is warranted, and the design will have to include PK of the single agent(s) as well as of the combination. This means that a single agent dose will have to be a part of the design, and inpatient and interpatient variabilities will have to be taken into account (Figure 1).



**Figure 1** | Single agent as well as drug combination pharmacokinetic analysis enabling inpatient evaluation. Drug A is administered i.v. every 3 weeks and drug B is administered orally every day.

If the hypothesised interaction would lead to an anticipated increased drug exposure, the first dose-level should be defined cautiously low. Given the hypothesis, dose-escalation to the next cohort can only take place once the PK-analysis in the previous cohort is completed and can be taken into account. The outcome of this analysis can upfront be incorporated in the projected dose-levels, by introducing PK-based dose-escalation rules, such as, for instance, 'If steady state of drug A increases less than factor X due to the addition of drug B, and no dose-limiting toxicity (DLT) is observed: escalate to dose-level 3. Otherwise escalate to dose-level 2.'

### ***Type 2 hypothesis: interaction at PD level without interaction at PK level***

In some combinations no PK interaction is anticipated based on the respective single agent data, but evidence supports a potential PD interaction, like an increase in a specific toxicity or an additional effect on a mechanism-related biomarker. In these studies neither extensive PK-sampling nor a run-in single agent phase would be required. However, limited PK-sampling may be advisable to enable verification that the right hypothesis was chosen and to allow exclusion of a totally unexpected PK interaction as a cause in case of observed excessive toxicity. Such limited sampling can be done at each dose-level or only in dose-expansion cohorts at the maximally tolerated dose (MTD).

Obviously, the follow-up assessments of patients should be scheduled in a way that optimal monitoring of the toxicity for which an interaction is anticipated can be ensured.

The follow-up schedule can be different for a combination in which QT-interval prolongation is expected during the first 5 days after intravenous administration of drugs A and B as compared to a combination in which a prolonged neutropaenia between day 10 and day 20 is anticipated after intravenous administration of drug A and daily oral administration of drug B.

***Type 3 hypothesis: no interaction at PK or at PD level***

This hypothesis would render the phase I part of a developmental path extremely short. All we need to know is the feasibility of the drugs involved given at their respective recommended single agent doses. The challenge will thus be to factor this feasibility part into the phase II trial design.

In case of a Type 1 or 2 hypothesis a phase I study has to define one or more MTDs (see paragraphs 4, 5 and 6), in contrast to a Type 3 hypothesis in which just the feasibility at one or limited dose levels has to be shown (paragraph 7).

**Defining the MTD in Types 1 and 2 studies**

In theory the number of MTDs of two drugs is infinite and does describe a curve, which we can call an envelope of tolerability. Obviously, the full envelope of tolerability of a drug combination will never be described, but defining multiple MTDs as derivatives of the envelope will generate knowledge on the dosing-range of the combination (Figure. 2).

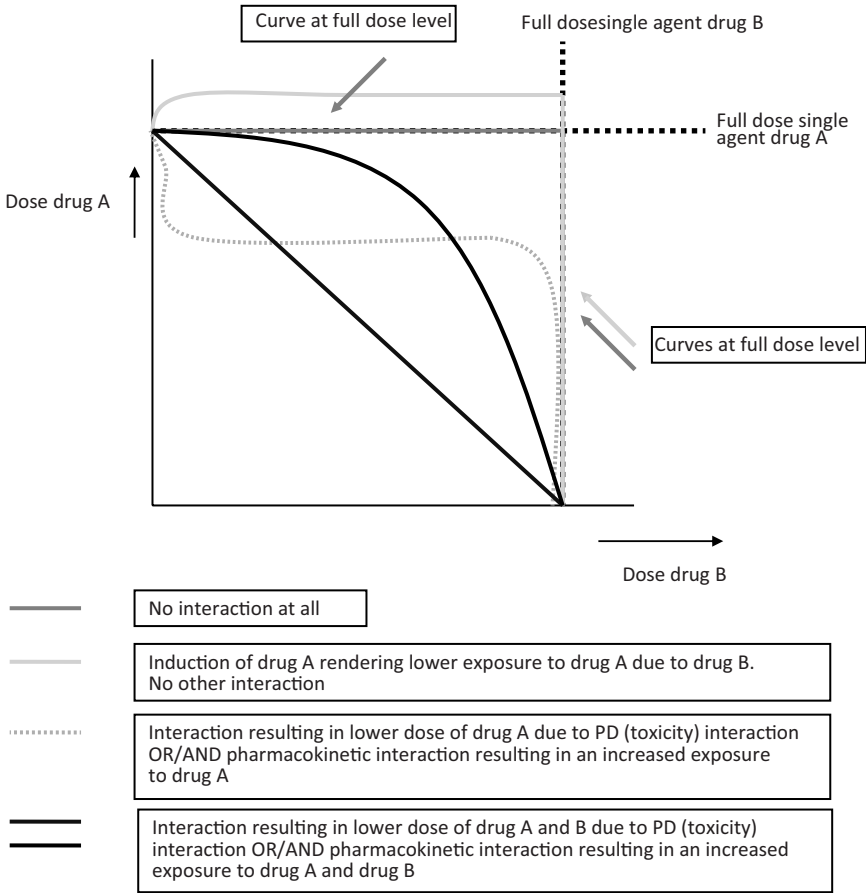
At first glance, the MTD often seems to be determined by the data generated during the trial. However, some choices made with regard to the pre-defined dose and dose-escalation steps are crucial and will have an impact on the MTD(s) defined: the drug schedule and administration sequence, order of pre-defined escalation steps and whether or not to compromise on a dose of an active agent.

***Drug schedule and administration sequence***

Additive or synergistic effects of combinations of agents may be dependent on specific drug scheduling and will have to be taken into account. For example, combining sunitinib (standard schedule 4 weeks on, 2 weeks off) with capecitabine (standard schedule 2 weeks on, 1 week off) will have to lead to choices regarding the drug-administration schedules. In this example, if a direct drug-drug interaction is anticipated with respect to exposure to either drug due to the other (or its metabolites), then a constantly changing drug exposure might result if the common single agent on/off schedules are applied. Other potential schedules would lead to more consistent drug exposures and the choice of which schedules to explore in a phase I trial should preferably be supported by data from preclinical studies. Similar issues could arise when an intravenously administered drug is combined with an oral agent.



Furthermore, the sequence in which the drugs are administered might impact on tolerability and/or efficacy. Particularly for drugs with a short half-life a PK interaction could necessitate specific sequences of administration. But also PD interactions will have to be taken into account, when selecting the best sequence of administration.

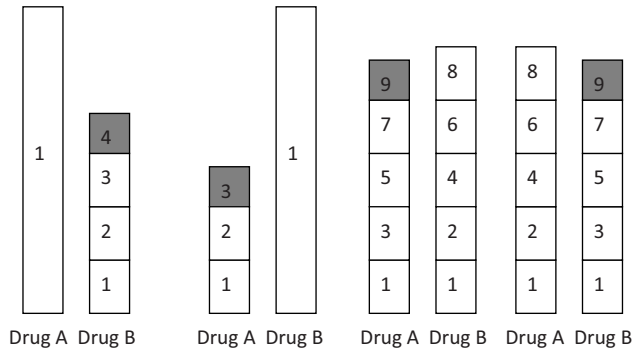


**Figure 2 |** Sample of envelopes of tolerability (as types of envelopes are infinite) of a two-drug combination. An established MTD will be a single point on its curve. And although often only a single MTD is determined, many more MTDs do exist. The location of the determined MTD on its envelope is often a result of trial design.

**MTD depends on escalation steps**

As previously described, solely on the basis of a different use of the pre-defined dose-escalation steps, at least four different MTDs can be determined in a 'simple' combination phase I trial involving only two drugs (Figure 3).<sup>3</sup> As mentioned earlier, the true number of MTDs is infinite, so even this represents a crude way to define the envelope of tolerability.

In considering issues such as schedule, sequence and escalation steps, one might not want to limit to the identification of only a single MTD. It might even be preferred to study different schedules and drug-sequences and thus identify a multitude of MTDs in a single phase I combination study. Given the fact that phase I trials are aiming to define tolerated doses, and cannot identify the most optimal schedule, the identified multiple MTDs can subsequently be studied in a randomised phase 2 trials to pick the winner.



**Figure 3** | Four maximally tolerated doses (MTDs) determined with two drugs, solely depending on escalation steps. (Numbers are dose levels. Grey blocks are the dose-levels exceeding the MTD.)

### ***Compromising on the dose of an active agent?***

Intuitively, physicians are reluctant to lower the dose of an agent known to be active. Although this is understandable in daily oncology practise, it may in theory be incorrect and hamper the options of exploiting a possible synergistic or additive effect. So, for most combinations, one should be prepared to (initially) compromise on the dose of an active agent, however, there may be rational exceptions to this. First of all, in a curative multimodality treatment in which a systemic agent is used as a radiosensitiser, the study population will not be a standard phase I population without further treatment options, but will consist of patients in whom cure is the goal. Lowering the dose of radiation might reduce the chances of cure, in return for an unknown benefit. On the other hand, no other population can be identified to study potential beneficial combinations during radiotherapy, so a very trial-specific approach should be defined.

Secondly, if the mechanism of action of the added agent, for example, a poly(ADP-ribose) polymerase (PARP)-inhibitor, is clearly dependent on the in vivo effects induced by the standard treatment, it is logical not to compromise on the dose of the standard treatment. The rationale of adding a PARP-inhibitor to conventional cytotoxic therapy, like a schedule of carboplatin and paclitaxel, is the inhibitory effect of a PARP-inhibitor on the repair of



DNA-damage caused by alkylating agents. Applying the full dose of the cytotoxic therapy ascertains that the circumstances to determine the most rational MTD are optimised.

A third exception applies to studies incorporating agents, known to be almost inactive below a given standard dose, such as ifosfamide, and can fix the dose of that agent, but the involved protocols should include a clause allowing a decreased dose of that agent if pharmacokinetic studies show an increased exposure due to drug-drug interactions.

### **Interaction at PD level focusing on toxicity**

#### ***MTD/DLT-incidence in perspective of known toxicity profiles***

Combination phase I studies should preferably be initiated if knowledge is available on the single agent toxicity profile of the involved agents. Combination phase I trials should aim to model the toxicity as function of dose and PK. While defining the limits of 'tolerability' based on an incidence of unacceptable side-effects is relatively straight-forward in single agent phase I trials, this turns out to be more difficult for combination phase I studies. And yet defining MTD and DLT is critical to the outcome of these studies.

A few rather philosophical questions need to be addressed for combinations in which one of the two agents already has a high incidence of dose-limiting toxicities: how to handle a limited increase in DLT-incidence that crosses the classical phase I DLT-incidence cut off (33.3%) due to the addition of the second drug? For example, docetaxel has a high incidence of febrile neutropaenia (25–35% of patients) and most of these events occur during the first cycles of treatment. In combination with docetaxel, even agents with a very limited febrile neutropaenia rate in theory can raise the incidence of this dose limiting side-effect above the classical upper boundary of acceptability. So for each trial a choice has to be made whether or not it is acceptable to shift this boundary upwards.

Another issue is whether the definition of tolerability should be approached differently if the two drugs have overlapping limiting side-effects as compared to a combination with non-overlapping toxicity. If, for instance, drugs A and B are both dose limited by diarrhoea at an incidence of 25% and 20%, respectively, it is highly likely that diarrhoea will be the DLT of the combination as well (Figure 4). In this circumstance, there is no rationale to be more liberal in setting the limits of acceptability of toxicity, by allowing a higher incidence of this specific toxicity to define DLT/MTD. If more liberal criteria would be applied in this case, they could just as well be applied for defining DLT/MTD of the respective single agents.

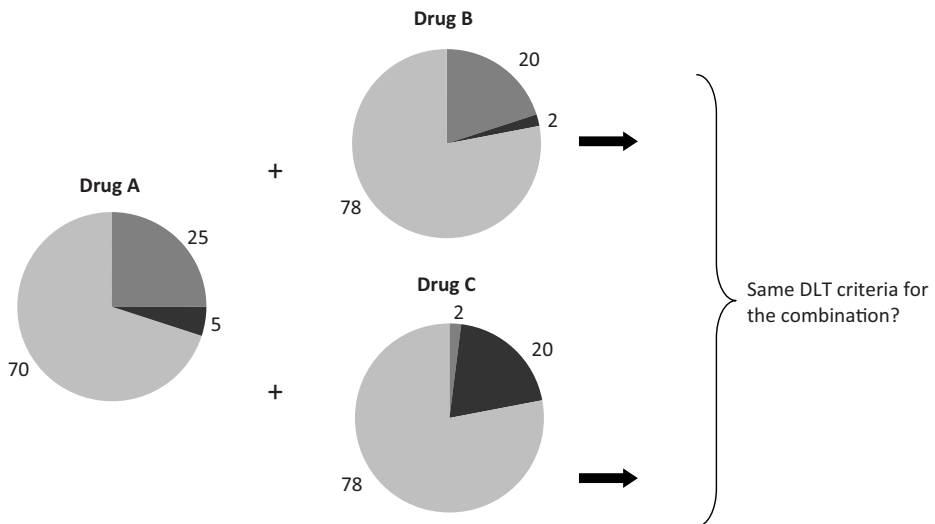
More difficult are situations with non-overlapping toxicity, adding drug C (with a hypertension incidence of 20%) to the same drug A. No standard recipe can be given here and the design should anticipate on two different scenarios: non-overlapping toxicity can occur in the same patients (rendering an incidence of toxicity of 25%) or in other patients with a potential incidence of toxicity up to or even above 45% (Figure 4).



**Type of toxicity and duration of observation**

It remains a matter of debate, also in single agent phase I studies, whether short lasting and more chronic toxicity should have the same impact in defining the DLT and therewith the MTD. In combination phase I trials, this is even more complex, especially given the fact that many phase I trials are studies combining cytotoxic therapy with agents more specific to the cancer (cell). Due to the chronic exposure during treatment with the latter, the resulting toxicity usually also has a chronic character and can affect the tolerability by other means than the more acute toxicity related to cytotoxic therapy.

Cumulative toxicity is known for some conventional cytotoxic agents such as doxorubicin (cardiotoxicity), taxanes, oxaliplatin (both neuropathies) and etoposide (leukaemogenic) and is just as important in determining the possible maximal total duration of therapy as is the dosing per cycle. Long-term toxicities are frequently only recognised just prior to or after registration of an agent. This may also be true for the more modern cancer (cell) specific agents (e.g. cardiotoxicity due to sunitinib<sup>4</sup>) and in early stages of drug development it will be unknown if lowering the dose per administration will allow drug administration for a more prolonged period of time. Incorporating such evaluation time frames will render phase I trials undoable. This type of toxicity can best be explored in (randomised) phase II trials.



**Figure 4 | Toxicity in the same domain versus non-overlapping toxicity.**  
DLT, dose-limiting toxicity.



### Avoiding background noise on toxicity

One of the challenges in conducting combination drug phase I trials is to carefully weigh whether or not the frequency of observed toxicity is representative for the novel combination or that, due to chance, the toxicity attributable to just one of the drugs occurs in the trial in a higher than usual incidence.

Two instruments may be helpful by valuing the toxicity data generated more carefully: (1) the 3 + 3 + 3 design and (2) introducing control groups into phase I studies.

### The 3 + 3 + 3 design

The classical 3 + 3 design allows dose-escalation based on the frequency of encountered DLTs. If in a cohort of three patients no DLTs are encountered, the next dose-level will be explored. In the case of two DLTs out of three patients, the MTD is considered exceeded, and a lower dose level is further evaluated for its MTD potential. In the case of one DLT out of three patients, an extra three patients are enrolled at that dose-level. If two or more DLTs occur in six patients, the MTD is considered exceeded.

By using the classical 3 + 3 design, implicitly an incidence of <33.3% of severe toxicity is considered acceptable, whereas determining this incidence is based on a very limited number of patients.

The chance that dose-escalation is 'falsely' halted is intrinsically related to the incidence of the severe toxicity of the new drug combination. This incidence is of course unknown, but prior data may point towards a relatively high incidence of severe toxicity, making the investigators eager to address this issue before commencing the trial.

By using the classical 3 + 3 design in a drug combination phase I trial with an unknown but true incidence of severe toxicity of 5% the chance of a 'falsely' halted dose-escalation can be calculated by using formula 1 and is: 3%, but it rapidly increases to 29% and 51% if the unknown but true incidence of severe toxicity increases to 20% and 30%, respectively (Table 1).

$$\text{Formula 1a: } x^3 + 3 * (x^2 * y + x^2 * y^2 + x^2 * y^3 + x^2 * y^4)$$

OR

$$\text{Formula 1b: } 1 - \{y^3 + 3 * y^5 * x\}$$

(x = unknown but true incidence of severe toxicity; y = 1 - x).

The recently briefly mentioned 3 + 3 + 3 design<sup>3</sup> decreases the chance of 'falsely' halting dose-escalation by means of the addition of enrolment of three extra patients at the same cohort as soon as two DLTs in six patients are observed. The extra three patients do allow the investigators a more refined grip on the incidence of severe toxicity of the treatment under evaluation. This strategy will result in a decrease of 'falsely' halting dose-escalation from 29% to 19% (by itself a reduction of approximately one-third!) if the unknown but true incidence of severe toxicity is 20% as can be calculated from formula 2 (Table 1).

$$\text{Formula 2: } 1 - \{y^3 + 3 * y^5 * x + 12 * y^7 * x^2\}$$

A specific issue that needs some extra thought is the situation of two DLTs occurring in nine patients. From a mathematical viewpoint, it does not matter if these two DLTs occur in the first three patients or in the last three patients. The latter situation will never occur, given the fact that after 0 DLTs in the first three patients, the dose will already be escalated after these three patients. It is more delicate how to handle the situation of two DLTs in the first three patients. In the classic 3 + 3 design, this would be the signal for exceeding the MTD, as a further expansion of three patients will not get the incidence below 33.3%. But in the 3 + 3 + 3 design, there is a chance that the following six patients are without a DLT rendering an incidence of severe toxicity below the threshold of 33.3%. In our opinion this is not worthwhile, based on the following two arguments.

First of all, if in two of three patients a DLT has occurred, while the true but unknown incidence of severe toxicity is 30%, the chance of ending up with just two of nine is 12%. So even if the incidence is within the acceptable range, only 1 of 8 trials will succeed to prove so.

The second argument against dose-expansion in the presence of two DLTs in the first three patients is that it facilitates 'falsely' continuing dose-escalation to a greater extent than it limits 'falsely' halting dose-escalation. The chance of 2 of 3 will occur in 14 of 100 trials in which the true but unknown incidence is 25%, whereas this occurs in 29 of 100 trials if the unknown incidence of severe toxicity is 40%.

So, applying the 3 + 3 + 3 design excluding the '2-out-of-the-first-3-situation' is the same as applying the 3 + 3 design and only in the case in which after expansion to six patients two DLTs are observed, a further expansion to nine patients will be done. The advantage of this approach can be calculated with formula 3, and examples are shown in the last column of Table 1.

$$\text{Formula 3: } 1 - \{y^3 + 3 * y^5 * x + 9 * y^7 * x^2\}$$

It is clear that the 3 + 3 + 3 design is only of additional value if the incidence of severe toxicity is anticipated to be in the upper range of what may be considered acceptable. On the other hand, there is no harm done by applying this to all phase I trials, as it will only be used at a point where the classic phase 3 + 3 design already definitely has halted dose-escalation.

### ***Introducing controls in phase I studies***

Diminishing the effect of chance by introducing a control population is a standard procedure in phase II and III trials, rendering results better interpretable for a larger population. In phase I studies in the field of oncology controls have not been used up to now. However, particularly in combination phase I studies they might help in distinguishing added toxicity related to the added drug, from the toxicity related to the backbone standard.



**Table 1** | Chances of falsely halting dose-escalation as a function of the Incidence of unknown but true severe toxicity and the trial design

Incidence of unknown but true severe toxicity	Formula parameters	Chance of 'falsely' halting dose-escalation using classic 3 + 3 design; (formula 1)	Chance of 'falsely' halting dose-escalation using 3 + 3 + 3 design (incl 2 of 3*); (formula 2)	Chance of 'falsely' halting dose-escalation using 3 + 3 + 3 design (excl 2 of 3*); (formula 3)
5%	x=0.05 y=0.95	0.03	0.01	0.01
20%	x=0.20 y=0.80	0.29	0.19	0.22
30%	x=0.30 y=0.70	0.51	0.42	0.44

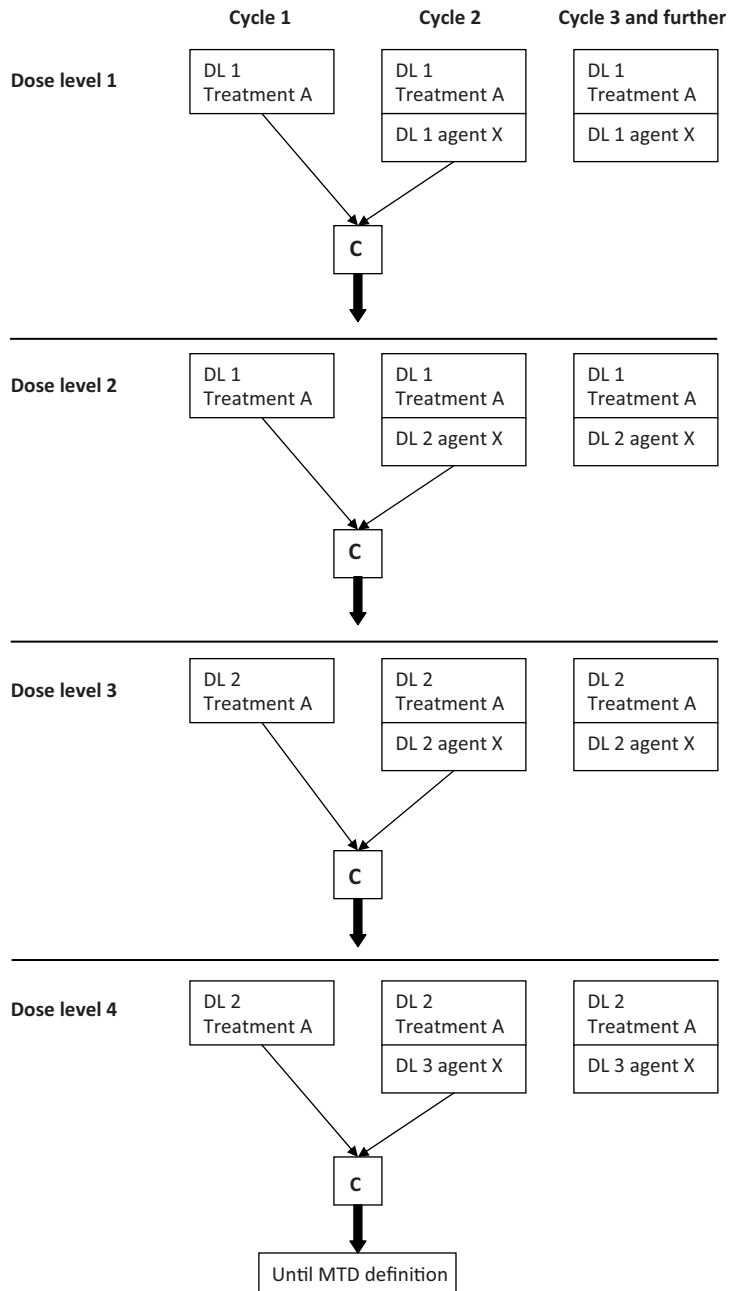
\* 2 of 3 refers to two dose-limiting toxicities (DLTs) occurring in the first three patients in a specific dose-level.

### ***Each patient its own control***

Using every patient as his or her own control is a simple way to introduce a controlled situation. In cycle 1, for instance, the patients can be treated with drug A, followed by a second cycle in which the combination of drug A + B is administered, without changing the dose of drug A (Figure 5). This approach will generate information on additional toxicity due to the added agent and leans on the premise, as discussed in Section 4, that thoughts are given to the increment in toxicity that is allowed. In this model, dose-escalation decisions should relate to the increment in toxicity between cycles 1 and 2 but also include rules with regard to the absolute incidence of toxicity. If this latter is not incorporated, the following might occur: 3 of 3 patients experience febrile neutropaenia during cycle 1 (not imaginary as this will happen in 2% of cohorts of three patients treated with docetaxel). No matter how many episodes of febrile neutropaenia do occur in cycle 2, there will be no increment in toxicity and dose-escalation will be done.

This model has some major limitations that need to be addressed in each specific trial to which it is applied. For example, most usually a preventive measure (like dose-reduction) is taken to avoid repetition of toxicity observed in the previous cycle, whereas in this model the single difference between cycles 1 and 2 should only be the addition of drug B and not a change in dose. Replacing these patients, on the basis of non-evaluability, is not appropriate as it results in an observation bias rendering an underestimation of toxicity.

If this model is applied, then only agents can be used of which the toxicity has completely disappeared at the start of cycle 2 and that do not have cumulative toxicity. For example, combining oxaliplatin with placebo might result in an increment in neurotoxicity that falsely would be assigned to the placebo.

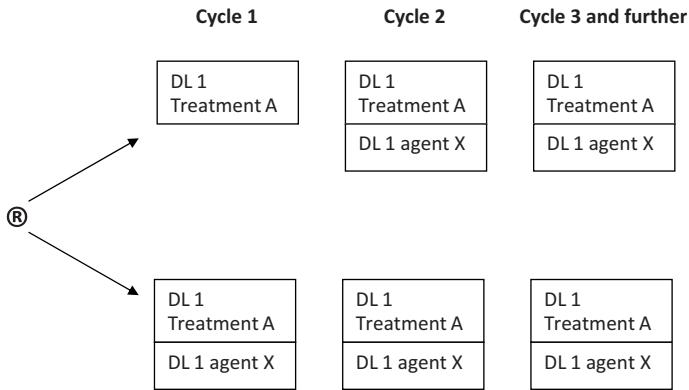


**Figure 5** | Using each patient as its own control to decide on dose-escalation. Treatment A can be either standard treatment or an investigational drug.

C: compare and if within pre-defined criteria: escalate; DL, dose-level; MTD, maximally tolerated dose.

**Each patient its own control plus randomisation**

A second control-method can be added to the model described in the previous paragraph by using a randomisation between a group of patients treated according to the model described in paragraph 5.2.1 and a group of patients treated with the combination from the start. This generates inpatient information as well as information between the two cohorts of patients. Obviously defining the dose-escalation rules for this double-controlled phase I trial will be equally challenging (Figure 6).



**Figure 6 |** At each dose level there is an inpatient comparison (like that in Figure 4) and an interpatient comparison (between the two arms) before deciding on dose-escalation.

R: randomisation

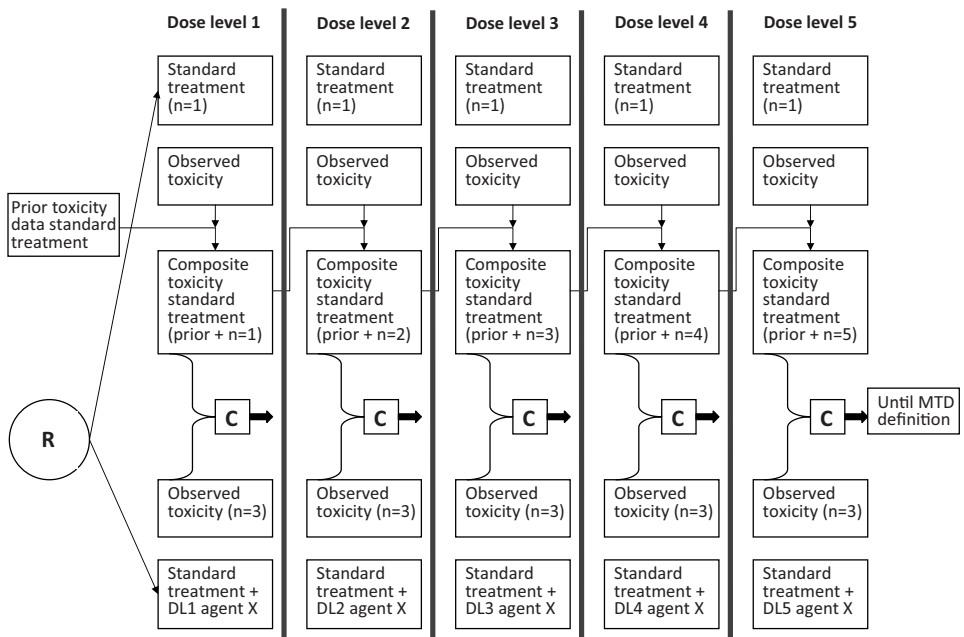
**Bayesian approach**

This strategy is proposed to deal with the issue of background toxicity if an agent is added to standard treatment, especially for treatments with curative intent, for example, chemo-radiotherapy for locally advanced head and neck cancer, given the fact that alterations in the standard treatment can compromise survival. Due to coinciding differences in patient group characteristics, the observed incidence of severe toxicity might be higher than that expected, even if the added agent was dosed at placebo-level. To circumvent the issue of background noise, the Bayesian approach introduces, next to enrolling controls at each dose-level, adaptive (continual reassessment) design.

However, if a new agent is added to the standard treatment, the incidence of observed toxicity in the trials designating the standard treatment as standard can be used as the a priori probability of severe toxicity by means of an adaptive design, by invoking a Bayesian approach. Differences in the settings of the current and the pivotal trial can be accounted for in the prior probability. The prior probability should be clearly defined before enrolling the first patient.

As a small number of controls are enrolled at each dose-level (Figure 7), safety data generated in the control group, across the dose-levels, are combined with the prior, resulting in a more stable and importantly a more robust estimate of the background toxicity in that specific population treated solely with the standard treatment. So, the impact of the initial prior will thus decrease as more control data are gathered.

Then, at each dose-level that estimate is used to relate the observed toxicity in the group treated with the additional agent added to the standard treatment, to correct for coincidence as well as patient selection issues. It is important to note that the adaptive design of the Bayesian model uses accumulated data of patients treated solely with the standard treatment at all previous dose-levels to decide on dose-escalation (Figure 7).



**Figure 7** | Randomised phase I drug combination trial in which an agent is added to standard treatment: continual reassessment of toxicity of the standard treatment in order to compare the toxicity of the new combination to controls.

C: compare and if within pre-defined criteria: escalate. DL, dose-level; MTD, maximally tolerated dose; R: randomisation. n=1 and 3 are arbitrarily chosen to support the illustration.

A Bayesian approach renders probabilities, and that is an important difference that definitely needs a change in mind-set of phase I investigators. Such a probability is presenting the data in the opposite way as we are used to in phase I trials, as usually we interpret incidence

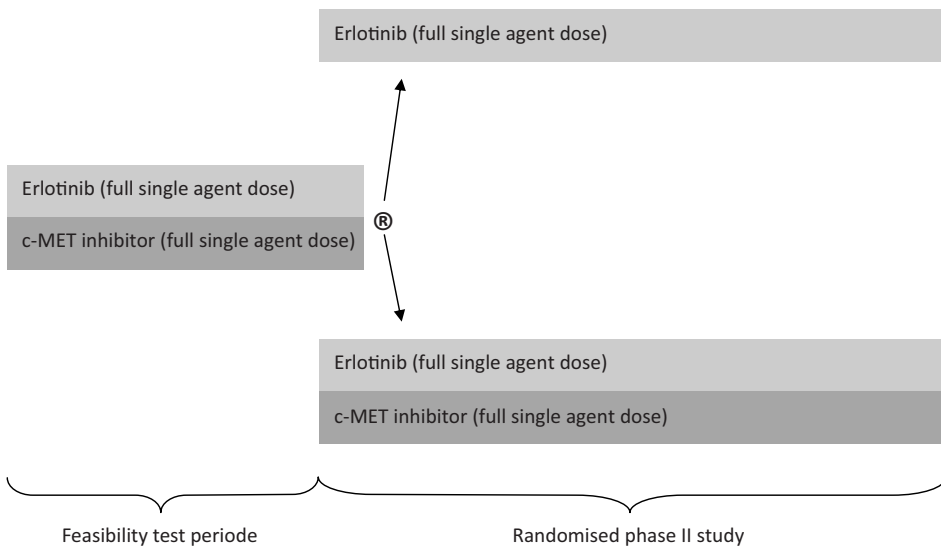


data in small groups of patients, deliberately ignoring the large uncertainties inherently related to a limited sample size.

**Determining feasibility in Type 3 studies**

If no PK or PD interaction is anticipated, the feasibility of a combination can be proven in a kind of phase I/II trial. If we assume that drug A is already regarded as standard treatment for a certain tumour type, then patients with this tumour type can be enrolled in a trial in which all patients start with a combination of drug A and drug B, both at full dose. Such an approach is feasible in, for example, the non-small lung cancer patient population in which the epidermal growth factor inhibitor erlotinib is combined with a novel c-MET inhibitor of which single agent MTD already has been determined. After the first treatment period (the feasibility-test period) randomisation can be performed between drug erlotinib plus or minus the c-MET inhibitor rendering a randomised phase II population (Figure 8). Instead of the classical DLT rules applied in phase I trials, this type of trials should incorporate go/no go rules based on a safety-interim evaluation observed in the first treatment period in a pre-defined number of patients.

A more conservative approach would utilise two cohorts, beginning with full dose of drug A combined with slightly lower dose of drug B. If the safety-interim analysis does not detect an excess of toxicity, then subsequent patients can be enrolled in the cohort using full dose of both drugs.



**Figure 8** | Testing of feasibility in drug combinations in which no pharmacokinetic or pharmacodynamic interaction is anticipated and one of the agents is regarded as standard treatment for this population. As example: erlotinib, regarded as standard therapy, is combined with a novel c-MET inhibitor.

Ⓡ: randomisation.



## CONCLUSION

Dose-finding studies are the first step in the clinical development of new combination treatment strategies. Combination phase I studies are extremely complex. Designing phase I studies is as important as the conduct itself and should be done by dedicated phase I researchers since a standard template cannot be made.

If there is a rationale to develop a specific drug combination, a hypothesis should be generated. This hypothesis, based on the anticipated levels of interaction, will be guiding for the design of the phase I study, although one should always be open to detect the unexpected. A strong consideration is to design studies aiming to potentially determine multiple MTDs with final dose determination in randomised phase II trials. Introducing controls and the 3 + 3 + 3 design are strategies allowing more grip on combinations with high incidence of severe toxicity. Key opinion leaders in the field of phase I oncology trials should make joint considerations towards future trial design in combination phase I studies.



## REFERENCES

1. Cheung YK, Chappell R. Sequential designs for phase I clinical trials with late-onset toxicities. *Biometrics* 2000; **56**:1177-82.
2. [http://www.phrma.org/files/attachments/meds\\_in\\_dev/09-046PhRMACancer09\\_0331.pdf](http://www.phrma.org/files/attachments/meds_in_dev/09-046PhRMACancer09_0331.pdf) [accessed 24.06.10].
3. Hamberg P, Verweij J. Phase I drug combination trial design: walking the tightrope. *J Clin Oncol* 2009; **27**:4441-3.
4. Chu TF, Rupnick MA, Kerkela R, et al. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet* 2007; **370**:2011-9.

Chapter

# 4

## **Decreased exposure to sunitinib due to concomitant administration of ifosfamide: results of a phase I and pharmacokinetic study on the combination of sunitinib and ifosfamide in patients with advanced solid malignancies**

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## ABSTRACT

**Background:** This study aimed to define the maximally tolerated dose (MTD) of sunitinib combined with two different infusion schedules of ifosfamide.

**Methods:** Patients with advanced solid tumours, good performance score, good organ function, and no standard therapy available were eligible. Continuous once daily sunitinib, in escalating doses per cohort, was combined with ifosfamide, 9 g/m<sup>2</sup> for 3 days or 6 g/m<sup>2</sup> for 5 days, administered every 3 weeks. Pharmacokinetic (PK) and pharmacodynamic (PD) assessments were performed.

**Results:** With growth-factor support, the MTD of sunitinib combined with either ifosfamide schedule was 12.5 mg in 32 patients enrolled. Neutropenia-related adverse events were dose-limiting toxicities. Sunitinib did not affect ifosfamide PK. Ifosfamide significantly decreased exposure to sunitinib and increased exposure to its metabolite, SU12662. No consistent changes in PD parameters were observed.

**Conclusion:** With growth-factor support, the MTD of sunitinib with both ifosfamide schedules was 12.5 mg. Ifosfamide produced decreased sunitinib blood levels because of CYP3A induction. As PK interactions cannot explain the relatively low sunitinib doses that can be combined with ifosfamide, synergy in toxicity is likely. Whether this also holds true for anti-tumour activity needs to be further explored.

## INTRODUCTION

The introduction of tyrosine kinase inhibitors (TKIs) specifically inhibiting tumour-driving factors was accompanied with high expectations with regard to their activity against solid malignancies. Their single-agent activity in most tumour types is, however, modest, with obvious exceptions in tumours such as renal cell carcinoma (RCC) and gastrointestinal stromal tumours (GIST).<sup>1,2</sup>

One potential way to augment the activity of TKIs is to combine them with conventional cytotoxic agents. In particular, combinations of TKIs targeting the vascular endothelial growth factor (VEGF) pathway and conventional cytotoxic agents seem attractive, given several potential mechanisms that may yield synergistic anti-tumour effects. Vascular endothelial growth factor produced by tumour cells results in the formation of new vasculature that is abnormal in structure and more permeable than normal vasculature. This causes a high interstitial pressure within the tumour, hindering the penetration of drugs into tumours.<sup>3</sup> Inhibition of VEGF-mediated effects has been shown to decrease intra-tumoural interstitial pressure, thereby enhancing the delivery of concomitantly administered drugs.<sup>3-5</sup> Other mechanisms that may contribute to synergistic interaction between VEGF-pathway inhibitors and conventional cytotoxic drugs include prevention of endothelial progenitor cell mobilisation from the bone marrow induced by chemotherapy and decreased production of tumour factors conferring resistance against chemotherapy.<sup>6-10</sup>

Sunitinib is a potent inhibitor of VEGF receptors (VEGFR)1–3, KIT, platelet-derived growth factor receptor- $\alpha$  and - $\beta$ , and Fms-like tyrosine kinase-3 (Flt3), and is one of the first and most commonly used VEGFR-TKIs. It is currently registered for the treatment of advanced RCC and imatinib-refractory GIST<sup>2,11</sup> and is being explored for its anti-tumour activity in a wide range of other tumour types. A potential attractive agent to combine with sunitinib is ifosfamide, an alkylating agent with established activity against a similar wide range of tumour entities including breast cancer, testicular cancer, lung cancer, sarcomas, and central nervous system (CNS) tumours such as medulloblastomas.

This study aimed to define the MTD of ifosfamide combined with sunitinib. Two different infusion schedules of ifosfamide were explored. In addition, extensive PK and PD studies were conducted.

## PATIENTS AND METHODS

### Patient selection

Patients with histologically or cytologically confirmed advanced or metastatic solid tumours, for whom no standard therapy was available, with an Eastern Cooperative Oncology Group (ECOG) performance status  $<2$ , were eligible. Other inclusion criteria were evaluable or



measurable disease according to RECIST version 1,<sup>12</sup> age 18 years, life expectancy 12 weeks, adequate bone marrow (neutrophil count  $1.5 \times 10^9$  cells per L; platelets  $100 \times 10^9$  cells per L; and haemoglobin 6.0 mmol/L), liver (serum bilirubin 1.5 x upper limit of normal (ULN) and serum ASAT and ALAT 2.5 x ULN or, if liver metastases were present, 5 x ULN), and renal function (serum creatinin 1.5 x ULN and creatinine clearance 60 ml/min), two functioning kidneys, systolic blood pressure <150 mm Hg, and diastolic blood pressure <90 mm Hg (treatment with two anti-hypertensive drugs was allowed). Main exclusion criteria were history of cardiovascular disease, known HIV seropositivity, and signs or symptoms of CNS metastases.

The study was designed and conducted under the approval of appropriate institutional review boards (METC 2006–273 and CME 06–273) and in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from each participant.

### **Study design and drug dosing, escalation, and administration**

Daily oral sunitinib was planned to be evaluated in three dose cohorts, 12.5 mg, 25 mg, and 37.5 mg, in combination with a fixed dose of ifosfamide, according to one of the standard schedules of monotherapy ifosfamide: 9 g/m<sup>2</sup> administered as 3-day continuous intravenous infusion (CIV) at 3-weekly intervals. After establishing the MTD of sunitinib with this dose and schedule of ifosfamide, this sunitinib dose was evaluated with ifosfamide at 6 g/m<sup>2</sup> given as 5-day CIV. The latter ifosfamide schedule was chosen as in multidrug cytotoxic schedules; ifosfamide is frequently administered for 5 days, for example, in combination with cisplatin and etoposide. Additional patients were treated at the MTD of sunitinib with both ifosfamide regimens to get a better insight into the safety profile of the combination and to study PK drug-drug interactions. With regard to the latter, patients enrolled in these dose-expansion cohorts initiated sunitinib at day 8 of the first cycle instead of at day 1, which enables the investigation of the PK of sunitinib alone. On the basis of the mean half-life of sunitinib (40–60 h), it was anticipated that steady state of sunitinib levels was reached before initiating the second cycle of ifosfamide. Samples for PK evaluation were collected during the first two treatment cycles. After a protocol amendment because of prolonged neutropenia, granulocyte-colony stimulating factor (pegfilgrastim 6 mg once per cycle) was administered to all patients. Twelve days before the first administration of study treatment and throughout the whole study, concurrent treatment with known CYP3A4 inhibitors or inducers was not allowed.

Using the Common Terminology Criteria for adverse events (CTCAE), version 3.0, dose-limiting toxicity (DLT) was defined as the following toxicity during the first treatment cycle: grade 4 neutropenia 7 days, febrile neutropenia, grade 4 thrombocytopenia, serum creatinine 2 x ULN, and any drug-related grade 3 or 4 non-haematological toxicity excluding the following events: nausea and vomiting without optimal supportive care,

grade 3 fatigue <7 days, and hypertension not refractory to anti-hypertensive medication. If patients developed a systolic blood pressure >160 mm Hg, a diastolic blood pressure >100 mm Hg, or an increase in diastolic blood pressure >20 mm Hg, which (despite anti-hypertensive medication with an ACE inhibitor and a calcium-channel blocker) was not adequately controlled within 2 weeks, treatment with sunitinib was stopped. In case of grade 4 hypertension, sunitinib was also discontinued. A dose delay or interruption for longer than 2 weeks was also classified as DLT.

A classic 3+3 design was applied, implying that if a DLT was observed in one patient, three additional patients were recruited at that dose level, with the dose level escalating if no further DLT occurred at that level. If a DLT was observed in 2 patients in a cohort, it could be concluded that the MTD had been exceeded. MTD was defined as the highest dose level with a DLT incidence of <33%.

Before commencing each ifosfamide cycle, patients had to have neutrophils  $\times 1.5 \times 10^9$  cells per L and platelets  $100 \times 10^9$  cells per L. If a patient experienced an ifosfamide-related DLT, the dose of ifosfamide was reduced by 25%. A dose reduction of more than 50% of the initial ifosfamide dose was not allowed. In those patients experiencing a DLT related to sunitinib, sunitinib was withheld for a maximum of 2 weeks. If toxicity resolved to grade 0 or 1, continuation at the next lower dose cohort level was allowed for the subsequent courses. Patients were treated for a maximum of six ifosfamide cycles. Those patients who experienced a benefit from the combination of sunitinib and ifosfamide were allowed to continue treatment with sunitinib monotherapy. Treatment was continued until disease progression or unacceptable toxicity.

### PK sampling and analysis

In patients enrolled in the expansion cohorts, blood samples for PK analysis were collected.

For ifosfamide and its most important metabolites, 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide, and 4-hydroxy-ifosfamide blood samples were collected in the presence of lithium heparin as anti-coagulant before infusion and 3, 6, 10, and 24 h after the start of ifosfamide infusion, and thereafter every 12 h until the end of infusion, before the end of infusion and 1, 3, 6, 12, and 24 h after the end of infusion during the first two treatment cycles. Blood samples were centrifuged within 15 min after collection for 10 min at 3000 g at 4°C. Subsequently, an aliquot of exactly 1 ml of the plasma supernatant was transferred into a vial containing 100 µl of a 2M semicarbazide solution and was stored at <70°C until analysis of 4-hydroxy-ifosfamide. The remaining plasma was stored at <70°C, without any additive, until the simultaneous analysis of ifosfamide and its 2-dechloroethyl and 3-dechloroethyl metabolites. Ifosfamide and the 2-dechloroethyl and 3-dechloroethyl metabolites were simultaneously quantitated by a validated liquid chromatography tandem triple quadrupole mass spectrometry (LC-MS/MS) assay. Analytes were extracted by liquid-liquid extraction from 10 l aliquots of plasma with cyclofosfamide as internal standard. For



4-hydroxy-ifosfamide, a separate LC-MS/MS method was developed and validated. Aliquots of 50 µl of semicarbazide-stabilised plasma were extracted by liquid-liquid extraction with the same internal standard. Peak area ratios were a function of the concentration from 50.0 to 5000 ng/ml for all analytes, with the within and between-run precisions 4.9 and 5.2%, respectively, and the average accuracy ranging from 90.0 to 105.4%. Individual PK parameters for ifosfamide, 2-dechloroethyl-ifosfamide and 3-dechloroethylifosfamide, and 4-hydroxy-ifosfamide were estimated using noncompartmental analysis (1/y weighting factor) using the software programme WinNonLin 5.0 (Pharsight, Mountain View, CA, USA).

For the analysis of sunitinib and its active metabolite SU12662, blood samples were taken before dosing every 3–4 days and every day during the second ifosfamide cycle. Blood samples were centrifuged within 15 min after collection for 10 min at 3000 g at 4°C. The plasma was stored at <-70°C, in tubes wrapped with aluminium foil, until the simultaneous analysis of sunitinib and SU12662, as recently published.<sup>13</sup>

### **Statistical data analysis**

Statistical analysis, using software package SPSS (version 15 (Softonic International, San Francisco, CA, USA)), of the changes in sunitinib concentration was carried out by Wilcoxon signed ranks test using the pre-ifosfamide sunitinib concentration as comparator. Changes in ifosfamide concentration have been evaluated using the same test. Correlation of the auto-induction rate of ifosfamide and changes in sunitinib concentrations was carried out by the Pearson's correlation test.

### **Biomarker analysis**

Circulating endothelial cell (CEC) enumeration, considered to reflect vascular damage, was determined using the CellSearch system (Veridex, LCC, Raritan, NJ, USA).<sup>14</sup> Plasma concentrations of VEGF and soluble VEGFR2 (sVEGFR2) were determined using ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

## **RESULTS**

### **Dose escalation, MTD, and dose intensity**

In total, 32 patients were enrolled (Table 1). At the first dose level (sunitinib 12.5mg and ifosfamide 9 g/m<sup>2</sup> for 3 days), a DLT occurred in two out of six patients; both experienced a prolonged, uncomplicated neutropenia (>7 days). This was pre-specified as exceeding the MTD. The protocol was amended and subsequent patients were treated with pegfilgrastim. The first dose level was repeated and no DLTs were observed. However, in the subsequent 25mg sunitinib cohort, three DLTs occurred in five patients (two grade 4 febrile neutropenia; in one patient, hypertension accompanied by chest pain), indicating that the MTD was



exceeded, rendering 12.5mg sunitinib plus ifosfamide 9 g/m<sup>2</sup> for 3 days combined with pegfilgrastim to be the MTD. After confirming the tolerability at this dose level (in total, one DLT in nine patients: grade 3 febrile neutropenia), the safety of this sunitinib dose was tested with the second ifosfamide regimen (6 g/m<sup>2</sup> for 5 days), also supported with pegfilgrastim. In nine patients, one DLT was observed (grade 3 ifosfamide-induced encephalopathy).

The dose intensities of sunitinib at the MTD were 93 and 98% during combination treatment with the 3-day and 5-day schedule, respectively. A median of 4 and 3.5 cycles of ifosfamide was administered, resulting in an ifosfamide dose intensity of 93 and 96% for the 3-day and 5-day schedule, respectively.

**Table 1 |** Demographics and baseline characteristics (in numbers (%) if not otherwise specified)

<b>Age</b> (median, years)	53 (range 29–74)
<b>Gender</b>	
Male	16 (50)
Female	16 (50)
<b>WHO performance status</b>	
0	11 (34)
1	21 (66)
<b>Tumour type</b>	
Sarcoma	15 (47)
Chondrosarcoma	2 (6)
Leiomyosarcoma	2 (6)
Liposarcoma	2 (6)
Ewing sarcoma	2 (6)
Pleomorphic sarcoma	2 (6)
Other sarcoma types	5 (16)
Carcinoma of unknown primary tumour	3 (9)
Neuroendocrine carcinoma	2 (6)
Non-small-cell lung cancer	2 (6)
Melanoma (uveal and mucosal)	2 (6)
Miscellaneous <sup>a</sup>	8 (26)
<b>Previous non-hormonal systemic anticancer treatment</b>	
0	5 (16)
1	18 (56)
2	5 (16)
3	2 (6)
4	2 (6)

Abbreviation: WHO, World Health Organisation. <sup>a</sup>Bile duct carcinoma, breast cancer, adenoid cystic carcinoma, endometrial cancer, urothelial cancer, pancreatic cancer, chordoma and cervical carcinoma; for all the cases n, 1.

## Toxicity

The non-haematological toxicity of sunitinib and ifosfamide was mainly grade 1–2 toxicity. Haematological toxicity was more pronounced, as grade 3–4 neutropenia was observed in 19 patients (59%), resulting in one or more episodes of febrile neutropenia in 7 patients (22%) (Table 2). At the MTD, in almost every patient in the 3-day schedule (eight out of nine; 89%), grade 3–4 neutropenia, and in four patients febrile neutropenia, occurred during combination therapy, whereas only three out nine (33%) patients treated with the 5-day schedule had grade 3–4 neutropenia, and no episodes of febrile neutropenia were observed.

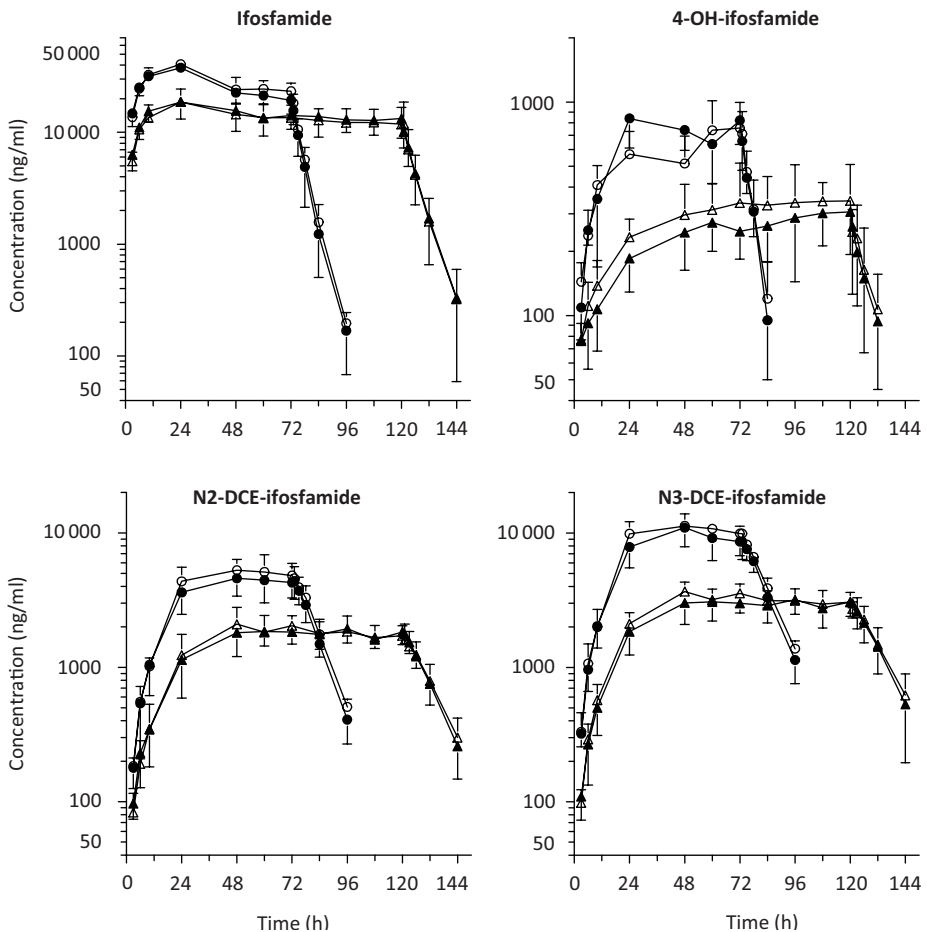
**Table 2** | Adverse events during combination therapy of sunitinib and ifosfamide (number of patients (%))

	First cycle adverse events		Adverse events at all combination cycles	
	All grades	Grade 3/4	All grades	Grade 3/4
<b>Haematological toxicity</b>				
Anaemia	28	0	31	2
Thrombocytopenia	19	4	22	9
Neutropenia	14	10	21	19
Febrile neutropenia	3	3	7	7
<b>Non-haematological toxicity</b>				
Fatigue	24	2	29	4
Nausea	20	0	24	0
Constipation	20	0	24	0
ALAT and/or ASAT	20	0	21	0
Alopecia	19	0	26	0
Vomiting	14	0	19	0
Creatinine/renal	9	0	12	1
Hypo-/hyperkalaemia	5/1	1/0	5/1	2/0
Bilirubin	5	0	6	0
Diarrhoea	4	0	5	0
Pyrosis	3	0	5	0
TSH increase/decrease	2/2	0	2/2	0
Neurotoxicity	2	1	8	1
Anorexia	2	0	11	1
Haemorrhage	1	1	1	1

ALAT, alanine transaminase; ASAT, aspartate aminotransferase; TSH, thyroid-stimulating hormone.

## Pharmacokinetics

Pharmacokinetic data of sunitinib and ifosfamide were obtained in six patients per ifosfamide schedule at the MTD. Plasma concentration time curves of ifosfamide, 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide, and 4-hydroxy-ifosfamide were not affected by sunitinib (Figure 1). Ifosfamide concentrations were significantly lower at 48 h ( $C_{48h}$ ) compared with 24 h ( $C_{24h}$ ) in the 3-day and 5-day schedule (40% and 17%, respectively; both  $P=0.028$ ), in line with the known ifosfamide capacity to induce its own CYP3A-mediated metabolism.<sup>15</sup>



**Figure 1** | Mean concentrations (plus s.d.) of ifosfamide, 4-hydroxy-ifosfamide (4-OH-ifosfamide), 2-dechloroethyl-ifosfamide (N2-DCE-ifosfamide), and 3-dechloroethyl-ifosfamide (N3-DCE-ifosfamide) administered alone (open symbols: bars up) or in combination with sunitinib (closed symbols, bars down) during the 3-day (open and closed circles) and 5-day (open and closed triangles) continuous infusions.

Sunitinib trough concentrations decreased during ifosfamide infusions during the 3-day and 5-day schedule (Figure 2 upper graphs). As presented in Figure 3 (upper panel), trough concentrations were significantly lower at 1, 2, and 3 days ( $P < 0.05$ ) after the start of ifosfamide infusion in the 3-day schedule, whereas during the 5-day schedule, the decrease was less pronounced, not reaching statistical significance. The decrease in sunitinib was paralleled by an increase in the trough concentrations of its pharmacologically active metabolite SU12662 (Figure 2, lower graphs), which reached statistical significance in both the 3-day and 5-day ifosfamide schedules (Figure 3, middle panel). However, the sum of sunitinib and its pharmacologically active metabolite, SU12662, slightly decreased during the ifosfamide infusions, reaching significance only 1 day after the start of ifosfamide infusion in the 3-day schedule ( $P < 0.05$ ) (Figure 3, lower panel).

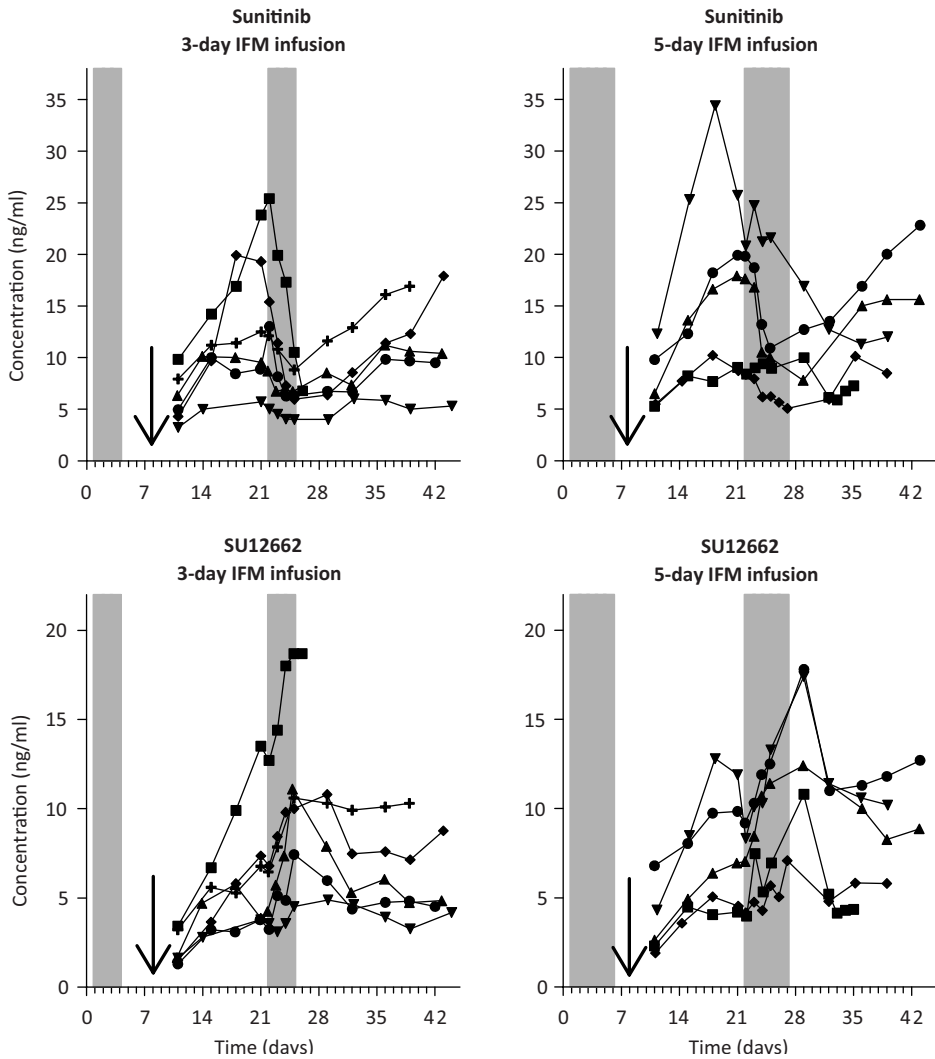
The auto-induction rate of ifosfamide, expressed as  $C_{48h} / C_{24h}$ , was correlated to the decrease in sunitinib trough concentrations, expressed as  $C_{day2} / C_{day0}$  ( $R^2 = 0.47$ ;  $P = 0.019$ ) (Figure 4).

### Biomarker analysis

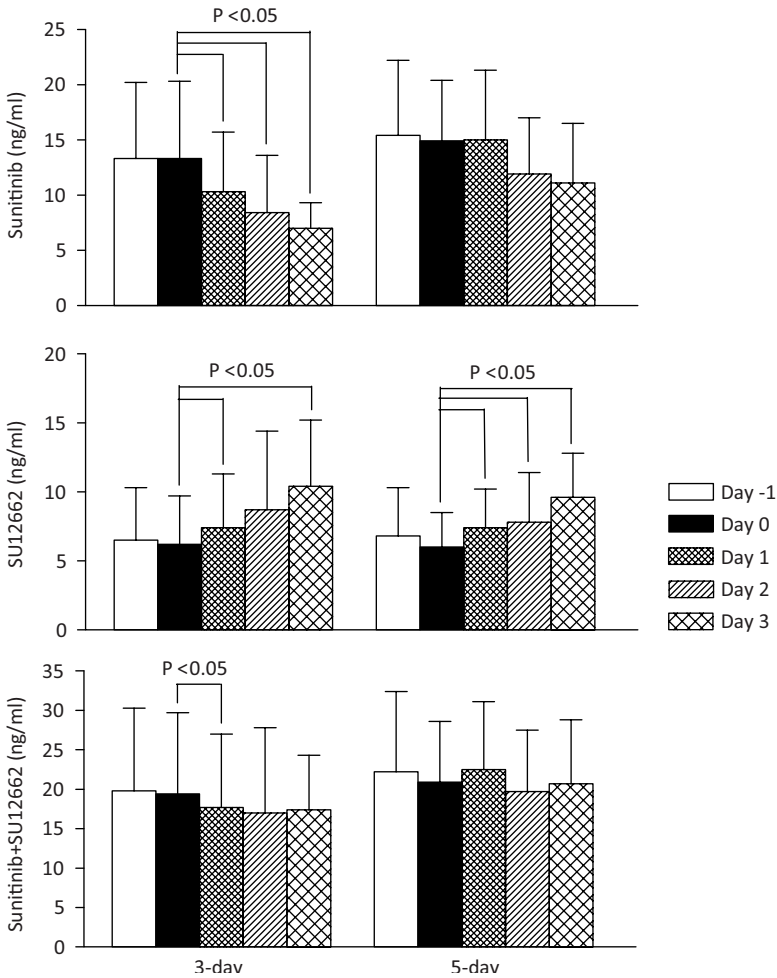
Circulating endothelial cell enumeration and determination of plasma concentrations of VEGF and sVEGFR2 with serial sampling were successful in 22 patients. A wide range in relative changes in the number of CECs from baseline to post-cycle 2 (0.05–35.2) and post-cycle 5 (0.09–5.2) was observed. Relative changes from baseline to post-cycle 2 and post-cycle 5 were widespread for VEGF (0.04–14.1 and 0.25–5.6, respectively) and less outspoken for sVEGFR2 (0.63–1.4 and 0.46–1.1, respectively). No consistent change in time, correlation with dose levels, with the occurrence of febrile neutropenia, or tumour response was observed for VEGF or sVEGFR2 plasma concentrations or number of CECs.

### Anti-tumour activity

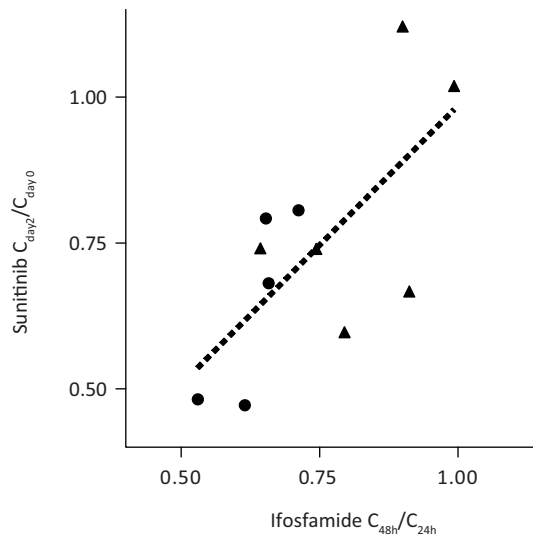
All 32 patients were assessable for efficacy. Partial responses were seen during the 3-day schedule in three patients (one patient with adenocarcinoma of unknown primary, one patient with a sarcoma NOS, and one patient with a liposarcoma). Disease stabilisation was observed in 17 patients in whom a prolonged  $> 3$  months) disease stabilisation was observed in 9 patients (7 out of 23 patients during the 3-day schedule and 2 out of 9 patients during the 5-day schedule). The latter group consisted of patients with uveal melanoma, chordoma, chondrosarcoma, Ewing sarcoma, uterus sarcoma, endometrial carcinoma, small-cell neuroendocrine carcinoma, non-small-cell lung cancer, and carcinoma of unknown primary (all entities  $n = 1$ ).



**Figure 2** | Individual plasma concentration time curves of sunitinib in combination with 3-day ( $n=6$ ) and 5-day ( $n=5$ ) ifosfamide schedule (upper graphs) and of SU12662 in combination with 3-day ( $n=6$ ) and 5-day ( $n=5$ ) ifosfamide schedule (lower graphs). One patient in the 5-day schedule has not been included in the figure as cycle two was delayed by 2 weeks. Bars represent the ifosfamide infusion schedules and arrows the first sunitinib administrations.



**Figure 3** | Absolute concentration of sunitinib (upper panel), SU12662 (middle panel), and the sum of sunitinib plus SU12662 (lower panel) in patients after the 3-day and 5-day continuous infusions of ifosfamide. P-values (Wilcoxon signed rank test) of significant lower or higher relative trough concentrations compared with the trough concentrations observed before the start of ifosfamide infusions (that is, day 0) are presented. Day – 1 is the sample taken the day before the start of ifosfamide infusion and days 1, 2, and 3 are the samples taken 24, 48, and 72 h after the start of ifosfamide infusions, respectively. Data are presented as the mean±s.d. of six observations;  $n=5$  for day 2 in the 3-day schedule.



**Figure 4** | Linear relationship ( $R^2=0.47$ ;  $P=0.019$ ) between auto-induction of ifosfamide and decrease in sunitinib trough concentrations during the 3-day (closed red circles) and 5-day (closed blue triangles) ifosfamide infusions.

## DISCUSSION

This is one of the first full reports on a combination of sunitinib with a conventional cytotoxic agent and the first on the combination of ifosfamide with a VEGFR-inhibiting TKI. Combined with ifosfamide at  $9 \text{ g/m}^2$  given continuously over 3 days, it seemed that the MTD of sunitinib is mainly determined by neutropenia-related events. With growth factor support, sunitinib at the lowest evaluated dose of  $12.5 \text{ mg}$  daily was feasible in combination with ifosfamide at  $9 \text{ g/m}^2$  for 3 days and at  $6 \text{ g/m}^2$  for 5 days. Importantly, our data on the occurrence of neutropenia-related adverse events should be appreciated in the context of toxicity from ifosfamide monotherapy. Ifosfamide given at  $9 \text{ g/m}^2$  for 3 days, as an established treatment schedule, has an observed incidence in a phase III trial of 62.7% grade 3–4 neutropenia when given as first-line therapy. In addition, during a mean number 3.7 cycles of therapy, 19.6% of the patients encountered febrile neutropenia.<sup>16</sup> Furthermore, sunitinib administered as a single agent produces grade 3–4 neutropenia in 12% of patients.<sup>2</sup> Given these figures, it comes as no surprise that neutropenia-related adverse events were the determining toxicity for the MTD of the combination. On the basis of the findings in this study, it is clear that the occurrence of neutropenia-related events is not attributable to changes in drug exposure. Recently, several trials on sunitinib combined with conventional cytotoxic agents have been reported, although only in abstract form. The majority of the tested combinations

also yielded a high incidence of neutropenia-related events, which were frequently dose-limiting toxicities. This held true for the combinations of sunitinib with docetaxel, irinotecan, FOLFIRI, carboplatin/ paclitaxel, or gemcitabine/cisplatin, preventing sunitinib from being administered at full single-agent.<sup>17-25</sup> The only two combinations not hampered by neutropenia-related adverse events were sunitinib with capecitabine or with gemcitabine.<sup>26,27</sup>

What becomes apparent from these studies is the fact that the schedule in which sunitinib is administered is likely to largely impact the tolerability of combinations in terms of neutropenia-related events. The recommended dose of sunitinib as a single agent was initially reported as 50 mg administered daily for 28 days every 6 weeks. However, we choose to administer sunitinib continuously. Evidence accumulates that persistent inhibition of the VEGF pathway might be advantageous over intermittent dosing, although both dosing schedules have not been directly compared yet. For combinations, however, toxicity might be more pronounced using sunitinib continuously rather than intermittently, as recovery from toxicity induced by the cytotoxic agent might be hampered in the presence of sunitinib. Accordingly, preliminary data on the combination of sunitinib with FOLFIRI suggest that continuous dosing of sunitinib was not feasible because of neutropenia, whereas in contrast, sunitinib administered in a 4-week on, 2-week off schedule could be applied at a dose of 37.5 mg in combination with FOLFIRI.<sup>22</sup> From a mechanism of action point of view, the least desirable administration schedule of sunitinib might be best combinable with chemotherapy. In contrast, sunitinib continuously administered, as well as administered in the 4-week on 2-week off schedule in combination with capecitabine, was tolerated at the same sunitinib dose (37.5 mg per day); however, as mentioned before, neutropenia was not a major issue in that specific combination.<sup>27</sup>

Besides the neutropenia-related adverse events, the combination of (a relatively low dose of) sunitinib, using continuous administration, and ifosfamide was well tolerated (Table 2).

Assessing the PK of both drugs, a clear influence of ifosfamide on the PK of sunitinib was observed. This interaction resulted in a decreased systemic exposure to sunitinib and an increased exposure to its active metabolite, SU12662. Although the exact contribution of the active metabolite SU12662 to the toxicity and efficacy pattern of sunitinib in humans is unknown, preclinical data point towards equipotent inhibitory capacities.<sup>28</sup> Ifosfamide is a potent inhibitor of CYP3A, the enzyme mainly responsible for conversion of sunitinib into SU12662.<sup>29</sup> This observed drug-drug interaction is in line with findings in healthy subjects showing a decreased systemic exposure to sunitinib when concomitantly treated with the potent CYP3A inducer rifampicin<sup>30</sup> and an increased exposure in the presence of ketoconazole, a potent CYP3A inhibitor.<sup>31</sup> Pharmacokinetic drug-drug interactions have been studied combining sunitinib with gemcitabine, capecitabine, paclitaxel, and



docetaxel with or without trastuzumab, but no relevant PK interactions were observed in these studies.<sup>19,21,26,27</sup>

As a relatively low dose of sunitinib in combination with a standard dose of ifosfamide already results in DLT, which cannot be explained by PK interactions, synergy in toxicity seems most likely. An explanatory hypothesis for the occurrence of the described neutropenia-related events while combining ifosfamide with sunitinib might consist of a dual hit. Initially, neutrophils decrease because of the administration of ifosfamide. The physiological response of mobilisation and proliferation of haematopoietic progenitor cells to restore the neutrophil count is hampered because of inhibition by sunitinib of tyrosine kinases involved in haematopoietic progenitor cell survival and proliferation, such as Flt3 and colony-stimulating factor receptor (CSF-1R). Importantly, a multiple drug combination should not be discarded as a result of its low combinability because of synergy in toxicity alone, as this synergistic interaction may also occur at tumour cell level.

As PD parameters, CEC numbers and plasma concentrations of VEGF and sVEGFR2 were assessed. Alterations in CEC numbers is likely to reflect vascular damage,<sup>32</sup> but no consistent changes in CEC numbers could be seen, either during therapy or between the two sunitinib doses explored. In contrast to monotherapy with sunitinib,<sup>33</sup> no consistent pattern in the changes in plasma concentrations of VEGF and sVEGFR2 was observed.

In conclusion, the MTD of daily sunitinib combined with continuously infused ifosfamide (9 g/m<sup>2</sup> for 3 days) supported by pegfilgrastim is 12.5 mg. The same dose of sunitinib is feasible using an ifosfamide schedule of 5 days (total dose per cycle 6 g/m<sup>2</sup>). Concomitant treatment with ifosfamide significantly decreased the systemic exposure to sunitinib, whereas the exposure to its active metabolite, SU12662, increased because of CYP3A induction. As PK interactions cannot explain the fact that ifosfamide can be combined safely only with relatively low sunitinib doses, synergy in toxicity is likely. Whether this holds true for anti-tumour activity needs to be determined, and it is particularly attractive to explore this further in tumour types against which both sunitinib and ifosfamide as monotherapy exhibit anti-tumour activity, such as soft tissue sarcomas, as well as lung and breast cancer.



## REFERENCES

1. Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal tumours with high-dose imatinib: randomised trial. *Lancet* 2004; **364**:1127-1134.
2. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**:1115-124.
3. Boucher Y, Jain RK. Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse. *Cancer Res* 1992; **52**:5110-5114.
4. Heldin CH, Rubin K, Pietras K, et al. High interstitial fluid pressure – an obstacle in cancer therapy. *Nat Rev Cancer* 2004; **4**:806-813.
5. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**:145-147.
6. Simakajornboon N, Szerlip NJ, Gozal E, et al. In vivo PDGF beta receptor activation in the dorsocaudal brainstem of the rat prevents hypoxia-induced apoptosis via activation of Akt and BAD. *Brain Res* 2001; **895**:111-118.
7. Tran J, Master Z, Yu JL, et al. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. *Proc Natl Acad Sci USA* 2002; **99**:4349-4354.
8. Riedel F, Götte K, Goessler U, et al. Targeting chemotherapy-induced VEGF up-regulation by VEGF antisense oligonucleotides in HNSCC cell lines. *Anticancer Res* 2004; **24**:2179-2183.
9. Shaked Y, Henke E, Roodhart JM, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: implications for antiangiogenic drugs as chemosensitizing agents. *Cancer Cell* 2008; **14**:263-273.
10. Dias S, Shmelkov SV, Lam G, et al. VEGF(1 promotes survival of leukemic cells by Hsp90-mediated induction of Bcl-2 expression and apoptosis inhibition. *Blood* 2002; **99**:2532-2540.
11. Demetri GD, van Oosterom AT, Garrett CR, et al. Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. *Lancet* 2006; **368**:1329-1338.
12. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**:205-216.
13. De Bruijn P, Sleijfer S, Lam MH, et al. Bioanalytical method for the quantification of sunitinib and its n-desethyl metabolite SU12662 in human plasma by ultra performance liquid chromatography/tandem triple-quadrupole mass spectrometry. *J Pharm Biomed Anal* 2010; **51**:934-941.
14. Rowand JL, Martin G, Doyle GV, et al. Endothelial cells in peripheral blood of healthy subjects and patients with metastatic carcinomas. *Cytometry A* 2007; **71**:105-113.
15. Boddy AV, Cole M, Pearson AD, et al. The kinetics of the auto-induction of ifosfamide metabolism during continuous infusion. *Cancer Chemother Pharmacol* 1995; **36**:53-60.
16. Lorigan P, Verweij J, Papai Z, et al. Phase III trial of two investigational schedules of ifosfamide compared with standard-dose doxorubicin in advanced or metastatic soft tissue sarcoma: a European Organisation for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Study. *J Clin Oncol* 2007; **25**:3144-3150.
17. Reck M, Frickhofen N, Gatzemeier U, et al. A phase I dose escalation study of sunitinib in combination with gemcitabine + cisplatin for advanced non-small cell lung cancer (NSCLC). *J Clin Oncol* 2007; **25**:18s.
18. Robert F, Sandler A, Schiller JH, et al. A phase I dose-escalation and pharmacokinetic study of sunitinib plus docetaxel in patients with advanced solid tumors. *J Clin Oncol* 2007; **25**:18s.
19. Mariani G, Cardoso F, Besse-Hammer T, et al. Sequential administration of sunitinib and docetaxel in women with advanced breast cancer: an exploratory evaluation. *J Clin Oncol* 2008; **26**:18s.
20. Boven E, Massard C, Armand JP, et al. Phase I, dose-finding study of sunitinib in combination with irinotecan in patients with advanced solid tumors. *Ann Oncol* 2008; **19**:viii156.

21. Cardoso F, Canon JL, Amadori D, et al. Sunitinib plus docetaxel and trastuzumab as first-line therapy for HER2+ advanced breast cancer. *Eur J Cancer* 2009 Suppl 7:283 .
22. Starling N, Vazquez F, Cunningham D, et al. Phase I study to evaluate the safety and efficacy of sunitinib in combination with FOLFIRI in treatment-naïve metastatic colorectal cancer (mCRC). *J Clin Oncol* 2008; **26**:18s.
23. Zurita AJ, Liu G, Hutson T, et al. Sunitinib in combination with docetaxel and prednisone in patients with metastatic hormone-refractory prostate cancer. *J Clin Oncol* 2009; **27**:15s.
24. Friedman HS, Vredenburgh JJ, Desjardins A, et al. A phase I study of sunitinib plus irinotecan in the treatment of patients with recurrent glioma. *J Clin Oncol* 2009; **27**:e13024.
25. Heath EI, Blumenschein GR, Cohen RB, Sunitinib in combination with paclitaxel and carboplatin in patients with advanced solid tumors; updated phase I study results. *J Clin Oncol* 2009; **27**:e14509.
26. Michaelson MD, Schwarzbach A, Ryan DP, et al. A phase I dose-finding study of sunitinib (SU) in combination with gemcitabine (G) in patients (pts) with advanced solid tumors. *Ann Oncol* 2008; **19**: viii156.
27. Royce M, Chiorean EG, Sweeney C, et al. A phase I dose-finding study of sunitinib in combination with capecitabine in patients with advanced solid tumors. *Breast Cancer Symposium Proceedings* 2008: **169**.
28. Baratte S, Sarati S, Frigerio E, et al. Quantitation of SU11248, an oral multi-target tyrosine kinase inhibitor, and its metabolite in monkey tissues by liquid chromatography with tandem mass spectrometry following semi-automated liquid-liquid extraction. *J Chromatogr A* 2004; **1024**:87-94.
29. Faivre S, Delbaldo C, Vera K, R et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**:25-35.
30. Bello C, Houk B, Sherman L, et al. Effect of rifampin on the pharmacokinetics of SU11248 in healthy volunteers. *J Clin Oncol* 2005; **23**:3078.
31. Washington C, Eli M, Bello C, et al. The effect of ketoconazole, a potent CYP3A4 inhibitor, on SU011248 pharmacokinetics in Caucasian and Asian Healthy subjects. *J Clin Oncol* 2003; **22**:553.
32. Strijbos MH, Gratama JW, Kraan J, et al. Circulating endothelial cells in oncology: pitfalls and promises. *Br J Cancer* 2008; **98**:1731-1735.
33. Deprimo SE, Bello CL, Smeraglia J, et al. Circulating protein biomarkers of pharmacodynamic activity of sunitinib in patients with metastatic renal cell carcinoma: modulation of VEGF and VEGF-related proteins. *J Transl Med* 2007; **5**:32.





Chapter

# 5

## **(Pre-)Clinical pharmacology and activity of pazopanib, a novel multikinase angiogenesis inhibitor**

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## ABSTRACT

Pazopanib is a recently approved, novel tyrosine kinase inhibitor specifically designed to impair angiogenesis by abrogating vascular endothelial growth factor receptor 2 (VEGFR-2) to exert its function. Pazopanib inhibits VEGF-induced endothelial cell proliferation *in vitro* and angiogenesis *in vivo* and demonstrates antitumor activity in mouse models. Furthermore, the pazopanib concentration resulting in maximal inhibition of VEGFR-2 phosphorylation *in vivo* was in line with the steady-state concentration required to inhibit growth of tumor xenografts, suggesting that pazopanib's mechanism of action is indeed through VEGFR-2 inhibition.

In a phase I trial, a generally well-tolerated dose was identified at which the majority of patients achieved pazopanib plasma concentrations above the concentration required for maximal *in vivo* inhibition of VEGFR-2 phosphorylation in preclinical models. Administered as monotherapy, evidence of antitumor activity was observed in phase II studies in several tumor types, including soft tissue sarcoma, renal cell cancer (RCC), ovarian cancer, and non-small cell lung cancer (NSCLC). Recently, the U.S. Food and Drug Administration granted approval for treatment with pazopanib in patients with RCC based on the longer progression-free survival time observed with this agent in a placebo-controlled, randomized trial. This review summarizes the preclinical and clinical pharmacokinetics and pharmacodynamics of pazopanib, as well as data on clinical activity, that ultimately resulted in its recent approval.

## INTRODUCTION

The advent of tyrosine kinase inhibitors (TKIs) has considerably changed the daily practice of oncology. This type of anticancer agent can be classified within the larger group of the so-called cancer(-cell)-specific therapies, and several of these compounds also target infiltrating host cells supporting tumor growth, such as endothelial cells and fibroblasts. Over the last several years, major advances have been made in elucidating the pathogenesis of tumor growth and metastasis. This has resulted in the identification of numerous tumor growth-driving factors, such as VEGFR-2 and platelet-derived growth factor receptor (PDGFR). By inhibiting the activity of these factors, it was aimed to specifically intervene in tumor pathogenesis and to avoid untoward effects on normal cells.

After the introduction of imatinib, the first TKI used in solid tumors, the therapeutic armamentarium in solid malignancies was expanded by registration of several other TKIs. These include the epidermal growth factor receptor (EGFR) inhibitors erlotinib and gefitinib, the dual EGFR and human epidermal growth factor receptor (HER)-2 inhibitor lapatinib, and the VEGFR inhibitors sunitinib, sorafenib, and, recently, pazopanib. Importantly, none of the TKIs are entirely specific for one target. In particular, the VEGFR inhibitors target a wide spectrum of kinases, including the PDGFR and fibroblast growth factor receptor (FGFR). Additionally, sorafenib is also a strong Raf inhibitor.

In addition to different mechanisms of action with regard to antitumor activity, TKIs are also characterized by a toxicity pattern that is substantially different from that of conventional cytotoxic agents. Furthermore, the need to administer these agents more or less continuously necessitates a different assessment of tolerability than with classic cytotoxic drugs. Compliance with anticancer therapy, and therefore its success, is, to a large extent, determined by its toxicity and tolerance. Therefore, getting insight into the mechanisms accounting for TKI-mediated toxicity and its manageability is of great importance.

Pazopanib (GW786034), a synthetic indazolympyrimidine, is a novel multitargeted TKI targeting several tumor and tumor environment factors thought to play an important role in a broad spectrum of tumor types. The first outcomes of several phase II studies have been reported, suggesting antitumor activity in patients with diverse tumor types and showing that pazopanib is generally well tolerated. Recently, the first phase III data became available, resulting in approval of this agent by the U.S. Food and Drug Administration (FDA) for the treatment of patients with RCC. Currently, phase III trials in other tumor entities are ongoing. As described, multiple kinases are inhibited by pazopanib. However, the observation that bevacizumab, a pure VEGF inhibitor, also has activity in patients with RCC strongly suggests that pazopanib's mechanism of action in RCC is largely through VEGFR-2 inhibition. Importantly, it is conceivable that its antitumor effect in other types of cancer depends on inhibition of receptors other than VEGFR-2.



This review summarizes the preclinical and clinical pharmacokinetics (PK) and pharmacodynamics of pazopanib as well as data on its clinical activity.

## PRECLINICAL DATA

### Mechanism of Action

Angiogenesis plays a critical role in the progression of solid malignancies from tumor volumes as small as 1–2 mm<sup>3</sup>.<sup>1</sup> Numerous proangiogenic factors are involved in this process, with the VEGF family being the most important. The human VEGF family consists of VEGF-A (referred to as VEGF), VEGF-B, VEGF-C, VEGF-D, and placenta growth factor. Members of the VEGF family bind to the cell surface receptors VEGFR-1, VEGFR-2, and VEGFR-3 on endothelial cells to initiate cellular signaling. Of these, VEGFR-2 is the primary tyrosine kinase receptor mediating VEGF signaling.

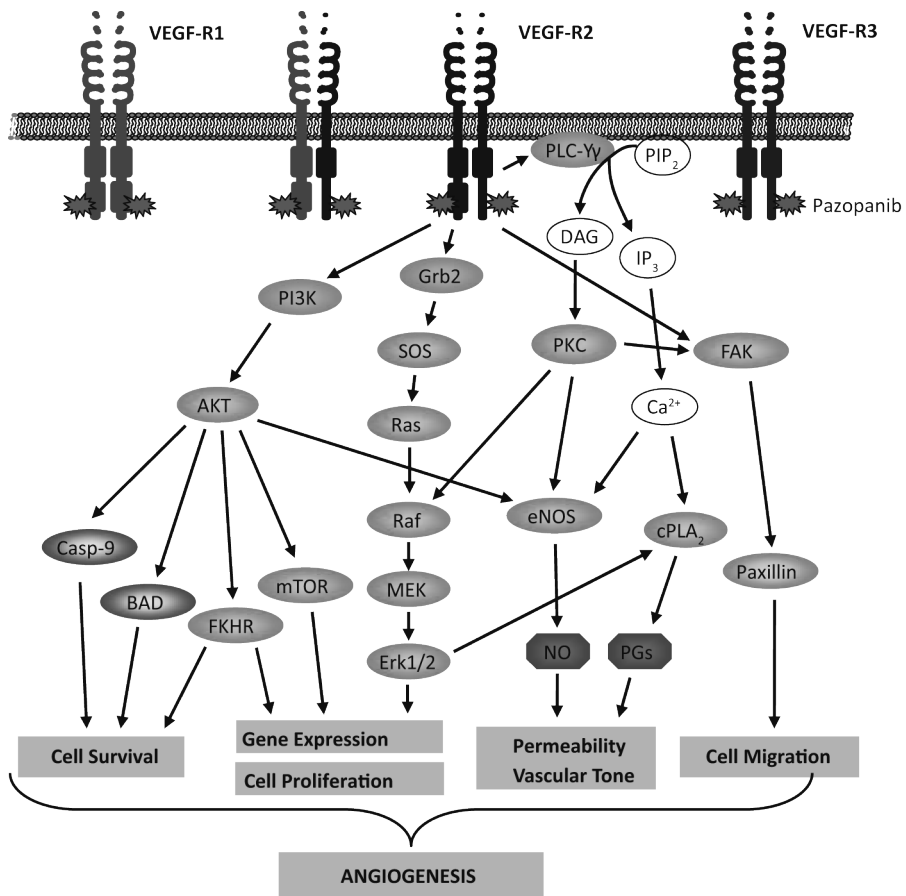
Because of its central role in angiogenesis, VEGF is considered a pivotal factor in the pathogenesis of many tumor types. Increased expression of VEGF has been found in many tumor types, including breast cancer (BC), colorectal cancer (CRC), and lung cancer, and is associated with a poor prognosis and response to therapy.<sup>2,3</sup> Moreover, inhibition of the VEGF pathway has been demonstrated to exhibit antitumor activity in clinical studies in a wide range of tumor types, including RCC, CRC, NSCLC, and BC.<sup>4-7</sup>

Under physiological conditions, VEGFR is only activated by ligand binding. Subsequently, ATP is recruited and binds in the so-called ATP-binding pocket of the tyrosine kinase region of the receptor. This is followed by the transfer of a phosphate group from ATP to VEGFR itself and to various other substrates in a process called phosphorylation. Through phosphorylation, downstream signaling pathways become activated, ultimately resulting in cellular effects, including proliferation of endothelial cells and recruitment of endothelial progenitor cells derived from the bone marrow, both of which are pivotal for angiogenesis.

Inhibition of VEGF-VEGFR driven processes can be achieved by several approaches. Monoclonal antibodies either target the extracellular domain of the VEGFR or trap VEGF, thereby preventing VEGF binding to VEGFRs. Another mechanism is through TKIs competitively binding to the ATP-binding pocket of the intracellularly located tyrosine kinase domain of VEGFR. Consequently, the binding of ATP to VEGFR is hampered, resulting in inhibition of the signal transduction from VEGFR.

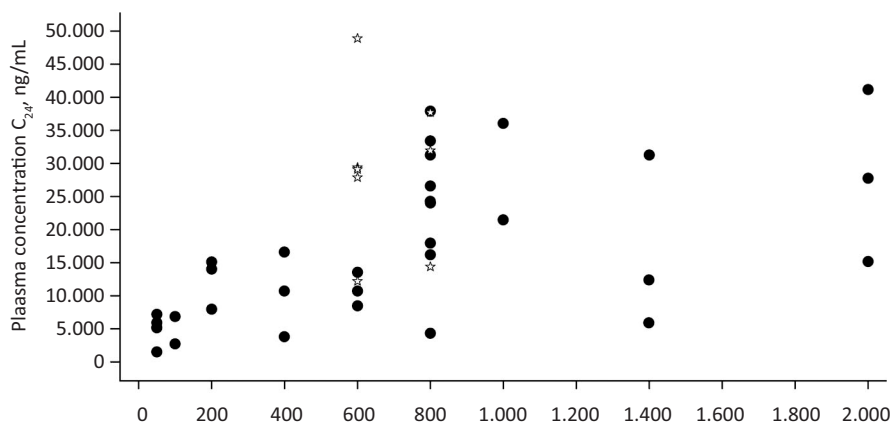
Pazopanib is a TKI designed to inhibit angiogenesis by abrogating VEGFR-2 function (Figure 1). A widely used parameter reflecting the inhibitory effects of a drug on the kinase activity of a certain factor is the inhibition of the autophosphorylation of that factor *in vitro*. The pazopanib concentration required to produce 50% inhibition (IC<sub>50</sub>) of human VEGFR-2 kinase activity is 0.03 μM (Table 1).<sup>8</sup>





**Figure 1 | VEGFR-2 downstream pathway.** By binding to the intracellular domain of VEGFR-2, pazopanib abrogates this pathway.

Abbreviations: BAD, Bcl-2-associated death promoter; Casp-9, caspase 9; cPLA<sub>2</sub>, cytosolic phospholipases A<sub>2</sub>; DAG, diacylglycerol; eNOS, endothelial nitric oxide synthase; Erk, extracellular signal-related kinase; FAK, focal adhesion kinase; FKHR, forkhead box O1; Grb2, growth factor receptor-bound protein 2; IP<sub>3</sub>, inositol 1,4,5-trisphosphate; MEK, mitogen-activated protein kinase/extracellular signal-related kinase; mTOR, mammalian target of rapamycin; PG, prostaglandin; PIP<sub>2</sub>, phosphatidylinositol-bisphosphate; PI<sub>3</sub>, phosphoinositide 3-kinase; PKC, protein kinase C; PLC-γ, phospholipase C γ; SOS, son of sevenless; VEGFR, vascular endothelial growth factor receptor.



**Figure 2** | Steady-state exposure to pazopanib in the individual patients in the phase I study. Reprinted from Hurwitz HI, Dowlati A, Saini S et al. Phase I trial of pazopanib in patients with advanced cancer. *Clin Cancer Res* 2009;15:4220-4227, with permission of the American Association for Cancer Research.

In comparison, sorafenib and sunitinib, other TKIs inhibiting VEGFR-2, have IC<sub>50</sub> values of 0.09 and 0.009  $\mu\text{M}$  for inhibiting VEGFR-2 activity, respectively.<sup>9,10</sup> Furthermore, pazopanib inhibited VEGF-induced proliferation in a human umbilical vein endothelial cell (HUVEC) culture in vitro (IC<sub>50</sub>, 0.02  $\mu\text{M}$ ). As observed with other TKIs, pazopanib is not entirely specific for one target. Besides VEGFR-2, comparable inhibitory effects were found against VEGFR-1, VEGFR-3, PDGFR- $\alpha$ , PDGFR- $\beta$ , and *c-Kit* (Table 1).<sup>8</sup> Pazopanib inhibition of VEGF-induced proliferation of HUVECs in vitro was more pronounced than that of basic FGF-induced HUVEC proliferation (IC<sub>50</sub>, 0.72  $\mu\text{M}$ ). Furthermore, VEGF-induced as well as basic FGF-induced angiogenesis in a mouse corneal micropocket model was impaired by pazopanib, although the inhibition was more pronounced when VEGF was used as the stimulant.<sup>8</sup>

### Preclinical PK Data

Pazopanib is orally available, with 49% bioavailability in dogs.<sup>11</sup> In mice, the level needed for maximal inhibition of VEGFR-2 phosphorylation occurs in vivo at approximately 40  $\mu\text{M}$ .<sup>8</sup> The discrepancy between in vivo and in vitro requirements can be attributed to >99.9% protein binding for pazopanib. PK analysis showed that tumor growth inhibition in a xenograft model using a CRC cell line is correlated with the steady-state concentration ( $C_{\text{trough}}$ ) and not with the peak plasma concentration ( $C_{\text{max}}$ ). In addition, the  $C_{\text{trough}}$  required for in vivo inhibition of tumor growth in a xenograft model was almost equivalent to the concentration required for in vivo inhibition of VEGFR-2 phosphorylation (approximately 40  $\mu\text{M}$ ), suggesting that the drug concentration of pazopanib required for in vivo VEGFR-2

inhibition can predict the PK requirements for in vivo antitumor activity of pazopanib.<sup>8</sup> In mice, a single dose of 30 mg/kg resulted in plasma concentrations >40  $\mu\text{M}$  for >8 hours.<sup>8</sup>

**Table 1** | IC<sub>50</sub> of the indicated enzyme activity in a cell-free assay

Enzyme	Kinase IC <sub>50</sub> ( $\mu\text{M}$ )
VEGFR-1	0.010
VEGFR-2	0.030
VEGFR-3	0.047
PDGFR- $\alpha$	0.071
PDGFR- $\beta$	0.084
c-Kit	0.074
FGFR-1	0.14
FGFR-3	0.13
FGFR-4	0.8
c-Fms	0.146

Abbreviations: c-Fms, colony-stimulating factor receptor; FGFR, fibroblast growth factor receptor; IC<sub>50</sub>, 50% inhibitory concentration; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

Adapted from Kumar R, Knick VB, Rudolph SK et al. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther* 2007;6:2012-2021, with permission of the American Association for Cancer Research.

## Preclinical Efficacy and Toxicity

The antitumor activity of pazopanib was demonstrated in several human tumor xenograft models in mice, most prominently in an RCC model but also in CRC, NSCLC, and multiple myeloma (MM) models. Less potent inhibition was observed in melanoma, BC, and prostate cancer models.<sup>8,12</sup> Sunitinib and sorafenib, as antiangiogenic class members of pazopanib, do share preclinical activity in several tumor types, including RCC, thyroid cancer, pancreatic cancer, and hepatocellular cancer (HCC). Furthermore, sorafenib has activity in MM, melanoma, and osteosarcoma, whereas sunitinib also exerts antitumor activity in small cell lung cancer, urothelial cancer, and acute myeloid leukemia models.

After cessation of pazopanib, rapid regrowth of MM cells was seen, underlining the importance of continuous exposure.<sup>12</sup> Because pazopanib has no significant effect on the proliferation of most tumor cell lines in vitro, inhibition of angiogenesis is likely the mechanism underlying the antitumor effects observed in vivo.

In addition to its preclinical efficacy as a single agent, synergistic cytotoxic effects of low-dose pazopanib combined with conventional chemotherapy (melphalan) or other molecular targeted agents (bortezomib) were observed in MM cell lines.<sup>12</sup>

Currently, no published data are available on preclinical toxicity with pazopanib.



## CLINICAL DATA

### PK Data

Clinical PK data on single-agent pazopanib are available from 63 patients who were enrolled in a phase I study.<sup>13</sup> Three-times-a-week (50 mg), once-a-day (OD) (50–2,000 mg), and twice-a-day (BID) (300 mg or 400 mg) schedules were evaluated at 13 dose levels. Pazopanib was absorbed orally with median time to maximum concentration ( $t_{max}$ ) values in the range of 2.0–4.0 hours and 2.0–8.0 hours following single and multiple dosing, respectively. Although of less relevance because preclinical data strongly suggest that, for antitumor activity  $C_{trough}$  levels are more important,  $C_{max}$  increased with higher doses of pazopanib. By comparing  $C_{trough}$  at day 22 with the concentration 24 hours after a single dose, accumulation appeared to be 1.2- to 4.5-fold.<sup>13</sup> The steady-state exposure was dependent on the dose and frequency of administration (Figure 2). Steady-state exposure plateaued at doses  $\geq 800$  mg/day and was  $\geq 15$   $\mu\text{g/ml}$  ( $\approx 34$  J.M) in 93% of patients receiving a dose of 800 mg OD. This  $C_{trough}$  plasma pazopanib concentration of  $\geq 15$   $\mu\text{g/ml}$  appeared to correlate with clinical activity in patients with RCC as well as with the pharmacodynamic effect of hypertension.<sup>13</sup> A separate phase I, PK study was performed in HCC patients, showing that, at the maximum-tolerated dose (MTD) of 600 mg daily (QD),  $C_{trough}$  was  $\geq 15$   $\mu\text{g/ml}$  in 67% of patients.<sup>14</sup>

Pazopanib was eliminated slowly, with mean half-life values in the range of 20.3–52.3 hours.<sup>13</sup>

### Drug-Drug Interactions

Two interaction studies are available exploring several doses of pazopanib in combination with lapatinib and with paclitaxel. Concurrent administration with lapatinib (750–1,500 mg OD), an orally available potent ErbB-1 and ErbB-2 TKI, alters the PK of pazopanib, given an increased  $C_{trough}$  of pazopanib. Lapatinib concentrations were similar to those observed after monotherapy.<sup>15</sup> In contrast, pazopanib administered concomitantly led to a higher mean  $C_{max}$  and area under the curve of paclitaxel, by approximately 40% and 45%, respectively.<sup>16</sup> Whether paclitaxel affects the PK of pazopanib has not yet been reported.

### Recommended Dose for Further Studies

In the phase I study, pazopanib was generally well tolerated with continuous daily dosing of pazopanib  $\leq 2,000$  mg OD. An MTD was not determined. Four patients experienced dose-limiting toxicities (DLTs) at 50 mg OD ( $n=2$ ), 800 mg OD ( $n=1$ ), and 2,000 mg OD ( $n=1$ ). The two DLTs occurring at 50 mg OD were gastrointestinal hemorrhage from a metastatic lesion in the small bowel in a patient with RCC and grade 3 extrapyramidal involuntary movements resulting from a potential drug-drug interaction between trazadone and pazopanib. Grade 3 hypertension and subsequently recurring grade 3 proteinuria were seen at the 800-mg

pazopanib OD dose despite dose reductions, whereas a DLT comprising grade 3 fatigue occurred at the 2,000-mg OD dose, which improved to grade 1 after a dose reduction to 800 mg OD.<sup>13</sup> Despite the DLTs at 50 mg OD and 800 mg OD, dose escalation to 2,000 mg was feasible. In the absence of a MTD, the choice of the 800-mg dose as the recommended dose for further studies was based on the observation of a plateau in  $C_{\text{trough}}$  at doses  $\geq 800$  mg/day, significant changes in dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) at doses of 300–400 mg BID, a threshold concentration that correlates with preclinical activity in patients, and pharmacodynamic effects of hypertension, as discussed below. OD administration was recommended for further studies because the fluctuation between  $C_{\text{max}}$  and  $C_{\text{trough}}$  with OD dosing was low ( $\sim 2$ ), rendering drug exposure similar to that with continuous infusion.<sup>13</sup>

In contrast to pazopanib, for which no MTD has been defined based on toxicity, the MTD of sunitinib was set at 50 mg daily for 28 days every 6 weeks, given an excess in toxicity (grade 3 asthenia and grade 3 hypertension) observed at doses  $> 50$  mg/day.<sup>17</sup> For sorafenib, skin and gastrointestinal toxicities were dose limiting, rendering an MTD of 400 mg BID.<sup>18</sup>

The MTD of pazopanib in patients with HCC has been determined to be 600 mg QD, although the observed toxicity has not been reported yet.<sup>19</sup>

### Antitumor Activity

One phase III trial showed a beneficial effect of pazopanib in patients with RCC<sup>19</sup> and resulted in approval by the FDA. In other tumor types, some interesting signs of anti-tumor activity with pazopanib were observed, though it should be kept in mind that activity data from phase I/II trials should be interpreted with extreme caution.

In the phase I study, of the 63 patients included, a partial response (PR) was observed in three patients and 14 patients achieved stable disease (SD) for  $> 6$  months (Table 2).<sup>13</sup> Of interest is the activity seen in the 10 included patients with RCC: two patients achieved a PR (at the 300-mg BID and 1,400-mg OD doses), SD was observed in four patients (at the 300-mg BID, 800-mg OD [ $n=2$ ], and 2,000-mg OD doses), and progressive disease (PD) occurred in four patients, all at doses  $\leq 400$  mg OD. A  $C_{\text{trough}} \geq 15$   $\mu\text{g/ml}$  was achieved in 83% of patients with RCC who achieved a PR or SD, whereas all four patients experiencing PD had a  $C_{\text{trough}} < 15$   $\mu\text{g/ml}$ .<sup>13</sup>

In a randomized discontinuation phase II trial, 225 patients who had received, maximally, one prior line of systemic therapy for RCC received pazopanib at a dose of 800 mg OD for 12 weeks. Pazopanib was continued if a response was achieved at 12 weeks, but in cases of SD at 12 weeks, patients were randomized between placebo and continuation of pazopanib. Following an interim analysis by the independent data monitoring committee that showed a high response rate (38%) in the first 60 patients at week 12, all randomized patients were unblinded and allowed to cross over to pazopanib.<sup>20</sup> Sixty-nine percent of patients had received no prior systemic therapy and 31% had failed one prior systemic therapy (cytokine-



or bevacizumab-based therapy).<sup>20</sup> In all 225 patients, the complete response (CR) + PR rate at 12 weeks was 35%, whereas an additional 45% of patients achieved SD.<sup>20</sup> Subsequently, a placebo-controlled, randomized phase III trial ( $n=435$ ) was conducted in therapy-naïve or cytokine-pretreated patients with RCC.<sup>18</sup> At the interim analysis, a significantly longer progression-free survival (PFS) interval was observed (9.2 months versus 4.2 months). The response rate (CR + PR) was also more favorable in the pazopanib-treated patients (30% vs 3%), and a response had a median duration of 59 weeks.<sup>19</sup> The difference in overall survival was statistically not significant, given the interim O'Brien-Fleming boundary.<sup>19</sup> Furthermore, clean survival data will most likely not become available because patients on placebo could, upon progression, receive pazopanib. No worsening of quality of life was observed in the patients treated with pazopanib, versus placebo.<sup>19</sup>

**Table 2 | Signs of activity of single-agent pazopanib**

Tumor type	<i>n</i> of patients	Outcome
<b>Phase I</b>		
Solid malignancy [13]	63	3 patients with PR–2 RCC, 1 pancreatic islet cell tumor; 14 patients with SD $\geq$ 6 mos
HCC [14]	17	SD in 6 of 10 patients treated at MTD; PR in 1 of 10 patients treated at MTD
<b>Phase II</b>		
Advanced or metastatic RCC [20]	225	Response at 12 wks—CR/PR, 35%; SD, 45%; PD, 11%; unknown 10%
Relapsing STS after or during chemotherapy [21] 49%; adipocytic STS, 26%; other STS, 39%	142	PFR12wk—leiomyosarcoma, 44%; synovial sarcoma,
Advanced ovarian cancer [23]	36	CA-125 response in 11 patients (31%)
Stage I–II NSCLC (preoperative treatment) [24]	35	Median treatment duration, 18 days; PR, 9%; SD, 89%; PD, 3%; 20 (87%) patients had a reduction in tumor volume
Relapsed or refractory multiple myeloma [26]	21	Median TTP, 52 days; at 6 wks: SD, 47%; PD, 53%
<b>Phase III</b>		
Advanced or metastatic RCC [19]	435	Pazopanib versus placebo: PFS, 9.2 mos versus 4.2 mos ( $P<.001$ ); RR (CR + PR), 30% versus 3%

Abbreviations: CA, cancer antigen; CR, complete remission; HCC, hepatocellular carcinoma; MTD, maximum-tolerated dose; NSCLC, non-small lung cancer; PD, progressive disease; PFR, progression-free rate; PFS, progression-free survival; PR, partial response; RCC, renal cell cancer; RR, response rate; SD, stable disease; STS, soft tissue sarcoma; TTP, time to progression.

The phase II European Organization for Research and Treatment of Cancer 62043 trial explored pazopanib (800 mg OD) in patients with relapsing or refractory soft tissue sarcoma (STS).<sup>21</sup> The primary endpoint of that study was the 12-week progression-free rate (PFR12wk), because the response rate is thought to not adequately reflect the antitumor activity of many drugs in STS.<sup>22</sup> An interesting PFR12wk, meeting the predefined criteria of a potentially active agent, was found in patients with leiomyosarcomas, patients with synovial sarcomas, and the group of patients with other eligible STSs (44%, 49%, and 39%, respectively). In contrast, there was insufficient activity in adipocytic STS.<sup>21</sup>

Furthermore, preliminary data from a small phase II study in women with progressive, platinum-pretreated ovarian cancer showed a cancer antigen 125 response (defined as a confirmed decrease  $\geq 50\%$  from baseline) in 11 (31%) patients, with a median duration of response of 113 days.<sup>23</sup>

In preliminary data from a proof-of-concept phase II study evaluating preoperative treatment with pazopanib (800 mg OD; median duration, 18 days) in stage I–II NSCLC patients, 87% of patients had a reduction in tumor volume, with volume changes in the range of -86% to +17%, whereas three patients achieved a PR.<sup>24</sup>

In patients with advanced HCC, signs of activity were observed at the MTD, with six of 10 patients achieving prolonged SD and one of 10 patients achieving a PR. Furthermore, using DCE-MRI, a decline of 40% in imaging markers was seen in those 10 patients.<sup>15</sup>

No data on single-agent pazopanib in BC patients are available. However, the combination of pazopanib (400 mg OD) and lapatinib (1000 mg/day) was recently compared with single-agent lapatinib (1,500 mg/day) in a randomized, phase II trial in patients with advanced or metastatic HER-2-positive BC. Previous chemotherapy or HER-2-directed therapy for advanced or metastatic disease was not allowed. A predefined interim analysis after 62 patients were accrued showed PD rates at 12 weeks, the primary endpoint, of 19% versus 27%, whereas the response rates were 44% versus 30%, both favoring the combination.<sup>26</sup>

Insufficient activity of pazopanib in patients with MM, as shown by a complete lack of response at 6 weeks and a termination of the phase II trial.<sup>25</sup>

## Adverse Events

The most common adverse events in the phase I study were hypertension, diarrhea, hair depigmentation, and nausea.<sup>13</sup> A similar toxicity profile was seen in the phase II and phase III studies.<sup>19,20,21,23,24</sup>

Similar to other agents targeting the VEGF–VEGFR pathway, hypertension is frequently reported during pazo-panib treatment.<sup>6,13</sup> In the phase I study, a study-specific hypertension definition was used in order to not underestimate the incidence:  $\geq 15$  mmHg rise from baseline in mean arterial blood pressure on three separate occasions and/or the initiation or escalation of antihypertensive medications. Antihypertensive medications were started or increased if blood pressure exceeded 160/100 mmHg on three occasions over any 2-week



period. By this definition, the incidence was 62%; according to National Cancer Institute Common Toxicity Criteria, version 2.0, 29% had grade 3 hypertension. The overall incidence of hypertension was similar in patients with and without a history of hypertension (71% vs 62%).<sup>13</sup> All episodes of hypertension in the phase I study were easily manageable with antihypertensive medication; however, temporary interruption or dose reduction because of hypertension was needed in two patients.<sup>13</sup> Hypertension seems to be correlated with a  $C_{\text{trough}} \geq 15 \mu\text{g/ml}$ , because 77% of the patients above this cutoff developed hypertension, versus only 39% of the patients below this level.<sup>14</sup> In the three largest studies conducted to date, the incidence of hypertension was 37%–40% (grade 3 or 4, 4%–8%).<sup>19–21</sup> Of interest, cumulative incidence analysis revealed that, in general, patients develop hypertension within 4 weeks after treatment initiation, and only a few patients develop it thereafter.<sup>21</sup>

Hair depigmentation, as reported for other agents targeting *c-Kit*, was observed in 32%–38% of patients and seen at pazopanib doses  $\geq 600 \text{ mg/day}$ .<sup>13,19,27</sup> Other frequent, mostly mild, toxicities comprised fatigue, anorexia, diarrhea, and skin discoloration. Laboratory findings show mild and infrequent bone marrow suppression, whereas elevations in aspartate aminotransferase and/or alanine amino-transferase are relatively common but rarely a reason for treatment discontinuation (Table 3). Moreover, pazopanib-induced isolated hyperbilirubinemia is, in most cases, a benign manifestation of Gilbert's syndrome, as shown by *UGT1A1* polymorphism in 84% of patients.<sup>28</sup> Collectively, it appears that pazopanib is generally well tolerated, which is important given the necessity of administering this agent for prolonged periods of time. In the two largest phase II studies of pazopanib, 6%–15% of patients discontinued treatment because of adverse events.<sup>20,21</sup>

## DISCUSSION

Pazopanib is one of the novel drugs belonging to the rapidly expanding class of TKIs and was recently approved for patients with advanced RCC. Although other mechanisms may contribute, the main mechanism underlying its antitumor activity in RCC is thought to be the inhibition of VEGFR-2, although other inhibited factors will play a role as well and might even be of greater relevance in tumor types other than RCC. In vivo, a threshold for inhibition of VEGFR-2 by pazopanib has been established, a level paralleling its antitumor activity in vivo in preclinical models. Furthermore, it was revealed that a  $C_{\text{trough}}$  above the threshold associated with VEGFR-2 inhibition, rather than  $C_{\text{max}}$ , is associated with antitumor activity. In humans, a comparable  $C_{\text{trough}}$  level is reached in the majority of patients treated at pazopanib doses of 800 mg/day. Based on the finding that doses  $>800 \text{ mg}$  do not result in higher  $C_{\text{trough}}$  values, increasing the dose to  $>800 \text{ mg}$  is unlikely to yield better outcomes.



**Table 3** | Reported toxicity in studies on single-agent pazopanib in patients with solid tumors<sup>13,14,19-21,23,24,26</sup>

Toxicity	Incidence (%)	
	All grades	Grade 3 or 4
Nausea	26–42	0–2
Vomiting	17–24	0–2
Anorexia	22–25	0–2
Diarrhea	30–63	4–8
Abdominal cramps/pain	11–16	0–3
Fatigue	19–46	2–11
Headache	10–20	0
Hemorrhage	13	2
Hypertension	40–62 <sup>a</sup>	3–29 <sup>a</sup>
Dysgeusia	13–24	0
Hair discoloration	32–43	1
Skin hypopigmentation	8–37	0
Rash	6–16	<1
Alopecia	8–10	0
Dizziness	5–12	<1
Neutropenia	21–34	1–4
Lymphopenia	19–46	4–14
Thrombocytopenia	10–32	1–2
Bilirubin	13–38	0–6
Aspartate aminotransferase	38–54	2–8
Alanine aminotransferase	24–53	0–19
Creatinine	13–32	0–4
Hypoglycemia	17–33	0–5
Hyperglycemia	33–41	0–3
Hypocalcemia	33–38	0–3
Hyperkalemia	17–26	3–5
Hypophosphatemia	16–34	2–4

<sup>a</sup>Study-specific criteria in one trial resulting in the highest incidences.

At a dose of 800 mg, pazopanib is well tolerated, even with long-term use. The side-effect profile seems consistent with the tyrosine kinases inhibited. In several phase II trials conducted in STS, RCC, relapsing ovarian cancer, and NSCLC, early hints of antitumor activity of pazopanib were seen. In the first phase III study, a longer PFS time was observed in patients with RCC, resulting in the FDA approval of pazopanib.

Putting pazopanib into perspective and comparing it with other VEGFR TKIs that have been approved for a longer time are difficult and have to be done on the basis of indirect comparisons, given the lack of data from comparative trials among the several treatment options. With respect to the first-line treatment of patients with RCC, with the clear cell subtype and belonging to the so-called good and intermediate prognostic groups, according to the Memorial Sloan-Kettering Cancer Center classification,<sup>29</sup> three treatment options are currently approved: sunitinib, pazopanib, and the combination of bevacizumab and interferon. Sorafenib failed to show superiority relative to interferon- $\alpha$  in treatment-naïve patients.<sup>30</sup> Approval of all three treatment options was granted on the basis of longer PFS times; however, in the meantime, sunitinib was shown to lead to longer overall survival than with interferon- $\alpha$  (26.4 months versus 21.8 months).<sup>31</sup> The two pivotal trials on the combination of bevacizumab and interferon- $\alpha$  showed superiority over monotherapy interferon- $\alpha$  as a result of a longer PFS time, 8.5 months versus 5.2 months and 10.2 months versus 5.4 months, respectively.<sup>32,33</sup> An indirect comparison was made between sunitinib and the combination of bevacizumab and interferon- $\alpha$ , suggesting superiority for sunitinib; however, no firm conclusions can yet be drawn.<sup>34</sup> In contrast to the other two treatment options, pazopanib has not been compared with interferon- $\alpha$  but with placebo, hindering a direct comparison. This issue soon will be clarified because a phase III study comparing pazopanib with sunitinib is currently enrolling patients. Altogether, based on the current data, pazopanib can be regarded as an alternative first-line therapy for these patients, in particular for those not tolerating sunitinib or the combination of bevacizumab and interferon- $\alpha$ .

For second-line treatment of RCC patients following cytokine-based therapy, three treatment options have gained approval: sunitinib, sorafenib, and pazopanib. Approval for sunitinib was based on two single-arm studies,<sup>35,36</sup> whereas sorafenib and pazopanib were approved on the basis of placebo-controlled trials rendering longer PFS intervals with these agents.<sup>19,37</sup>

In the two single-arm studies of sunitinib in RCC patients pretreated with cytokine-based therapy, sunitinib resulted in objective response rates of 34%–40% and a PFS time of 8.3–8.7 months.<sup>35,36</sup> Pazopanib in second-line treatment was tested in the placebo-controlled trial that also examined first-line treatment; the proportion of patients who were cytokine pretreated (47%) had a longer PFS duration, 7.4 months versus 4.2 months, while receiving pazopanib as compared to placebo.<sup>19</sup> Sorafenib treatment led to a longer PFS interval, 5.5 months versus 2.8 months, in a cytokine-pretreated population.<sup>37</sup> Indirectly comparing these two trials, neither agent is clearly superior to the other with regard to survival, making arguments like differences in the toxicity profiles more important.

In conclusion, the recent approval of pazopanib for patients with advanced RCC is a result of a rational stepwise development using translational research. Based on preclinical and early clinical data on pazopanib, further exploration of pazopanib is warranted in tumor

types other than RCC. The currently ongoing phase III studies and studies examining the feasibility of pazopanib in combination with other antitumor agents will be instrumental in defining the place of pazopanib as a novel antitumor agent.



## REFERENCES

1. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. *Nat Rev Cancer* 2003; **3**:401-410.
2. Foekens JA, Peters HA, Grebenchtchikov N et al. High tumor levels of vascular endothelial growth factor predict poor response to systemic therapy in advanced breast cancer. *Cancer Res* 2001; **61**:5407-5414.
3. Toi M, Matsumoto T, Bando H. Vascular endothelial growth factor: Its prognostic, predictive, and therapeutic implications. *Lancet Oncol* 2001; **2**:667-673.
4. Kabbinar F, Hurwitz HI, Fehrenbacher L et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**:60-65.
5. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006; **355**:2542-2550.
6. Motzer RJ, Hutson TE, Tomczak P et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**:115-124.
7. Miller K, Wang M, Gralow J et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med* 2007; **357**:2666-2676.
8. Kumar R, Knick VB, Rudolph SK et al. Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. *Mol Cancer Ther* 2007; **6**:2012-2021.
9. Mendel DB, Laird AD, Xin X et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: Determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003; **9**:327-337.
10. Wilhelm SM, Carter C, Tang L et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**:7099-7109.
11. Sonpavde G, Hutson TE. Pazopanib: A novel multitargeted tyrosine kinase inhibitor. *Curr Oncol Rep* 2007; **9**:115-119.
12. Podar K, Tonon G, Sattler M et al. The small-molecule VEGF receptor inhibitor pazopanib (GW786034B) targets both tumor and endothelial cells in multiple myeloma. *Proc Natl Acad Sci USA* 2006; **103**:19478-19483.
13. Hurwitz HI, Dowlati A, Saini S et al. Phase I trial of pazopanib in patients with advanced cancer. *Clin Cancer Res* 2009; **15**:4220-4227.
14. Yau CC, Chen PJ, Curtis CM et al. Phase I study of pazopanib in hepato-cellular carcinoma: Evaluation of clinical activity, pharmacokinetics and dynamic contrast enhanced MRI (DCE MRI). *Eur J Cancer* 2009; **7**(suppl 2):122.
15. De Jonge M, Savage S, Verweij J et al. A phase I open-label study of the safety and pharmacokinetics of pazopanib and lapatinib administered concurrently [abstract 3088]. *Proc Am Soc Clin Oncol* 2006; **24**:142.
16. Tan AR, Jones SF, Dowlati A et al. Phase I study of the safety, tolerability, and pharmacokinetics (PK) of weekly paclitaxel administered in combination with pazopanib (GW786034) [abstract 3552]. *Proc Am Soc Clin Oncol* 2008; **26**:166.
17. Faivre S, Delbaldo C, Vera K et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006; **24**:25-35.
18. Strumberg D, Richly H, Hilger RA et al. Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. *J Clin Oncol* 2005; **23**:965-972.
19. Sternberg CN, Davis ID, Mardiak J et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: Results of a randomized phase III trial. *J Clin Oncol* 2010; **28**:1061-1068.

20. Hutson TE, Davis ID, Machiels JP et al. Efficacy and safety of pazopanib in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2010; **28**:475–480.21 Sleijfer S, Ray-Coquard I, Papai Z et al. Pazopanib, a multikinase angio-genesis inhibitor, in patients with relapsed or refractory advanced soft tissue sarcoma: A phase II study from the European Organisation for Research and Treatment of Cancer–Soft Tissue and Bone Sarcoma Group (EORTC study 62043). *J Clin Oncol* 2009; **27**:3126-3132.
21. Van Glabbeke M, Verweij J, Judson I et al.; EORTC Soft Tissue and Bone Sarcoma Group. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. *Eur J Cancer* 2002; **38**:543–549.
22. Friedlander M, Hancock KC, Benigno B et al. Pazopanib (GW786034) is active in women with advanced epithelial ovarian, fallopian tube and peritoneal cancers: Final results of a phase II study. *Ann Oncol* 2008; **19**(suppl 8):viii211.
23. Altorki N, Heymach J, Guarino M et al. Phase II study of pazopanib (GW786034) given preoperatively in stage I-II non-small cell lung cancer (NSCLC): A proof-of-concept study. *Ann Oncol* 2008; **19**(suppl 8):viii89.
24. Slamon D, Gomez H, Kabbinawar F et al. Randomized study of pazopanib + lapatinib vs. lapatinib alone in patients with HER2 positive advanced or metastatic breast cancer [abstract 1016]. *Proc Am Soc Clin Oncol* 2008; **26**:45.
25. Prince HM, Hönemann D, Spencer A et al. Vascular endothelial growth factor inhibition is not an effective therapeutic strategy for relapsed or refractory multiple myeloma: A phase 2 study of pazopanib (GW786034). *Blood* 2009; **113**:4819-4820.
26. Routhouska S, Gilliam AC, Mirmirani P. Hair depigmentation during chemotherapy with a class III/V receptor tyrosine kinase inhibitor. *Arch Dermatol* 2006; **142**:477-479.
27. Xu CF, Reck BH, Xue Z et al. Pazopanib-induced hyperbilirubinemia is associated with Gilbert's syndrome UGT1A1 polymorphism. *Eur J Cancer* 2009; **7**(suppl 2):119.
28. Motzer RJ, Mazumdar M, Bacik J et al. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol* 1999; **17**:2530-2540.
29. Escudier B, Szczylik C, Hutson TE et al. Randomized phase II trial of first-line treatment with sorafenib versus interferon alfa-2a in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**:1280-1289.
30. Motzer RJ, Hutson TE, Tomczak P et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**:3584-3590.
31. Escudier B, Pluzanska A, Koralewski P et al. Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: A randomised, double-blind phase III trial. *Lancet* 2007; **370**:2103-2111.
32. Rini BI, Halabi S, Rosenberg JE et al. Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol* 2008; **26**:5422-5428.
33. Thompson Coon JS, Liu Z, Hoyle M et al. Sunitinib and bevacizumab for first-line treatment of metastatic renal cell carcinoma: A systematic review and indirect comparison of clinical effectiveness. *Br J Cancer* 2009; **101**:238-243.
34. Motzer RJ, Rini BI, Bukowski RM et al. Sunitinib in patients with meta-static renal cell carcinoma. *JAMA* 2006; **295**:2516-2524.
35. Motzer RJ, Michaelson MD, Redman BG et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006; **24**:16-24.
36. Escudier B, Eisen T, Stadler WM et al. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**:125-134.





Chapter

# 6

## **Pazopanib exposure decreases as a result of an ifosfamide-dependent drug-drug interaction: results of a phase I study**

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## ABSTRACT

**Background:** The vascular endothelial growth factor receptor (VEGFR) pathway plays a pivotal role in solid malignancies and is probably involved in chemotherapy resistance. Pazopanib, inhibitor of, among other receptors, VEGFR1-3, has activity as single agent and is attractive to enhance anti-tumour activity of chemotherapy. We conducted a dose-finding and pharmacokinetic (PK)/ pharmacodynamics study of pazopanib combined with two different schedules of ifosfamide.

**Methods:** In a 3 + 3 + 3 design, patients with advanced solid tumours received escalating doses of oral pazopanib combined with ifosfamide either given 3 days continuously or given 3-h bolus infusion daily for 3 days (9 g/m<sup>2</sup> per cycle, every 3 weeks). Pharmacokinetic data of ifosfamide and pazopanib were obtained. Plasma levels of placental-derived growth factor (PlGF), vascular endothelial growth factor-A (VEGF-A), soluble VEGFR2 (sVEGFR2) and circulating endothelial cells were monitored as biomarkers.

**Results:** Sixty-one patients were included. Pazopanib with continuous ifosfamide infusion appeared to be safe up to 1000 mg per day, while combination with bolus infusion ifosfamide turned out to be too toxic based on a variety of adverse events. Ifosfamide-dependent decline in pazopanib exposure was observed. Increases in PlGF and VEGF-A with concurrent decline in sVEGFR2 levels, consistent with pazopanib-mediated VEGFR2 inhibition, were observed after addition of ifosfamide.

**Conclusion:** Continuous as opposed to bolus infusion ifosfamide can safely be combined with pazopanib. Ifosfamide co-administration results in lower exposure to pazopanib, not hindering biological effects of pazopanib. Recommended dose of pazopanib for further studies combined with 3 days continuous ifosfamide (9 g/m<sup>2</sup> per cycle, every 3 weeks) is 800 mg daily.



## INTRODUCTION

Given the heterogeneity of cancer, it is conceivable that combinations of anti-tumour agents render the best outcomes for patients with advanced solid malignancies.

The combination of conventional cytotoxic therapy with inhibition of the vascular endothelial growth factor receptor (VEGFR) pathway is attractive for several reasons. The VEGFR inhibition reduces the interstitial pressure of tumours rendering higher intratumoural levels of concomitantly administered cytotoxic agents and enhances effects of several cytotoxic agents at the tumour cell level.<sup>1-3</sup> In addition, most VEGFR-inhibiting agents have no overlapping toxicities with conventional cytotoxics.

Pazopanib is a tyrosine kinase inhibitor targeting the VEGFR1-3, the platelet-derived growth factor receptors  $\alpha$  and  $\beta$  and *c-kit*.<sup>4</sup> It received marketing approval for patients with metastatic renal cell carcinoma and for patients with advanced non-adipocytic soft tissue sarcomas.<sup>5,6</sup> The recommended dose of pazopanib is 800 mg once daily with fatigue, diarrhoea, nausea, hypertension and elevated liver-enzymes as most common toxicities.<sup>6</sup>

Ifosfamide is standard of care for different tumour types including advanced soft tissue sarcomas, where a continuous and bolus infusion schedule have shown equivalent activity.<sup>7</sup> Myelosuppression, febrile neutropenia (FN) and encephalopathy are the most relevant toxicities.<sup>7</sup>

Given the potential of the combination of pazopanib and ifosfamide, we performed a phase I study to determine the recommended dose of pazopanib combined with ifosfamide in two different schedules.

## PATIENT AND METHODS

### Patient selection

Patients with advanced or metastatic solid tumours for whom ifosfamide-based therapy was considered appropriate or for whom no standard therapy was available were eligible. Other inclusion criteria included: ECOG performance status  $<2$ , evaluable or measurable disease (RECIST 1.1),<sup>8</sup> age 18 years, adequate bone marrow, liver, and renal function, and systolic blood pressure (BP)  $<160$  mm Hg and diastolic BP  $<90$  mm Hg (two antihypertensive drugs allowed). Main exclusion criteria were: history of cardiovascular disease other than hypertension and signs/symptoms of central nervous system metastases.

The study was approved by the institutional review boards and conducted in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from each participant prior to enrollment.



### Study design

Daily oral pazopanib was evaluated in combination with a fixed dose of ifosfamide 9 g/m<sup>2</sup> per cycle, either given as 3 days continuous intravenous infusion (CIV) or as 3 h bolus intravenous infusion (BIV) for 3 consecutive days, both at 3-weekly intervals. Pazopanib was escalated in serial cohorts at a dose of 400,800 and 1000 mg daily. If the maximal tolerated dose (MTD) would be exceeded at 400 mg then an extra cohort exploring pazopanib at 200 mg daily was added.

The 3 + 3 + 3 design, a novel model recently proposed aiming to reduce falsely halting dose escalation in combination phase I trials, was applied in the original protocol.<sup>10</sup> If a dose-limiting toxicity (DLT) was observed in one patient, three additional patients were recruited at that dose level, with dose escalation proceeding if no further DLT occurred at that dose level. If DLT was observed in two out of six, three additional patients were enrolled. If a DLT occurred in 2 out of 3, >2 out of 6 or >2 out of 9 patients in a cohort, MTD had been exceeded. The MTD was defined as the highest dose level with a DLT incidence of <33%.

In order to be exposed to steady-state concentrations of pazopanib and to determine the effects of ifosfamide administration on pazopanib pharmacokinetics (PK), patients in the dose-escalation phase started on pazopanib 7 days prior to the first cycle of ifosfamide. At the MTD, six additional patients were treated in an expansion cohort to get better insight into the safety profile, to confirm the MTD, and to further study the PK interaction. For the latter, pazopanib was started 7 days after the first ifosfamide cycle in the patients in the expansion phase, which enabled an intra-patient comparison of ifosfamide PK with or without the presence of pazopanib (Figure 1).

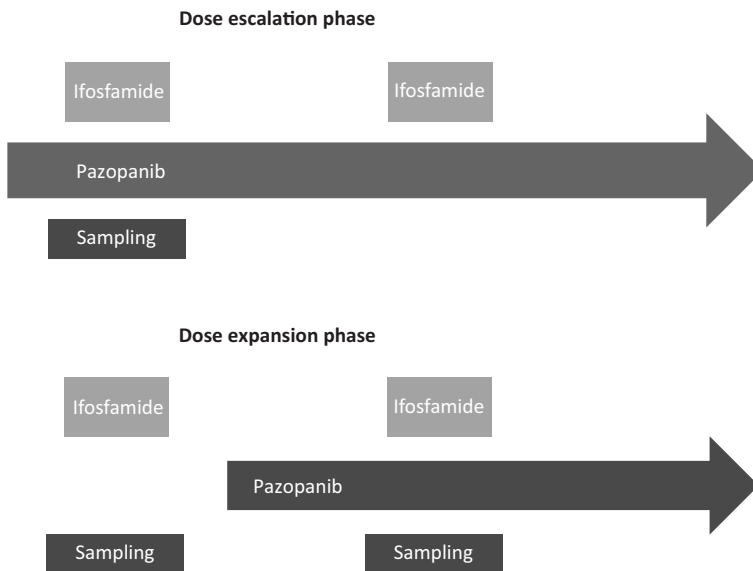


Figure 1 | Study design.

Using the Common Terminology Criteria for adverse events, version 3.0, DLT during the first treatment cycle (in the dose expansion phase during the first two treatment cycles) was defined as: grade 4 neutropenia 7 days, FN, grade 4 thrombocytopenia, creatinine clearance 50 ml/min, grade 3–4 proteinuria or any drug-related grade 3 or 4 non-haematological toxicity. Hypertension was considered DLT in case of symptomatic hypertension; persistent (>24 h) and asymptomatic systolic BP >170 mm Hg and/or diastolic BP >100 mm Hg; systolic BP 160–170 and/or diastolic BP 90–100 that could not be controlled within 2 weeks; or an increase of diastolic BP >20 mm Hg, which despite antihypertensive medication was not adequately controlled within 2 weeks. A dose delay or interruption exceeding 2 weeks was classified as DLT. If neutropenia comprised the predominant DLT at a certain dose level, that and subsequent levels were explored in combination with granulocyte cell stimulating factor (pegfilgrastim 6 mg once per cycle).

Patients were treated for a maximum of six ifosfamide cycles. Patients experiencing clinical benefit from the combination of pazopanib and ifosfamide were allowed to continue treatment thereafter with pazopanib until disease progression or unacceptable toxicity.

### **PK sampling and analysis**

Concentrations of ifosfamide and its most important metabolites, 2-dechloroethyl-ifosfamide, 3-dechloroethyl-ifosfamide and 4-hydroxy-ifosfamide were quantitated as previously reported in 11–16 samples per ifosfamide cycle.<sup>11</sup>

For the analysis of pazopanib, 15 samples per patient were drawn and quantitated as previously reported.<sup>12</sup>

### **Statistical data analysis**

Plasma concentrations of ifosfamide and its metabolites were plotted as a function of time. Area under the curve (AUC) was calculated by the trapezoidal method. Non-compartmental PK analysis including half-life ( $T_{1/2}$ , h) was calculated using the software package WinNonlin version 6.1. Total body clearance of ifosfamide was calculated by dividing the administered dose by the AUC of ifosfamide. Statistical analysis were made using the software package SPSS (v20). Correlation of the changes in AUC or clearance and half-life was evaluated by a two-sided paired t-test for subjects in the expansion cohorts.

Median plasma pazopanib concentration-time profiles were generated for subjects in the dose escalation cohorts. The area under the plasma pazopanib concentration–time curve from 0 to 24 h (AUC (0–24)) was calculated using nominal blood sample collection times after administration of pazopanib on day 21 cycle 1 (pazopanib alone) and day 3 cycle 2 (pazopanib plus ifosfamide) for subjects in the expansion cohorts.



### **Biomarker analysis**

Biomarker samples were collected in all patients at baseline and prior cycle 2 day 1. During the escalation phase, an additional sample was drawn prior to day 1 cycle 1 and during the expansion prior to the first pazopanib dose. Circulating endothelial cell (CEC) enumeration was determined with a flow-cytometry-based method.<sup>13</sup> Plasma concentrations of VEGF-A, soluble VEGFR2 (sVEGFR2) and placental-derived growth factor (PIGF) were determined using ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions.

## **RESULTS**

### **Dose escalation, MTD and dose intensity**

In total, 61 patients were enrolled (Table 1), 29 in the CIV schedule and 32 on the BIV schedule. In all, 15 out of 61 patients were not evaluable for determination of DLT of the combination and were replaced. The most common reason was early progression ( $n=5$ ). Two other patients were registered but did not receive a dose of study drug at all. Three patients appeared not to tolerate the single agent treatment they received before the second agent was added. As the aim of this study was to identify the MTD of the combination of pazopanib and ifosfamide, it was decided to replace these patients. Five others were not evaluable due to a diversity of reasons including withdrawal of consent (not based on toxicity), and an allergic reaction to mesna, which was prophylactically administered with ifosfamide.

In the CIV arm, no DLTs were observed in the three evaluable patients at the first dose level, whereas 1 DLT (FN) was observed at a dose level with 800 mg pazopanib (six evaluable patients). At the highest pre-defined dose level of 1000 mg pazopanib two DLTs occurred (FN and encephalopathy) in nine patients. During expansion phase ( $n=6$ ) at this dose level, no additional DLTs were observed. At the MTD in the CIV arm, the dose intensity of ifosfamide and pazopanib was 92% and 93%, respectively, with a median number of ifosfamide cycles of 4.

In the first two patients treated with 400 mg pazopanib in the BIV arm, an episode of FN was encountered. Adding G-CSF to the 400 mg pazopanib dose level three DLTs were observed in nine patients (grade 3 encephalopathy, grade 3 proteinuria and grade 3 pneumonia during neutropenia). The dose of pazopanib was de-escalated to 200 mg and supported with G-CSF. In two out of the first nine evaluable patients DLTs occurred (one case each of grade 3 encephalopathy and FN). The subsequent dose expansion in another six patients resulted in three more DLTs (pneumonia during neutropenia and grade 5 cardiac arrest in one patient, renal toxicity and grade 3 fatigue).

**Table 1** | Demographics and baseline characteristics

<b>Age (median, years)</b>	56 (range 18–76)
<b>Gender</b>	
Male	40 (65)
Female	21 (35)
<b>WHO</b>	
0	23 (38)
1	38 (62)
<b>Tumour type</b>	
Sarcoma (incl GIST)	19
Melanoma	6
Urothelial carcinoma	6
Ovarian carcinoma	5
Carcinoma of unknown primary	5
Prostate	4
Gastric carcinoma	3
Oesophageal carcinoma	3
Miscellaneous	10
<b>Previous non-hormonal systemic anticancer treatment</b>	
0	12
1	29
2	11
3	3
4	4
5	1

Abbreviation: GIST, gastro-intestinal stromal tumour.

## Toxicity

In addition to hematological toxicity, the main grades 3–4 toxicities during combination therapy were fatigue and hypophosphatemia, irrespective of treatment schedule. Grade 3–4 vomiting was more pronounced in the BIV-treated patients, whereas all grades of hypertension and grade 3/4 neutropenia occurred more often in the CIV group. (Table 2).

## Pharmacokinetics

Pazopanib had no impact on plasma half-life, AUC or clearance, of ifosfamide or any of its metabolites. Figures 2 and 3 depicts the ifosfamide concentrations with and without concomitant administration of pazopanib.



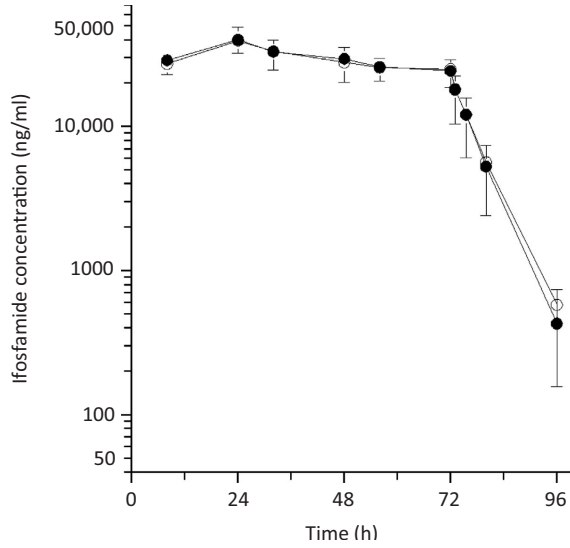
**Table 2** | Most frequent adverse events during the first and during all combination cycles

	CIV (n=26)				BIV (n=27)			
	First cycle adverse events		Adverse events at all combination cycles		First cycle adverse events		Adverse events at all combination cycles	
	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4	All grades
Anaemia	1	21	5	26	0	23	4	27
Thrombocytopenia	1	7	3	13	3	14	5	17
Neutropenia	23	24	25	25	13	16	15	19
Fatigue	2	24	6	25	2	20	5	23
Nausea	1	24	2	25	2	22	2	23
Vomiting	0	15	0	21	5	20	6	21
Mucositis	0	8	0	11	0	4	0	8
Anorexia	0	17	0	22	1	15	4	18
Constipation	0	17	0	19	0	13	0	15
Diarrhoea	0	10	0	15	1	7	1	11
Hypertension	1	10	2	13	1	3	2	4
Encephalopathy	1	10	1	14	2	9	3	14
ALAT	0	13	0	19	0	11	1	12
ASAT	0	14	1	19	0	12	0	16
Hypophosphatemia	2	11	5	13	1	12	5	17
Proteinuria	1	11	1	12	0	9	0	15

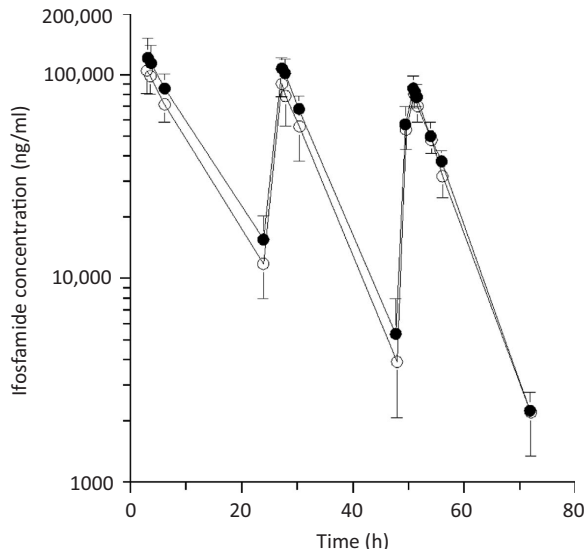
Abbreviations: ALAT, alanine aminotransaminase; ASAT, aspartate aminotransferase; BIV, bolus intravenous infusion; CIV, continuous intravenous infusion.

In contrast, pazopanib concentrations declined by ~35% within 72 h during concomitant ifosfamide infusion in patients in the dose escalation phase during CIV (Figure 4). There seems to be a time-dependent effect, resulting in comparable median plasma pazopanib concentrations at the end of ifosfamide infusion across dose-levels of pazopanib (Figure 4). There seems to be a time-dependent effect, resulting in comparable median plasma pazopanib concentrations at the end of ifosfamide infusion across dose-levels of pazopanib (Figure 4).

PK analysis in patients treated in the dose expansion phase revealed that the mean AUC (0–24) of pazopanib was reduced by ~27% upon co-administration of ifosfamide as compared with the AUC of pazopanib single agent (Figure 5).

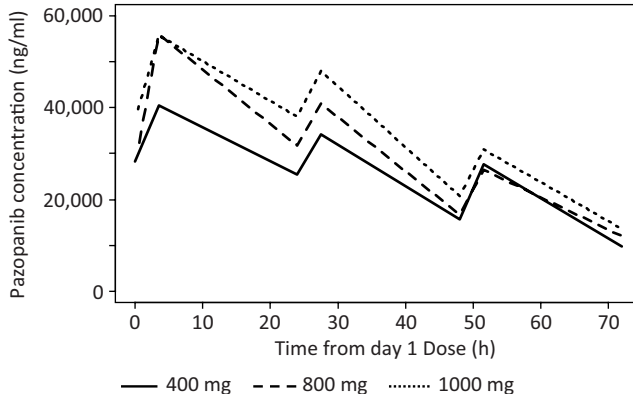


**Figure 2** | Mean concentrations (plus SD) of ifosfamide administered alone (closed symbols: bars up) or in combination with pazopanib (open symbols, bars down) during the 3-days continuous infusions.

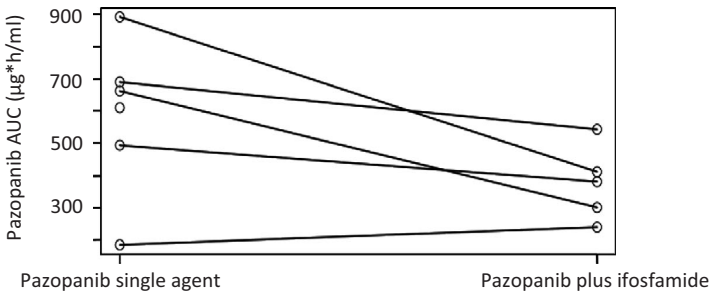


**Figure 3** | Mean concentrations (plus SD) of ifosfamide administered alone (closed symbols, bars up) or in combination with pazopanib (open symbols, bars down) during 3 consecutive bolus infusion days.





**Figure 4 |** Median plasma pazopanib concentration time profile (dose escalation, in patients with continuously given ifosfamide).



**Figure 5 |** Pazopanib area-under-curve (AUC) administered as single agent (day 21, cycle 1) versus combined administration with continuous ifosfamide (day 3, cycle 2) in the dose expansion phase.

**Biomarker analysis**

During the treatment with pazopanib, there was a dose-dependent increase in PIGF and VEGF-A with a concurrent decline in sVEGFR2 (Table 3). Importantly, this phenomenon remained intact after the addition of ifosfamide. No consistent pattern was seen by enumeration of CEC (data not shown).

**Anti-tumour activity**

Of 45 patients evaluable for response, 10 partial responses were observed: 4 in the CIV-treated patients (2 patients with synovial sarcoma, 1 each with ovarian and prostate cancer) and 6 patients in the BIV schedule (urothelial cancer (*n*=2), one each with sarcoma not otherwise specified, mesothelioma, ovarian cancer and an cancer of unknown primary).



Prolonged disease stabilisation defined as stable disease for at least 3 months was noted in nine and five patients in the CIV and BIV group, respectively.

**Table 3** | Biomarkers in the dose escalation cohorts (baseline normalised to 1)

Ifosfamide schedule	Biomarker	Pazopanib dose	Baseline	After 7 days pazopanib but prior to first ifosfamide	Prior to second ifosfamide
CIV	PIGF	400	1	1.75	1.35
		800	1	6.87	8.46
		1000	1	7.13	4.39
	VEGF-A	400	1	1.08	0.54
		800	1	5.26	5.72
		1000	1	3.85	2.73
	sVEGFR2	400	1	0.89	0.88
		800	1	0.77	0.70
		1000	1	0.76	0.69
BIV	PIGF	200	1	1.81	1.75
		400	1	2.40	3.24
	VEGF-A	200	1	1.72	3.12
		400	1	1.96	2.20
	sVEGFR-2	200	1	0.89	0.81
		400	1	0.79	0.77

Abbreviations: BIV, bolus intravenous infusion; CIV, continuous intravenous infusion; PIGF, placental-derived growth factor; sVEGFR2, soluble VEGFR2; VEGF-A, vascular endothelial growth factor-A.

## DISCUSSION

In this study, we explored safety and tolerability of the combination of pazopanib and ifosfamide, and its possible dependence on the ifosfamide infusion schedule. Pazopanib in combination with BIV-administered ifosfamide turned out to be intolerable. In contrast, if ifosfamide is continuously administered over 3 days, pazopanib could be escalated to dose levels exceeding the registered single agent dose of 800 mg daily. The inclusion of a dose higher than the registered single agent dose was done, since in a previous study a drug-drug interaction between ifosfamide and sunitinib resulted in a decreased exposure to sunitinib.<sup>11</sup> This observation was confirmed in the current study, showing a PK interaction between pazopanib and ifosfamide.

Our study clearly underlines that the administration schedule of one of the drugs of a combination can have a major impact on the tolerability of the combination. One of the

reasons that could account for the observed differences in tolerability between the two schedules of ifosfamide might be a schedule-dependent drug-drug interaction. Previously, pazopanib has demonstrated PK interactions with conventional chemotherapy, resulting in higher exposure to paclitaxel or the combination of paclitaxel and carboplatin.<sup>14-16</sup> In contrast, combining pazopanib with full-dose gemcitabine was feasible up to 800 mg pazopanib and no PK interaction was found.<sup>17</sup> Currently, extensive PK analysis did not show an impact of pazopanib on serum levels of ifosfamide or its metabolites.

In contrast, pazopanib exposure was clearly lowered by concurrent infusion of ifosfamide. This effect of ifosfamide on pazopanib levels yielded in similar pazopanib concentrations at the end of the ifosfamide infusion, regardless of the dose of pazopanib given. A potential underlying mechanism might be ifosfamide's inductive effects on CYP3A<sup>18</sup> for which pazopanib, in addition to being a weak inhibitor, is also a substrate.<sup>19</sup> If the induction of CYP3A by ifosfamide is indeed the cause of the decreased pazopanib exposure, this could imply that other drugs able to induce CYP3A should be avoided during pazopanib therapy but this warrants further exploration.

Yet, this PK interaction does not explain the striking difference in tolerability between the CIV and BIV schedule. In a study in patients with soft tissue sarcoma, BIV and CIV administrations of ifosfamide were compared demonstrating CIV to be slightly less toxic in terms of a lower rate of treatment discontinuation due to toxicity and a lower incidence of grade 3–4 dyspnoea and infection. However, CIV induced a higher incidence of grade 3–4 anaemia and grade 2–3 nausea than BIV ifosfamide.<sup>7</sup> The higher incidences of grade 3–4 neutropenia in the CIV schedule (88%; 23 out of 26 patients) compared with the BIV (48%; 13 out of 27 patients) observed in this study were in contrast with the study by Lorigan et al in which comparable incidences in CIV- and BIV-treated patients for grade 3–4 neutropenia incidence (62.7% vs 60.0%) were found.<sup>7</sup> This strongly suggests that the potentiating effect of pazopanib on the occurrence of neutropenia depends on the chosen infusion schedule. Auto-induction of ifosfamide is 52% higher during bolus infusion as compared with continuous infusion<sup>20</sup> and as a result higher concentrations of ifosfamide are generated. Accordingly, the AUC<sub>0–72</sub> of ifosfamide in our trial was indeed higher during bolus infusions as compared with the continuous infusion (3149 µg\*h/ml vs 2234 µg\*h/ml, respectively).

Three cytokines known to reflect biological effects from VEGFR-TKIs showed alterations in PlGF, VEGF-A and sVEGFR levels following exposure to pazopanib consistent with inhibition of VEGFR2 activity.<sup>21</sup> Ifosfamide did not nullify these pazopanib-induced biological effects, so ifosfamide does not hinder pazopanib to exert biological effects despite its lowering effects on pazopanib-levels.

This study shows that the upper boundary of safe dosing is, at least, 1000 mg per day pazopanib with CIV ifosfamide 9 g/m<sup>2</sup>. Like the single-agent pazopanib dose-finding study, the tolerable dose is higher than the currently approved dose, chosen on the basis of PK parameters and analyses of biological activity.<sup>11</sup> On the basis of the facts that the exposure

to pazopanib during ifosfamide is comparable in patients using 1000 mg per day and 800 mg per day after 48 h (Figure 4) and that the pharmacodynamic parameters showed that the short period of lower pazopanib levels using 800 mg compared with 1000 mg pazopanib did not have any effects on the pazopanib-induced biological effects, both in line with findings from the phase I trial on pazopanib monotherapy,<sup>12</sup> we recommend as dose for further studies 800 mg pazopanib and CIV ifosfamide 9 g/m<sup>2</sup>.

Importantly, this is the first phase I study to apply the 3 + 3 + 3 design. This pre-planned design for combination phase I trials aims to eliminate chances of falsely halting dose escalation, based on a high a priori chance of developing a DLT of one of the drug.<sup>10</sup> In the CIV arm, two out of the first six patients at the 1000 mg pazopanib dose level experienced a DLT. According to the conventional 3 + 3 design, this would have been interpreted as toxicity exceeding MTD. However, no DLTs were encountered in the other nine patients enrolled in this dose level. This strongly underscores the clinical applicability of this 3 + 3 + 3 approach in establishing the tolerability of drug combinations.

In conclusion, this study has clearly demonstrated that tolerability of pazopanib and ifosfamide is dependent on the infusion schedule. An evident explanation for the observed differences is not readily available. Furthermore, ifosfamide appeared to lower pazopanib levels, but despite the lower levels of pazopanib, it still exerted biological activity. In addition, this study stresses the importance of the 3 + 3 + 3 design for exploring drug combinations in phase I studies when one of the agents is known to induce high rates of toxicity. Last, based upon our data the dose recommended for pazopanib when combined with CIV ifosfamide 9 g/m<sup>2</sup> is 800 mg, while a combination with bolus ifosfamide is not feasible. Further studies on the combination of pazopanib and ifosfamide are currently being designed.



## REFERENCES

1. Boucher Y and Jain RK. Microvascular pressure is the principal driving force for interstitial hypertension in solid tumors: implications for vascular collapse. *Cancer Res* 1992; **52**:5110-14.
2. Heldin CH, Rubin K, Pietras K, et al. High interstitial fluid pressure- an obstacle in cancer therapy. *Nat Rev Cancer* 2004; **4**:806-13.
3. Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**:145-7.
4. Hamberg P, Verweij J, Sleijfer S. (Pre-)clinical pharmacology and activity of pazopanib, a novel multikinase angiogenesis inhibitor. *The Oncologist* 2010; **15**:539-47.
5. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2010; **28**:1061-8.
6. Van der Graaf WT, Blay JY, Chawla SP, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double blind, placebo-controlled phase 3 trial. *Lancet* 2012; **379**:1879-86.
7. Lorigan P, Verweij J, Papai Z, et al. Phase III trial of two investigational schedules of ifosfamide compared with standard-dose doxorubicin in advanced or metastatic soft tissue sarcoma: a European Organisation for Research and Treatment of Cancer soft tissue and bone sarcoma group study. *J Clin Oncol* 2007; **25**:3144-50.
8. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**:228-47.
9. Hamberg P, Ratain MJ, Lesaffre E, Verweij J. Dose-escalation models for combination phase I trials in oncology. *Eur J Cancer* 2010; **46**:2870-8.
10. Hamberg P, Steeghs N, Loos WJ, et al. Decreased exposure to sunitinib due to concomitant administration of ifosfamide: results of a phase I and pharmacokinetic study on the combination of sunitinib and ifosfamide in patients with advanced solid malignancies. *Br J Cancer* 2010; **102**:1699-1706
11. Hurwitz HI, Dowlati A, Saini S, et al. Phase I trial of pazopanib in patients with advanced cancer. *Clin Cancer Res* 2009; **15**:4220-7.
12. Kraan J, Strijbos MH, Sieuwerts AM, et al. A new approach for rapid and reliable enumeration of circulating endothelial cells in patients. *J Thromb Haemost* 2012; **10**:931-9.
13. Tan AR, Dowlati A, Jones SF, et al. Phase I study of pazopanib in combination with weekly paclitaxel in patients with advanced solid tumors. *Oncologist* 2010; **15**:1253-61.
14. Burris HA3rd, Dowlati A, Moss RA, et al. Phase I study of pazopanib in combination with paclitaxel and carboplatin given every 21 days in patients with advanced solid tumors. *Mol Cancer Ther* 2012; **11**: 1820-8.
15. Du Bois A, Vergote I, Wimberger P, et al. Open-label feasibility study of pazopanib, carboplatin, and paclitaxel in women with newly diagnosed, untreated, gynaecologic tumours: a phase I/II trial of the AGO study group. *Br J Cancer* 2012; **106**:629-32.
16. Plummer R, Madi A, Jeffels M, et al. A phase I study of pazopanib in combination with gemcitabine in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 2013; **71**:93-101.
17. Boddy AV, Cole M, Pearson AD, et al. The kinetics of the auto-induction of ifosfamide metabolism during continuous infusion. *Cancer Chemother Pharmacol* 1995; **36**:53-60.
18. Goh BC, Reddy NJ, Dandamudi UB, et al. An evaluation of the drug interaction potential of pazopanib, an oral vascular endothelial growth factor receptor tyrosine kinase inhibitor, using a modified cooperstown 5+1 cocktail in patients with advanced solid tumors. *Clin Pharmacol Ther* 2010; **88**:652-9.
19. Kerbusch T, Mathot RAA, Keizer HJ, et al. Influence of dose and infusion duration on pharmacokinetics of ifosfamide and metabolites. *Drug Metab Dispos* 2001; **29**:967-75.
20. Sleijfer S, Gorlia T, Lamers C, et al. Cytokine and angiogenic factors associated with efficacy and toxicity of pazopanib in advanced soft tissue sarcoma: an EORTC-STBSG study. *Br J Cancer* 2012; **107**:639-45.

Chapter

7

**Impact of pazopanib on docetaxel exposure: results of a phase I combination study with two different docetaxel schedules**

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## ABSTRACT

**Background:** There are several reasons why combining an inhibitor of the vascular endothelial and the platelet derived growth factor receptor (VEGFR and PDGFR) with a taxane might induce synergistic anti-tumor activity. This phase I study aimed to determine the maximal tolerated dose (MTD) of the combination of pazopanib with two different schedules of docetaxel.

**Patients and Methods:** In a 3+3+3 design, patients with advanced solid tumors received escalating doses of oral pazopanib combined with docetaxel either given every 3 weeks (D3w) or weekly at days 1,8 and 15 every 28 days (D1w). Pharmacokinetic data of docetaxel and pazopanib were obtained through extensive sampling and WinNonlin modelling.

**Results:** Forty-six patients were enrolled to 6 dose-levels. Both schedules of docetaxel could be combined with 400 mg/day pazopanib. The MTD of D3w docetaxel was 50 mg/m<sup>2</sup>, while for D1w MTD it was 20 mg/m<sup>2</sup>. In the D3w schedule, the administration of pazopanib led to a 33% lower docetaxel clearance (mean 31.5 *versus* 21.1 L/h/m<sup>2</sup>; *P*=0.019) and >50% increase in AUC<sub>0-∞</sub> (mean 1,602 *versus* 2,414 ng\*h/mL; *P*=0.029) compared to docetaxel single agent data. Data for the D1w schedule were comparable.

**Conclusion:** Both treatment schedules of docetaxel combined with pazopanib are feasible but at doses for both drugs that are considerably lower than the recommended single agent doses. This is largely due to a clinically relevant pharmacokinetic interaction with pazopanib, substantially increasing docetaxel exposure. This interaction is most likely due to CYP3A4 and OATP1B1 inhibition.

## INTRODUCTION

Activation of the signalling transduction pathways of the vascular endothelial growth factor receptor (VEGFR)- as well as the platelet derived growth factor receptor (PDGFR) are thought to be involved in tumor initiation and progression in various tumor types. In the last decade, several compounds targeting these pathways have been approved in several tumor types. Combining such agents with conventional cytotoxic agents has been explored quite extensively, as it is believed that based upon the unique mechanisms of action, these combinations might have synergistic anti tumor activity. Reduction of interstitial pressure, (pseudo-)normalisation of tumor-vasculature increasing drug penetration and blocking the mobilisation of endothelial progenitor cells induced by cytotoxics have been considered to be biological mechanisms underlying this synergism.<sup>1</sup>

Pazopanib is an orally available potent tyrosine kinase inhibitor known to inhibit VEGFR1-3, PDGFR  $\alpha$  and  $\beta$ , and *c-Kit*<sup>2</sup> and has been approved for treatment of patients with metastatic renal cell carcinoma and advanced non-adipocytic soft tissue sarcomas.<sup>3,4</sup> The recommended dose of pazopanib is 800 mg once daily with fatigue, diarrhea, nausea, hypertension and elevated liver-enzymes as most common toxicities.<sup>4</sup> Pazopanib is metabolized mainly through CYP3A4 mediated conversion to inactive metabolites. Patients with impaired liver functions do not tolerate pazopanib at its recommended dose, although this cannot fully be explained by pharmacokinetics.<sup>5</sup> Co-medication, influencing the absorption and metabolism of pazopanib is known to dramatically alter the area under the curve (AUC) of this drug.<sup>6</sup>

Docetaxel, a semisynthetic taxane, is one of the most commonly used antitumor agents and acts by disrupting the essential microtubular network in cells. Docetaxel is an intravenously administered anticancer drug with marketing approval for the treatment of a range of solid tumors including breast, prostate, lung and gastric carcinoma. Although highly active, docetaxel is well known for its side-effects, including (febrile) neutropenia and neurotoxicity. In addition, docetaxel is prone for an altered exposure due to concomitantly administered medication. This is partly due to its phase I metabolism (mainly through CYP3A iso-enzymes), and probably also because of its hepatic transport via OATP1B.<sup>7-9</sup>

This study aimed to determine the feasibility and the maximal tolerated doses (MTD) of the combination of pazopanib with two different schedules of docetaxel, and to assess potential interactions between these drugs.



## PATIENTS AND METHODS

Patients with advanced or metastatic solid tumors for whom docetaxel-based therapy was considered appropriate, or for whom no standard therapy was available, were eligible. Other inclusion criteria included: ECOG performance status <2, evaluable or measurable disease according to RECIST 1.1,<sup>10</sup> age ≥18 years, and systolic blood pressure <160 mmHg and diastolic blood pressure <90 mmHg (two antihypertensive drugs were allowed to control blood pressure). At laboratory evaluation an adequate bone marrow (hemoglobin ≥5 mmol/L; platelets ≥100 × 10<sup>9</sup>/L, absolute neutrophil count ≥1.5 × 10<sup>9</sup>/L), liver (bilirubin <1.25 upper limit of normal (ULN); serum alanine and aspartate aminotransferase <2 × ULN; alkaline phosphatase <2.5 × ULN), and renal function (creatinine clearance ≥ 50 mL/min and proteinuria grade 0 or 1) was also an entry criterion. Main exclusion criteria were: history of cardiovascular disease other than hypertension, and signs/symptoms of central nervous system metastases.

The study was approved by the institutional review board (MEC-2009-462) and conducted in accordance with the principles embodied in the Declaration of Helsinki. Written informed consent was obtained from each participant prior to enrolment.

### Study design

Daily oral pazopanib was evaluated in combination with intravenously administered docetaxel, either given every 3 weeks (D3w) or weekly on days 1, 8 and 15 every 28 days (D1w). First, the MTD with D3w was determined while alternately escalating pazopanib and docetaxel (see Table 1 for the chosen dose-escalation steps). In D1w the starting dose of pazopanib was one dose level lower than the MTD in D3w. For docetaxel the starting dose was also based upon the recommended dose of the D3w schedule using a conversion table. For both the D3w and D1w schedules, dose de-escalation guidelines were incorporated if MTD was exceeded at the first dose level.

The 3+3+3 design, a novel model recently proposed aiming to reduce falsely halting dose escalation in combination phase I trials, was included in the original protocol.<sup>11</sup> Briefly, if a dose limiting toxicity (DLT) was observed in one patient, three additional patients were recruited at that dose level. The dose escalation proceeded, if no further DLT occurred at that specific dose level. If a DLT was observed in 2 out of 6, another three additional patients were enrolled. If a DLT occurred in 2 out of 3, >2 out of 6, or >2 out of 9 patients in a cohort, the MTD had been exceeded. The MTD was defined as the highest dose level with a DLT incidence < 33% out of at least 9 patients.

In order to be exposed to steady-state concentrations of pazopanib and to adequately determine effects of docetaxel administration on pazopanib PK, patients in the dose-escalation phase started on pazopanib seven days prior to the first docetaxel administration. At the MTD, six additional patients were treated in an expansion cohort



to get better insight into the safety profile, to confirm the MTD, and to further study the putative pharmacokinetic interaction. For the latter, pazopanib was started one day after the first docetaxel administration, enabling intra-patient comparison of docetaxel PK with and without pazopanib.

Using the National Cancer Institute – Common Terminology Criteria (NCI-CTC) for adverse events, version 3.0, a DLT during the first treatment cycle (in the dose expansion phase during the first two treatment cycles) was defined as: any toxicity giving rise to a dose omission on day 8 or 15 docetaxel in the weekly schedule; febrile neutropenia (FN); grade 4 neutropenia  $\geq 7$  days; grade 4 thrombocytopenia; grade 3–4 proteinuria or any drug-related grade 3–4 non-haematological toxicity. Hypertension was considered DLT in case of symptomatic hypertension; persistent ( $>24$  hours) and asymptomatic systolic blood pressure (BP)  $\geq 170$  mmHg and/or diastolic BP  $\geq 110$  mmHg; systolic BP 160–170 and/or diastolic BP 90–110 that could not be controlled within two weeks. A dose delay or interruption for longer than two weeks was classified as DLT.

Patients were treated for a maximum of six docetaxel cycles. Patients experiencing clinical benefit from the combination of pazopanib and docetaxel were allowed to continue treatment thereafter with pazopanib until disease progression or unacceptable toxicity.

### Pharmacokinetic sampling and analysis

For the analysis of docetaxel 11 (in the escalation phase) or 14 (in the expansion phase) plasma samples per patient were obtained and quantitated as previously reported.<sup>12</sup>

For the analysis of pazopanib, 7 (in the escalation phase) or 14 (in the expansion phase) samples per patient were drawn and quantitated as previously reported.<sup>13</sup>

### Statistical Data Analysis

Plasma concentrations of docetaxel were plotted as a function of time. Areas under the curves extrapolated to infinity ( $AUC_{0-\infty}$ ) were calculated using the trapezoidal method. Non-compartmental PK analysis was calculated using the software package Phoenix WinNonlin (Pharsight, Mountain View, CA). Total body clearance of docetaxel was calculated by dividing the administered dose by the  $AUC_{0-\infty}$  of docetaxel. Statistical analyses were made using the software package SPSS version 20 (SPSS Inc). Correlation of the changes in AUC, peak plasma concentration ( $C_{max}$ ) or clearance was evaluated by a two-sided paired *t*-test for subjects in the expansion cohorts.

Pazopanib plasma concentration data was analyzed via non-compartmental analysis using Phoenix WinNonlin. Parameters recovered were  $C_{max}$  and  $AUC_{0-\tau}$  (area under the plasma concentration-time curve within a 24h dosing interval). Parameters were summarized as means  $\pm$  SD.



**Table 1** | Dose escalation steps (D3w: docetaxel every 3 weeks; D1w: docetaxel weekly on days 1, 8 and 15 every 28 days; DLTs: dose-limiting toxicities)

	Pazapanib-dose mg/day	Docetaxel-dose mg/m <sup>2</sup>	Number of evaluable patients	DLTs
<b>D3w</b>				
Dose level 1	400	60	5	3
Dose level 2	400	50	6	0
Dose level 3	600	50	3	2
Dose level 2 expansion	400	50	6	1
<b>D1w</b>				
Dose level 1	200	20	6	1
Dose level 2	400	20	6	1
Dose level 3	400	25	3	2
Dose level 2 expansion	400	20	6	2

## RESULTS

### Dose escalation, MTD and dose intensity

Overall, 46 patients (equally distributed over D3w and D1w) were enrolled. Five patients were not evaluable (Table 2). Most of the non-evaluable patients did not receive the combination of drugs, for example because of early progression or clinical deterioration.

Table 1 summarizes the dose levels and the observed DLTs per dose level. In D3w, 3 DLTs were observed in five patients at the first dose level (400 mg pazopanib plus docetaxel 60 mg/m<sup>2</sup>): one episode of febrile neutropenia, two episodes of mucositis grade 3. At the dose level of 400 mg pazopanib plus docetaxel 50 mg/m<sup>2</sup> no DLT was observed in six patients. Subsequent escalation of pazopanib to 600 mg plus docetaxel 50 mg/m<sup>2</sup> resulted in two out of three patients with a DLTs: one episode of grade 3 increased transaminases and one episode of prolonged neutropenia grade 4 for more than eight days. Dose expansion was performed with six patients at 400 mg pazopanib plus docetaxel 50 mg/m<sup>2</sup>, resulting in one additional DLT: cardiac failure resulting in therapy-refractory cardiogenic shock (grade 5) in a patient with pre-existent hypertension.

At the MTD, the relative dose intensity of docetaxel and pazopanib was 98.6% and 95.5% respectively, with a median number of four combination therapy cycles.

In the D1w schedule, one DLT (grade 3 increased transaminases) was observed in the first cohort of 200 mg pazopanib plus docetaxel 20 mg/m<sup>2</sup>. At the second dose level (400 mg pazopanib plus docetaxel 20 mg/m<sup>2</sup>) one DLT consisting of ataxia grade 2 resulting in dose omission of docetaxel was observed. The third dose level (400 mg pazopanib plus

docetaxel 20 mg/m<sup>2</sup>) exceeded the MTD based upon the observation of two DLTs (one episode of grade 3 encephalopathy considered to be related to pazopanib and one episode of grade 3 nausea). Six patients were enrolled in the dose expansion (400 mg pazopanib plus docetaxel 20 mg/m<sup>2</sup>) giving rise to two additional DLTs: one episode of grade 3 fatigue and one episode of grade 3 haemorrhage, resulting in 3 DLTs in 12 patients in total at this dose level.

**Table 2 |** Demographics and baseline characteristics

<b>Age (median, years)</b>	<b>58 (31–73)</b>
<b>Gender</b>	
Male	29
Female	17
<b>WHO performance score</b>	
0	6
1	40
<b>Tumor type</b>	
Sarcoma	
Gastro-intestinal stromal tumor	5
Other types sarcoma	4
Urothelial Cancer	6
Gastric cancer	6
Oesophageal cancer	3
Pancreatic cancer	4
Colorectal cancer	2
Head and neck squamous cell cancer	4
Breast Cancer	2
Adenoid cystic carcinoma	2
Cutaneous squamous cell cancer	2
Miscellaneous	6
<b>Previous number of non-hormonal systemic anticancer treatment</b>	
0	8
1	18
2	14
3	3
4	0
5	3



At the MTD the relative dose intensity of docetaxel and pazopanib was 92.6% and 88.5% respectively, with a median number of two combination therapy cycles.

## Toxicity

Neutropenia was the most frequently observed grade 3/4 toxicity in D3w, while it hardly occurred in D1w. (Table 3) Other toxicities were comparable between D3w and D1w, with fatigue, gastrointestinal toxicity (nausea, vomiting, constipation and diarrhea), anorexia, increased transaminases and proteinuria occurring most frequently. The majority of toxicities occurring during combination therapy became apparent during the first cycle. (Table 3)

**Table 3** | Most frequent adverse events during the first and during all combination cycles (D3w: docetaxel every 3 weeks; D1w: docetaxel weekly on days 1, 8 and 15 every 28 days)

	D3w (n=20)				D1w (n=21)			
	First cycle adverse events		Adverse events at all combination cycles		First cycle adverse events		Adverse events at all combination cycles	
	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4	All grades	Grade 3/4	All grades
Anaemia	0	14	1	19	0	14	0	15
Thrombocytopenia	0	1	0	3	0	3	0	3
Neutropenia	14	16	14	17	0	1	1	1
Fatigue	1	18	5	19	1	20	3	20
Nausea	0	9	0	14	0	11	1	16
Vomiting	1	7	2	10	0	5	1	8
Mucositis	2	8	2	11	0	6	0	8
Anorexia	0	12	1	18	0	11	0	15
Constipation	0	8	0	11	0	14	0	14
Diarrhea	0	11	0	13	0	11	0	15
Hypertension	2	2	2	3	2	7	2	9
Sensory neuropathy	0	6	0	11	0	11	0	12
ALAT	1	9	1	11	3	11	4	12
ASAT	1	11	1	14	1	12	2	13
Proteinuria	0	14	0	14	0	13	0	16

Abbreviations; ALAT, alanine aminotransaminase; ASAT aspartate aminotransferase

## Pharmacokinetics

In D3w the concurrent administration of pazopanib resulted in a 33% lower docetaxel clearance (mean 31.5 versus 21.1 L/h/m<sup>2</sup>;  $P=0.019$ ) and more than 50% increase in AUC<sub>0-∞</sub> (mean 1,602 versus 2,414 ng\*h/mL;  $P=0.029$ ) compared to docetaxel single agent data (Table 4 and Figure 1A). Also in D1w, a clinically relevant (39%) decrease in docetaxel clearance during concomitant administration of pazopanib was observed (mean 25.4 versus 18.3 L/h/m<sup>2</sup>;  $P=0.043$  (Table 4 and Figure 1B).

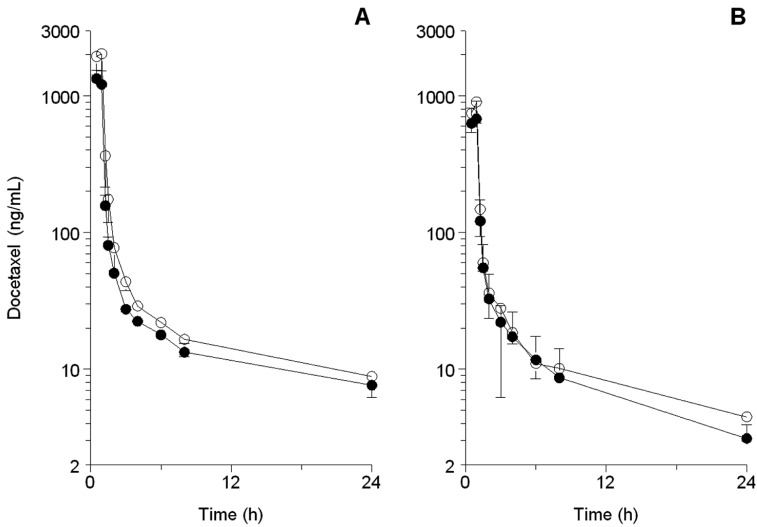
The mean pazopanib C<sub>max</sub> and AUC<sub>0-τ</sub> did not change significantly ( $P>0.405$ ) when dosed in combination with docetaxel, compared to pazopanib monotherapy. (Table 4 and Figure 2). A ~30% increase in docetaxel exposure when co-administered with pazopanib was confirmed by a geometric mean ratio approach.

**Table 4** | Pharmacokinetic parameters (mean and ranges within brackets) of docetaxel and pazopanib in de dose expansion cohorts at the maximally tolerated dose (docetaxel 50 mg/m<sup>2</sup> given every 3 weeks (D3w) or docetaxel 20 mg/m<sup>2</sup> weekly on days 1, 8 and 15 every 28 days (D1w); 400 mg per day pazopanib for D3w and D1w).

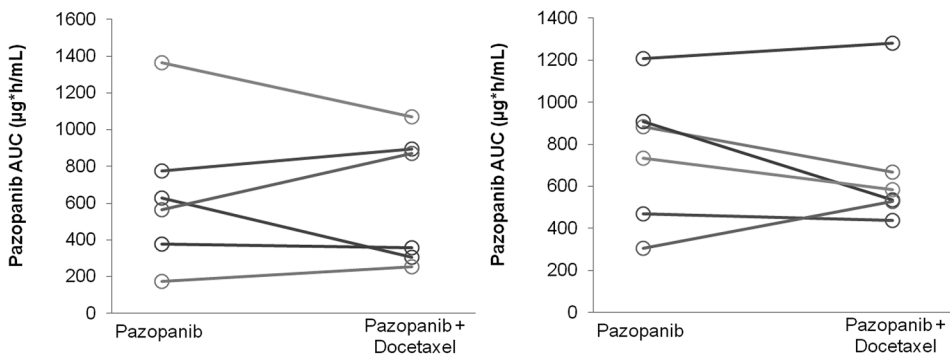
Docetaxel PK	D3w			D1w		
	Without pazopanib	With pazopanib	<i>p</i> -value	Without pazopanib	With pazopanib	<i>p</i> -value
Docetaxel clearance (L/h/m <sup>2</sup> )	31.5 (28.5–34.2)	21.1 (18.0–24.3)	0.019	25.4 (14.1–32.3)	18.3 (16.0–23.9)	0.043
AUC <sub>0-∞</sub> (ng*h/mL)	1602 (1462–1735)	2414 (2060–2774)	0.029	857 (620–1417)	1113 (837–1249)	0.072
C <sub>max</sub> (ng/mL)	1415 (1195–1625)	2085 (1394–2558)	0.075	694 (457–1073)	903 (543–1336)	0.101
Pazopanib PK	Without docetaxel	With docetaxel		Without docetaxel	With docetaxel	
C <sub>max</sub> (μg/mL)	34.1 (9.66–63.9)	38.5 (12.2–53.0)	0.429	35.7 (21.1–67.8)	39.3 (16.3–60.1)	0.506
AUC <sub>0-τ</sub> (μg*h/mL)	647 (176–1363)	625 (254–1070)	0.837	672 (437–1280)	752 (305–1208)	0.405

Abbreviations: AUC, area under the curve; C<sub>max</sub>, peak plasma concentration





**Figure 1** | Mean concentrations (plus SD) of docetaxel administered alone (closed symbols, bars up) or in combination with pazopanib (open symbols, bars down) once daily pazopanib in combination with docetaxel administered at 3-weekly interval (A) and 1-weekly interval (B).



**Figure 2** | Individual pazopanib  $AUC_{0-t}$  after administration of 400 mg pazopanib alone, or in combination with docetaxel (Panel 2A: docetaxel 50 mg/m<sup>2</sup> given every 3 weeks (D3w) or docetaxel 20 mg/m<sup>2</sup> weekly on days 1, 8 and 15 every 28 days (D1w)

**Antitumor activity**

Of the 15 evaluable patients in D3w one patient with metastatic breast cancer, previously treated with 5 different chemotherapy schedules including paclitaxel, achieved a partial response. In eight other patients, a progression-free survival of at least 3 months was

observed (2 patients with adenoid cystic carcinoma, and one patient with each one of the following entities: Gastro-intestinal stromal tumor; myoepithelial carcinoma; synovial sarcoma; pancreatic cancer; esophageal cancer and submandibular gland tumor). Of the 15 evaluable patients allocated to the D1w schedule, one patient with metastatic urothelial cancer had a partial response, while two patients had a prolonged disease stabilisation for longer than three months: one patient with a gastro-intestinal stromal tumor and one patient with gastric cancer.

## DISCUSSION

The current trial evaluated the safety, tolerability, and pharmacokinetics of intravenously administered docetaxel, given in two different schedules, combined with orally taken pazopanib. For both schedules, MTDs for both pazopanib and docetaxel could be established.

Importantly, in combination with docetaxel given either D3w or D1w, the MTD of pazopanib in this study was 400 mg/day, which is substantially lower than the MTD of single agent pazopanib at 800 mg daily. The same held true for docetaxel with MTDs of 50 mg/m<sup>2</sup> and 20 mg/m<sup>2</sup> for D3w and D1w, respectively, being significantly lower than the approved single agent dosages (75 to 100 mg/m<sup>2</sup> for D3w and 30 to 35 mg/m<sup>2</sup> for D1w, respectively). This significantly lower dose intensity of docetaxel in combination with pazopanib can be explained by a relevant drug-drug interaction, resulting in an up to 50% increased exposure to docetaxel when combined with pazopanib.

Our findings on this clinically relevant drug-drug interaction, although based on a small sample size, are in line with two other phase I studies, combining docetaxel with either sorafenib or axitinib, other VEGFR-targeting tyrosine kinase inhibitors.<sup>14,15</sup> Similar to the current study, both trials found increased docetaxel AUCs upon co-administration of the tyrosine kinase inhibitor.<sup>14,15</sup> Fortunately, in contrast to the impact of pazopanib on docetaxel PK, no consistent effect of docetaxel on the exposure to pazopanib could be detected.

Docetaxel and pazopanib are both substrates for CYP3A4, and therefore prone for possible drug-drug interactions if combined with each other.<sup>16</sup> In pre-clinical studies pazopanib was shown to be a weak CYP3A4 inhibitor. Similar to the current findings, concomitant administration of another taxane, paclitaxel, and pazopanib (800 mg) also resulted in a profound PK interaction, with an observed 18% decrease in clearance, a 26% increase in AUC, and a 36% increase in  $C_{max}$  of paclitaxel. This interaction is considered to be the result of the inhibition of CYP3A4 and/or CYP2C8.<sup>17</sup>

In contrast to paclitaxel, which is metabolized by two iso-enzymes, docetaxel is solely and almost completely metabolized by CYP3A4.<sup>9</sup> Consequently, it can be hypothesized that pazopanib increases the exposure to taxanes mainly by inhibiting CYP3A4 metabolism as



the increase in exposure to docetaxel as observed in our trial is in the same range as found for paclitaxel when combined with pazopanib. In addition, also the transport of docetaxel in the liver by OATP1B-type carriers may be influenced by pazopanib.<sup>9</sup> Recent *in vitro* studies, using HEK293 cells that expressed OATP1B1, explored the inhibiting effects of several tyrosine kinase inhibitors on this transporter. Also pazopanib was studied and showed a strong inhibition of OATP1B1.<sup>18</sup>

Remarkably, and despite previous indications of clinically relevant drug-drug interactions between TKIs and taxanes, two dose-finding studies on the triple combination of pazopanib, paclitaxel, and carboplatin started with full single agent doses for each of the drugs in the first dose level. Not surprisingly, both trials observed excessive toxicity, even after lowering the pazopanib dose to 400 mg.<sup>19,20</sup> One of these trials continued to explore full dose carboplatin with full dose paclitaxel with 200 mg pazopanib and this combination appeared to be feasible.<sup>20</sup> PK-analyses showed a pazopanib-induced increase in  $C_{\max}$  of paclitaxel and carboplatin of >40% and >54%, respectively.<sup>20</sup>

The observed patterns of DLTs and toxicity profile of the combination of pazopanib and docetaxel were in accordance with the known single agent side effects of both study drugs. No remarkable patterns were detected and in general this combinations at the MTD were well tolerated. It might be worthwhile to test the combination of pazopanib with docetaxel for its efficacy in patients with for example breast, lung or gastric cancer.

In summary, 400 mg of pazopanib can be safely combined with docetaxel when given at either 50 mg/m<sup>2</sup> D3w or 20 mg/m<sup>2</sup> D1w. A significant effect of pazopanib on docetaxel exposure, as observed by a 34% increase in AUC, was found. This effect is most likely based on CYP3A4 and OATP1B inhibition by pazopanib. However, at the recommended doses obtained from this study, the combination of pazopanib and docetaxel can safely be administered and further evaluation assessing potential antitumor activity is proceeding.



## REFERENCES

1. Boere IA, Hamberg P, Sleijfer S, It takes two to tango: combinations of conventional cytotoxics with compounds targeting the VEGF-VEGFR pathway in patients with solid malignancies. *Cancer Sci* 2010; **101**:7-15
2. Hamberg P, Verweij J, Sleijfer S. (Pre-)clinical pharmacology and activity of pazopanib, a novel multikinase angiogenesis inhibitor. *Oncologist* 2010; **15**:539-47.
3. Sternberg CN, Davis ID, Mardiak J, et al. Pazopanib in locally advanced or metastatic renal cell carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2010; **28**:1061-8.
4. Van der Graaf WT, Blay JY, Chawla SP, et al. Pazopanib for metastatic soft-tissue sarcoma (PALETTE): a randomised, double blind, placebo-controlled phase 3 trial. *Lancet* 2012; **379**:1879-86.
5. Shibata SI, Chung V, Synold TW, et al. Phase I study of pazopanib in patients with advanced solid tumors and hepatic dysfunction: a National Cancer Institute Organ Dysfunction Working Group study. *Clin Cancer Res* 2013; **19**:3631-9.
6. Van Leeuwen RW, van Gelder T, Mathijssen RH, et al. Drug-drug interactions with tyrosine kinase inhibitors: a clinical perspective. *Lancet Oncol*, 2013; In press.
7. Engels FK, ten Tije AJ, Baker SD, et al. Effect of cytochrome P450 3A4 inhibition on the pharmacokinetics of docetaxel. *Clin Pharmacol Ther* 2004; **75**:448-454;
8. Goey AK, Meijerman I, Rosing H, et al. The effect of St John's wort on the pharmacokinetics of docetaxel. *Clin Pharmacokin* 2013 sept 26 [epub]
9. De Graan AJ, Lancaster CS, Obaidat A, et al. Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. *Clin Cancer Res* 2012; **18**:4433-40.
10. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer* 2009; **45**:228-47.
11. Hamberg P, Ratain MJ, Lesaffre E, et al. Dose-escalation models for combination phase I trials in oncology. *Eur J Cancer* 2010; **46**:2870-8.
12. De Graan AJ, Loos WJ, Friberg LE, et al. Influence of smoking on the pharmacokinetics and toxicity profiles of taxane therapy. *Clin Cancer Res* 2012; **18**:4425-32.
13. Hurwitz HI, Dowlati A, Saini S, et al. Phase I trial of pazopanib in patients with advanced cancer. *Clin Cancer Res* 2009; **15**:4220-7.
14. Awada A, Hendlisz A, Christensen O, et al. Phase I trial to investigate the safety, pharmacokinetics and efficacy of sorafenib combined with docetaxel in patients with advanced refractory solid tumours. *Eur J Cancer* 2012; **48**:465-74.
15. Martin LP, Kozloff MF, Herbst RS, et al. Phase I study of axitinib combined with paclitaxel, docetaxel or capecitabine in patients with advanced solid tumours. *Br J Cancer* 2012; **107**:1268-76.
16. Harris JW, Rahman A, Kim BR, et al.. Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res* 1994; **54**:4026-35.
17. Tan AR, Dowlati A, Jones SF, et al. Phase I study of pazopanib in combination with weekly paclitaxel in patients with advanced solid tumors. *Oncologist* 2010; **15**:1253-61.
18. Hu S, Mathijssen RHJ, de Bruijn P, et al. Inhibition of OATP1B1 by tyrosine kinase inhibitors: in vitro-in vivo correlations. *Br J Cancer* 2013. In press
19. Du Bois A, Vergote I, Wimberger P, et al. Open-label feasibility study of pazopanib, carboplatin and paclitaxel in women with newly diagnosed, untreated, gynaecologic tumours: a phase I/II trial of the AGO study group. *Br J Cancer* 2012; **106**:629-32.
20. Burris HA 3<sup>rd</sup>, Dowlati A, Moss RA, et al. Phase I study of pazopanib in combination with paclitaxel and carboplatin given every 21 days in patients with advanced solid tumors. *Mol Cancer Ther* 2012; **11**: 1820-8.





Chapter

# 8

**Randomized phase II study comparing efficacy and safety of combination-therapy trastuzumab and docetaxel vs sequential therapy of trastuzumab followed by docetaxel alone at progression as first-line chemotherapy in patients with HER2<sup>+</sup> metastatic breast cancer: HERTAX trial**

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## ABSTRACT

**Background:** Because chemotherapy for metastatic breast cancer (MBC) is associated with relevant toxicity, sequential monotherapy trastuzumab followed by cytotoxic therapy at disease progression might be an attractive approach.

**Methods:** In a multicenter phase II trial, 101 patients with overexpression of human epidermal growth factor receptor 2 (HER2<sup>+</sup>) MBC were randomized between combination-therapy trastuzumab (Herceptin) plus docetaxel (H+D) and sequential therapy of single-agent trastuzumab followed at disease progression by docetaxel alone (H→D) as first-line chemotherapy for metastatic disease. The primary endpoint was progression-free survival (PFS) after completed sequential or combination therapy.

**Results:** For the H+D group the median PFS was 9.4 vs. 9.9 months for the H→D group and 1-year PFS rates were 44% vs. 35%, respectively. However the overall response rates (ORRs) were 79% vs 53%, respectively ( $P=.016$ ), and overall survival was 30.5 vs 19.7 months, respectively ( $P=.11$ ). In the H→D group, response rates to monotherapy trastuzumab and subsequent docetaxel were 34% and 39%, respectively, with a median PFS during single-agent trastuzumab of 3.9 months. The incidence and severity of neuropathy were significantly higher in the H+D group. Retrospective analysis of trastuzumab treatment beyond progression (applied in 46% of patients in the H+D group and 37% in the H→D group) showed a correlation with longer overall survival in both treatment arms (36.0 vs 18.0 months and 30.3 vs. 18.6 months, respectively).

**Conclusion:** First-line treatment in patients with MBC with H→D resulted in a similar PFS compared with H+D, but the response rate was lower and the overall survival nonsignificantly shorter.

## INTRODUCTION

Metastatic breast cancer (MBC) remains an incurable entity. The goals of therapy therefore are aimed at symptom palliation by control of disease progression with preservation of quality of life (QoL) and prolongation of survival. Given the fact that chemotherapy is associated with relevant side effects, treatment modalities whereby the introduction of chemotherapy may be delayed are worthwhile to explore.

Overexpression and/or amplification of human epidermal growth factor receptor 2 (HER2<sup>+</sup>) occurs in approximately 20% of breast cancers. It is associated with worse outcomes, including shortened overall survival (OS), compared with HER2<sup>-</sup> breast cancer.<sup>1,2</sup> Blocking HER2 by means of the monoclonal antibody trastuzumab is beneficial in patients with HER2<sup>+</sup> MBC and results in survival benefit.<sup>3-6</sup> Three trials have evaluated the activity of single-agent trastuzumab as first-line therapy for MBC,<sup>4,5,7</sup> whereas many more have investigated a combination regimen of trastuzumab and a variety of chemotherapeutic agents as first- to third-line therapy, mostly however in a nonrandomized setting.<sup>3,6-10</sup> Currently the combination of trastuzumab (Herceptin) and docetaxel (H+D) is considered one of the standard first-line cytotoxic treatment modalities for patients with HER2<sup>+</sup> MBC. The value of adding trastuzumab to docetaxel, however, has been studied only in the pivotal phase II trial (M77001) that randomly assigned 186 women to docetaxel with or without trastuzumab. This study showed superior outcomes for the combination regimen on all efficacy parameters, including a significant OS benefit (31.2 vs 22.7 months).<sup>6</sup>

For a long time, the issue of combination vs. sequential chemotherapy has been controversial. As this issue has not been well studied, it remains a matter of debate. In general the combination of different drugs yields an increased overall response rate (ORR) in comparison with monotherapy but at the cost of more toxicity and, unfortunately, does not always result in a prolonged time to progression and/or overall survival.

With respect to the sequential therapy of trastuzumab and chemotherapy, a single study was recently published on the value of monotherapy trastuzumab followed at progression by the combination of trastuzumab and docetaxel (H→D) in comparison with initial combination therapy H+D as first-line therapy for HER2<sup>+</sup> MBC.<sup>7</sup>

The current randomized phase II study aimed to determine whether it is worthwhile to explore a sequential approach in women with HER2<sup>+</sup> MBC, assessed by progression-free survival (PFS) as well as the rate of PFS at 1 year (PFS<sub>1yr</sub>). Secondary endpoints were response rate (RR), OS, and toxicity of both treatment regimens.



## PATIENTS AND METHODS

Women with progressive HER2<sup>+</sup> advanced breast cancer or MBC requiring first-line chemotherapy were eligible. HER2<sup>+</sup> was defined as score 3+ assessed by immunohistochemistry (test, Dako Denmark A/S, Glostrup, Denmark ) and/or gene amplification by fluorescence in situ hybridization (positive if  $\geq 2.2$  copy numbers per cell).<sup>11</sup> Other eligibility criteria were WHO performance status 0 to 2, life expectancy >12 weeks, hemoglobin value  $\geq 9.7$  g/dL, white blood cell count  $\geq 3.0 \times 10^9$ /L, neutrophil count  $\geq 1.5 \times 10^9$ /L, platelet count  $\geq 100 \times 10^9$ /L, creatinine clearance  $\geq 60$  mL/min or creatinine  $\leq 1.5 \times$  upper limit of normal (ULN); bilirubin level  $\leq 1.0 \times$  ULN; alkaline phosphatase level  $\leq 5.0 \times$  ULN; ALT and/or AST levels  $\leq 1.5 \times$  ULN; left ventricular ejection fraction (LVEF) >50%.

Exclusion criteria were previous anti-HER2 or taxane-containing therapy, uncontrolled serious illness, history or presence of central nervous system metastases, radiation therapy within 2 weeks or myocardial infarction within 6 months of study entry, and previous invasive malignancy.

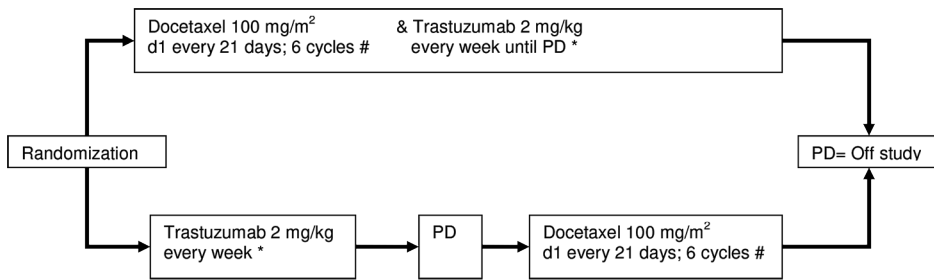
The HERTAX trial was a randomized, phase II trial conducted in 22 centers in the Netherlands and coordinated by the Dutch Breast Cancer Trialists' Group (BOOG). Patients were centrally randomized in a 1:1 ratio between H+D and H→D. Block randomization was used (2, 4, or 6 patients per block in order to avoid selective enrollment of patients based on previous treatment allocation in a center) after stratification for a participating center. Doses and regimens are shown in Figure 1. Docetaxel was administered over a 60-minute period. Corticosteroids were administered according to the standard procedure of each institution. No primary prophylaxis with growth factor support was allowed and antibiotics were not routinely administered. After the recognition that once every 3 weeks trastuzumab administration (6 mg/kg) was likely equivalent to the weekly schedule,<sup>5</sup> once every 3 weeks regimen was allowed for patients not receiving docetaxel concurrently. It was planned that docetaxel in either arm would be given for 6 cycles or longer at the discretion of the investigator. Either treatment was continued until disease progression, intolerable toxicity, or patient withdrawal.

The ethical review boards of the participating centers approved the study, and written informed consent was obtained from all patients. The study has been conducted in accordance with the principles embodied in the Declaration of Helsinki.

### Study Evaluations

Prestudy evaluations performed  $\leq 3$  weeks before randomization included tumor assessments, electrocardiography, LVEF measurement, blood counts, and clinical chemistry examinations. During study, laboratory evaluations and toxicity assessments (using the National Cancer Institute-Common Toxicity Criteria version 2) were performed every 3 weeks. Tumor assessment and LVEF measurements were performed every 6 and 9 weeks, respectively. Tumor response assessment was done by the local investigator according to

response evaluation criteria in solid tumors (RECIST).<sup>12</sup> Complete response (CR) or partial response (PR) had to be confirmed no less than 6 weeks after the first observation. Overall response assessment was reviewed centrally at data analysis.



**Figure1** | Treatment Flow Chart of the HERTAX Trial: PD, Progressive Disease. (\*) After a Loading Dose of 4 mg/kg (#) in the Absence of Progressive Disease or Intolerable Toxicity, Additional Cycles Added at the Discretion of the Treating Physician.

PFS in the H+D group (PFS<sub>comb</sub>) was defined as time from start of treatment to the date of documented disease progression, initiation of another systemic anticancer treatment without documented objective (radiologic) tumor progression, or death, whichever occurred first. In the H→D group, the following definitions were used: (1) PFS on trastuzumab (PFS<sub>tras</sub>) by using the same criteria as already mentioned; (2) PFS on sequential therapy (PFS<sub>seq</sub>) being the time between start of treatment and the date of documented progressive disease (PD) on subsequent single-agent docetaxel therapy, initiation of another systemic anticancer treatment, or death, whichever occurred first. If patients did not receive docetaxel when disease progressed on trastuzumab, the PFS<sub>seq</sub> was defined as the PFS<sub>tras</sub> in order not to overestimate the PFS<sub>seq</sub>. The PFS<sub>1yr</sub> was estimated on the basis of Kaplan-Meier plots. OS was measured from start of study treatment until death from any cause, with censoring at the last visit date for patients still alive.

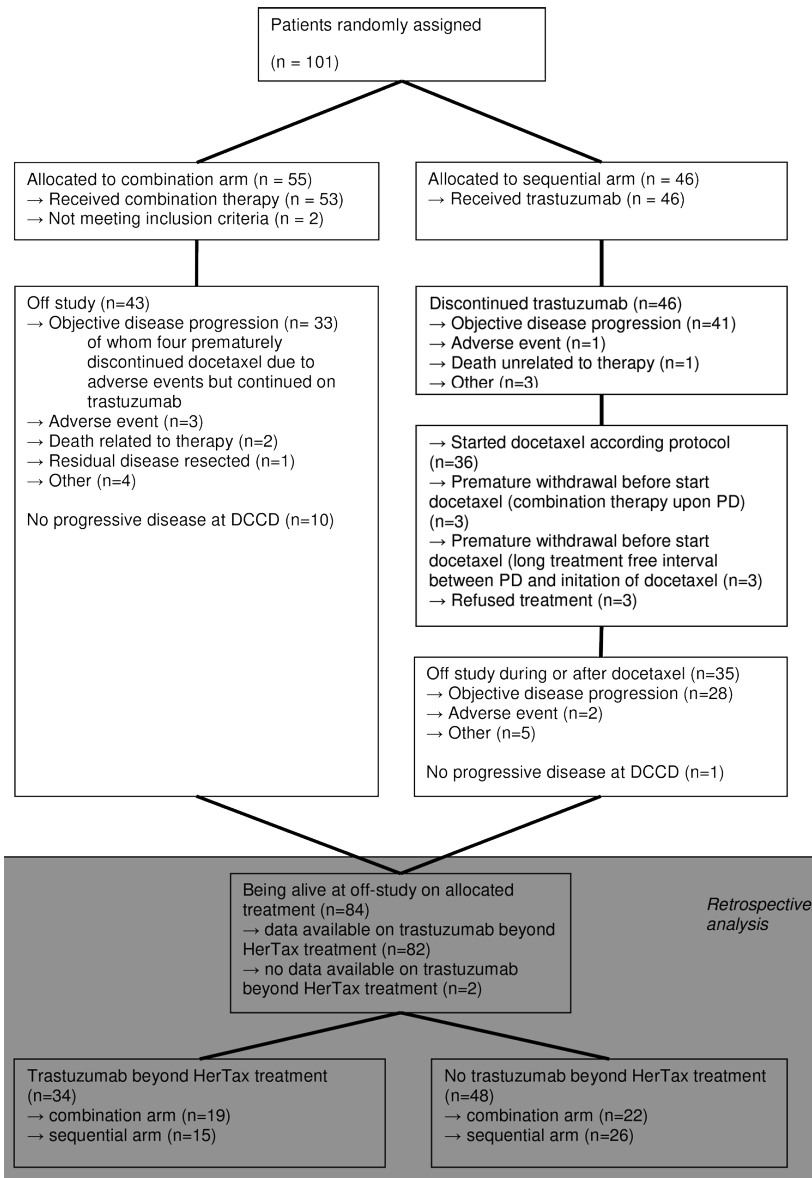
Data on the administration and clinical outcome of trastuzumab treatment beyond progression on the protocol-specified treatment were collected retrospectively because this issue is controversial and potentially might have affected study results.

### Statistical Methods

Estimating the PFS<sub>1yr</sub> with sufficient precision in both arms was the primary objective. Estimating a PFS<sub>1yr</sub> of 15% (based on data from patients with MBC irrespective of HER2 status from the Rotterdam Breast Cancer Database [pretrastuzumab era]), with a standard error (SE) of 5%, a sample size in each group of 50 patients was calculated. If trastuzumab treatment in general would increase the PFS<sub>1yr</sub> to around 50%, the SE of 7% with 50 patients would still be acceptable.



PFS and OS were analyzed using the Kaplan-Meier method and the log-rank test. Hazard ratios and their 95% confidence intervals were calculated with an univariate Cox regression model. Response rates and grade 3/4 toxicities were compared by means of X2 tests or Fisher’s 2-sided exact test when expected numbers were low. Ordinal variables, such as scored grades of toxicity, were tested with the Mann-Whitney *U* test.



**Figure 2.** HERTAX CONSORT Flowchart

Abbreviations: DCCD, data collection cut off date; PD, progressive disease.



## RESULTS

Between February 2003 and December 2007, a total of 101 patients were randomized (Figure 2). Two ineligible patients were excluded, in 1 patient because brain metastases became symptomatic before the start of treatment, and in the other patient because final workup did not reveal metastatic disease. Because of block randomization within centers, a slight imbalance occurred in the treatment arm allocation. On August 1, 2008 (the data collection cutoff date for the current analysis), 11 patients were still in the study (10 in the H+D group and 1 in the H→D group) (Figure 2), and 33 patients were still alive (21 and 12 in the H+D and H→D groups, respectively).

### Patient Characteristics and Treatment Delivered During Study

Baseline characteristics and demographics were well balanced between the treatment arms (Table 1). In both arms the median number of docetaxel cycles administered was 6 (range, 1-18). There were no statistically significant differences in dose delays (25% vs 26%;  $P=.86$ ) or dose reductions (11% vs 17%;  $P=.39$ ) between the H+D group and the H→D group, respectively. During the study period, trastuzumab was administered significantly longer in the H+D group (9.3 vs 4.1 months;  $P=.001$ ). Seven patients in the H+D group switched to receiving single-agent trastuzumab 3 times a week; this occurred in 3 patients in the H→D group. In the H→D group, 4 patients did not receive docetaxel: 3 patients refused this therapy, and 1 patient died from infectious endocarditis during trastuzumab treatment (Figure 2).

### Efficacy

At central review of response assessment, it appeared that 3 patients in the H→D group prematurely discontinued trastuzumab and started with docetaxel because PD was presumed by the local investigator, but according to RECIST ought to have been classified as stable disease (SD) ( $n=2$ ) or PR ( $n=1$ ). In contrast, 2 patients were classified as having PD and had continued on trastuzumab until the next tumor assessment because the local investigator had assessed SD. On achieving a long-lasting PR, 1 patient who was receiving combination therapy underwent resection of residual disease (primary tumor, axillary lymph nodes, liver metastases) after 21 months of treatment and was classified as having a PR for the current analysis. Initiation of another systemic anticancer treatment without documented objective (radiologic) tumor progression was applicable in 2 patients in H+D and in 3 patients in H→D.



Table 1 | Patient Characteristics

	Combination Arm (H+D) (n=53)	Sequential Arm (H→D) (n=46)
<b>Age Median</b>	50 years	54 years
<b>Range</b>	32–74 years	36–74 years
<b>Menopausal State</b>		
Premenopausal	19%	22%
Postmenopausal	68%	74%
Unknown	13%	4%
<b>WHO PS</b>		
0	49%	49%
1	49%	49%
2	2%	2%
<b>ER<sup>+</sup> and/or PR<sup>+</sup> Tumor</b>	49%	52%
<b>Previous Adjuvant Treatment</b>		
Chemotherapy	55%	52%
Anthracycline-based	45%	43%
Hormonal treatment	26%	35%
<b>Previous Hormonal Treatment for Advanced/ Metastatic Disease</b>	35%	37%
<b>Involved Tumor Site</b>		
Lung	38%	35%
Liver	51%	46%
Bone	45%	52%
<b>Predominant Metastatic Site</b>		
Single visceral organ	55%	43%
Multiple visceral organs	15%	15%
Lymph nodes	17%	11%
Soft tissue	8%	15%
Bone	6%	15%
<b>Interval Between BC Diagnosis and Randomization</b>		
Median	27.7 months	32.1 months
Range	0.2–213.6 months	0.4–151.3 months

Abbreviations: BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; WHO PS, World Health Organization performance status.

The median PFS in the H→D group during monotherapy with trastuzumab (PFS<sub>tras</sub>) was 3.9 months with an ORR of 34%. The median PFS in the H+D group and H→D group was 9.4 and 9.9 months for PFS<sub>comb</sub> and PFS<sub>seq</sub>, respectively, which was not significantly different

( $P=.20$ ) (Table 2; Figure 3B). Of note, the median PFStras was 3.9 months (Table 2; Figure 3A). PFS<sub>1yr</sub> rates for the H+D group and the H→D group were 44% (SE 7%) and 35% (SE 7%), respectively.

The ORR (CR + PR) was significantly higher in the H+D group than in the H→D group (ie, in the latter the best response was during either single-agent trastuzumab or single-agent docetaxel) (79% and 53%, respectively;  $P=.016$ ) (Table 2). Conversely, more patients in the H→D group achieved SD (13% vs. 38%, respectively). In the H→D group, the separate ORR during single-agent trastuzumab treatment was 34% and during subsequent monotherapy docetaxel treatment was 39% (Table 2).

The median OS in the H+D group was 30.5 months, and in the H→D group it was 19.7 months. Although there was a difference of 10.8 months in favor of the H+D group, this was not statistically significant ( $P=.12$ ) (Table 2; Figure 3C).

**Table 2.** Efficacy Results of the HERTAX Trial

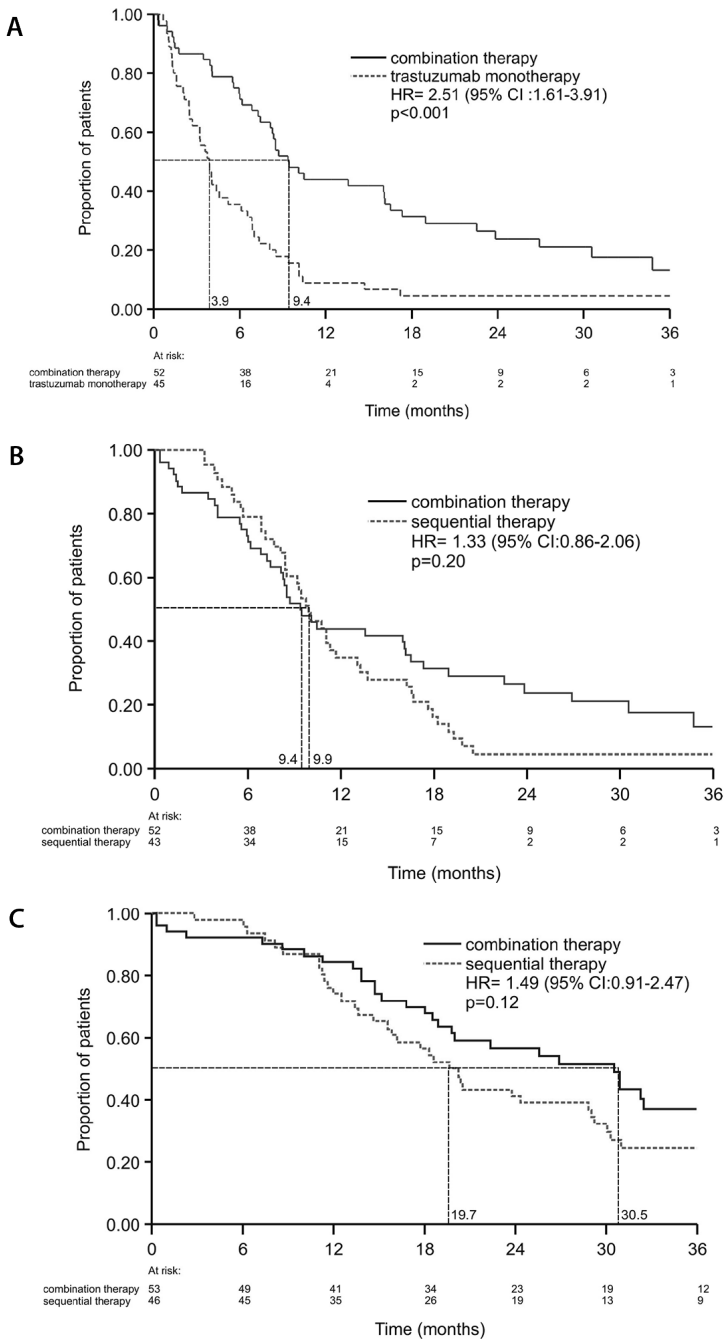
	Combination Arm (H+D)	Sequential Arm (H→D)	P-Value*	Within Sequential Arm	
				Trastuzumab Monotherapy	Docetaxel Monotherapy
<b>CR</b>	15%	3%	.016	0%	3%
Confirmed	13%	3%		0%	3%
Unconfirmed	2%	0%		0%	0%
<b>PR</b>	64%	50%		34%	36%
Confirmed	58%	38%		27%	23%
Unconfirmed	6%	12%		7%	13%
<b>SD</b>	13%	38%		39%	37%
<b>PFS<sub>1yr</sub></b>	44%	35%	NA	NA	NA
<b>PFS, Median</b>	9.4 months	9.9 months	.20	3.9 months	NA
<b>OS, Median</b>	30.5 mo	19.7 mo	.12	NA	NA

Abbreviations: CR, complete remission; NA, not applicable; OS, overall survival; PFS, progression-free survival; PFS<sub>1yr</sub>, progression-free survival at 1 year; PR, partial remission; SD, stable disease. \* P value on the comparison between the combination arm and the sequential arm.

## Toxicity

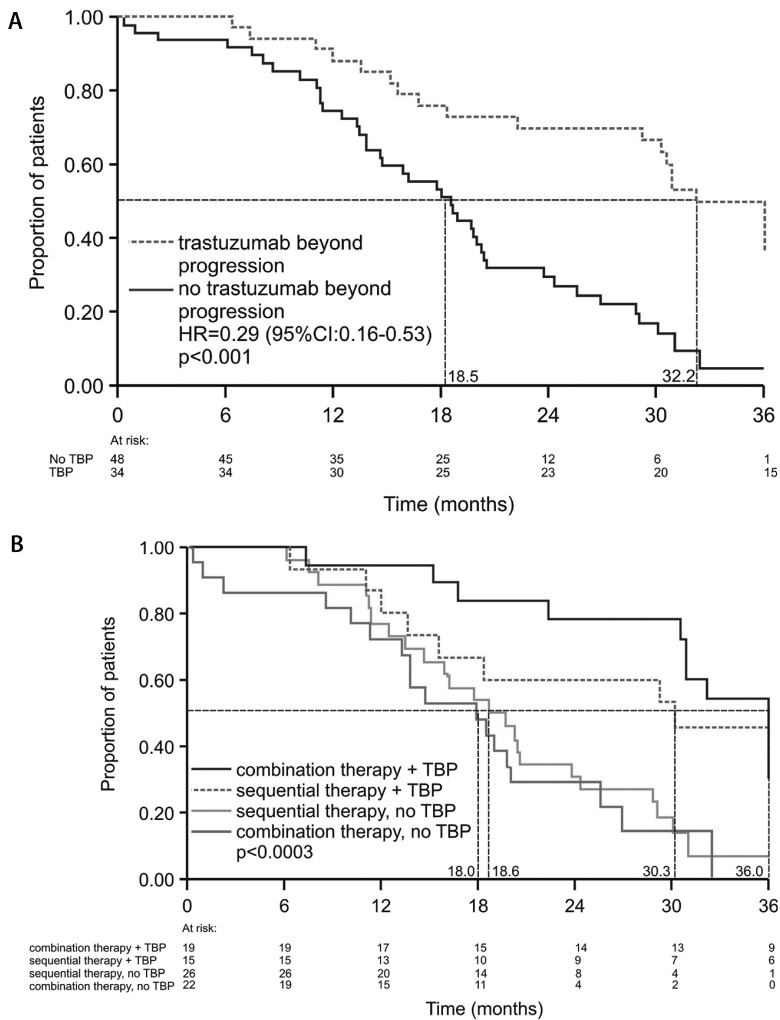
All 99 eligible patients were included in the safety analysis. Adverse events typically were chemotherapy related. The toxicity profiles of the 2 regimens were comparable in the majority of domains (Table 3). However a significantly higher incidence of sensory neuropathy was observed in the H+D group (grade 1/4: 62% vs 31%;  $P=.003$ ). Also there seemed to be a shift in severity of this untoward event, as no grade 3/4 sensory neuropathy was noticed in the H→D group, whereas this occurred in 8% of the patients in the H+D group ( $P=.053$ ) (Table 3).





**Figure 3.** Comparison of estimated (A) progression-free survival (PFS) of trastuzumab plus docetaxel vs trastuzumab alone, (B) PFS of trastuzumab plus docetaxel (H+D) vs trastuzumab alone and on progression, docetaxel alone arm H→D, and (C) overall survival between trastuzumab plus docetaxel (H+D) vs trastuzumab alone and on progression, docetaxel alone arm H→D (Kaplan-Meier plots)

Abbreviations: HR, hazard ratio; CI, confidence interval



**Figure 4.** Comparison of estimated overall survival in patients who were alive at off-study between (A) those patients receiving trastuzumab after HERTAX trial treatment vs. those who did not receive any trastuzumab after HERTAX trial treatment and (B) after further stratification for previous HERTAX treatment arm (Kaplan-Meier Plots)

Abbreviations: HR, hazard ratio; CI, confidence interval; TBP, trastuzumab beyond progression.

The incidence of febrile neutropenia (FN) was 23% in the H+D group compared with 15% in the H→D group ( $P=.35$ ) (Table 2). Furthermore, no significant difference in decline in LVEF between the 2 treatment arms was observed (Table 3).

Three patients died during the study: 2 in the H+D group (both being attributable to study treatment [interstitial lung disease; diarrhea during FN]), whereas the third patient died of infectious endocarditis during monotherapy with trastuzumab, which was considered unrelated to study treatment.



**Table 3.** Incidence of Adverse Events

	All Grades			Grade 3/4		
	Combination Arm (H+D)	Sequential Arm (H→D)	P-Value	Combination Arm (H+D)	Sequential Arm (H→D)	P-Value
<b>Nonhematologic Toxicity</b>						
Nausea	39%	42%	.86	4%	4%	1.00
Vomiting	31%	22%	.28	4%	7%	.67
Stomatitis	53%	38%	.25	4%	2%	1.00
Diarrhea	39%	33%	.50	4%	2%	1.00
Sensory neuropathy	62%	31%	<b>.003</b>	8%	0%	.053
Fatigue	88%	67%	.20	8%	9%	1.00
Myalgia	40%	29%	.24	0%	0%	NA
Peripheral edema	47%	42%	.97	0%	2%	.48
<b>Left Ventricular Ejection Fraction</b>						
Median relative decrease (worst value compared with baseline)				10%	10%	
Relative decrease ≥15% (worst value compared with baseline)				36%	28%	.47
LVEF <40%				4%	5%	1.00
Discontinuation of trastuzumab because of cardiotoxicity				8%	2%	.37
<b>Febrile Neutropenia</b>						
Incidence of febrile neutropenia				23%	15%	.35

Abbreviations: LVEF, left ventricular ejection fraction; NA, not applicable.

### Use of Trastuzumab Beyond Protocol Treatment

Data on the use of trastuzumab beyond protocol treatment were available from 82 of the 84 patients who were alive when PD resulted in withdrawing from the study (Figure 2). In 41% of patients (34/82), trastuzumab treatment was given beyond the study protocol (46% [19/41] in the H+D group vs 37% [15/41] in the H→D group;  $P=.37$ ). The median duration of trastuzumab administration beyond HERTAX study treatment was 7.5 months (range, 1–32 months). Median total trastuzumab administration for the whole group ( $n=82$ ) was 18.3 months (range, 2.4–42.3 months), whereas it was 21 vs. 12.7 months for those previously treated with H+D and H→D, respectively ( $P=.048$ ). OS in the patients receiving trastuzumab beyond progression ( $n=34$ ) was 32.2 months vs. 18.5 months in patients ( $n=48$ ) not receiving trastuzumab beyond HERTAX study treatment ( $P<.0001$ ) (Figure 4A).

The difference remained significant after stratification of the 2 groups according to the preceding HERTAX treatment regimen (Figure 4B).

## DISCUSSION

Combined treatment regimens are compared mostly with single-drug therapy and rarely with sequential therapy using the same drugs, although the latter in fact is a more robust and fair comparison. To our knowledge, the current study is the only one exploring the value of a sequential regimen of trastuzumab alone followed at disease progression by cytotoxic chemotherapy with docetaxel alone vs. initial combination therapy with trastuzumab and docetaxel in patients with HER2<sup>+</sup> MBC. We observed a comparable median PFS in both groups, whereas a different ORR was observed; there was an overall survival of 30.5 months in the H+D group vs. 19.7 months in the H→D group ( $P=.11$ ).

Crossing of the PFS and the OS curves is caused by different reasons. Crossing of the PFS curve is most likely caused by the absence of early progression in the sequential arm (H→D), as the PFS curve for this treatment arm shows progression on docetaxel after previous progression on trastuzumab, resulting in the observation that the PFS curve, by definition, starts to decline at a later point in time compared with the PFS curve of the combination arm (H+D). Although it might be fairer to compare the slopes of both curves, in the field of biostatistics there is as yet no solution to this problem.

The crossing of the OS curve is partially caused by the early occurrence of treatment-related deaths in 2 patients treated in the H+D group. The arrears of the combination therapy is then made up during treatment, causing the H+D OS curve to cross the H→D curve.

Recently an important study was published reporting on the outcome of sequential therapy with trastuzumab alone as initial treatment followed by combination therapy in comparison with initial combination therapy.<sup>10</sup> Despite the similarities, the Japanese study is at several major levels different from the HERTAX study because patients allocated to sequential treatment in our study were treated with single-agent docetaxel at disease progression during single-agent trastuzumab (H→D) therapy, whereas the Japanese trial used the combination of docetaxel and trastuzumab at disease progression (H→H+D). Furthermore the docetaxel dosage applied in our study was 100 mg/m<sup>2</sup> once every 3 weeks compared with 60 mg/m<sup>2</sup> once every 3 weeks in the Japanese study. Trastuzumab treatment beyond progression with first-line chemotherapy using docetaxel was applied during subsequent lines of chemotherapy in 90% of the patients in the Japanese study and 41% of patients in our study.<sup>7</sup>

Importantly, the JO17360 Trial Group decided to use a primary endpoint that explicitly did not include sequential therapy as it compared the PFS of patients treated with trastuzumab



to the PFS of patients treated with the combination of docetaxel and trastuzumab.<sup>10</sup> The JO17360 trial was terminated prematurely based on a significant difference in PFS favoring the combination therapy of trastuzumab and docetaxel compared with single-agent trastuzumab. This difference could have been anticipated because the known PFS of single-agent trastuzumab is about 3.5 months<sup>4,5</sup> and the PFS of the same combination therapy used in the study of Marty and colleagues was 11.7 months.<sup>6</sup>

The comparable PFS found in both treatment arms of our study is in accordance with the data from the Japanese study, although the PFS in both treatment arms of the latter trial was longer than expected.

In our study, monotherapy with trastuzumab resulted in a PF-Stras of 3.9 months and an ORR of 34%, in accordance with previous reports and confirming the single-agent activity of trastuzumab as first-line treatment for HER2<sup>+</sup> MBC.<sup>4,5</sup> The Japanese study showed a strikingly lower ORR (14.8%), but a comparable PFS was observed.<sup>7</sup>

The ORR observed in the H+D group (79%) is in line with previous findings of first-line docetaxel and trastuzumab treatment in patients with HER2<sup>+</sup> MBC,<sup>13</sup> whereas the remarkably lower ORR observed in the Japanese trial (47%) most likely is attributable to the lower dose of docetaxel.<sup>10</sup> Although a substantial proportion of the patients from the H→D group achieved remission on subsequent docetaxel treatment (39%), the ORR remained significantly better in the H+D group; however, this did not translate to a superior PFS.

The median OS of patients in the H+D group is comparable to the findings of the pivotal M77001 trial in regard to the combination regimen.<sup>6</sup> The difference in OS between the 2 treatment groups in our study might be related to the significantly longer duration of trastuzumab treatment in the H+D group, not only during the HERTAX therapy period (9.3 months in the H+D group vs 4.1 months in the H→D group) but also beyond protocol treatment (21 months in the H+D group vs 12.7 months in the H→D group). Also, the observation that not all patients received docetaxel therapy at disease progression on trastuzumab monotherapy might have affected outcome. At the interim analysis of the JO17360, the difference in OS did not reach the prespecified level of significance<sup>7</sup> and the exact OS data have not been reported yet because of a lack of events, although a hazard rate of 2.72 favoring initial combination therapy was observed.<sup>10</sup>

After the early termination of the study, combination treatment was allowed before disease progression in patients treated with single-agent trastuzumab, with the consequence that clear data on the true difference in OS will never be available for the total group of enrolled patients.

Inoue and associates<sup>7</sup> reflected that the difference in docetaxel dosing strategy between the HERTAX and JO17360 trials did allow an increasing number of docetaxel cycles to be administered. However it merely reflects the differences between Europe and the United States, where chemotherapy is usually discontinued after 6 cycles of docetaxel (75–100 mg/m<sup>2</sup>) in line with the pivotal trial by Marty and colleagues,<sup>6</sup> in contrast to the



common use of a lower docetaxel dose and administering more cycles of chemotherapy in Japan.

One of the driving thoughts behind the HERTAX trial was to explore whether it is valid to delay chemotherapy and thereby chemotherapy-induced adverse events in patients with HER2<sup>+</sup> MBC. Because observed toxicity was mainly chemotherapy related, most of the toxicity encountered in the sequential approach occurred during the time docetaxel was administered, which most likely had an impact on QoL.

The few differences observed in toxicity were all in favor of the H→D group. Interestingly, next to a higher frequency of sensory neuropathy, an increased frequency of grade 3 neuropathy was observed in the H+D group. Although a higher incidence of paresthesias in patients taking docetaxel and trastuzumab was reported in the M77001 phase II trial,<sup>6</sup> the remarkable shift in severity of neuropathy, as found in our study, has not been reported before and deserves further attention. Also, 15% of women treated with docetaxel alone and 23% of those treated with H+D experienced an episode of FN, which is in line with the results found by Marty and associates<sup>6</sup> (17% and 23%, respectively) and is consistent with the finding of Buzdar and colleagues<sup>14</sup> showing a doubling of the incidence of grade 4 neutropenia during trastuzumab and paclitaxel compared with paclitaxel alone. The lower rate of FN (6%–8%) observed in JO17360 compared with our study is attributable to the lower docetaxel dose per cycle used in Japan.<sup>7</sup>

In view of the controversy of trastuzumab beyond progression treatment, as well as the potential impact on the results of the current trial, the data on the use of trastuzumab beyond progression and the outcome thereof are informative but need to be interpreted with caution, as it was an unplanned and explorative analysis. Obviously a posthoc analysis is open to selection bias, as the reason for not receiving trastuzumab beyond progression may have been guided by lack of response to trastuzumab as well as by the clinical condition of the patient not allowing further systemic treatment and thus implicitly relating to a shorter survival. However despite these limitations, the data of the HERTAX study cohort are valuable given the fact that the various trials prospectively evaluating this issue have been closed prematurely because of slow accrual and the registration of lapatinib.<sup>15-17</sup> The only prospectively collected data were obtained from a trial evaluating 156 patients with HER2<sup>+</sup> MBC who progressed during or relapsed after previous trastuzumab therapy and were randomized for capecitabine with or without trastuzumab as second-line treatment.<sup>15</sup> A significant prolongation in PFS for the combination therapy (8.2 vs 5.6 months) was recently reported, without a translation into a statistically significant OS benefit (25.5 vs 20.4 months).<sup>15</sup> As noted by von Minckwitz and associates,<sup>15</sup> the genuine activity of trastuzumab beyond progression most likely will not be assessed again in a randomized study. Other retrospectively collected data are not consistent on the value of trastuzumab treatment beyond progression, although in the majority of reports a benefit is suggested.<sup>18-24</sup>



One of the limitations of our study, related to the phase II character, is the relatively small sample size, hindering a definite conclusion with regard to OS. Furthermore, it would have been interesting if on finishing protocol treatment, patients were enrolled in a subsequent randomized study evaluating the continuation of trastuzumab beyond progression. QoL assessments were not incorporated, although it would have been informative to compare the QoL of patients during the different treatment strategies.

Altogether, the data of the current study and the Japanese study are pointing in the same direction showing superiority of combination-treatment trastuzumab and docetaxel compared with initial treatment with single-agent trastuzumab followed by docetaxel either with or without trastuzumab, so this should remain the treatment of choice in daily clinical practice. However the concept of delaying cytotoxic therapy still should not be repudiated, although strategies other than single-agent trastuzumab should be explored. Combinations of trastuzumab with other targeted agents are potential candidates, including vascular endothelial growth factor inhibitors and epidermal growth factor receptor blockers as well as hormonal therapy. For example, anastrozole in combination with trastuzumab in patients with estrogen receptor (ER)<sup>+</sup>, HER2<sup>+</sup> breast cancer evaluated in the TAnDEM study, resulted in a doubling of the median PFS compared with patients treated with single-agent anastrozole.<sup>25</sup>

## CONCLUSION

In summary, despite a comparable PFS in both the sequential treatment arm consisting of single-agent trastuzumab followed by single-agent docetaxel and the combination therapy arm, the ORR and OS data did not support the sequential approach. Strategies delaying the introduction of chemotherapy should focus on combining trastuzumab with other agents.

## REFERENCES

1. Ross JS, Slodkowska EA, Fraser Symmans W, et al. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. *Oncologist* 2009; **14**:320-68.
2. Berns EM, Klijn JG, van Staveren IL, et al. Prevalence of amplification of the oncogenes c-myc, HER2/neu, and int-2 in one thousand human breast tumours: correlation with steroid receptors. *Eur J Cancer* 1992; **28**:697-700.
3. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; **344**:783-92.
4. Vogel CL, Cobleigh MA, Tripathy D, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002; **20**:719-26.
5. Baselga J, Carbonell X, Castañeda-Soto NJ, et al. Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. *J Clin Oncol* 2005; **23**:2162-71.
6. Marty M, Cognetti F, Maraninchi D, et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 2005; **23**:4265-74.
7. Inoue K, Nakagami K, Mizutani M, et al. Randomized phase III trial of trastuzumab monotherapy followed by trastuzumab plus docetaxel versus trastuzumab plus docetaxel as first-line therapy in patients with HER2-positive metastatic breast cancer: the JO17360 Trial Group. *Breast Cancer Res Treat* 2010; **119**:127-36.
8. Burstein HJ, Keshaviah A, Baron AD, et al. Trastuzumab plus vinorelbine or taxane chemotherapy for HER2-overexpressing metastatic breast cancer: the trastuzumab and vinorelbine or taxane study. *Cancer* 2007; **110**:965-72.
9. Gasparini G, Gion M, Mariani L, et al. Randomized phase II trial of weekly paclitaxel alone versus trastuzumab plus weekly paclitaxel as first-line therapy of patients with Her-2 positive advanced breast cancer. *Breast Cancer Res Treat* 2007; **101**:355-65.
10. Bartsch R, Wenzel C, Altorjai G, et al. Capecitabine and trastuzumab in heavily pretreated metastatic breast cancer. *J Clin Oncol* 2007; **25**:3853-8.
11. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol* 2007; **25**:118-45.
12. Therasse P, Arbuuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000; **92**:205-6.
13. Wardley AM, Pivot X, Morales-Vasquez F, et al. Randomized phase II trial of first-line trastuzumab plus docetaxel and capecitabine compared with trastuzumab plus docetaxel in HER2-positive metastatic breast cancer. *J Clin Oncol* 2010; **28**:976-83.
14. Buzdar AU, Ibrahim NK, Francis D, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. *J Clin Oncol* 2005; **23**:3676-85.
15. Von Minckwitz G, du Bois A, Schmidt M, et al. Trastuzumab beyond progression in human epidermal growth factor receptor 2-positive advanced breast cancer: a German Breast Group 26/ Breast International Group 03-05 study. *J Clin Oncol* 2009; **27**:1999-2006.
16. Spanish Breast Cancer Research Group. Benefit of adding trastuzumab to second line chemotherapy in breast cancer patients previously treated with trastuzumab. Available at <http://www.clinicaltrials.gov/ct/show/NCT00130507>. Accessed March 15, 2011.
17. Montemurro F, Faggiuolo R, Redana S, et al. Continuation of trastuzumab beyond disease progression. *J Clin Oncol* 2005; **23**:2866-8; discussion 2868-9.



18. Fountzilias G, Razis E, Tsavdaridis D, et al. Continuation of trastuzumab beyond disease progression is feasible and safe in patients with metastatic breast cancer: a retrospective analysis of 80 cases by the Hellenic Cooperative Oncology Group. *Clin Breast Cancer* 2003; **4**:120-5.
19. Tripathy D, Slamon DJ, Cobleigh M, et al. Safety of treatment of metastatic breast cancer with trastuzumab beyond disease progression. *J Clin Oncol* 2004; **22**:1063-70.
20. García-Sáenz JA, Martín M, Puente J, et al. Trastuzumab associated with successive cytotoxic therapies beyond disease progression in metastatic breast cancer. *Clin Breast Cancer* 2005; **6**:325-9.
21. Bartsch R, Wenzel C, Hussian D, et al. Analysis of trastuzumab and chemotherapy in advanced breast cancer after the failure of at least one earlier combination: an observational study. *BMC Cancer* 2006; **6**:63.
22. Canello G, Montagna E, D'Agostino D, et al. Continuing trastuzumab beyond disease progression: outcomes analysis in patients with metastatic breast cancer. *Breast Cancer Res* 2008; **10**: R60. Epub 2008 Jul 16.
23. Montemurro F, Redana S, Nolè F, et al. Vinorelbine-based salvage therapy in HER2-positive metastatic breast cancer patients progressing during trastuzumab-containing regimens: a retrospective study. *BMC Cancer* 2008; **8**:209.
24. Fabi A, Metro G, Ferretti G, et al. Do HER-2 positive metastatic breast cancer patients benefit from the use of trastuzumab beyond disease progression? A mono-institutional experience and systematic review of observational studies. *Breast* 2008; **17**:499-505.
25. Kaufman B, Mackey JR, Clemens MR, et al. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III TAnDEM study. *J Clin Oncol* 2009; **27**:5529-37.

Chapter

9

Summary

Through advances in molecular biology, insight into the mechanisms driving malignancies has improved immensely. As a result, various factors playing an essential role in the biology of numerous tumor types have been revealed. By using agents that specifically block the function of a single factor being crucial for tumor pathogenesis, it was hoped to exert anti-tumor activity while avoiding toxicities characteristic for conventional chemotherapy. One of the processes of crucial importance in the development of cancer thereby rendering it a potential target for treatment, is angiogenesis, the formation of new tumor vessels. In recent years, several key factors for angiogenesis have been identified, including ligands, receptors and transduction signalling factors. Of these, the vascular-endothelial growth factor (VEGF) pathway was found to be activated in numerous tumor types and considered one of the main drivers of angiogenesis. Roughly, VEGF-mediated angiogenesis can be inhibited by two approaches; either by monoclonal antibodies directed towards VEGF or its corresponding receptors or by kinase inhibitors targeting the signal transduction of the VEGF-receptors. As monotherapy, several kinase inhibitors exert anti-tumor activity in tumor types such as renal cell carcinoma and soft tissue sarcoma. However in most tumor types, anti-tumor activity of compounds targeting the VEGF pathway is limited. In recent years, evidence is mounting that the paradigm of one single factor driving malignant behaviour applies rarely and is an oversimplification for most tumors in which there are multiple driving pathways being activated in parallel. Consequently, multi-targeting rather than single-targeting approaches are required. One of the means to accomplish this is by combining targeted agents with conventional cytotoxics. As the VEGF pathway also affects the sensitivity of tumor cells to chemotherapeutics, combinations of compounds targeting this pathway and conventional cytotoxics are attractive to be explored. In **chapter 2** a review summarizes and puts into perspective the clinical data on the use of the combination of cytotoxic therapy combined with agents inhibiting of the VEGF-VEGFR-pathway published so far. Though such a combination is theoretically attractive, combination strategy at the time of the writing of the review has been proven for a few indications (metastatic colorectal cancer and metastatic breast cancer) with a modest benefit. A lot of effort is still needed to find optimal combinations as well as to identify predictive factors that are able to identify patients most likely to benefit from combination therapy.

Before a combination is put in the armamentarium of the medical oncologist as regular treatment, there are many challenges that should be tackled towards a final robust strategy with proven efficacy. Dose-finding studies are the first step in the clinical development of new combination treatment strategies. **Chapter 3** describes several challenges encountered in dose-finding studies. Additionally, several potential solutions to critical issues in the design of phase I anti-cancer drug combination studies are suggested. It has become increasingly complex to construct a trial, due to the increasing diversity in classes of agents, mechanisms of action, safety profiles and drug-administration schedules. With over 850 agents currently in development for cancer treatment, it is evident that combination development must

be prioritized based on a specific hypothesis (for example on the basis of an expected pharmacokinetic and/or pharmacodynamic interaction and/or expected anti-tumor activity against a particular molecularly defined tumor type), as well as on a projected development path for the involved combination. If there is a rationale to develop a specific drug combination, a hypothesis with respect to the additional value of the combination of the drugs as compared to single agent (sequential) therapy should be generated. This hypothesis, based on anticipated levels of interaction, should guide the design of the phase I study, although one should always be open to detect the unexpected. Clearly, a dose-finding study on a single anti-tumor agent will result in a maximally tolerated dose (MTD). However, in drug combination studies there are several factors that does increase the number of potentials MTDs, like the sequence of drug-administration as well as the steps made in drug-escalation (for example keeping one of the drugs at a low dose-level while increasing the other and vice versa). A strong consideration is to design studies aiming to potentially determine multiple MTDs with final dose determination in randomised phase II trials. Introducing controls as well as the novel 3 + 3 + 3 design (in contrast to the classic 3 + 3 design) are strategies allowing more grip on combinations with high incidence of severe toxicity. The 3 + 3 + 3 design reduces the chance of falsely halting dose-escalation in studies that use drugs with high rate of severe toxicity, by allowing the number of patients at a dose level to be increased to 9, if 2 out of 6 patients experience dose-limiting toxicity (DLT). Importantly, we should continuously realise that the MTD of an anti-tumor regimen is not necessarily also the optimal biological dose underlining the importance of appropriate PD markers adequately reflecting the anti-tumor effects from the regimen under study.

In **Chapter 4** the first phase I trial in this thesis is reported, aiming to define the MTD of sunitinib, in combination with two different infusion schedules of ifosfamide. Sunitinib is a potent inhibitor of the VEGF-receptors (VEGFR)1-3, KIT, platelet derived growth factor receptor- $\alpha$  and - $\beta$ , and Fms-like tyrosine kinase-3 (Flt3) and is one of the first and most commonly used VEGFR-TKIs. It is currently registered for the treatment of advanced RCC and imatinib-refractory GIST. Patients with advanced solid tumors, good performance score, good organ function, and no standard therapy available, were eligible. Continuous once daily sunitinib, orally given, in escalating doses per cohort, was combined with a standard dose ifosfamide, 9 g/m<sup>2</sup>/3 days or 6 g/m<sup>2</sup>/5 days, given every 3 weeks. Next to evaluation of safety and toxicity, pharmacokinetic (PK) and pharmacodynamic assessments were performed. With growth-factor support, the MTD of sunitinib combined with either ifosfamide schedule was 12.5 mg once daily, in contrast to the single agent dose sunitinib of 50 mg during 28 days followed by 14 days rest. Neutropenia-related adverse events were the dose-limiting toxicity. No consistent changes in VEGF or sVEGFR2 that might reflect biological activity of sunitinib or changes in circulating endothelial cells were observed. Sunitinib did not affect ifosfamide PK. In contrast, ifosfamide significantly decreased exposure to sunitinib, and increased exposure to its active metabolite, SU12662, due to CYP3A-induction. As PK-



interactions cannot explain the relatively low sunitinib doses that can be combined with ifosfamide, synergy in toxicity at a cellular level is more likely. Whether this also holds true for anti-tumor activity needs to be further explored in randomized studies.

**Chapter 5** is a review focusing on the novel tyrosine-kinase inhibitor pazopanib. This drug is specifically designed to impair angiogenesis by abrogating vascular endothelial growth factor receptor 2 (VEGFR2) to exert its function. Pazopanib inhibits VEGF-induced endothelial cell proliferation *in vitro* and angiogenesis *in vivo* and demonstrates anti-tumor activity in mouse models. Furthermore, the pazopanib concentration resulting in maximal inhibition of VEGFR2 phosphorylation *in vivo* was in line with the steady state concentration required to inhibit growth of tumor xenografts suggesting that pazopanib's mechanism of action is indeed through VEGFR2 inhibition. In a prior study a generally well tolerated dose was identified at which the majority of patients achieved pazopanib plasma concentrations above the concentration required for maximal *in vivo* inhibition of VEGFR2 phosphorylation in preclinical models. Administered as monotherapy, evidence of anti-tumor activity has been observed in phase III studies in several tumor types, including soft tissue sarcoma and renal cell cancer (RCC).

A dose-finding and pharmacokinetic/pharmacodynamics study of pazopanib combined with two different schedules of ifosfamide is described in **chapter 6**. In this trial the 3 + 3 + 3 design has been applied for the first time. Patients with advanced solid tumors received escalating doses of oral pazopanib combined with ifosfamide either given 3-days continuous or 3-hours bolus infusion daily for three days (9 g/m<sup>2</sup> per cycle, every 3 weeks). Pazopanib with continuous ifosfamide infusion appeared to be safe up to 1000 mg/day, while combination with bolus infusion ifosfamide turned out to be too toxic based on variety of adverse events. In the PK analyses, an ifosfamide-induced decline in pazopanib exposure was observed. Despite this, increases in placental derived growth factor (PlGF) and VEGF-A with concurrent decline in sVEGFR2 levels, consistent with pazopanib-mediated VEGFR2 inhibition, were observed after addition of ifosfamide. The recommended dose of pazopanib for further studies combined with 3-days continuous ifosfamide (9 g/m<sup>2</sup> per cycle, every 3 weeks) is 800 mg daily.

In another phase I trial pazopanib was combined with two different schedules of intravenously administered docetaxel and reported in **chapter 7**. Patients with advanced solid tumors received, next to daily oral pazopanib, docetaxel intravenously either given as a 3-weekly infusion or a weekly infusion for three weeks in a row followed by one week rest. Both drugs were escalated in serial cohorts by applying the 3 + 3 + 3 model. This study showed that both intravenous schedules could be combined with pazopanib at a dose of 400 mg. The MTD of 3-weekly infused docetaxel was 50 mg/m<sup>2</sup>, while for the other schedule it was 20 mg/m<sup>2</sup>. These doses are lower than the registered single agent dose of docetaxel. This is largely due to a clinically relevant pharmacokinetic interaction with pazopanib, substantially increasing docetaxel exposure caused by a 33% lower clearance of docetaxel



during concomitant administration of pazopanib. This interaction is most likely due to CYP3A4 and OATP1B1 inhibition.

After determining dose levels in dose-finding studies subsequent phase II studies will need to be performed to get a glance on whether or not a specific combination is worthwhile to be evaluated in a larger randomised phase III trial. Where in the previous chapters of this thesis combinations of two drugs that are given simultaneously are assessed, two drugs can also be administered sequentially. A sequential approach, in general, is better tolerated than combination therapy. Therefore as long as overall survival is the treatment goal and is comparable, sequential therapy is to be preferred over combination therapy, especially if the introduction of cytotoxic therapy with a higher rate of severe toxicity can be delayed.

**Chapter 8** describes this important issue of sequencing versus combining in a randomized phase II trial using one of the first targeted drugs in solid malignancies: trastuzumab, here in combination with docetaxel. Both drugs as single agents have been shown to exert anti-tumor activity in patients with metastatic breast cancer (MBC) expressing HER2, but both drugs are usually given in combination with each other. As this combined regimen associated with relevant toxicity, a sequential regimen with monotherapy trastuzumab followed by cytotoxic therapy upon disease progression was thought to be an attractive approach in terms of quality-of-life provided that the outcome of this sequential approach is not worse compared to the two drugs concomitantly administered. Patients with HER2-overexpressing MBC were randomized between combination therapy trastuzumab plus docetaxel (H+D) and sequential therapy of single agent trastuzumab followed upon disease progression by docetaxel alone (H→D) as first line chemotherapy for metastatic disease. The primary end point was progression free survival (PFS) after completed sequential or combination therapy.

The trial showed that for the H+D group and H→D group the median PFS was 9.4 vs 9.9 months and 1-year PFS rates were 44% vs 35%, respectively. However, the overall response rates were 79% and 53%, respectively ( $p=0.016$ ), and overall survival was 30.5 versus 19.7 months ( $p=0.11$ ) favouring the H+D group. In the H→D group, response rates to monotherapy trastuzumab and subsequent docetaxel were 34% and 39%, respectively, with a median PFS during single agent trastuzumab of 3.9 months. The incidence and severity of neuropathy were significantly higher in the H+D group. Retrospective analysis of trastuzumab treatment beyond progression, applied in 46% and 37% in the H+D group and the H→D group, respectively, showed a correlation with longer overall survival in both treatment arms (36.0 versus 18.0 months and 30.3 versus 18.6 months, respectively). From this trial it can be concluded that first line treatment in patients with MBC with H→D resulted in a similar PFS as compared to H+D, but the response rate was lower and the overall survival non-significantly shorter.



## FUTURE PERSPECTIVE

Given the enormous numbers of drugs in development that can in theory be applied in countless numbers of various schedules and regimens, it will not be possible to perform combination trials on all potential combinations. The process of designing a dose-finding study is a cornerstone in the evaluation towards a proven effective therapy. From the very first beginning of the development of a drug combination rigorous hypothesis-driven selections should be made to end up with combinations that have a high likelihood to eventually give benefit to the cancer patient. Hypothesis can be based on presumed synergy based on theoretical or preclinical data. Before launching a dose-finding trial educated guesses have to be made whether or not PK-interaction is expected. As presumed synergy is a PD-interaction (regarding efficacy) the issue of potential interaction at toxicity level should be tackled as well.

Computational models, build upon preclinical and early clinical data on known inhibitory and inductive effects of drugs on the most relevant enzymes in drug metabolism, might help to identify the combinations with a relevant potential pharmacokinetic interaction.

Furthermore, it is worthwhile to try to increase the gain of information retrieved from exposing a specific patient to a combination of drugs. For example the possibilities of intra-patient dose escalation can be explored. Although, there is a bias, given the fact that before a dose can be escalated the patient has to prove that the assigned dose-level is well-tolerated, it gives additional information regarding upper-boundaries of dosing. A potential pitfall might be toxicity that only reveals after the MTD-observation window has been elapsed, however in current phase I models this is not covered either. Another issue is that cumulative toxicity might be attributed to the wrong dose-level. So primarily data gained after intra-patient dose-escalation should not be considered a definitive answer, but a first signal of tolerance.

Not only the information gained per enrolled patient should be increased, also the gain per dose-finding study must be raised. It should be mandatory that all combination dose finding trials combinations should have a methodology that potentially defines at least two MTDs. Furthermore, it should be compulsory that these trials also explore differences in administration sequence. Once again, an intra patient change in administration sequence might be attractive to be applied.

Strategies stimulating the enrolment of patients with a proven benefit of one of the drugs in the combination might successfully accelerate the trial. Enriching patient populations in dose-finding trials may also give phase II-like signal. Intertwining phase I and II trials (as well as phase II and III) might give early signals of information. However, we should keep in mind that these kinds of signals are not a definitive answer but merely a kick start for future phase II and subsequent phase III trials.

Key opinion leaders in early drug development should move forward and agree on novel models, like the ones suggested above, that construct and implement designs

that adequately define MTD-envelopes for promising combinations as well as defining a minimum set of quality parameters for dose finding studies.

However, despite all theoretical novel models that might identify promising combinations, in the end, and it also apply to combining anti-cancer drugs, the proof of the pudding is in the eating.







# Appendix

SAMENVATTING

DANKWOORD

CURRICULUM VITAE

LIST OF PUBLICATIONS

PHD PORTFOLIO



## Samenvatting

Door vooruitgang in de moleculaire biologie zijn de inzichten in sturende mechanismen van maligniteiten enorm toegenomen. Hierdoor zijn verschillende factoren die een essentiële rol in het biologische gedrag van verschillende tumoren ontdekt. Door geneesmiddelen in te zetten die het functioneren van een specifieke factor die cruciaal is voor de tumorpathogenese blokkeren, werd gehoopt dat er antikanker activiteit van deze middelen uitging waarbij de bijwerkingen die karakteristiek zijn voor klassieke chemotherapie konden worden vermeden. Een van de belangrijke processen in het ontstaan van kanker en daarmee een potentieel aangrijpingspunt voor therapie is angiogenese, de formatie van nieuwe bloedvaten. Recentelijk zijn verschillende factoren die angiogenese beïnvloeden geïdentificeerd zoals ligand, receptoren en signaaltransductie factoren. Het vascular-endothelial growth factor (VEGF) -pad blijkt geactiveerd te zijn in veel tumor-types en wordt beschouwd als een van de belangrijkste aansturende paden voor angiogenese. Grofweg kan VEGF-gemedieerde angiogenese geblokkeerd worden op 2 manieren, te weten door monoclonale antilichamen gericht tegen VEGF of de VEGF-receptor (VEGFR) of door kinasereemers gericht tegen de signaal transductie van de VEGFR. Ingezet als monotherapie hebben meerdere kinasereemers antikanker effect laten zien in tumor types zoals nierkanker en het weke delen sarcoom, echter in de meeste tumoren is de activiteit van geneesmiddelen die het VEGF-pad als aangrijpingspunt hebben, beperkt. In de afgelopen jaren is steeds duidelijker geworden dat het paradigma dat slechts één factor relevant is voor het maligne gedrag van een tumor slechts zelden opgaat en dat er vaak meerdere sturende paden parallel aan elkaar geactiveerd zijn. Als gevolg hiervan is een aanpak gericht op meerdere aangrijpingspunten een logischer strategie, waarbij dit bijvoorbeeld mogelijk is door het combineren van zogeheten doelgerichte middelen met klassieke chemotherapie. Omdat het VEGF-pad ook de gevoeligheid van tumorcellen voor klassieke chemotherapie beïnvloedt, is het combineren van remmers van het VEGF systeem met klassieke chemotherapie aantrekkelijk. In **hoofdstuk 2** worden in een review de klinische data met betrekking tot deze combinatie doorgenomen en in perspectief geplaatst. Ook al is het in theorie een werkzame combinatie, de daadwerkelijke toepassing op het moment van schrijven van het review was beperkt tot een beperkt aantal indicaties, te weten gemetastaseerd borstkanker en gemetastaseerd dikke darm kanker. Veel inspanning is nog nodig om de optimale combinatie te vinden en tevens voor het vinden van factoren die adequaat patiënten kunnen identificeren die baat hebben bij deze therapie.

Voordat een combinatie van antikanker geneesmiddelen daadwerkelijk als reguliere therapie ingezet kan worden, zijn er verschillende obstakels die genomen moeten worden om te komen tot een strategie met aangetoonde meerwaarde. Een studie gericht op het vinden van de toe te passen dosering is de eerste stap in de klinische ontwikkeling van een

nieuwe combinatie strategie. **Hoofdstuk 3** beschrijft de verschillende uitdagingen die er zijn voor de studies die proberen de optimale dosering te vinden. Verschillende oplossingen worden aangedragen die behulpzaam kunnen zijn in de aanpak van belangrijke zaken bij het ontwerpen van fase I studies die meerdere antikanker middelen combineren. De complexiteit is aan het toenemen door de diversiteit in verschillende soorten geneesmiddelen, werkingsmechanismen, bijwerkingen-profielen en toedieningsschema's. Met meer dan 850 geneesmiddelen in ontwikkeling is het overduidelijk dat er geen mogelijkheid is om elke willekeurige combinatie te testen en dat het ontwikkelen van combinaties gebaseerd moet zijn op basis van een specifieke hypothese (bijvoorbeeld op basis van een verwachte farmacokinetische en/of farmacodynamische interactie en/of verwachte antikanker activiteit tegen een moleculair gedefinieerd subtype tumor) gecombineerd met een beoogd ontwikkelingstraject voor de te onderzoeken combinatie. Als er een rationale is om een bepaalde combinatie te gaan ontwikkelen, dan zou een hypothese met betrekking tot de aanvullende waarde van de gecombineerde therapie in vergelijking met (sequentiële) monotherapie opgesteld moeten worden. Deze hypothese, die gebaseerd moet zijn op geanticipeerde interacties, zou het ontwerp van de fase I studie moeten bepalen, alhoewel men altijd open moet staan om het onverwachte wel te kunnen detecteren. Vanzelfsprekend is het eindresultaat van een studie die de maximaal tolereerbare dosis (MTD) van een antikanker medicijn evalueert, één specifieke dosering. Echter, in medicatie combinatie studies zijn er verschillende factoren die het potentiële aantal MTDs beïnvloeden, met als voorbeeld de volgorde van medicatie toediening als ook de gekozen dosis-escalatie stappen (bijvoorbeeld één van de medicamenten op een bepaald dosis-level houden en de ander juist escaleren en vice versa). Het introduceren van controles in fase I studies als ook het nieuwe 3 + 3 + 3 studie-ontwerp (ten opzichte van het klassieke 3 + 3 ontwerp) zijn strategieën om meer grip te krijgen op combinaties met een *a priori* hoge kans op niet-tolereerbare toxiciteit. Het 3 + 3 + 3 ontwerp vermindert de kans op het onterecht staken van de dosis-escalatie in studies die medicijnen combineren met al een betrekkelijk hoge incidentie van niet-tolereerbare bijwerkingen, ook wel dosis limiterende toxiciteit (DLT). Dit model staat toe dat indien er in 2 van de 6 patiënten een DLT is gezien, het totaal aantal patiënten in dat cohort opgehoogd wordt naar 9. Belangrijk is dat we ons steeds blijven realiseren dat een MTD niet noodzakelijkerwijs ook de optimale biologische dosis hoeft te zijn. Daarmee nog eens het belang benadrukkend van het verkrijgen van de geschikte farmacodynamische parameters die een relatie zouden kunnen hebben met een potentieel antitumor effect van de combinatie.

In **hoofdstuk 4** wordt de eerste fase I studie van dit proefschrift beschreven. Het doel van deze studie was om de MTD te definiëren van de combinatie van sunitinib met verschillende infusie schema's van ifosfamide. Sunitinib is een krachtige remmer van de VEGFR1-3, KIT, de bloedplaatjes groeifactor receptor  $\alpha$  and  $\beta$  en Fms-like tyrosine kinase-3 en het is één van de veel gebruikte VEGFR-tyrosine kinase remmers. Sunitinib is momenteel geregistreerd



voor de behandeling van het gevorderd heldercellig niercelcarcinoom en imatinib-ongevoelig geworden gastro intestinale stroma tumoren. Patiënten met een gevorderde solide maligniteit, een goede conditie en een goede orgaanfunctie voor wie geen standard therapie beschikbaar was, waren geschikt voor het onderzoek. Dagelijks oraal in te nemen sunitinib in escalerende dosering per patiënt-cohort werd gecombineerd met een standaard dosering van ifosfamide van 9 g/m<sup>2</sup> in 3 dagen of 6 g/m<sup>2</sup> in 5 dagen, elke 3 weken toe te dienen. Naast de evaluatie van veiligheid en verdraagzaamheid werd materiaal afgenomen voor farmacokinetiek en farmacodynamiek. Met groeifactor (G-CSF) ondersteuning, blijkt de MTD van sunitinib in combinatie met elk van de ifosfamide-schema's 12.5 mg per dag te zijn, terwijl de dosering van sunitinib als enkelvoudige therapie 50 mg per dag is gedurende 28 dagen gevolgd door 14 dagen rust. Neutropenie-gerelateerde bijwerkingen zijn dosis-limiterend voor deze combinatie. Er werden geen structurele veranderingen gezien in VEGF of de oplosbare VEGFR2 die zouden kunnen wijzen op biologische activiteit en ook geen veranderingen in aantal circulerende tumorcellen. Sunitinib heeft geen invloed op ifosfamide farmacokinetiek. Echter ifosfamide verlaagt significant de expositie aan sunitinib met daarbij een toegenomen expositie aan de actieve metabooliet (SU12662) als gevolg van CYP3A4-inductie. Aangezien de farmacokinetische interactie niet de relatief lage dosering van sunitinib in de MTD kan verklaren, is het meer waarschijnlijk dat er een synergie in toxiciteit op cellulair niveau is. Of dit ook op gaat voor antikankeractiviteit zal verder uitgezocht moeten worden.

**Hoofdstuk 5** is een overzichtsartikel over de relatief nieuwe tyrosine kinase remmer pazopanib. Dit medicijn is ontworpen om angiogenese tegen te gaan door het blokkeren van de functie van VEGFR2. Pazopanib remt VEGF-geïnduceerde endotheliale proliferatie *in vitro* en angiogenese *in vivo* en heeft aangetoonde antitumor activiteit in het muismodel. Dat de concentratie die *in vivo* resulteerde in de maximale VEGFR2-fosforylatie overeen kwam met de concentratie die nodig is voor het inhiberen van tumorgroei in xenografts, suggereert dat pazopanib inderdaad werkt door remming van VEGFR2. In een voorgaande studie is de dosering pazopanib geïdentificeerd die zowel goed getolereerd werd als ook die in een groot deel van de mensen tot plasmaconcentraties leidt tot boven het niveau die *in vivo* nodig bleek voor maximale VEGFR2-fosforylatie in de preklinische modellen. Pazopanib heeft aangetoonde antitumorwerking in onder andere weke delen tumoren als ook niercelkanker.

Een studie gericht ter bepaling van de MTD van de combinatie van pazopanib gecombineerd met twee verschillende ifosfamide schema's is beschreven in **hoofdstuk 6**. Dit is de eerste studie die gebruikt maakte van het eerder beschreven 3 + 3 + 3 ontwerp. Patiënten met een gevorderde maligniteit kregen een vaste dosering ifosfamide, als een 3 dagen continu-infuus dan wel als een 3-uur durende bolus op 3 dagen achter elkaar (9 g/m<sup>2</sup> per kuur, elke 3 weken). Oraal in te nemen pazopanib werd in opvolgende cohorten geëscaleerd. Tijdens continue ifosfamide infusie kon de dosering pazopanib opgehoogd

worden tot 1000 mg/dag, terwijl tijdens bolus infusie zelfs de laagste dosering pazopanib te schadelijk was op basis van uiteenlopende toxiciteit. De farmacokinetiek gegevens toonde een ifosfamide geïnduceerde vermindering van blootstelling aan pazopanib aan. Maar desondanks werd na de toevoeging van ifosfamide een toename van placentale groeifactor en VEGF-A met een gelijktijdige daling in oplosbaar VEGFR2 gezien, passend bij pazopanib gemedieerde VEGFR2-remming. De aanbevolen dosering voor vervolgstudies is 800 mg pazopanib per dag gecombineerd met 3-dagen durende continue-infuus van ifosfamide van 9 g/m<sup>2</sup> per kuur.

In een volgende fase I studie werd pazopanib gecombineerd met twee verschillende schema's van docetaxel en deze studie staat beschreven in **hoofdstuk 7**. Patiënten met een solide maligniteit kregen naast dagelijks oraal in te nemen pazopanib, docetaxel via het infuus toegediend, om de 3 weken dan wel wekelijks met na drie weken een week geen toediening. Beide medicijnen werden geëscaleerd in opeenvolgende cohorten, waarbij opnieuw gebruik werd gemaakt van het 3 + 3 + 3 model. Deze studie toonde aan dat beide infusie schema's gecombineerd konden worden met een dosis van 400 mg pazopanib per dag. De MTD van het 3-wekelijks toegediende docetaxel was 50 mg/m<sup>2</sup>, terwijl voor het andere schema dit 20 mg/m<sup>2</sup> was. Beide doseringen zijn lager dan de dosering die wordt toegepast als docetaxel als enige antikanker medicijn wordt toegediend, waarbij dit grotendeels verklaard wordt door een klinisch relevante toename in docetaxel blootstelling als gevolg van een gedaalde docetaxel klaring met circa 33% door de gelijktijdige toediening van pazopanib. Meest waarschijnlijk is deze interactie een gevolg van CYP3A4 en OATP1B1 remming.

Nadat doseringen van combinaties zijn vastgesteld, moeten fase II studies verricht worden die inzicht geven of er een potentiële antikanker werking van een combinatie uitgaat, die het waard maakt de combinatie in een grote fase III studie te evalueren. In de voorgaande hoofdstukken van dit proefschrift zijn steeds 2 antikanker medicijnen tegelijkertijd gebruikt door de patiënten, echter een combinatie kan ook gebruikt worden door eerst het ene middel een langere tijd toe te dienen en vervolgens over te stappen op een 2<sup>e</sup> middel indien daar aanleiding toe is. In het algemeen wordt sequentie therapie beter verdragen dan combinatie therapie. Als vervolgens de totale overleving met sequentie therapie even groot is als met combinatie therapie, dan heeft sequentie therapie de voorkeur, zeker als dit resulteert in het uitstel van de introductie van klassieke chemotherapie en er mee samenhangende bijwerkingen. **Hoofdstuk 8** beschrijft het onderwerp van sequentie versus combinatie gebruik makend van een van de eerste doelgerichte antikanker middelen in de solide oncologie: trastuzumab; in deze studie in combinatie met docetaxel. Beide middelen hebben van zich zelf al bewezen antitumor effect in patiënten met een gemetastaseerd Her2Neu-positief mammacarcinoom, echter doorgaans worden beide middelen in combinatie gegeven. Aangezien dit gecombineerde schema geassocieerd is met relevante toxiciteit, is een sequentiële aanpak met eerst toedienen van trastuzumab

en dit wijzigen in docetaxel zodra er tumor progressie is opgetreden, een mogelijk zinnige strategie om de kwaliteit van leven te bevorderen. Chemotherapie-naïeve patiënten met een Her2Neu-positief gemetastaseerd mammacarcinoom werden gerandomiseerd tussen combinatie behandeling trastuzumab plus docetaxel (H+D) versus sequentiële behandeling trastuzumab en bij ziekteprogressie alleen docetaxel (H → D), met als primaire eindpunt progressie vrije overleving (PFS). De studie liet zien dat de mediane PFS voor de H+D groep en voor de H → D groep 9.4 vs 9.9 maand was met een 1-jaar PFS kans van 44% vs 35%, respectievelijk. Echter de kans op respons was 79% en 53% ( $p=0.016$ ) met een overleving van 30.5 vs 19.7 maand in het voordeel van de H+D groep. In de H → D groep was de respons op trastuzumab 34% met een kans van 39% op respons tijdens de vervolgbehandeling met docetaxel, met een mediane PFS op trastuzumab alleen van 3.9 maand. De incidentie en ernst van neuropathie was significant hoger in de H+D groep. Retrospectieve analyse van continueren van trastuzumab na progressie, toe gepast in 46% en 37% van de H+D groep en de H → D groep, respectievelijk, liet een correlatie zien met een langere overleving in beide behandelgroepen (36.0 vs 18.0 maanden en 30.3 versus 18.6 maanden, respectievelijk). Op basis van deze studie kan geconcludeerd worden dat 1<sup>e</sup> lijns behandeling bij patiënten met een gemetastaseerd Her2Neu-positief mammacarcinoom met H → D resulteerde in een vergelijkbare progressie vrije overleving in vergelijking met H+D, maar dat de kans op respons kleiner was en de overleving niet-significant korter.

## Toekomst

Gegeven het enorme aantal nieuwe medicamenten in ontwikkeling die toe gepast kunnen worden in een vrijwel oneindig aantal aan schema's, is het onmogelijk om combinatie studies te verrichten met elke potentiële combinatie. Het proces van studie ontwerp van de studies gericht op het vinden van de MTD is een hoeksteen in de evaluatie richting de implementatie van een bewezen effectieve combinatie therapie. Vanaf het allereerste begin van de ontwikkeling van een combinatie van antikanker middelen, moet strenge selectie op basis van hypothesen worden toe gepast om te komen tot combinaties die een hoge mate van waarschijnlijkheid hebben om uiteindelijk te kunnen bij dragen aan de tijd en/ of kwaliteit van leven van de patiënt met kanker. Hypothesen kunnen gegenereerd worden op basis van verwachte synergie naar aanleiding van theoretische of preklinische data. Voordat een dergelijke studie van start gaat moet een inschatting gemaakt worden of er een farmacokinetische interactie te verwachten is. Omdat verhoogde antitumor activiteit een farmacodynamische interactie is, moet ook op het gebied van potentiële interactie met betrekking tot toxiciteit geanticipeerd worden. Computermodellen, gevoed met data van preklinische als ook van vroeg-klinische studies met gegevens over remmende en inducerende effecten van de geneesmiddelen op de meest relevante enzymen betrokken

bij geneesmiddelmetabolisme, kunnen behulpzaam zijn bij het identificeren van de combinaties met relevante farmacokinetische interacties.

Voorts is het te overwegen de opbrengst per patiënt die bloot gesteld wordt aan een bepaalde combinatie van geneesmiddelen te verhogen. Er zou bijvoorbeeld intra-patiënt dosis escalatie plaats kunnen vinden. Dit introduceert uiteraard wel een bias, omdat een dosis alleen opgehoogd zal kunnen worden als logischerwijs de toegewezen startdosering verdragen wordt, maar het geeft wel aanvullende informatie met betrekking tot de bovenste doseer limiet. Een valkuil kan zijn dat de toxiciteit zich juist toont nadat de vooraf gedefinieerde MTD-observatie termijn verstreken is, echter deze valkuil bestaat ook in de huidige fase I modellen. Een andere zwakte is dat cumulatieve toxiciteit toegeschreven wordt aan een verkeerd dosis-niveau. Dus, data gegenereerd op basis van intra-patiënt dosis escalatie kunnen geen definitief antwoord geven, maar hooguit een signaal over de tolerantie afgeven.

Maar niet alleen de informatie per geïncludeerde patiënt moet toe nemen, ook de informatie per fase I studie moet worden geoptimaliseerd. Het zou verplicht moeten worden voor fase I combinatie studies om een strategie toe te passen die potentieel tenminste 2 MTDs voor die combinatie definieert. Tevens zou het aan te raden zijn om veranderingen in toedieningsvolgorde te onderzoeken en ook hierbij kan een intra-patiënt vergelijking leerzaam zijn.

Strategieën die de inclusie bevorderen van patiënten die een aangetoond voordeel hebben van de toepassing van een van beide middelen, kunnen de studie versnellen. Het verrijken van de patiëntpopulatie kan er voor zorgen dat een fase I studie tevens fase II-achtige informatie oplevert. Het vervlechten van fase I en II studies (net als fase II en III studies) kan zorgen voor het eerder beschikbaar komen van relevante informatie voor verdere ontwikkeling van de combinatie. Het is dan wel belangrijk dat dit type signalen niet gezien wordt als het definitieve antwoord, maar louter dient om een snelle startpositie voor de vervolgfase II studie en later de fase III studie te verkrijgen.

De leiders in het veld van vroeg klinische medicatie ontwikkeling zouden in gezamenlijkheid moeten besluiten tot nieuwe modellen zoals bijvoorbeeld bovenstaande voorstellen, die het mogelijk maken om fase I studies uit te voeren die snel en adequaat resulteren in het definiëren van MTD-curves voor veelbelovende combinaties als mede ook in het vast stellen van een minimum set aan kwaliteitseisen voor dergelijk studies.

Het blijft echter ook bij het toepassen van een combinatie van antikanker middelen zo dat, hoe behulpzaam alle nieuwe theoretische modellen ook kunnen zijn in het identificeren van veelbelovende combinaties, uiteindelijk de “proof of the pudding is in the eating”.

## Dankwoord

Het boekje is af. Er is weer een mijlpaal bereikt en die heb ik kunnen bereiken door de ondersteuning van velen. Allereerst gaat mijn dank uit naar de patiënten en hun naasten die hebben deel genomen aan de studies beschreven in dit proefschrift. Door de aard van de studies, te weten de begin fase van de ontwikkeling van medicamenteuze therapie, was er voor velen geen bewezen effectieve therapie meer beschikbaar. Om uiteenlopende redenen is door de patiënten gekozen voor deelname aan deze projecten, waarbij er een soms heftige behandeling ondergaan moest worden terwijl er vaak maar een beperkte tijd van leven was. Het was indrukwekkend om betrokken te zijn bij de (poli)-klinische begeleiding.

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## Curriculum vitae

Aart Paul Hamberg (1971; Erle) started medical school in 1990 after graduating *het Christelijk Lyceum* in Apeldoorn. He qualified as MD at the University of Utrecht in 1999. During the medical curriculum he was a board member of the Medical Student Society MSFU "Sams" as well as member of *het Jonge Heeren Gezelschap "Single Handed"*.

He started his internship in *het Antoni Van Leeuwenhoek*, (Amsterdam), followed by an internship in *het Diakonessenhuis* (Utrecht) with a subsequent residency in Internal Medicine in the same hospital (Supervisor Prof. Dr. JBL Hoekstra and later on Dr. WNM Hustinx). The academic phase of his residency was supervised by Prof. Dr. HAP Pols and subsequently Prof. Dr. JLCM van Saase (Erasmus Medical Center, Rotterdam). He was trained as medical oncologist at the Department of Medical Oncology in the Daniel den Hoed Cancer Center (currently the Erasmus MC Cancer Institute) of the Erasmus Medical Center, Rotterdam (Supervision Prof. Dr. G Stoter and later on Prof. Dr. J Verweij)

During the latter training, he started on the scientific work described in this thesis coached by Prof. Dr. J Verweij and Prof. Dr. S Sleijfer. After he became a board certified medical oncologist, he joined the staff of the Department of Medical Oncology in the Daniel den Hoed Cancer Center. Subsequently, he moved for a short period of time to *Medisch Centrum Haaglanden* (The Hague), before finding his current position in 2010 as medical oncologist in *het Sint Franciscus Gasthuis* (Rotterdam).

He married Minouche ten years ago and they live with their three children, Kasper, Julianne & Justus in Scheveningen, The Hague.





## List of publications

*"Serum S100 is suitable for prediction and monitoring of response to chemo-immunotherapy in metastatic malignant melanoma"*

**Hamberg P**, Korse CM, Bonfrer JM, de Gast GC.

Melanoma Res 2003; 13(1): 45-49.

*"Central nervous system toxicity induced by irinotecan"*

**Hamberg P**, Donders RCJM, ten Bokkel Huinink D

J Natl Cancer Inst 2006; 98: 219.

*"Non-islet-cell tumour induced hypoglycaemia in patients with advanced gastrointestinal stromal tumour, possibly worsened by imatinib"*

**Hamberg P**, de Jong FA, Boonstra JG, van Doorn J, Verweij J, Sleijfer S

J Clin Oncol 2006; 18: e30-31.

*"Cytotoxic therapy for the elderly with metastatic breast cancer: a review on safety, pharmacokinetics and efficacy"*

**Hamberg P**, Verweij J, Seynaeve C.

Eur J Cancer 2007; 43: 1514-1528.

*"Irinotecan-induced central nervous system toxicity. Report on two cases and review of the literature"*

**Hamberg P**, de Jong FA, Brandsma D, Verweij J, Sleijfer S.

Acta Oncol 2008; 47(5): 974-978.

*"When to start cytotoxic therapy in asymptomatic patients with hormone refractory prostate cancer?"*

**Hamberg P**, Verhagen PCMS, de Wit R.

Eur J Cancer 2008; 44:1193-1197.

*"Phase I drug combination trial design: Walking the tightrope"*

**Hamberg P**, Verweij J

J Clin Oncol 2009; 27: 4441-4443.

*"Chemotherapy in patients with castration-resistant prostate cancer"*

Meulenbeld HJ, **Hamberg P**, de Wit R

Eur J Cancer 2009; 45 Suppl1: 161-171.

*"It takes two to tango: combinations of conventional cytotoxics with compounds targeting the VEGF-VEGFR pathway in patients with solid malignancies"*

Boere IA, **Hamberg P**, Sleijfer S

Cancer Sci 2010; 101: 7-15.

*"Physiological effects of aging on treatment of older women with breast cancer. Impact on pharmacokinetics and pharmacodynamics of anticancer treatment"*

**Hamberg P**, de Jonge M, Seynaeve C

In: Management of breast cancer in older women (Reed MW and Audisio RA, editors), Springer Verlag London Ltd, 2010; pp 75-99.

*"Decreased sunitinib exposure due to concomitant administration of ifosfamide: results of a phase I and pharmacokinetic study on the combination of sunitinib and ifosfamide in patients with advanced solid malignancies"*

**Hamberg P**, Steeghs N, Loos WJ, van der Biessen D, den Hollander M, Tascilar M, Verweij J, Gelderblom H, Sleijfer S.

Br J Cancer 2010; 102: 1699-1706.

*"(Pre-)clinical pharmacology and activity of pazopanib, a novel multi-tyrosine kinase inhibitor"*

**Hamberg P**, Verweij J, Sleijfer S.

The Oncologist 2010; 15: 539-547.

*"Dose escalation models for combination phase I trials in oncology"*

**Hamberg P**, Ratain MJ, Lesaffre E, Verweij J.

Eur J Cancer 2010; 46: 2870-2878.

*"Phase I safety, pharmacokinetics and inhibition of Src activity study of saracatinib in patients with solid tumors"*

Baselga J, Martinelli E, Chirivella I, Hoekman K, Hurwitz HI, Jodrell DI, **Hamberg P**, Casado E, Elvin P, Swaisland A, Iacona RB, Tabernero J.

Clin Cancer Res 2010; 16: 1476-1483.

*"Randomized phase II study comparing efficacy and safety of trastuzumab/docetaxel combination therapy versus sequential therapy trastuzumab followed by docetaxel at progression as first line chemotherapy in patients with HER2-overexpressing metastatic breast cancer: HerTax trial"*

**Hamberg P**, Bos MEM, Braun HJJ, Stouthard JML, van Deijk GA, Erdkamp FLG, van der Stelt-Frisen IN, Bontenbal M, Creemers GJM, Portielje JEA, Pruijt JFM, Loosveld OJL, Smit WM, Muller EW, Schmitz PIM, Seynaeve C, Klijn JGM, on behalf of the Dutch Breast Cancer Trialists' Group (BOOG)

Clin Breast Cancer 2011; 11: 103-113.

*"Effect of ketoconazole-mediated CYP3A4 inhibition on clinical pharmacokinetics of panobinostat (LBH589), an orally active histone deacetylase inhibitor"*

**Hamberg P**, Woo MM, Chen LC, Verweij J, Porro MG, Zhao L, Li W, van der Biessen D, Sharma S, Hengelage T, de Jonge M.

Cancer Chemother Pharmacol 2011; 68: 805-813.

*"Topical psoralen plus UV-A therapy for Tyrosine kinase inhibitor induced hand foot syndrome"*

Bos WE, Nijsten TE, de Jonge MJ, **Hamberg AP**

Arch Dermatol 2012; 148(4): 546-547.

*"Effects of CYP induction by rifampicin on tamoxifen exposure"*

Binkhorst L, van Gelder T, Loos WJ, de Jongh FE, **Hamberg P**, Ghobadi Moghaddam-Helmantel IM, de Jonge E, Jager A, Seynaeve C, van Schaik RHN, Verweij J, Mathijssen RHJ.

Clin Pharmacol Ther 2012; 92(1): 62-62.

*"Phase I and pharmacokinetic study of pazopanib and lapatinib combination therapy in patients with advanced solid tumors"*

De Jonge MJA, **Hamberg P**, Verweij J, Savage S, Suttle AB, Hodge J, Arumugham T, Pandite LN, Hurwitz HI.

Investigational New Drugs 2013; 31(3): 751-759.

*"Sunitinib associated thrombocytopenia: not always what it seems"*

Albersen A, Porcelijn L, Schilders J, Zuetenhorst H Njo T, **Hamberg P**

Platelets 2013; 24(7): 566-570.

*"Cyanide intoxication by apricot kernel ingestion as complimentary cancer therapy"*

Seghers L, Walenbergh-van Veen, Salome J, **Hamberg P**

Neth J Med 2013; 71(9): 496-498.

*"Clinical activity and Tolerability of Enzalutamide (MDV3100) in Metastatic Castration Resistant Prostate Cancer Patients progressing after Docetaxel and Abiraterone Treatment"*

Badrising S, van Oort IM, van den Berg HP, Los M, **Hamberg P**, Coenen JL, van den Eertwegh AJM, de Jong IJ, Kerver ED, van Tinteren H, van der Noort V, Bergman AM

Cancer 2014; 120 (7): 968-975.

*"Pazopanib exposure decreases as a result of an ifosfamide-dependent drug-drug interaction: results of a phase I study"*

**Hamberg P**, Boers-Sonderen M, van der Graaf WTA, de Bruijn P, Suttle AB, Eskens FALM, Verweij J, van Herpen CML, Sleijfer S

Br J Cancer 2014; 110 (4): 888-893.

*"A randomized phase I bayesian dose escalation design for the combination of anti-cancer drugs"*

Dejardin D, Lesaffre E, **Hamberg P**, Verweij J

Pharmaceutical Statistics 2014 In press.

## **Abstracts**

*"Serum S100 is suitable for prediction and monitoring of response to chemo-immunotherapy in metastatic malignant melanoma"*

**Hamberg P**, Korse CM, Bonfrer JM, de Gast GC.

Melanoma Res 2001; 11, suppl 1; S134.

*"Serum S100 is suitable for prediction and monitoring of response to chemo-immunotherapy in metastatic malignant melanoma"*

**Hamberg P**, Bonfrer JGM, Korse CM, de Gast GC.

The Netherlands Journal of Medicine 2001; 58: A48 (93).

*"Phase I study of AZD0530, an oral potent inhibitor of Src kinase: first demonstration of inhibition of Src activity in human cancers"*

Tabernero J, Cervantes A, Hoekman K, Hurwitz HI, Jodrell DI, **Hamberg P**, Stuart M, Green TP, Iacona RB, Baselga J.

Proceedings Annual Meeting Am Soc Clin Oncol 2007; abstract # 3520.

*"Combined trastuzumab (HER)/docetaxel (TAX) versus sequential trastuzumab followed by docetaxel at progression as first line chemotherapy for Her2-positive metastatic breast cancer: preliminary results (multicenter BOOG-study; 2002-02)"*

**Hamberg P**, Bontenbal M, Vernhout RM, Bos MM, Braun HJ, Erdkamp F, Stouthard JML, van Deijk G, Schmitz PIM, Seynaeve C, Klijn JGM.

Breast Cancer Res Treat 2007; 106 suppl 1, abstract # 1077.

*"Randomized study comparing efficacy/toxicity of monotherapy trastuzumab followed by monotherapy docetaxel at progression and combination trastuzumab/docetaxel as first line chemotherapy in HER2-neu positive, metastatic breast cancer (MBC)(HERTAX study)"*

Bontenbal M, Seynaeve C, Stouthard JML, Bos MM, Braun HJ, Erdkamp F, van Deijk G, Schmitz PIM, van der Stelt-Frissen I, Seynaeve C, **Hamberg P**, Klijn JGM.

Proceedings Annual Meeting Am Soc Clin Oncol 2008; abstract # 1014.

*"Phase I safety and pharmacokinetics study of sunitinib in combination with ifosfamide in patients with advanced solid tumors"*

**Hamberg P**, Steeghs N, Loos WJ, van der Biessen AJ, den Hollander M, Tascilar M, Verweij J, Gelderblom H, Sleijfer S.

Proceedings Annual Meeting Am Soc Clin Oncol 2009; abstract # E13520.

*"A drug interaction study between ketoconazole and panobinostat (LBH589), an orally active histone deacetylase inhibitor, in patients with advanced cancer"*

de Jonge M, Woo MM, van der Biessen D, **Hamberg P**, Sharma S, Chen LC, Myke N, Zhao S, Hirawat S, Verweij J.

Proceedings Annual Meeting Am Soc Clin Oncol 2009; abstract # E2501.

*"Pharmacokinetic interaction between intravenous ifosfamide and oral sunitinib"*

Loos WJ, **Hamberg P**, Steeghs N, Kroep J, Tascilar M, Verweij J, den Hollander M, van der Biessen D, Gelderblom H, Sleijfer S.

Clin Pharmacol Ther 2010; 87: 578.

*"Predictive and mechanistic factors of tyrosine kinase inhibitor (TKI) induced macrocytosis"*

**Hamberg P**, Dulfer R, Oosten AW, von Lindern MM, Boere IA, van Doorn L, van Meerten E, Sleijfer S, Verweij J, Kruit WH

Proceedings Annual Meeting Am Soc Clin Oncol 2010; abstract 42589.

*"Incidental pulmonary embolism in oncology patients; a frequent finding with unknown prognosis"*

van der Veer M, **Hamberg P**, Hanselaar WEJJ

Proceedings Annual Meeting Netherlands Association of Internal Medicine 2011; abstract 134.

*"Bayesian dose escalation in phase I studies of combinations of drugs with control"*

Dejardin D, **Hamberg P**, Verweij J, Lesaffre E.

Proceedings of the 26<sup>th</sup> International Workshop on Statistical Modelling, (editors: Conesa D, Forte A, Loppers-Quiles A, Munos F.) page 169-172.

*"Bayesian dose escalation in phase I studies of combinations of drugs with control"*

Lesaffre E, **Hamberg P**, Verweij J, Dejardin D

Proceedings of the 2<sup>nd</sup> Conference of the Central European Network (CEN2011), 2011; abstract 239.

*“Induction of tamoxifen metabolism by rifampicin: a worrying drug-drug interaction”*

Binkhorst L, Loos WJ, de Jongh FE, **Hamberg P**, Ghobadi Moghaddam-Helmantel IM, Jager A, Seynaeve C, Verweij J, van Gelder T, Mathijssen RHJ.

San Antonio Breast Cancer Conference 2011; abstract P2-17-04.

*“Thrombotic thrombocytopenic purpura-Haemolytic Uremic syndrome (TTP-HUS) following administration of docetaxel and zoledronic acid in a patient with castrate refractory prostate cancer (CRPC)”*

van Oosten MJM, Schrama YC, Boeve ER, **Hamberg P**

10<sup>th</sup> European Congress on Internal Medicine 2011; European Journal of Internal Medicine 2011; 22: S97.

*“Sunitinib associated pseudothrombocytopenia: a cause of erroneous platelet transfusion”*

Albersen A, Porcelijn L, Schilders J, **Hamberg P**, Njo T

Ned Tijdschr Klin Chem Labgeneesk 2012; 37: 123 (Abstract nr 60).

*“Sunitinib associated pseudothrombocytopenia: a cause of erroneous platelet transfusion”*

Albersen A, Porcelijn L, Schilders J, Zuetenhorst H, **Hamberg P**, Njo T

Int J Lab Hem 2012; 34 S1: 85 abstract nr AM82.

*“Sunitinib associated pseudothrombocytopenia: not always what it seems”*

Schilders J, Albersen A, Porcelijn L, Zuetenhorst H, Njo T, **Hamberg P**

Proceedings Annual Meeting Netherlands Association of Internal Medicine 2012; abstract 52.

*“Phase I and pharmacokinetic (PK) study of pazopanib (P) in combination with two schedules of ifosfamide (I) in patients (pts) with advanced solid tumors (STs)”*

**Hamberg P**, Boers-Sonderen M, Loos WJ, de Jonge MJA, van der Graaf WT, Eskens FA, Verweij J, van Herpen CM, Sleijfer S

Proceedings Annual Meeting Am Soc Clin Oncol 2012; abstract 2593.

*“Gene expression profiles of circulating tumor cells in metastatic breast cancer patients”*

Mostert B, Sieuwerts AM, Kraan J, Bolt-de Vries J, Peeters D, Dirix LY, Seynaeve CM, Jager A, de Jongh FE, **Hamberg P**, Stouthard JML, Kehrler DFS, Look MP, Smid M, Jiang Y, Wang Y, Gratama JW, Foekens JA, Martens JWM, Sleijfer S

Proceedings Annual Meeting Am Soc Clin Oncol 2012; abstract 10504.

*"Induction of tamoxifen metabolism by rifampicin: a worrying drug-drug interaction"*

Binkhorst L, Loos WJ, de Jongh FE, **Hamberg P**, Ghobadi Moghaddam-Helmantel IM, Jager A, Seynaeve C, Verweij J, van Gelder T, Mathijssen RHJ.

Meeting of the Dutch Society of Clinical Pharmacokinetics and biofarmacia 2012.

*"Evaluation of the economic burden and impact on patients' quality of life of metastatic castration resistant prostate cancer: implementation of an observational study in 6 European countries"*

Dass RN, **Hamberg P**, Spencer M, Wheatley Price P

Mirrors of Medicine 2012: Proceedings of the Global congress on prostate cancer 2012; page 12, abstract nr 25.

*"Quality of life of patients with metastatic castration-resistant prostate cancer: Results from an observational study in 6 European countries"*

Dass RN, **Hamberg P**, Spencer M, Wheatley Price P

European Society of Medical Oncology, 2012; abstract e954.

*"The evaluation of healthcare resource utilization in patients with metastatic castration resistant prostate cancer: Results of a multinational observational study"*

Dass RN, **Hamberg P**, Spencer M, Wheatley Price P

ISPOR 15<sup>th</sup> Annual European Congress, 2012; abstract PCN 140.

*"Mapping FACT-P to EQ-5D in a large cross-sectional study of metastatic castrate-resistant prostate cancer patients"*

Diels J, Spencer M, **Hamberg P**, Wheatley Price P, Dass RN

ISPOR 15<sup>th</sup> Annual European Congress, 2012; abstract PCN116.

*"Phase I and pharmacokinetic (PK) study of pazopanib in combination with two schedules of docetaxel (D) in patients (pts) with advanced solid tumors"*

**Hamberg P**, Mathijssen RH, de Bruijn P, v/d Biessen D, Loos WJ, Sleijfer S, Verweij J, de Jonge MJ.

24<sup>th</sup> EORTC-NCI-AACR Symposium on 'Molecular Targets and Cancer Therapeutics', 2012; abstract 579.

*"mRNA expression profiles in circulating tumor cells (CTCs) of patients with metastatic breast cancer (MBC) treated with aromatase inhibitors (AI)"*

Reijm E, Sieuwerts AM, Bol-de Vries J, Mostert B, Onstenk W, Peeters D, Dirix LY, Seynaeve CM, Jager A, de Jongh FE, **Hamberg P**, van Galen A, Kraan J, Jansen MPH, Gratama JW, Foekens JA, Martens JM, Berns EMJJ, Sleijfer S.

Proceedings Annual Meeting Am Soc Clin Oncol 2013; abstract 11045.

*"Baseline bone mineral density in men with prostate cancer commencing with androgen deprivation therapy (ADT): Characteristics of Quantitative Ultrasound (QUS) of the heel as triage test to measure compared to Dual energy X-ray Absorptiometry (DXA)"*

Van Casteren-Messidoro C, Huisman AM, Bangma CH, Birnie E, van Gelder M, van de Geijn FE, **Hamberg P**

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 2942. P449.

*"Effect of dosing time on sunitinib pharmacokinetics"*

Kloth JSL, Binkhorst L, de Bruijn P, Burger H, **Hamberg P**, Wiemer EAC, van der Horst GTJ, Mathijssen RHJ

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 692. P193.

*"Tamoxifen pharmacokinetics influenced by circadian rhythm?"*

Binkhorst L, Kloth JSL, de Bruijn P, Burger H, van Alphen RJ, **Hamberg P**, van Gelder T, Wiemer EAC, van der Horst GTJ, Mathijssen RHJ

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 2095. P268.

*"Circulating tumor cells (CTCs) in castration-resistant prostate cancer patients treated with cabazitaxel; a Dutch Uro-Oncology Studygroup side-study"*

Onstenk W, Nieuweboer AJM, **Hamberg P**, Creemers GJM, MMEM Bos, Gratama JW, de Wit R, Mathijssen AHJ, Sleijfer S.

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 644. P139.

*"Gene expression profiles of circulating tumor cells (CTCs) in patients with metastatic breast cancer (MBC) treated with aromatase inhibitors (AI)"*

Reijm E, Sieuwerts AM, Bol-de Vries J, Mostert B, Onstenk W, Peeters D, Dirix LY, Seynaeve CM, Jager A, de Jongh FE, **Hamberg P**, van Galen A, Kraan J, Jansen MPH, Gratama JW, Foekens JA, Martens JM, Berns EMJJ, Sleijfer S.

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 698. P193.

*"A retrospective study on cabazitaxel and abiraterone acetate sequential treatment (CAST) in docetaxel treated metastatic castrate-resistant prostate cancer patients"*

Wissing M, Coenen JLLM, van den Berg P van Ort IM, de Wit R, Bergman AM, Westgeest HM, **Hamberg P**, Los M, Gelderblom H.

European Cancer Congress 2013 (ECCO-ESMO-ESTRO), abstract 2904. P411.



*"Quantitative Ultrasound (QUS) of the heel as triage test compared to Dual energy X-ray Absorptiometry (DXA) in men with prostate cancer commencing with androgen deprivation therapy (ADT)."*

Van Casteren-Messidoro C, Huisman AM, Birnie E, van Gelder M, van de Geijn FE, **Hamberg P**  
Scientific meeting Dutch Rheumatology Society (NVR) 2013, abstract PV35.

*"Estimating prevalence of prostate cancer clinical states using a dynamic patient progression model"*

Dass RN, Spencer M, Prutz C, Ahlgren G, Berruti A, Ford D, **Hamberg P**, Klier J, Ribal MJ, Schrijvers D, Wolff JM, Durand A.

ISPOR 16<sup>th</sup> Annual European Congress 2013, Value in Health 2013; 16(7): A397.

*"Citation analysis of the 'Big Six' journals in Internal Medicine"*

Van der Veer T, Baars JE, Birnie E, **Hamberg P**

12<sup>th</sup> European Congress on Internal Medicine 2013; European Journal of Internal Medicine 2013; 24:e233: abstract nr 460.

*"Gene expression profiles of primary tumors versus circulating tumor cells in metastatic breast cancer"*

Onstenk W, Sieuwerts AM, Weekhout M, Mostert B, Reijm EA, van Deurzen CHM, Kraan J, Jager A, Seynaeve C, Peeters D, **Hamberg P**, de Jongh FE, Smid M, van Galen A, Ramirez Moreno R, Dirix LY, Gratama J-W, Martens JWM, Foekens JA, Sleijfer S.

Proceedings Annual Meeting Am Soc Clin Oncol 2014; abstract 11017.

*"Mannose-binding lectin (MBL) serum level and genotype and risk of chemotherapy induced febrile neutropenia (FN) in patients with solid tumors"*

Goudzwaard JA, Fiets WE, van de Geijn GJM, Njo TL, Zuetenhorst JM, Birnie E, van Schaik RHN, van der Putten MM, Polee MB, **Hamberg P**

Proceedings Annual Meeting Am Soc Clin Oncol 2014; abstract e20653.



# PhD Portfolio

## Summary of PhD training and teaching

	Year	Workload (Hours/ ECTS)
<b>General courses</b>		
– FECS-AACR-ASCO Workshop: Methods in Clinical Cancer Research		2,0 ECTS
– BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2009	1,0 ECTS
– Good Clinical Practise	2013	1,0 ECTS
– Development of Business plan		1,0 ECTS
<b>Specific courses (e.g. Research school, Medical Training)</b>		
– Medical Specialist Training		2,0 ECTS
<b>Seminars and workshops</b>		
– OMBO workshops Daniel den Hoed		1,5 ECTS
<b>Presentations</b>		
– BOOG presentation HerTax trial		0,5 ECTS
– ASCO 2010 poster presentation	2010	1,5 ECTS
– ASCO 2012 poster presentation	2012	1,5 ECTS
<b>(Inter)national conferences</b>		
– Comprehensive Cancer Network Conferences		1,0 ECTS
– European Society of Medical Oncology		0,8 ECTS
– "Oncologie dagen" NVMO	2010	0,4 ECTS
– Annual meeting of American Society Clinical Oncology	2010	1,2 ECTS
– Jaar symposium Continuum Oncologie	2010	0,4 ECTS
– "Oncologie dagen" NVMO	2011	0,4 ECTS
– Post ASCO	2011	0,4 ECTS
– WIN-O symposium renal cell cancer	2011	0,4 ECTS
– Annual meeting of American Society Clinical Oncology	2012	1,2 ECTS
– "Oncologie dagen" NVMO	2012	0,4 ECTS
– "Oncologie dagen" NVMO	2013	0,4 ECTS
– Dutch Uro-oncology Meetings		0,6 ECTS
<b>2. Teaching</b>		
<b>Lecturing</b>		
– Several postgraduate lectures		1,2 ECTS
– Education AIOS/ANIOS Oncology Erasmus MC		2,0 ECTS
– Education AIOS/ANIOS Internal Medicine SFG		2,0 ECTS
<b>Supervising practicals and excursions, Tutoring</b>		
– Supervisor phase I unit		1,0 ECTS
– Mentor Specialist Oncology Nurse		2,0 ECTS
<b>Other</b>		
– EORTC-NCI-AACR meeting, poster preparation		0,5 ECTS
– Research meetings Oncology SFG		1,5 ECTS

