# Targeted therapy in oncology: mechanisms and toxicity

Steeghs, N.

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

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# Abstract

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

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

# Introduction

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

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

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

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

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

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

# Patients and Methods

## Eligibility criteria

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

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

## Dose-levels and Dose Escalation Procedure

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

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

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

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

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

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

## Pre-treatment Evaluation and Safety Assessment

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

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

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

## Pharmacokinetic Evaluation

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

## Blood sample collection

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

## Analysis of ifosfamide and its metabolites

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

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

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

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

### Pharmacokinetic Data Analysis

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

### Circulating Endothelial Cells

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

## Results

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

### Safety and Tolerability

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

**Table 1.** Baseline demographics and patient characteristics.

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

WHO: World Health Organization

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

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

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

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

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

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

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

## Pharmacokinetics

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

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

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

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

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

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

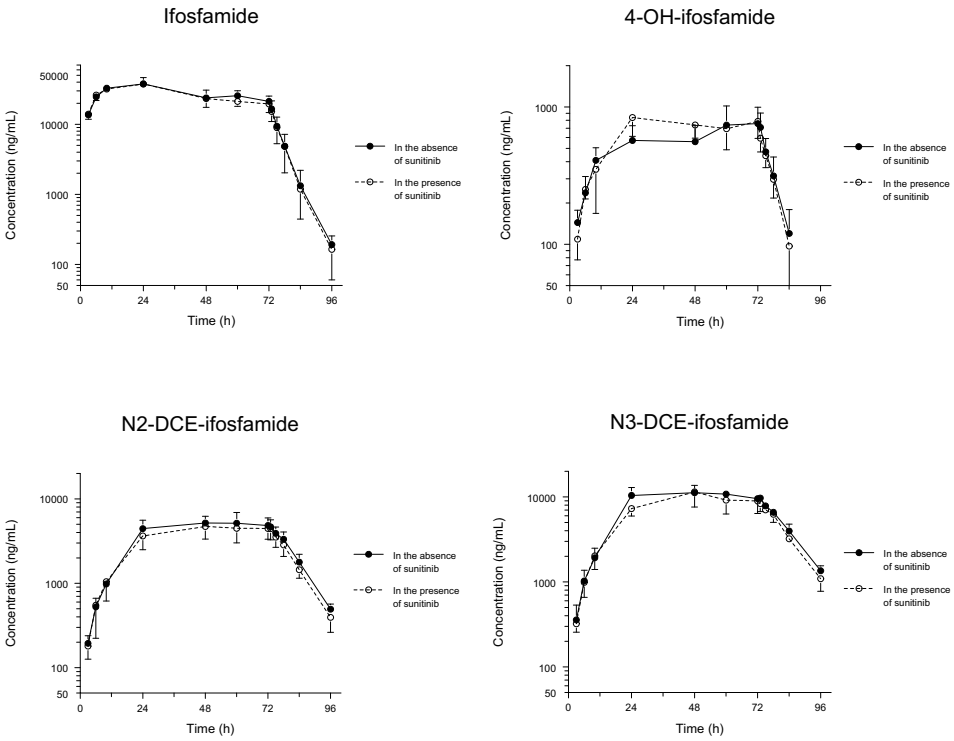


**Fig. 1.** Mean concentrations of ifosfamide and its metabolites of patients treated with the sunitinib 12.5 mg and ifosfamide 3g/m2/days1-3 combination.

## Circulating Endothelial Cells

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

## Anti tumor activity

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

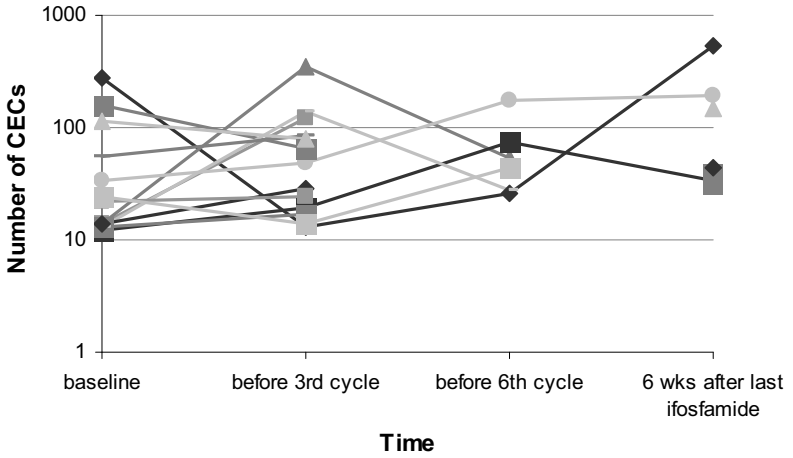


**Fig. 2.** Circulating endothelial cells during treatment with sunitinib in combination with ifosfamide 3g/m2/days1-3.

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

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

* 1 patient ongoing, no evaluation performed yet.

# Discussion

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

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

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

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

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

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

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

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

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

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

## Acknowledgements

The technical assistance of Peter de Bruijn and Inge Ghobadi Moghaddam-Helmantel for the analysis of ifosfamide and its metabolites is kindly acknowledged.

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