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ORIGINAL ARTICLE

A phase II study of sunitinib in patients with recurrent and/or metastatic non-nasopharyngeal head and neck cancer

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Abstract
Purpose Patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck (RM-SCCHN) bear a grave prognosis. There are unmet needs for the development of novel agents for this incurable disease. Angiogenesis is an important biological process in SCCHN. We, therefore, evaluated the activity and safety of sunitinib, an oral tyrosine kinase inhibitor that targets multiple receptors, in patients with RM-SCCHN.
Patients and methods Seventeen patients were treated with sunitinib 50 mg per day administrated in 4-week cycles followed by a rest period of 2 weeks. Sunitinib and SU012662 plasma levels were determined based on a validated liquid chromatography-tandem mass spectrometry method and pharmacokinetic data were fitted in a non-compartmental analysis.
Results Totally, 28 6-week cycles of treatment with sunitinib were administered (median, 2 cycles). Only three patients demonstrated stabilization of the disease; therefore, the study had to be terminated prematurely due to futility. Grade 3 toxicities, apart from fatigue, were infrequent. Other frequently reported side effects were skin discoloration, neutropenia, and thrombocytopenia. Ten various bleeding complications were reported in seven patients. Mean maximum concentrations (C_{max}) were reached during the first day of treatment for sunitinib at 38.98 (±22.66) ng/ml and for SU012662 at 11.12 (±24.57) ng/ml. Our results showed that SU012662 has a longer half-life and a larger volume of distribution than the parent drug sunitinib. None of the biological markers tested was of any prognostic value.
Conclusions According to our findings, sunitinib monotherapy was not proven active in RM-SCCHN, and no further development of the drug in this indication is warranted.

Keywords Head and neck cancer · Pharmacokinetic analysis · Sunitinib · Angiogenesis

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Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is the eighth most common cause of cancer-related death worldwide. In North America and the European Union, it accounts for 3–4% of all new cancer cases annually [1, 2].

Patients with recurrent and/or metastatic disease have a grave prognosis. Regardless of the chemotherapeutic

regimen used, median survival remains less than 1 year [3]. Platinum derivatives and taxanes increase response rate (RR) but have little impact on survival [4]. One exception is the addition of cetuximab to platinum-based chemotherapy plus 5-FU, which improved progression-free and overall survival when given as first-line treatment in patients with recurrent or metastatic disease [5]. Therefore, there are unmet needs for novel agents to be tested in this incurable disease.

Sunitinib (SU01148, Sutent®, Pfizer) is an oral tyrosine kinase inhibitor (TKI) that targets multiple tyrosine kinase receptors, including platelet-derived growth factor receptors (PDGFR-a and PDGFR-b), vascular endothelial growth factor receptors (VEGFRs, VEGFR1, VEGFR2 and VEGFR3), stem cell factor receptor (KIT), glial cell-line derived neurotrophic factor receptor, FMS-like tyrosine kinase-3 (FLT3), rearranged during transfection (RET) kinase, and colony-stimulating factor-1 receptor (CSF1R) [6]. Inhibition of the above receptor tyrosine kinases has a major impact on essential tumor cellular functions, such as cell growth, metastasis, apoptosis, and angiogenesis [7–9].

Angiogenesis is considered to be an important biological process in SCCHN. A recent meta-analysis of clinical studies evaluating the correlation between VEGF-A expression (assessed by immunohistochemistry, IHC) and survival of patients with SCCHN indicated that VEGF-A expression was associated with worse survival [10].

Clinical studies have suggested that the synchronous production of multiple cytokines, such as VEGF-A, PDGF, beta fibroblast growth factor, granulocyte colony stimulating growth factor (G-CSF), or granulocyte macrophage CSF (GM-CSF) by the tumor was strongly correlated with poorer prognosis of patients with SCCHN [11]. Furthermore, it has been previously shown by several investigations that VEGF-A, VEGF-R2 and VEGFR3 are frequently overexpressed in head and neck tumors [12]. However, Neuchrist et al. [13] were unable to demonstrate an association between VEGF-C expression and clinical parameters when investigating the expression of VEGFR3 and its ligand VEGF-C in established cell lines and tumors from patients with SCCHN.

Building upon this background, we conducted a phase II study of single-agent sunitinib in patients with recurrent and/or metastatic SCCHN. The primary objective of the study was to evaluate the objective response rate as a result of sunitinib monotherapy. Secondary objectives were time to tumor progression (TTP), survival, safety, and tolerability of sunitinib and the assessment of plasma SU011248 and SU012662 levels. Paraffin-embedded tumor tissue was prospectively collected in order to explore associations of potential biological markers with clinical outcomes.

Patients and methods

Eligibility

Eligibility criteria included histological confirmation of SCCHN, measurable or evaluable disease, performance status (PS) of 0–2 of the Eastern Cooperative Oncology Group (ECOG) scale, age > 18 years, adequate hepatic, renal and hematological function, willingness and ability to comply with protocol requirements, and provision of study-specific written informed consent. Patients were excluded for carcinomas of the nasopharynx, nasal or paranasal sinuses, salivary glands or thyroid, isolated recurrences amenable to local therapy, prior systemic treatment for recurrent or metastatic disease, major surgical operation or radiation therapy (RT) within 4 weeks prior to study entry, diagnosis of other malignancies, except for non-melanoma skin cancer that had been completely excised, history of myocardial infarction within 12 months prior to study entry, congestive heart failure or uncontrolled hypertension or angina, other medical or psychiatric illness that could impair the ability of the patient to receive protocol treatment, and any third space accumulation of fluid.

The protocol was approved by the Bioethics Committee of Aristotle University of Thessaloniki School of Medicine and by the National Organization for Medicines, Division of Pharmaceutical Studies and Research (EudraCT number 2005-004939-22).

Treatment

The starting dose of sunitinib was 50 mg per day administered in 6-week cycles consisting of 4 weeks of daily treatment followed by a rest period of 2 weeks. Sunitinib was self-administered orally once a day at approximately the same time without regard to meals. The investigational drug was provided by Pfizer, free of charge. The study medication was administered to all patients participating in the study until disease progression, unacceptable toxicity, or consent withdrawal.

All patients were closely monitored for toxicity and the drug dose was adjusted according to individual patient tolerance. Intrapatient dose reduction by 1, and if needed 2, dose level(s) (to 37.5 mg/day and then to 25 mg/day) may be required depending on the type and severity of toxicity encountered provided that criteria for patient withdrawal from study treatment have not been met.

In case of non-hematological toxicity, the dose of sunitinib was not reduced for grade 1 or 2 toxicity, unless otherwise judged by the investigator. For grade 3 non-hematological toxicity, however, the dose was withheld until toxicity was at maximum grade 1, or had returned to baseline. The treatment was then resumed at the same dose

level or the dose was reduced by 1 level at the discretion of the investigator. Similarly, in case of any non hematological grade 4 toxicity the dose was withheld until toxicity was of maximum grade 1 severity, or had returned to baseline. The dose was then reduced by 1 level and treatment was resumed, or discontinued at the discretion of the investigator.

Regarding hematological toxicity, no dose reduction of sunitinib was required for grade 1 or grade 2 toxicities. In case of grade 3 toxicity, the dose was withheld until toxicity was at maximum of Grade 2 severity, or it had returned to baseline and then the treatment was resumed at the same dose level. In the case of grade 4 hematological toxicity, the dose was withheld until toxicity was below Grade 2; then the dose was reduced by 1 level and the treatment was resumed treatment. Patients with grade 3 or grade 4 lymphopenia were allowed to continue with treatment without interruption. Similarly, patients who developed grade 3 or 4 hyperlipasemia or hyperamylasemia without clinical or other evidence of pancreatitis, or grade 4 hyperuricemia and/or grade 3 hypophosphatemia without clinical symptoms were allowed to continue treatment with sunitinib without interruption, with the reduction of the dose left at the discretion of the investigator. Patients were discontinued from the study if they were at the dose level of 25 mg and if toxicity guidelines indicated that a further dose reduction was necessary. The dosing period could not be extended to compensate for interruptions in study treatment due to any cause. Patients requiring more than 4 weeks of dose interruption (it could have been in addition to 2-week rest period for a maximum of 6 weeks) were discontinued from the study, unless continuation of treatment was still clinically indicated. Intrapatient re-escalation back to the previous dose level was permitted in the absence of greater than grade 3 hematological or greater than grade 2 non-hematological treatment-related toxicity in the previous cycle. Extra caution was recommended when sunitinib was administered with inhibitors or inducers of the CYP3A4 family. Of note, concomitant treatment with anti-arrhythmic drugs was not permitted. Nausea/vomiting was managed with antiemetics and diarrhea with loperamide. The use of hematopoietic growth factors was allowed at the discretion of the treating physician. Toxicity was graded using the National Cancer Institute Common Terminology Criteria for adverse events (NCI CTC) Version 3.0.

Evaluation

Baseline evaluation included medical history, physical examination, complete blood count (CBC), blood chemistry, bone scan, and CT scan of the head and neck region, chest, abdomen, and pelvis within 3 weeks prior to the start of treatment. Cardiac function was evaluated by electrocardiogram and echocardiogram or MUGA scan. Physical examination, CBC, blood chemistry, and clinical tumor assessment were repeated at the end of each 6-week cycle. CT scans of all tumor areas were repeated every two cycles. Objective tumor response was assessed by Response Evaluation Criteria in Solid Tumors (RECIST). All imaging material pertinent to tumor response was reviewed by one of the authors (A. K–F).

Sample collection for the pharmacokinetic studies

The pharmacokinetic parameters of sunitinib (SU011248) and its active metabolite SU012662 were assessed in 17 patients, on the first day of treatment and during the first cycle of therapy. Blood samples (4 ml) were drawn into EDTA tubes before treatment and at the following hours post-dose: 1, 2, 4, 8, 24 (day 1), 48 (day 2), 96 (day 4), 192 (day 8), 288 (day 12), 384 (day 16), 480 (day 20), 576 (day 24), and 672 (day 28). All samples were centrifuged immediately at 3,500 rpm at 4°C for 10 min. Plasma was separated, divided into two aliquots and placed into 1.5 ml Nalgene cryovials (Nalgene, Rochester, New York, USA). Plasma samples were stored at −80°C protected from light until analysis.

Pharmacokinetic analysis

A validated liquid chromatography-tandem mass spectrometry (LC-MS-MS) was used in order to determine plasma concentrations of sunitinib (SU011248) and SU012662. The analytical method, previously described by Motzer et al. [14], was developed at Bioanalytical Systems (West Lafayette, IN, USA). Calculation of sunitinib and SU012662 was based on a standard calibration curve in the range of 1–200 ng/ml ($r^2 = 0.9983$) and of 1–100 ng/ml ($r^2 = 0.9975$), respectively. Method validation was based on quality control (QC) samples ranging from 3 ng/ml (low concentration), 100 ng/ml (median concentration), and 150 ng/ml (high concentration) for SU011248 and from 3 ng/ml (low concentration), 50 ng/ml (median concentration), and 75 ng/ml (high concentration) for SU012662. The lowest limit of quantification (LLOQ) was determined at 1 ng/ml for both substances.

Assay accuracy expressed as bias (%) of quality control samples (QC) ranged from -3.7 to 2% and from −3.2 to 2% for SU011248 and SU012662, respectively. Assay reproducibility, expressed as coefficient of variation (CV%) of QC samples, ranged from 1.5 to 4.3% for SU011248 and from 2.3 to 5.3% for SU012662.

Plasma pharmacokinetic parameters were calculated by non-compartmental analysis of concentration versus time data using the WinNonlin software version 2.1 (Pharsight, Palo Alto, CA, USA). The following parameters were calculated: maximum concentration (C_{max}), time of occurrence

of C_{max} (T_{max}), $AUC_{0\text{-}24}$, $AUC_{0-\infty}$, terminal half-life ($t_{1/2}$), volume of distribution (V_z), and oral clearance (Cl/F).

C_{max} and T_{max} were determined directly from plasma concentration data. The area under the concentration curve versus time (AUC_{0-24}) was calculated using the linear trapezoidal rule from time zero to the time of the last measured day 1 concentration at 24 h. The $AUC_{0-\infty}$ was expressed as AUC extrapolated to infinity using the terminal elimination rate constant λ_z, which was calculated from log-linear regression of the terminal portion of the plasma concentration-time curve. Clearance (Cl) was calculated as dose/AUC and $t_{1/2}$ as $0.693/\lambda_z$.

Tissue samples

Formalin-fixed paraffin-embedded (FFPE) tumor tissue from 16 patients was used for protein expression by immunohistochemistry (IHC). Representative slides (H&E) from the tissue blocks were reviewed by an experienced pathologist (M.B.) for confirmation of the diagnosis, adequacy of material, and calculation of the percentage of tumor cells in each case. In 14 cases, the tumor tissue on the paraffin block was adequate for the construction of tissue microarrays (TMAs). The specimens were arrayed (2 cores per case, 1.5 mm in diameter) into a recipient paraffin block (Paraplast®, McCormick, USA) using a manual arrayer (Beecher Instruments, Sun Prairie, WI, USA). The TMA block also included six cores of the same diameter in the first column and three cores in the last column from placenta, tonsil, kidney, thyroid, endometrium, ovarian, and endometrial carcinomas, which served as positive and negative controls of the tested antibodies. After construction, the array block was placed in an incubator (58°C) for 15 min in order to assist the filling of the gap between the core-wax interface that could prevent the loss of the cores during cutting.

Immunohistochemistry (IHC)

Serial 3 μm thick sections form the original blocks or the TMA block, mounted on adhesion microscope slides, were cut at the Department of Pathology of the Aristotle University of Thessaloniki School of Medicine, and used for immunohistochemical labeling using the following antibodies: CD117 (c-kit), HIF-1a, VEGF-A, VEGF-C, VEGFR1, VEGFR2, and VEGFR3. Immunohistochemistry for PDGFR-a and PDGFR-b was performed at the Institut de Cancerologie Gustave Roussy, Villejuif, France. All slides were stained in one run for each antibody, 2–10 days after tissue sectioning. The deparaffinization, antigen retrieval, and staining procedures were performed according to standard protocols with slight modifications. Endogenous peroxidase activity and non-specific protein reaction were blocked by treatment of the tissue slides with H_2O_2 and protein-blocking agent, respectively. The antibodies, immunohistochemical procedures, and interpretation criteria used are presented in Table 1. The antigen-antibody complex was visualized using diaminobenzidine (DAB) as a chromogen. Slides were counterstained with Mayer's hematoxylin, washed in fresh water, dehydrated, and mounted.

The evaluation of all stained sections was done by experienced pathologists blinded to the patients' clinical characteristics and survival data. In all cases, the staining intensity, percentage of stained cells, and the cellular staining pattern were indicated. Furthermore, the presence or absence of staining in other non-neoplastic tissue elements (vessels, inflammatory cells, fibroblasts) was also noted. CD117 (c-kit) expression was evaluated according to Miettinen et al. [15]. HIF-1 alpha immunoreactivity was assigned as positive if the tumor samples contained neoplastic cells with positively stained nuclei [16]. Any expression of PDGFRs, VEGFs, and VEGFRs by tumor

Table 1 Antibodies and staining procedures used in the present study

Antigen (antibody)	Clone (source)	Pretreatment/time	Ab dilution	Detection system	Incubation time	Staining pattern
CD117, c-kit	pl (1)	EDTA, pH 8.8/20′	1:100	Bond™ Polymer (8)	30′	C, C/M
HIF-1α	H1alpha67 (2)	EDTA, pH 8.8/25′	1:500	Bond™ Polymer (8)	o/n	N
PDGFR-α	AF-307-NA (3)	CB, pH 7.3/30′	1:100	Envision™ (1)	60′	C
PDGFR-β	P-20 (4)	CB, pH 7.3/30′	1:150	Envision™ (1)	60′	C
VEGF-A	VG1 (1)	EDTA, pH 8.8/20′	1:75	Bond™ Polymer (8)	60′	C
VEGF-C	18-2255, pl (5)	CB, pH 6.0/20′	1:250	HRP (9)	o/n	C
VEGFR1	RB-9049-P1 (2)	CB, pH 6.0/15′	1:450	HRP (9)	o/n	C
VEGFR2	55B11 (6)	CB, pH 6.0/20′	1:450	Bond™ Polymer (8)	o/n	C
VEGFR3	KLT9 (7)	CB, pH 6.0/15′	1:50	HRP (9)	o/n	C

C cytoplasm, *CB* citric acid pH 6.0, *EDTA* ethylenediaminetetraacetate, pH8.8, *M* membrane, *N* nuclear; *o/n* overnight, *pl* polyclonal

Antibody sources: (1) Dako, Glosrtup, DK; (2) Thermo Fisher Scientific, Fremont, CA, USA; (3) R&D Systems, Minneapolis, MN, USA; (4) Santa Cruz, Santa Cruz, CA, USA; (5) Zymed™, Invitrogen, Carlsbad, CA, USA; (6) CST, Beverly, MA, USA; (7) Novocastra™, Newcastle Upon Tyne, UK; (8) Leica Biosystems, Wetzlar, Germany; (9) BioGenex, San Ramon, CA, USA

cells or other tissue elements was considered as positive. High expression of VEGFs and VEGFRs was considered if over 50% of tumor cells showed moderate or intense staining patterns [modified according to Gunningham et al. [17].

Statistical analysis

The sample size was estimated based on the primary endpoint of the study, the response to treatment. According to Simon's two stage minimax design, 31 patients were needed to be accrued in the first stage. If at least 7 responses would be observed, an additional 22 patients would then be recruited in the second stage. Sunitinib would be considered active in this patient population if at least 14 responses were to be observed among the 53 patients. The sample size was derived using the following values: $P_0 = 0.20$, $P_1 = 0.35$, $\alpha = 0.05$, and $\beta = 0.80$.

Seventeen patients entered the first stage and no response was observed. At this point an unplanned futility analysis was performed to decide whether to continue accruing in the first stage. Under the scenario of a response rate of at least 20%, the probability of observing enough responses to proceed to the second stage (i.e. at least 7 responses in the remaining 14 patients) was only 1.2%. Even under the best-case scenario, of the response rate being at least 35%, the conditional probability of continuing was 18%. These results led to the decision of discontinuing the first-stage accrual due to futility.

Responses are presented as frequencies and percentages along with 95% exact confidence intervals. Comparisons between selected biomarkers were performed using the Fisher's exact test. Secondary endpoints were survival and toxicity. The Mann–Whitney test was used to compare the distribution of the maximum concentrations (C_{max}) and the areas under the concentration curve versus time ($AUC_{0-\infty}$) for sunitinib and its metabolite in terms of the reported toxicity (severe vs. mild). Survival was measured from the date of randomization to the date of last contact or until death. Time to progression (TTP) was defined as the time interval from the date of entry to the date of tumor progression or death. Survival and time to progression were estimated using the Kaplan–Meier method and differences between survival curves were assessed with the log-rank test. For all comparisons the level of significance was set at $a = 0.05$. The statistical analysis was conducted using SPSS 15 for Windows.

Results

Patient characteristics

Selected tumor and patient characteristics are shown in Table 2. Totally, 17 patients entered the study. There were 15 men and 2 women with a median age of 61 years and median PS of 1. Twelve patients (71%) presented at study entry with locally recurrent disease only and 5 (29%) with both local and metastatic disease. Primary treatment consisted of some type of surgical operation in ten patients,

Table 2 Patients and tumor characteristics

N	17
Age (years)	
Median	61
Range	45–75
	N (%)
Sex	
Male	15 (88)
Female	2 (12)
ECOG performance status	
0	5 (29)
1	12 (71)
Extent of disease	
Locoregional	
Tumor	13 (76)
Nodes	12 (71)
Skin	2 (12)
Metastatic	
Lung	5 (29)
Nodes	2 (12)
Only locoregional	12 (71)
Only metastatic	0 (0)
Both	5 (29)
Tumor grade	
Well differentiated	2 (12)
Moderately differentiated	11 (65)
Poorly differentiated	4 (24)
Primary tumor	
Larynx	11 (65)
Oral cavity	5 (29)
Oropharynx	1 (6)
Primary surgery	
No	7 (41)
Yes	10 (59)
Total leryngectomy	7 (41)
Hemiglossectomy	1 (6)
Fuctional neck dissection	1 (6)
Chordectomy	1 (6)
Primary radiation	
No	12 (71)
Yes	5 (29)
Primary chemoradiotherapy	
No	9 (53)
Yes	8 (47)

radiation therapy (RT) in five, induction chemotherapy followed by concomitant chemoradiotherapy in three and concomitant chemoradiotherapy in eight.

Treatment characteristics

A total of 28 six-week cycles of treatment with sunitinib were administered, with a median of 2 cycles per patient (range, 1–3). The median duration from the time of first to last sunitinib dose (including 14 days following the last dose) was 8.1 weeks (range, 2.6–18.1). Reduction of the dose of sunitinib was not needed in any of our patients. Treatment delays were required for six patients, in one of the cycles. One patient stopped treatment because of dysphagia caused by the tumor and restarted treatment 3 weeks later. Treatment was eventually discontinued in all 17 patients. The main reason for treatment discontinuation was progression of the disease, which occurred in 12 patients. Additionally, three patients withdrew consent, one of them following a transient interruption of treatment because of grade 3 neutropenia and the other two because the results of their treatment did not meet their expectations. Furthermore, administration of sunitinib had to be discontinued in two patients due to bleeding around the tracheostoma in one and reactivation of lung tuberculosis in the other. At the time of the present analysis no patient was still on treatment with sunitinib.

Efficacy

Response was evaluated radiologically and/or clinically in 14 patients. However, response could not be evaluated in three patients, since one of them died from disease, one discontinued treatment because of toxicity, and one withdrew his consent prior to the first response evaluation. Overall, three patients (18%, 95% exact CI 3.8–43.4%) demonstrated stabilization of disease and 11 (65%, 95% exact CI 38.3–85.79%) showed progressive disease.

After a median follow-up time of 12.8 months (range 6–19.7, 95% CI 3.7–22.0), 14 patients (82%) had tumor progression and 12 (70%) died. Median TTP was 2.3 months (range 0.6–16.6, 95% CI 1.0–3.6) and median survival 4.0 months (range 1.0–19.7, 95% CI 3.2–4.9). Six patients continued their treatment with some type of chemotherapy, four of them with platinum-based combinations and two with methotrexate monotherapy.

Toxicity

The most frequent side effects encountered in the study which were at the same time possibly related to the administration of sunitinib are depicted in Table 3. In general, treatment was well tolerated. Grade 3 toxicities were infrequent, apart from fatigue, which occurred in 7 out of 17 patients. Other frequently reported side effects were skin discoloration, neutropenia, and thrombocytopenia. Ten various bleeding complications were reported in seven patients, one of them was bleeding around the tracheostomia of grade III, and the rest of grade II or I. One unusual side effect, reported in one of our patients, was a tracheoesophageal fistula formed within the tumor, probably a result of the progression of the tumor. However, possible relation with the administration of sunitinib cannot be excluded. No hypertension was reported, probably due to the relatively short period of treatment with sunitinib in the present study.

Other reported grade I side effects not shown in Table 3 were insomnia, rigors/chills, dry skin, hand-foot syndrome, wound complications (non-infectious), vomiting, muscle weakness, watery eye, elevated or decreased laboratory values, such as creatinine, alkaline phosphatase, bilirubin, and GGT, hyperuricemia, hyperglycemia, hypoalbuminemia, hyponatremia, hypothyroidsm, and edema in the head and neck region, all of them reported once.

Table 3 Most frequent adverse events possibly related to sunitinib

Toxicity	Any grade		Grade III	
	N	%	*N*	%
Yellow coloration of skin	11	65	0	0
Fatigue	10	59	7	41
Hemorrhage/bleeding	10	59	1	6
Infection	7	41	0	0
Thrombocytopenia	6	35	3	18
Anorexia	6	35	2	12
Stomatitis	6	35	0	0
Leukopenia	5	29	1	6
Neutropenia	5	29	1	6
Dysphagia	5	29	0	0
Pain	5	29	0	0
Anemia	4	24	0	0
Dizziness	4	24	0	0
Hypertransaminasemia	4	24	0	0
Skin breakdown	3	18	0	0
Taste alteration	3	18	1	6
Lymphopenia	2	12	0	0
Fever	2	12	0	0
Rash	2	12	0	0
Constipation	2	12	0	0
Diarrhea	2	12	0	0
Nausea	2	12	0	0
Somnolence	2	12	0	0
Cough	2	12	0	0
Mood alteration	2	12	0	0
Tracheoesophageal fistula	1	6	1	6

Pharmacokinetics

Representative chromatograms of the standard solutions of sunitinib and SU012662 are shown in Fig. 1a, b, respectively. Pharmacokinetic (PK) data for sunitinib (SU011248) and its active metabolite, SU012662, were available for 17 patients. The mean concentration over time profiles of sunitinib and SU012662 on day 1 are shown in Fig. 2a, b. Moreover, Fig. 3a, b represent the mean concentration of each substance during the first cycle of therapy, while Fig. 4 represents the mean concentration over time profile of the combination of sunitinib and SU012662. The mean and standard deviation values of the PK parameters for sunitinib and SU012662, listed in Table 4, are in accordance with previous published data [18, 19]. Times of maximum concentrations (T_{max}) for sunitinib and SU012662 were 7.06 (±1.70) h and 6.12 (±2.0) h, respectively. Mean maximum concentrations (C_{max}) were estimated to be 38.98 (±22.66) ng/ml for sunitinib and 11.12 (±24.57) ng/ml for SU012662, both achieved on the first day of treatment. Thereafter, concentrations declined with a mean half-life of approximately 39.65 (±17.62) h for sunitinib and a longer half-life approximately 83.50 (±42.93) h for SU012662. The area under the curve (AUC_{0-24}) on day 1 for sunitinib and SU012662 was determined to be 580.50 (±284.64) and 152.30 (±250.63) ng h/ml, respectively. The mean values for clearance expressed as Cl/F were 34.38 (±12.60) l/h for sunitinib and 117.77 (±54.12) l/h for SU012662. Sunitinib showed a much lower volume of distribution (1817.03 ± 825.36 l) than its active metabolite SU012662 (13378.75 ± 8594.94 l).

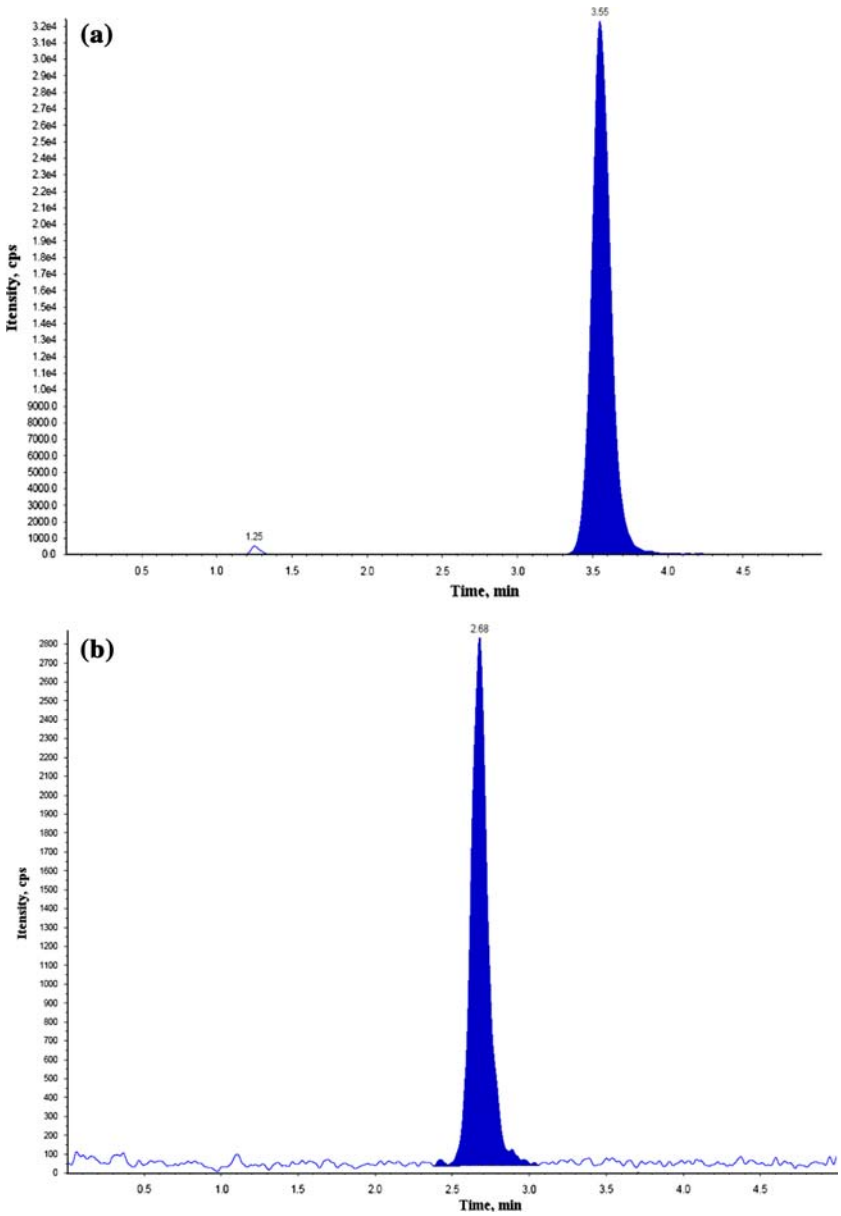

Fig. 1 Representative chromatograms of sunitinib (**a**) and SU012662 (**b**) standard solutions at 1 ng/ml

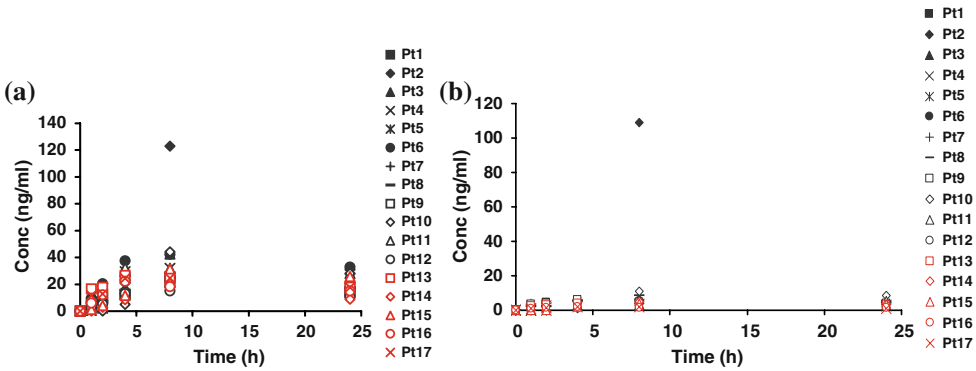

Fig. 2 Sunitinib (**a**) and SU012662 (**b**) plasma concentrations in 17 patients receiving sunitinib orally (50 mg)

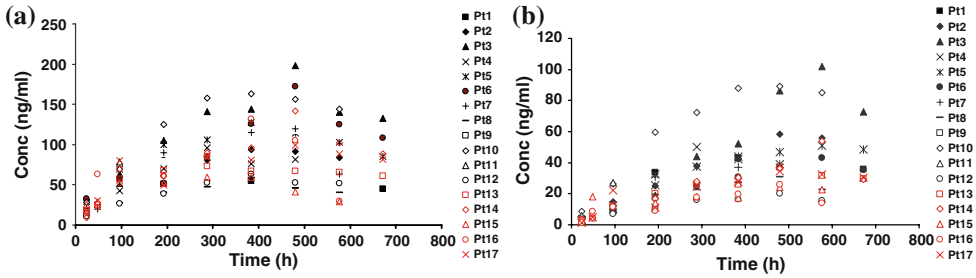

Fig. 3 Sunitinib (**a**) and SU012662 (**b**) plasma concentrations in 17 patients receiving sunitinib orally (50 mg total daily dose) for 28 days (672 h)

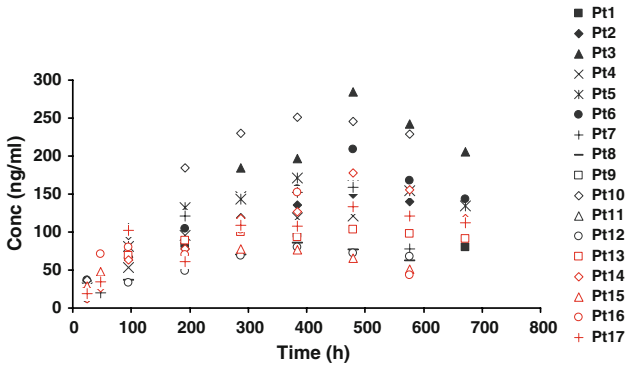

Fig. 4 SU011248 and SU012662 plasma concentrations in 17 patients receiving sunitinib orally (50 mg total daily dose) for 28 days (672 h)

Table 4 Pharmacokinetic parameters of sunitinib (SU011248) and its metabolite SU12662 in 17 patients enrolled in pharmacokinetic analysis

$N = 17$	Sunitinib (SU011248)	SU012662
T_{max} (h)	7.06 ± 1.70	6.12 ± 2.00
C_{max} (ng/ml)	33.98 ± 23.66	11.12 ± 24.57
C_{last} (ng/ml)	20.10 ± 6.74	3.65 ± 1.68
AUC_{0-24} (ng h/ml)	580.50 ± 284.64	152.30 ± 250.63
$t_{1/2}$ (h)	39.65 ± 17.62	83.50 ± 42.93
$AUC_{0-\infty}$ (ng h/ml)	1728.54 ± 845.89	557.62 ± 316.73
Cl/F (l/h)	34.38 ± 12.60	117.77 ± 54.12
V_z/F(l)	1817.03 ± 825.36	13378.75 ± 8594.94

Data presented as mean values ± standard deviation (SD)

No significant associations were found between observed toxicity and C_{max} of sunitinib and its metabolite ($p = 0.758$ and $p = 0.918$, respectively) or $AUC_{0-\infty}$ for the parent compound and its metabolite ($p = 0.999$ and $p = 0.758$, respectively).

IHC

C-kit receptor was detected in 3 out of 16 tumor samples (Table 5). The stain was cytoplasmic in two cases (Fig. 5a) and cytoplasmic/membraneous in one case. The mast cells and dendritic cells, when present, highly expressed the above receptor. HIF-1α nuclear staining was observed in 7 out 16 tumors (Table 5; Fig. 5b). For PDGFR-a, we did not observe any staining in tumor cells in the 12 cases tested (Fig. 5c). Control tissues were positively stained, as well as many inflammatory cells and some stromal cells. One out of the 13 cases tested showed weak cytoplasmic positivity for PDGFR-b (Table 5; Fig. 5d), while immunoexpression was moderate to intense in inflammatory and stromal cells. In some cases, the endothelial cells showed weak cytoplasmic staining for the above receptor. VEGF-A and VEGF-C expression was detected in the majority of tumor samples (Table 5). Specifically, VEGF-A was variably expressed in tumor cells, in 13 out of 14 samples. High expression of VEGF-A, i.e., over 50% of tumor cells, combined with moderate or intense staining was observed in 7 out of 13 cases (Fig. 5e). Inflammatory cells and fibroblasts showed variable positivity in all tested tissue samples. In the vast majority of the cases the staining intensity of endothelial cells was similar to that of tumor cells. VEGF-C expression

Table 5 IHC protein expression

	N (%)
CD117, c-kit	
Negative	13 (81)
Positive	3 (19)
HIF-1a	
Negative	9 (56)
Positive	7 (44)
PDGFR-a	
Negative	13 (81)
Positive	–
NE	3 (19)
PDGFR-b	
Negative	12 (75)
Positive	1 (6)
NE	3 (19)
VEGF-A	
Negative	1 (6)
Low	6 (38)
High	7 (44)
NE	2 (12)
VEGF-C	
Negative	1 (6)
Low	10 (62)
High	4 (25)
NE	1 (6)
VEGFR1	
Negative	5 (31)
Low	6 (38)
High	4 (25)
NE	1 (6)
VEGFR2	
Negative	4 (25)
Low	10 (62)
High	1 (6)
NE	1 (6)
VEGFR3	
Negative	2 (12)
Low	2 (12)
High	11 (69)
NE	1 (6)

was detected in 14 out of 15 tumor samples. Four cases (25%) showed high VEGF-C cytoplasmic staining (Fig. 5f). The inflammatory cells were positive in all cases. VEGFR1 evaluation was successful in 15 out of 16 cases (Table 5). The receptor was expressed in 10 out of 15 tumor samples (Fig. 5g). High levels of VEGFR1 were observed in four tumor samples (25%). Stromal tissue elements (inflammatory cells, endothelial cells, and fibroblasts) showed at least moderate staining intensity. VEGFR2 was positive in 11 out of 15 tumor samples and highly expressed in one case (Fig. 5h). In all cases the endothelial cells showed cytoplasmic staining, even when the receptor was not expressed in the tumor cells. Plasma cells, when present, highly expressed VEGFR2. VEGFR3 was expressed in tumor cells in 13 out of 15 cases. High expression of the receptor was observed in 11 out of 13 positive cases (Fig. 5i). Only in positive cases the endothelial cells co-expressed the receptor. The stromal plasma cells and fibroblasts showed in all cases moderate to intense expression.

No significant associations were found between the expression of HIF-1a and VEGF-A and between VEGF-A or VEGF-C and VEGFR1, and R2 (data not shown). The prognostic value of HIF-1a and VEGF-A was examined. Among seven HIF-1a positive patients six deaths were recorded, all of them in the first 4 months, versus five deaths among nine patients with HIF-1a negative expression (log-rank, $p = 0.016$). Similarly, all VEGF-A positive patients died, six of them in the first 4 months, compared with four deaths among the seven VEGF-A negative patients (log-rank, $p = 0.105$).

Discussion

In the present study we evaluated the role of sunitinib in patients with recurrent and/or metastatic SCCHN. Even though the drug was given as first-line treatment for recurrent or metastatic disease, efficacy results were disappointing. Among the first 17 patients, no objective response was observed and the trial had to be discontinued prematurely. In a study presented at the 2008 ASCO Meeting [20], investigators from the University of Chicago evaluated the efficacy and tolerability of sunitinib, given exactly in the same way as in the present study, to patients with recurrent/metastatic SCCHN. Among 19 evaluable for response patients, only one partial response was recorded. The toxicity profile was similar to that observed in our patients, with myelotoxicity and fatigue being the most common side effects.

In a study similar to that of ours, sorafenib, another TKI of B-Raf, C-Raf and receptor tyrosine kinases of VEGFR2, VEGFR3, PDGF, FLT3, and c-kit, failed to demonstrate a meaningful activity in recurrent and/or metastatic SCCHN [19]. Indeed, among 26 eligible for response patients, one achieved a PR (3.7%) and 10 patients (37%) achieved disease stabilization. Median TTP was 1.8 months and median survival 4.2 months, similar to that reported in our study. Biomarker analysis of paired tumor samples, performed in a small cohort of patients, clearly showed a successful disruption of the Ras-Raf, MEK, ERK pathway and in a lesser extent a pro-apoptotic effect by the use of sorafenib. Our

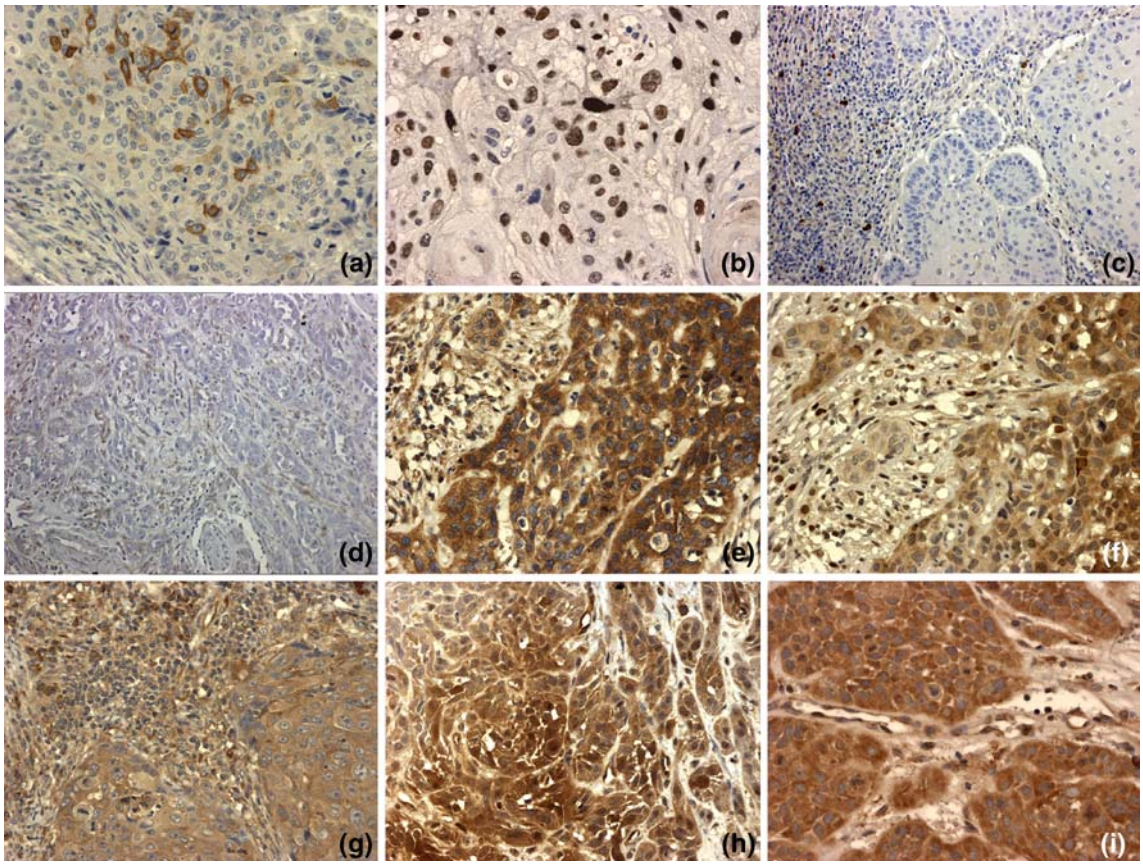

Fig. 5 (**a**) c-kit receptor intense cytoplasmic staining by a fraction of tumor cells; **(b)** HIF-1α nuclear positivity in tumor cells; **(c)** PDGFR-a receptor absence of staining in tumor cells, whereas the inflammatory cells stained positive; **(d)** PDGFR-b mild expression by tumor cells; **(e)** VEGF-A strong cytoplasmic expression in tumor cells, fibroblasts, and endothelial cells; **(f)** VEGF-C expression in tumor cells; **(g)** VEGFR1 staining in tumor cells, inflammatory, endothelial cells and fibroblasts; **(h)** VEGFR2 intense expression by tumor cells and endothelial cells; **(i)** The tumor cells and vascular lining cells express VEGFR3. Original magnification ×200

findings concerning the expression of the VEGF receptors 1–3 and their ligands VEGF-A and VEGF-C, as well as HIF-1α are similar to the reported literature in SCCHN [12, 21, 22]. For kit receptor, PDGFR-a and PDGFR-b the discrepancies in protein expression between our study and a previous study [23] possibly reflect differences in the polyclonal antibodies used and in the epitope retrieval modalities applied. The reasons of this profound lack of activity of both TKIs, sunitinib or sorafenib, in this group of patients are not clear at present. There is a large body of evidence suggesting that the molecular targets of both drugs play an important role in the biology of SCCHN and affect progression-free survival and survival of patients [12, 24–26]. However, proliferation of tumor cells may not exclusively depend on these pathways, while there may be constitutively intrinsic operative mechanisms of resistance to antiangiogenic treatments reviewed in ref. [27]. Obviously, we need to further investigate these mechanisms before a meaningful clinical benefit from the use of these agents in head and neck cancer could be achieved. Whether the inefficacy of sunitinib (and sorafenib as well) also applies to other antioangiogenic treatments, such as monoclonal antibodies, is unknown at the present time. Ongoing clinical trials with the anti-VEGF monoclonal antibody bevacizumab in combination with chemotherapy or concomitantly with RT will partly clarify this issue.

Overall, treatment was well tolerated. The safety profile of sunitinib in the present study was similar to that reported in other tumor types with single-agent sunitinib at the daily dose of 50 mg [14, 28–30]. Grade 3 side effects were infrequently observed, apart from fatigue, probably due to the short duration of the sunitinib treatment.

The pharmacokinetic data of sunitinib and its active metabolite (SU012662) have already been evaluated by other groups in patients with metastatic renal, colorectal, and breast cancer [14, 28, 29] treated with similar dosage schedules. Our findings are in accordance with the previous studies [18, 19]. Sunitinib displayed a long half-life and a large volume of distribution. Once daily dosing for 28 days resulted in an approximately 4.2-fold accumulation of sunitinib, an 11-fold accumulation of SU012662, and a 5.3-fold accumulation of total drug (sunitinib and SU012662).

Among our patients, there was one whose sunitinib and SU012662 plasma levels were exceptionally high, already on the first day of treatment (123 and 109 ng/ml, respectively). This finding could not be attributed to any known drug interactions or to any other apparent factors. As for the rest of the 16 patients treated, the interpatient variability was moderate for sunitinib C_{max} and AUC_{0-24}, reaching 30 and 29%, respectively. For SU012662, the inter-patient variability of C_{max} was higher at 45% and that of AUC_{0-24} at 41%.

It is noteworthy that the mean C_{min} of sunitinib and SU012662 on the fourth day of treatment was 70.91 ng/ml. Moreover, on the eighth day (192 h), the combined levels of sunitinib and SU012662 were within or above the range of 50–100 ng/ml in all patients, which has been shown to be the active concentration for the inhibition of target tyrosine kinases in preclinical models [31].

The increased survival observed in HIF-1a negative patients is in agreement with previously published data in SCCHN patients [32, 33]. However, these data should be interpreted with caution, due to the small number of patients, and should be viewed merely as hypothesis generating that would need to be validated in larger SCCHN patient cohorts.

In conclusion, sunitinib, as given in the present study, has limited activity in unselected patients with recurrent or metastatic SCCHN. Further development of this agent, at least as monotherapy, in this indication is not warranted.

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