UniversitätsKlinikum Heidelberg

Name of Sponsor/Company: Universitätsklinikum Heidelberg		Sponsor-Code of Study:	(For National Authority Use only)
Name of (Finished) Product: Nexavar		Name of Active Ingredient: Sorafenib	
EudraCT-Nr.: 2008-002667-13	BfArM Vorlage-Nr.: 4034390	Ethik Antrags-Nr.: AFmo-224/2008	

SYNOPSIS

Title of Study:
A single-center controlled pilot study to investigate pre- and perioperative therapy with Sorafenib (Nexavar®) in patients who are candidates for a curative surgery of renal cell cancer (PREST = preoperative Sorafenib Therapy in RCC)

Investigators:
Principle Investigator:
Prof. Dr. Markus Hohenfellner
University Clinic for Urology
University Heidelberg
Im Neuenheimer Feld 110
69120 Heidelberg
Phone: +49-6221-56-6320
Fax: +49-6221-56-53566
E-mail: Markus.Hohenfellner@med.uni-heidelberg.de

Study Centre(s):
Department of Urology
University Hospital
Im Neuenheimer Feld 110
69120 Heidelberg

Publication (reference):

Hatiboglu G, Hohenfellner M, Arslan A, Hadaschik B, Teber D, Radtke JP, Hallscheidt P, Tolstov Y, Roth W, Grüllich C, Huesing J, Duensing S, Pahernik S. Effective downsizing but enhanced intratumoral heterogeneity following neoadjuvant sorafenib in patients with non-metastatic renal cell carcinoma.
Langenbecks Arch Surg. 2017 Jun;402(4):637-644. doi: 10.1007/s00423-016-1543-8. Epub 2016 Dec 23. PubMed PMID: 28012035.

Study period: (date of first enrolment) (date of last completed)	Date of first enrolment: 2008-10-09 Date of last patient visit: 10/2013	**Study Phase: II**

Objectives:
- To compare the gene expression profiles (RNA and protein expression) of primary RCC tumor after neoadjuvant Sorafenib treatment compared with placebo treatment- To determine safety profile of neoadjuvant and adjuvant Sorafenib with respect to hemorrhage as well as wound healing after performing surgery for renal cell cancer

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Methodology:

Methods in design and analysis

Randomization and blinding
Patients are randomized in a 3:1 ratio between Sorafenib and Placebo. Patient registration and randomization is performed centrally at the study site. A patient number is assigned by the randomization unit at the site and the pharmacy prepares study medication according to the randomisation number and a copy of the randomization list, which is prepared in the KKS.

All patients now have been unblinded. Patients 04/ 06 and 09 received placebo while all other patients received Sorafenib.

Variable definitions

Primary endpoint
The primary endpoints are taken as the gene expression profile of the excised primary tumour in comparison between treatment arms, and as the safety profile. The safety profile will be assessed as

- all adverse events of CTC ≥ 3, listed by randomization number and study day of start
- all laboratory values outside the reference ranges, listed by randomization number and study day.

Secondary study objectives:

- To determine early susceptibility of RCC to Sorafenib by radiological volumetry as compared to assessment by RECIST
- To determine early susceptibility of RCC to Sorafenib by MRI with Vasovist® after randomization and immediately prior to RCC resection
- To determine effect of preoperative Sorafenib therapy on intra-tumor vasculature density and necrosis volume in the resected material
- To assess treatment effects on other tissue biomarkers and blood biomarkers
- To determine the concentration of circulating RCC tumor cells in peripheral blood prior to Sorafenib during Sorafenib therapy prior to surgery, during surgery, after surgery and at study end.
- Tissue biomarker results will be correlated with imaging results in both groups
- To determine safety of Sorafenib
- To determine the relapse rate 2 years after surgery, in particular progression free survival.

Analysis sets
The main analysis of the expression profile was planned to be carried out in an intention-to-treat fashion, while the safety set would contain all patients who have received study medication at least once. As for the current report the functional genomic data are not analyzed, and the blind is not broken, only patients who received study medication at least once are considered.

Number of patients (planned and analysed):
Planned 40
Analysed 10

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Diagnosis and main criteria for inclusion:

Inclusion criterias:

- Patients with stage I/II or III renal cell carcinoma who are eligible for curative standard surgery of the primary tumor without any prior treatment at or immediately after primary diagnosis
- At least 18 years of age
- Sufficient bone marrow function : neutrophils 1500/µl, haemoglobin 10g/dl, and platelets $100x10^{9}/l$
- Patients with performance status of ECOG ≤ 1
- AST and ALT ≤ 2.5 x ULN (≤5x ULN for patients with liver involvement of their cancer), total bilirubin ≤ 1.5xULN; alkaline phosphatase ≤ 4xULN, PT-INR/PT < 1.5xULN
- Serum creatinine ≤ 1.5xULN; MDRD (GFR =186 x (Serum Creatinine [mg/dl])$^{-1.154}$ x(age)$^{-0.203}$ x 0.742 (female patients) □□40ml
- Ability of subject to understand character and individual consequences of the clinical trial
- Given written informed consent with the federal and institutional guidelines before any study treatment
- Life expectancy of at least 12 weeks
- Women and men with reproductive potential enrolled in this trial must use safe and effective barrier birth control measures during the course of the trial and for at least three months after the last administration of Sorafenib

Exclusion criterias:

- History of cardiac disease: congestive heart failure (NYHA II-IV), active CAD (MI more than 6 months prior to study entry is allowed), cardiac arrhythmias requiring antiarrhythmic therapy (betablockers or digoxin are permitted) or uncontrolled hypertension
- History of would healing complications
- History of HIV infection or chronic hepatitis B or C
- Active clinically serious infections (> Grade 2 NCI-CTC version 3.0)
- Symptomatic brain metastasis or meningeal tumors
- Pregnant or breast feeding women
- Patients with seizure disorders requiring medication (such as steroids or anti-epileptics)
- History of organ allograft
- Patients with evidence or history of bleeding diathesis
- Patients undergoing renal dialysis
- Known or suspected allergy or hypersensitivity to the investigational agent or to any excipient present in the pharmaceutical form or any agent given in association with this trial
- Any previous or concurrent malignancy that is distinct in primary site or histology from the cancer being evaluated in this study except treated basal cell carcinoma or any cancer curatively treated >3 years prior to study entry
- Drug or alcohol abuse. Psychological or social conditions that may interefere with the patient's participation in the study or evaluation of the study results
- Any condition that is unstable or could jeopardize the safety of the patient and their compliance in the study

Excluded therapies and medications, previous and concomitant

- Prior systemic therapy for RCC
- Prior surgery for RCC (RCC resection as part of study permitted). Major other surgery within 4 weeks prior to start of study or incomplete would healing from previous other major surgery
- Radiotherapy during study or within 3 weeks of start of study drug
- Enrollment in another clinical trial within the last 4 weeks
- Severe renal impairment that does not allow MR contrast agents (glomerular filtration rate < 30mL/min/1.73m2)

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Test product, dose and mode of administration, batch number:
Patients take 2 tablets of Sorafenib (200 mg tablets)/Placebo twice daily, orally on a Continuous basis for 12 weeks. The study drug is supplied as 200-mg tablets. The tablets should be swallowed whole with approximately 250 mL of water, each morning and evening (ie, twice daily). Study drug may be taken either with a low/moderate fat meal or without food. After a dose, patients do not have to wait before eating. In case of high fat meal the study drug has to be taken at least 1 hour before or 2 hours after eating. Patients are to continue Sorafenib until one of the criteria for "criteria for stopping therapy" were met (see 3.3.1). After four weeks of treatment patients are scheduled for surgery. No interruption of study medication was planned during surgery, although dose modification according for safety reasons is permitted in two steps of frequency reduction (two tablets per day, two tablets every other day), or even delayed. Patients who need further treatment after study end will be treated according to current standard of care.

Duration of treatment:
12 weeks

Reference therapy, dose and mode of administration, batch number:
Sorafenib (200 mg tablets) twice daily

Batch number:
Sorafenib 200mg (bulk): S26701
Placebo: S26702

Criteria for evaluation: (efficacy, safety)

Primary endpoint
The primary endpoints are taken as the gene expression profile of the excised primary tumour in comparison between treatment arms, and as the safety profile. The safety profile will be assessed as

- all adverse events of CTC ≥ 3, listed by randomization number and study day of start
- all laboratory values outside the reference ranges, listed by randomization number and study day.

The gene expression profile will be obtained after blood samples have been collected from all patients. It is outside the scope of this status report. The times when blood samples were collected for circulating tumour cells are listed in the appendix.

Statistical methods:
The main analysis of the expression profile was planned to be carried out in an intention-to-treat fashion, while the safety set would contain all patients who have received study medication at least once. As for the current report the functional genomic data are not analyzed, and the blind is not broken, only patients who received study medication at least once are considered. For comparing different groups, non-parametric Mann-Whitney test was used. Statistical significance was regarded as $p < 0.05$.

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Summary – Conclusions:

Efficacy Results:

Clinical benefit can be summarized as a possible downstaging of the tumor. In these patients, that have been scheduled for nephrectomy, a nephron sparing approach was mostly possible. Adverse events could be handled with dose reduction or supportive medication. All together, 6 adverse events could be observed in 5/9 verum-patients and 4 adverse events in 3 placebo patients. In 3 verum-patients and 1 placebo-patients a dose modification was done.

As the study is still blinded, the seen effects can only be estimated and will have to be re-evaluated after unblinding the study population. We did not observe any wound healing problems. After unblinding the cohort we found a 29% reduction of tumor volume compared to control after 4 weeks of neoadjuvant therapy with sorafenib.

Eight of the nine patients of the sorafenib group experienced tumor volume reduction during 4 weeks treatment. No significant tumor volume reduction was seen in placebo group . Mann-Whitney-U-Test showed a p=0,02. There was no RECIST evaluation as no non-target lesions were observed (therefore no secondary endpoint

None of the patients developed distant metastasis under study medication or during follow-up.

During study period, blood samples have been collected and mononuclear cells have been isolated. These samples will be evaluated for circulating tumor cells. We have investigated with our collegues the method to measure CTC from blood. After several validation experiments, it was concluded that reliable measurements of CTC is not possible. Analysis of genome sequencing is not available up to now. Therefore, evaluation of secondary endpoints are not available.

Morphological heterogeneity of treatment responses

We first analyzed representative tumor areas of sorafenibtreated patients for signs of necrosis. Of nine patients treated with TKI, only one patient showed an expansive central necrosis comprising over 90% of the residual tumor area. Two patients showed a focal necrosis whereas two patients showed focal hemorrhage. Interestingly, four patients showed areas of tumor cell depopulation and replacement with loose connective tissue.

To assess functional ITH in response to sorafenib on a cellular level, we performed immunohistochemical stainings for Ki-67, cleaved caspase-3, and CD31 (Figs. 3 and 4). Median expression of the proliferation marker Ki-67 was 7.0 cells per HPF in the TKI group vs. 11.4 cells per ×40 HPF in the placebo group ($p > 0.05$). The range for Ki-67 positive cells in the TKI group was 1.8–75.3 cells per HPF vs. 2.3–13.3 cells per HPF in the placebo group. One specimen with the highest number of Ki-67-positive cells was from a sorafenib-treated patient (75.3 cells per HPF), who also showed tumor progression under TKI treatment. The median frequency of cells positive for cleaved caspase-3 in the treatment and the control group, respectively, was 0.25 cells per HPF (range = 0–25.3) vs. 3.25 cells per HPF (range = 0.4–15.7). Statistical analysis showed no significant difference ($p > 0.05$). The median number of CD31-positive blood vessel cross-sections was 15.5 per HPF in the treatment group (range = 5.3–33.0) and 5.25 per HPF in the placebo group (range = 1.1–17.8; $p > 0.05$). In order to directly compare the level of functional ITH between patient subgroups and markers, we used the standard deviation of the mean number of positive cells per HPF Langenbecks Arch Surg (2017) 402:637–644 639 (standard deviation, STDEV) as a measuring tool as previously described [6]. In the placebo group, STDEVs for Ki-67, cleaved caspase-3, and CD31 were 5.88, 8.13, and 8.70, respectively.

In the TKI group, STDEVs for Ki-67, cleaved caspase-3, and CD31 were consistently increased to 23.18, 10.76, and 11.27, respectively. Collectively, these results underscore that neoadjuvant sorafenib enhances functional ITH.

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Safety Results:

Regarding AE and SAE no new safety issues were identified. All seen adverse events were expected due to the known adverse event profile of study medication (sorafenib). In the majority of cases, these adverse events could be handled with dose reduction or supportive medication. In three patients, the study medication was withdrawn.

Conclusion:

Sorafenib in standard dosage, given preoperatively for 28 days, was clinically active in downsizing tumors in patients with locally confined, non-metastatic RCC together but led to an enhanced functional ITH in the residual tumor tissue.

Date of report: 07.02.2019

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List of AEs:

Subject ID	Treatment group	AE term	Serious AE?	Study Day of AE start	Study Day of AE end	Ongoing ?	CTC Toxicity Grade	Pattern	Action taken	Association with study drug	Outcome
SCRNO:12 / RNDNO:10	Sorafenib	Hand-food-Syndrome 4.+5. toe right food	.	22	63	.	3	intermittend	study drug reduced	certain	disappeared
SCRNO:14 / RNDNO:11	Sorafenib	Rash maculopapular	.	12	32	.	3	intermittend	Study drug interrupted	certain	disappeared
SCRNO:14 / RNDNO:11	Sorafenib	Gastroenteritis	yes	63	67	.	3	isolated	Concom. medic. given + hospitalisation	probable	disappeared
SCRNO:15 / RNDNO:12	Sorafenib	Rash maculopapular	.	14	24	.	3	isolated	Study drug reduced	certain	disappeared
SCRNO:3 / RNDNO:3	Sorafenib	Worsening of hypertension	.	33	33	.	3	isolated	Concomitant medication given	probable	disappeared
SCRNO:6 / RNDNO:6	Placebo	NSTEMI	yes	37	75	.	4	isolated	Study drug stopped, conmeds given	possible	death
SCRNO:6 / RNDNO:6	Placebo	Anemia	.	33	75	.	3	intermittend	blood transfusion given	probable	present
SCRNO:6 / RNDNO:6	Placebo	Worsening of pulmonary hypertension	.	35	75	.	3	intermittend	Concom. medication given	.	.
SCRNO:6 / RNDNO:6	Placebo	Anuria	.	34	.	.	3	intermittend	Hemodialepsis	unlikely	.
SCRNO:8 / RNDNO:8	Sorafenib	Generalized exanthema and enanthema	yes	12	.	yes	3	isolated	Decortin 60 mg, Fenistil, ideos, hospitalization of patient	certain	present

Lesion Volume:

		Lesion volume					
		N	Min	Median	Max	Mean	StdDev
Treatment group	**Visit**						
Placebo	**SC**	3	108.4	439.6	535.2	361.1	224.0
	D28	3	134.7	438.7	545.4	372.9	213.1
Sorafenib	**SC**	9	38.7	65.9	128.7	76.8	35.7
	D28	9	18.8	37.4	107.4	53.0	33.4

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Treatment group	Subject ID	Volume (cm^3) at screening	Volume (cm^3) at 28 days post	Volume diff (cm^3)
Placebo	SCRNO:4 / RNDNO:4	108.43	134.71	26.28
Placebo	SCRNO:6 / RNDNO:6	439.63	438.68	-0.95
Placebo	SCRNO:9 / RNDNO:9	535.18	545.39	10.21
Sorafenib	SCRNO:1 / RNDNO:1	65.94	37.43	-28.51
Sorafenib	SCRNO:12 / RNDNO:10	78.97	30.69	-48.28
Sorafenib	SCRNO:14 / RNDNO:11	116.22	82.47	-33.75
Sorafenib	SCRNO:15 / RNDNO:12	128.65	107.44	-21.21
Sorafenib	SCRNO:17 / RNDNO:13	38.73	40.62	1.89
Sorafenib	SCRNO:20 / RNDNO:14	42.08	18.75	-23.33
Sorafenib	SCRNO:3 / RNDNO:3	52.45	26.52	-25.93
Sorafenib	SCRNO:7 / RNDNO:7	48.58	35.04	-13.54
Sorafenib	SCRNO:8 / RNDNO:8	119.25	97.91	-21.34