Debates on Bladder Cancer

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Original article

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Phase I dose-escalation study of intravesical instillation of antisense oligonucleotide FFC15-01 against Ki-67 in patients with non-muscle invasive bladder cancer

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Abstract

Background: In this phase I dose escalation study, we evaluated the pharmacokinetic and toxicity profiles of intravesical instillation of the FFC15-01 Ki-67 antisense oligonucleotide in patients with bladder cancer.

Methods: Six patients with bladder cancer were included. The Ki-67 antisense oligonucleotide was administered intravesically with a starting dose of 50 mg at an escalated dose according to an accelerated dose titration design a day before surgical intervention and during the intervention after resection of the tumour. Retention time in the bladder was two hours. Duration of treatment was altogether 48 hours. Maximum tolerated dose (MDT), dose limiting toxicity (DLT) and pharmacokinetics were determined.

Results: Treatment was clinically well tolerated in patients. Adverse events were predominantly grade I according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC). Dose limiting toxicity was not observed. The dose escalation was terminated at the predetermined MDT of 500 mg. At the 3-month control cystourethroscopy 3 out of 4 patients were stable without signs of tumour.

Discussion: The accessibility of the bladder mucosa makes intravesical therapy an excellent route of administration in bladder cancer. Previous clinical studies with intravenous application of antisense agents in other oncological indications were associated with marked side effects. Here, a systemic uptake of the antisense oligonucleotides was not found.

Conclusions: Instillation of the Ki-67 antisense oligonucleotide FFC15-01 is a safe and well tolerated treatment. A phase II study was initiated.

Key words: Phase I, Ki-67, antisense oligonucleotide, bladder cancer, dose escalation

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BACKGROUND

Bladder cancer is the second most common malignancy of the urinary tract in the Western world with an estimated 28,750 new cases diagnosed each year in Germany [1].

In the current treatment of non muscle-invasive bladder cancer, the limitations of the therapies available are manifested in high recurrence and progression rates. 50-70% of non muscle-invasive bladder cancers will recur after transurethral resection (TUR), with or without chemotherapy [2], and 50% of patients with carcinoma in situ (CIS) will develop invasive bladder cancer within 5 years [3]. Therefore, novel therapeutic options especially for high-risk non muscle-invasive bladder cancer should be explored to prevent recurrence and progression, and improve cancer-specific survival.

The expression of the human Ki-67 protein is strictly associated with cell proliferation. Detailed cell cycle analysis revealed that the antigen is present in nuclei of proliferating (G1-, S-, G2-phase and mitosis) cells, but not in nuclei of quiescent or resting cells (G0-phase) [4]. It was demonstrated that the Ki-67 protein belongs to the family of MPM-2 antigens and that phosphorylation of the Ki-67 protein during mitosis is associated with the condensation of the chromosomes and the separation of sister chromatids [5,6]. Furthermore, a C-terminal domain of Ki-67 protein (Kon21) is able to bind to all three members of the mammalian heterochromatin protein 1 (HP1) family in vitro and in vivo suggesting a role for Ki-67 protein in the control of higher order chromatin structure [7].

The function of the Ki-67 protein provided a clue for its use as a target in antisense mediated cancer therapy. In vitro and in vivo experiments provide evidence for the efficacy of a Ki-67 antisense oligonucleotide against cancer cells [8,9]. The efficacy of FFC15-01 was also proven *in vitro* and *in vivo*. The therapeutic efficacy is dependent on the drug concentration at the site of action. Pharmacologic experiments have shown that after local administration of the oligonucleotide in the urinary bladder, high local concentrations in the surrounding tissue could be achieved [9]. This led to the rationale for the treatment of non muscle-invasive bladder carcinomas with FFC15-01 by topical application of the drug in the human bladder.

METHODS

This phase I study was designed as an open-label trial with dose escalation according to an accelerated titration design. The trial was conducted in accordance with the Declaration of Helsinki and all its revisions. The protocol was approved by the local ethic committee of the Charité Berlin in July 2003. All patients provided written informed consent before study entry. The first patient was included in April 2004, and the last patient completed in October 2004.

It was planned to include 20 patients to assess the maximum tolerated dose (MTD); in total, 6 patients were included when the study goal was met.

Inclusion criteria were normal bone marrow (WBC ≥ 4.0/nl, platelets ≥ 100/nl and haemoglobin ≥ 10 g/dl) function, no hepatic impairment (bilirubin > 1,5 and/or AST, ALT, γ-GT, PT or PTT >2 times the upper normal limit) or renal impairment (creatinine > 2,0 mg/dl), no cancer treatment within four weeks of study entry and a life expectancy of ≥ 3 months. Exclusion criteria were any cancer medication during the last 4 weeks prior to study entry, participation in a study 3 months prior to the beginning of this

Table 1. General Grading of Toxicity

0	No toxicity
1+	Mild toxicity, usually transient, requiring no special treatment and generally not interfering with usual daily activities
2+	Moderate toxicity which may be ameliorated by simple therapeutic manoeuvres; impairs usual activities
3+	Severe toxicity which requires therapeutic intervention and interrupts usual activities, hospitalisation may be required
4+	Life-threatening toxicity which requires hospitalisation. If the toxicity caused a drug-related death, it must be graded 4F

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study, poorly controlled coexisting medical conditions, major organ dysfunction in a site of known or assumed toxic effects (e.g. potential renal tubular dysfunction indicated by increased proteinuria), a history of any haematological disease or disorder, recurrent infections of the urogenital system, prostate hyperactivity, uterus dislocation and chronic bladder disease or disorder, conditions requiring the administration of anticoagulants, underlying disease associated with active bleeding or a past history of coagulopathy or complement abnormality, psychiatric or emotional problems, which would invalidate the giving of informed consent or limit the ability of the patient to comply with study requirements.

Primary objective was to evaluate the maximum tolerated dose (MTD) and the dose limiting toxicity (DLT) of FFC15-01 as an intravesical application and to establish an optimal dosage for further phase II trials, to assess qualitative and quantitative toxic effects of FFC15-01 and the duration, intensity, date of onset thereof as well as their reversibility and dose dependency. The study would be terminated at a predetermined MDT of 500 mg.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC). The MTD was defined as the highest dose studied for which the incidence of DLT was less than 33%. DLT was defined as any of the criteria below:

- any NCI-CTC grade ≥ 3 non-haematologic toxicity (excluding alopecia)
- NCI-CTC grade 4 complicated neutropenia (neutrophils < 0,5/nl and temperature >38,5°C or clinical signs of infection)
- NCI-CTC grade 4 neutropenia lasting ≥ 7 days
- NCI-CTC grade ≥ 3 thrombocytopenia +/- hemorrhagic complications

For the general scaling of toxicities see Table 1.

Secondary objectives were to assess the safety and tolerability of FFC15-01 in terms of clinical findings, adverse events (AE), vital signs, routine blood chemistry, haematological values, urin analysis and other relevant physiological parameters that can be measured in the trial centre. After tumour resection, analysis of Ki-67 index was performed using immunohistochemical Ki-67 staining as previously described [10].

Enrolled patients received different dosages of FFC15-01 with a starting dose of 50 mg up to 500 mg in several escalation steps according to an accelerated titration design [11]. The accelerated phase would have been terminated, if a patient exhibited dose limiting toxicity (DLT) or two patients experienced toxicities grade 2+, according to NCI-CTC during the treatment. Afterwards, the design would have been changed to the standard method of dose escalation i.e. the modified Fibonacci scheme [12]. If any patient exhibited a significant oligonucleotide plasma level (>50 µg/ml), the study would have also been terminated.

The antisense oligonucleotide FFC15-01 against the mRNA encoding the Ki-67 nuclear protein, consists of 23 desoxyribonucleotides based on a phosphorothioate backbone, to prevent the oligonucleotide from degradation. This oligonucleotide hybridises to nucleotides 197 to 220 of the Ki-67 mRNA, covering the translational start codon. The FFC15-01 oligonucleotide sequence is 5'- ACC AGG CGT CTC GTG GGG CAC AT – 3'. The nucleic acid was chemically synthesised and made available in its sodium salt form. Identity was verified by mass spectroscopy (theoretical mass +/- 0.1 %). Sulphur substitution existed in more than 99 % of the phosphodiester groups. The material contained at least 88 % of full-length oligonucleotides as determined by HPLC analysis. The remainder were chain-shortened products. Heavy metal concentrations were below 20 ppm and residual solvents matched the criteria given by European and United States Pharmacopeia.

The trial drug was administered to patients via a transurethral catheter as an instillation of 100 ml into the urinary bladder over 2 hours,

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one day before surgical intervention and after resection of the tumour.

Blood samples were taken from one patient on each dose level immediately before first intake of the substance; during the intervention at 30, 60 and 90 min and the end of application; and at 15, 30, 45, 60 min, 2, 4 and 6 hours after the end of substance application. The same blood samples were taken for the second intake of the substance. A final sample was taken 24 hours after the second application. Descriptive summary statistics were used to evaluate MTD and DLT, toxic effects and/or adverse events.

RESULTS

6 patients received at least one dose of the study drug and were eligible for safety evaluation. All patients had no tumour therapy within 6 months before study inclusion. The predetermined MDT of 500 mg was reached after 6 patients were treated. Changing to the modified Fibonacci scheme was not necessary. One patient (FFC15-01-01-03) received 200 mg of the antisense nucleotide and had to be excluded from the study due to acute virus hepatitis; therefore the next patient (FFC15-01-01-04) also received 200 mg of FFC15-01. For further patients characteristics and dosage, see Table 2.

FFC15-01 was well tolerated in the doses studied. Adverse events (AE), for which a relationship to the study medication could not be excluded outright, were observed in some patients and were predominantly grade I according to NCI-CTC.

Most common AEs were bladder cramps and in one case, a mild exanthema on an extremity, however further treatment was not necessary.

DLT was not observed. The dose escalation was terminated at a final predetermined dose of 500 mg.

Table 2. Patients tumour grading after resection and the received absolute dose of FFC15-01

Patient number	Patient age (years)	Patient sex	Histopathology of the bladder tumours after resection	Received absolute dose of FFC15-01 in mg
FFC15-01-01-01	59	female	TaG1	50
FFC15-01-01-02	78	male	TaG2	100
FFC15-01-01-03	60	male	TaG2	200
FFC15-01-01-04	64	male	TaGx	200
FFC15-01-01-05	77	male	T2G3	400
FFC15-01-01-06	55	male	T2G3	500

Table 3. Plasma and urine concentrations of FFC15-01

Patient number	Plasma concentration of FFC15-01 in µg/ml	Urine concentration of FFC15-01 in µg/ml
FFC15-01-01-01	blq*	59
FFC15-01-01-02	blq*	blq*
FFC15-01-01-03	blq*	22,45
FFC15-01-01-04	blq*	476
FFC15-01-01-05	blq*	138
FFC15-01-01-06	blq*	blq*

* blq: below limit of quantification (1 µg/ml)

The analysis of plasma samples of patients after treatment with FFC15-01 revealed no significant oligonucleotide levels in any of the plasma samples (Limit of quantification (LOQ) = 1,0 µg/ml). High levels of FFC15-01 were found in 6 of the 23 urine samples (1,48-476,2 µg/ml) taken 24 hours after instillation of the antisense oligonucleotide (see Table 3).

Table 4. Immunohistochemical Ki-67 staining index

Patient number	Ki-67 staining index in %
FFC15-01-01-01	35
FFC15-01-01-02	40
FFC15-01-01-03	30
FFC15-01-01-04	45
FFC15-01-01-05	60
FFC15-01-01-06	35

Immunohistochemical Ki-67 staining of the resected tumours revealed Ki-67 levels between 35 and 60 % (see Table 4).

DISCUSSION

Antisense oligonucleotides against different target genes and routes of administration (subcutaneous, intraperitoneal, intravenous and inravesical) are currently being evaluated in numerous oncological studies [13,14].

This is the first clinical phase I study with intravesical application of an antisense oligonucleotide. The accessibility of the bladder mucosa makes the intravesical therapy an excellent route of administration of the medication. No studies exist to assess the

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efficacy, toxicity and dose limiting biological effect and possible antisense oligonucleotide uptake after intravesical instillation in humans; it is therefore difficult to extrapolate from previous phase I studies.

This study however, could show that an intravesical instillation of Ki-67 antisense oligonucleotides was very well tolerated. In general, clinical trials where antisense oligonucleotides were administered intravenously have also shown good toleration of antisense oligonucleotides, with initial fever and chills being the most common side effects. More severe reported side effects included hypotension, liver dysfunction and myelosupression. None of the above-mentioned side effects occurred in this trial. The most common side effects with possible connection to the trial medication were bladder cramps and a mild exanthema on one extremity.

Immunohistochemical staining of the Ki-67 antigen could give an impression of target expression.

A systemic uptake of the antisense oligonucleotides was not found. Control cystoscopy showed preliminary signs of efficacy. Nevertheless, proof of intracellular uptake of FFC15-01 and reduction of Ki-67 positivity is still outstanding.

CONCLUSIONS

The Ki-67 antisense oligonucleotide FFC15-01 could be an effective tool in the treatment of non muscle-invasive bladder cancer. It has been well tolerated and the results support further trials.

A phase II protocol with intravesical instillation of 100 mg FFC15-01 was initiated.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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