Bladder cancer therapy with Alpha1H: From preclinical development to successful Phase II trials

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Abstract

In a single center, placebo controlled, double blinded Phase I/II interventional clinical trial of non-muscle invasive bladder cancer, all primary end points of safety and efficacy of alpha1-oleate treatment are reached, as evaluated in an interim analysis. Intra-vesical instillations of alpha1-oleate triggers massive shedding of tumor cells and the tumor size is reduced but no drug-related side effects are detected (primary endpoints). Shed cells contain alpha1-oleate, treated tumors show evidence of apoptosis and the expression of cancer-related genes is inhibited (secondary endpoints). The results are especially encouraging for bladder cancer, where therapeutic failures and high recurrence rates create a great, unmet medical need. FDA has granted fast track designation for its drug candidate Alpha1H for the treatment of non-muscle invasive bladder cancer.

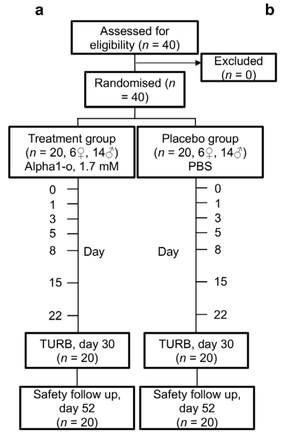
Introduction

The power of conformational fluidity is illustrated by HAMLET (Human Alpha-lactalbumin Made LEthal to Tumor cells), a tumoricidal complex serendipitously discovered in human milk[1-3]. Alpha-lactalbumin is crucial for the survival of lactating mammals. In its native state, the protein defines the nutritional content and fluidity of milk where the native protein serves as a substrate specifier in the lactose-synthase complex[4]. Partially unfolded alpha-lactalbumin, in contrast, forms oleic acid complexes, named HAMLET, kills a range of tumor cells with rapid kinetics and shows therapeutic efficacy in animal models of colon cancer, glioblastoma and bladder cancer[5-8]. Early, investigator-driven clinical studies demonstrated that HAMLET is active topically, against skin papillomas and induces tumor cell shedding into the urine in patients with bladder cancer[5,8].

This study presents a synthetic, peptide-based drug candidate derived from alpha-lactalbumin, which reproduces the tumoricidal properties of HAMLET and allows for full translation of these findings into the clinic. Therapeutic efficacy of the complex is demonstrated in patients with non-muscle invasive bladder cancer (NMIBC), in a fully controlled clinical trial.

Methods and Materials

a



b

Parameters	Visit	0	1	2-6*	7	FU 1
Visit window (days)				±1	±5	±5
Informed consent		X				
Physical examination			X		X	
Height and weight			X			
Pregnancy test			X		X	
Medical diagnosis			X			
Vital signs			X		X	X
ECG			X		X	
Blood sample			X		X	X
In-/exclusion criteria			X			
Subject demographics			X			
Randomisation			X			
Cystoscopy + photo			X		X	
Treatment / Intravesical instillation at hospital			X	X		
Concomitant medication			X	X	X	X
Urinalysis (safety)			X		X	X
Urinalysis (efficacy)			X	X		
Adverse event(s)			X	X	X	X
TURB					X	

** Time window for visit 2-3 is ±1 day, visit 4-5 is ±2 days*

c

	Adverse events					
	Alpha1-o			Placebo		
	No of events	No of patients	%	No of events	No of patients	%
Related to IMP	0	0	0	0	0	0
Related to procedure	4	4	20.0	8	8	40.0
Related to underlying disease	3	3	15.0	3	1	5.0
Serious adverse events	0	0	0	3	2	10.0
Resulted in discontinuation	0	0	0	0	0	0
Any adverse events	13	12	60.0	16	11	55.0

Fig. 1. Clinical study protocol, demographic data, and adverse events.
a, Study CONSORT diagram. b, Study protocol. After diagnosis and informed consent, the subjects received intra-vesical instillations of either alpha1-oleate or placebo on six occasions during one month preceding a scheduled TURB. A safety follow-up was performed 52 days after the first instillation. c, Number of adverse events in the active and placebo group. No drug related adverse events were recorded.

Results

- Tumor cell shedding and release of tumor cell clusters

Cells with uroepithelial morphology were quantified in urine at each visit, before instillation and about 2 hours after the instillation of alpha1-oleate or placebo. Alpha1-oleate triggered a rapid increase in cell shedding compared to the pre-instillation sample in all treated patients, at all visits.

Results

- Effects on tumor size

The endoscopic appearance of each tumor was recorded at the time of diagnosis and prior to surgery using a flexible cystoscope with white light- and narrow band imaging. A reduction tumor size (45%) was detected in the treatment group. No difference in superficial necrosis or vascularization was observed and there was no change in lesion size in the placebo group (n = 20).

- Evidence of tumor cell apoptosis

A significant increase in TUNEL fluorescence was detected in the treatment group, compared to placebo.

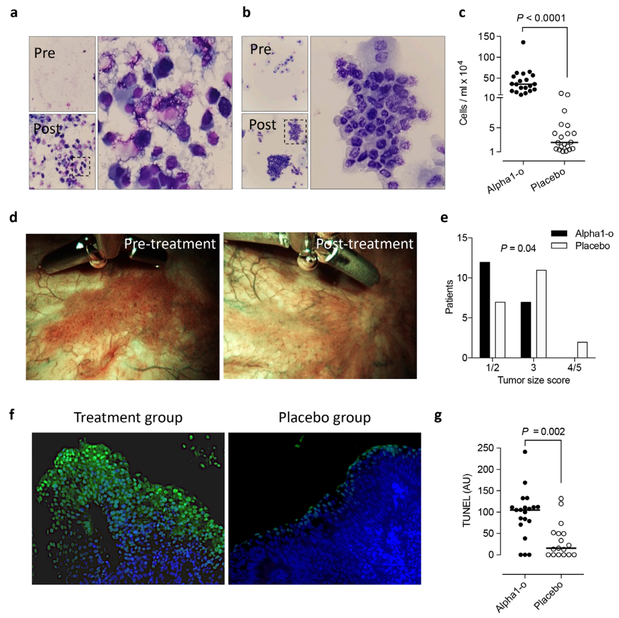


Fig. 2. Primary end points: shedding of tumor cells and reduction in tumor size by alpha1-oleate. **a-c**, Cell shedding increased significantly after alpha1-oleate instillation. **d, e,** Reduction in tumor size in patients receiving alpha1-oleate treatment. Images were compared between the time of diagnosis and the time of TURB (χ^2 test for trend, compared to placebo). Examples of cystoscopy photographs obtained at the time of diagnosis and after treatment at the time of TURB. **f,** Representative images of TUNEL staining in tumor tissue from patients receiving alpha1-oleate instillations. **g,** TUNEL staining intensity.

Discussion

Bladder cancer is the 4th most common malignancy in the US and the 5th in Europe, with a prevalence of about 1/4,000. More than 80% recur after complete surgical removal of the first tumor and 15% progress to muscle invasive disease. Chemotherapy and immunotherapy are often suboptimal due to significant side effects and limited efficacy. Therapeutic options are also limited by inadequate supply of immunotherapy and chemotherapy drugs. In this study, we identify Alpha1H complexes as additional tools in cancer therapy and show that intra-vesical inoculation of alpha1-oleate is safe and effective in patients with bladder cancer.

Treatment triggered shedding of cells and tissue fragments into the urine and alpha1-oleate internalization by tumor cells confirmed the affinity of the complex for the tumor. Further analysis of tissue biopsies suggested a lasting effect of the alpha1-oleate instillations, as several tumor samples showed a gradient-like pattern of apoptosis. Dysfunctional apoptosis has been identified as a key to tumor development. The ability of alpha1-oleate to stimulate apoptosis in the majority of bladder tumors is therefore encouraging and consistent with the apparent lack of toxicity for bladder tissue.

Conclusions

The protein-lipid complexes studied here are attractive to cancer cells, which actively internalized them, but end up being killed by an apoptosis-like mechanism. Healthy cells are less responsive and extensive toxicity studies have failed to detect adverse effects[13]. This low toxicity was confirmed here, as no drug-related side effects were observed in the treatment group. The results therefore identify alpha1-oleate treatment of non-muscle invasive bladder cancer as an interesting new therapeutic option. In view of the low toxicity observed so far, liberal intra-vesical administration in early stage NMIBC might be an interesting approach to postponing the introduction of more toxic and invasive therapeutic options.

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