

A scientific journey from discovery to validation of efficacy in cancer patients: HAMLET and alpha1-oleate

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ABSTRACT

The protein-lipid complex alpha1-oleate, derived from HAMLET (Human Alpha-lactalbumin Made LEthal to Tumor cells), is identified as a molecular entity with significant therapeutic potential. Structural characterization of the complex and results of a successful placebo-controlled clinical trial are presented.

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The N-terminal, 39-residue alpha-helical domain of alpha-lactalbumin forms a tumoricidal complex with oleic acid (alpha1-oleate), which is attractive to cancer cells, which internalize it, but end up being killed.¹

The complex reproduces the tumoricidal properties of HAMLET (Human Alpha-lactalbumin Made LEthal to Tumor cells), first identified as the cause of unexpected tumor cell death.^{2,3} Lung carcinoma cells were killed by a fraction of human milk containing HAMLET and the effect was reproduced in a number of different carcinoma and lymphoma cell lines. The purified complex, formed by partially unfolded alpha-lactalbumin and oleic acid, triggered rapid tumor cell death by inserting itself into the plasma membrane, activating ion fluxes, perturbing Ras signaling and affecting membrane-enclosed organelles, including cell nuclei.^{4,5} Extensive data in tumor models and clinical studies later confirmed that protein-lipid complexes such as HAMLET have a significant therapeutic potential and low toxicity.^{6,7}

Characterization and large-scale production of a synthetic, peptide-based alpha1-oleate complex has allowed for full translation into the clinic. Potent effects of the alpha1-oleate complex were demonstrated in a single center, placebo controlled, double blinded Phase I/II interventional clinical trial of non-muscle invasive bladder cancer (NMIBC).¹ Patients with suspected NMIBC were randomized 1/1 to receive alpha1-oleate or placebo during a period of 22 days, prior to endoscopic removal of the tumor by transurethral resection. Alpha1-oleate (1.7 mM) or placebo (phosphate-buffered saline) was administered intra-vesically on six occasions (30 ml, days 1, 3, 5, 8, 15 and 22). Demographic data, medical history and vital signs did not differ between the treatment and placebo groups.

We recorded highly significant differences between the alpha1-oleate-treated patients and the placebo group for several crucial efficacy variables. All primary end points of safety and efficacy of alpha1-oleate treatment were reached, as evaluated in an interim analysis.¹ Intra-vesical instillations of alpha1-oleate triggered massive shedding of tumor cells and shed cells contained alpha1-oleate, confirming uptake by tumor tissue. The tumor size was reduced and treated tumors showed evidence of apoptosis, with a gradient from the lumen toward the interior of the tumor. By gene expression analysis of tumor tissue, massive inhibition of multiple cancer biofunctions was observed in the treatment group (Figure 1).

Native protein structure is often regarded as a prerequisite for biological function, through epitope-specific interactions and molecular fitness for specific cellular targets. Yet, this study suggests that a lack of structural definition may result in a gain of function.¹ Native alpha-lactalbumin serves as a substrate specifier in the lactose-synthase complex but when partially unfolded, the protein forms oleic acid complexes with potent tumoricidal activity as first observed for the HAMLET complex.^{2,3,8}

Now the atomic-level structural basis for this gain-of-function was defined for the alpha1 peptide, by complementary nuclear magnetic resonance (NMR) analysis. Accompanying all-atom computational simulations were able to provide corroborating shallow free-energy landscapes and three-dimensional structural motifs that influenced fatty acid binding efficiency and tumoricidal activity. It was also shown that the apparently unrelated sar1alpha peptide can form oleic acid complexes with shared structural characteristics, involving a flexible peptide moiety and a fatty acid cluster.

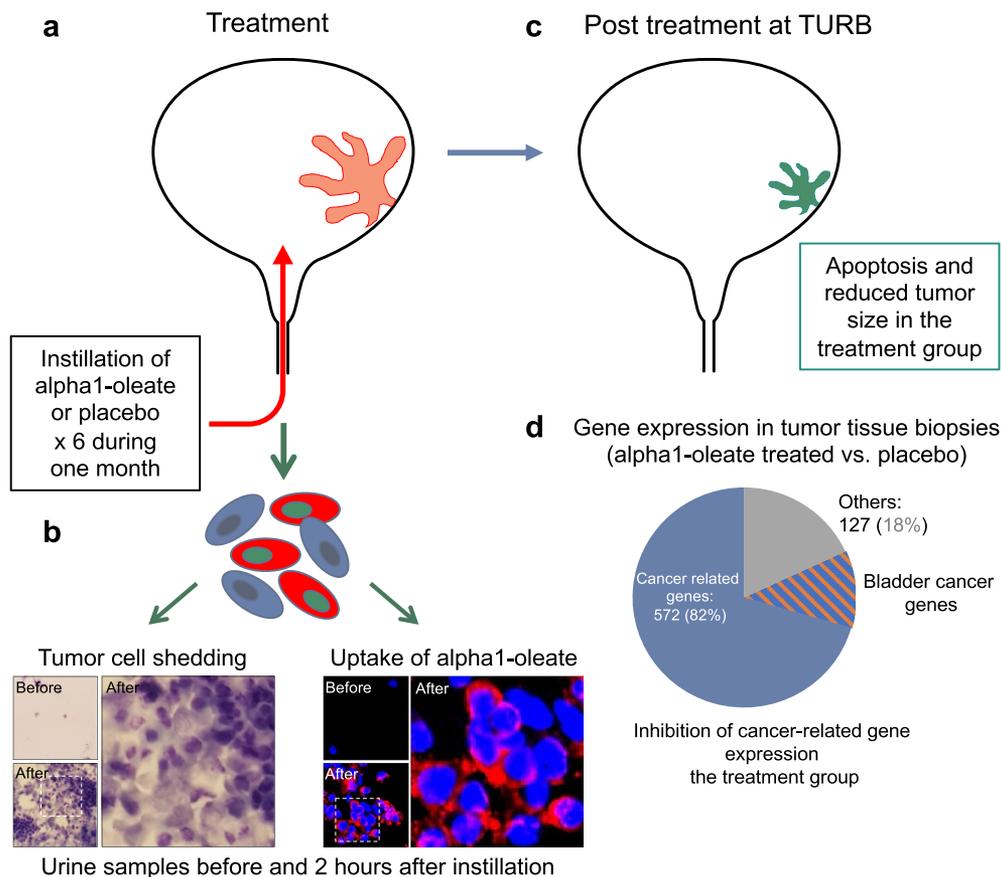


Figure 1. Overview of the study protocol and outcomes. (a) Patients diagnosed with non-muscle invasive bladder cancer were enrolled in the study. Intra-vesical instillation of alpha1-oleate or placebo was performed on six occasions. (b) By comparing urine samples from each patient before and after the instillation of alpha1-oleate or placebo, we detected a significant increase in tumor cell shedding in the treatment group and an accumulation of cells containing alpha1-oleate (red). (c) A reduction in tumor size and an increase in apoptosis was detected at transurethral resection of the bladder tumor (TURB), after about one month. (d) By gene expression analysis, treated tumor tissue showed a marked reduction in cancer related gene expression, including bladder-cancer-specific genes.

The results suggest that a lack of structural definition may be key to the biological activity of the complex, in part by uncovering different conformations and exposing peptide motifs that are unavailable in the native state.

It may be speculated that a loss of tertiary structural definition may enable a number of proteins to gain essential functions in different tissue environments, where fatty acid co-factors are present.¹⁻³

A plausible scenario is that gain-of-function may allow protein-lipid complexes to target aberrant cells, in a process similar to tumor immune surveillance.

NMIBC has been declared a great, unmet medical need, due to high recurrence rates, a lack of new effective treatments and limited supply of recommended Bacillus Calmette-Guérin (BCG) immunotherapy or chemotherapy (Mitomycin) for intravesical use.^{9,10} In view of the low toxicity of alpha1-oleate observed so far, liberal intravesical administration in early stage NMIBC might be an interesting approach to postponing the introduction of more toxic and invasive therapeutic options. The long-term goal is to attack tumors with better precision, thus avoiding some of the toxicity that accompanies treatments currently used for bladder cancer.

Disclosure statement

C.S. holds shares in HAMLET Pharma, as a representative of scientists in the HAMLET group. Patents protecting the use of the alpha1-oleate complex have been granted. Other authors declare no conflict of interest.

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