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# Amanitin-based ADCs targeting PSMA as novel therapeutic modality for prostate cancer therapy

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Heidelberg PHARMA
Focused Cancer Therapies

## Introduction

Tumor-specific delivery of cytotoxic small molecules can be achieved by conjugation to monoclonal antibodies which bind with high affinity to a specific target thereby enhancing antitumor activity and reducing off-target toxicity. Heidelberg Pharma focuses on **ATAC**s (**A**ntibody **T**argeted **A**manitin **C**onjugates), a new class of antibody-drug conjugates (**ADCs)** with amanitin as toxic payload (1). α-Amanitin, the principal toxin found in *Amanita phalloides* - the notorious "death-cap" mushroom – is a highly selective allosteric inhibitor of eukaryotic RNA polymerase II (RNAP2). As RNAP2 is essential for cellular growth and homeostasis, α-amanitin **kills dividing and quiescent cells** by inhibiting RNAP2, which leads to rapid proteolytic degradation and finally cell death (2, 3). This unique mechanism of action distinguishes α-amanitin from nearly all other toxic payloads which act primarily on rapidly growing cells and holds potential for overcoming drug resistance and improving patient outcome.

By using **site-specific conjugation** to attach the amatoxin-linkers to engineered cysteines at defined positions of the antibody (THIOMAB®† strategy) heterogeneity of drug-antibody species is dramatically reduced, and highly stable conjugates with broad therapeutic window are generated.

In the current study, *in vitro* and *in vivo* data of **ATACs targeting** prostate specific membrane antigen (**PSMA**) are presented. PSMA is a type II integral membrane glycoprotein and used as tumor marker due to its predominant expression on malignant prostate cells in prostate carcinoma. As PSMA expression increases with tumor aggressiveness, metastatic and disease recurrence, it is considered an ideal target for amanitin-based ADCs.

## Methods

**Anti-PSMA antibody**: The monoclonal anti-PSMA antibody was developed at Albert Ludwig University Freiburg, humanized and a THIOMAB® derivative thereof was produced by Heidelberg Pharma Research GmbH using ExpiCHO cells (Life Technologies) and transient transfection methods.

**Synthesis of conjugates**: Cysteine reactive amanitin-linker constructs containing either a non-cleavable or a cleavable Val-Ala linker were synthesized at Heidelberg Pharma and conjugated site-specifically to engineered cysteine residues of the anti-PSMA antibody. DAR (Drug-Antibody Ratio) for both conjugates was 2.0 ± 0.1 as determined by LC-MS analysis.

**Cell lines**: The PSMA$^+$ cancer cell lines LNCap and 22RV1 were obtained from the University of Freiburg and DSMZ, respectively. The PSMA$^-$ cell line PC-3 was obtained from DSMZ.

**Binding assays:** Binding activity of anti-PSMA antibody to hPSMA$_{44-750}$ (ACROBiosystems) was analyzed by an indirect ELISA using HRP conjugated goat anti-human F(ab')2-Fragment (Jackson Immuno-Research) for detection. Cell binding of anti-PSMA antibody and ATAC to PSMA$^+$ LNCap cells was measured by flow cytometry using a goat Fab anti-human IgG-AlexaFluor488 secondary antibody (Dianova) on a FACSCalibur system (BD Biosciences).

**Cell proliferation assay**: Quantitative determination of cell viability was performed by BrdU-based chemiluminescent cell proliferation ELISA (Roche).

**Animal models**: Subcutaneous mouse xenograft tumor models with LNCap cells were performed using male CB-17 SCID mice (Janvier) inoculated with 2.5 x 10$^6$ LNCap cells and treated with a single dose i.v. application at an established tumor volume of approx. 140mm$^3$. Tolerability was assessed in mice (Heidelberg Pharma) and in non-human primates (Alta Sciences, Everett, USA).

## Results

### 1. Conjugation of anti-PSMA Antibody Targeted Amanitin Conjugates (ATACs)

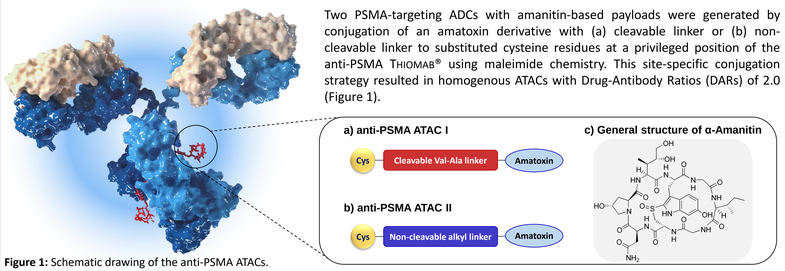
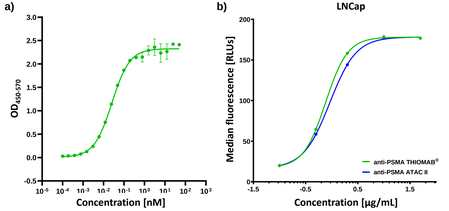



**Figure 1:** Schematic drawing of the anti-PSMA ATACs.

Two PSMA-targeting ADCs with amanitin-based payloads were generated by conjugation of an amatoxin derivative with (a) cleavable linker or (b) non-cleavable linker to substituted cysteine residues at a privileged position of the anti-PSMA THIOMAB® using maleimide chemistry. This site-specific conjugation strategy resulted in homogenous ATACs with Drug-Antibody Ratios (DARs) of 2.0 (Figure 1).

### 2. High affinity of anti-PSMA antibody to human PSMA and to PSMA$^+$ cell lines

Binding of humanized anti-PSMA antibody to human PSMA$_{44-750}$ was analyzed in an indirect ELISA. The anti-PSMA THIOMAB® showed high affinity to immobilized hPSMA$_{44-750}$ with a EC50 value of 25pM (Figure 2a). For FACS analysis, the PSMA$^+$ cell line LNCap was incubated with the anti-PSMA THIOMAB® or anti-PSMA ATAC II. Antibody and ATAC showed specific binding to the PSMA$^+$ cell line LNCap (Figure 2b).

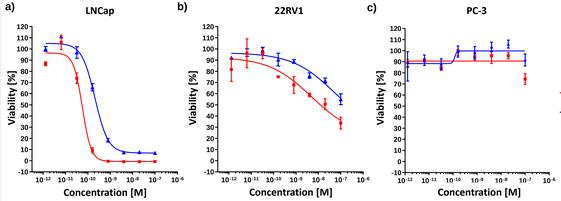



| | EC50 [µg/ml] on LNCap cells |
|---|---|
| anti-PSMA THIOMAB® | 0.7659 |
| anti-PSMA ATAC II | 0.9209 |

**Figure 2:** (a) Binding ELISA of the anti-PSMA antibody to human PSMA antigen (b) Flow cytometry analysis of anti-PSMA THIOMAB® antibody and anti-PSMA ATAC with non-cleavable linker on LNCap cells.

### 3. Cytotoxic activity of anti-PSMA ATACs on PSMA$^+$ and PSMA$^-$ cell lines

The PSMA$^+$ cell lines LNCap and 22RV1, as well as the PSMA$^-$ cell line PC-3 were used to test the cytotoxic potency of anti-PSMA conjugates. Both ATACs - with cleavable linker (anti-PSMA ATAC I, red) and with non-cleavable linker (anti-PSMA ATAC II, blue) – showed favorable *in vitro* cytotoxicity with nano- to picomolar activity on PSMA$^+$ cell lines (Figures 3a,b) and absence of cytotoxic activity on PSMA$^-$ cells (Figure 3c).




| ATAC | LNCap EC50 [nM] | 22RV1 EC50 [nM] | PC-3 EC50 [nM] |
|---|---|---|---|
| anti-PSMA ATAC I | 0.056 | 4.98 | >100 |
| anti-PSMA ATAC II | 0.207 | 51.2 | >100 |

**Figure 3:** Cytotoxic activity of anti-PSMA ATACs on (a) LNCap, (b) 22RV1 and (c) PC-3 cells using BrdU ELISA after incubation for 96h.

### 4. Efficacy in mouse subcutaneous prostate cancer xenograft models

The antitumor activity of anti-PSMA ATACs with cleavable linker and non-cleavable linker was evaluated in mouse LNCap s.c. xenograft models *in vivo*. At equitoxic doses of ¼ MTD complete tumor remission was achieved after single dose administration of the ATACs (Figure 4a). Low dose treatment of 1/8 MTD resulted in partial remission after single dose application of the ATAC with cleavable linker. A significant increase in median survival with 5/8 tumor free animals was observed after application of the ATAC with non-cleavable linker (Figure 4b).

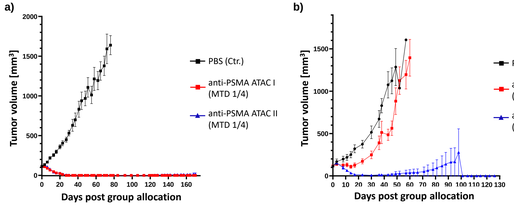
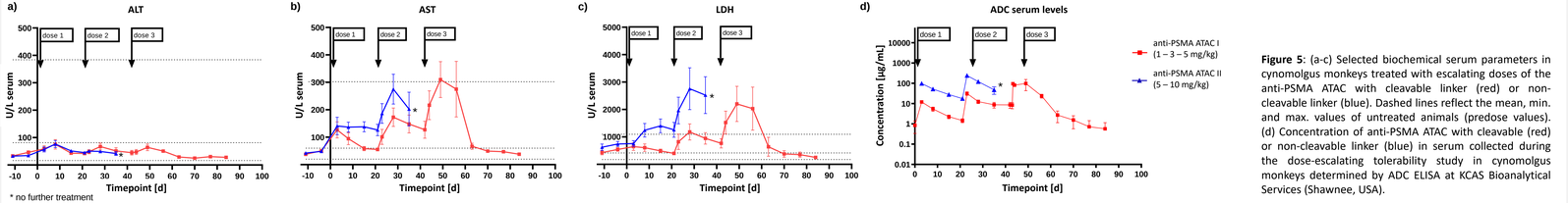



**Figure 4:** Subcutaneous LNCap xenograft model. Single dose i.v. application of anti-PSMA ATACs with cleavable (red) or non-cleavable linker (blue) treated with (a) high ATAC doses and (b) low ATAC doses. Shown is mean tumor volume ± SEM.

### 5. Tolerability of anti-PSMA ATACs with cleavable and non-cleavable linker in cynomolgus monkeys

Two anti-PSMA ATACs with cleavable and non-cleavable linker were assessed for a dose-escalating tolerability study in cynomolgus monkeys. Each group consisted of equal numbers of female and male animals. The ATAC with cleavable linker was applied sequentially at doses of 1 mg/kg, 3 mg/kg and 5 mg/kg while the ATAC with non-cleavable linker was applied sequentially at doses of 5 mg/kg and 10 mg/kg. Biochemical and hematological blood parameters were evaluated extensively during the study (Figure 5a-c). The anti-PSMA ATAC with cleavable linker (red) was well tolerated up to 3mg/kg while the anti-PSMA ATAC with non-cleavable linker (blue) was well tolerated up to 5mg/kg dose level. Transient and mild increase in liver-damage-relevant biochemical parameters (ALT, AST and LDH) were observed after application of dose 2 in case of the ATAC with non-cleavable linker (blue) and after dose 3 of the ATAC with cleavable linker (red). The sequential administration of increased ATAC doses is reflected by the serum levels (Figure 5d).




* no further treatment

**Figure 5**: (a-c) Selected biochemical serum parameters in cynomolgus monkeys treated with escalating doses of the anti-PSMA ATAC with cleavable linker (red) or non-cleavable linker (blue). Dashed lines reflect the mean, min. and max. values of untreated animals (predose values). (d) Concentration of anti-PSMA ATAC with cleavable (red) or non-cleavable linker (blue) in serum collected during the dose-escalating tolerability study in cynomolgus monkeys determined by ADC ELISA at KCAS Bioanalytical Services (Shawnee, USA).

## Conclusion

Prostate cancer is one of the most common types of cancer in men worldwide. PSMA is a type II membrane glycoprotein which is expressed on secretory cells within the prostatic epithelium and in nearly all prostate cancers (4). Elevated expression of PSMA correlates with tumor aggressiveness and tumor stage rendering PSMA an ideal target for prostate cancer treatment.

In the current study, *in vitro* and *in vivo* data of amanitin based ADCs (ATACs) targeting PSMA with both a cleavable linker and a non-cleavable linker are presented. The promising potential of ATACs targeting PSMA is reflected by the high cytotoxic potency of anti-PSMA THIOMABS® on different PSMA$^+$ cell lines. Data presented underscores an effective and persistent anti-tumor activity of anti-PSMA ATACs in mouse xenograft models: Single dose treatment with ATACs employing different linker designs caused dose-dependent tumor regression including complete tumor remission. Safety profiling in cynomolgus monkeys revealed good tolerability and therapeutic index for anti-PSMA ATACs.

The use of amatoxins and the inhibition of RNA polymerase II as novel mode of action for ADCs not only results in apoptosis of dividing cells, but also of slowly growing and dormant cells. Based on its activity on drug resistant cells independent of the expression of multi-drug resistance transporters amatoxins possess the ability to overcome resistance as prerequisite for a long-lasting antitumor efficacy even in slow proliferating prostate cancers. The positive findings of these experiments warrant the clinical development of anti-PSMA ATACs.

## References

(1) Anderl J, Echner H, Faulstich H. Chemical modification allows phallotoxins and amatoxins to be used as tools in cell biology. Beilstein J Org Chem. 2012; 8:2072-84

(2) Moldenhauer G, Salnitov AV, Lüttgau S et al. Therapeutic potential of amanitin-conjugated anti-epithelial cell adhesion molecule monoclonal antibody against pancreatic carcinoma. J Natl Cancer Inst. 2012; 104(8):622-34

(3) Liu Y, Zhang X, Han C, et al. TP53 loss creates therapeutic vulnerability in colorectal cancer. Nature 2015; 520(7549):697-701

(4) Tsourlakis MC, Klein F, Kluth M, et al. PSMA expression is highly homogenous in primary prostate cancer. Appl. Immunohistochem. Mol. Morphol. 2015; 23(6):449-55

† THIOMAB is a registered US trademark owned by Genentech (no. 88606375)

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