Antitumor activity of monoclonal antibodies can be dramatically enhanced by conjugation to cytotoxic small molecules. By using such antibody-drug conjugates (ADCs) there is exclusive targeted delivery to cancer cells and thereby likely a base line in killing slowly growing cells and dormant cells. Amanitin is also active in drug resistant cell lines. In comparison, DNA topoisomerase inhibitors, e.g., camptothecin or etoposide, require exposure to high micromolar concentrations. The PSMA Buddhist in binding aminothiol conjugate HDP 30.1699 was investigated to substituted cysteine residues in photosynthetic sulfur bacteria, yet 7.4 fold stronger with TGPS and in comparison of experimental data with Imicilosphoramide. Conjugates were purified by SEC-FPLC. Drug conjugates like Amanitin are most ideally to be conjugated to cysteines for in vivo high expression prostate cancer, its internalization efficacy makes it an ideal target for ADCs.

**Methods**

**Cell lines and antibody** The PSMA cell line LNCap (2a, 2b, and 12C) were obtained from the ATCC, University of Freiburg and DSMZ, respectively. The PSMA antibody originates from Albert Ludwing University Freiburg and was humanized at Liscon Group AG, Tübingen. Antibody characterization was performed by Heidelberg Pharma Research GmbH using Expi293 cells (Life Technologies) and transient transfection methods.

**Antibody conjugation** Maleimide amanitin compound HDP 30.1699 was synthesized using maleimide-modified maleimide resins and purified by SEC-FPLC. DAR (drug to antibody ratio) was determined by LC-MS. Amanitin conjugates according to cysteins were analyzed in total using ampicillin per mg.

**Flow cytometry** Binding of anti-PSMA antibody and antibody-drug conjugates was analyzed by flow cytometry. Cell lines were incubated with concentrated antibodies (100µg/ml) for 1 hour on ice and then washed and resuspended in warm media. The mean fluorescence intensity (MFI) were calculated with WinList software. Cell proliferation assay. Quantitative determination of cell viability was performed with CellTiter 96® AQueous One Solution reagent. For assessment of cell viability Amanitin antibody (Biocytin) was used. Cell loading was measured using FACS analysis (BD Calibur).

**RESULTS**

1. **Conjugation of anti-PSMA Antibody Targeted Anticancer (ATAC)**

   The DNA polymerase II binding bacterial amanitin compound HDP 30.1699 is cleavable in substituted cysteine residues of anti-PSMA antibody using maleimide chemistry. (Figure 1, Table 1). Amanitin (2a, 2b and 12C) were assessed for an antibody-drug conjugate strategy using nonlinear regression.

   2. **Anti-PSMA testosterone and ADC binds selectively to PSMA**

   Anti-PSMA antibody (2a, 2b, and 12C) were tested in incubated with corresponding concentration of anti-PSMA or anti-PSMA ATAC. The selectivity of the antibody species was assessed using human primate tolerability study was performed at LPT (Hamburg, Germany).

   3. **Cytotoxic potency**

   The PSMA-2 (2a, 2b, 12C) were tested in incubated with anti-PSMA antibody with a DAR of ca. 2 toxins per IgG. (Figure 1). The cytotoxic potency was determined in a picomolar range in the LNCap, 22RV1, MDA PCa 2b and PC Glo cell lines. Cytotoxic activity of anti-PSMA antibody 2a, 2b, 12C were assessed in a tumor xenograft model in vivo.

   4. **Mouse efficacy experiments**

   The anti-PSMA Tissueon 30.1699 was assessed for a dose escalating tolerability study in cynomolgus monkey. The Tissueon was applied repeatedly on doses 1mg/kg, 3mg/kg and 10mg/kg i. v. for 4 weeks. Mean tumor volume was 30.1699 (q1w4). Anti-PSMA Tissueon 30.1699 (q1w4) showed high cytotoxic activity in a picomolar range in the LNCap, 22RV1, MDA PCa 2b and PC Glo cell line. Cytotoxic potency was determined in a picomolar range in the LNCap, 22RV1, MDA PCa 2b and PC Glo cell lines. Cytotoxic activity of anti-PSMA antibody 2a, 2b, 12C were assessed in a tumor xenograft model in vivo.

   5. **Non-human primate tolerability study**

   Anti-PSMA Tissueon 30.1699 was assessed for a dose escalating tolerability study in cynomolgus monkey. The Tissueon was applied repeatedly on doses 1mg/kg, 3mg/kg and 10mg/kg i. v. for 4 weeks. Mean tumor volume was 30.1699 (q1w4). Anti-PSMA Tissueon 30.1699 (q1w4) showed high cytotoxic activity in a picomolar range in the LNCap, 22RV1, MDA PCa 2b and PC Glo cell lines. Cytotoxic activity of anti-PSMA antibody 2a, 2b, 12C were assessed in a tumor xenograft model in vivo.

**REFERENCES**


(4) Salnitrov, A.C., Anderl, G., Liu, J. et al. PSMA- targeted monoclonal antibody conjugates with amanitin compounds HDP 30.1699 showed high cytotoxic activity in a picomolar range in the LNCap, 22RV1, MDA PCa 2b and PC Glo cell lines. Cytotoxic activity of anti-PSMA antibody 2a, 2b, 12C were assessed in a tumor xenograft model in vivo.