Preclinical evaluation of an anti-PSMA Antibody Targeted Amanitin Conjugate (ATAC)

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Introduction

Antitumor activity of monoclonal antibodies can be dramatically enhanced by conjugation to cytotoxic small molecules. By using such antibody-drug conjugates (ADCs) the toxin is exclusively delivered to target cells and thereby kills only those cells. Beside the approval of Mylotarg (CD33), Adcetris (CD30), Kadcyla (HER2) and Besponsa (CD20), nearly 60 ADCs have entered clinical trials, promising to strengthen the therapeutic capabilities for cancer treatment in the next decade. Most ADCs are based on a few cytotoxic compounds only with microtubule- or DNA-targeting toxins as payloads. Accordingly, the use of new drugs that function via alternative toxicity mechanisms could enhance the therapeutic potential of ADCs. Heidelberg Pharma focuses on Amanitin based ADCs, so called ATACs (C), comprising a new class of ADCs with Amanitin as toxic payload (1). Amanitin is the well-known toxin of the amatoxin family and binds specifically to the eukaryotic RNA polymerase II thereby inhibiting the cellular transcription process (2, 3). Heidelberg Pharma also pursues the strategy of site-specific conjugation to limit heterogeneity of drug-antibody species, to improve conjugate stability, and to increase the therapeutic window of ADCs. For this purpose, antibodies are engineered at specific locations to incorporate cysteines resulting in so called THIOMABS.

In the current study, *in vitro* and *in vivo* data of THIOMAB based ATACs targeting prostate specific membrane antigen (PSMA) are presented. PSMA, a type II membrane glycoprotein, is expressed on secretory cells within the prostatic epithelium and in mainly all prostate cancers. The expression is elevated with tumor aggressiveness and tumor stage. In addition to its high expression in prostate cancer, its internalization efficacy makes it an ideal target for ATACs.

Methods

Cell lines and antibody: The PSMA$^+$ cell lines MDA-PCa-2b, LNCap and 22RV1 were obtained from the ATCC, University of Freiburg and DSMZ, respectively. The PSMA$^-$ cell line PC-3 was obtained from DSMZ. The anti-PSMA antibody originates from Albert Ludwig University Freiburg and was humanized at Lonza Group AG. THIOMAB derivative thereof was produced by Heidelberg Pharma Research GmbH using Expi293 cells (Life Technologies) and transient transfection methods.

Synthesis of conjugates: Maleimide Amanitin compound HDP 30.1699 was conjugated to substituted cysteine residues in phosphate buffered saline, pH 7.4 after reduction with TCEP and re-oxidation of interchain disulfides by DHAA. Conjugates were purified by SE-FPLC. DAR (drug-antibody ratio) for conjugates according to LC-MS analysis was around 2.0 Amanitins per IgG.

Flow cytometry: Binding of anti-PSMA antibody and ATAC was analyzed with increasing concentrations. Goat Fab anti-human IgG-AlexaFluor488 secondary antibody (Dianova) was used. Cell binding was measured using FACSCalibur (BD Biosciences).

Cell proliferation assay: Quantitative determination of cell viability was performed by CellTiter Glo 2.0 (Promega) or BrdU-ELISA chemiluminescent (Roche) assays.

Animal models: Subcutaneous mouse xenograft tumor models with PSMA$^+$ cell line LNCap were performed in single dose and multiple-dosing experiments. Six to eight-week-old male CB-17 SCID mice were obtained from Janvier. 2.5 x 10^6 LNCap cells were injected into the right flank of each mouse for the development of a tumor. When tumors were well-established (ca. 150mm^3), treatment was started by either a single dose or multiple dose (q1wx4) i.v. application. Tumor volume was calculated as V = a x b^2 x 0.5, where a is the length and b is the width. NHP tolerability study was performed at LPT (Hamburg, Germany).

Results

1. Conjugation of anti-PSMA Antibody Targeted Amanitin Conjugate (ATAC)

Amanitin compound HDP 30.1699 with cleavable linker was conjugated to substituted cysteine residues of anti-PSMA THIOMAB antibody by maleimide addition, resulting in homogenous ATAC (anti-PSMA THIOMAB-30.1699) with a DAR of app. 2 toxins per IgG (Figure 1).

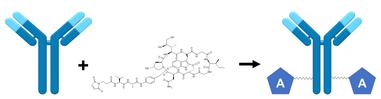

Figure 1: Schematic drawing of conjugation of anti-PSMA antibody with Amanitin compound HDP 30.1699 resulting in an anti-PSMA ATAC with DAR of 2.

2. Anti-PSMA THIOMAB and ATAC binds selectively to PSMA$^+$ cell lines

For FACS analysis of the binding properties, PSMA$^+$ cell lines LNCap, 22RV1 and MDA-PCa-2b as well as the PSMA$^-$ cell line PC-3 were incubated with increasing concentrations of the anti-PSMA THIOMAB or anti-PSMA ATAC. The antibody as well as anti-PSMA ATAC showed specific binding to the PSMA$^+$ cells whereas no binding was observed on the PSMA$^-$ cell line (Figures 2a+b).

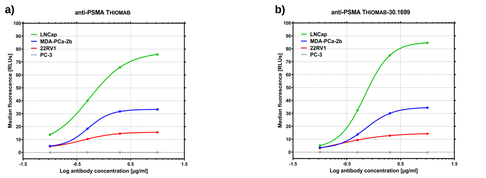

Figure 2: FACS analysis of (a) anti-PSMA THIOMAB antibody and (b) anti-PSMA ATAC (anti-PSMA THIOMAB-30.1699) on LNCap, 22RV1, MDA-PCa-2b and PC-3 cells in increasing concentrations.

3. Cytotoxic potency

The PSMA$^+$ cell lines LNCap, 22RV1, MDA-PCa-2b and PC-3 were used to test the cytotoxic potency of anti-PSMA ATAC with cleavable linker. In LNCap, 22RV1 and MDA-PCa-2b cell lines, the anti-PSMA ATAC (anti-PSMA THIOMAB-30.1699) showed high cytotoxic activity in a picomolar range whereas no cytotoxicity was observed on PC-3 cells (Figures 3a+b).

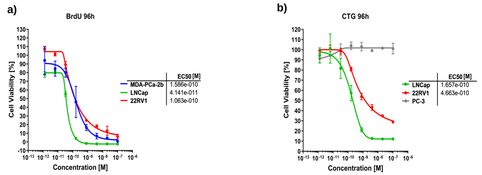

Figure 3: Cytotoxic potential of anti-PSMA-Amanitin ATAC on LNCap, 22RV1, MDA-PCa-2b and PC-3 cells using (a) BrdU ELISA and/or (b) Cell Titer Glo (CTG) assay after incubation for 96h.

4. Mouse efficacy experiments

The antitumor activity of single dose and multiple dose treatment of anti-PSMA ATAC was determined in LNCap subcutaneous (s.c.) xenograft models *in vivo*.

Single dose subcutaneous model
Anti-PSMA ATAC showed dose-dependent tumor regression and partial or complete remissions after single dose application of 2mg/kg and 4mg/kg, respectively (Figure 4a). In the 4mg/kg group, all animals were alive and four out of nine animals were tumor free at the end of the study.

Multiple dosing subcutaneous model
Subcutaneous LNCap tumors were treated weekly with multiple doses of either 1, 2 and or 4mg/kg anti-PSMA THIOMAB-30.1699 (q1wx4). The ATAC showed slight tumor regression at 1mg/kg. Complete remissions were achieved with 2mg/kg and 4mg/kg anti-PSMA ATAC (Figure 4b). In the 2mg/kg group, seven out of eight animals were alive and six out of eight animals were tumor free at the end of the study at day 110. In the 4mg/kg group, all animals were alive and tumor free at the end of the study.

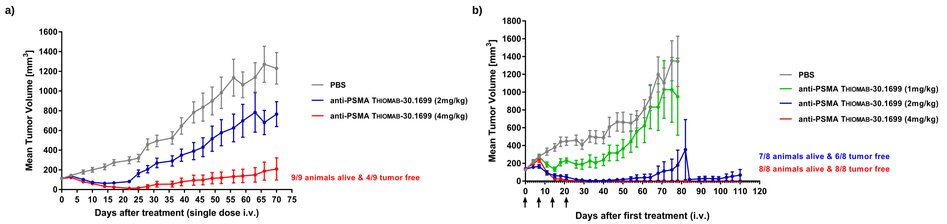

Figure 4: Subcutaneous LNCap xenograft. (a) Single dose i.v. application. (b) . Multiple doses i.v. applications (indicated by arrows). Shown is mean tumor volume ± SEM.

5. Non-human primate tolerability study

Anti-PSMA THIOMAB-30.1699 was assessed for a dose-escalating tolerability study in cynomolgus monkeys. The ATAC was applied sequentially at doses of 0.3mg/kg, 1.0mg/kg and 3.0mg/kg to the same animals. Biochemical and hematological blood parameters were evaluated extensively during the study (selected parameter: Figure 5).

The anti-PSMA THIOMAB-30.1699 was well tolerated up to the 3mg/kg dose level:
- Transient and mild increase in liver-relevant biochemical parameters in one out of three animals after application of 3mg/kg.
- no signs of systemic intolerance
- no sign of kidney damage by serum parameters.
- food consumption and body weight remained unaffected at doses 0.3 and 1mg/kg and was lowered at dose 3mg/kg

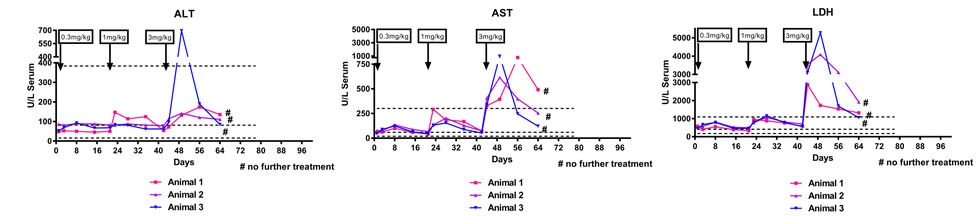

Figure 5: Selected biochemical serum parameters in cynomolgus monkeys treated with escalating doses of anti-PSMA THIOMAB-30.1699. Dashed lines reflect the mean, min. and max. values of untreated animals (pre-dose values).

Conclusion

Prostate cancer in one of the most common type of cancer in men world-wide. PSMA is a type II membrane glycoprotein and is expressed on secretory cells within the prostatic epithelium and in mainly all prostate cancers (4). The expression is elevated with tumor aggressiveness and tumor stage. Thus, PSMA is an ideal target for prostate cancer.

In the current study, *in vitro* and *in vivo* data of an Amanitin based ADC (ATAC) with cleavable linker targeting PSMA are presented. On different PSMA$^+$ cell lines anti-PSMA THIOMAB-30.1699 showed strong cytotoxic potential. In mouse xenograft models anti-PSMA THIOMAB-30.1699 conjugate showed clear anti-tumor activity. Complete tumor remission was achieved with 4mg/kg at single dose treatment and at 2 and 4mg/kg at multiple dosing. Safety profiling in cynomolgus monkey revealed a good tolerability and therapeutic index for anti-PSMA-THIOMAB-30.1699. Amanitin as a toxic warhead for ADCs targeting PSMA seems to be a suitable therapeutic option because of the unique mode of action and the molecular characteristics of the toxin. By inhibition of RNA polymerase II, Amanitin-binding leads not only to apoptosis of dividing cells, but also of slowly growing cells and dormant cells. Amanitin is also active in drug resistant cells, independent of the expression of multi-drug resistance transporters. This is a potential advantage for amatoxin payloads in slow proliferating prostate cancers.

The positive findings of these experiments warrant the clinical development of anti-PSMA ATACs.

References

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