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Preclinical evaluation of HDP-101, an anti-BCMA antibody-drug conjugate

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INTRODUCTION

Beside the recent approval of Kadcyla (T-DM1) and Adcetris (SGN-35) more than 30 antibody-drug conjugates (ADCs) have entered clinical trials, promising to strengthen the therapeutic capabilities for cancer treatment in the next decade. Surprisingly most ADCs are based on one of few toxic compounds only and on an even smaller number of toxicity mechanisms: Most antibodies are coupled to microtubuli- or DNA-targeting toxins (auristatins and maytansines or duocarmycins and PBDs). 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 or ATACs (antibody-targeted Amanitin conjugates) comprising a new class of antibody-drug conjugates with amanitin as toxic payload. Amanitin is the most well-known toxin of the amatoxin family and binds to the eukaryotic RNA pol II thereby inhibiting the cellular transcription process at very low concentrations.
In the current study, *in vitro* and *in vivo* data of HDP-101, an ATAC targeting BCMA (B cell maturation antigen), are presented. BCMA (also known as CD269) is expressed on cells of the B cell lineage, predominantly on plasma blasts and plasma cells. It is not expressed on naïve B cells, germinal center B cells and memory B-cells. BCMA is highly expressed on malignant plasma cells like Multiple Myeloma, a non-Hodgkin B cell lymphoma of the bone marrow. Since Multiple Myeloma is a usually incurable malignancy of plasma cells, new therapies are urgently needed. Using ADCs in the cure of Multiple Myeloma could be a promising approach, especially with a toxin whose mode of action was not applied before, like the amanitin based ADCs.
In mouse xenograft models, HDP-101 caused dose-dependent tumor regression and complete remission after a single i.v. dose of 2.0 mg/kg and 4.0 mg/kg in subcutaneous xenografts and after single i.v. doses from 0.1 mg/kg to 2.0 mg/kg in disseminating xenografts.
Safety profiling in Cynomolgus monkeys revealed a good tolerability and therapeutic index after sequentially applied doses of 0.3, 1.0, and multiple dose application of 4 x 3.0 mg/kg. Hematology and clinical chemistry parameters were unaffected except liver enzymes and LDH: A mild to moderate and transient increase was observed. The half-life of the ADC in serum was ~12 days; the free toxin was detectable at levels close to the lower limit of quantification only (LLOQ = 1.2nM).
Targeted cytotoxic drug delivery to BCMA positive MM cell lines was achieved by using HDP-101, an anti-BCMA-ATAC. The mode of action of the payload Amanitin led to an efficient anti-tumor potential *in vitro* and *in vivo* with good tolerability in non-human primate studies. Using ADCs in the therapy of multiple myeloma is a promising approach, especially by using a cytotoxic agent whose mode of action differs from other commonly used toxins, like ATACs. First-in-human trial with HDP-101 is expected to start in 2018.

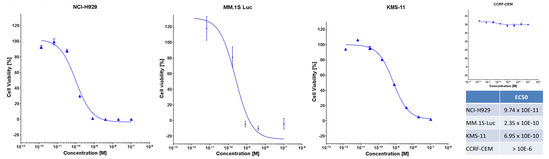
METHODS

Cell lines and antibodies: The human Multiple Myeloma cell line NCI-H929 was obtained from the ATCC, KMS-11 was obtained from JCRB (Japan), the Luciferase-transfected Multiple Myeloma cell line MM.1S Luc was provided by the Max Delbrück Center for Molecular Medicine (MDC), Berlin.
Antibodies are based on anti-BCMA antibodies developed at the MDC. Thiomab derivatives thereof were produced by HDP using Expi293 cells (Life Technologies) and transient transfection methods.
Synthesis of HDP-101: Maleimido amanitin compound HDP 30.2115 was conjugated to engineered cysteine residues in PBS pH 7.4 after reduction with TCEP and re-oxidation of interchain disulfides by dehydroascorbic acid (dhAA). The Conjugate was purified by SE-FPLC and dialysis. DAR (drug-antibody ratio) according to LC-MS analysis was 1.90 to 2.05 amanitins per IgG.
Cell proliferation assay: Quantitative determination of cell proliferation was performed by WST-1 assay (Roche).
Animal models: *NCI-H929 s.c:* NCI-H929 cells (5.0 x 10^6) were injected into the flank of female NMRI nude mice mouse for the development of a tumor. When tumors were well-established (100-200 mm^3), animals received a single intravenous dose. Tumor volume was calculated as $V = a \times b^2 \times 0.5$, where a is the length and b is the width.
MM.1S-Luc i.v.: 6 to 8-wk-old female SCID beige mice were obtained from Charles River. Animals were implanted with 1 x 10^7 MM.1S-Luc cells intravenously into their tail vein. Once a mean total flux of around 1.5 x 10^6 - 1 x 10^7 (~14 days after implantation) was reached, animals received a single intravenous dose. Luciferase activity was monitored by non-invasive bio-imaging (Caliper IVIS).
*KMS-11 i.v.:*7 to 8-wk-old female NOD/SCID mice (Janvier, France) were implanted with 5 x 106 KMS-11 cells intravenously into their tail vein. When tumors were well-established (100-200 mm^3), animals received a single intravenous dose. Readoutparameter: Overall survival.
NHP studies were performed in treatment-naive female cynomolgus monkeys at LPT (Germany, Hamburg).

RESULTS

Cytotoxic potency

The Multiple Myeloma cell lines NCI-H929, MM.1S Luc and KMS-11 (BCMA positive) were used to test the cytotoxic potency of HDP-101. CCRF-CEM (BCMA negative) served as negative control.
In all BCMA positive cell lines cell lines, the anti-BCMA Thiomab-Amanitin ADC HDP-101 showed high activity in pico- to nanomolar range (Figure 1). No cytotoxic effect up to a concentration of 10^{-6} M was seen in the BCMA negative cell line CCRF-CEM.



	EC50
NCI-H929	9.74 x 10E-11
MM.1S-Luc	2.35 x 10E-10
KMS-11	6.95 x 10E-10
CCRF-CEM	> 10E-6

Figure 1: Cytotoxic activity of HDP-101 in BCMA-positiv cell lines NCI-H929, MM.1S Luc amd KMS-11 after incubation for 96h. CCRF-CEM (BCMA-negativ) served as control.

In vivo efficacy

Antitumor activities of ADCs were determined in an NCI-H929 subcutaneous xenograft model and an MM.1S Luc intravenous xenograft model *in vivo*.

NCI-H929 subcutaneous xenograft model

HDP-101 showed dose-dependent tumor regression and complete remissions after single dose application of 2mg/kg and 4mg/kg, respectively (Figure 2). The increasing tumor volume in the 4.0 mg/kg group is due to only one single animal (figure 2; right panel).

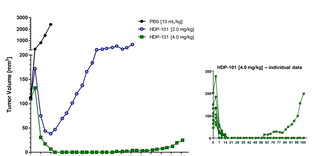


Figure 2: Subcutaneous xenograft model based on **NCI-H929** cells. Single dose i.v. application.

MM.1S Luc intravenous xenograft model

HDP-101 shows tumor regression and complete remissions (to baseline signal, dashed line, Fig. 3) after single dose application down to 0.1 mg/kg (Figure 3). The re-occurence of the tumor signal is dose-dependent. Please note that only one animal in both treatment groups with 1.0 and 2.0 mg/kg showed elvated luminescence signals at the latest timepoint of observation (d93).

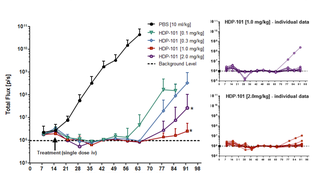


Figure 3: Dose dependent efficacy of HDP-101 on the tumor burden in the intravenous **MM.1S Luc** xenograft model. Single dose i.v. application. Data signals represent luciferase-dependent bioluminescence intensity and reflect the tumor burden of the whole animal. Left panel: Mean values of groups +/- SD. Right panel: Graphs show luminescence intensity of all individual animals in the groups treated with 1.0 mg/kg (uper right) and 2.0 mg/kg (lower right). Dashed line represents background level.

MM.1S Luc intravenous xenograft model (continued)

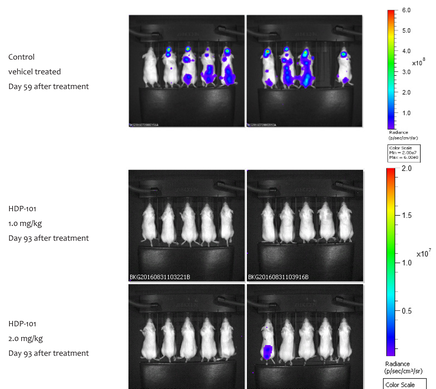


Figure 4: Tumor burden in the intravenous **MM.1S Luc** xenograft model. Single dose i.v. application. Data and signals represent luciferase-dependent bioluminescence intensity and reflect the number of tumor cells in the signal area. Upper panel represents vehicle treated control group (total flux data on day 59). Lower pannels represent treatment groups (1.0 mg/kg and 2.0 mg/kg; luminescence data on day 93). Please note the different scale bars in the two parts of the figure.

KMS-11 intravenous xenograft model

HDP-101 shows overall survival of 100% after single dose application of 4 mg/kg and 90% at 1 and 2 mg/kg over an observation period of 123 days (Figure 5a). At the 0.1 and 0.3 mg/kg dose, overall survival was still significantly increased compared to the control group.
Figure 5 b depicts all control groups within this study: The toxin moiety coupled to a non-binding Antibody (DIG-Thiomab-30.2115), standard of care (Carfilzomib) and a reference toxin (MMAF) conjugated to the same antibody used in HDP-101 (Anti-BCMA-mcMMAF). Even at highest concentration, this reference ADC showed significantly lower overall survival than the corresponding dose of HDP-101. Note that the DAR in the reference ADC is 3-4, while the DAR of HDP-101 is 1.9 - 2.05.
HDP-101 was well tolerated up to 4 mg/kg.

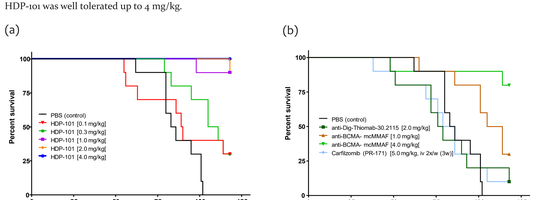
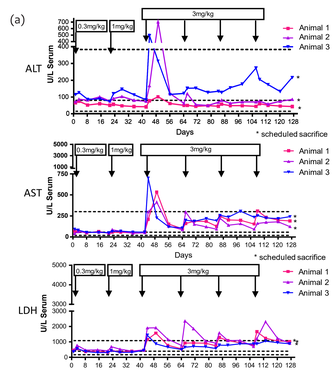


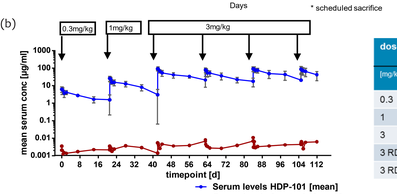
Figure 5: Kaplan-Meier Plot of dose dependent overall survival of HDP-101-treated animals in the intravenous **KMS-11** xenograft model. Single dose i.v. application. (a) Control group (PBS) and HDP-101 treatment groups; doses: 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg, 2.0 mg/kg and 4.0 mg/kg. (b) Combined graph of all control groups within this study.

Non-human primate tolerability study

HDP-101 was tested in a dose-escalating tolerability study in cynomolgus monkeys. The ADC was applied sequentially at doses of 0.3 mg/kg, 1.0 mg/kg and 3.0 mg/kg to the same animals. The 3 mg/kg-dose was chosen for a repeated dosing (four times at a three week interval).
Before and after application biochemical and hematological blood parameters are evaluated extensively (selected parameters: figure 6a).
Concentration of HDP-101 and free toxin were assayed in serum samples at different time intervals ranging from 5 min up to 21 days after injection (figure 6b). The increased serum concentrations correlate with injected doses of 0.3, 1 and 3 mg. In addition, maximum serum concentrations in each of the repeated dose injections of 3 mg/kg (3, 3RD1, and 3RD2; table in figure 6b) are comparable.
Wash-out times of 21 days between repeated applications of 3 mg/kg resulted in drop of mean serum ADC to approximately 20 µg/ml.
The table on the right (figure 6b) summarizes mean PK parameters for HDP-101. Half-life of HDP-101 was calculated to average 12 days.
Cmax mirrors repeated dosing in group 10 and peaks to maximum 30 µg/ml per dose.

Up to 3 mg/kg, HDP-101 was well tolerated:
- No severe increase in liver-relevant biochemical parameters, no patho-morphological findings.
- No signs of kidney damage by serum parameters.
- Food consumption and body weight remained unaffected at doses applied.





dose	T1/2	Cmax
[mg/kg]	[day]	[µg/ml]
0.3	9.13	6.47
1	12.8	26.3
3	17.6	94.9
3 RD1	11.2	77.6
3 RD2	15.6	93.7

Figure 6: (a) Selected biochemical serum parameters in cynomolgus monkeys treated with escalating doses of HDP-101 up to 3 mg/kg. Dashed lines reflect the mean, min. and max. values of untreated animals (predose values). (b) Serum concentration of HDP-101 and the corresponding free toxin in cynomolgus monkeys for dose levels of 0.3 mg/kg, 1.0 mg/kg and 4 x 3 mg/kg. The table on the right shows the main pharmacokinetic parameters: T1/2 and Cmax.

CONCLUSION

Multiple Myeloma is the second most common hematologic cancer after Non-Hodgkin-Lymphoma. It ranks as the 15th most common type of cancer in the United States. While significant therapeutic advances have been made, therapeutic options remain unsatisfactory. Amanitin-based ADCs (ATACs) using an anti-BCMA antibody showed high antitumor activity in preclinical models of oncology. Amanitin as a toxic warhead for ADCs in Multiple Myeloma seems to be a suitable therapeutic option because of the unique mode of action and the molecular characteristics of the toxin. Amanitin is highly active in drug-resistant cells, independent of the status of expression of multi-drug resistance transporters because of its hydrophilic structure. By inhibition of RNA polymerase II Amanitin-binding leads not only to apoptosis of dividing cells, but also of slowly growing cells, as observed in dormant cells and cancer-initiating cells. HDP has constructed more than 70 different linkers allowing the conjugation of Amanitin to lysine-residues, cysteine-residues (both, genetically engineered and interchain cysteines) as well as non-natural amino acids of antibodies. In the presented work we characterized the efficacy and tolerability of Heidelberg Pharmas development candidate HDP-101 in preclinical models. HDP-101, an anti-BCMA-Thiomab ADC with synthetic Amanitin linked via a cleavable linker showed a very good efficacy profile in disseminating xenograft models at doses as low as 0.1 mg/kg. Despite the fact that subcutaneous tumors are much harder to treat, we see tumor regression at 2 mg/kg (single dose) and complete remission in 9 out of 10 animals at a dose of 4 mg/kg (single dose) in the subcutaneous NCI-H929 multiple myeloma model (intravenous model ongoing).
Furthermore, HDP-101 was assessed for its safety profiles in a dose escalating study in cynomolgus monkeys and revealed very good tolerability at a repeated-dose setting of 4 times 3 mg/kg. The serum levels of HDP-101 correlate with the injected doses of 0.3 mg/kg, 1 mg/kg and 3 mg/kg.
The positive findings encouraged Heidelberg Pharma to proceed with HDP-101 towards clinics. Currently, GMP material is produced, meetings with competent authorities are scheduled and the first-in-human clinical trial is planned for 2018.

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