Preclinical Evaluation of HDP-101, a Novel anti-BCMA Antibody-Drug Conjugate, in Multiple Myeloma

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Introduction

- Multiple myeloma (MM) is a malignancy characterized by the clonal proliferation of plasma cells in the bone marrow and makes up ca. 1% of all neoplastic diseases and 13% of all hematologic malignancies.
- Despite improvements in treatment, MM remains a generally incurable disease.
- Most antibody-drug conjugates (ADCs) in current clinical trials are based on few toxic compounds, mainly targeting proliferating cells potentially leading to limited efficacy in multiple myeloma.
- In the current study, *in vitro* and *in vivo* data of HDP-101, an ATAC (Antibody-Targeted Amanitin Conjugate) targeting B-cell maturation antigen (BCMA), are presented.
- Amanitin binds to the eukaryotic RNA polymerase II thereby inhibiting the cellular transcription process at very low concentrations irrespective of the proliferation status of the target cell.
- BCMA (also known as CD269) is highly expressed on malignant plasma cells and not expressed on naïve B cells, germinal center B cells and memory B cells, therefore considered an ideal target in multiple myeloma.
- Using ADCs in the cure of multiple myeloma could be a promising approach, especially with amanitin whose mode of action was not applied before.

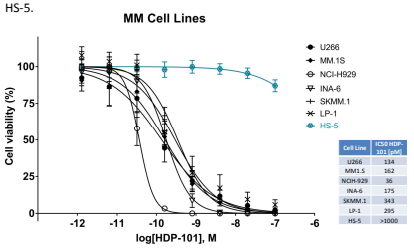
Methods

Cell lines and antibodies: The human multiple myeloma cell lines U266, MM.1S, NCI-H929 as well as the bone marrow stroma cell line HS-5 were obtained from the ATCC, SKMM.1 was obtained from the DSMZ (Germany), LP-1 was obtained from the myeloma center of the university hospital Heidelberg (Germany), INA-6 was obtained from Renate Burger at the department of stemcell and immunotherapy in Kiel (Germany), 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.
Patients: Patient samples from patients diagnosed with MM according to the criteria of the International Myeloma Working Group were included.
Primary MM cells: Primary CD138+ cells were isolated from MM patient whole bone marrow samples with magnetic CD138 microbeads (Miltenyi Biotech). Purity was examined by flow cytometry (ACCURI C6, BD Biosciences) using CD45 (low), CD38 and CD138 (bright) markers.
Bone marrow stroma cells (BMSC): BMSCs were isolated from MM patient whole bone marrow samples by taking mononuclear cells (MNC) from sample in culture in Dulbecco's Modified Eagle Medium (DMEM) + 10% Fetal Calf Serum (FCS).
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.
Synthesis of anti-DIG ATAC: The Anti-DIG ATAC was prepared similar to HDP-101 by conjugating HDP 30.2115 to engineered cysteine residues resulting in an ATAC with a DAR of 2.0.
Cell viability assay: Quantitative determination of cell viability was performed by CellTiter-Glo 2.0 assay (Promega).
Flow cytometry: Epitope density of BCMA on cells was determined with PE-labelled BCMA-antibody (Heidelberg Pharma) and QuantiBRITE PE Beads (BD) as a reference using ACCURI C6 (BD Biosciences).
Animal models: *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).
NHP studies were performed in treatment-naive female cynomolgus monkeys at LPT (Germany, Hamburg).

Results

Cytotoxic potency

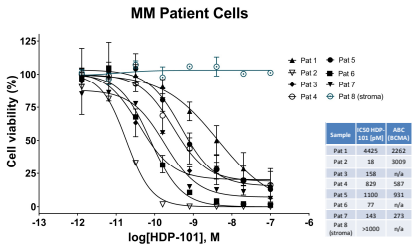
MM cell lines U266, MM.1S, INA-6, SKMM.1, NCI-H929 and LP-1 were used to test the cytotoxicity of HDP-101 (Figure 1). HS-5 (BCMA-negative) served as negative control. In all BCMA positive cell lines, the anti-BCMA Thiomab-ATAC HDP-101 showed high activity in pico- to nanomolar range. No cytotoxic effect up to a concentration of 10^{-6} M was seen in the BCMA negative cell line HS-5.



Cell Line	IC50 HDP-101 [pM]
U266	134
MM.1S	162
NCH-929	36
INA-6	175
SKMM.1	143
LP-1	295
HS-5	>1000

Figure 1: Cytotoxic activity of HDP-101 in BCMA-positive cell lines U266, MM.1S, INA-6, SKMM.1, NCI-H929, LP-1 (black) and HS-5 (BCMA-negative, blue) after incubation for 96h. The table on the right shows the respective IC50 values.

Primary MM cells (CD138+) from patients with newly diagnosed or refractory MM extracted from the bone marrow were tested with HDP-101. Patient bone marrow stroma cells (BMSC) served as negative control (Figure 2).



Sample	IC50 HDP-101 [pM]	ABC (BCMA)
Pat 1	4425	2262
Pat 2	18	3009
Pat 3	158	n/a
Pat 4	829	587
Pat 5	1100	931
Pat 6	77	n/a
Pat 7	143	273
Pat 8 (stroma)	>1000	n/a

Figure 2: Cytotoxic activity of HDP-101 in purified CD138+ MM patient cells from bone marrow samples (black) and patient BMSC (BCMA-negative, blue) after incubation for 96h. The table on the right shows the respective IC50 values and anti-BCMA antibodies bound per cell (ABC, see also Fig. 3).

BCMA expression and drug response

BCMA expression on MM cell lines and patients cells was detected by flow cytometry. Even low BCMA-expression was sufficient for effective cell killing in the concentration range of 0,1-10nM (Figure 3).

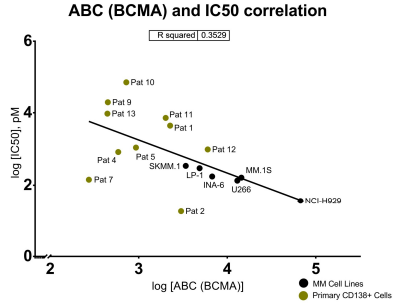


Figure 3: Correlation of epitope density and IC50 HDP-101 on cell lines (black) and primary CD138+ patient cells (green). ABC – Antibodies bound per cell.

Specificity

MM cell lines U266, MM.1S, INA-6, SKMM.1, NCI-H929 and LP-1 were drugged with an anti-Digoxigenin (DIG) control ATAC (Figure 4). DIG is a steroid only found in certain plant species and absent in the human organism. Control DIG ATAC showed no cytotoxic activity up to a concentration of 10^{-6} M for most cell lines, reduced viability of U266 and SKMM.1 at 10^{-7} M.

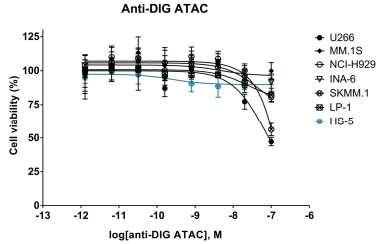
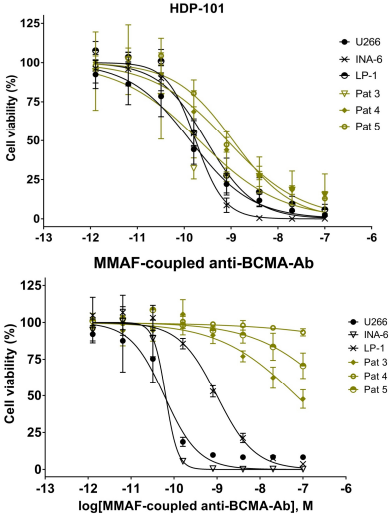


Figure 4: Viability assay of non-target DIG ATAC on U266, MM.1S, INA-6, SKMM.1, NCI-H929, LP-1 (black) and HS-5 (BCMA-negative, blue) after incubation for 96h.

Targeting non-proliferating primary cells

MM cell lines U266, INA-6 and LP-1 were compared to primary CD138+ cells from three different patients in their sensitivity towards an anti-BCMA Thiomab antibody either coupled to amanitin (HDP-101) or monomethyl auristatin F (MMAF) (Figure 5). HDP-101 showed greater efficacy in primary CD138+ cells in the same dose range as compared to the MMAF-coupled anti-BCMA antibody.



Sample	U266	INA-6	LP-1	Pat 3	Pat 4	Pat 5
IC50 HDP-101 [pM]	134	175	295	158	829	1100
IC50 MMAF-BCMA-Ab [pM]	59	63	991	70160	> 100000	> 100000

Figure 5: Viability assay of HDP-101 and a MMAF-coupled anti-BCMA antibody on U266, INA-6, LP-1 (MM cell lines, black) and primary CD138+ cells from MM patients (green) after 96h incubation. Table shows the respective IC50 values.

In vivo efficacy

Antitumor activities of ADCs were determined in a MM.1S Luc intravenous xenograft model and a NCI-H929 subcutaneous xenograft model (data not shown) *in vivo*.

MM.1S Luc intravenous xenograft model

HDP-101 shows tumor regression and complete remissions (to baseline signal, dashed line, Figure 7) after single dose application down to 0.1 mg/kg (Figure 7). 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 elevated luminescence signals at the latest time point of observation (day 93).

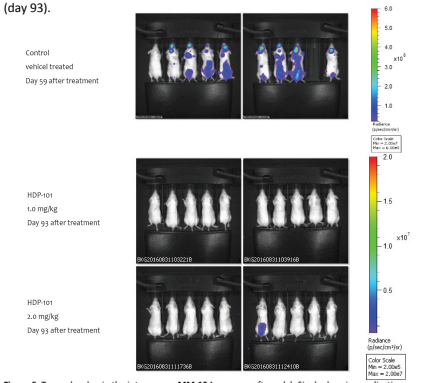


Figure 6: Tumor burden in the intravenous **MM.1S Luc** xenograft model. Single dose i.v. application. Data and signals represent luciferase-dependent bioluminescence intensity and reflect the number of tumor cells in the signal area. Upper panel represents vehicle treated control group (total flux data 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.

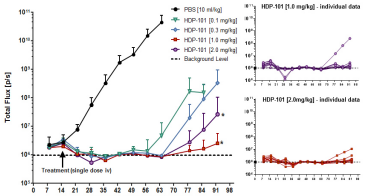


Figure 7: Dose dependent efficacy of HDP-101 on the tumor burden in the intravenous **MM.1S Luc** xenograft model. Single dose i.v. application. Data signals represent luciferase-dependent bioluminescence intensity and reflect the tumor burden of the whole animal. Left panel: Mean values of groups +/- SD. Right panel: Graphs show luminescence intensity of all individual animals in the groups treated with 1.0 mg/kg (upper right) and 2.0 mg/kg (lower right). Dashed line represents background level.

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).

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.

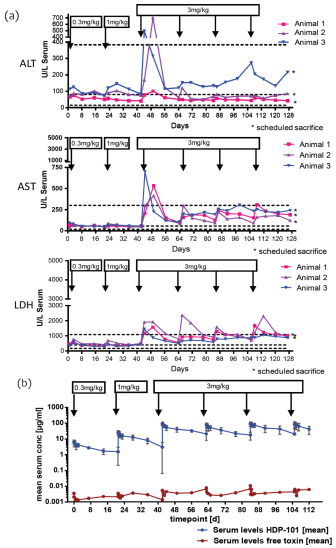


Figure 8: (a) Selected biochemical serum parameters in cynomolgus monkeys treated with escalating doses of HDP-101 up to 3 mg/kg. Dashed lines reflect the mean, min. and max. values of untreated animals (predose values). (b) Serum concentration of HDP-101 and the corresponding free toxin in cynomolgus monkeys for dose levels of 0.3 mg/kg, 1.0 mg/kg and 4 x 3 mg/kg.

Conclusion

- HDP-101 showed *in vitro* cytotoxic potency on several BCMA-positive myeloma cell lines and non-proliferating primary CD138+ cells at pico- to nanomolar concentrations.
- HDP-101 conferred high cytotoxicity in low-BCMA-expressing cells.
- Non-target controls confirmed high specificity of HDP-101.
- In contrast to MMAF, amanitin effectively targets non-proliferating primary CD138+ cells.
- In mouse xenograft models of human myeloma, HDP-101 single-dose caused dose-dependent tumor regression including complete remissions.
- Safety profiling in cynomolgus monkeys revealed a good tolerability and favorable therapeutic index.
- A first-in-human trial with HDP-101 is expected to start in 2018.
- **Amanitin-based ADCs (ATACs) in the therapy of multiple myeloma are a novel promising approach with a distinct mode of action to overcome drug resistance and improve patient outcome.**

References

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(2) Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, Gross JA, Greipp PR, Jelinek DF. Expression of BCMA, TACI, and BAFF-R in Multiple Myeloma: a mechanism for growth and survival. Blood. 2004 Jan 15;103(2):689-94.

Disclosures

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