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# CD269 – A Promising Target for Amanitin Based ADCs

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## INTRODUCTION

Antitumor activity of monoclonal antibodies can be dramatically enhanced by conjugation to toxic small molecules. Beside the recent approval of Kadcyla (T-DM1) and Adcetris (SGN-35) more than 30 antibody-drug conjugates (ADC) 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 the microtubuli-targeting auristatins and maytansines. Toxins that operate through such a mechanism could suffer from limited activity in different cancer indications and in cells expressing resistance mechanisms. Accordingly, the use of new drugs that function via alternative toxicity mechanisms could enhance the therapeutic potential of ADCs. Heidelberg Pharma focuses on Amanitin, the most well-known toxin of the amatoxin family. Amanitin binds to the eukaryotic RNA pol II and thereby inhibits the cellular transcription at very low concentrations.

In the current study, in vitro and in vivo Data of Amanitin-ADCs targeting CD269 (B cell maturation antigen, BCMA) are presented. 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 (Darce et al. (2007) J Immunol 179:7276-7286). CD269 is highly expressed on malignant plasma cells like multiple myeloma and B cell non Hodgkin lymphoma of the bone marrow (Novak et al. (2004) Blood 103:689–94). 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 by using a toxin whose mode of action was not applied before, like amanitin based ADCs.

## METHODS

**Cell lines and antibodies:** The human multiple myeloma cell line NCI-H929 was obtained from the ATCC, 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 CD269 antibodies developed at the MDC. Thiomab derivaitves thereof were produced by HDP using Expi293 cells (Life Technologies) and transient transfection methods.

**Synthesis of amanitin-ADCs:** Maleimide amanitin compounds HDP 30.2115 and HDP 30.1699 were conjugated to substituted cysteine residues in phosphate buffer, pH 7.4 after reduction with TCEP and re-oxidation of interchain disulfides by dehydroascorbic acid (dhAA). Conjugates were purified by SEC-HPLC and dialysis. DAR (drug-antibody ratio) according to LC-MS analysis was ca. 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:** Six to 8-wk-old athymic female NMRI nude mice (nu/nu) were obtained from Janvier. NCI-H929 cells ($5.0 \times 10^6$) were injected into the flank of each mouse for the development of a tumor. When tumors were well-established (100-200 $mm^3$), treatment was started by a single-dose i.v. application. Tumor volume was calculated as $V = a \times b^2 \times 0.5$, where $a$ is the length and $b$ is the width.

NHP studies were performed by Dr. Jost Leuschner, LPT (Germany, Hamburg).

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## RESULTS

### Engineered Thiomab has Comparable Binding Properties to CD269

For FACS analysis of the binding properties, CD269-positive NCI-H929 cells were incubated with increasing concentrations of either anti-CD269 (wild type, WT) or the thiomab variant thereof. The mean fluorescence of both antibodies did not show any significant difference. The binding properties of the thiomab variant is comparable to the wild type antibody (Figure 1).

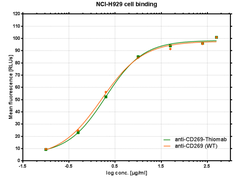


Figure 1: FACS analysis: anti-CD269 Antibodies (thiomab and wild type (WT)) were incubated with NCI-H929 cells in increasing concentrations.

### Cytotoxic potency

The multiple myeloma cell lines NCI-H929 and MM.1S Luc were used to test the cytotoxic potency of anti-CD269-Amanitin ADCs. Following ADCs were assessed:

| ADC | Amanitin source | linker type | DAR (MS) |
|---|---|---|---|
| anti-CD269-Thiomab-30.1699 | biological | protease cleavable | ~2.0 |
| anti-CD269-Thiomab-30.2115 | synthetic | protease cleavable | ~2.0 |

In both cell lines, the anti-CD269-Amanitin ADCs showed high activity in pico to nanomlar range. Anti-CD269-30.1699 („natural" Amanitin) showed slightly superior cytotoxic potential compared to the synthetic Amanitin ADC variant anti-CD269-30.2115 (Figure 2).

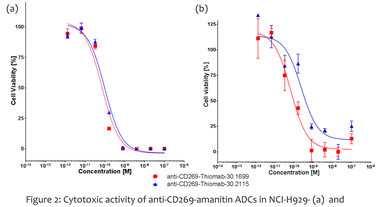


Figure 2: Cytotoxic activity of anti-CD269-amanitin ADCs in NCI-H929- (a) and MM.1S Luc-cells (b) after incubation for 96h.

### Stability of CD269-Amanitin ADCs

To test the stability of anti-CD269-ADCs, compounds were incubated in murine and human plasma for up to 10 days. After the respective incubation period, the compounds were either analyzed using an anti-Amanitin Western-Blot or checked for remaining cytotoxic potential in an in vitro cytotoxicity assay (NCI-H929 cells). The Western Blot analysis showed higher stability of the synthetic Amanitin ADC variant anti-CD269-Thiomab-30.2115 compared to the „natural" variant anti-CD269-Thiomab-30.1699 (Figure 3a and b). This finding was confirmed by the decreased cytotoxic efficacy of the plasma incubated ADCs in NCI-H929 cells (Figure 3 c and d).

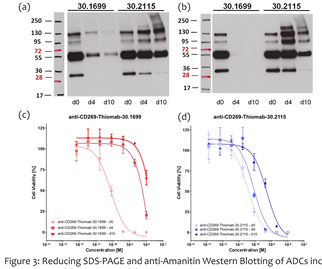


Figure 3: Reducing SDS-PAGE and anti-Amanitin Western Blotting of ADCs incubated in murine (a) and human (b) plasma. 30.1699 = anti-CD269-Thiomab-30.1699; 30.2115 = anti-CD269-Thiomab-30.2115. (c) (d): Cytotoxic activity of anti-CD269-amanitin ADCs in NCI-H929 cells after incubation of ADCs in human plasma for up to 10 days.

### Mouse efficacy and tolerability experiments

Antitumor activities of ADCs were determined in a NCI-H929 subcutaneous xenograft model *in vivo*. anti-CD269-Thiomab-30.1699 and anti-CD269-Thiomab-30.2115 show dose-dependent tumor regression and complete remissions after single dose application between 2mg/kg and 4mg/kg (Figure 4). The ADCs differ in the Amanitin moiety: 30.1699 represents the "natural" alpha-Amanitin while 30.2115 is a fully synthetic variant thereoff. At the 2mg/kg dose, the synthetic Amanitin-variant 30.2115 had superior antitumor efficacy compared to the "natural" amanitin based ADC anti-CD269-Thiomab-30.1699. Both ADCs were well tolerated at 4 mg/kg.

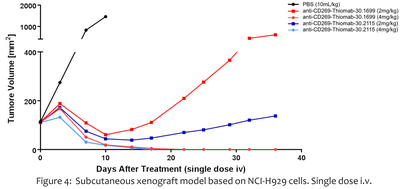


Figure 4: Subcutaneous xenograft model based on NCI-H929 cells. Single dose i.v. application.

### Non-human primate tolerability study

Anti-CD269-Thiomab-30.1699 and anti-CD269-Thiomab-30.2115 are currently assessed for a dose-escalating tolerability study in cynomolgus monkeys. The ADCs are applied sequentially at doses of 0.3mg/kg and 1.0mg/kg; further dose levels are planned: 3.0mg/kg and 10.0mg/kg. The study is currently ongoing. Before and after application biochemical and hematological blood parameters are evaluated extensively (selected parameter: Figure 5).

Until now, both ADCs were well tolerated:

- No significant increase in liver-relevant biochemical parameters.
- There was no sign of kidney damage by serum parameters.
- Food consumption and body weight remained unaffected at doses applied so far

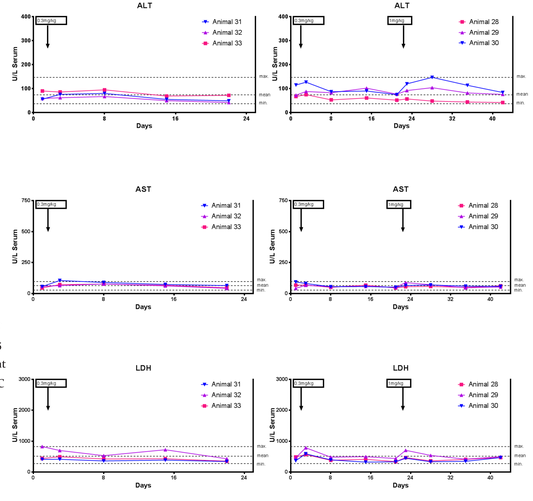


Figure 5: Biochemical serum parameters in cynomolgus monkeys treated with escalating doses of anti-CD269-Thiomab-30.1699 (left panel) and anti-CD269-Thiomab-30.2115 (right panel).
Dashed lines reflect the mean, min. and max. values of untreated animals (predose values).

## CONCLUSIONS

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 using an anti-CD269 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 resistant 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 cysteins) as well as non-natural amino acids of antibodies. In the presented work we characterized ADCs directed against CD269 (B cell maturation antigen, BCMA). Anti-CD269 antibodies with synthetic and "natural" Amanitin linked via a cleavable linker were compared in vitro an in vivo for efficacy and showed very good efficacy in cell based assays as well as in first mouse xenograft experiments. Both ADCs, anti-CD269-Thiomab-30.1699 and anti-CD269-Thiomab-30.2115 are currently assessed for their safety profiles in a dose escalating study in cynomolgous monkeys and revealed very good tolerability so far.

These positive findings of these initial experiments encourage Heidelberg Pharma to further proceed with anti-CD269-Amanitin-based ADCs towards clinics.

## REFERENCES

(1) Darce JR, Arendt BK, Wu X, Jelinek DF. Regulated Expression of BAFF-Binding Receptors during Human B Cell Differentiation. J Immunol. 2007 Dec 1;179(11):7276-86.

(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.

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