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Preclinical evaluation of anti-CD37 Antibody Targeted Amanitin Conjugates (ATAC) in B-cell malignancies

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Introduction

Antitumor activity of therapeutic antibodies can be significantly 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 increasing number of approved ADCs including Mylotarg (CD33), Adcetris (CD30), Kadcyla and Enhertu (HER2), Besponsa (CD20), Polivy (CD79b), Padcev (Nectin-4), Trodelvy (Trop-2) and Blenrep (BCMA), 189 ADCs have entered clinical trials, promising to strengthen the therapeutic capabilities for cancer treatment. 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 **ATAC**s (**A**ntibody **T**argeted **A**manitin **C**onjugates), comprising a new class of ADCs with amanitin as toxic payload (1). Amanitin is the well-known toxin of the amatoxin family which specifically binds 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 *THIOMAB*®.

In the current study, *in vitro* and *in vivo* data of ATACs targeting CD37 are presented. CD37 is a transmembrane protein expressed exclusively on cells of the immune system and mainly on mature B-cells, as well as in many B-cell malignancies, including Richter's Syndrome (RS) or Richter´s transformation. RS is a transformation of B cell chronic lymphocytic leukemia, refractory to treatment and carries a poor prognosis, and hence is considered an ideal target for amanitin-based ADCs.

Methods

Cell lines and antibody: CD37$^+$ cell lines MEC-1, MEC-2, Ramos and Raji and CD37$^-$ cell line Nalm-6 were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ).
The *THIOMAB*® derivative of the anti-CD37 antibody was produced by Heidelberg Pharma Research GmbH using Expi293 cells (Life Technologies) and transient transfection methods.
Synthesis of conjugates: Cysteine-reactive amanitin-linker constructs were synthesized at Heidelberg Pharma and were conjugated site-specifically by *THIOMAB* conjugation. Drug-antibody ratio (DAR) for amanitin conjugates according to LC-MS analysis was +/- 2.0 amanitins per IgG.
Flow cytometry: Binding of anti-CD37 antibody 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 WST-1 (Roche) assays.

Animal models: Disseminating mouse xenograft tumor models with CD37$^+$ cell lines MEC-2 and Raji-Luc were used in single dose experiments.
Six to eight-week-old female CB-17 SCID mice were obtained from Janvier. 2.5 x 10^6 MEC-2 or Raji-Luc cells were injected i.v. in each mouse. 3 days post inoculation, high or low doses of anti-CD37 ATACs with cleavable or non-cleavable linker were applied i.v. In MEC-2 xenograft study, median survival of each group was determined.
In Raji-Luc xenograft study, tumor burden was measured weekly by bioluminescence. 150mg/kg of D-luciferin was injected i.p. in each mouse. After 10 minutes, bioluminescence was measured with IVIS Lumina II (Caliper).
Non-human primate tolerability study was performed at Alta Sciences (Everett, USA).

Results

1. Conjugation of CD37 Antibody Targeted Amanitin Conjugate (ATAC)

RNA polymerase II binding toxin amanitin compounds with cleavable linker (blue) or non-cleavable linker (orange) were conjugated to substituted cysteine residues of anti-CD37 *THIOMAB*® antibody using maleimide chemistry, resulting in homogenous ATAC with a DAR of 2 toxins per IgG (Figure 1).

Figure 1: Schematic drawing of conjugation of anti-CD37 antibody with amanitin compounds with cleavable (blue) or non-cleavable linker resulting in an anti-CD37 ATAC with DAR of 2.

2. Anti-CD37 *THIOMAB*® binds selectively to CD37$^+$ cell lines

The binding property of anti-CD37 *THIOMAB*® was analyzed by flow cytometry. Therefore, CD37$^+$ cell lines MEC-1, MEC-2, Ramos and Raji as well as the CD37$^-$ cell line Nalm-6 were incubated with increasing concentrations of the anti-CD37 *THIOMAB*®. The antibody showed specific binding to all CD37$^+$ cells lines while no binding was observed on the CD37$^-$ cell line (Figure 2).

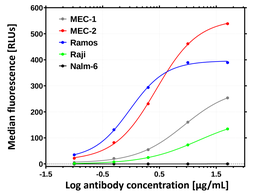

Figure 2: Binding capacity of anti-CD37 *THIOMAB*® antibody on MEC-1, MEC-2, Ramos, Raji and Nalm-6 cell lines was assessed by flow cytometry.

3. Cytotoxic activity of anti-CD37 ATACs on CD37$^+$ and CD37$^-$ cell lines

The CD37$^+$ cell lines MEC-1, MEC-2, Ramos and Raji were used to test the cytotoxic potency of anti-CD37 ATACs with cleavable linker (CD37-ATAC I; Figure 3a) and non-cleavable linker (CD37-ATAC II; Figure 3b). Both anti-CD37 ATACs showed full blown cytotoxicity with EC_{50} values in the low nanomolar range on all used cell lines.

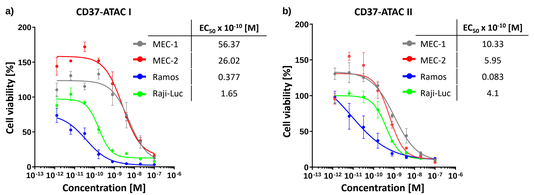

Figure 3: Cytotoxic activity of anti-CD37 ATAC with a cleavable (a) or non-cleavable (b) linker on MEC-1, MEC-2 and Ramos cells using Cell Titer Glo (CTG) assay after incubation for 96h. Cytotoxic activity on Raji-Luc cells was assessed by WST-1 ELISA.

4. Efficacy in mouse disseminating xenograft models

The antitumor activity of single dose treatment of anti-CD37 ATAC I with a cleavable and anti-CD37 ATAC II with a non-cleavable linker was determined in disseminating MEC-2 and Raji-Luc xenograft models *in vivo*.

MEC-2 xenograft model:
The high and low dose treatment with anti-CD37 ATACs with either cleavable or non-cleavable linker led to a significantly increased median survival compared to PBS control. After 180 days, even the lowest treatment led to 70-80% overall survival.

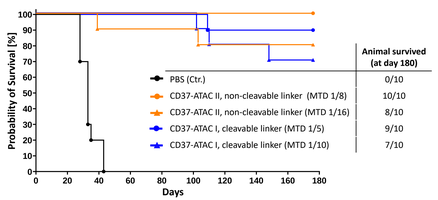

Figure 4: Disseminating MEC-2 xenograft model. Single dose i.v. application of anti-CD37 ATACs with non-cleavable (orange) or cleavable linker (blue) at high and low doses. Shown is median survival.

Raji-Luc xenograft model:
At day 15 post inoculation, all mice of vehicle control group (PBS) showed a high tumor burden and were sacrificed while all mice treated with anti-CD37 ATACs were tumor-free (Figure 5a). Even 124 days after treatment, 9/10 and 10/10 animals treated with anti-CD37 ATACs with cleavable (CD37 ATAC I) or non-cleavable linker (CD37 ATAC II), respectively survived (Figure 5b).

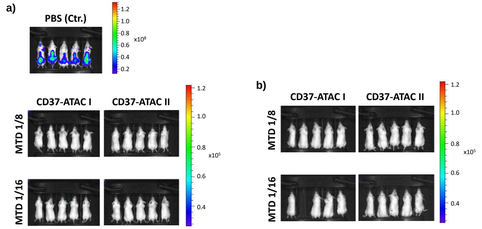

Figure 5: Disseminating Raji-Luc xenograft model. 3 days post inoculation, mice were treated with anti-CD37 ATACS with cleavable (left column) or non-cleavable linker (right column). 12 days (a) and 124 days (b) after treatment, tumor burden was measured by bioluminescence.
Color scale shows radiance (p/sec/cm^2/sr).

Richter Syndrome PDX model:
Cell suspension of Richter Syndrome cells of two different patients were injected *i.v.* into NOG mice. 10-16 days post inoculation, mice were treated with anti-CD37 ATAC with non-cleavable linker. Animals treated with high or low doses of anti-CD37 ATAC survived > 2-times longer compared to animals treated with vehicle control (data not shown).

5. Tolerability of anti-CD37 ATACs in non-human primates

Two anti-CD37 ATACs with cleavable and non-cleavable linker were assessed for a dose-escalating tolerability study in cynomolgus monkeys. Each group consists of equal number of female and male animals. The ATAC with cleavable linker or non-cleavable linker was applied sequentially with increasing concentrations to the same animals. Biochemical and hematological blood parameters were evaluated extensively during the study.

The anti-CD37 ATAC with cleavable linker (blue) was well tolerated up to 3mg/kg while the anti-CD37 with non-cleavable linker (orange) was well tolerated up to 10mg/kg dose level (Figure 6):

- Transient and mild increase in liver-damage-relevant biochemical parameters (ALT, AST and LDH) after application of dose 2.
- The MTD of the anti-CD37 ATAC with the cleavable or non-cleavable linker was determined with 3mg/kg and 10mg/kg, respectively.
- Macroscopic changes in kidney, liver, skin and spleen were observed during necropsy of prematurely terminated animals

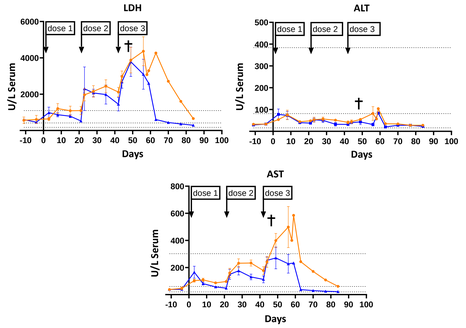

Figure 6: Female and male animals were treated with escalating doses of 1mg/kg, 3mg/kg and 5mg/kg of the anti-CD37 ATAC with cleavable linker or with escalating doses of 5mg/kg, 10mg/kg and 15mg/kg of the anti-CD37 ATAC with non-cleavable linker. Selected biochemical serum parameters (LDH, ALT, AST) were measured. Dashed lines reflect the mean, min. and max. values of untreated animals (defined in previous NHP studies).

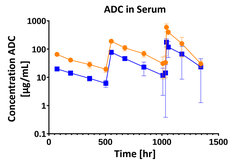

Figure 7: Concentration of anti-CD37 ATAC with cleavable (blue) or non-cleavable linker (orange) in serum collected during the dose-escalating tolerability study in cynomolgus monkeys was determined by ADC ELISA at KCAS Bioanalytical Services (Shawnee, USA).

Conclusion

Lymphoma is the most common lymphoid malignancy (4). It is a heterogenous entity including B-cells which overexpress CD37. This transmembrane protein of the tetraspanin superfamily is involved in different biological processes (5). Because of its abundant overexpression on different blood malignancies which include B-cells, CD37 is an ideal target to treat B-cell lymphoma.
In the current study, *in vitro* and *in vivo* data of an amanitin based ADC (ATAC) with cleavable and stable linker targeting CD37 are presented. On different CD37$^+$ cell lines, both anti-CD37 ATACs showed strong cytotoxic potential with EC_{50} values in the nanomolar range.
In two independent mouse xenograft models the used anti-CD37 ATACs showed strong and persistent anti-tumor activity. Even the treatment with low doses of both ATACs (MTD 1/16 of non-cleavable linker; MTD 1/10 of cleavable linker) resulted in an 80% and 70% survival rate, respectively.
Given the results of the safety profiling in cynomolgus monkeys which have proven a good tolerability and therapeutic index for both anti-CD37 ATACs, amanitin as a toxic warhead for ADCs targeting CD37 on B cells could represent a viable therapeutic option to treat B-cell malignancies. Through its unique mode of action, ATACs are not only suited to target dividing cells, but also slowly growing cells and dormant cells. Amanitin is also effective in drug resistant cells, independent of the expression of multi-drug resistance transporters. This is a potential advantage for amatoxin payloads in slow proliferating B-cell malignancies, including mantel cell lymphoma.
The findings of these experiments warrant further clinical development of anti-CD37 ATACs.

References

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