Amanitin-based ADCs targeting Guanylyl cyclase C (GCC) as novel therapeutic modality for treatment of colorectal cancer

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Introduction

Tumor-specific delivery of cytotoxic small molecules can be achieved by conjugation to monoclonal antibodies which bind with high affinity to a specific target thereby enhancing anti-tumor activity and reducing off-target toxicity. Heidelberg Pharma focuses on **ATAC®**s (amatoxin-based antibody-drug conjugates), a new class of antibody-drug conjugates (**ADCs)** with amatoxin as payload (1). α-Amanitin, the principal toxin found in *Amanita phalloides* - the notorious "green death-cap" mushroom – is a highly selective allosteric inhibitor of eukaryotic RNA polymerase II (RNAP2). As RNAP2 is essential for cellular growth and homeostasis, α-amanitin **kills dividing and quiescent cells** by inhibiting RNAP2, which leads to rapid proteolytic degradation and finally cell death (2, 3). This unique mechanism of action distinguishes α-amanitin from nearly all other toxic payloads which act primarily on rapidly growing cells and holds potential for overcoming drug resistance and improving patient outcome.

By using **site-specific conjugation** to attach the amatoxin-linkers to engineered cysteines at defined positions of the antibody heterogeneity of drug-antibody species is dramatically reduced, and highly stable conjugates with broad therapeutic window are generated.

In the current study, *in vitro* and *in vivo* data of **ATAC®**s **targeting** GCC (guanylyl cyclase C) are presented. GCC is a cell surface receptor expressed in >95% of colorectal cancer, and in approximately 65% of esophageal, gastric, and pancreatic tumors (4). In healthy conditions GCC expression is restricted to the gastrointestinal tract, and more specific to the apical brush border of the intestinal epithelium (luminal site). Thus, GCC in healthy tissue is not exposed to the circulation, but upon tumor progression in gastrointestinal malignancies, it becomes accessible for i.v. injected targeted therapeutics. This tumor-specific accessibility for drugs in circulation makes GCC a highly attractive target for amanitin-based ADCs.

Methods

Synthesis of conjugates: Cysteine reactive amanitin-linker constructs containing either a non-cleavable or a cleavable Val-Ala linker were synthesized at Heidelberg Pharma and conjugated site-specifically to engineered cysteine residues of the humanized proprietary anti-GCC antibody. DAR (Drug-Antibody Ratio) for all conjugates was 2.0 ± 0.1 as determined by LC-MS analysis.

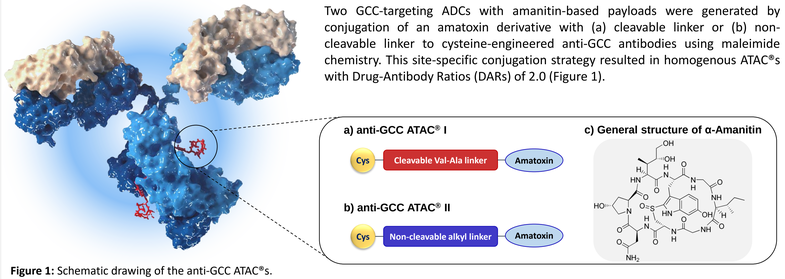
Cell lines: The GCC overexpressing cell line HEK293-GUCY2C was produced at Heidelberg Pharma Research GmbH using the human embryonal kidney cell line HEK293 obtained from German Collection of Microorganisms and Cell Cultures (DSMZ).

Cell proliferation assay: Quantitative determination of cell viability was performed by BrdU-based chemiluminescent cell proliferation ELISA (Roche).

Animal models: Subcutaneous (s.c.) mouse xenograft tumor models with the GCC overexpressing cell line HEK293-GUCY2C were used. Six to eight-week-old female NOD SCID mice were obtained from Janvier. 8-10 mice per group were inoculated s.c. with 5 x 10^6 HEK293-GUCY2C tumor cells and treated with a single dose i.v. application at an established tumor volume of approx. 150mm³. Tumor growth and median survival of each group was determined. Colon cancer PDX models (Charles River) were performed in single-dose and multiple-dosing experiments. Tolerability was assessed in female NOD SCID mice and in non-human primates (NHP). Studies in NHP were performed at Wuxi using 2-to-4-year-old monkeys were treated with a single i.v. dose in an escalating dose regiment.

Results

1. Conjugation of anti-GCC Antibody Targeted Amanitin Conjugates (ATAC®s)

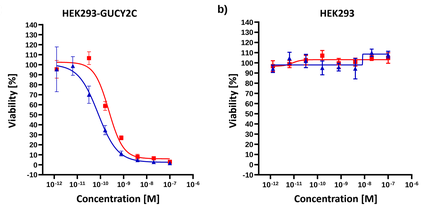


Two GCC-targeting ADCs with amanitin-based payloads were generated by conjugation of an amatoxin derivative with (a) cleavable linker or (b) non-cleavable linker to cysteine-engineered anti-GCC antibodies using maleimide chemistry. This site-specific conjugation strategy resulted in homogenous ATAC®s with Drug-Antibody Ratios (DARs) of 2.0 (Figure 1).

Figure 1: Schematic drawing of the anti-GCC ATAC®s.

2. Cytotoxic activity of anti-GCC ATAC®s on GCC⁺ and GCC⁻ cell lines

The GCC⁺ cell line HEK293-GUCY2C as well as the GCC⁻ cell line HEK293*wt* were used to test the cytotoxic potency of anti-GCC conjugates. Both ATAC®s – with cleavable linker (anti-GCC ATAC® I, red) and with non-cleavable linker (anti-GCC ATAC® II, blue) – showed favorable *in vitro* cytotoxicity with picomolar activity on the GCC⁺ cell line (Figure 2a) and absence of cytotoxic activity on GCC⁻ cells (Figure 2b).



ATAC®	HEK293-GUCY2C EC50 [pM]	HEK293 EC50 [pM]
anti-GCC ATAC® I	78.1	>1000
anti-GCC ATAC® II	228.5	>1000

Figure 2: Cytotoxic activity of anti-GCC ATAC®s on (a) HEK293-GUCY2C and (b) HEK293 cells using BrdU ELISA after incubation for 96h.

3. Efficacy in mouse subcutaneous colon cancer xenograft models

The anti-tumor activity of anti-GCC ATAC®s with cleavable linker and non-cleavable linker was evaluated in mouse HEK293-GUCY2C s.c. xenograft models *in vivo*. Single dose administration of the ATAC® with cleavable linker at low doses resulted in temporarily complete tumor remission (Figure 3a). Similar anti-tumor activity was observed after application of the ATAC® with non-cleavable linker at a dose of 6 mg/kg (Figure 3b).

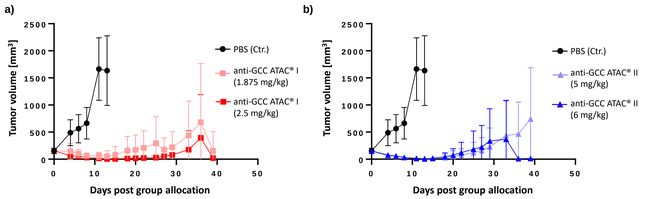


Figure 3: Subcutaneous HEK293-GUCY2C xenograft model. Single dose i.v. application of two different doses of anti-GCC ATAC® with cleavable (a) or non-cleavable linker (b). Shown is mean tumor volume ± SEM.

4. Efficacy in colon cancer PDX models

The efficacy of both anti-GCC ATAC®s after single dose treatment was compared to multiple dosing in two PDX models. Single dose treatment of anti-GCC ATAC® with cleavable linker (anti-GCC ATAC® I; red; MTD: 7.5 mg/kg) and anti-GCC ATAC® with non-cleavable linker (anti-GCC ATAC® II; blue; MTD: 50 mg/kg) at ⅓ MTD resulted in partial tumor remission. Multiple dosing of both ATAC®s at a lower dose of ¼ MTD administered q7dx4 significantly improved the anti-tumor efficacy in both colon cancer PDX models (Figure 4a, b).

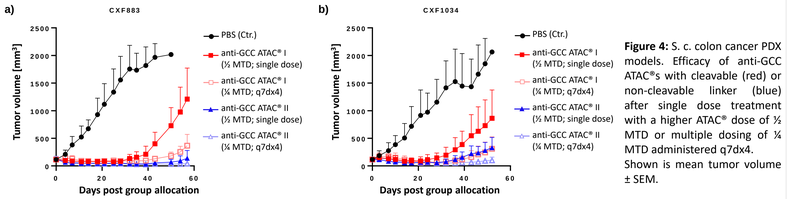
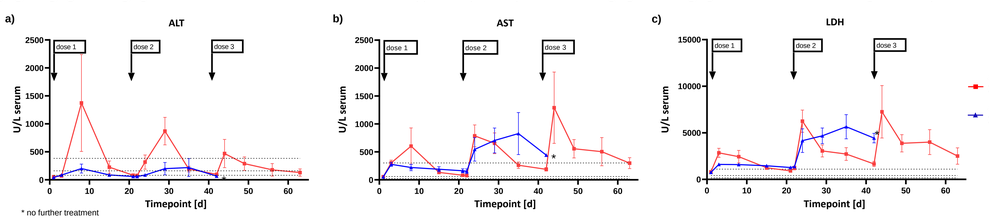


Figure 4: S. c. colon cancer PDX models. Efficacy of anti-GCC ATAC®s with cleavable (red) or non-cleavable linker (blue) after single dose treatment with a higher ATAC® dose of ½ MTD or multiple dosing of ¼ MTD administered q7dx4. Shown is mean tumor volume ± SEM.

5. Tolerability of anti-GCC ATAC®s with cleavable and non-cleavable linker in cynomolgus monkeys

Two anti-GCC ATAC®s with cleavable and non-cleavable linker were assessed for a dose-escalating tolerability study in cynomolgus monkeys. Each group consisted of equal numbers of female and male animals. The ATAC® with cleavable linker was applied sequentially at doses of 1 mg/kg, 2 mg/kg and 3 mg/kg while the ATAC® with non-cleavable linker was applied sequentially at doses of 5 mg/kg and 10 mg/kg. Biochemical and hematological blood parameters were evaluated extensively during the study (Figure 5a-c).



The anti-GCC ATAC® with cleavable linker (anti-GCC ATAC® I; red) was well tolerated up to 2 mg/kg while the anti-GCC ATAC® with non-cleavable linker (anti-GCC ATAC® II; blue) was well tolerated up to 5 mg/kg dose level.
Transient and mild increase in liver-damage-relevant biochemical parameters (ALT, AST and LDH) were observed after application of dose 2 in case of the ATAC® with non-cleavable linker (blue) and after dose 3 of the ATAC® with cleavable linker (red).

Figure 5: (a-c) Selected biochemical serum parameters in cynomolgus monkeys treated with escalating doses of the anti-GCC ATAC® with cleavable linker (red) or non-cleavable linker (blue). Dashed lines reflect the mean, min. and max. values of untreated animals (predose values). Animals treated with dose 3 of the ATAC® with cleavable linker were prematurely terminated because of declining conditions and moribundity.

Conclusion

Guanylyl cyclase C (GCC) is a transmembrane cell surface receptor that functions in the maintenance of intestinal fluid, electrolyte homeostasis, and restriction of cell proliferation. GCC expression is maintained upon neoplastic transformation of intestinal epithelial cells, with expression in >95% of primary and metastatic colorectal tumors and in 60–70% of gastric, esophageal, and pancreatic cancers (4). The tissue-restricted expression and consistent association with GI malignancies make GCC a highly attractive target for ATAC®s.
In the current study, *in vitro* and *in vivo* data of amanitin based ADCs (ATAC®s) targeting GCC with both a cleavable linker and a non-cleavable linker are presented. The promising potential of ATAC®s targeting GCC is reflected by their high cytotoxic potency on GCC⁺ cells and no cytotoxic activity on target-negative cells. Data presented underscores an effective and persistent anti-tumor activity of anti-GCC ATAC®s in mouse models: Single dose treatment with ATAC®s employing different linker designs caused dose-dependent tumor regression including complete tumor remission in HEK293-GUCY2C s.c. xenografts. Even in colorectal cancer PDX models, anti-GCC ATAC®s led to a substantial anti-tumor effect. Multiple dosing improved this anti-tumor efficacy even further without negative impact on tolerability. Safety profiling in cynomolgus monkeys revealed good tolerability and therapeutic index for anti-GCC ATAC®s.
The use of amatoxins and the inhibition of RNA polymerase II as novel mode of action for ADCs not only results in apoptosis of dividing cells, but also of slowly growing and dormant tumor cells. Based on their activity on drug resistant cells, independent of the expression of multi-drug resistance transporters, amatoxins possess the ability to overcome resistance mechanisms as prerequisite for a long-lasting anti-tumor efficacy even in slow proliferating colon cancers. The positive findings of these experiments warrant the clinical development of anti-GCC ATAC®s.

References

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