Treatment with ATAC® based ADCs induces tolerability in preclinical animal models without triggering tolerance

#1761

AACR Annual Meeting 2022

Kristin Decker, Can Araman, Franziska Ebeling, Marisa Schmitt, Anikó Pálfi, Irina Dranova, Andreas Pahl, Torsten Hechler and Michael Kulke
Heidelberg Pharma Research GmbH, Gregor-Mendel-Straße 22, 68526 Ladenburg, Germany

Heidelberg PHARMA
Focused Cancer Therapies

Introduction

With HDP-101[1],the first antibody-drug conjugate (ADC) based on ATAC® technology (short ATAC), is currently being tested in a first-in-human phase I/II clinical trial in multiple myeloma patients. HDP-101 is an ADC that contains an anti-BCMA antibody conjugated to an amanitin variant. ATAC® based ADCs are distinguished from other ADCs as they function via a novel mode of action. While currently approved ADCs (e.g. Enhertu (HER2), Trodelvy (Trop-2) and Blenrep (BCMA)) are based on only a few cytotoxic mechanisms with mainly microtubule- or DNA-targeting toxins as payloads, amanitin, the toxin used in ATACs, specifically inhibits the eukaryotic RNA polymerase II thus stalling the cellular transcription process[2, 3]. Through this novel mode of action, ATACs have several advantages compared to other ADC: Firstly, they also affect non-proliferating and quiescent cells[1]. Secondly, amatoxins are no substrate to known efflux transporters. Thus, ATAC® based ADCs are also efficacious in tumors that are resistant to other lines of therapy[4, 5]. Thirdly, due to the high importance of RNA polymerase II, there is no resistance mechanisms known to amanitin poisoning.
Taken together, ATAC® based ADCs are highly potent ADCs with great potential for cancer treatment. The ongoing clinical trial with HDP-101 will provide further insights into mechanisms and tolerability in humans. One aspect that will be investigated in this first-in-human study is, how multiple dosing affects safety and anti-tumor efficacy. The data presented in this study show that repeated dosing improves the tolerability and anti-tumor efficacy of ATACs in pre-clinical models.

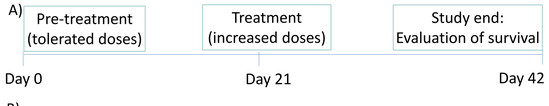
Methods

Antibodies: anti-BCMA, anti-PSMA and anti-DIG antibodies were produced by Heidelberg Pharma Research GmbH (HDP).
Synthesis of ATACs: cysteine-reactive amanitin-linker constructs, synthesized at HDP, were conjugated site-specifically to the antibodies. Drug-antibody ratio (DAR) according to LC-MS analysis was ~2.0 amanitins per IgG.
Mouse tolerability study: female CB17-SCID mice were treated with 10 or 25 mg/kg of an anti-DIG ATAC or PBS as single i.v. dose on day 0. On day 21, all mice were treated with 40 or 50 mg/kg of the same ADC.
Efficacy study in LNCaP prostate xenograft model: 2.5x10^6 LNCaP cells were implanted s.c. into male CB17-SCID mice. Once a mean tumor volume of 97 mm^3 was reached, mice were randomized (n = 15) and were treated i.v. with PBS or an anti-PSMA ATAC either as single dose or in a q7dx4 treatment regiment.
Tolerability studies in Cynomolgus monkeys: studies in Cynomolgus monkeys were performed at LPT or AltaSciences. 2-to-4-year-old monkeys were treated with HDP-101 via a 30 min i.v. infusion as single dose, in a multiple dose or escalating dose treatment scheme. Blood was collected pre-dose, 3, 7, 14 and 21 days after the treatment. LDH, AST and ALT levels in the serum were measured using the methods recommended by the IFCC.

Results

1. Repeated treatment improves tolerability of a non-targeting ATAC® based ADC in mice

The impact of repeated dosing on the tolerability of ATAC® based ADCs was investigated in immuno-compromised mice. Previously, an anti-DIG ATAC was found to be tolerated up to 30 mg/kg as single dose intravenous (i.v.) treatment in naïve mice. Therefore, mice were pre-treated with a tolerated dose of 10 or 25 mg/kg of the anti-DIG ADC followed by a second higher dose of 40 or 50mg/kg 21 days later. Survival of the mice and macroscopic liver changes were analyzed (Figure 1).



B)

Treatment Day 21 [mg/kg]	Pre-treatment PBS		Pre-treatment ATAC 10 mg/kg	
	Macroscopic findings in liver	Survival	Macroscopic findings in liver	Survival
40	1/3 (marbeled)	0/3	p = 0.11 0/2‘	2/3

Treatment Day 21 [mg/kg]	Pre-treatment PBS		Pre-treatment ATAC 25 mg/kg	
	Macroscopic findings in liver	Survival	Macroscopic findings in liver	Survival
40	4/5 (enlarged; dark colored)	1/5	p = 0.07 0/5	4/5
50	2/2‘ (swollen)	0/5	p= 0.05 1/4‘ (dark colored)	3/5

Figure 1: *Pre-treatment with a tolerated dose of a non-targeting anti-DIG ATAC improves the survival of mice upon application of higher ADC doses.*
A) Experimental study design of a tolerability study in CB17-SCID mice with and without ATAC pre-treatment.
B) Two independent experiments with 3 (upper panel) or 5 (lower panel) mice/group were performed using different doses of the anti-DIG ADC. Number of mice with macroscopic changes in the liver as well as survival is depicted. ‘: due to autolytic state, not all mice could be examined for liver abnormalities. p-value: Gehan-Breslow-Wilcoxon-test;

Pre-treatment with a tolerated dose of an anti-DIG ATAC improves the tolerability of a higher dose of the same conjugate that is toxic if applied at single dose in naïve animals and leads to less macroscopic liver abnormalities and improved survival.

2. Liver toxicity is reduced after multiple dose treatment with an ATAC® based ADC compared to single dose in Cynomolgus monkeys

Non-human primates are the most relevant species for toxicity testing of ATAC® based ADCs as accepted by US and European authorities. Therefore, toxicity was assessed in Cynomolgus monkeys. Interestingly, doses that are tolerated as single dose application did not become toxic even if applied several times, suggesting that tolerability may also be induced in Cynomolgus.

This hypothesis was investigated by analyzing the liver damage markers AST, ALT and LDH in monkey serum after administration of HDP-101 as these markers correlate with ATAC® toxicity. Serum levels of liver damage markers were compared after single dose and multiple dose treatment (Figure 2).

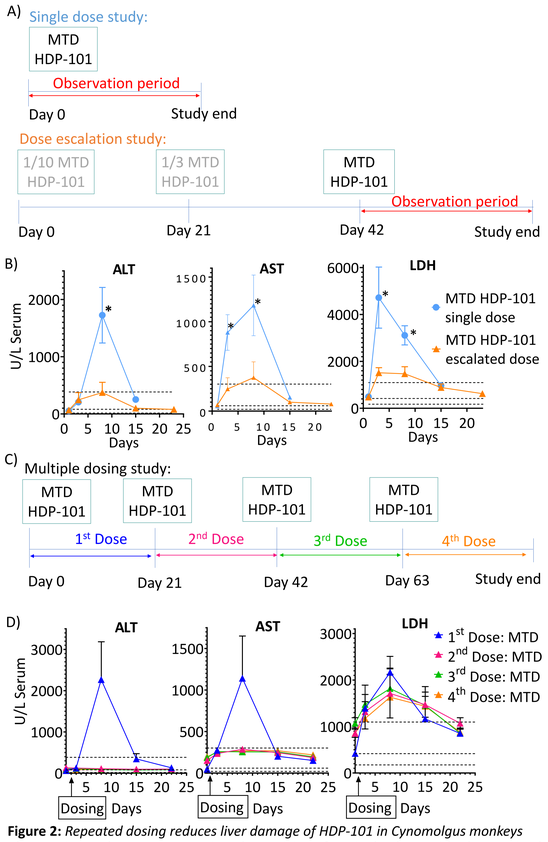


Figure 2: *Repeated dosing reduces liver damage of HDP-101 in Cynomolgus monkeys*
A) Experimental set-up comparing the toxicity of a single dose with the maximal tolerated dose (MTD) of HDP-101 with the toxicity occurring after the same dose was given as final step of a dose escalation scheme.
B) AST, ALT and LDH levels in the serum of Cynomolgus monkeys following i.v. treatment with the MTD of HDP-101 either given as single dose (blue) or as final step of a dose escalation scheme (orange). Values after application of 1/10 and 1/3 MTD doses are not shown. Dotted lines: baseline levels in untreated animals. Mean of three biological replicates with SEM is shown. p-value: Welch´s t-test; *: p ≤ 0.05
C) Experimental set-up comparing toxicity of the MTD of HDP-101 given as different rounds of treatment.
D) AST, ALT and LDH levels in the serum of Cynomolgus monkeys following i.v. treatment with the MTD of HDP-101 given as first (blue), second (pink), third (green) or fourth (orange) treatment round. Mean of three biological replicates with SEM is shown.

Treatment with HDP-101 leads to reduced levels of the liver damage markers ALT, AST and LDH in Cynomolgus monkeys if applied either following a dose escalation treatment or as second, third or fourth treatment round as compared to single and first treatment, respectively. These data indicate that multiple dose treatment increases the tolerability of ATAC® based ADCs also in Cynomolgus monkeys.

3. Multiple dosing increases the anti-tumor efficacy of ATAC® based ADCs

Having demonstrated that repeated treatment improves the tolerability of ATAC® based ADCs in mice and Cynomolgus, it was investigated whether multiple dosing affects the anti-tumor efficacy of ATACs.
Mice bearing subcutaneous (s.c.) human prostate adenocarcinoma LNCaP tumors were treated with a single dose or repeated doses (q7dx4) of an anti-PSMA ATAC. Tumor growth and survival was analyzed (Figure 3).

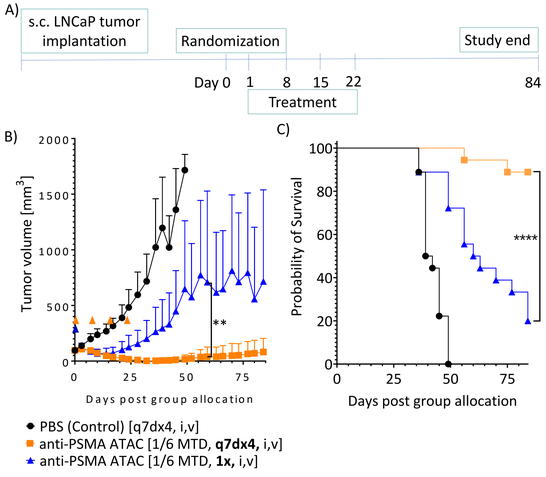


Figure 3: *Repeated dosing with an anti-PSMA ATAC improves the anti-tumor efficacy in a s.c. LNCaP CDX model*
A) Experimental set-up of an *in vivo* efficacy study in a s.c. human prostate adenocarcinoma LNCaP CDX model comparing single dose with multiple dose (q7dx4) treatment of an anti-PSMA ADC based on ATAC® technology .
B) Tumor growth of s.c. LNCaP tumors was measured by caliper for 84 days (n = 18 animals/group). Single dose treatment is shown in blue, multiple dose treatment in orange. Means with SD are shown. p-value: Welch t-test with multiple testing correction by Holm-Sídák Method; statistically significant from day 21 to 56. ** p < 0.01
C) Survival probability of mice carrying LNCaP s.c. tumors and either treated with PBS, a single dose (blue) or multiple doses (orange) of the anti-PSMA ATAC. p-value: Gehan-Breslow-Wilcoxon-test; ****: p < 0.001

Multiple dosing (q7dx4) improves the anti-tumor effect of an anti-PSMA ATAC in a s.c. human prostate adenocarcinoma LNCaP CDX model significantly as compared to single dose treatment as demonstrated by reduced tumor growth and improved survival of the mice.

Conclusion

ATAC® based ADCs are a novel and promising class of ADCs that are currently being tested in a first-in-human phase I/II clinical trial. Patients are being treated with HDP-101 in several treatment rounds consisting of i.v. drug administration on day 0, followed by a 21-days washout phase[6].
This clinical trial will provide valuable insights into the impact of repeated dosing on the tolerability of HDP-101 in humans. Two scenarios are possible: (1) repeated dosing can lead to accumulative toxicity, in which case the side effects will increase with each treatment round. (2) the liver can adapt to the treatment e.g., by upregulation of regenerative processes leading to induced tolerability and thus reduced side effects with each treatment round.
The pre-clinical investigations, presented in this study, suggest that repeated treatment leads to improved tolerability of ATAC® based ADCs as shown by improved survival in mice as well as by reduced liver damage in Cynomolgus monkeys. As these results were obtained in different animal species it is likely that it is a general effect that might be reproducible in humans.
At the same time, repeated dosing increased the anti-tumor efficacy of an ATAC® based ADC in a prostate cancer CDX model in mice.
This combination of reduced toxicity and improved efficacy upon repeated dosing holds great potential for the treatment with ATAC® based ADCs as it suggests improvement of the therapeutic index through repeated dosing. If these results translate into humans, it might impact the treatment regimen and significantly enhance the clinical success of ATAC® based ADCs.

References

(1) Figueroa-Vazquez V *et al.;* Mol Cancer Ther 2021; 20:367–78
(2) Anderl J *et al.;* Beilstein J Org Chem. 2012; 8:2072-84
(3) Moldenhauer G *et al.* J Natl Cancer Inst. 2012; 104(8):622-34
(4) Hechler T, Kulke M, Anderl J; Future Medicine; 2015
(5) Kume *et al.*; *Scientific Reports 2016;* ***Vol. 6****, 25895*
(6) Clinical study NCT04879043

Contact

Heidelberg Pharma Research GmbH
Gregor-Mendel-Straße 22
68526 Ladenburg
Germany

Phone: +49-6203-1009 0
Email: info@hdpharma.com
https://www.heidelberg-pharma.com