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 immunocompetent mice. Pretreatment of tumor xenografts with AMAN tin, an alkaloid isolated from a non-targeting ATAC® based ADCs was found to be tolerated to up to 30 mg/kg as single dose intravenous (i.v.) treatment in naive mice. Therefore, mice were pre-treated with doses of 10 or 25 mg/kg of the anti-DSG ADC followed by a second higher dose of 40 or 50 mg/kg 21 days later. Survival of the mice and macroscopic liver changes were analyzed (Figure 1).

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

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 are evaluated with ATAC® toxicity. Serum levels of liver damage markers were compared after single dose and multiple dose treatment (Figure 2).

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

Having demonstrated that repeated treatment improves the tolerability of ATPA® in mice and Cynomolgus, it was investigated whether multiple dosing would also increase the anti-tumor activity of HDP-101. Mice bearing subcutaneous (s.c.) human prostate adenocarcinoma LNCaP xenografts were treated with ATAC® ((1/6 MTD, 1x, i.v.) of an anti-PSMA ATAC. Tumor growth and survival was analyzed (Figure 3).

**RESULTS**

**METHODS**

**Antibodies:** anti-PSMA, ATAC® and anti-PSMA and anti-DIG antibodies were produced by Heidelberg Pharma AG.

**Synthesis of ATACs:** cytokine-active eukaryotic linker constructs, synthesized at HDP, were conjugated stepwise to the appropriate ADCs.

**Dosing schedule:** Once-daily dosing (q1d) or twice-daily dosing (q2d) based on prior PK studies. Doses were not administered on day 2.

**Efficacy study in LNCaP prostate xenograft model:** Male C3H.SD mice were inoculated s.c. with LNCaP cells (50,000 cells/mouse). Treatment started at the same day as tumor inoculation. At day 14 all animals were treated with a single dose of 15 mg/kg. Tumor growth was measured 7 days after treatment.

**Macroscopic liver changes:** Following single and multiple dose administration, liver tissue was collected after sacrifice and subjected to macroscopic analysis. Liver coloration was used to assess liver damage.

**Histology:** Liver tissue was collected after sacrifice and subjected to histology and immunohistochemistry. Tissue sections were stained with H/E, Sirius red and specific antibodies against e.g. Fibulin 5 and Factor VIII. Images were analyzed using the software Adobe Photoshop.

**Tumor burden:** Tumor burden was assessed by serial caliper measurement. The tumor volume was calculated with the following equation: Volume = 4/3 * pi * (d1 * d2 * d3)/3, where d1, d2, d3 are the diameters of the tumor in millimeters.

**Conclusion:** ATAC® based ADCs are novel and promising class of ADCs that are currently entering clinical development in humans (I/II clinical trial). Patients are being treated with HDP-101 in several treatment regimens consisting of single drug administration (a day 1 bolus followed by a 21-day washout phase).

This initial 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 changes the tolerability profile of a drug; changes in device side effects will increase with each treatment round. (2) the liver can be treated by the treatment, e.g. by improving the regenerative processes leading to increased tolerability and thus a reduced need for drug administration. Mice bearing subcutaneous tumors were used as experimental models. The pre-clinical investigations, presented in this study, suggest that repeated treatment leads to improved tolerability of anti-PSMA ADCs by improving liver toxicity and survival in mice as well as by reduced liver damage in Cynomolgus monkeys. Currently, there is no good animal model for different animal species it is likely that it is a general effect that might be reproducible in humans. At the same time, repeated dosing the increased the anti-tumor efficacy of an ATAC® based ADC in a prostate cancer xenograft model in mice.

This combination of reduced toxicity and improved efficacy upon repeated dosing provides strong evidence for the treatment with ATAC® based ADCs as it suggests improving the therapeutic index through repeated dosing. If these results can be translated to humans, it might impact the treatment regimen and significantly enhance the clinical success of ATAC® based ADCs.