Building an ADC Platform with Amanitin as a New Payload and a Novel Mode of Action

Kristin Decker, PhD | ADC Toxicity Summit | July 27th 2023
Safe Harbor

Forward looking statements

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Heidelberg Pharma – a Clinical Stage Company

Our Company

- 113 employees
- Headquarters in Heidelberg area, Germany
- Listed on Frankfurt Stock Exchange: HPHA
- Clinical stage biotech
  Complete in-house research capabilities
- Cash reach until mid-2025 (as of July 2023)

Our Mission

- Provide new options in cancer therapy
- Overcome resistance mechanisms
- Kill dormant tumor cells
- Develop biomarker for patient stratification

Our Approach

- Inhibition of RNA Polymerase II
- Targeted delivery via antibodies (ADC technology)
  Use Amanitin as toxic payload (ATAC technology)
ATACs\textsuperscript{1}: ADCs with Amanitin as a Payload

Amanitin as Warhead
- Differentiated mechanism of action: \textit{inhibition of RNA Polymerase II}
  - Kills dormant tumor cells
  - Overcomes resistance
  - Predictive biomarker
- Synthetic amanitin derivatives with improved properties
- GMP manufacturing through fully synthetic process

Antibody
- Targeting tumor antigen

Site-specific Conjugation
- Proprietary conjugation sites
- Excellent stability in circulation
- Drug-Antibody Ratio (DAR) = 2.0

\textsuperscript{1}ATAC\textsuperscript{®} is a registered trademark of Heidelberg Pharma Research GmbH in the EU and USA.
## ATACs Promise Significant Clinical Benefits

### Unique preclinical features of ATACs
- Efficacious against dormant tumor cells
- Efficacious in ultra-low target-expressing tumor cells
- Novel MoA to which all patients will be naïve
- Enhanced efficacy in high-risk del(17p) tumors
- Ocular toxicity not seen for Amanitin or ATACs

### Potential clinical benefit
- Longer PFS
- Deeper responses and higher ORR
- Overcome resistance
- Breakthrough designation and accelerated approval
- Superior safety profile

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**ATACs have best-in-class potential**
Amatoxins – A Unique Class of Natural Toxins

• Group of toxins from the poisonous green death cap (Amanita phalloides)
• Bicyclic octapeptide structure
• Exceptionally stable & unique biophysical properties

The unique biophysical properties of amatoxins impact the toxicity mechanisms of ATACs

Drawing: Tamara Clark; tamaraclark.com

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Adapted from http://chemistry.elmhurst.edu/vchembook/655cancer2.html
Toxicity Mechanism of \( \alpha \)-amanitin in Humans

Upon mushroom intoxication \( \alpha \)-amanitin leads to hepatotoxicity by specific uptake of the toxin into hepatocytes via the OATP1B3 transporter.

After: M. Aylin Arici, Yesim Tuncok, Chapter 43; Toxicology, Academic Press, 2021

https://doi.org/10.1016/B978-0-12-819092-0.00044-3
## α-amanitin and Amanitin-based ADCs Have Very Similar Clinical Manifestations

Amanitin and Amanitin-based ADCs have very similar clinical manifestations. Here are some of the clinical symptoms after mushroom poisoning:

- Yellow; of creamy consistency
- Hemorrhagic foci
- Edematous hepatic stroma
- Centrilobular hepatic necrosis
- Vacuolar degeneration of hepatocytes
- Kidney
  - Multiple hemorrhages
  - Necrosis of convoluted tubules
  - Hyaline tubular casts
  - Hydropic protein dystrophy in epithelium

### Macroscopic and microscopic findings in patients after intoxication with amanitin:

<table>
<thead>
<tr>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow; of creamy consistency</td>
<td>Multiple hemorrhages</td>
</tr>
<tr>
<td>Hemorrhagic foci</td>
<td>Necrosis of convoluted tubules</td>
</tr>
<tr>
<td>Edematous hepatic stroma</td>
<td>Hyaline tubular casts</td>
</tr>
<tr>
<td>Centrilobular hepatic necrosis</td>
<td>Hydropic protein dystrophy in epithelium</td>
</tr>
<tr>
<td>Vacuolar degeneration of hepatocytes</td>
<td></td>
</tr>
</tbody>
</table>

Eventually off-target toxicity of ATACs is caused by amatoxins.

I.B. Il’in et al. / Sud Med Ekspert. 2019; single-case report
J. Garcia et al. / Food and Chemical Toxicology, 2015
How is Liver Toxicity Caused by ATACs?

Three potential uptake mechanisms:
1. Uptake of ATACs via the OATP1B3 transporter
2. Release of the free payload in circulation
3. Unspecific uptake of ATACs via yet unknown mechanisms

Adapted from: https://doi.org/10.3390/toxins13060417

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1. Uptake of ATACs via the OATP1B3 Transporter?

Cytotoxicity of Amanitin and ATACs on OAT1B3 expressing HEK cells compared to wt HEK cells:

Tolerability of Amanitin and ATACs in Oatp1a/b KO mice:

<table>
<thead>
<tr>
<th>Test item</th>
<th>MTD [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In wild type mice</td>
</tr>
<tr>
<td>α-Amanitin</td>
<td>0.3</td>
</tr>
<tr>
<td>Active metabolite 1</td>
<td>1.25</td>
</tr>
<tr>
<td>HDP-101</td>
<td>6</td>
</tr>
</tbody>
</table>

Off target toxicity of ATACs is not mediated by uptake of the ADC via the OATP1B3 transporter
2. Release of the Free Payload in Circulation?

ADC vs total antibody in Cynomolgus monkeys after a single i.v. ATAC dose of 3 mg/kg:

DAR analysis in serum of mice:

Amanitin-based ADCs are stable in circulation
- No significant release of amatoxins in serum

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2. Release of the Free Payload in Circulation?

Time course of ADC in mice after a single i.v. dose:

**Serum**

- Administration of DIG-ATAC 1

**Liver (perfused)**

Quantification of the DAR species in mice 24h after a single i.v. dose DIG-ATAC 1:

ATACs seem to be taken up into liver cells and be deconjugated.

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2. Release of the Free Payload in Circulation?

The kinetics of amatoxin uptake into liver cells is different after ATAC and amatoxin administration.
How is Liver Toxicity Caused by ATACs?

Liver toxicity of amanitin-based ADCs is caused by unspecific uptake of the ATAC into liver cells.

Thus, liver toxicity is determined by the amount of ADC (1) that is taken up into liver cells and ADC backbone (2) that reaches the liver over time. PK profile.

Adapted from: https://doi.org/10.3390/toxins13060417

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Liver Toxicity is Determined by the Amount of ADC that is Taken Up by Liver Cells

Known unspecific uptake mechanisms of ADCs:

- Non-specific endocytosis
- Receptor-mediated endocytosis
- Deconjugation
- Bystander effect

Binding of an ATAC to FcγR:

Amanitin-based ADCs bind to FcγR
- can the tolerability be increased by reducing FcγR binding?

Adapted from https://doi.org/10.1016/j.pharmthera.2019.04.008
Liver Toxicity is Determined by the Amount of ADC that is Taken Up by Liver Cells

Binding of the antibody to the FcγR is mediated by Leu234 and Leu235 in the FC part

→ Mutations at these positions reduce the binding

<table>
<thead>
<tr>
<th>ADC</th>
<th>DAR</th>
<th>( K_D ) [nM]</th>
<th>( \frac{K_D}{K_{D, LALA}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-ATAC 2</td>
<td>1.96</td>
<td>1.53 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>HER2-LALA-ATAC 2</td>
<td>2.02</td>
<td>2950 ± 160</td>
<td>~2000</td>
</tr>
</tbody>
</table>

Detection of a HER2-ATAC +/- LALA mutation in murine livers after i.v. administration:

- LALA
+LALA

Introduction of a LALA mutation reduces the FcγR binding of amanitin-based ADCs
Liver Toxicity is Determined by the Amount of ADC that is Taken Up by Liver Cells

T tolerability of ATACs in mice dependent on the antibody backbone:

<table>
<thead>
<tr>
<th>Test item</th>
<th>MTD [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-LALA</td>
</tr>
<tr>
<td>HER2-ATAC 2</td>
<td>8</td>
</tr>
<tr>
<td>PSMA-ATAC 2</td>
<td>4</td>
</tr>
<tr>
<td>PSMA-ATAC 1</td>
<td>10</td>
</tr>
</tbody>
</table>

Introduction of a LALA mutation improves the tolerability while maintaining the efficacy.
- The LALA mutation improves the TW of ATACs

Anti-tumor efficacy in a HER2+ JIMT-1 model:

Liver Toxicity is Determined by the Amount of ADC that is Taken Up by Liver Cells
How is Liver Toxicity Caused by ATACs®?

Liver toxicity of amanitin-based ADCs is caused by unspecific uptake of the ATAC into liver cells.

Thus, liver toxicity is determined by the amount of ADC:
1. that is taken up into liver cells
   → ADC backbone
2. that reaches the liver over time
   → PK profile

Adapted from: https://doi.org/10.3390/toxins13060417
Optimizing the PK Profile to Reduce the Amount of ATAC that Reaches the Liver

How can the PK profile be changed (reduced Cmax; maintained AUC) without changing the molecular structure of the ADC?

1. Change the treatment schedule: dose fractionation instead of single dose
2. Change the administration route: subcutaneous dosing instead of intravenous

Working hypothesis:
- Cmax drives tolerability
- AUC drives efficacy

How can the PK profile be changed (reduced Cmax; maintained AUC) without changing the molecular structure of the ADC?

ADC Concentration in serum after i.v. administration

C_{max} = \text{max ADC conc. reached immediately after injection}

AUC = \text{area under the curve}
Subcutaneous Dosing Refines the PK Parameters of HDP-103 in Mice and Cynomolgus Monkeys

Serum PK of HDP-103 in mouse and Cynomolgus monkeys after single i.v. or s.c. administration of HDP-103:

<table>
<thead>
<tr>
<th>Test Item</th>
<th>mouse</th>
<th>monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route</td>
<td>s.c.</td>
<td>i.v.</td>
</tr>
<tr>
<td>Half-Life</td>
<td>12.5</td>
<td>9.5</td>
</tr>
<tr>
<td>Cmax_D</td>
<td>13.0</td>
<td>12.8</td>
</tr>
<tr>
<td>AUCINF_D_obs</td>
<td>210</td>
<td>216</td>
</tr>
</tbody>
</table>

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Subcutaneous Dosing Refines the PK Parameters of HDP-103 in Mice and Cynomolgus Monkeys

Tolerability of HDP-103 in Cynomolgus monkeys:

<table>
<thead>
<tr>
<th>Test item</th>
<th>Route</th>
<th>MTD [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDP-103</td>
<td>i.v.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Therapeutic window of HDP-103:

\[ TW = \frac{MTD_{\text{monkey}}}{MED_{\text{mouse}}_{\text{converted}}} \]

<table>
<thead>
<tr>
<th>Test item</th>
<th>Route</th>
<th>TW</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDP-103</td>
<td>i.v.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>12</td>
</tr>
</tbody>
</table>

Efficacy of HDP-103 after s.c. or i.v. administration of the same dose in a prostate cancer mouse CDX model:

Subcutaneous dosing improves the tolerability while maintaining the efficacy - S.c. dosing improves the TW of ATACs

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Conclusion

• Although the toxicity of α-amanitin and amanitin-based ADCs is eventually caused by the amatoxin in hepatocytes, the molecular mechanism leading to the uptake into liver cells is distinct.

• While α-amanitin is actively transported into hepatocytes via the OATP1B3 transporter, amanitin-based ADCs are unspecifically taken up into liver cells.

In general, the TW of an ADC can be increased by:
• improving efficacy while maintaining tolerability
• improving tolerability while maintaining efficacy

The knowledge about the toxicity mechanism of ATACs can be used to improve the TW of Amanitin-based ADCs:

<table>
<thead>
<tr>
<th>Improvements</th>
<th>Tolerability</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>LALA mutation</td>
<td>Improved by reduced uptake of ATACs into liver cells via the FcγR</td>
<td>Maintained</td>
</tr>
<tr>
<td>Subcutaneous dosing</td>
<td>Improved by reduction of Cmax levels</td>
<td>Maintained</td>
</tr>
</tbody>
</table>

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Thank you for your attention!

Our collaborators:

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