Discovery and first structural disclosure of AZD5305, a next generation, highly selective PARP1 inhibitor and trapper

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Disclosure Statement

- Jeffrey Johannes and Elisabetta Leo are employees and shareholders of AstraZeneca
Efficacious monotherapy PARP inhibitors trap PARP1 and PARP2 on DNA single strand breaks and selectively kill cancer cells.
First generation PARP inhibitors act on PARP1 and PARP2

<table>
<thead>
<tr>
<th>Company</th>
<th>Olaparib</th>
<th>Veliparib</th>
<th>Rucaparib</th>
<th>Niraparib</th>
<th>Talazoparib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase</td>
<td>Approved</td>
<td>III</td>
<td>Approved</td>
<td>Approved</td>
<td>Approved</td>
</tr>
<tr>
<td>PARP1 binding IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>0.007</td>
<td>0.013</td>
<td>0.007</td>
<td>0.035</td>
<td>0.009</td>
</tr>
<tr>
<td>PARP2 binding IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>0.006</td>
<td>0.225</td>
<td>0.172</td>
<td>1.8</td>
<td>0.030</td>
</tr>
<tr>
<td>Fold Selectivity PARP1/2</td>
<td>1</td>
<td>18</td>
<td>25</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>PARP3/5a/6 IC&lt;sub&gt;50&lt;/sub&gt; (µM)</td>
<td>0.2/70/1.8</td>
<td>1/&gt;100/&gt;100</td>
<td>0.5/37/&gt;100</td>
<td>&gt;39/&gt;40/&gt;99</td>
<td>0.2/1.9/1.1</td>
</tr>
<tr>
<td>Sec Pharm (# &lt;30 µM/total)</td>
<td>1/93</td>
<td>8/95</td>
<td>29/94</td>
<td>30/94</td>
<td>2/93</td>
</tr>
<tr>
<td>Response PhII Ovarian</td>
<td>45% (23/50)</td>
<td>5% (1/20) (non-trapper)</td>
<td>45%</td>
<td>40% (8/20)</td>
<td>44% (11/25)</td>
</tr>
<tr>
<td>%Anemia ≥Gr3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>16-19</td>
<td>-</td>
<td>19</td>
<td>25</td>
<td>39</td>
</tr>
<tr>
<td>%Neutropenia ≥Gr3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>5-9</td>
<td>-</td>
<td>7</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>%Thrombocp ≥Gr3&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0</td>
<td>-</td>
<td>5</td>
<td>34</td>
<td>15</td>
</tr>
</tbody>
</table>

The opportunity and challenge of a PARP1 selective inhibitor

Opportunity

1. First generation PARPi are generally dual PARP1-PARP2 inhibitors, while some have additional secondary pharmacology hits.

2. Only PARP1 trapping is required for synthetic lethality in homologous recombination repair deficient (HRD) settings.*

3. PARP2 has been linked to hematological effects,† the main clinical adverse events observed with first generation PARPi.

4. A selective PARP1 inhibitor and DNA trapper may improve the therapeutic index vs first generation PARPi.

Challenge

• PARP1 and PARP2 are highly homologous around the NAD+ cofactor binding site.

† Farrés, J. et al., Blood 2013, 122 (1), 44–54.
Identification of a PARP1 selective series

<table>
<thead>
<tr>
<th>FR257516</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP1 binding IC50 (µM)</td>
<td>0.065</td>
</tr>
<tr>
<td>PARP2 binding IC50 (µM)</td>
<td>20</td>
</tr>
<tr>
<td>PARP3,5a,6 IC50 (µM)</td>
<td>&gt;97, &gt;100, &gt;100</td>
</tr>
<tr>
<td>hERG IC50 (µM)</td>
<td>2.6</td>
</tr>
<tr>
<td>LogD pH 7.4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Screen of known PARP inhibitors for PARP1 vs PARP2 selectivity

Confirmed FR257516 as a highly selective PARP1 binder
Nicotinamide mimetic core optimization

Structure of the nicotinamide mimetic core has a profound impact on PARP1 binding, BRCAm cell potency, hERG, and logD.

<table>
<thead>
<tr>
<th></th>
<th>PARP1 IC50 (µM)</th>
<th>PARP2 IC50 (µM)</th>
<th>BRCAm cell IC50 (µM)</th>
<th>hERG IC50 (µM)</th>
<th>Solubility pH 7.4 (µM)</th>
<th>LogD pH 7.4</th>
<th>Hu Mics (µl/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.009</td>
<td>4</td>
<td>&gt;24</td>
<td>1.6</td>
<td>4</td>
<td>2.3</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>0.009</td>
<td>0.595</td>
<td>0.018</td>
<td>3.9</td>
<td>&lt;12</td>
<td>3.3</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>0.030</td>
<td>9.1</td>
<td>0.018</td>
<td>11</td>
<td>15</td>
<td>2.5</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>0.051</td>
<td>1.1</td>
<td>0.033</td>
<td>30</td>
<td>136</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>0.029</td>
<td>4.2</td>
<td>0.005</td>
<td>12</td>
<td>71</td>
<td>2.7</td>
<td>25</td>
</tr>
</tbody>
</table>

Cores:
Keep Imidazole, Vary Bicyclic Core

Compound 5 (cyan) bound to PARP1 (blue)
Overlay with 1 (magenta) in PARP1
Optimization of the aryl group on piperazine

Pyridine carboxamide group improved the issue of hERG while maintaining selectivity and BRCAm cell potency
Aza core delivers AZD5305

In vivo DMPK data for AZD5305

<table>
<thead>
<tr>
<th></th>
<th>Mouse</th>
<th>Rat</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (mL/min/kg)</td>
<td>0.23</td>
<td>1.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Vss (L/kg)</td>
<td>0.17</td>
<td>0.38</td>
<td>0.3</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>8</td>
<td>4.6</td>
<td>10</td>
</tr>
<tr>
<td>Oral Bioavailability (%)</td>
<td>77</td>
<td>132</td>
<td>73</td>
</tr>
</tbody>
</table>

In vitro DMPK data for AZD5305

<table>
<thead>
<tr>
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<th>Mouse</th>
<th>Rat</th>
<th>Dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP inhibition (µM)</td>
<td>All &gt;30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4, 2D6, 2C9, 2C19, 1A2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP Time Dependent Inhibition (TDI)</td>
<td>&lt;20 % ihib. @ 50 µM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

More details in #296
PARylation: AZD5305 selectively inhibits PARP1 in cells

PARylation inhibition is most proximal biomarker for PARPi

<table>
<thead>
<tr>
<th>PARylation contribution</th>
<th>WT</th>
<th>PARP1</th>
<th>PARP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARP1-KO</td>
<td>/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PARP2-KO</td>
<td>PARP1</td>
<td>/</td>
<td></td>
</tr>
</tbody>
</table>

Representative PARylation profiles of clinical PARPi

Olaparib
in A549 isogenic for PARP1 or PARP2

Talazoparib
in A549 isogenic for PARP1 or PARP2

AZD5305 PARylation
in A549 isogenic for PARP1 or PARP2

More details in #1272
Trapping: AZD5305 selectively traps PARP1 onto the chromatin

Representative trapping profiles of clinical PARPi

High throughput; quantitative trapping assay

Seed cells in 96 well plate
Drug treatments
In-Situ Extraction
Fixation and IF staining

AZD5305

Olaparib
Veliparib

More details in #1272
PARP inhibitors are synthetic lethal with homologous recombination repair deficient (HRD) cancers (e.g. BRCAm)

AZD5305 retains and expands synthetic lethality with HRD

AZD5305 in DLD1 isogenic cell line pair

More details in #1272

<table>
<thead>
<tr>
<th>PARPi</th>
<th>BRCA2&lt;sup&gt;-/-&lt;/sup&gt; GI&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>WT GI&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Δ Log Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD5305</td>
<td>0.4</td>
<td>33000</td>
<td>5</td>
</tr>
<tr>
<td>Talazoparib</td>
<td>0.5</td>
<td>25</td>
<td>1.7</td>
</tr>
<tr>
<td>Olaparib</td>
<td>11</td>
<td>22800</td>
<td>3.32</td>
</tr>
<tr>
<td>Rucaparib</td>
<td>30</td>
<td>13000</td>
<td>2.64</td>
</tr>
<tr>
<td>Niraparib</td>
<td>43</td>
<td>5800</td>
<td>2.13</td>
</tr>
<tr>
<td>Veliparib</td>
<td>500</td>
<td>&gt;10000</td>
<td>/</td>
</tr>
</tbody>
</table>

Mean of ≥4 independent experiments

Selectivity window

More details in #1272
AZD5305 is highly efficacious and has durable responses in vivo

After cessation of treatments, tumor regrowth was not observed in animals that received effective doses of AZD5305.

More details in #1270
No haematological toxicity observed with AZD5305 treatments in vivo

Rats were tested at matched exposure of PARPi

Neutrophils and platelets were also unaffected with AZD5305

More details in #1374
AZD5305 carboplatin combination opportunity

**Efficacy in HBCx-9 TNBC PDX**
(methylated BRCA1)

- **Vehicle**
- **Carboplatin 50mg/kg QW**
- **AZD5305 1mg/kg QD**
- **Combination**

- **88% TGI *****
- **72% TGI *****
- **87% reg *****

Rat bone marrow cellularity (d15)

- **olaparib**
- **AZD5305**

More details in #1374 and #1270
Summary of AZD5305:

• Potent, **selective inhibitor** and PARP1-DNA trapper.
• **Excellent** secondary pharmacology, physicochemical properties and oral bioavailability.
• Synthetic lethality observed with defects in **DNA repair pathways**.
• **Minimal effects** on **haematopoiesis** in pre-clinical studies at predicted clinically efficacious doses.
• **More potent and efficacious** vs first generation PARP inhibitors, with greater **depth** and **duration** of effect in xenografts and PDX models.
• **Next generation PARP inhibitor** with multiple clinical options as monotherapy and in combination.
• **AZD5305 is in Phase 1 clinical trials**
Acknowledgments

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