Nonclinical Evaluation of Rucaparib in Tumors with Mutations in Non-$BRCA1/2$ Homologous Recombination Repair (HRR) Genes

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Abstract 1260

Supplementary Material
Methods

- **siRNA transfection and RNA analysis:** Cells were seeded at 1–2×10^6 cells/10 cm dish, and transfected the next day with siRNA pools using DharmaFECT reagent (Thermo Fisher) following the manufacturer’s instructions. A minimum of 3 replicates/transfections per gene was performed. Cells transfected with a non-targeting siRNA were used as a control. Cells were collected 24 hours later and used for either a 6-day cell viability assay or immediate RNA analysis. RNA was extracted using the PureLink RNA kit (Invitrogen), and cDNA was generated using SuperScript III Super Mix (Invitrogen) following the manufacturer’s instructions. Quantitative Real-Time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) reactions were run using Taqman Universal Master Mix II (Applied Biosystems) and performed on the ViiA 7 PCR System (Applied Biosystems). RNA expression was knocked down on average by 80% (61–96% loss when compared to control). Statistical analysis was performed using two-sided t-test, and P<0.05 was considered significant.

- **Tumor efficacy studies:** PDX tumor fragments were implanted subcutaneously in the right flank of immunocompromised female mice, and dosing of 50 or 150 mg/kg rucaparib BID PO began when an average tumor volume of 106–234 mm^3 was reached. Body weights and tumor volume were measured twice weekly, and tumor growth inhibition (TGI) was calculated as \[1-(V_t-V_i)/(V_c-V_i)]\times100.\] Percent body weight loss (BWL) was calculated as \[1-(BWL_t/BWL_c)]\times100,\] where BWL_c and BWL_t are the BWL of control and treated groups at the time point. Statistical analysis was performed using two-sided t-test, and P<0.05 was considered significant.

- **Genomic analysis:** DNA mutation analysis was performed using HRD Plus testing (Myriad). Methylation analysis was performed using the pyrosequencing method as per manufacturer’s protocol (Qiagen).
## Summary of Rucaparib Tumor Efficacy in PDX Tumors and Their BRCA1/2 Mutations

<table>
<thead>
<tr>
<th>Model</th>
<th>Cancer type</th>
<th>Genomic alteration</th>
<th>Biallelic</th>
<th>Dose (mg/kg)</th>
<th>TGI (%)</th>
<th>P-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL3325</td>
<td>Bladder</td>
<td>BRCA2 c.C5750A + c.6462delTCTC;p.S1917X + p.Y2154fs</td>
<td>Yes</td>
<td>150 BID</td>
<td>101.45%</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>CTG-0136</td>
<td>Esophageal</td>
<td>BRCA2 copy loss</td>
<td>Yes</td>
<td>150 BID</td>
<td>72.00%</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>CTG-1061</td>
<td>Bladder</td>
<td>Wild-type</td>
<td>NA</td>
<td>150 BID</td>
<td>-4.31%</td>
<td>0.409</td>
<td>10</td>
</tr>
<tr>
<td>CTG-1076</td>
<td>Bladder</td>
<td>BRCA1 3265_3266dupTT;p.L1089fs</td>
<td>No</td>
<td>150 BID</td>
<td>65.13%</td>
<td>0.150</td>
<td>3</td>
</tr>
<tr>
<td>GXA 3036</td>
<td>Gastric Adeno</td>
<td>BRCA1 c.668delA;p.K223X</td>
<td>No</td>
<td>150 BID</td>
<td>-12.64%</td>
<td>0.364</td>
<td>3</td>
</tr>
<tr>
<td>GXF 97</td>
<td>Gastric Adeno</td>
<td>BRCA2 c.6482delACAA;p.D2161X</td>
<td>Yes</td>
<td>150 BID</td>
<td>114.25%</td>
<td>&lt;0.0001</td>
<td>10</td>
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<tr>
<td>HBCx-17</td>
<td>Tumor</td>
<td>BRCA2 c.6033_6034delTT;p.F2013Qfs4aa</td>
<td>Yes</td>
<td>150 BID</td>
<td>100.62%</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>HBCx-6</td>
<td>Tumor</td>
<td>BRCA1 Hypermethylation</td>
<td>NA</td>
<td>150 BID</td>
<td>109.43%</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
<tr>
<td>LU-01-0010</td>
<td>Lung Adeno</td>
<td>BRCA1 c.C5503T;p.R1835X</td>
<td>Yes</td>
<td>150 BID</td>
<td>88.20%</td>
<td>&lt;0.0001</td>
<td>3</td>
</tr>
<tr>
<td>LU-01-0340</td>
<td>Lung Squamous</td>
<td>BRCA2 c.5066_5067insA;p.A1689fs</td>
<td>No</td>
<td>150 BID</td>
<td>28.32%</td>
<td>0.266</td>
<td>3</td>
</tr>
<tr>
<td>LU-01-0407</td>
<td>Lung Squamous</td>
<td>BRCA2 c.C7480T;p.R2494X</td>
<td>Yes</td>
<td>150 BID</td>
<td>100.10%</td>
<td>&lt;0.0001</td>
<td>10</td>
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<tr>
<td>LUS152</td>
<td>Lung Cancer</td>
<td>BRCA2 c.2951insA;p.E984fs</td>
<td>No</td>
<td>150 BID</td>
<td>24.87%</td>
<td>0.130</td>
<td>3</td>
</tr>
<tr>
<td>LXF 2415</td>
<td>Lung Squamous</td>
<td>BRCA2 c.A1402T;p.R468X</td>
<td>No</td>
<td>150 BID</td>
<td>15.02%</td>
<td>0.343</td>
<td>3</td>
</tr>
<tr>
<td>PAXF 1876</td>
<td>Pancreatic</td>
<td>BRCA2 c.9090delA;p.T3030X</td>
<td>No</td>
<td>150 BID</td>
<td>30.94%</td>
<td>0.054</td>
<td>10</td>
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<tr>
<td>PAXF 2005</td>
<td>Pancreatic</td>
<td>BRCA2 c.6392delAATT;p.K2131X</td>
<td>Yes</td>
<td>150 BID</td>
<td>94.40%</td>
<td>&lt;0.0001</td>
<td>10</td>
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<tr>
<td>PAXF 2094</td>
<td>Pancreatic</td>
<td>BRCA2 c.1315insAG;p.F439X</td>
<td>No</td>
<td>150 BID</td>
<td>69.12%</td>
<td>0.0008</td>
<td>10</td>
</tr>
<tr>
<td>ST-02-0328</td>
<td>Gastric Adeno</td>
<td>BRCA2 c.1806delA;p.G602fs</td>
<td>No</td>
<td>150 BID</td>
<td>7.70%</td>
<td>0.421</td>
<td>3</td>
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<tr>
<td>ST-02-0360</td>
<td>Gastric Adeno</td>
<td>BRCA2 c.3854dupA;p.E1285fs</td>
<td>No</td>
<td>150 BID</td>
<td>25.98%</td>
<td>0.192</td>
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<tr>
<td>ST-02-0386</td>
<td>Gastric Adeno</td>
<td>BRCA2 c.4279dupT;p.T1426fs</td>
<td>No</td>
<td>150 BID</td>
<td>34.28%</td>
<td>0.036</td>
<td>3</td>
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<tr>
<td>ST-02-0393</td>
<td>Gastric Adeno</td>
<td>BRCA2 c.5195delT;p.L1732fs</td>
<td>Yes</td>
<td>150 BID</td>
<td>104.34%</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
</tbody>
</table>

TGI, tumor growth inhibition; n, number of animals per treatment group; TNBC, triple-negative breast cancer
<table>
<thead>
<tr>
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<th>Genomic alteration</th>
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<th>Dose (mg/kg)</th>
<th>TGI (%)</th>
<th>P-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR-05-0044</td>
<td>Breast</td>
<td>RAD51C Hypermethylation</td>
<td>NA</td>
<td>50 QD</td>
<td>107</td>
<td>&lt;0.0001</td>
<td>10</td>
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<tr>
<td>BR1180</td>
<td>Breast</td>
<td>PALB2 c.758dupT;p.S254fs</td>
<td>Yes</td>
<td>150 BID</td>
<td>107</td>
<td>&lt;0.0001</td>
<td>10</td>
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<tr>
<td>CH-17-0038</td>
<td>Cholangiocarcinoma</td>
<td>PALB2 c.1444_1448del.p.L482fs</td>
<td>Unknown*</td>
<td>150 BID</td>
<td>26</td>
<td>0.169</td>
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<td>CTG-2208</td>
<td>Breast</td>
<td>RAD51C c.653_654del;p.E218Vfs</td>
<td>Yes</td>
<td>150 BID</td>
<td>131</td>
<td>0.001</td>
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<tr>
<td>GA6822</td>
<td>Gastric</td>
<td>BRIP1 c.2507_2508del;p.R836Kfs</td>
<td>Yes</td>
<td>150 BID</td>
<td>-25.4</td>
<td>0.275</td>
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<tr>
<td>LI-03-0655</td>
<td>Liver</td>
<td>RAD51B c.G40T;p.E14X</td>
<td>Yes</td>
<td>150 BID</td>
<td>79.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>LU2529</td>
<td>Lung (Clear)</td>
<td>Wild type</td>
<td>NA</td>
<td>150 BID</td>
<td>-17</td>
<td>0.335</td>
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<tr>
<td>MC2296</td>
<td>Neuroendocrine carcinoma</td>
<td>RAD51D c.C898T;p.R300X</td>
<td>Uncertain#</td>
<td>150 BID</td>
<td>51</td>
<td>0.0005</td>
<td>10</td>
</tr>
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<td>OVXF 1023</td>
<td>Ovarian</td>
<td>RAD51C Hypermethylation</td>
<td>NA</td>
<td>50 QD</td>
<td>59.4</td>
<td>0.0018</td>
<td>10</td>
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<tr>
<td>ST1659</td>
<td>Colon</td>
<td>BRIP1 c.2392G&gt;A;p.R798X</td>
<td>Yes</td>
<td>150 BID</td>
<td>-46</td>
<td>0.506</td>
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<td>ST2067</td>
<td>Ovarian</td>
<td>NBN c.1645T&gt;A;p.K549X</td>
<td>Uncertain#</td>
<td>150 BID</td>
<td>101</td>
<td>0.001</td>
<td>10</td>
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<tr>
<td>ST2073</td>
<td>Endometrial</td>
<td>PALB2 c.2257G&gt;A;p.R753X</td>
<td>Yes</td>
<td>150 BID</td>
<td>55</td>
<td>0.013</td>
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<tr>
<td>ST2636</td>
<td>Breast</td>
<td>BARD1 c.614dupA;p.Q206Afs</td>
<td>Yes</td>
<td>150 BID</td>
<td>21</td>
<td>0.215</td>
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<tr>
<td>T298</td>
<td>Breast</td>
<td>PALB2 c.1424C&gt;G;p.S475X</td>
<td>Yes</td>
<td>150 BID</td>
<td>109</td>
<td>&lt;0.0001</td>
<td>10</td>
</tr>
</tbody>
</table>

*DNA sample unavailable for confirmation; #LOH cannot be determined due to splice variants.

TGI, tumor growth inhibition. n, number of animals per treatment group.
Disclosures

- Liliane Robillard, Kevin K. Lin, Andrea Loehr, Tanya Kwan, Rachel Dusek, Andrew D. Simmons, Thomas C. Harding, and Minh Nguyen are employees of Clovis Oncology, Inc. and may own stock or have stock options in that company.
- Brieuc Sautois has served in a consulting or advisory role for Clovis Oncology, Astellas, BMS Belgium, and Janssen; and received honoraria from Janssen and MSD.