Identification and characterization of potent RAD51 inhibitors targeting RAD51-BRCA2 interaction

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**Background:**

The DNA damage response (DDR) is a complex cellular network that acts as a cell defense mechanism and exerts signal for repair of DNA lesions. It repairs different types of DNA damage such as single-strand (ssDNA) breaks, DNA inter-strand crosslinks and double strand breaks (DSB) caused by endogenous and exogenous factors. These damages are mainly repaired by homologous recombination (HR) repair, and non-homologous end joining (NHEJ). DNA double-strand breaks (DSBs) are the most severe DNA damage and predominantly repaired by HR. As the DDR pathway is critical for tumorigenesis, a high degree of interest has been generated in the recent past to target this network. RAD51 is one of the pivotal enzymes for DNA double-strand break repair by the HR pathway. While PARP inhibitors are extensively used for BRCA2 mutated cancers, their responses in BRCA2 WT is limited. Targeting RAD51 will open a new avenue to treat BRCA2 WT cancer patients. BRCA2 interaction with RAD51 is a prerequisite for RAD51 nuclear translocation to exert its function by binding to DNA. The disruption of this interaction leads to inhibition of DNA repair and in turn inhibits proliferation and tumor growth. Disruption of RAD51:BRCA2 interaction leads to BRCA-ness in an otherwise BRCA2 WT background and is thus expected to have synergy with PARP1 inhibitors. We report the identification of novel and potent RAD51 inhibitors which disrupt the RAD51:BRCA2 interaction. Our hit compounds show anticancer activity in relevant cancer cell lines across various indications. Further, our compounds inhibit RAD51 nuclear translocation upon induction of exogenous DNA damage. Multiple series have been identified, with a good SAR trend and correlating well with cell based and PD activity. Further profiling of these compounds is ongoing.
Speaker Name: Sanjita Sasmal

I have the following financial relationships to disclose:
Employee of: Satya\textsubscript{rx} Pharma Innovations Private Limited

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Anti-Proliferation Activity:

- Compounds show anti-proliferation potency with IC$_{50}$ of 2-5 µM in MDA-MB-231 (BRCA2 WT TNBC), and 10 nM – 1 µM in AID-over-expressing Daudi

<table>
<thead>
<tr>
<th>Compound</th>
<th>MDA-MB-231 IC$_{50}$ (µM)</th>
<th>Daudi IC$_{50}$ (µM)</th>
</tr>
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<tbody>
<tr>
<td>SAT-13</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>SAT-14</td>
<td>14</td>
<td>4.7</td>
</tr>
<tr>
<td>SAT-36</td>
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<td>SAT-40</td>
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<td>5.2</td>
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<tr>
<td>SAT-46</td>
<td>43</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46</td>
</tr>
</tbody>
</table>
Potentiation of Olaparib with SATYA’s RAD51 inhibitor

Colony Forming Assay for combination effect with Olaparib in MDA-MB-231:

- The compounds show potentiation of Olaparib activity in the BRCA2-WT MDA-MB231 cell line, in a Colony Forming Assay.
Selectivity over BRCA2 mutant cells (Capan-1):

- RAD51-BRCA2 inhibitors are significantly more potent in the BRCA2 proficient MDA-MB231 cells as compared to the BRCA2-mutant Capan-1 cells showing better fold selectivity.

**ADME profile:**

<table>
<thead>
<tr>
<th>Compound SAT-</th>
<th>MDA-MB231 (WT) IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>Capan-1 (mut) IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>2</td>
<td>~30</td>
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<td>14</td>
<td>4.7</td>
<td>11.1</td>
</tr>
<tr>
<td>46</td>
<td>2.5</td>
<td>16.4</td>
</tr>
</tbody>
</table>

**Thermodynamic solubility (µM):**

- Compound SAT-13: 3.97
- Compound SAT-14: 17.69
- Compound SAT-36: 1.10
- Compound SAT-43: 33.2
- Compound SAT-46: 6.75

**Intrinsic clearance (MLM):**

- Compound SAT-13: 41.9 µL/min/mg protein
- Compound SAT-14: 43.2 µL/min/mg protein
- Compound SAT-36: 11.9 µL/min/mg protein
- Compound SAT-43: 14 µL/min/mg protein
- Compound SAT-46: 29.1 µL/min/mg protein
Reduction in RAD51 Nuclear Foci in MDA-MB-231 (BRCA2 WT) vs. Capan-1 (BRCA2 mut):

- Treatment with SAT-13, 14 or 46 reduce RAD51 nuclear localization in Cisplatin-treated MDA-MB-231 cells
- While significant peri-nuclear RAD51 expression is observed in BRCA2 mutant Capan-1 cells, there is near-absence of nuclear foci formation upon treatment of BRCA2 mut Capan-1 cells with Cisplatin with/ without SAT-46

**RAD51-BRC4 peptide disruption assay (ELISA):**

- Compound 14 shows disruption of the interaction of RAD51 protein and BRC4 peptide (interacting motif from BRCA2 protein) in an ELISA format
**Summary**

- Novel disruptors of the RAD51-BRCA2 PPI have been identified
  - Lead compounds show potent activity in BRCA2-WT TNBC cell line (MDA-MB-231), and are selective over BRCA2-mutant pancreatic cancer cell line Capan-1
  - In addition, the compounds show very potent activity in AID-over-expressed Lymphoma cell line Daudi
  - Significant potentiation of activity upon combination with PARP1 inhibitor Olaparib is seen in a Colony Forming Assay (CFA)
- RAD51-BRCA2 disruption potential of the compounds is established in an ELISA assay
- The lead compounds prevent RAD51 nuclear localization upon induction of exogenous damage by Cisplatin in BRCA2-WT MDA-MB-231 cells; however, as expected, nuclear localization of RAD51 is insignificant in Cisplatin treated BRCA2-mut Capan-1 cells
- Satya’s compounds show reasonable solubility with low to medium intrinsic clearance in MiLM making them viable for further in vivo studies