Mechanism of APR-246 and Sensitization of Cells to Targeted Agents

Allyson Drawdy, Shady Tantawy and Varsha Gandhi

Introduction

APR-246 (APR) is a novel agent proposed to reactivate mutant p53 and consequential downstream biological effects, including induction of apoptosis.

Role of p53 in APR-246 Cytotoxicity

Does p53 status relate to APR-246 induced cytotoxicity?

Figure 2. HG3 cell line p53 protein expression was visualized via immunoblot. HG3WT had minimal expression of p53. HG3R175H and HG3R248Q both had increased in p53 expression when compared to WT.

Table 1. IC50 values for HG3 and Mec1 cell lines after 24- and 48-hour treatment with APR-246

<table>
<thead>
<tr>
<th>Time</th>
<th>HG3-WT</th>
<th>HG3-KO</th>
<th>HG3-R175H</th>
<th>HG3-R248Q</th>
<th>Mec1</th>
</tr>
</thead>
<tbody>
<tr>
<td>24hr</td>
<td>68.29</td>
<td>66.02</td>
<td>61.55</td>
<td>76.97</td>
<td>24.18</td>
</tr>
<tr>
<td>48hr</td>
<td>39.06</td>
<td>32.36</td>
<td>29.73</td>
<td>30.96</td>
<td>19.91</td>
</tr>
</tbody>
</table>

Materials and Methods

Cell lines: CLL isogenic HG3 cell lines: wild-type (WT), knock-out (KO), two lines with hot-spot induced mutations: R175H and R248Q, and Mec1 cell line.

Cell viability quantified using FITC–Annexin/Propidium Iodide Flow Cytometry.

GSH and Glutathione (GSH)-associated protein expression confirmed via Immunoblot.

Cellular ROS detected by DCFDA fluorescence through flow cytometry.

Intracellular GSH measured by Promega GSH-Glo Assay via luminometer.

Figure 1. Schematic of Isogenic HG3 cell lines

Figure 6. Mec1 cells were treated with 50µM of APR-246 for six hours. Relative illumination at 1 hour was 124, while illumination at 5 hours was 64, indicating that intracellular GSH concentration decreased by half.

Does APR-246 treatment induce oxidative stress?

Figure 4. HG3 cell lines were treated with TBrT and increasing doses of APR-246. APR50µM produced the highest DCFDA fluorescence, indicating the highest levels of cellular ROS.

Figure 5. Mec1 cell line was treated with increasing doses of APR-246 and analyzed for DCFDA fluorescence. Treatment with APR50µM produced the highest levels of ROS in comparison to lower doses.

Is there a cytotoxicity-related decline in glutathione after treatment with APR-246?

Figure 7. Mec1 cells were treated with APR10µM and increasing amounts of glutathione. After 24-hour incubation, cell viability was analyzed showing that Mec1 cells were rescued from cell death as dosage of GSH increased.

Role of APR-246 in Sensitization of Cells

Does combining APR-246 with each venetoclax (VTX) and ibrutinib (IBR), sensitize all HG3 cell lines to treatment?

Figure 8. HG3 Cell lines were administered increasing doses of both alone and in combination with 20µM APR-246 for 72hrs and triplicated. In all four cell lines with varying p53 status, ibrutinib combined with APR20µM induced significantly more cell death than venetoclax as a single agent. This effect was more pronounced at higher VTX doses.

Figure 9. HG3 Cell lines were administered increasing doses of both ibrutinib alone and in combination with APR-246 for 72hrs and triplicated. In all four cell lines with varying p53 status, ibrutinib combined with APR20µM induced significantly more cell death than ibrutinib as a single agent.

Table 2. IC50 and R2 Values for VTX, APR, IBR, and APR+IBR in HG3 cell lines

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VTX</th>
<th>APR</th>
<th>IBR</th>
<th>APR+IBR</th>
<th>VTX+APR</th>
<th>VTX+IBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HG3-WT</td>
<td>3.46</td>
<td>3.34</td>
<td>3.42</td>
<td>1.989</td>
<td>3.695</td>
<td>3.403</td>
</tr>
<tr>
<td>HG3-KO</td>
<td>3.42</td>
<td>3.42</td>
<td>3.42</td>
<td>1.989</td>
<td>3.695</td>
<td>3.403</td>
</tr>
<tr>
<td>HG3-R175H</td>
<td>3.42</td>
<td>3.42</td>
<td>3.42</td>
<td>1.989</td>
<td>3.695</td>
<td>3.403</td>
</tr>
<tr>
<td>HG3-R248Q</td>
<td>3.42</td>
<td>3.42</td>
<td>3.42</td>
<td>1.989</td>
<td>3.695</td>
<td>3.403</td>
</tr>
<tr>
<td>R2</td>
<td>0.9753</td>
<td>0.9272</td>
<td>0.9758</td>
<td>0.8392</td>
<td>0.9196</td>
<td>0.8361</td>
</tr>
</tbody>
</table>

Conclusions

- APR-246 is equally efficacious in cells with proficient, deficient, or mutated p53.
- Mode of action of APR-246 is independent of p53 status, and glutathione depletion appears to be the primary mechanism.
- APR-246 sensitizes CLL malignant B-cells to treatment with targeted therapies, venetoclax and ibrutinib.

Why do these findings matter?

1. APR-246 may be useful in treating all CLL patients rather than solely p53-mutated.
2. APR-246 shows promise for treatment of other malignancies.
3. A phase III clinical trial has been designed for patients with CLL to test APR-246 with ibrutinib or venetoclax.

References