EP100 SYNERGIZES WITH PACLITAXEL IN OVARIAN, BREAST AND PROSTATE CANCER CELL LINES

Carola Leuschner, Cody Giardina, Hector Allia
Esperance Pharmaceuticals, Inc., Baton Rouge, LA

Abstract 3715

EP100 is a targeted anti-cancer drug composed of Luteinizing Hormone Releasing Hormone (LHRH) fused to a tyrosine kinase (TK) and is currently being investigated in human clinical trials. EP100 weakly and dose-dependently inhibits LHRH receptor positive cancer cells via a novel mechanism of action involving direct membrane insertion. Studies were conducted to determine the effects of EP100 and Paclitaxel on cell viability in vitro using multi-drug resistant LHRH receptor positive ovarian (SKOV-3), breast (MDA-MB-231), prostate (PC-3) and LHRH receptor negative ovarian (OVCAR-3) cells. The IC₅₀ values for EP100 and Paclitaxel were measured as Combination Index (CI) using the Compusyn Software analysis program. EP-100 alone killed LHRH receptor positive OVCAR-3 and MDA-MB-231 cells at low micromolar concentrations after 72 h incubation (IC₅₀ values 2.18 ± 0.07 µM and 2.02 ± 0.11 µM, respectively). Both cell lines showed resistance to Paclitaxel (IC₅₀ values 13.6 ± 0.7 µM for OVCAR-3 and 62.6 ± 5.9 µM for MDA-MB-231). The IC₅₀ values for EP100 alone and Paclitaxel alone were 1.41 ± 0.13 µM and 1.9 ± 0.07 µM, respectively. After 48 h incubation, the IC₅₀ values for the combination were 0.27 ± 0.10 µM and 4.3 ± 0.19 µM for OVCAR-3 and MDA-MB-231 cells, respectively. The IC₅₀ values for EP-100 were 0.66 and 0.42 µM for OVCAR-3, MDA-MB-231, and PC-3, respectively, indicating synergistic responses in the cell lines. No synergy was observed in the LHRH receptor negative cell line (OVCAR-3). These results indicate that a combination of EP100 and Paclitaxel results in synergistic responses in LHRH receptor positive and multi-drug resistant cancer cells.

Materials & Methods

Traditional non-targeted treatments are often associated with serious side effects, are systemically active and do not discriminate between cancer and normal cells in vivo organs. Esperance Pharmaceuticals is developing a targeted Calicort Lytic Peptide (CLYP) technology for killing cancer cells. It involves small targeted peptides that seek and destroy cancer cells without harming normal cells. The peptides are linear, alpha-helical, and calicort peptides that directly interact with negatively charged membranes resulting in their disruption and cell death.

Major advantages of membrane disrupting peptides over traditional non-targeted treatments include the following: 1. Preferentially destroy negatively charged cells, such as cancer cells, at concentrations up to 500 times lower than comparable peptide to normal cells 2. Fast acting - causing necrosis within minutes through direct membrane rupture 3. No toxicity to normal cells 4. Activity is independent of multidrug resistance. 5.Short half life in vivo; destroying cancer cells before new resistance development. These advantages are combined with desensitization

As a result of this technology, Esperance Pharmaceuticals is developing a new generation of lipopeptide TK molecules that are over-expressed on cancer cells. Esperance’s lead candidate, EP100, tested in Phase 1 clinical trial, is now entering Phase 2 clinical phase for treating LHRH receptor positive cancers. LHRH receptors are over-expressed in a wide range of cancers including breast, prostate, endometrial, ovarian, renal, testicular; liver adenomas and hematological malignancies. EP100 consists of LHRH linked to a novel 18 amino acid lytic peptide (CLPY) to form a 29 amino acid EP100 (LHRH has 10 amino acids). Due to its small size EP100 is manufactured by standard solid phase chemistry to produce highly homogeneous and pure product that is highly water soluble. EP100 is not immunogenic and was well tolerated in humans. Previously, lycopenes and pepsic acid showed potentiation in combination with desensitization

P-gps, also known as MDR1 (ABCB1), is a 170 kDa integral membrane protein that functions as an ATP-dependent drug efflux pump. Compounds that interact with P-gps can be identified as substrates for transport or inhibitors of its ATPase activity. The effect of EP100 on P-gp dependent multidrug resistance was tested in Paclitaxel (Pgp) resistant cell lines in vitro. Cell lines expressing LHRH receptors (SKOV-3, OVCAR-3, PC-3, MDA-MB-231) and the LHRH receptor negative cell line SKO-2 were compared for single agent EP-100 and Paclitaxel and in combination. These results suggest that a combination of EP100 with Paclitaxel in an ongoing Phase 2 clinical trial (NCT01485548).

Results – in vitro Studies

Figure 3: EP100 sensitizes Paclitaxel resistant cancer cells expressing LHRH receptors resulting in a highly synergistic, potentiation of cell killing effect. Activities were measured with increasing concentrations of Paclitaxel (0.05-250 nM) in the absence or presence of EP100 concentrations of 0.1, 0.5, 1.5 and 100 nM. Cell viability was determined after 72 hours using a luminometric viability assay. Satellites and 0.1% Triton served as controls.

Table 1: Combination Studies of EP100 and Paclitaxel in LHRH receptor expressing cancer cell lines.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>SKOV-3 - LHRH R (+)</th>
<th>OVCAR-3 - LHRH R (+)</th>
<th>OVCAR-3 - LHRH R (+)</th>
<th>PC-3 - LHRH R (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ [nM]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>48.6 ± 1.7</td>
<td>33.1 ± 4.2</td>
<td>33.4 ± 4.3</td>
<td>60.3 ± 2.7</td>
</tr>
<tr>
<td>D</td>
<td>54.9 ± 6.6</td>
<td>22.0 ± 3.6</td>
<td>25.2 ± 3.6</td>
<td>129.9 ± 5.3</td>
</tr>
</tbody>
</table>

The potentiality of the combination of EP100 and Paclitaxel is dependent on the order of administration and is highest for long term exposure.

Figure 4: P-gp ATPase activity on human recombinant Pgp protein. The activity of P-gpg dependent ATP hydrolysis was determined through measurement of remaining ATP in a luminometric assay. n=6. EP100 and CLP-71 (unligated tyrosine peptide) were added at concentrations of 0.5, 5, 50 and 100 nM. Cytarabine (50 nM) served as control for non-P-gp substrate. Vespramine served as positive control (2 µM). Vespramine represented baseline activity (non-P-gp dependent).

Table 2: Inhibitory effects of SKOV-3, OVCAR-3, MES-SA-Dx5, MDA-MB-231 and PC-3 cell lines in vitro with Paclitaxel for 48 hours with single agent EP100 or paclitaxel and in combination at non-synergistic ratios. Cell lines were cultured in the presence of EP100 (0.5-32 µg/ml) and Paclitaxel (0.005-250 nM) alone or in combination and incubated for 48 h or 72 hours. Combination indices were determined from experiments in control cell lines (MES-SA-Dx5, MDA-MB-231 and SKOV-3) and IC₅₀ (OVCAR-3). The combination index (CI) was determined by the Software analysis program. The combinations of EP100 and Paclitaxel were highly synergistic, positive for LHRH receptors – LHRH negative cell lines (SKOV-2) showed potentiation in combination.

Conclusions

The combination of EP100 and Paclitaxel is highly synergistic in cancer cells that combine both TK and TKI activity. This marks a significant departure from previous reports of EP100 with Paclitaxel as potential treatment of ovarian, breast, prostate cancers.

References

4. Popko et al Cancer Res, 64, 5757-5759, 2004