The stapled peptide ALRN-6924, a dual inhibitor of MDMX and MDM2, enhances antitumor efficacy of paclitaxel and Nab-paclitaxel in TP53 wild-type breast cancer models

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Background

MDM and MDM2 are endogenous inhibitors of the p53 tumor suppressor protein. MDMX levels are frequently elevated in luminal breast cancer, which generally express wild-type (wt) p53. ALRN-6924, a c-helical stapled p53 peptide, is the first and only dual inhibitor of MDMX and MDM2 currently in clinical trials for solid tumors and hematological malignancies. We sought to determine the antitumor efficacy of the combination of ALRN-6924 with taxanes in wild-type p53 estrogen receptor positive (ER+) models of human breast cancer.

Methods

Sulfotetrahydrobmine B (SRB) colorimetric assay was used to assess the cytotoxicity of the combination of ALRN-6924 with taxanes in vitro. Atxytic nude mice were implanted with wt p53 and ER+ MCF-7 and ZR-75-1 cells and treated for 4 weeks with ALRN-6924 alone and in combination with paclitaxel in cremophor (Taxol®, study #1) or a nanoparticle-albumin-bound (nab) formulation (Abraxane®, study #2). In study #1, ALRN-6924 (5, 10 mg/kg) was dosed twice weekly and paclitaxel (10, 15 mg/kg) was dosed weekly, with paclitaxel administered 6 h prior to ALRN-6924. In study #2, ALRN-6924 alone (5 mg/kg) was dosed twice weekly while nab-paclitaxel (15 mg/kg) was administered weekly in combination at -24h, -6h, 0h, +6h, or +24h relative to ALRN-6924 administration.

Results

Sensitivity of hormone positive breast cancer cells to ALRN-6924 and Paclitaxel

Cells were seeded in 96-well plates and treated with various drugs at different drug ranges for 3 days. The cell survival was measured from optical density (OD) value after SRB staining. IC50 were calculated from dose curves using CalcuSyn.

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Paclitaxel

Table 1. Sensitivity of hormone positive breast cancer cells to ALRN-6924 and Paclitaxel

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ALRN-6924</th>
<th>Paclitaxel</th>
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<tbody>
<tr>
<td>MCF-7</td>
<td>117 nM</td>
<td>0.03 nM</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>606 nM</td>
<td>0.2 nM</td>
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Paclitaxel

Figure 1. The combination of ALRN-6924 and Paclitaxel demonstrates synergistic activity on cell survival in MCF-7 and ZR-75-1 breast cancer cells. Cells seeded in 96-well plates were incubated with various drugs at a variable concentration of ALRN-6924 or paclitaxel along with single drug treatment. IC50s were obtained from each dose curve using CalcuSyn.

Figure 2. Dose-dependent inhibition of colony formation by MCF-7 breast cancer cells by ALRN-6924 and Paclitaxel. Cells seeded in 96-well plates were incubated with various drugs at different drug ranges for 10 days followed by fixation of cells and crystal violet staining.

Figure 3. Combining ALRN-6924 and Paclitaxel demonstrates synergistic activity on colony formation assay of MCF-7 cells. Cells were treated with single or combined drugs at the indicated doses for 10 days. Total colony area was quantitated using ImageJ. Combination index was calculated using Bliss model (CI<1: synergistic; CI=1: additive, CI>1: antagonistic effect respectively).

Figure 4. Growth inhibition of breast cancer tumors by ALRN-6924 and Paclitaxel in mice. The combination of ALRN-6924 and paclitaxel significantly inhibited MCF-7 tumor growth compared to either agent alone (p<0.001). Paclitaxel 15 mg/kg + ALRN-6924 5 mg/kg in MCF-7 resulted in the greatest tumor inhibition with average tumor size decreased by 13% at four weeks versus the starting size.

Table 2. Combination indices across wt p53 hormone positive breast cancer cells to ALRN-6924 and Paclitaxel

<table>
<thead>
<tr>
<th>Cell line</th>
<th>ALRN-6924 + Paclitaxel combination indices</th>
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<tr>
<td>MCF-7</td>
<td>0.41</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>0.52</td>
</tr>
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Conclusion

1. There is a significant increase in antitumor efficacy observed with ALRN-6924 in combination with paclitaxel in preclinical breast cancer tumor models.
2. Combining ALRN-6924 with nab-paclitaxel resulted in significant increase in number of tumor regressions.
3. These findings support further evaluation of described combinations in patients with breast cancer.

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