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
MDMX 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, an α-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
Sulforhodamine B (SRB) colorimetric assay was used to assess the cytotoxicity of the combination of ALRN-6924 with taxanes in vitro. Athymic nude mice were implanted with wt p53 and ER+ MCF-7 and ZR-75-1 cells and treated for four weeks with ALRN-6924 alone and in combination with paclitaxel in cremophor (Taxol®), or a nanoparticle-albumin-bound (nab) formulation (Abraxane®, 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.

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>
</tr>
</thead>
<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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</tbody>
</table>

Results

![Figure 1. The combination of 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 by ImageJ. Combination index was calculated using Bliss model (CI=1: synergistic; CI>1: additive; CI<1: antagonistic effect respectively).](image1)

Figure 1. 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 by ImageJ. Combination index was calculated using Bliss model (CI=1: synergistic; CI>1: additive; CI<1: antagonistic effect respectively).

![Figure 2. Dose-dependent inhibition of colony formation of hormone positive breast cancer cells by ALRN-6924 and Paclitaxel. Combination index (CI) was calculated using CalcuSyn. CI < 1: Synergistic; CI=1: Additive; CI > 1: Antagonistic effect respectively.](image2)

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>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>0.41</td>
</tr>
<tr>
<td>ZR-75-1</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Figure 2. Dose-dependent inhibition of colony formation of hormone positive breast cancer cells by ALRN-6924 and Paclitaxel. Combination index (CI) was calculated using CalcuSyn. CI < 1: Synergistic; CI=1: Additive; CI > 1: Antagonistic effect respectively.

![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 by ImageJ. Combination index was calculated using Bliss model (CI=1: synergistic; CI>1: additive; CI<1: antagonistic effect respectively).](image3)

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 by 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 10 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.](image4)

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 10 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.

![Figure 5 and 6. The combination of Nab-Paclitaxel with ALRN-6924 to MCF-7 xenograft models resulted in improved antitumor efficacy over either single agent. ALRN-6924 was administered 6h to +24h relative to nab-paclitaxel, significant increase in the number of tumor regressions (up to 30 with 3 consecutive measurements <50% of starting volume) compared to nab-paclitaxel alone (1:0, p<0.005). When ALRN-6924 was administered 24h prior to nab-paclitaxel, there was a marked decrease in efficacy and no tumor regressions were observed.](image5)

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 a significant increase in number of tumor regressions.
3. These findings support further evaluation of described combinations in patients with breast cancer.

Funding
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