**Abstract**

EZN-3920, an ErbB3-locked nucleic acid-based RNA inhibitor, potently silences target gene expression in tumor cells grown in vitro and in vivo.

**Introduction**

The ErbB family consists of 4 transmembrane receptor tyrosine kinases, ErbB1 (EGFR), ErbB2, ErbB3, and ErbB4. Small molecules and antibodies that target ErbB1 and/or ErbB2 have proven anticancer activity in patients. ErbB3 is unique since it has little or no kinase activity, and therefore is not easily druggable by small molecules. However, inhibition of ErbB3 is likely to have antitumor effects since ErbB3 interacts with ErbB2 and ErbB1, (a key link in the PI3K-pro-survival signaling pathway), and 3 is activated in cells resistant to ErbB1 or ErbB2 therapeutics. In this report, a locked nucleic acid (LNA)-containing antisense oligonucleotide (LNA-ONA), designated EZN-3920, has been used to inhibit the expression of ErbB3. EZN-3920 is stable in plasma for greater than 72 hours and has exceptionally high binding affinity for ErbB3 mRNA. The compound was evaluated in vitro and animal models of cancer.

**Methods**

In vitro evaluation

The tumor cells were transfected with EZN-3920 ErbB3 LNA oligo (Santaris Pharma, see poster 312) with Lipofectamine-2000, and subjected to proliferation assay (MTS assay), apoptosis assay (Caspase 3/7 activity assay), and mRNA (qRT-PCR) and protein (western blot) examination two days after transfection. The EZN3046 scrambled oligo served as a negative control. 12 tumor cell lines were used for the evaluation.

In vivo evaluation

The nude mice bearing 15PC-3 (prostate), A549 (lung) and SKBR3 (breast) xenografts were intravenously injected by EZN3920 as multiple dose regimens. The mRNA and protein expression of ErbB3 were examined by qRT-PCR and western blot, respectively. EZN3046 scrambled oligo served as a control.

**Mechanism of Action**

LNA-ONA antagonists

<table>
<thead>
<tr>
<th>Oligo (nM)</th>
<th>0 nM</th>
<th>1 nM</th>
<th>5 nM</th>
<th>10 nM</th>
<th>20 nM</th>
<th>40 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>EZN3920</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EZN3920</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EZN3920</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**EZN-3920 inhibits ErbB3 expression and cell survival in multiple tumor lines**

**EZN-3920 potently inhibits ErbB3, p-AKT and proliferation, and induces apoptosis in 15PC-3 and SKBR3 cells in vitro**

**Conclusions**

- EZN-3920 potently knocked down ErbB3 expression and inhibits cell survival in multiple cell lines in vitro.
- EZN-3920 specifically downmodulates ErbB3 expression in liver and xenografts in three tumor models by systemic administration.
- ErbB3 downmodulation is associated with inhibition of PI3K/Akt pro-survival pathway, both in vitro and in xenograft tumor models.
- Targeting the ErbB3 signal transduction pathway is a very attractive therapeutic strategy for ErbB-driven tumors.

**References**