**INTRODUCTION**

The HER family consists of four tyrosine kinase receptors designated as EGFR, HER2, HER3 and HER4. Although HER3 has little or no kinase activity, it plays an important role in cancer since 1) it heterodimerizes with HER2, 2) is the primary link to the PI3K /AKT axis and 3) play a role in resistance to HER1 and HER2-targeted therapeutics.

We have used a HER3 mRNA locked nucleic acid (LNA) -based antisense antagonist, designated EZN-3920, to down regulate HER3. The aim of this study was to determine the anticancer activity of EZN-3920 in tumor cell lines and xenograft models, as well as evaluate the effect when combined within an EGFR inhibitor, gefitinib. The regulation of HER3 and downstream PI3K/akt signaling was correlated with the antitumor effects.

**METHODS**

In vitro evaluation

The tumor cells were treated with LNA-oligonucleotides (LNA-ON) without lipofection. Cell proliferation (MTS), mRNA (qRT-PCR), and protein (western blot) examinations were performed 3-5 days after drug incubation. A control oligonucleotide (EZN-SCR) served as a negative control.

**IN VITRO**

### Table 1. In vitro activity of EZN-3920

<table>
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<th>Tumor Type</th>
<th>Cell Line</th>
<th>HER3 expression</th>
<th>HER3 Knockdown</th>
<th>IC50 mRNA</th>
<th>HER3 protein</th>
<th>µM</th>
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<td>Breast</td>
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<tr>
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<td>NSCLC</td>
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<tr>
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ND: not done, *, *gefitinib resistant mutant developed in house (Tigges et al., KRTC 2003 poster #A07)

Human tumor cells were treated with LNA-ONS without lipofection at the same concentrations. Cell proliferation was examined by MTS assay. The optical densities were measured in a Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 490 nm. EZN-3920 was chosen because of its potency and selectivity among tested HER3 tumor cell lines. Cell proliferation (MTS), mRNA (qRT-PCR), and protein (western blot) examinations were performed 3-5 days after drug incubation.

### Figure 1. Characterization of EZN-3920 in vivo

(A) -150 mm³ tumor treated with EZN-3920 q3d x 10. i.v. (B) At day 31, HER3 mRNA in tumor measured by qRT-PCR, (C) target and downstream genes by Western blot and (D) apoptosis analysis by TUNEL.

**RESULTS**

### Table 3. Effect of EZN-3920 on NSCLC HCC827 xenograft

- Biweekly dosing: EZN-3920 40 mg/kg showed significant tumor growth inhibition compared to saline (20 mg/kg) and gefitinib (30 mg/kg).
- EZN-3920 combined with gefitinib showed additive or synergistic effects, with a significant reduction in tumor volume compared to gefitinib alone.
- EZN-3920 alone or in combination with gefitinib showed improved survival compared to saline and gefitinib alone.

### Figure 2. Down-modulation of target by EZN-3920

- Tumor cells were treated with EZN-3920 or its scrambled LNA (EZN-SCR) for 5 days. 200 µg of total protein of each treatment was analyzed by immunoblotting for HER2, pHER2, HER3, pHER3, AKT, and pAKT. γ-tubulin was also analyzed for specificity.

### Figure 3. Effect of EZN-3920 on breast cancer xenograft

- Human tumor cells were treated with LNA-ONS without lipofection at the same concentrations. Cell proliferation was examined by MTS assay. The optical densities were measured in a Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 490 nm. EZN-3920 was chosen because of its potency and selectivity among tested HER3 tumor cell lines. Cell proliferation (MTS), mRNA (qRT-PCR), and protein (western blot) examinations were performed 3-5 days after drug incubation.

### Figure 4. Effect of EZN-3920 on breast cancer xenograft

- Human tumor cells were treated with LNA-ONS without lipofection at the same concentrations. Cell proliferation was examined by MTS assay. The optical densities were measured in a Microplate Reader (Molecular Devices, Sunnyvale, CA, USA) at a wavelength of 490 nm. EZN-3920 was chosen because of its potency and selectivity among tested HER3 tumor cell lines. Cell proliferation (MTS), mRNA (qRT-PCR), and protein (western blot) examinations were performed 3-5 days after drug incubation.

**CONCLUSIONS**

A LNA-based antisense inhibitor of HER3 mRNA

- Potently down regulates HER3, the PI3K signaling axis, and inhibits cell proliferation in multiple cell lines grown in vitro.
- Specifically down regulates HER3, the PI3K/akt axis, as well as induces apoptosis and inhibits tumor growth in animal models.
- Enhances the effect of gefitinib in a NSCLC tumor model.
- May control the growth of tumors that are addicted to HER3 as well as those resistant to EGFR/HER1 TKIs or Herceptin.

EZN-3920 is being developed by Enzon under a licence with Santaris Pharma A/S.

**REFERENCES**

5. Cook RS et al. personal communication.