Reduced expression of HER3 with a specific RNA antagonist is associated with antitumor effects in preclinical models of cancer

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Abstract #144

INTRODUCTION

The HER family consists of four tyrosine kinase receptors designated as EGFR, HER2, HER3 and HER4. HER3 has the unique and potent ability to activate downstream PI3K and Akt pathway once it dimerizes with HER2, mediates resistance to HER-targeted therapeutics, and plays a critical role in cancer growth. Our study aimed at evaluating the efficacy of EZN-3920, a HER3 locked nucleic acid (LNA) antisense oligonucleotide (ON), in various tumor cell lines and xenograft models. The regulation on target HER3 and downstream PI3K/Akt signaling was explored.

METHODS

In vitro evaluation

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

In vivo evaluation

Nude mice bearing HCC827 (NSCLC), BT474M1 (breast) and Polyomavirus middle T (PyVmT)-driven mouse mammary tumors were intravenously injected with EZN-3920 and EZN-SCR on multiple dose regimens. Tumor apoptosis was determined by TUNEL assay. The mRNA level was examined by qRT-PCR, while protein levels were measured by western blot and immunohistochemistry. Tumor growth inhibition was calculated relatively to vehicle control.

LNA TECHNOLOGY

- 3rd generation LNA-based antisense technology
- Very high mRNA efficacy
- Excellent plasma stability
- Long tissue residence time (60 h)
- Short sequence (16-mer)
- In administration without delivery vehicle (in vivo RNA)

HER3 and PI3K/AKT SIGNALING

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

- EZN-3920 down modulates HER3 and PI3K axis signaling

RESULTS

IN VITRO

Table 1. Selection of EZN-3920

<table>
<thead>
<tr>
<th>Load LNA</th>
<th>Property</th>
<th>In vitro activity [%]</th>
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<td>EZN-3920</td>
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Specific target down-modulation by EZN3920. HCC827 cells were treated with 0.3 μM of EZN-3920 or a non-specific scrambled LNA (EZN-SCR) for 3 days without transfection (drug simply added to tissue culture media), HER3 (A), EGFR and HER2 (B) mRNA levels were determined by real-time PCR using TaqMan gene expression assay.

IN VIVO

Figure 1. Characterization of EZN-3920 in vivo

(A) ~150 mm\(^3\) 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.

- Tumor growth inhibition (TGI) coordinates with HER3 silencing and apoptosis induction

CONCLUSIONS

EZN-3920

- Potently ablates HER3/PI3K signaling axis and inhibits cell proliferation in multiple cell lines.
- Specifically downmodulates HER3 expression, induces apoptosis and inhibits tumor growth of three animal models.
- May control the growth of tumors that are addicted to HER3 as well as those resistant to EGFR/HER TKi or Herceptin.

See Poster #307 for more information.

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