Targeting HER3 mRNA by a locked nucleic antisense molecule enhances the antitumor activity of gefitinib *in vivo*

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Abstract #232

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

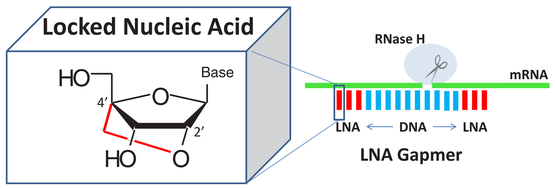
In vitro evaluation

The tumor cells were treated with LNA-oligonudleotides (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 vivo evaluation

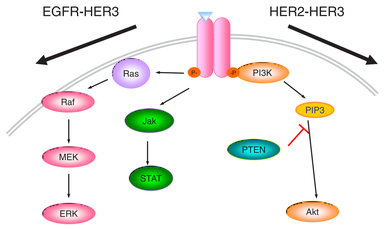
Nude mice bearing HCC827 (NSCLC), and BT474M1 (breast) tumors were intravenously injected with EZN-3920 and EZN-SCR on multiple dose regimens. Tumor apoptosis was determined by TUNEL assay. The mRNA level were examined by qRT-PCR, while protein levels were measured by western blot and immunohistochemistry.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

WHY USE LNA TECHNOLOGY?



- 3rd generation LNA-based antisense technology
- Very high mRNA affinity
- Excellent plasma stability
- Long tissue residence time (days)
- Short sequence (14-16-mer)
- IV administration without delivery vehicle (unlike siRNA)

HER3 and PI3K/AKT SIGNALING



RESULTS

IN VITRO

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

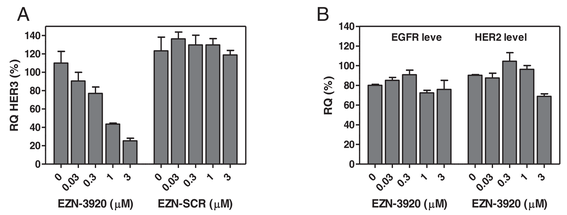
Tumor Type	Cell line	HER3 expression	HER3 Knockdown		IC50
			mRNA	HER3 protein	μM
Breast	BT474M1	+++	yes	yes	1
Breast	SKBR3	+++	yes	yes	0.5
NSCLC	HCC827	++	yes	yes	0.5
NSCLC	HCC827GR*	++	yes	yes	0.5
Ovarian	OVCAR-5	+	yes	ND	5
NSCLC	A549	+/-	yes	ND	>10

ND: not done ; *GR-gefitinib resistant developed in house (T.Qu et al, EORTC 2010 poster #307)

Human tumor cells were treated with LNA-ONs without lipofection at the various 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.

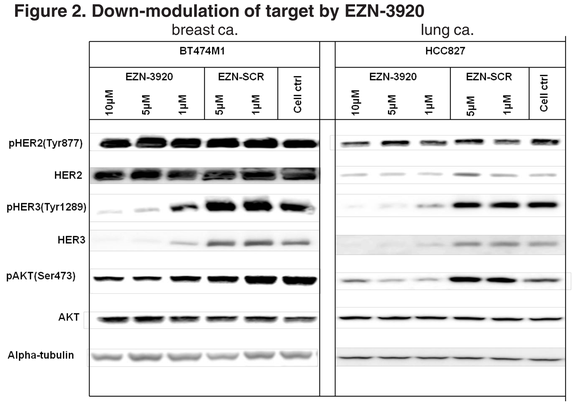
❖**EZN-3920 is potent and specific against HER3 mRNA**

Figure 1. Characterization of EZN-3920 *in vitro*



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 assays.

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

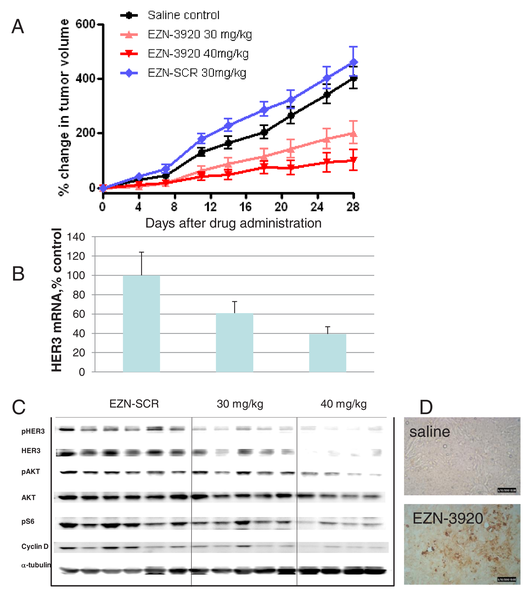


Tumor cells were treated with EZN-3920 or its scrambled EZN-SCR for 5 days. 200 μg of total protein of each treatment was analyzed by immunoblotting for HER2, pHER2, HER3, pHER3, AKT, and pAKT. α-tubulin was also analyzed for specificity.

❖**EZN-3920 down modulates HER3 and PI3K axis signaling**

IN VIVO

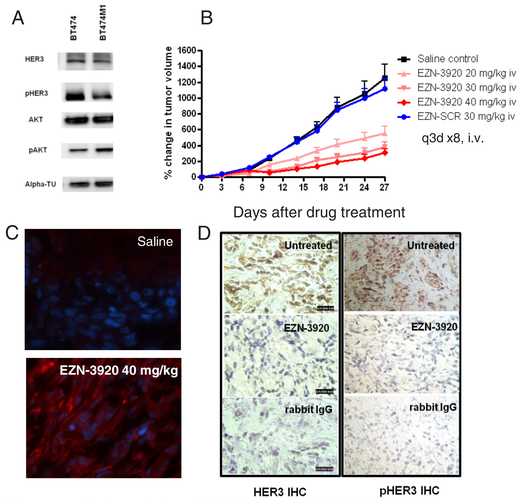
Figure 3. Effect of EZN-3920 on NSCLC HCC827 xenograft



(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.

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

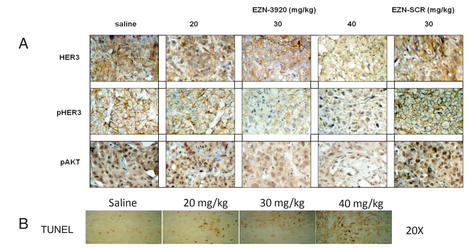
Figure 4. Effect of EZN-3920 on breast ca BT474M1 xenograft



(A) Immunoblot analysis of BT474 and BT474M1 in vitro,
(B) ~150 mm³ BT474M1 (n=10) treated with EZN-3920 or EZN-SCR,
(C) Localization of Cy5.5-EZN-3920 in xenograft tumor,
(D) Immunohistochemical analysis for target HER3 and pHER3.

❖**EZN-3920 downregulates both HER3 and pHER3, accumulates in tumors, and inhibits BT474M1 tumor growth**

Figure 5. Immunohistochemical analysis of EZN-3920-treated tumors



Animals bearing tumors derived from HCC827 cells were treated with EZN-3920 or EZN-SCR. 24 hr after last dose on day 32, the tumors were collected and analyzed by IHC (A) and TUNEL (B)..

❖**EZN-3920 down modulates HER3/PI3K axis and induces apoptosis in a dose-dependent manner**

Figure 6. Effect of EZN-3920 and gefitinib on NSCLC HCC827 xenograft

~150 mm³ HCC827 tumor bearing mice (n=10) treated with EZN-3920, gefitinib alone or combined EZN-3920 and gefitinib. 30 mg/kg of EZN-3920 was administered twice a week for 5 weeks. 15 mg/kg of gefitinib was daily dosed 5 days a week for 2 weeks.

❖**Combination of EZN-3920 and gefitinib shows tumor regression**

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/HER TKIs or Herceptin.**

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

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