EZN-3920, an LNA antisense oligonucleotide RNA antagonist, down modulates HER3 expression and PI3K/Akt signaling pathway and enhances antiproliferative effect of gefitinib in tumor cells

Baisong Liao, Yixian Zhang, Jolanta Kosek, Wenzhe Liu, Stephen Castaneda, Peifang Zhu, Qinghong Dai, Timothy Qu, Puja Sapra, Lee M. Greenberger, Ivan D. Horak

Enzon Pharmaceuticals, Inc., Piscataway, NJ

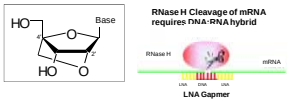
lee.greenberger@enzon.com

Abstract #4630

Introduction

The Her family consists of four tyrosine kinase receptors designated as EGFR, HER2, HER3 and HER4. Inhibition of HER3 is likely to have antitumor effects since HER3 (1) heterodimerizes with HER2 and EGFR, (2) is a key link to the PI3K pro-survival signaling pathway, and (3) is activated in cells resistant to EGFR or HER2 targeting therapeutics. This study aimed at evaluating the activities of EZN3920, an HER3 locked nucleic acid (LNA) antisense oligonucleotide (ON), in various tumor cells and xenograft models. The regulation on downstream PI3K/Akt pathway and combination with EGFR inhibitor were also explored. Moreover, the inhibitor effect of EZN-3920 on gefitinib-resistant cell proliferation was demonstrated.

LNA-based oligonucleotides



- 3rd generation LNA-based antisense technology
- Very high mRNA affinity
- Excellent plasma stability
- Long tissue residence time (days)
- Short sequence (14-16-mer)
- High cell potency (low nM comparable to siRNA)
- High potency may translate to better therapeutic window

Methods

In vitro evaluation

The tumor cells were treated with EZN-3920 with lipofactamine-2000. Proliferation assay (MTS assay), apoptosis assay (Caspase 3/7 activity assay), and mRNA (qRT-PCR) and protein (western blot) examination were performed two days after transfection. The EZN3046 scrambled oligonucleotide served as a negative control. 12 tumor cell lines were used for the evaluation. Gefitinib resistant HCC827 cells were generated by exposing cells to increasing amount of gefitinib for a period of three months.

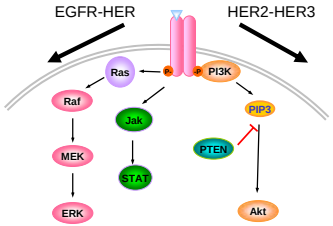
In vivo evaluation

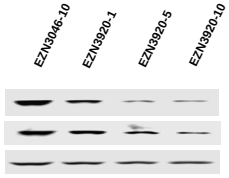
Nude mice bearing 15PC-3 (prostate), A549 (lung) and N87 (gastric) tumor xenografts were intravenously injected with EZN-3920 on multiple dose regimens. The mRNA and protein levels in liver and tumors were examined by qRT-PCR and western blot, respectively.

Objective

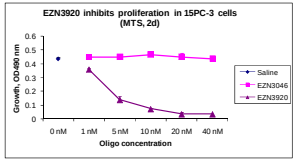
Demonstrate the utility of EZN-3920 in vitro and in vivo, including cells resistant to gefitinib.

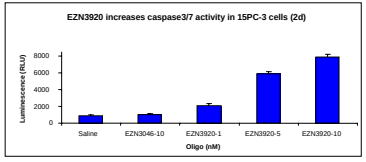
EZN-3920 inhibits HER3 activated PI3K signaling





EZN-3920 potently inhibits proliferation and induces apoptosis of 15PC-3



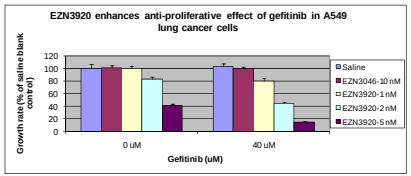


Growth inhibition and target mRNA knockdown by EZN-3920 in various cancer cell lines

Cell	15PC3	DU145	A549	H1993	H1373	Skbr3	HUH7	Skov3	A431	HT29	N87	Molt-4
Type	prostate	prostate	lung	lung	lung	breast	liver	ovary	epidermoid	colon	stomach	leukemia
mRNA KD	~1	<2	<2	<1	<5	<5	<1	<2	<1	>10	>10	>100
Growth inhibition	~2	<5	<2	<1	<10	<5	<1	<5	<1	>10	>10	>100
HER3 mRNA	+++	+	+	++	++	++++	+	+	+++++	+++++	+++++	-

(Based on relative ΔCt values from qRT-PCR)

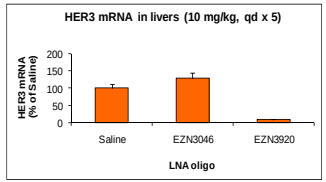
EZN-3920 potentiates the effect of gefitinib on the growth of 15PC-3



- A549 is resistant to gefitinib (>40 uM)
- EZN3920 potently inhibits cell growth
- EZN3920 treatment sensitizes A549 cells to gefitinib
- Similar effect observed with 15PC-3 cells

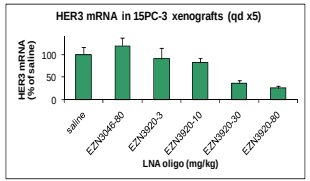
EZN-3920 specifically knocks down target mRNA in liver and tumors in 3 tumor models

In liver



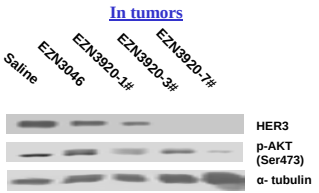
- EZN-3920 specifically knocks down HER3 mRNA in liver while a reference gene HIF-1α is not affected (data not shown)

In tumors



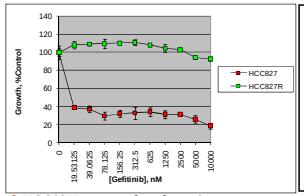
- EZN-3920 knocks down HER3 mRNA in tumor (A reference gene HIF-1α is not affected) (data not shown)
- Similar KD was observed in A549 and N87 animal tumor models

In tumors

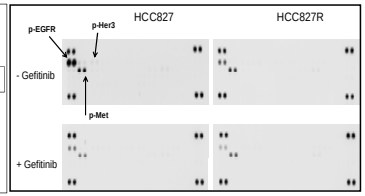


- EZN-3920 inhibits HER3 protein levels and phosphorylation of AKT in 15PC-3 tumors when animals were treated with 50 mg/kg (q3dx8) (#1, #3, and #7)

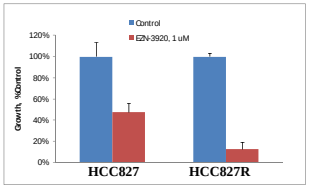
EZN-3920 inhibits the growth of gefitinib-resistant HCC827 cells



- HCC827R was made to be resistant to gefitinib through increasing higher exposure to gefitinib
- Resistant cells were maintained in 1 μM gefitinib



- HCC827R shows significantly reduced phospho-EGFR by receptor tyrosine kinase array
- No change in pMET detected
- EGFR level is also reduced (data not shown)



- EZN-3920 (without lipofection) significantly inhibits both parental and gefitinib-resistant cells in a colony formation assay (10 day)

Conclusions

- EZN-3920 potently knocks down target expression and inhibits cell survival in multiple cell lines
- EZN-3920 specifically silences HER3 expression in liver and xenografts in three tumor models by systemic administration
- HER3 down regulation by EZN-3920 inhibits downstream PI3K/Akt pathway both in vitro and in vivo
- EZN-3920 potentiates the effect of gefitinib
- EZN-3920 inhibits the growth of both parental HCC827 and gefitinib-resistant HCC827 cells despite the fact that p-EGFR and EGFR are reduced in resistant cells

References & Acknowledgments

(1) Engelman JA, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science. 2007; 316:1039-1043

(2) Sergina NV, et al. Mediates resistance of cancer cells to ErbB TKIs in breast cancer. Nature, 2007; 445:437-41

(3) Zhang H, et al. ErbB receptors: from oncogenes to targeted cancer therapies. *J Clin Invest.* 2007;117:2051-2058

The design and discovery of EZN-3920 has been done in collaboration with Santaris Pharma A/S. EZN-3920 is being developed by Enzon under a license with Santaris Pharma A/S