Abstract# 312

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

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Introduction

The ErbB family consists of 4 tyrosine kinase receptors designated 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 1) heterodimerizes with ErbB2 and ErbB1, 2) is a key link to the PI3K pro-survival signaling pathway(1), and 3) is activated in cells resistant to ErbB1 or ErbB2 therapeutics(2,3). In this report, a locked Nucleic Acid (LNA)-containing antisense oligonucleotide (LNA-ON), designated EZN-3920, has been used to inhibit the expression of ErbB3(4). 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

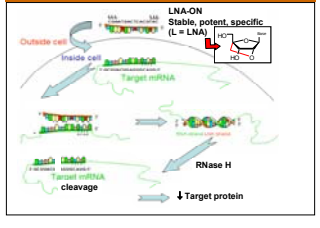
In vitro evaluation

The tumor cells were transfected by EZN-3920 ErbB3 LNA oligo (Santaris Pharma, see poster# 302) with lipofactamine-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 N87 (gastric) tumor xenografts were intravenously injected by EZN3920 on multiple dose regimens. The mRNA and protein levels in liver and tumors were examined by qRT-PCR and western blot, respectively. EZN3046 scrambled oligo served as a control.

Mechanism of Action: LNA-RNA antagonists



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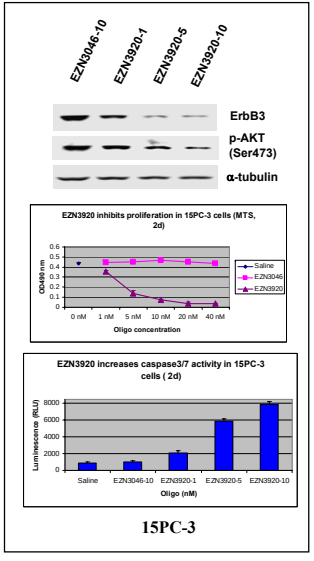
EZN-3920 inhibits ErbB3 expression and cell survival in multiple tumor cell lines

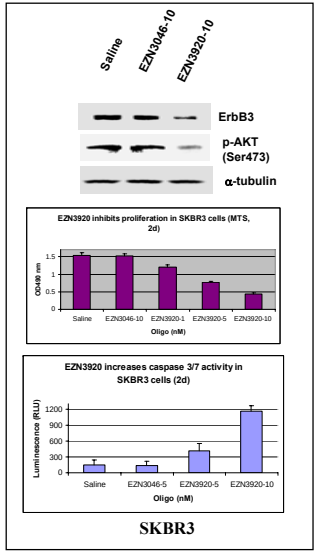
Growth inhibition and target mRNA knockdown by EZN-3920 (IC_{50}, nM)

Cell	15PC3	DU145	A549	H441	H1373	Skbr3	HUH-7	Skov3	A431	HT29	HCT116	N87
Type	prostate	prostate	lung	lung	lung	breast	liver	ovary	epidermoid	colon	colon	stomach
mRNA KD	<2	<2	<2	<5	<5	<5	<1	<2	>5	>10	>10	>10
Growth inhibition	<2	<5	<2	<10	<10	<5	<1	<5	>10	>10	>10	>10

EZN3046 control oligo dose not affect target mRNA and proliferation at 40 nM.

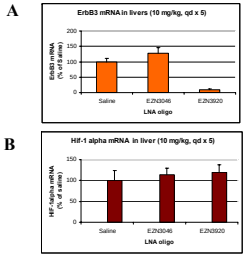
EZN-3920 potently inhibits ErbB3, p-AKT and proliferation, and induces apoptosis in 15PC-3 and SKBR3 cells in vitro



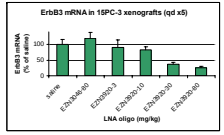


EZN-3920 specifically knocks down target mRNA in liver and tumors in 3 tumor models

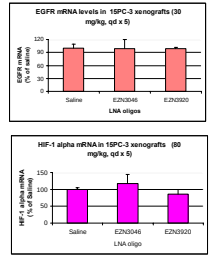
In liver



In tumors



In tumors



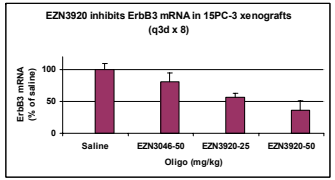
EZN-3920 specifically knocks down ErbB3 mRNA (A), without affecting HIF-1α and EGFR (B, C-control genes) levels in liver and 15PC-3 tumors (qd). Similar KD was observed in A549 and N87 animal tumor models (q3d).

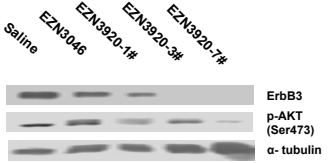
qd × 5: inject oligo daily, 5 doses in all.

q3d: inject oligo every 3 days.

ErbB3 knockdown by EZN-3920 inhibits PI3K/Akt pathway in 15PC3 tumors

ErbB3 mRNA in tumors





EZN-3920 knocks down both ErbB3 mRNA and protein, as well as inhibits phosphorylation of AKT in 15PC-3 tumors at 50 mg/kg (q3d x 8) in the treated animals (#1, #3 and #7).

Conclusions

- EZN-3920 potently knocks 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 prosurvival 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

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(3) Sergina NV, et al. Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature, 2007; 445(7126):437-41.
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