A novel locked nucleic acid oligonucleotide against survivin, EZN-3042, inhibits survivin expression and causes antitumor effects

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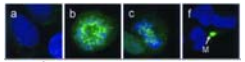
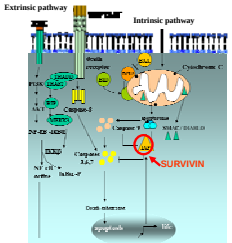
Introduction

SURVIVIN AS THE TARGET

Survivin is a protein with pivotal importance as a functional checkpoint for both mitosis and apoptosis in cancer biology. Since survivin is highly and selectively expressed in numerous cancers and linked to poor clinical outcome [1,2], inhibitors of survivin may have anticancer effects at tolerated doses [3]. A locked nucleic acid (LNA) antisense oligonucleotide (AsODN), EZN-3042 has been identified [4], which down-regulates the survivin mRNA and protein expression in cancer cells. The purpose of this report is to further explore the activity of EZN-3042 in models of cellular proliferation.

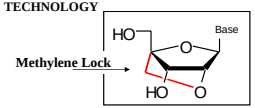


Survivin: High expression during mitosis *Ref.* 5



Survivin: central role in apoptosis pathway

LOCKED NUCLEIC ACID (LNA) PLATFORM TECHNOLOGY



Locked nucleic acid (LNA) monomer

LNA oligonucleotides have a ribose sugar ring in each monomer that is fixed in an RNA-like conformation by the introduction of a methylene bridge. This links the 2'-oxygen and 4'-carbon and gives the attributes of a higher affinity, specificity, and resistance against degradation compared with other oligonucleotides.

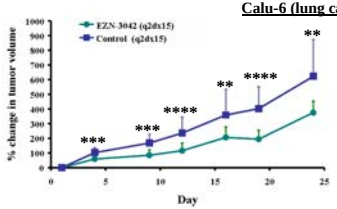
Objective

Here, we report the therapeutic efficacy of EZN-3042 in human cancer xenograft animal models and in a chemical-induced liver regeneration model.

Therapeutic efficacy

Therapeutic efficacy of EZN-3042 as a single agent and in combination with Taxol® (paclitaxel) was evaluated in xenograft models of human lung cancer (Calu-6 or A549). Treatment was initiated when tumors reached an average volume of 75-200 mm³.

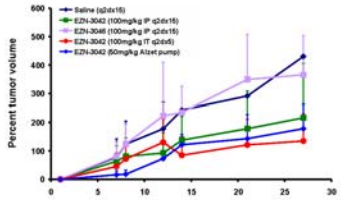
Calu-6 (lung cancer) xenograft

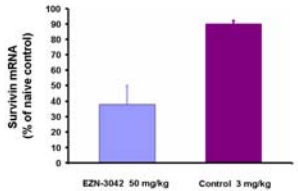


In the Calu-6 model, treatment with 100 mg/kg EZN-3042 q2d × 15 was well tolerated by mice and resulted in 37% tumor growth inhibition (TGI). The tumor volumes were significantly different from controls for the duration of the study ($P<0.01$ to 0.0005).

**$P<0.01$
***$P<0.005$
****$P<0.0005$

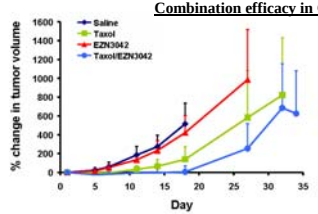
A549 (lung cancer) xenograft

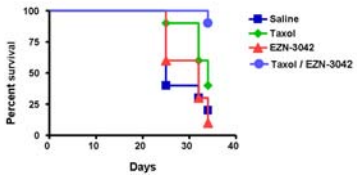




In the A549 xenograft model, EZN-3042 demonstrated significantly better therapeutic efficacy than EZN-3046 (scrambled LNA) and saline. EZN-3042 treatment at 100 mg/kg (q2d x 15 i.p.) resulted in a TGI of 42%. This inhibition was specific, as the tumors treated with EZN-3046 only had TGI of 7%. When EZN-3042 was injected intratumorally or given via a continuous infusion, a TGI of 55% and 45%, respectively, was obtained. Additionally, EZN-3042 treatment as a continuous infusion also resulted in a 61% knockdown (KD) of survivin mRNA, whereas the control showed no mRNA knockdown.

Combination efficacy in Calu-6 (lung cancer) xenograft

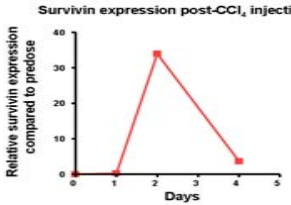


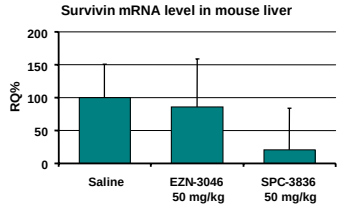


In the combination study in Calu-6 xenograft model, mice were treated with Taxol® 17 mg/kg q4d x 3 (starting on day 1) and/or EZN-3042 100 mg/kg q4d x 3 (starting on day 2). EZN-3042 and Taxol® as single agents produced a respective TGI of 13% and 57%. However, the combination of EZN-3042 and Taxol® demonstrated a significantly better TGI of 83% and also significantly improved the survival over either agents alone. Additionally, the combination group of EZN-3042 and Taxol® significantly improved survival over either agent alone.

mRNA KD in liver regeneration model

The effect of EZN-3042 was also evaluated in a carbon tetrachloride (CCl_4)-induced liver regeneration model.





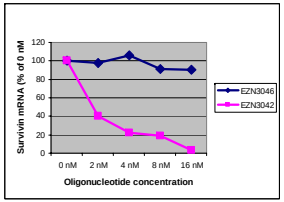
In this model, liver injury was induced by treatment with i.p. CCl_4 injection (1 mL/kg CCl_4 dissolved in olive oil). Expression of survivin mRNA was greatly elevated (34-fold) during the subsequent liver tissue repair (2 days after CCl_4) injection Because EZN-3042 (the LNA antagonist to human survivin) has six mismatches against mouse survivin, a murine surrogate with a perfect match to the same base positions in the mouse gene (SPC-3836) was made and used in this model system.

Treatment with SPC-3836 (murine surrogate of EZN-3042) at 50 mg/kg q12h was initiated 2 days before CCl_4 injection. On day 3, mice were injected i.p. with 1 mL/kg CCl_4 dissolved in olive oil (1:1 v/v). On day 6 (3rd day after CCl_4 treatment), mice were sacrificed, livers were harvested and homogenized, and survivin mRNA levels were analyzed via qRT-PCR. SPC-3836 treatment resulted in 80% knockdown of survivin mRNA. The KD of survivin mRNA achieved by SPC-3836 was specific as the scrambled LNA-oligonucleotide (EZN-3046) had no effect

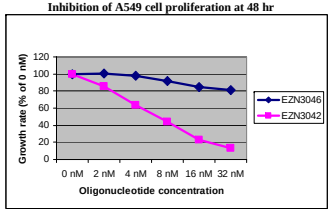
Inhibition of survivin expression and cell growth

EZN-3042 demonstrated potent *in vitro* knockdown of survivin mRNA levels and growth inhibition in several transfected tumor cell lines (A549, Calu-6, DU-145), as measured by qRT PCR and MTS assay, respectively. A representative example in A549 cells is demonstrated below:

Survivin mRNA inhibition in A549 cells at 24 hr



Inhibition of A549 cell proliferation at 48 hr



Indication	Cell Line	Target mRNA knockdown (IC_{50} nM)	Growth inhibition (IC_{50} nM)
Lung cancer	A549	<2	<8
Lung cancer	Calu-6	<2	<4
Prostate cancer	DU-145	<2	~16

EZN-3042 transfected A549 human lung cancer cells exhibited a potent *in vitro* knockdown of survivin mRNA levels (IC_{50} < 2 nM) and significant inhibition of growth (IC_{50} <8n nM).

Conclusions

1) EZN-3042 displayed potent *in vitro* knockdown of survivin mRNA and growth inhibition of several human cancer cell lines (IC_{50} in low nanomolar range).
2) Treatment with EZN-3042 as a single agent or in combination with Taxol® inhibited tumor growth and improved survival in lung cancer xenograft models.
3) In a liver regeneration model, treatment with EZN-3042 resulted in potent and specific knockdown of survivin mRNA.
4) EZN-3042 is a potent and selective LNA –RNA antagonist of survivin in preclinical models and thus merits further evaluation in the clinic.

References

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