LNA Oligo PE Glylation Reaction Yields & PEG-LNA ASO Stability in Rat Plasma

**Objective:**

**Comparison of in vivo target mRNA knockdown by Unmodified EZN-3920 & Pegylated EZN-3920 ASO in Xenografts**

**A. HER3 mRNA Knockdown in Liver**

**B. Tumor Growth Inhibition (TGI) in HCC27 Xenograft Model**

**Conclusions**

This study has demonstrated:

- Pegylated ASO oligonucleotides conjugates with releasable linkers were synthesized efficiently in high yields with comparable chromatographic profiles.
- Pegylation of EZN-3920 LNA oligonucleotide at 5'-end does not perturb hybridization to the RNA complement.
- Pegylated EZN-3920 showed prolonged circulation time and stability in plasma.
- Pegylated EZN-3920 oligonucleotide conjugate shows comparable mRNA target down modulation in the tumor to unmodified LNA oligonucleotide in vivo.
- Pegylated EZN-3920 LNA oligonucleotide at 20 mg/kg shows comparable tumor growth inhibition to unmodified EZN-3920 LNA oligonucleotide at 30 mg/kg in HCC27 tumor xenograft model.

The in vivo effects observed with PegLNA oligonucleotide conjugates may be due to EPR effect within the tumor, which has previously been observed with other Pegylated molecules.

**References**


**Novel Releasable PEG-Conjugates of LNA Antisense Oligonucleotides as Potent mRNA Antagonists to Cancer Targets**

Raj Bandara*, Jianwei Zhao, Melissa Richards, Dechen Wu, Jing Xia, Hong Zhao, Qing Dai, YiXian Zhang and Charles Conover

Enzon Pharmaceuticals, Inc., 20 Kingsbridge Road, Piscataway, NJ 08854

**Introduction**

Locked Nucleic Acid (LNA) antisense oligonucleotides (ASO), which can down-regulate mRNA expression in single digit nanomolar to high picomolar range have emerged as promising therapeutic agents for cancer treatment. Beyond that LNA ASOs have been shown to down-regulate target mRNA without detectable agent in vivo and in vitro, resulting in tumor growth inhibition. To take advantage of accumulation into solid tumors via enhanced permeability retention (EPR), conjugation of single stranded LNA ASO to branched polyethyleneglycol (PEG) with a releasable linker was developed. Here we report an efficient conjugation method in multi gram quantities and high purity without the need for an additional purification step after conjugation to minimize product loss and maximize yield. Purification and loading density of PEG to the PEGL conjugates vary by SEC-MALS and MALDI-TOF mass spectrometry, with the highest loading density of PEG to LNA ASOs that resulted in comparable potency in three different models. PEGL conjugates of LNA ASO resulted in comparable potency across three different models of target mRNA in vitro when compared to the unmodified LNA ASO. The additional PEG conjugates of LNA ASO targeting important cancer targets such as HER3 showed better tumor growth inhibition compared to the same dose observed when compared to the non free of unmodified LNA ASO. The further assessment of releasable PEG linkers for oligonucleotides therapeutic development and other agents such as small molecule drugs, antibody drug conjugates (ADC), peptides and proteins is warranted.

**Hypothesis**

- PEGLylation will prolong circulation time of LNA oligonucleotides in plasma.
- PEGLylation will improve tumor targeting of LNA oligonucleotides via enhanced permeability and retention (EPR).
- Conjugation of LNA oligonucleotides with PEG will extend circulation time in plasma and tumor uptake via EPR may translate to better therapeutic efficacy.

**PEGylation Reaction Scheme & SEC-MALS Analysis**

**Results**

- **PEG-LNA conjugates were purified by UF-DF without additional preparative HPLC to maximize reaction yield**
- In rat plasma 50% of free EZN-3920 ASO is released from Peg-EZN-3920 conjugate in ~18 hours

**Melting Temperature (Tm) of EZN-3920 and Peg-EZN-3920 PEGylation Reaction Yields**

**Objective:** Comparison of Unmodified and Peg-LNA ASO for in vivo Hybridization to RNA Complement

**Procedure:**

- A. Normalized temperature-dependent UV dissociation profiles for the EZN-3920 monitored as a function of concentration.
- B. van’t Hoff analysis of concentration-dependent dissociation of EZN-3920/RNA and Peg-LNA/RNA duplexes.
- C. Determination of concentration-dependent transition temperatures (Tm) for the EZN-3920/RNA and Peg-EZN-3920 RNA duplexes.

**Results**

- Analysis of the temperature dependent dissociation profiles for duplexes of unmodified EZN-3920 and Peg-EZN-3920 with complimentary RNA reveals comparable thermal stabilities (based on above Table, Tm = -6.9 ± 1 °C at CqH = 3.07 PM)
- Unmodified and Pegylated LNA and complimentary RNA duplexes showed comparable van’t Hoff dissociation enthalpies (ΔHv' = 104.3 ± 3.8 Kcal/mol) and van’t Hoff thermodynamic stabilities (ΔGv' = 27.8 ± 0.3 Kcal/mol)

**PEGylation Reaction Scheme & SEC-MALS Analysis**

**References**