Novel customized releasable polyethylene glycol (PEG) linkers improve tumor delivery and down regulation of target mRNA by locked nucleic acid oligonucleotides.

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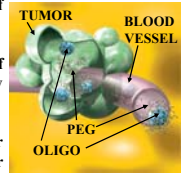
Introduction

Locked nucleic acid (LNA) antisense oligonucleotides (LNA-ONs) represent a new generation of RNA antagonists. Unlike previous chemistry, each LNA monomer contains a methylene bridge between the 2′-oxygen and 4′-carbon of the ribose sugar. This fixes the LNA residue in a favorable RNA-like conformation and enables LNA-ONs to have much higher affinity, specificity, and resistance against degradation compared with other oligonucleotides[1]. While unmodified LNA-ONs have activity in vivo, improved tumor targeting may further enhance efficacy. Previously we reported that simple PEGylation of antisense oligonucleotides using the releasable linkers improve their pharmacokinetic properties [2-3]. To address issue of the LNA-ON delivery, here we have used our Customized linker technology ™ to attach polyethylene glycol (PEG) to LNA-ONs via releasable linkers.

Hypothesis

- PEG will improve circulation time of LNA-ONs.
- PEG will improve tumor targeting of LNA-ONs via enhanced permeability and retention (EPR) phenomenon.
- Improved circulation and tumor retention will translate to better therapeutic efficacy.



Customized PEG-LNA conjugates

LNA Oligonucleotides

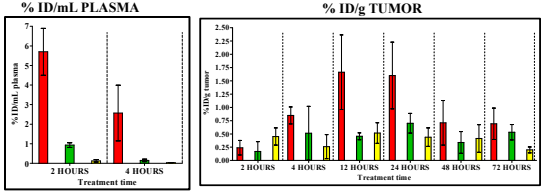
LNA1-ON : anti-survivin LNA
LNA2-ON : anti -erbB3 LNA

PEG-LNA conjugates with LNA2

Compound 1: 40kDa-PEG-LNA1-ON
Compound 2: 10kDa-PEG-LNA1-ON
Compound 3: 40kDa-PEG-LNA2-ON

Biodistribution of PEG-LNA1-ON in Tumor and Plasma

Objective: Compare circulation time and tumor retention of Naked-LNA1-ON vs PEG-LNA1-ON



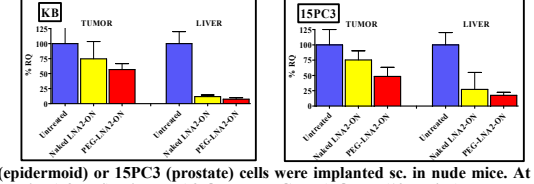
Procedure:
A549 cells were implanted sc. in athymic nude mice. At 75 mm^3 tumor size, the mice were randomly grouped and injected iv. with a single dose of either LNA1-ON or with 10kDa- or 40kDa-PEG-LNA1-ON (10 mg/kg equivalent dose of LNA1-ON). Tissue samples were collected from at the various time points, as indicated. Concentrations of equivalent-LNA1-ON in tumor or plasma were evaluated by an ELISA hybridization.

Results:
➢Compared to Naked-LNA1-ON, 40kDa-PEG-LNA1-ON had:
- much longer circulation time (>50-fold higher LNA1-ON circulating concentration at 2hrs and 4hrs).
- 3-fold more accumulation in tumors at 24 hours.

➢40KDa-PEG conjugates had >3.5 times more tumor accumulation at 12 hrs and maintained ≥1.5 times more accumulation up to 72 hrs compared to 10kDa-PEG conjugates.

PEG-LN2-ON knockdown of target mRNA in tumor xenograft model

Objective: Compare in vivo target mRNA knockdown by Naked-LNA2-ON vs PEG-LNA2-ON

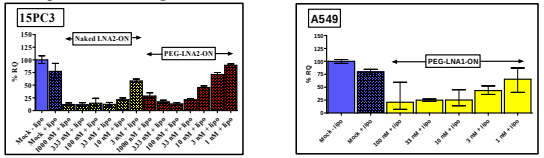


Procedure: KB (epidermoid) or 15PC3 (prostate) cells were implanted sc. in nude mice. At 75 mm^3 tumor size, the mice were injected iv. (q3d x4) with LNA2-ON or PEG-LNA-ON (10 mg/kg). Tumor and liver samples were collected 24 hrs after the last dose and analyzed by qRT-PCR for ErbB3 mRNA knockdown.

Results: In vivo, PEG-LNA2-ON increased tumor knockdown of ErbB3 mRNA by 2-fold in KB and 15PC3 xenografts models. Additionally, in the liver, PEG-LNA2-ON resulted in 83 to 92% knockdown of target mRNA compared to 73 to 88% for Naked LNA-ON.

In vitro efficacy studies of PEG-LNA-ON conjugates in cells

Objective: Compare in vitro target mRNA knockdown of Naked-LNA-ON vs PEG-LNA-ON



Procedure: In vitro, 15PC3 (prostate) or A549 (lung) cells were treated with Naked LNA2-ON or PEG-LNA2-ON or with PEG-LNA1-ON in the presence of lipofectamine, as indicated. The cells were harvested and analyzed by qRT-PCR for ErbB3 mRNA knockdown. The results were compared to untreated cells, with or without lipofectamine.

Results: Naked LNA2-ON, PEG-LNA2-ON or PEG-LNA1-ON resulted in equivalent and potent knockdown of ErbB3 mRNA in 15PC3 cells (IC50 = 5 nM or 8.5 nM, respectively) or in A549 (IC50 = 2 nM). The addition of PEG does not interfere with the potency of Naked LNA2-ON in vitro.

Conclusions

Releasable PEGylation of LNA-ONs enhances the tumor targeting and efficacy of Naked-LNA-ONs. This study has demonstrated:

- PEG-LNA-ON conjugates have higher plasma concentrations and longer circulating times compared to Naked-LNA-ON, with 40kDa-PEG performing significantly better than 10kDa-PEG.
- PEG-LNA-ON conjugates have 3-fold greater tumor accumulation compared to Naked-LNA-ON.
- PEG-LNA-ON conjugate induces 2-fold more target mRNA gene down-modulation in the tumor compared to Naked LNA-ON.
- In vitro, PEG-LNA-ON constructs have IC_{50} values comparable to Naked-LNA-ON, around 3 to 10 nM, in lipofectamine-treated human cancer cells.

The improved in vivo effects observed with PEG-LNA-ON conjugates may be due to the enhanced permeability and retention within the tumor, which has previously been observed with other PEGylated molecules.[4] Customized PEG linkers may enhance the in vivo delivery of RNA antagonists and subsequently improve efficacy.

References

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2. Zhao H, Greenwald RB, Reddy P, Xia J, Peng P. (2005) A new platform for oligonucleotide delivery utilizing the PEG prodrug approach. *Bioconjugate Chem. 16*:758-766.
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