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. 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. 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 into better therapeutic efficacy.

Customized PEG-LNA conjugates

PEG-LNA conjugates with LNA

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

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

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

Procedure:
A549 cells were implanted s.c. in athymic nude mice. At 75 mm³ 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 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-LNA-ON, 40KDa-PEG-LNA-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 s.c. in nude mice. At 75 mm³ tumor size, the mice were injected iv. with LNA2-ON or PEG-LNA2-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-LN2-ON increased tumor knockdown of ErbB3 mRNA by 2-fold in KB and 15PC3 xenografts models. Additionally, in the liver, PEG-LN2-ON resulted in 83 to 92% knockdown of target mRNA compared to 73 to 88% for Naked LNA-ON.

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 conjugates induce 2-fold more target mRNA gene down-modulation in the tumor compared to Naked-LNA-ON.
- In vitro, PEG-LNA-ON conjugates have IC50 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. Customized PEG linkers may enhance the in vivo delivery of RNA antagonists and subsequently improve efficacy.

References