Abstract

Primary Hyperoxaluria Type 1 (PH1) is an autosomal recessive disorder of glyoxylate metabolism. Hepatic glyoxylate detoxification is impaired due to mutation of the AGXT gene, which encodes for the liver peroxisomal alanine-glyoxylate aminotransferase (AGT) enzyme. Loss of AGT function to convert the intermediate metabolite glyoxylate to glycine causes accumulation and reduction of glyoxylate to glycolate which is oxidized to oxalate by the enzyme glycolate oxidase (GO). Excess oxalate in PH1 patients is unable to be fully excreted by the kidneys, leading to the formation and deposition of calcium oxalate crystals in the kidneys and urinary tract. Renal damage is caused by a combination of tubular toxicity from oxalate, nephrocalcinosis and renal obstruction by stones. Greater than 30% of patients advance to end stage renal disease (ESRD). RNA therapeutics are a new class of medicines that work by degrading a specific target mRNA to reduce the production of the disease causing protein. Here we explore the development of an RNAi therapeutic targeting GO using our clinically validated GalNAc-siRNA conjugate platform. Conjugation of siRNA to the sugar N-Acetylgalactosamine (GalNAc) mediates targeted delivery to the Asialoglycoprotein receptor (ASGPR) on the surface of hepatocytes, the major site of oxalate production. Using bioinformatics, we designed a panel of GalNAc-conjugates that were screened in vitro for their ability to silence the GO transcript. The best compounds were evaluated in vivo with subcutaneous dosing to identify the conjugate demonstrating the most potent silencing of liver GO mRNA. Finally, we tested the impact of GO knockdown on oxalate production in a rodent model of PH1. We conclude that liver silencing of GO with an RNAi therapeutic may have potential in the treatment of PH1.

Study overview

AGXT siRNA and
Go-GalNAc or PBS

1% Ethylene glycol in drinking water

Liver AGXT knockdown

D0 D1 D2 D3 D4 D5 D6 D7 D8

24h urine collection

Figure 1. GalNAc-siRNA investigational conjugates as RNAi therapeutics

Asialoglycoprotein Receptor (ASGPR)

• Highly expressed in hepatocytes
• High rate of uptake
• Recycling time ~15 minutes
• Conserved across species

GalNAc-siRNA Conjugates (ALN-TTRsc, ALN-AT3, ALN-CC5, ALN-PCSsc, other programs)

• siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand
• Efficient delivery to hepatocytes following subcutaneous administration
• *Enhanced stabilization chemistry* (ESC) used with ALN-AT3, ALN-CC5, ALN-PCSsc, and other programs
  - Significantly improved potency and durability compared with ALN-TTRsc

Figure 2. Oxalate synthesis in hepatocytes

Regulation of glycolate, the key precursor of oxalate, occurs at multiple cellular sites including the mitochondria, peroxisome and the cytosol. Transport processes are shown as the thin, dark arrows and enzymatic reactions as the thicker, lighter arrows. GO GalNAc-siRNA conjugates targets the enzyme glycolate oxidase (GO), also known as hydroxycitric acid (HCAO1). Silencing of GO1 with subcutaneous dosing should inhibit the production of oxalate and prevent crystal or stone formation in the bladder, urinary tract and kidneys. QMO, D-amino acid oxidase; GCS, glycine cleavage system; GO, glycolate oxidase; GOA, glutamate oxaloacetate aminotransferase; GR, glycolate reductase; HPGA, 4-hydroxy-2-ketogluconate lyase; LDH, lactate dehydrogenase; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolic acid.

Figure 3. In vitro screening of GO GalNAc-siRNA conjugates

(A) 72 GO GalNAc-siRNA conjugates were screened in vitro in primary monkey hepatocytes at 0.1 and 10nM concentrations using Max300. (B) A representative dose response with one of the most active conjugates (#31) from the primary two dose screen is shown. The IC50 was ~19pM.

Figure 4. In vivo evaluation of GO GalNAc conjugates in C57B6 mice. GO GalNAc conjugates were dosed subcutaneously in mice at 10, 5, 2.5 or 1.25 mg/kg and mRNA knockdown in liver was evaluated after 72 hours post dose using qPCR. The single dose EDS0s were approximately 1.25 and 2.5mg/kg for compound A and compound B respectively. In repeat dose studies, conjugates were dosed subcutaneously weekly (QW) for 4 weeks and liver GO mRNA levels were evaluated at 72 hours post the 4th dose. The repeat dose EDS0s were ~0.3mg/kg for both compounds.

Figure 5. Impact of GO knockdown on oxalate production in mouse PH1 model

Urinary oxalate

Time, Day

Urinary oxalate (mg/24h)

Figure 6. In vivo evaluation of GO-GalNAc conjugates in a rat AGXT knockdown model.

(A) To generate the rat PH1 model AGXT siRNA in an LNP was dosed at 1mg/kg intravenously on day 1 and day 7 to maintain knockdown of AGXT in rat liver and 1% Ethylene Glycol was added to the drinking water to further stimulate oxalate production. On day 1 and day 7, some rats were also dosed with a GO GalNAc-siRNA conjugate or PBS control. (B) Quantitation of liver AGXT mRNA levels 72 hours after a single 1 mg/kg dose of AGXT siRNA in an LNP. (C) Levels of urinary oxalate were quantified from 24-hour urines collected from day -1 to 0, day 3 to 4, day 5 to 6, and day 7 to 8. Data was normalized to creatinine to control for the diluteness of the urine.

Summary

GO GalNAc-siRNA conjugates demonstrate robust impact on oxalate in PH1 models

• GalNAc-conjugate RNAi platform is promising approach for knockdown of liver-expressed target genes
• Potent ESC-GalNAc-siRNA conjugates targeting AGXT, identified
  - Single dose EDS0 of 1.25 mg/kg
  - Repeat dose EDS0 of 0.3 mg/kg
• RNAi knockdown of liver-derived AGXT sufficient for therapeutic efficacy in rodent PH1 models
• Significant lowering of urinary oxalate levels
• Increase in urinary glycolate levels upstream of GO suggest robustness on target effect
• Defines innovative and promising therapeutic strategy for the treatment of PH1
• Plan to identify a development candidate by mid-2015 and file IND in 2016

References

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