

#2579

Complex Metabolism and Prolonged PK/PD of a GalNAc-Conjugated Editing Oligonucleotide (EON) in Mice

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OBJECTIVES

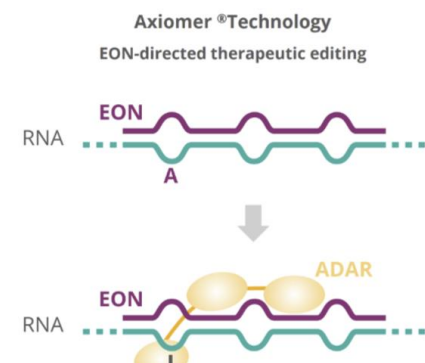
- To determine the plasma and tissue pharmacokinetics of GalNAc-EON11 in mice
- To assess tissue and plasma metabolite profiles

CONCLUSIONS

- Stable EON and GalNAc linker chemistry result in complex in vivo metabolite profile
 - Predominant analyte is EON11 with C6 linker attached (Metabolite 1)
 - Multiple beta-oxidation products of Metabolite 1 also observed in diseased and WT mice (not shown)
- Combined LCMS peak area PK profiles for all analytes are consistent with Hybridization-HPLC data
- Liver half-life of active oligonucleotide is very long, leading to significant accumulation with repeated dosing
- Plasma data are reflective of liver elimination profile, using hybridization-HPLC

BACKGROUND

Editing oligonucleotides (EONs) are single stranded oligonucleotides designed to utilize ADAR (Adenosine Deaminase Acting on RNA) to induce single nucleotide (A to I) changes in mRNA.



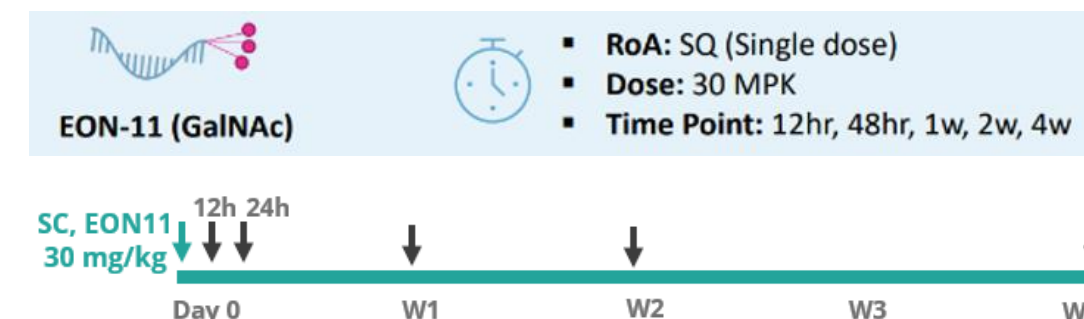
GalNAc-EON11 is a triantennary N-acetylgalactosamine-conjugated EON being explored for the treatment of a liver metabolic disorder.

- EON11 has demonstrated effective editing of the target mRNA in vitro and in vivo in mice.
- 29 NT single stranded oligonucleotide
- 3'- conjugated with GalNAc
- Heavily PS-modified backbone
- High 2'-F content + 2'MOE and 2'OME

STUDY DESIGN

Goals:

- Determine dose and regimen for definitive efficacy study in mouse disease model
- Assess preliminary PK/PD relationship and time-course
- Identify in vivo metabolites in target tissues



Endpoints Measured

- Drug concentration by hybridization-HPLC (Total EON + metabolites).
- Drug Concentration by LC-MS (Conjugated and unconjugated full-length EON + qualitative metabolite profile)
- Metabolite structure ID (High resolution MS)
- Pharmacodynamic endpoints (not shown)
 - mRNA editing
 - Protein functional readout

KEY RESULTS

EON11 was rapidly absorbed in liver, with a long half-life that was mirrored in plasma

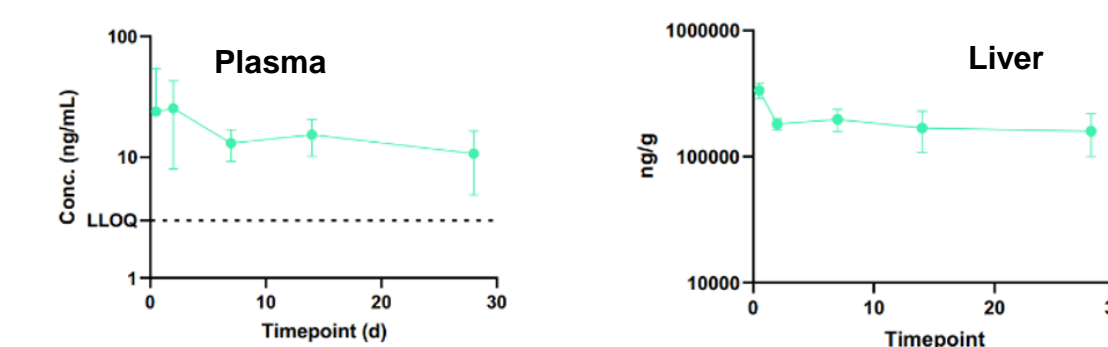
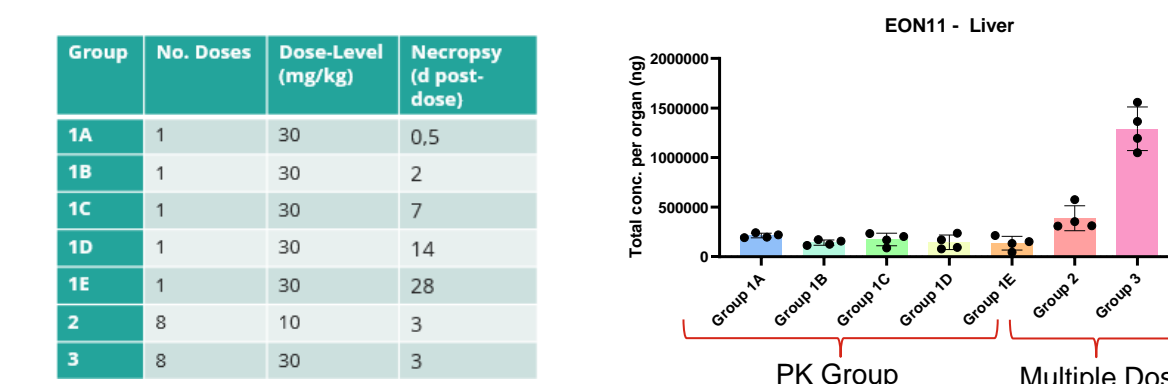


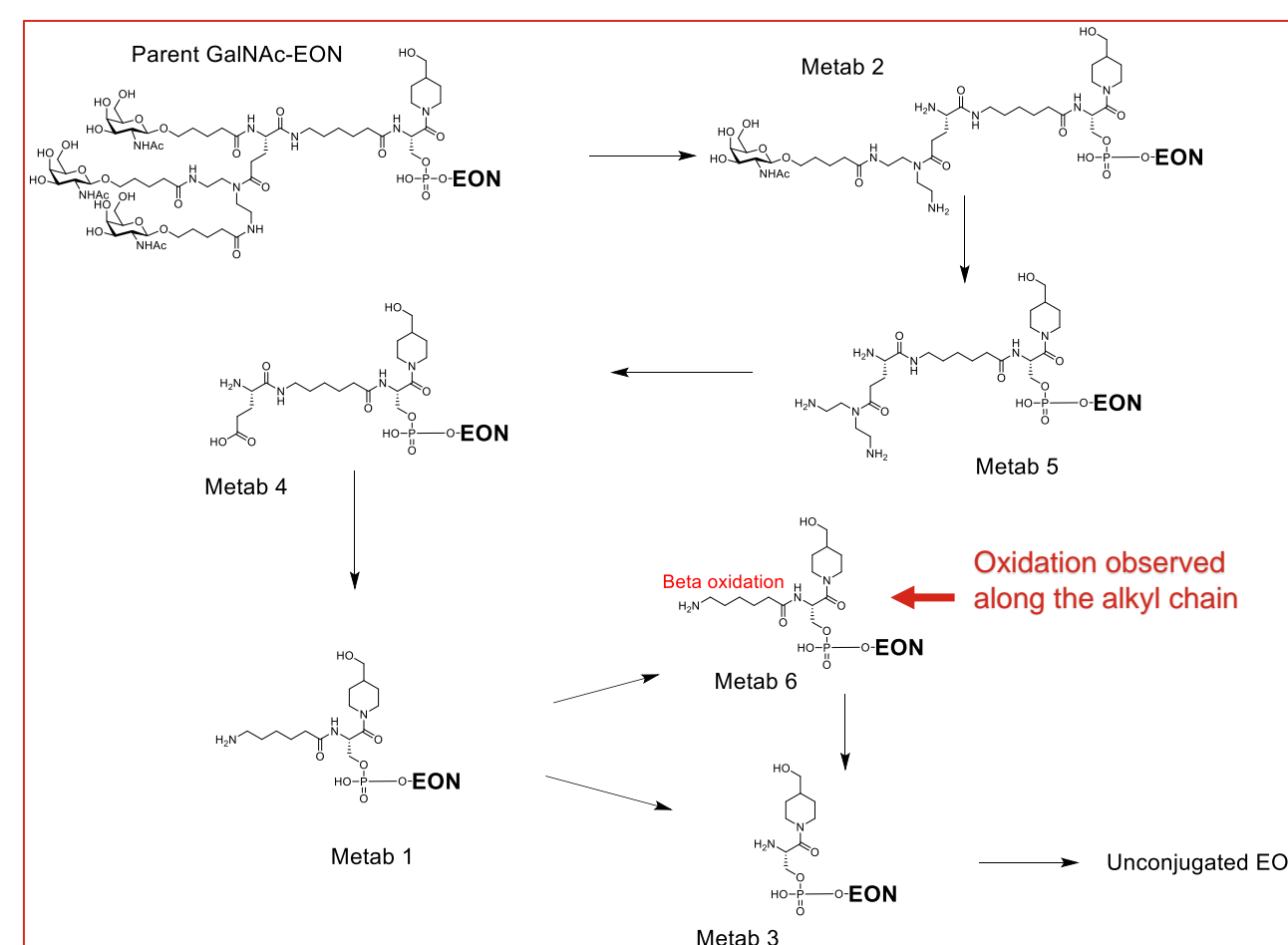
FIGURE 1 – Hybridization-HPLC results

- Plasma PK reflected the long t1/2 observed in liver (>30 days)
- Substantial accumulation in tissues was observed with repeat dosing (Q3d)



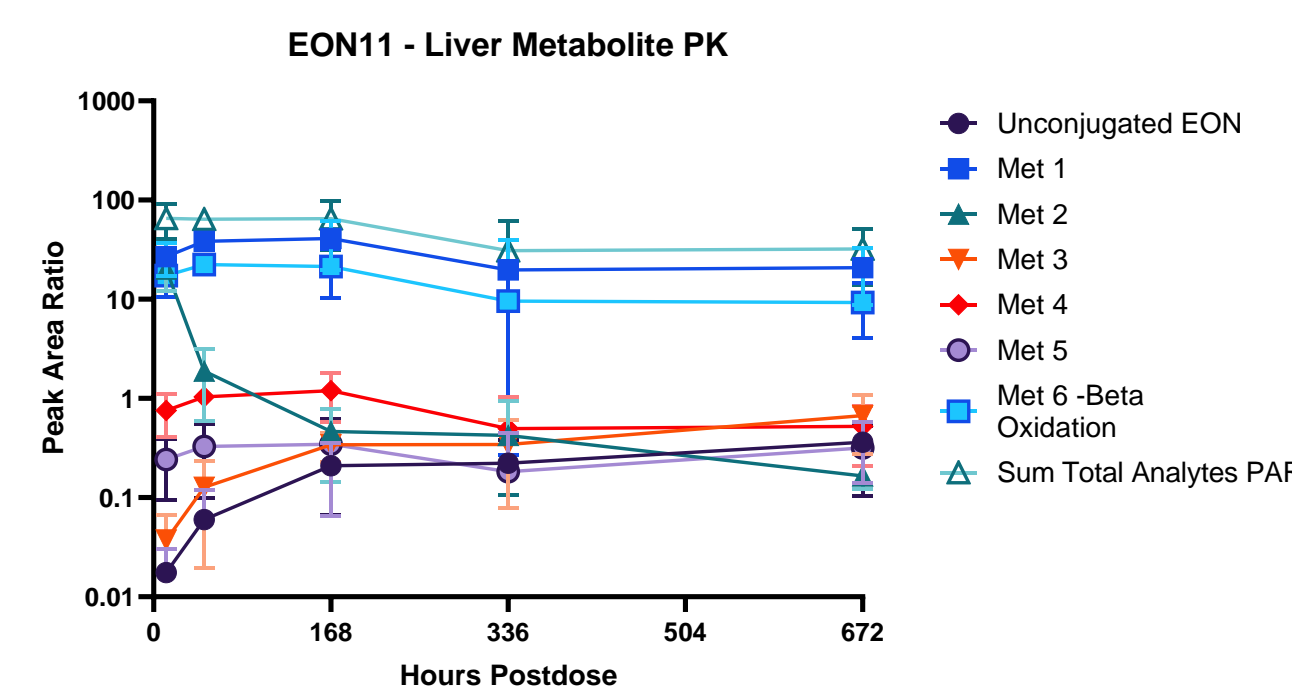
High resolution LC-MS data indicated multiple metabolites of the Lilly GalNAc moiety

In Vivo Metabolites of GalNAc-EON11



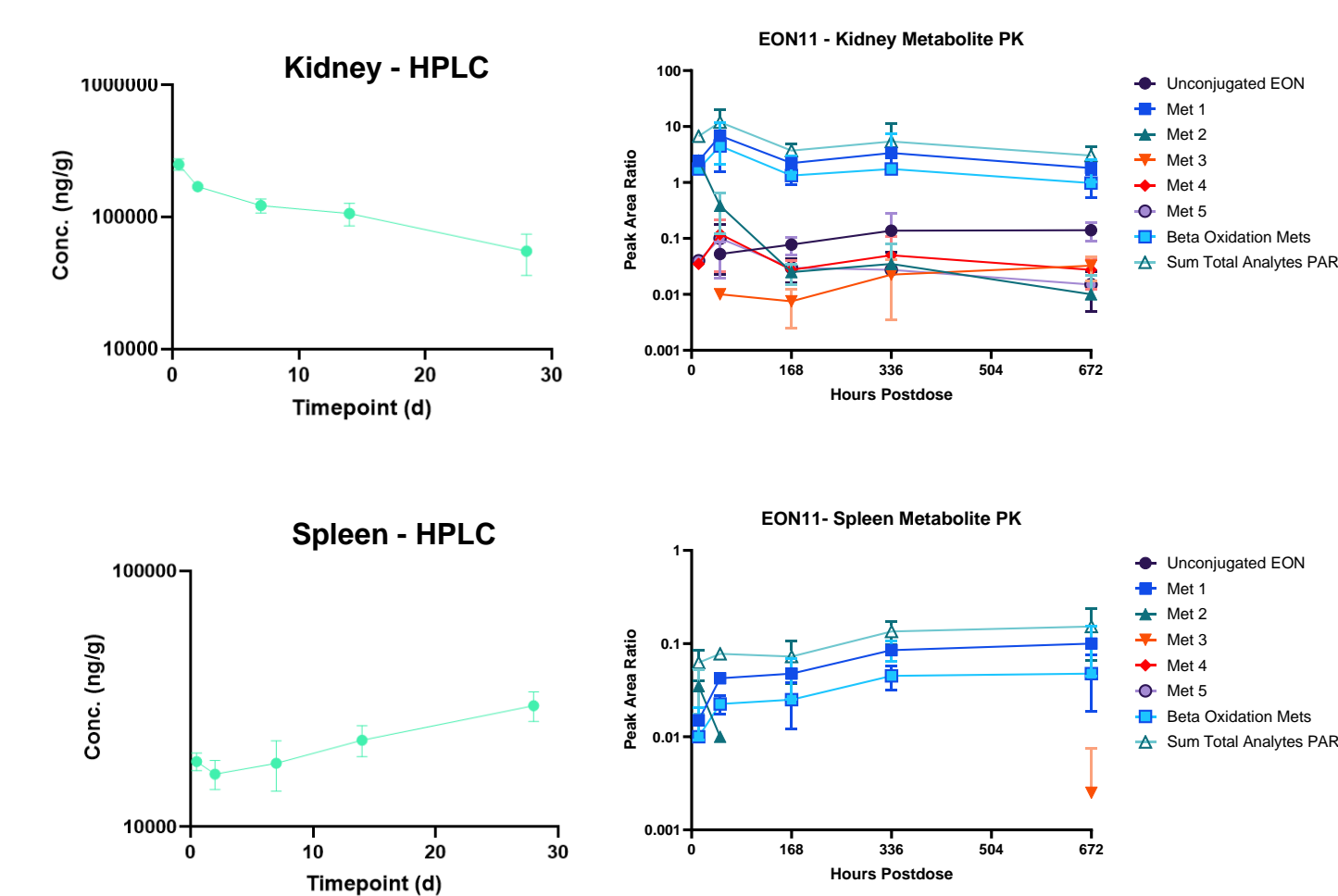
- Profile of metabolites indicates sequential cleavage of GalNAc and linker components remaining intact with the oligonucleotide
- No metabolites of the oligonucleotide portion were observed
- Only fully-conjugated GalNAc-EON was detected in plasma by LC-MS
- Wild-type mice had metabolite ratios similar to disease mouse (data not shown)

Combined profile of total metabolite peaks from LC-MS was consistent with total oligonucleotide HPLC data



The predominant tissue analyte was Metabolite 1 - EON11 with C6-piperidyl linker attached

- No fully intact GalNAc-EON11 was detected in tissues
- Multiple beta-oxidation products of the linker alky chain were observed (combined as Metab 6)
- Time-course profiles of metabolites is consistent with sequential cleavage
- Small amounts of fully unconjugated EON was detected in liver, relative to metabolites



- Profile of metabolites in kidney was similar to liver
- Total analytes in spleen continued to increase over the course of the study, with Metab 1 and Metab 6 observed as the predominant analytes