Preclinical Development of an RNAi Therapeutic Drug Candidate Targeting Hepatitis B Virus

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Abstract
Current HBV therapies, including polymerase inhibitors, effectively reduce viral titers in chronic hepatitis B by inhibiting viral replication, but these therapies fail to eradicate the infection in ~90% of treated patients. Additionally, in the absence of viral replication, high plasma levels of non-infectious, HBsAg-containing subviral particles are thought to mediate immunological tolerance. Reduction in HBsAg plasma levels is the single best predictor of immunological cure. An RNAi therapeutic targeting the HBV genome has the potential to achieve a "functional cure" by effectively decreasing expression of tolerogenic HBsAg, in addition to inhibiting all steps of the HBV life cycle. ALN-HBV, a therapeutic RNAi candidate consisting of a siRNA with Enhanced Stabilization Chemistry (ESC) design conjugated to a GalNAc-based targeting ligand and designed for SC administration has been developed. ALN-HBV effectively targets all viral transcripts as demonstrated by RNaseq. The sequence is pan-genotypic, targeting all major HBV genotypes and resides outside of an integration hotspot making it therefore unlikely to be disrupted by HBV genome integration. A single subcutaneous dose of ALN-HBV results in potent and durable silencing of tolerogenic HBsAg in preclinical models.

Figure 1. Ummet Need in Chronic Hepatitis B Infection

- Chronic HBV infection is a significant Worldwide problem
  - 280 million patients worldwide
  - 250M patients worldwide
  - 25M-285M patients with chronic infection
  - 1/3-50% of patients each in acute/chronic
  - ~90% of acute patients develop chronic infection

Figure 2. Disrupting the HBV Life Cycle by Targeting Viral mRNAs

- Essential for viral life cycle to inhibit any of the viral enzymes
- Prevents viral replication and subsequent spread to other cells
- ALN-HBV effectively targets multiple viral transcripts involved in different steps of the HBV life cycle

3.2 kb partially double stranded DNA genome containing 4 overlapping polycytronic viral transcripts encoding 7 viral proteins.

1. 5'-3' poly-G\(\text{OH}\) tail
2. Transcription of DNA to mRNA is mediated by RNA polymerase II and involves a process of RNA cap formation at the 5' end of the transcript and splicing at internal RNA elements.
3. The mRNA is then transported to the ribosome where it is translated into viral proteins and, in the case of HBV, viral replication.

Figure 3. HBV Genome Structure

- 4 overlapping polycytronic viral transcripts encoding 7 viral proteins
- 3.2 kb partially double stranded DNA genome
- Essential for viral life cycle to inhibit any of the viral enzymes
- Prevents viral replication and subsequent spread to other cells

Figure 4. ALN-HBV Targets all 4 Viral Transcripts in a Highly Conserved Sequence in HBV X

- ALN-HBV targets a sequence that is well conserved across HBV genotypes.
- The ALN-HBV binding site is highly conserved between all 10 HBV genotypes.
- ALN-HBV targets a region that does not overlap with known nucleos(t)ide resistance associated variants (NRAVs).
- ALN-HBV reduces expression of all transcripts in vitro and in vivo.

Figure 5. Reduction of HBsAg in Serum Following a Single Subcutaneous Dose of ALN-HBV

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 6. Reduced HBsAg in Liver After a Single Dose of ALN-HBV

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 7. ALN-HBV Silences All HBV Transcripts In Vivo

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 8. ALN-HBV Has Similar Activity Against All HBV Genotypes

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 9. The ALN-HBV Target Site is Conserved in HCC Samples Where Vrial Integration has Likely Occurred

- ALN-HBV targets a region that does not overlap with known nucleos(t)ide resistance associated variants (NRAVs).
- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 10. ALN-HBV Reduces HBsAg Level in Serum After a Single Dose of ALN-HBV

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 11. Some Polymorphisms in the ALN-HBV Binding Site Impact Silencing and Could Lead to Resistance

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Figure 12. ALN-HBV Binding Site Mutations Are Unlikely to Cause Resistance to Nucleoside Analogs

- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.

Summary
- We have developed ALN-HBV, an siRNA targeting a highly conserved HBV target site.
- ALN-HBV is potent and durable, with an IC50 of 20 pM and an approximately 1 log reduction of HBsAg in vivo.
- Single dose is in silico silencing for over 70 days at 9 mg/kg.
- ALN-HBV reduces expression of all transcripts in vitro and in vivo.
- ALN-HBV reduces the expression of all HBV transcripts by >95%.