Robust Antiviral Activity in Chronic HBV Infected Chimpanzees by RNAi Treatment

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Abstract

Background: Although nucleos(t)ide DNA polymerase inhibitors and interferon effectively reduce viral titers in chronic hepatitis B, these therapies fall to eradicate the infection in ~90% of treated patients. Even in the absence of viral replication, high plasma levels of non-infectious, HBsAg-containing, subviral particles are thought to mediate immunological tolerance, preventing immune mediated control of infection. Reduction in HBsAg plasma levels of >0.5 log is the single best predictor of immunological cure (viral antigen seroclearance and seroconversion to HBsAb+ve status). 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.

Methods and Results: Proof-of-concept pharmacology was generated in chronically-infected chimpanzees (n=4) treated with a siRNA targeting a conserved HBV region formulated as a lipid nanoparticle (LNP). When administered as a single 0.25 mg/kg IV dose, the RNAi therapeutic showed a mean 1.9 log decrease in viral DNA with >2 log reduction in the subject with the highest viral titer. The effects were RNAi-specific as determined with a control siRNA-LNP formulation, and mediated by an RNAi mechanism as detected by 5’RACE. In multi-dose, dose-escalation chimp studies, doses of 0.125 to 0.5 mg/kg achieved mean (and maximum) reductions of 2.9 (>4) log in viral titers and 2.0 (2.3) log in HBsAg. In one animal with >5X elevated ALT levels at baseline, administration of the RNAi therapeutic was associated with LFT normalization. In addition, two animals showed 2-3X ALT elevations ~1-2 months post dosing associated with increases in interferon-gamma and interleukin-6, suggestive of potential “therapeutic flares” related to immune clearance of infected hepatocytes. A therapeutic RNAi candidate, ALN-HBV, consisting of a GalNAc-targeted, Enhanced Stabilization Chemistry (ESC) siRNA conjugate designed for SC administration is being optimized and characterized for activity in vitro and in vivo.

Conclusion: A single siRNA targeting a conserved region in the HBV genome induced specific, potent and durable silencing of HBV viral transcripts and tolerogenic HBsAg. The clinical development strategy for ALN-HBV envisions finite treatment in combination with standard-of-care nucleos(t)ide analogs as a means for inducing a functional cure in CHB patients.
### Background

**Fig 1. Chronic Hepatitis B (CHB) Infection – Unmet Need and Product Opportunity**

**Chronic HBV infection is a significant worldwide problem**
- One third of world population infected
- 400M people with chronic disease
- Most unaware of infection
- High prevalence expected for next 3 decades

**Clinical manifestations severe**
- Chronic inflammation leading to cirrhosis and hepatocellular carcinoma
- Approved therapies are not curative: they reduce viral load, resulting in improved liver histology, decreased progression to cirrhosis and cancer, but do not eliminate the virus
- Tolerability and emergence of resistance limits use

Alnylam gained valuable assets across RNAi platform and programs, including HBV program, through Sirna acquisition in January 2014

**Fig 2. Compact HBV Genome Provides Multiple Opportunities for siRNA Targeting**

- 3.2 kb partially double stranded DNA genome (relaxed circle, rcDNA)
- 4 overlapping viral transcripts encode 7 viral proteins translated across 3 reading frames, and replication intermediate (pre-genomic RNA)
- siRNA is capable of silencing all steps in the viral life cycle, as well as reducing the expression of tolerogenic viral antigens such as HBsAg

**Fig 3. ALN-HBV RNAi Therapeutic for Treating Chronic Hepatitis B Infection**

**Goal:** Develop RNAi therapeutic, which in combination with established HBV treatment, enables a “functional cure”
- Negative for circulating HBV DNA, HBeAg, HBsAg; positive for anti-HBsAg Ab
- Limited duration of therapy ~6-12 months (monthly SC injections)
- Viral escape unlikely with RT inhibitors on board

**Strategy**
1. Select potent, selective siRNA targeting a highly conserved region of the HBV genome (Genotypes A-H)
2. Achieve proof-of-pharmacology with siRNA formulated as a lipid nanoparticle (LNP) in chronically-infected chimps
3. Develop hepatocyte-targeted Enhanced Stabilization Chemistry (ESC)-GalNAc siRNA conjugate
Fig 4. Alnylam’s ESC-GalNAc-siRNA Represents a Significant Advance in SC Delivery

- Hepatocyte-targeting via asialo-glycoprotein receptor (ASGPR)
- Enhanced metabolic stability results in increased potency and prolonged duration
- Wide therapeutic index (>100x) maintained; safe and efficacious in diseased liver
- Benefits: Decreased dose level and dose frequency, low volume dosing, decreased potential for ISR, improved COG
- ESC-conjugate design translates well across multiple siRNAs and targets
- ALN-AT3 Phase 1 data demonstrate translation of potency and duration in humans for ESC-GalNAc-conjugates

Metabolic Profiling in Liver 8 hr Post Dose

Liver Exposure [siRNA] (ng/g)

Efficacy (NHP)

<table>
<thead>
<tr>
<th>Days</th>
</tr>
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<tbody>
<tr>
<td>10</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>30</td>
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<tr>
<td>40</td>
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</tbody>
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Enzymatic cleavage site (thickness reflects frequency of corresponding cleavage products observed)
Methods

1. Identified single siRNA targeting highly conserved region across genotypes A-H
2. Formulated siRNA in a lipid nanoparticle formulation containing novel cationic lipid
3. Evaluated efficacy of HBV-specific siRNA in 4 chronically infected chimps after single IV infusion at 0.25 mg/kg
4. Measured plasma HBV titers, HBsAg and HBeAg, and HBV mRNA silencing, RISC-loading in liver biopsies
5. Demonstrated specificity of action with a control siRNA-LNP and RNAi mechanism by 5’RACE of slicing products
6. Confirmed siRNA site conservation via HBV genotype sequencing, pre and post dose
7. Extended results in follow-up, multiple ascending dose study; 0.125, 0.25, 0.5 mg/kg IV q3wk (n=4 chimps)
8. Determined clinical chemistry and plasma cytokine levels pre/post dosing

Results

Fig 5. Sequence-Specific Antiviral Response in HBV-Infected Chimpanzees

- Starting viral titers ranging from $10^4$ to $10^{10}$ copies/mL in 4 chronically infected chimps
- Mean $1.9 \log_{10}$ decrease in viral DNA on days 2-6 post single 0.25 mg/kg IV dose
- $>2 \log_{10}$ reduction in circulating viral DNA in highest titer animal
- Control siRNA-LNP confirms specificity

![Graph showing sequence-specific antiviral response in HBV-infected chimpanzees](image)
Fig 6. Confirmed RNAi Mechanism in HBV-Infected Chimps

- Robust and durable RISC-loading out to day 14 (last evaluable)
- Confirmation of RNAi mechanism at both days tested
- Sequencing of 5’RACE products confirmed cleavage site

Liver RISC Loading

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
<th>14</th>
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</thead>
<tbody>
<tr>
<td>HBV: siRNA / mi-16</td>
<td>100</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

5’ RACE RNAi Cleavage Products

Expected 182 bp, correct sequence

Fig 7. Sequence-Specific HBsAg Decrease in HBV-Infected Chimps

- HBsAg up to 107 pg/mL in serum
- Mean 0.7 log10 decrease in HBsAg 2-3 weeks post single 0.25 mg/kg IV dose
- Control siRNA-LNP confirms specificity

Absolute [HBsAg]

Normalized [HBsAg]
**Methods and Results:**

**Proof of Mechanism:**

Clinical manifestations severe enough to necessitate treatment:
- Chronic HBV infection is a significant worldwide problem.

**RNAi Design:**
- Clinical manufacturing process for tolerogenic RNAi-
  encoded formulations.
- Metabolic Profiling in Liver.
- Robust Antiviral Activity.
- GalNAc Conjugate
- Enhanced Stabilization Chemistry (ESC) Design
- Represents a Significant Advance in SC Delivery
- Limited duration of therapy (~6 months).

**Virologic Efficacy in HBV Rodent Models:**
- Hepatitis B surface antigen (HBsAg) negative for circulating HBV DNA.
- HBsAg+ HBV RNA+ infected tissue.

**Tolerability and emergence of resistance limits use:**
- **Results:**
  - ALN: Reduction in viral antigens such as HBeAg, HBxAg.
  - ALN: Activation of the PD1/PDL1 immune checkpoint is associated with viral clearance.

**Fig 1.**
- RNAi Levels
- Liver RISC Levels
- 0.125 mg/kg on day 0
- 0.25 mg/kg on day 21
- 0.50 mg/kg on day 42
- IV, n=4

**Fig 2.**
- Liver siRNA Levels (nmol/g)
- Days 2, 23, 44
- 0.125 mg/kg
- 0.25 mg/kg
- 0.50 mg/kg

**Fig 3.**
- Viral RNA Silencing
- Relative to Day -21 biopsy
- PCR site
- 2205
- 2535
- 3030
- 647
- 1620
- 1609

**Fig 4.**
- Plasma Concentration (pM)
- Low, Intermediate, High
- Treatment
- Fold Change
- ΔCt (Log2 Fold Change)
- Time (days, 48 hr post-dose)

**Fig 5.**
- Liver PK
- Homology
- Sequence
- On/Off Target
- Reporter Cell Line Silencing
- 0.1 to 1

**Fig 6.**
- AD-65403/AD-66110
- CHB
- Full therapeutic activity
- PCR site
- 2205
- 2535
- 3030
- 647
- 1620
- 1609

**Fig 7.**
- AD-65403/AD-66110
- HDV
- Full therapeutic activity
- PCR site
- 2205
- 2535
- 3030
- 647
- 1620
- 1609

**Fig 8.**
- Dose-Dependent Liver [siRNA], RISC Loading and Viral RNA Silencing After Ascending Doses in HBV Infected Chimps
Mean 2.9 log10 decrease in viral DNA day 2-6 post 0.5 mg/kg dose; >4 log10 reduction in circulating viral DNA achieved in highest titer animal

Mean 2.0 log10 reduction in HBsAg at 0.5 mg/kg dose; up to 2.3 log10 reduction achieved

**Fig 9. Dose-Dependent Antiviral Response in HBV-Infected Chimps Following Multiple Ascending Doses**

**Fig 10. ALT Normalization for Chimp with Most Robust Virologic Response; Potential Indication of Therapeutic Flare**

- High baseline ALT reversed in highest titer chimp
- 2x ALT increase post treatment in 2/4 chimps, possible therapeutic immune flare
- Includes increases in IL6 and IFNγ
On Track for ESC-GalNAc-siRNA Conjugate DC Selection in late 2014 for IND at ~ YE 2015

Enhanced Stabilization Chemistry (ESC)
Same target sequence, multiple chemical modifications

Reporter Cell Line Silencing

Viral RNA RT-PCR

Secreted HBsAg ELISA

HepG2.2.15 Cell Silencing

Liver-Restricted Immune Reactivation - ALN-PDL
- Obligate liver pathogens exploit the tolerant liver environment, further enhancing immune suppression via multiple mechanisms
- Activation of the PD1/PDL1 immune checkpoint is associated with establishment and maintenance of chronic infections
- Silencing liver PDL1 would enhance NK and T-cell activity against infected hepatocytes, while reducing the risk of broad systemic tolerance suppression
- POC studies conducted in a mouse model of Adenoviral infection demonstrated that PDL1 silencing was associated with increased viral clearance

Chronic Hepatitis D – ALN-HDV
- Co-/super-infection with HBV: delta virus relies on HBV S antigen for liver tropism and infectivity
- Severe clinical manifestations: cirrhosis develops in 70-80% of cases within 5-10 yr (3X higher risk and younger onset vs. CHB); mortality ~2-20% (10x higher than CHB)
- 15-20 M patients infected WW; 80 K patients in US

Dual RNAi therapeutic approach: ALN-HBV + ALN-HDV
- ALN-HBV: S antigen silencing will inhibit HDV life cycle
- ALN-HDV: direct anti-HDV effects by silencing RNA genome, anti-genome and viral transcripts

Adeno-Ova viral infection

Dolna et al Molecular Therapy Nucl Acids 2013, 2 e72
Conclusions

- Significant unmet need exists for novel HBV therapies resulting in a “functional cure”
- HBV RNAi therapeutic offers significant promise via a novel mechanism: silencing viral transcripts will inhibit all steps of the viral life cycle (replication, assembly, secretion of virus) and decrease the production of tolerogenic viral antigens, including highly abundant non-infective HBsAg particles
- Proof of concept data in naturally HBV-infected chimps suggests robust efficacy profile
  - Significant multi-log decrease in circulating virus and HBsAg
  - Confirmed specificity of effect and proof of RNAi mechanism
- ESC-GalNAc-siRNA conjugate against HBV enables hepatocyte-specific delivery, subcutaneous dosing, minimal toxicity and broad genotypic coverage
- ALN-HBV Development Candidate selection (DC) late ’14; IND ~YE ’15
- Multiple opportunities exist for RNAi drugs against Hepatitic Infectious Diseases. Initial emphasis on direct acting targets for HBV and HDV, and immune checkpoint blockade