Small Interfering RNAs

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Conflicts of Interest

- Gilead Sciences Inc
- Arrowhead Pharmaceutical
- SpringBank Pharmaceutical Inc
- AbbVie
- Abbott Diagnostics
RNAi Mechanism

1. Short interfering ds RNA can lead to transcriptional silencing (if homologous) and translational repression (if mismatched).

2. Involves Drosha and Dicer enzymes, the RNA-induced silencing complex (RISC) and the nuclease Ago.

3. Interference mediated by small RNA fragments ~21-25 nucleotides

HBV genome and siRNA target sites

- **HBV mRNA**
  - 3.5 kb pre-genomic RNA
  - 3.5 kb pre-core mRNA
  - 2.4 kb pre-S1 mRNA
  - 2.1 kb pre-S2/S mRNA
  - 0.7 kb X mRNA

- **HBV proteins**
  - Polymerase (with reverse transcriptase function)
  - Core (HBcAg), forms capsid
  - E antigen (HBeAg), also called pre-core, a secreted protein
  - Large, middle and small surface proteins (HBsAg), form envelope
  - X protein (Transactivator)

HBV life cycle and therapeutic intervention with NUCs or RNAi
A cccDNA centric model

NUC = reverse transcriptase inhibitors such as entecavir and tenofovir

Wooddell C, Schluep T, Given B. With permission, 2016
Groups Involved in RNAi Therapy and HBV

• Arrowhead Pharmaceuticals
  – ARC-520 (phase 2)
  – ARC-521 (phase 1/2)

• Arbutus Biopharma
  – ARB-1467 (phase 1/2)

• Alnylam Pharmaceuticals
  – ALN-HBV (phase 1)
ARC-520 for chronic HBV infection

ARC-520 consists of 2 vials

- Vial 1: ARC-520 Excipient
  - contains a masked, hepatocyte-targeted peptide (NAG-MLP) that aids in the delivery of the HBV chol-siRNAs.

- Vial 2: ARC-520 API
  - contains the HBV chol-siRNAs.

- The liquid in Vial 2 is used to dissolve the powder in Vial 1, resulting in ARC-520 for Injection (IV)

- DPC and the chol-siRNAs are targeted to the liver. When they are in the same endosome, the DPC facilitates chol-siRNA escape resulting in RNAi.

Wooddell et al, Mol Ther 2013 May; 21(5) 973-85
Dynamic Polyconjugate (DPC) Technology for siRNA Delivery \textit{in vivo}

- DPC polymer composition and physical characteristics
  - amphipathic peptide
  - peptide amines reversibly “masked” with CDM
  - slightly negatively charged

- cellular uptake of peptide is ligand-driven (N-acetyl galactosamine (NAG)) for hepatocytes)

- siRNA is made liver tropic by attachment of lipophilic ligand (e.g. cholesterol)

- \(\downarrow\) pH in endosomes drives peptide unmasking

- unmasked peptide disrupts endosomal membrane

- siRNA released to cytoplasm

Rozema, DB et al 2007. Proc Natl Acad Sci(USA);104:12982
RNAi treatment for chronic Hepatitis B

siRNA design and in vitro screening

- Designed 140 siRNAs targeting conserved regions of HBV genotypes A-D
- Confirmed conservation in genotypes E-H as well.

- Screened candidate siRNAs in a cell culture system
- 4 highly potent siRNAs chosen for further testing in animal models
- siHBV-74 and siHBV-77 chosen as leads

Wooddell C, Schluep T, Given B. With permission, 2016
Effect of ARC-520 on HBV core antigen expression in livers of HBV transgenic mice

Anti-HBcAg immunostain

Isotonic glucose  siControl  ARC-520

Strong reduction of core antigen in all liver hepatocytes in animals receiving ARC-520

Wooddell et al, Mol Ther 2013 May; 21(5) 973-85
HBV RNAi Program
Product ARB-1467 in Phase 2 Trials

The primary goal of ARB-1467 is to facilitate the loss of HBV surface antigen (HBsAg) in chronic HBV patients by:

- **Reducing levels of HBsAg** by inhibiting production (vs. blocking secretion)
- HBsAg promotes host immune tolerance of virus
- Removal should promote immune recognition and viral clearance

Kindly provided by Dr Mike Sofia
ARB-1467 Targets Multiple HBV Genomic Sites

- Primary viral target is HBsAg
- Target sites are regions of high conservation in HBV viral genomes
- Advantages of the **3-trigger combo:**
  - Increased potency
  - Coverage extension to 99.8% of HBV genotypes
  - Targets all HBV transcripts and prevents production of all antigens
  - 1 trigger directly targets the sAg coding region

Kindly provided by Dr Mike Sofia
ARB-1467 Reduces HBsAg in Multiple Genotypes

Kindly provided by Dr Mike Sofia

Primary Human Hepatocyte Model
**ARB-1467 Reduction in Multiple HBV Markers**

**HBV-Infected Humanized Mouse Model**

- Strong inhibition of HBsAg and HBeAg
- Viral DNA and cccDNA are also reduced by ARB-1467
In Vivo Combination Studies

ARB-1467 Complements NUC Standard of Care

**Serum HBV DNA**
- Saline
- Entecavir, daily oral
- ARB-1467, weekly iv
- Entecavir + ARB-1467

**Serum HBsAg**
- Saline
- Entecavir, daily oral
- ARB-1467, weekly iv
- Entecavir + ARB-1467

Weekly LNP

Daily Entecavir Treatment

Kindly provided by Dr Mike Sofia
**In Vivo Combination Studies**

RNAi Product ARB-1467 with Capsid Assembly Inhibitor AB-423

- Hydrodynamic injection mouse model
- AB-423 given BID for one week, ARB-1467 given on Day 0 only

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**Serum HBV DNA**

(% Day 0 Baseline)

**Day**

- Saline
- Vehicle
- AB-423 100 mg/kg
- ARB-1467 0.1 mg/kg
- AB-423 + ARB-1467

Kindly provided by Dr Mike Sofia
Alnylam RNAi (Preclinical)

- Delivery: **Multi-component lipid nanoparticles** for delivery to the liver via LDL receptor
- Triantennary Gal/Nac conjugated to 3’ end of sense strand of siRNA
- Two target regions:
  - 0.7 kb region overlapping across all 4 HBV transcripts.
  - 1.4 kb region overlapping across 3 transcripts
- Inhibits replication, assembly and secretion of virus as well as subviral antigens that overlaps across 3 HBV transcripts

http://www.alnylam.com/
Alnylam (ALN-HBV)

- effective in rodent HBV models when administered subcutaneously (SC)
- potent and durable knockdown of HBsAg (>1.6 log IU/ml) with a single dose
- multiple doses resulted in durable knockdown lasting over 4 months (3 weekly doses at 3 mg/kg)
- 2.3 log reduction in HBsAg in chronically HBV-infected chimpanzees
- well tolerated in 13 week GLP toxicology (rat and non-human primates)
- clinical studies aiming for once monthly dosing, SC
- clinical data expected mid-2017

http://www.alnylam.com/
Treatment of chimps with RNAi therapeutic ARC-520

- **Chimps**
  - 5 males, 4 females
  - 9-37 years old, HBV infected mostly since birth
  - 5 HBeAg+, 4 HBeAg- (1 became HBeAg- during NUC lead-in)

- **Treatment**
  - Daily oral NUCs
  - Up to 4 mg/kg ARC-520 dosed monthly

- **Monitor safety and efficacy**
  - Regular blood collection and periodic liver needle biopsies

Woodell C, Schluep T, Given B. With permission, 2016
HBsAg reduction correlated with HBeAg status

- Similar phenomenon was observed in human HBV patients
- What accounts for the difference in response between HBeAg positives vs. negatives?

Woodell C, Schluep T, Given B. With permission, 2016
Sustained response 31 weeks off all therapy

- HBeAg-negative and anti-HBe positive
- Final HBV serum DNA $5 \log_{10}$ fold lower than at pre-study
- Final HBsAg $1.7 \log_{10}$ fold lower than at pre-study
- Liver HBV RNA 99% lower than at pre-study

Wooddell C, Schlup T, Given B. With permission, 2016
Sustained response 31 weeks off all therapy followed ALT flare and coincided with T-cell responsive cytokine signals.

Chimp A4A014

- HBsAg in serum (µg/mL)
- ALT (U/L)
- INF γ (Luminex)
- TNFα (Luminex)
- CXCL9 (Luminex)
- CXCL10 (Luminex)

Wooddell C, Schluep T, Given B. With permission, 2016
ARC-520 Produces Deep and Durable Knockdown of Viral Antigens and DNA in a Phase II Study in Patients with Chronic Hepatitis B

HBV antigen reduction in ETV experienced HBeAg-positive patients with a single 4 mg dose (cohort 5)

HBsAg reduction in ETV naïve patients with a single 4 mg dose (cohort 7)

Direct antiviral effect lasted up to 57 days after a single dose of ARC-520, delayed response duration >85 days

- Small dose-related reduction in HBsAg
- Maximum effective dose not reached
- HBV DNA results pending in ETV naïve patients

Yuen M-F, et al. AASLD 2015, San Francisco. #LB-9
ARC-520 RNAi: clinical responses

**NUC naïve cohort (n=12):** 50% HBeAg positive, 1x 4mg dose

**HBsAg:**
- > 1log drop in HBsAg achieved by all HBe pos subjects (excluding 702; transitional HBe <0.1 PE IU/mL at BL)

**HBeAg:**
- > 1log decline in HBeAg achieved
Two Predictive Biomarkers of Functional HBV cure

HBsAg epitope mapping 19plex immunoassay to identify a Clearance Profile (CP) predictive of HBsAg clearance

**ASSAY 1**

1. Magnetic bead
2. Capture Ab: 19plex mouse anti-HBs mAbs to HBsAg ‘a’ determinant
3. Patient HBsAg sample
4. Reporter Ab: HRP conjugated Goat anti-Human IgG Fc

Complexed HBsAg/anti-HBs must be present in the tested sample to get reporter binding (absorbance)

Walsh R, et al. AASLD 2015

Immuno-detection of the developing anti-HBs response (complexed to HBsAg)

**ASSAY 2**

1. Magnetic bead
2. Capture Ab: mouse anti-HBs mAbs to control epitopes (C-term, Combo Loop1/2)
3. HBsAg/anti-HBs complexed (*patient sample*)
4. Reporter Ab: PE conjugated polyclonal anti-HBs

Walsh R, et al. EASL 2016

Assays validated against G103 cohort, TDF registration (Marcellin, P. et al. 2008. NEJM 359, 2442)
In a Treatment Naïve Cohort of Genotype A Chronic Hepatitis B (CHB) Patients Receiving Tenofovir Disoproxil Fumarate (TDF) Therapy (TF103 Trial):

**HBsAg clearance profile (CP)**

HBsAg epitope pressure (reduced recognition) at *both* loop 1 and loop 2 epitopes
- associated with HBsAg response/decline (>1 log) and potentially HBsAg loss/seroconversion

**HBsAg non-clearance (or escape) profile (NCP)**

No change in HBsAg epitope profile, OR reduced epitope binding at *only* one loop
- associated with no HBsAg response/decline (<1 log)

**Conclusion/Findings**

Significant association (p <0.02) between the development of a HBsAg CP and HBsAg Loss/Seroconversion [PPV 83%] by 48 weeks of treatment

*Walsh, R and Locarnini, S (2015), AASLD presentation*
## Synopsis: ARC-520 effect on HBsAg CP

### Identification of an HBsAg CP during the ARC-520 treatment cohorts trials 1-5:

<table>
<thead>
<tr>
<th>Cohort</th>
<th>HBeAg</th>
<th>BL (pre-treat)</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
<th>W4</th>
<th>W6</th>
<th>W8</th>
<th>W12</th>
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### Cohorts 1-5 BL (pre-treat)

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<th>BL (pre-treat)</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
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<td>2</td>
<td>0</td>
<td>1</td>
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</table>

| p-value | 0.730 | 0.038 | 0.019 | 0.003 | 0.145 | 0.007   | 1.000 | 1.000 |

There is a significant association between development of a HBsAg CP and ARC-520 RNAi treatment at multiple timepoints.

HBsAg reduction correlated with HBeAg status

Similar phenomenon was observed in human HBV patients

What accounts for the difference in response between HBeAg positives vs. negatives?
Novel finding: Predominant liver HBV DNA differs in HBeAg neg and HBeAg pos chimps

Liver biopsy at initiation of ARC-520 treatment revealed:

- Most HBV DNA in liver of HBeAg positive is cccDNA
- 500-fold less cccDNA in HBeAg negative animals
  - Only 5% of total HBV DNA in liver in HBeAg negative was cccDNA and total HBV DNA levels were *not* affected by NUCs

- **HBV DNA profile in HBeAg negative chimps is consistent with a high proportion of integrated HBV DNA**
The origin of linear HBV DNA during replication

Significant HBsAg mRNA can be produced from integrated HBV DNA
- These S transcripts contain complete HBsAg CDS
- Expected loss of ARC-520 target sites

HBV DNA integration events were detected in both HBeAg+ and HBeAg- chimps (Targeted DNA-sequencing Analysis)

- Integration in both HBeAg+ and HBeAg- chimps
- Integration hotspot near DR1 region

Woodell C, Schluep T, Given B. With permission, 2016
Fewer transcripts with HBV poly(A) signal in HBeAg- vs HBeAg+ chimps.

In HBeAg- chimps, frequency of reads is reduced in region near DR1, known for high frequency integration.

Are these transcripts coming from integrated HBV DNA?

Woodell C, Schluep T, Given B. With permission, 2016
HBV transcripts in HBeAg+ vs. HBeAg- chimp s prior to ARC-520 treatment

PacBio Single Molecule Real-Time (SMRT) Sequencing

HBeAg+
- Mostly non-fusion transcripts terminating near HBV poly(A) signal as expected

HBeAg-
- Mostly fusion transcripts encoding HBsAg with cryptic poly(A) signal at 3' end
- Fusion points typically between DR2 and DR1 as expected if transcripts arose from integrated dslDNA

Wooddell C, Schluep T, Given B. With permission, 2016
siRNA designed to target RNA derived from HBV integration products in HBeAg- chimps

- siHBV-i targets HBV RNA even if expressed from integrated HBV DNA
- siHBV-i gave deep reductions in HBsAg in HBeAg- chimps, similar to those observed using ARC-520 in HBeAg+ chimps

Woodell C, Schluep T, Given B. With permission, 2016
Conclusions

• ARC-520 well tolerated after multiple doses up to 4 mg/kg (highest dose tested)

• treatment with ARC-520 reduced HBsAg in all chimps
  – greatest response in HBeAg positive chimps: up to 2.7 log reduction
  – lower response in HBeAg negative chimps: up to 0.9 log reduction

• integrated HBV DNA is likely a significant source of HBsAg, especially in HBeAg negative chimps
  – liver HBV DNA profiles differ between HBeAg positive versus HBeAg negative chimps
  – HBV RNA profiles in HBeAg negative chimps consistent with transcripts arising from dsDNA

• siRNA targeting integrant-derived transcripts result in deep HBsAg reduction in HBeAg negative chimps
Summary

Key Virological Findings for ARC-520

- Direct antiviral effect on serum HBsAg, HBeAg, and HBcrAg levels which are substantial

- HBeAg-Pos CHB and HBeAg-Neg CHB have very different viral patho-physiologies

- This has important therapeutic and prognostic significance