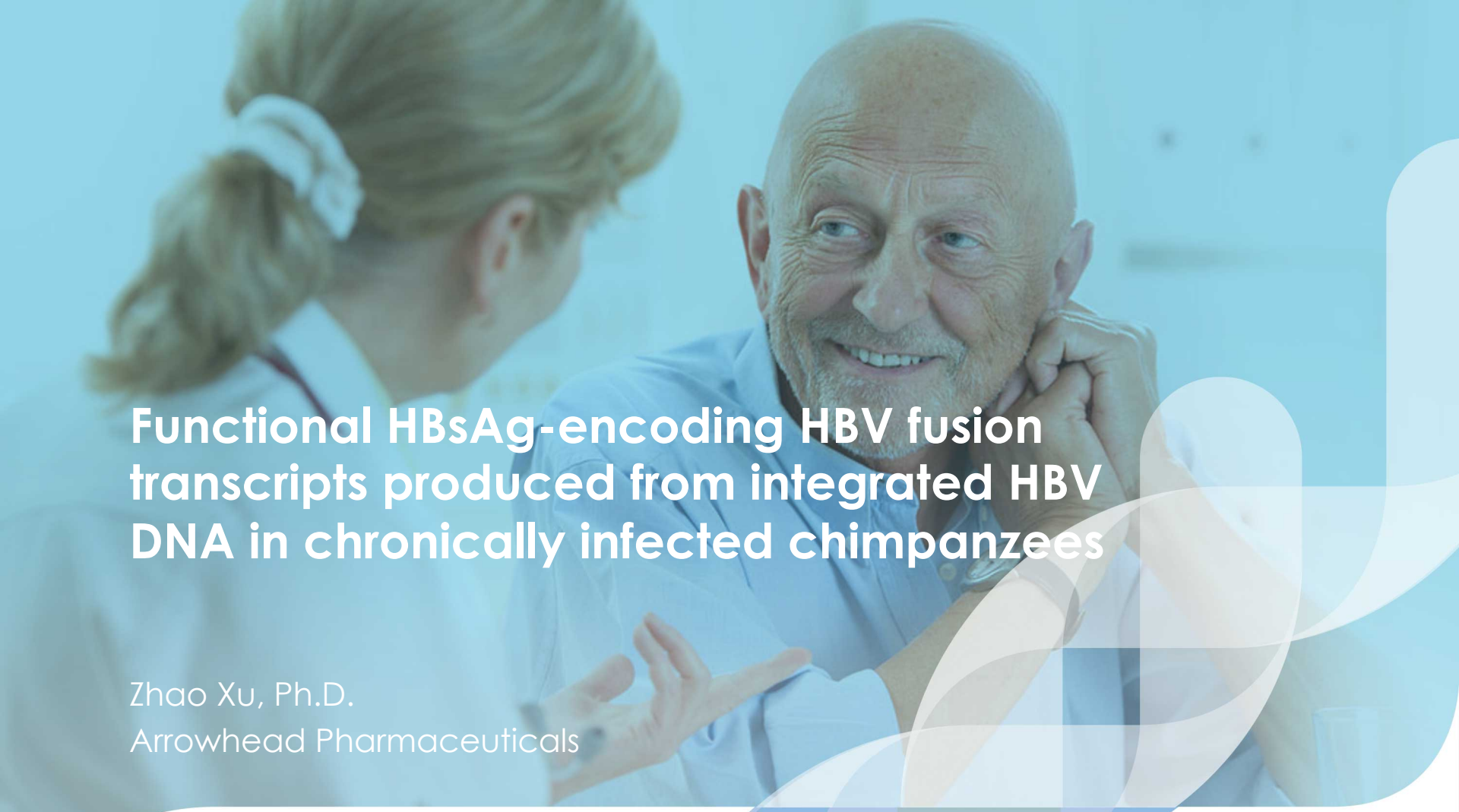

Functional HBsAg-encoding HBV fusion transcripts produced from integrated HBV DNA in chronically infected chimpanzees

Zhao Xu, Ph.D.
Arrowhead Pharmaceuticals

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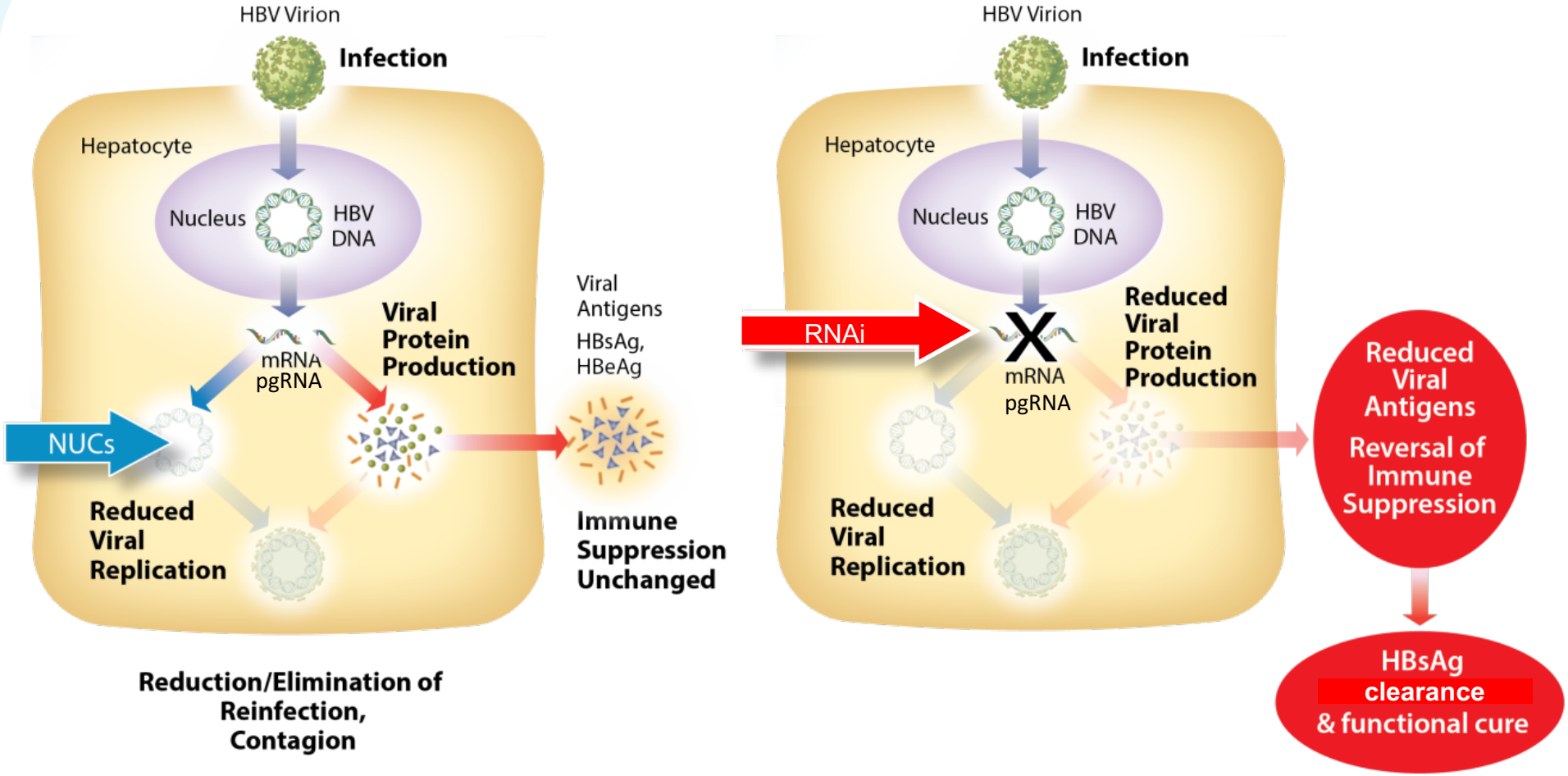
HBV life cycle and therapeutic intervention with NUCs or RNAi
A cccDNA centric model

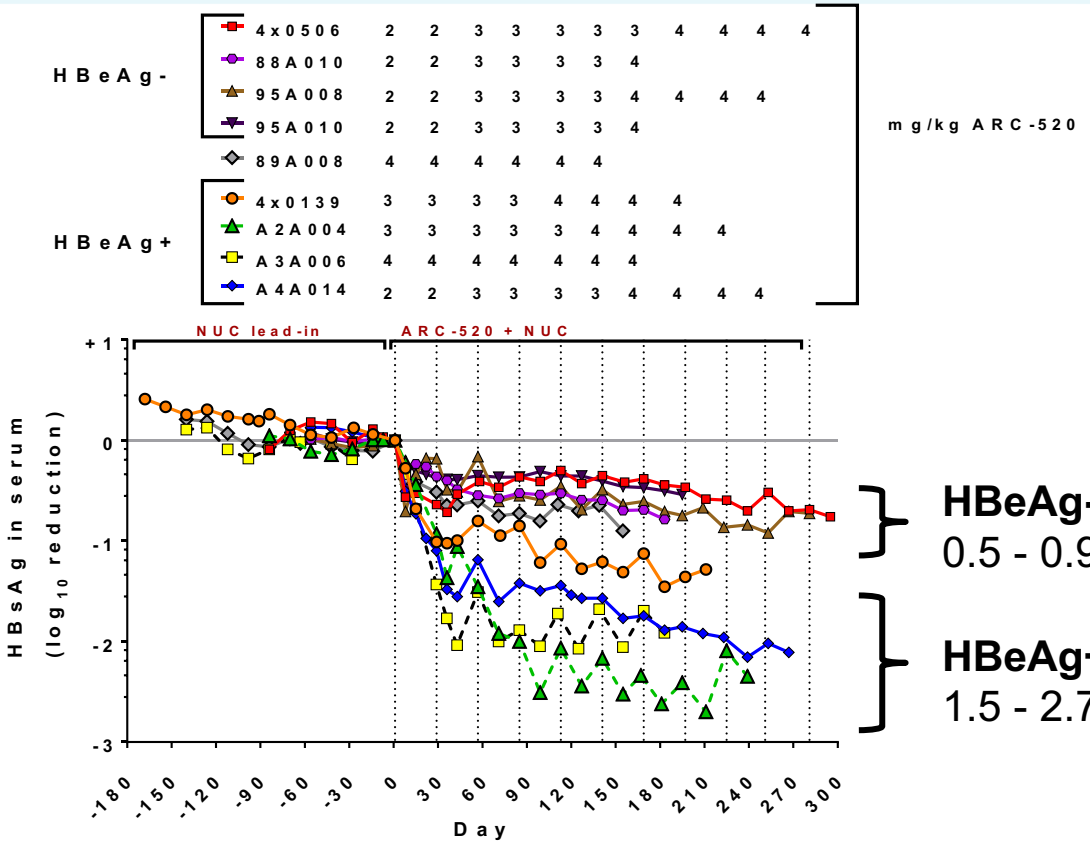


NUC = reverse transcriptase inhibitors such as entecavir and tenofovir

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

HBsAg reduction correlated with HBeAg status



HBeAg-
0.5 - 0.9 $\log_{10}$ reduction at nadir

HBeAg+
1.5 - 2.7 $\log_{10}$ reduction at nadir

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

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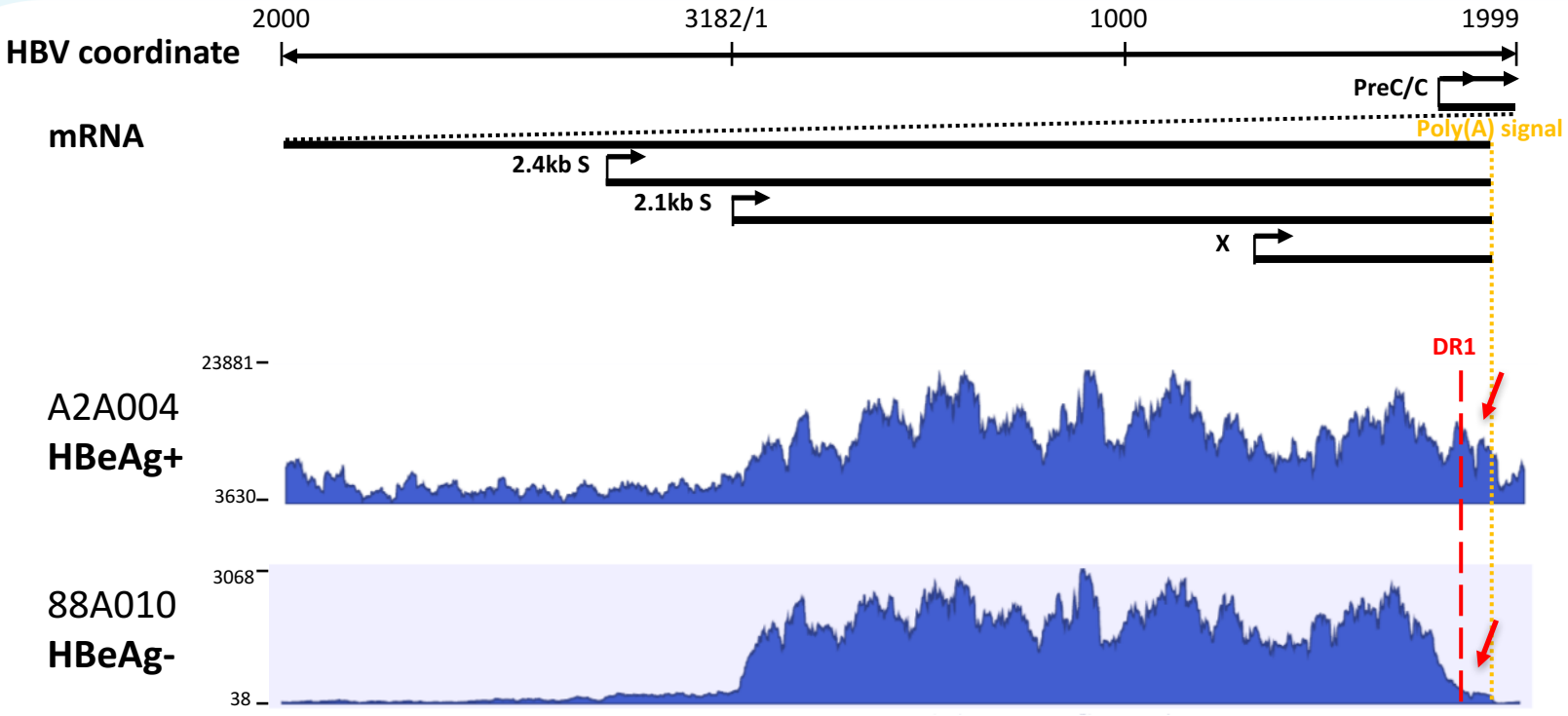
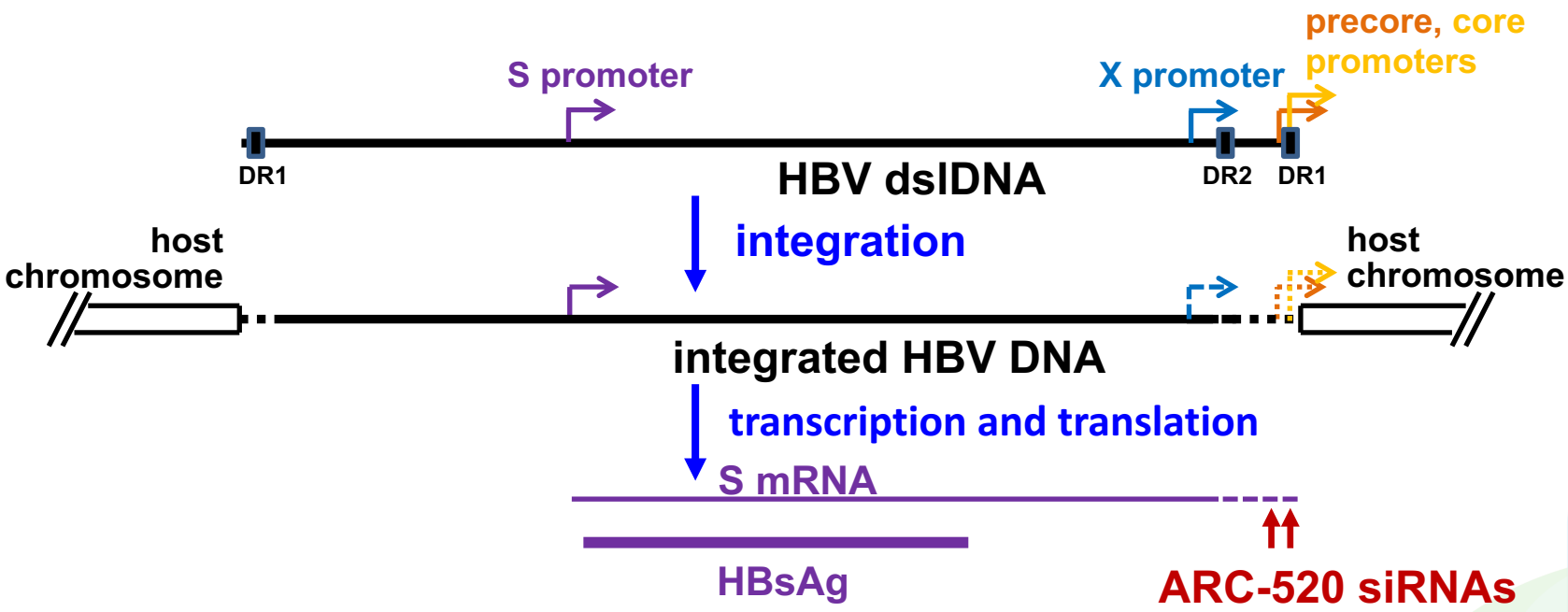
Representative HBV transcript profiles in HBeAg+ and HBeAg- chimps (Illumina RNA-seq analysis)



- 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?*

HBV integration into the host genome

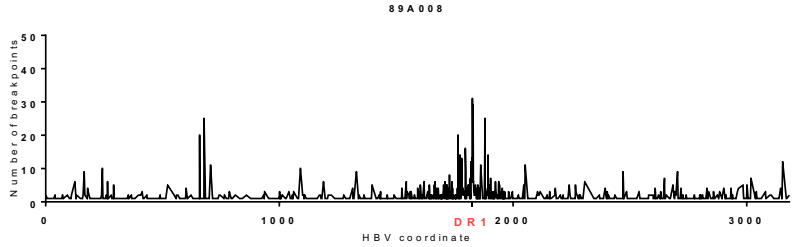


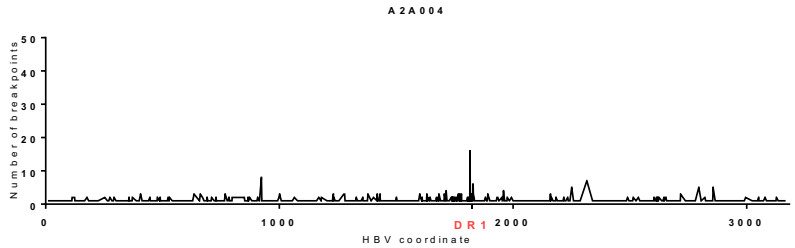
1. HBV DNA integrates into host chromosome, during which regions between DR2 and DR1 can be randomly deleted (not new!)

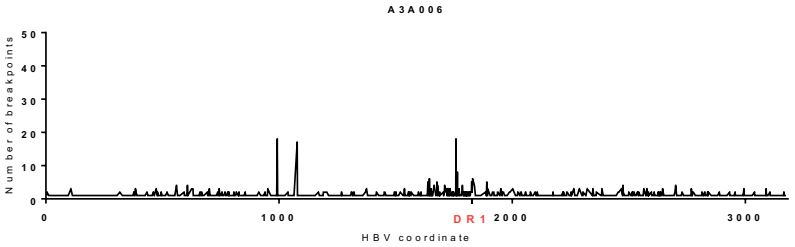
2. Significant HBsAg mRNA can be produced from integrated HBV DNA
   - These S transcripts contain complete HBsAg CDS
   - Expected loss of ARC-520 target sites in many

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

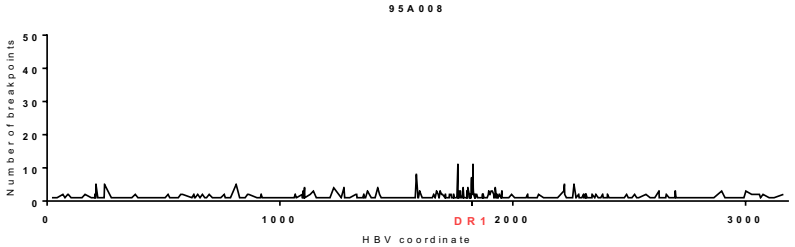
HBeAg+

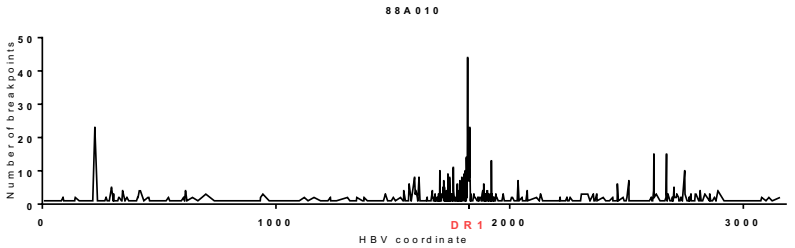


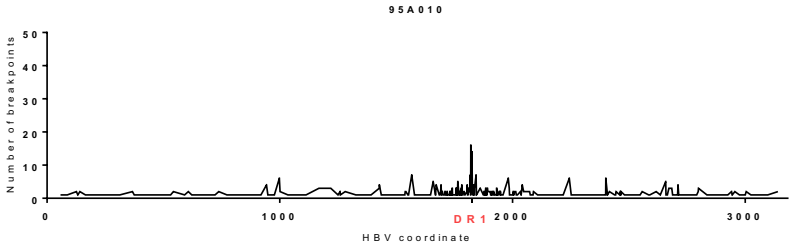




HBeAg-



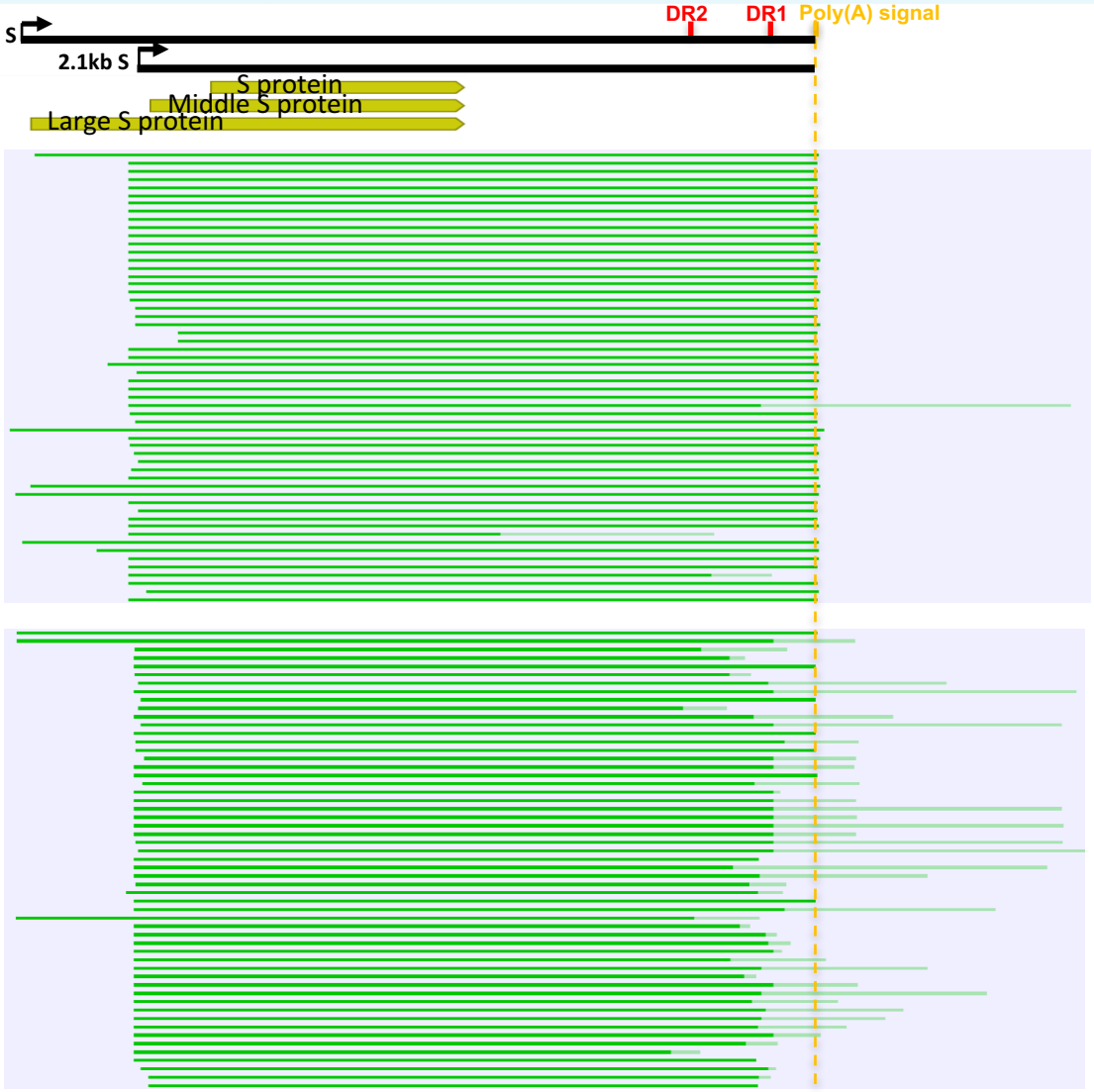




- Integration in both HBeAg+ and HBeAg- chimps
- Integration hotspot near DR1 region

HBV transcripts in HBeAg+ vs. HBeAg- chimps 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

HBV-aligning

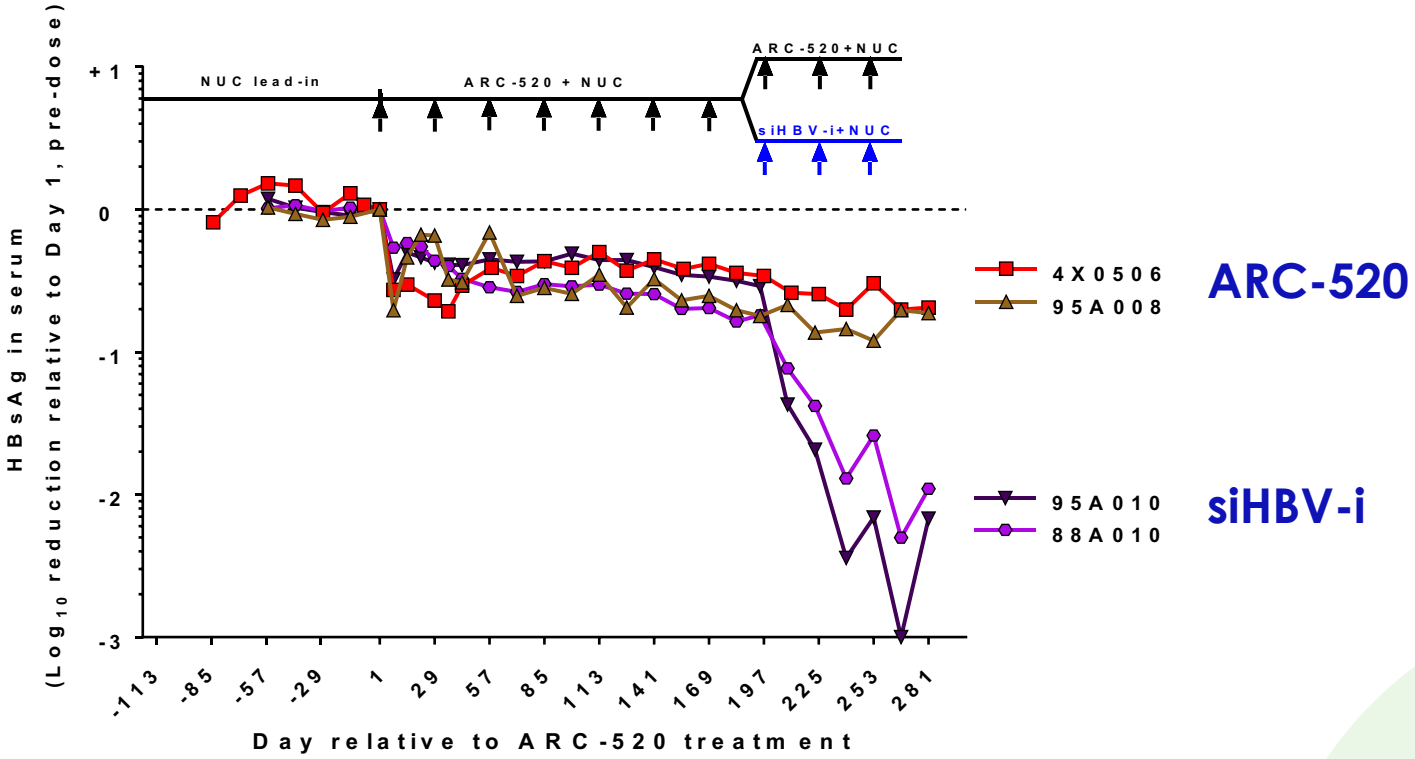
Non-aligning

Key observations from our next generation and SMRT sequencing analyses

1. HBV DNA integrates into host chromosome
   - Regions between DR2 and DR1 may be randomly deleted
2. HBsAg-encoding S mRNA are produced from integrated HBV DNA
   - Loss of ARC-520 target sites in some S fusion transcripts
3. Higher proportion of S fusion transcripts in an HBeAg- chimp than an HBeAg+ chimp

Can we effectively reduce HBsAg derived from integrated HBV DNA?

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

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Summary

1. As previously shown, we have demonstrated HBV integration into host chromosomal DNA in chronic HBV chimps

2. S mRNA transcribed from integrated HBV DNA represented the major form of S transcript in an HBeAg- chimp, but not in an HBeAg+ chimp; circulating HBsAg can be produced by these transcripts

3. Loss of ARC-520 target sites in some of these S transcripts likely accounts for less HBsAg reduction in HBeAg- chimps treated with ARC-520

4. siRNA designed to target RNA derived from HBV integration products (siHBV-i) gave deep reductions of HBsAg in HBeAg- chimps, similar to those observed using ARC-520 in HBeAg+ chimps.

5. Our findings in this chimp study have implications in treating chronically HBV-infected patients.

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