Integrated HBV DNA implicated in maintaining circulating HBsAg levels in chronically infected chimpanzees

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The Liver Meeting

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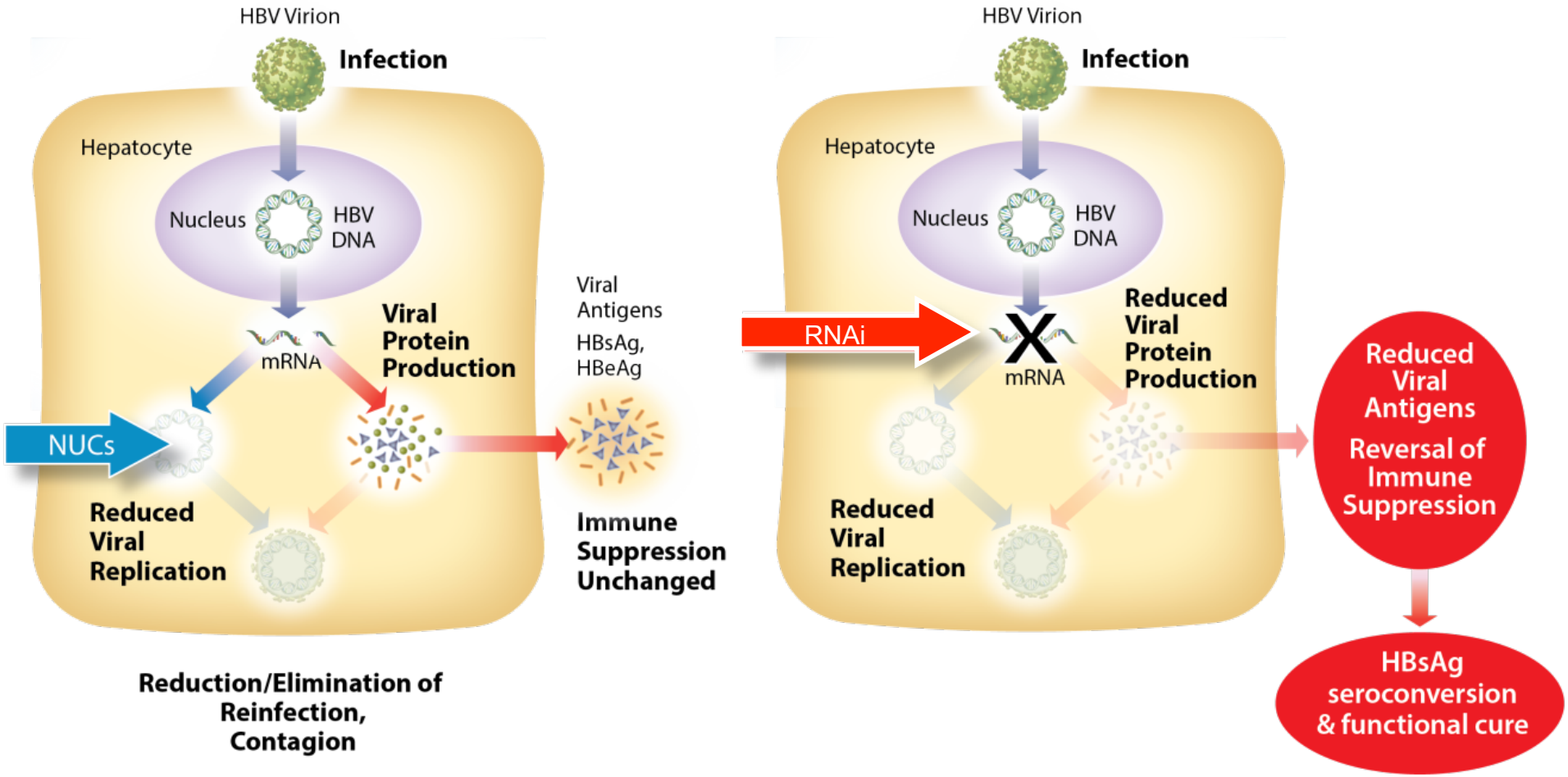
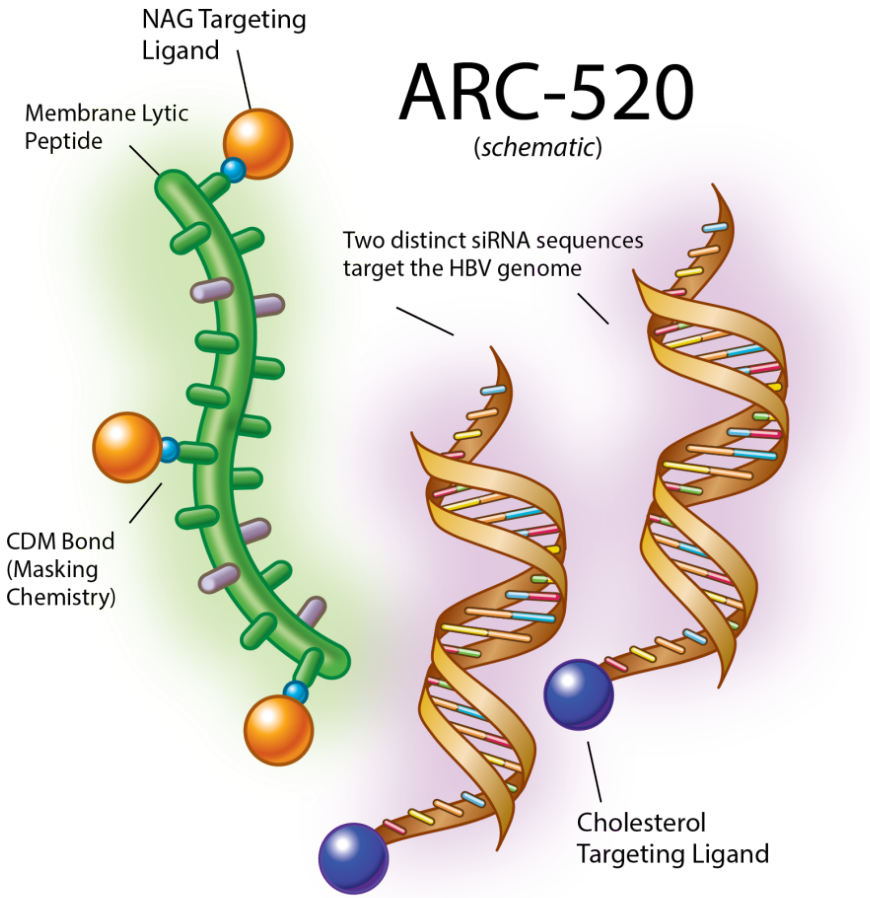
RNAi therapeutics vs. reverse transcriptase inhibitors (NUCs) for treatment of chronic HBV



RNA interference therapeutic ARC-520 for chronic HBV infection

Designed to reduce all transcripts from HBV cccDNA



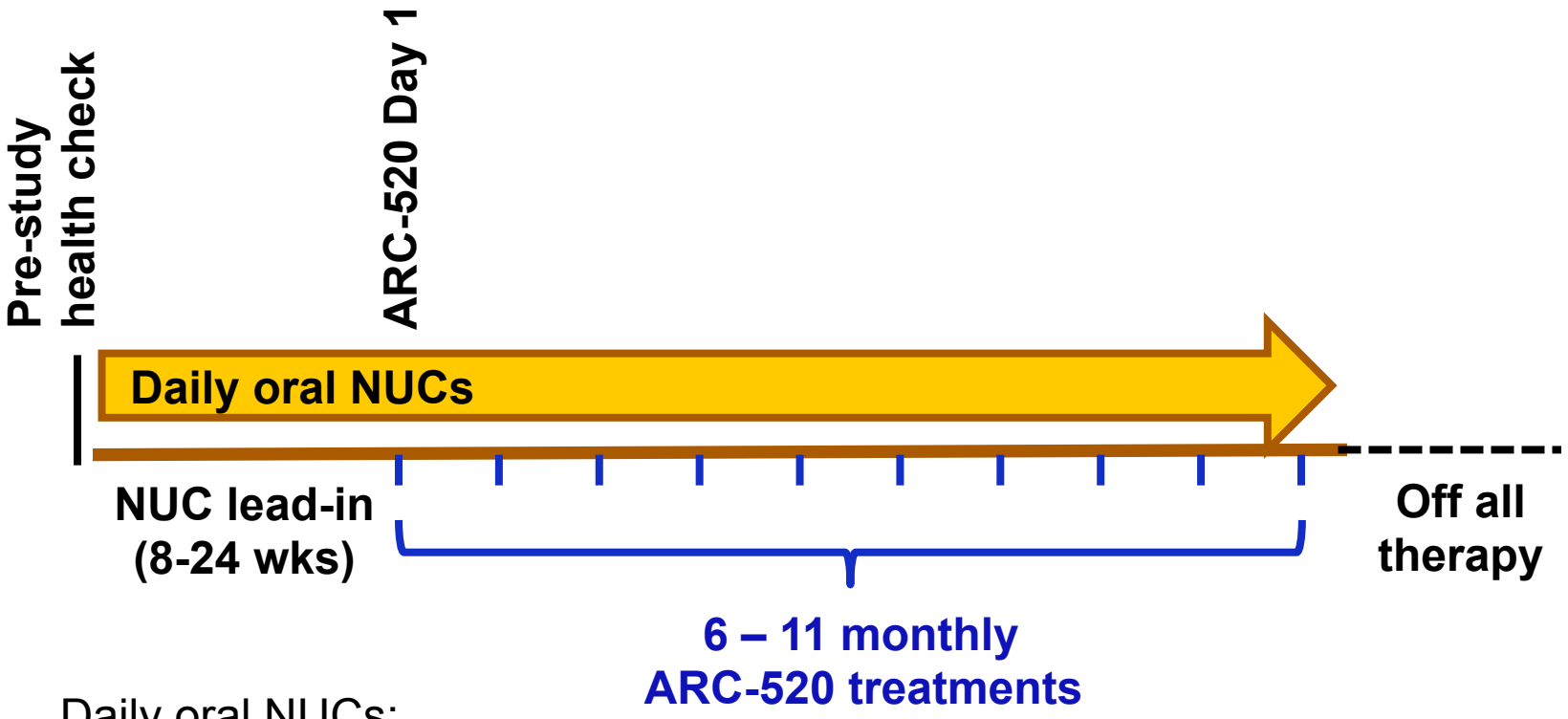
- **ARC-520 Excipient**
  - Hepatocyte-targeted DynamicPolyConjugate™ peptide (NAG-MLP) to enhance siRNA delivery
- **ARC-520 API**
  - Mixture of 2 cholesterol-conjugated siRNAs
  - Inclusion of two siRNAs gives broader genotype coverage (>99%)

Treatment of chimpanzees with RNAi therapeutic ARC-520

9 chimpanzees used in the study:

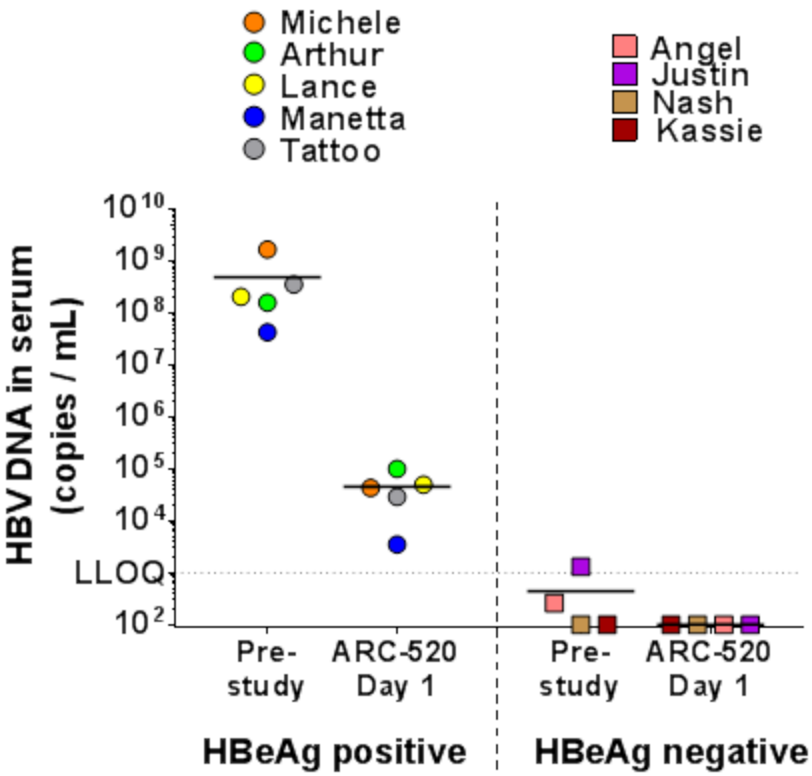
- 5 HBeAg positive and 4 HBeAg negative
- 5 males, 4 females; 9-37 years old at start of study
- Had been chronically infected many years, most from birth
- Deep sequencing and phylogenetic analysis indicated sequence was chimp variant of human HBV

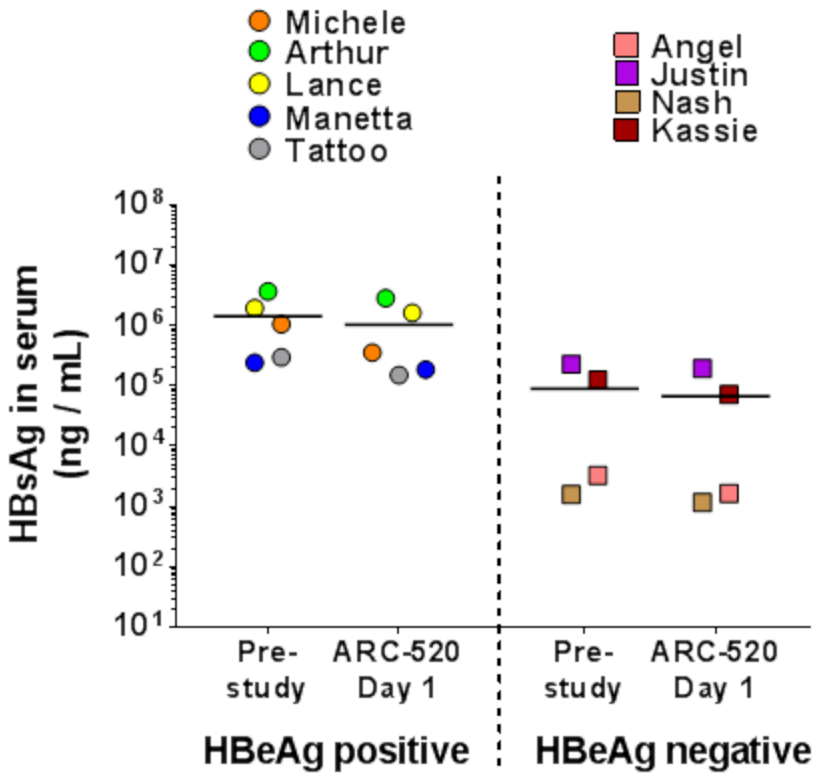
Chimp dosing and sampling timeline



- Daily oral NUCs:
  - 0.5 or 1.0 mg entecavir (ETV)
  - 300 mg tenofovir added at week 15 for chimp Michele
- 2, 3 or 4 mg/kg ARC-520 intravenous injections
- Monitor safety and efficacy
  - Blood collection performed regularly throughout study
  - Periodic liver needle biopsies

NUC lead-in: HBV serum DNA but not HBsAg decreased

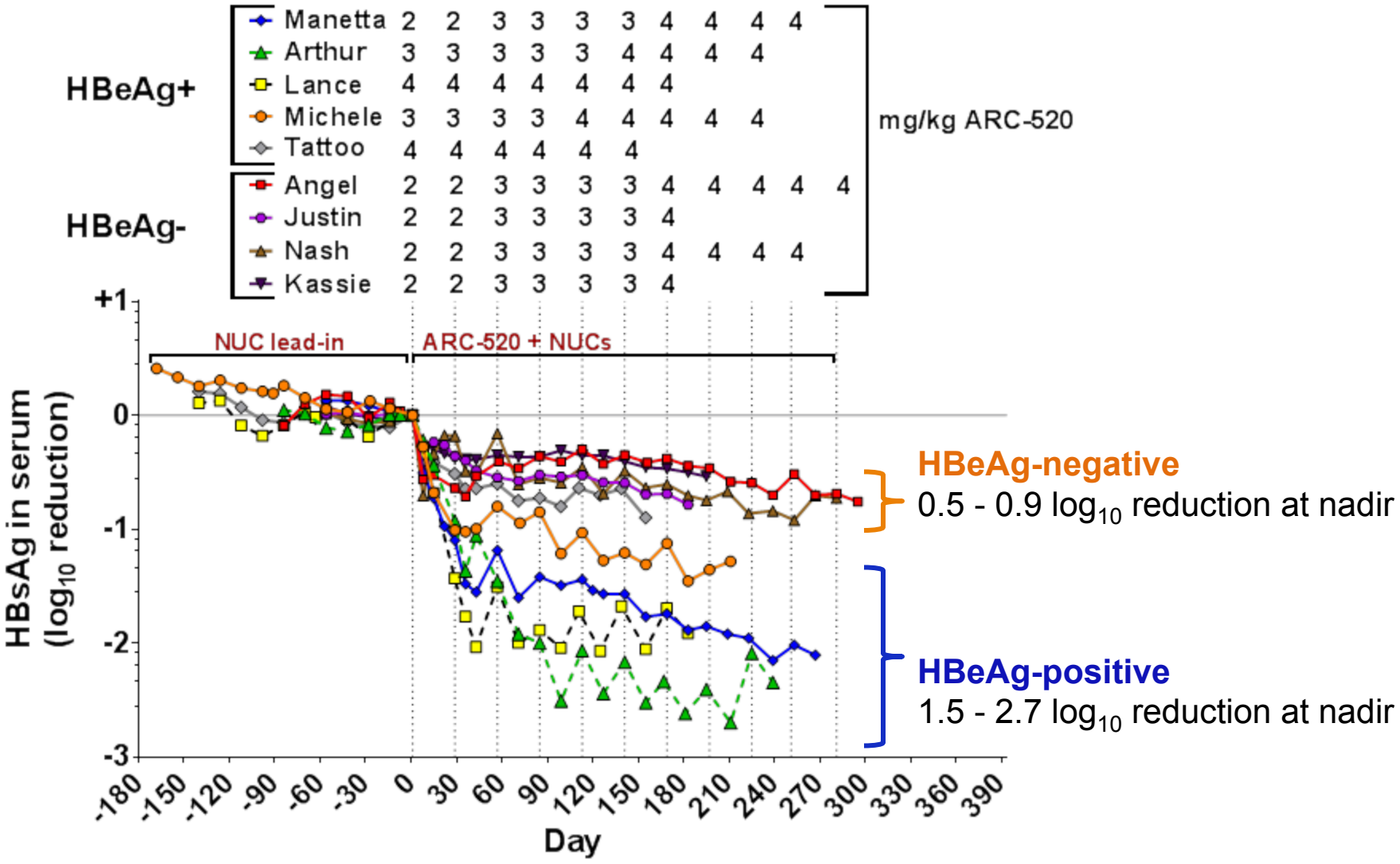




Chimp response to NUC therapy similar to humans:

- Deep decrease in serum HBV DNA in HBeAg+ chimps
- Serum HBV DNA in HBeAg- chimps dropped below the LLOQ
- NUC treatment had minimal effect on HBsAg levels

Differences in degree of HBsAg reduction correlated with HBeAg status



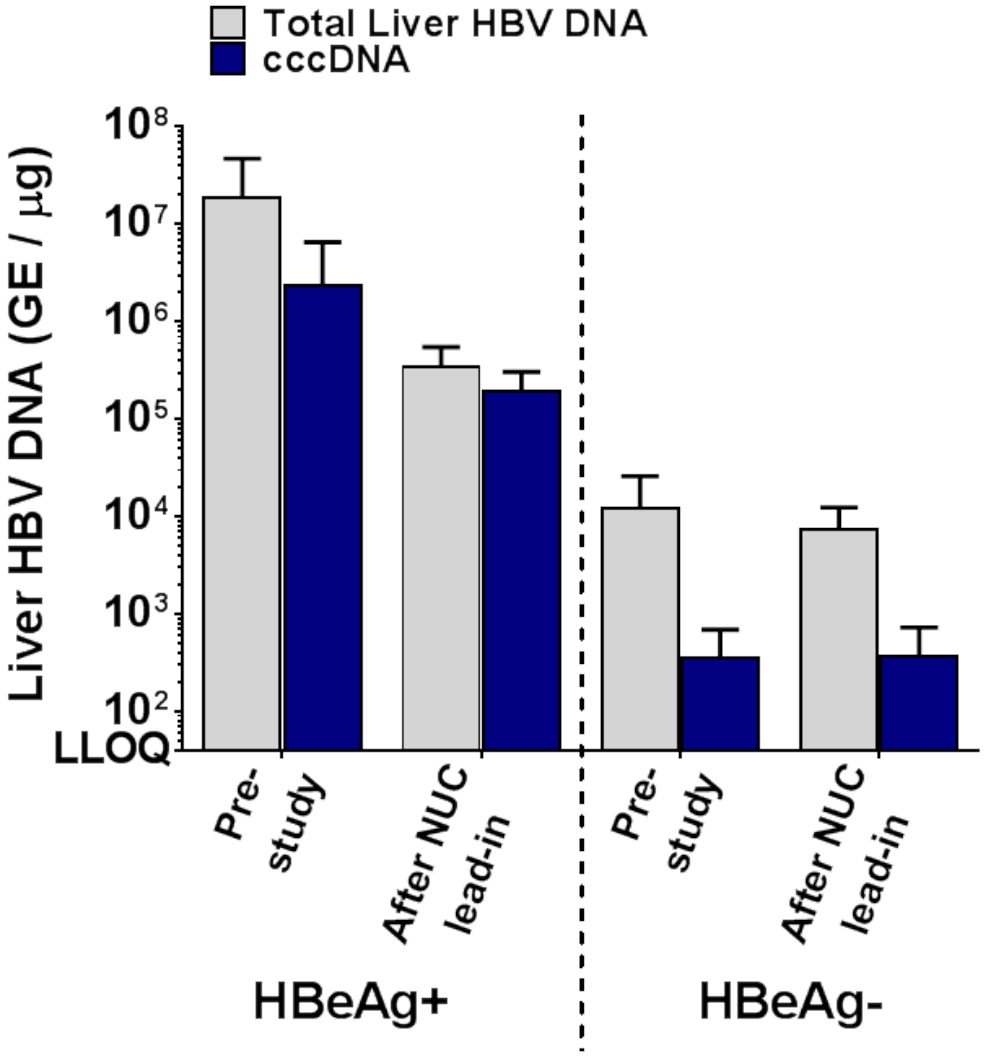
Analysis of HBV DNA from chimp liver biopsies

- Total HBV DNA (cccDNA, rcDNA and integrated HBV DNA) measured by qPCR using primers in the core gene.

- cccDNA measured by qPCR using same primers after digestion of rcDNA and linear (including chromosomal) DNA with Plasmid-Safe DNase.

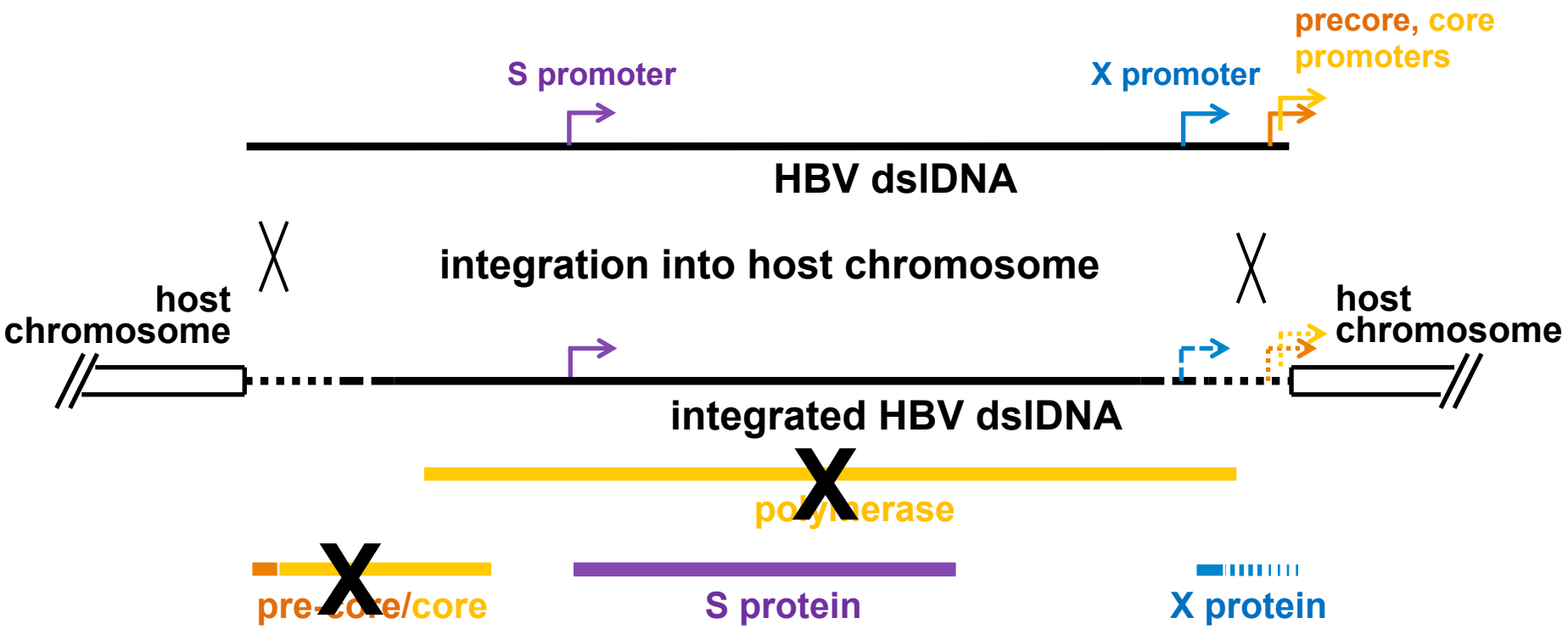
Method adapted from Bowden et al (2004), Methods in Molecular Medicine 95:41-50

Predominant form of liver HBV DNA differs in HBeAg- vs. HBeAg+



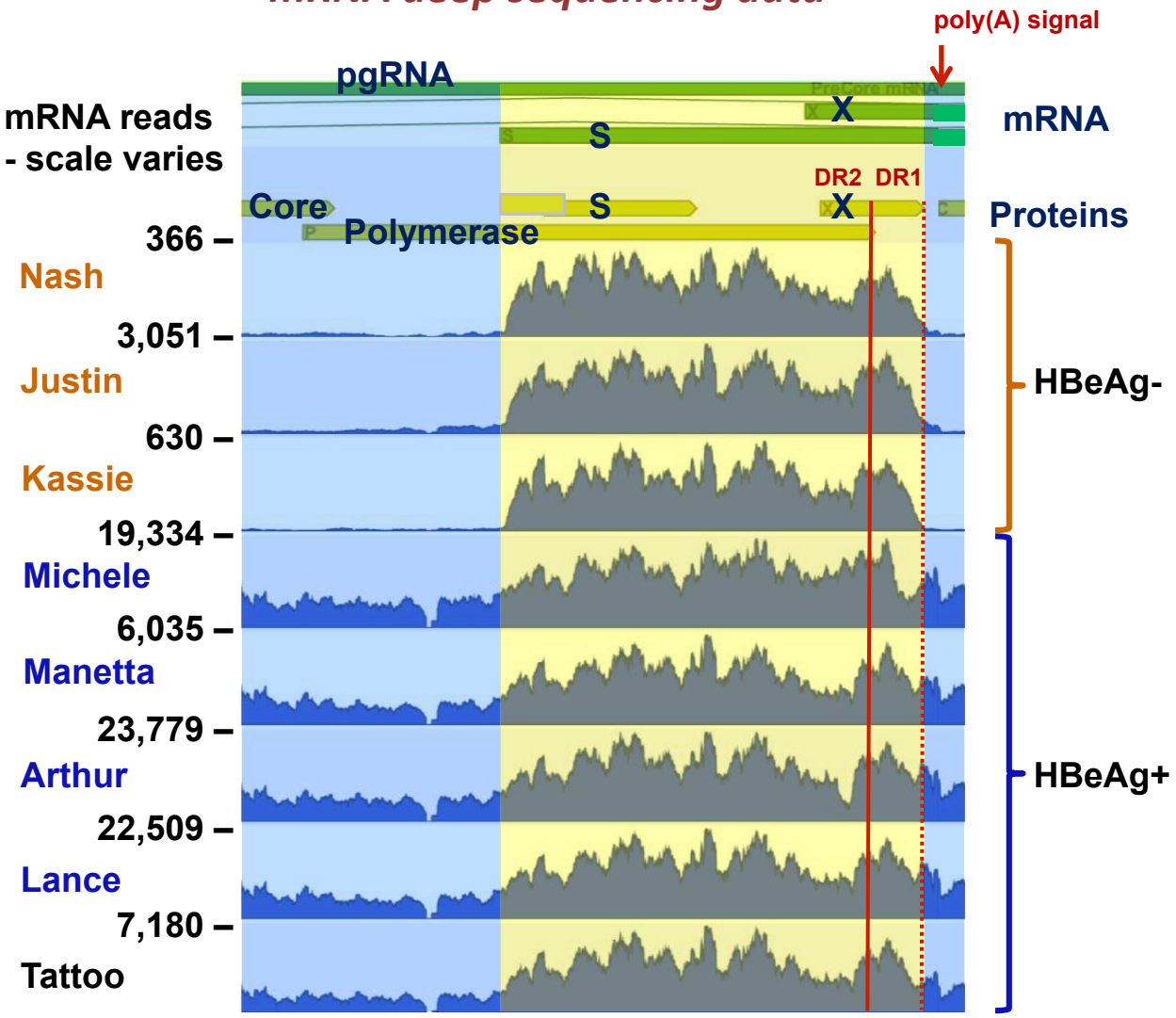
Conclusion: DNA profile in HBeAg- chimps suggests most of the HBV DNA is integrated.

Process of HBV dslDNA integration and theoretical production of HBsAg



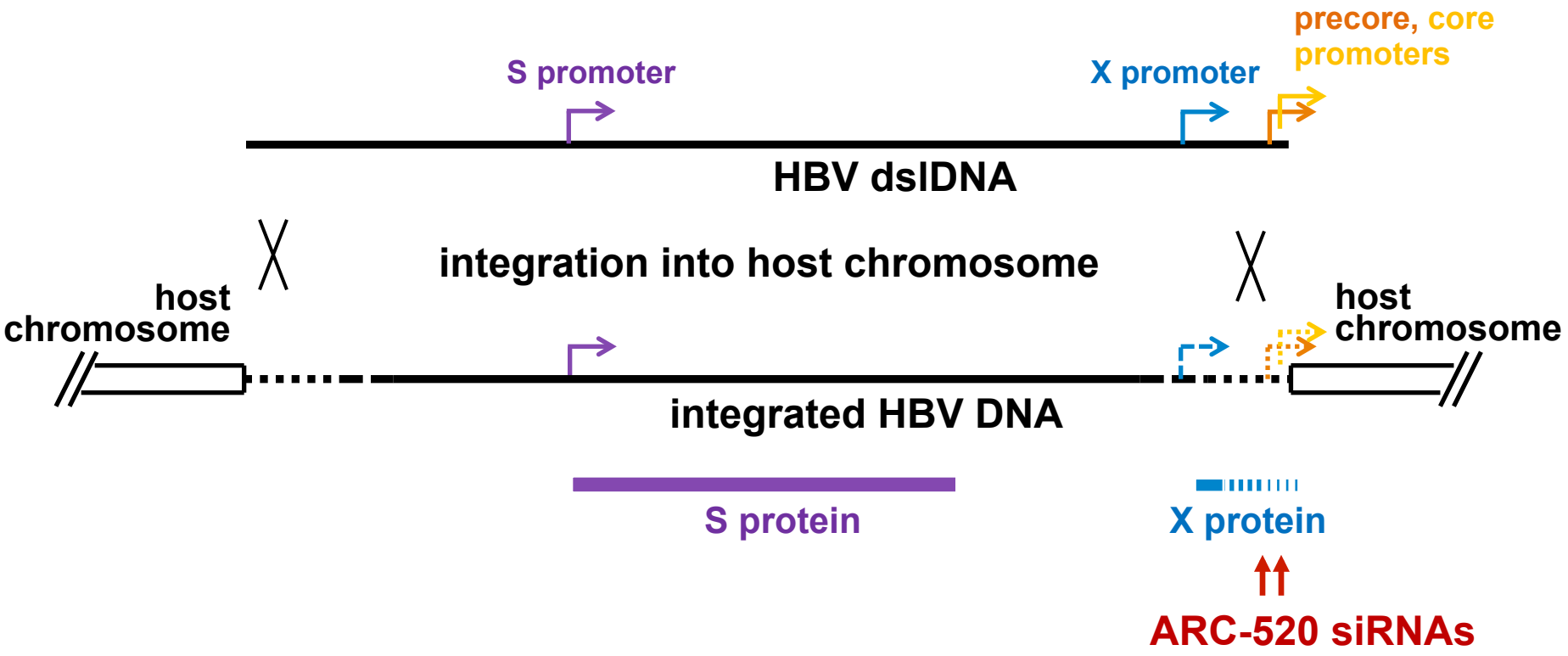
HBV transcript profiles differ between HBeAg- and HBeAg+ chimps

mRNA deep sequencing data



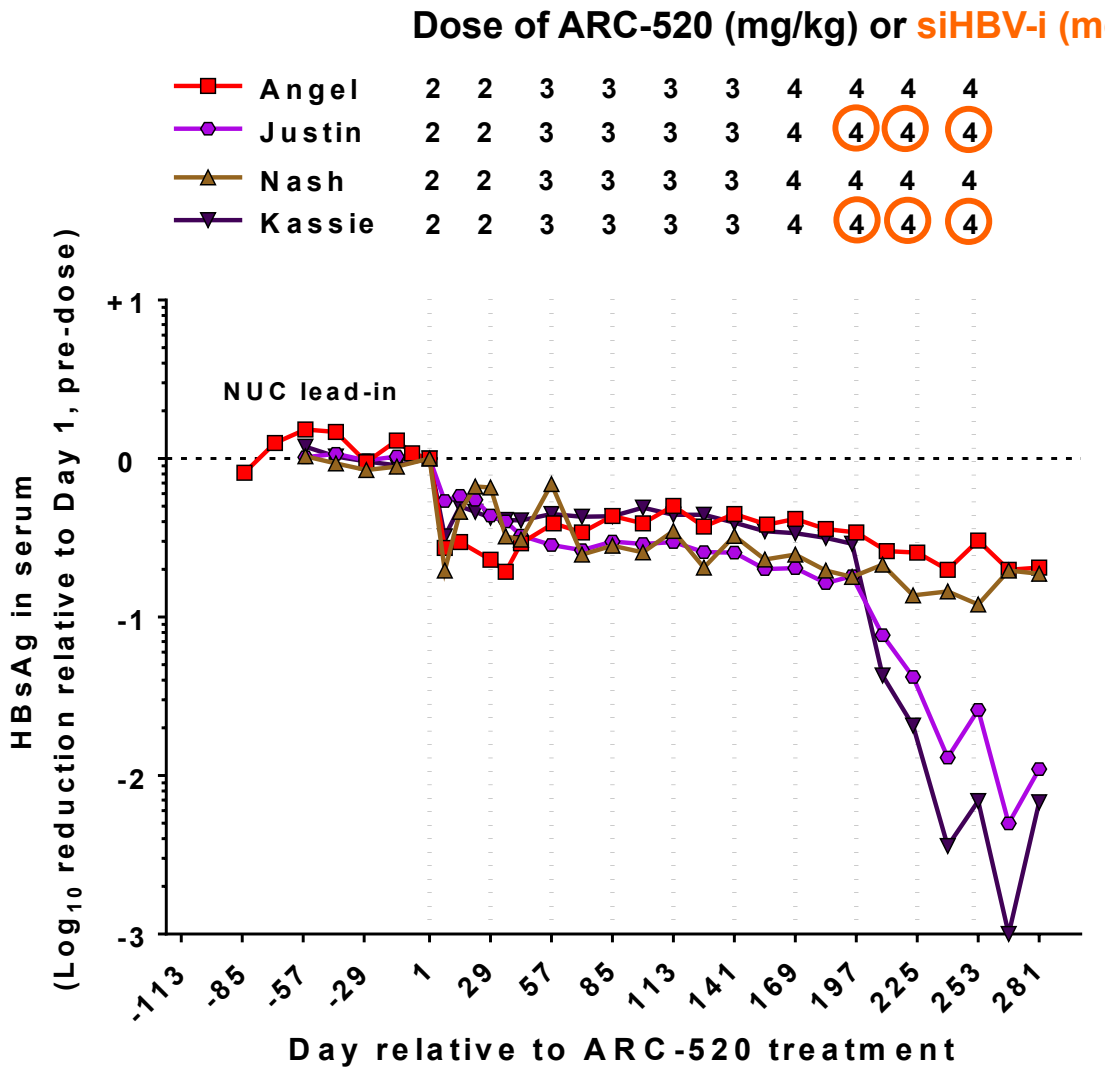
In HBeAg- chimps, HBsAg gene transcript reads are strongly reduced in a region surrounding the DR1 site.

Process of HBV dslDNA integration and theoretical production of HBsAg



- dslDNA integration explains persistent expression of HBsAg despite very low cccDNA in HBeAg- chimps.
- Loss of ARC-520 target sites explains lower KD of HBsAg in HBeAg- chimps.

siRNA designed to target RNA derived from HBV integration products in HBeAg- chimps



- siRNA with target sequence outside of DR1-DR2 (siHBV-i) was designed to target HBV RNA expressed from integrated HBV DNA.
- siHBV-i was administered to two HBeAg- chimps once a month for 3 months following ARC-520 therapy.
- siHBV-i gave deep reductions in HBsAg in HBeAg- chimps, similar to those observed using ARC-520 in HBeAg+ chimps.

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+ chimps: **up to 2.7 log reduction**
  - Lower response in HBeAg- 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+ vs. HBeAg- chimps
  - HBV RNA profiles in HBeAg- chimps consistent with transcripts arising from dslDNA
- **siRNA targeting integrant-derived transcripts results in deep HBsAg reduction in HBeAg- chimps**

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See poster #2022 on Tuesday