Integrated HBV DNA significantly contributes to serum HBsAg levels in chronically HBV injected chimpanzees

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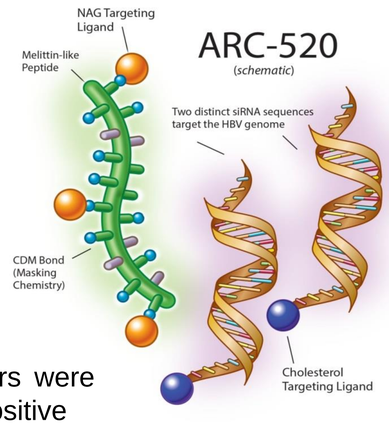
Background:

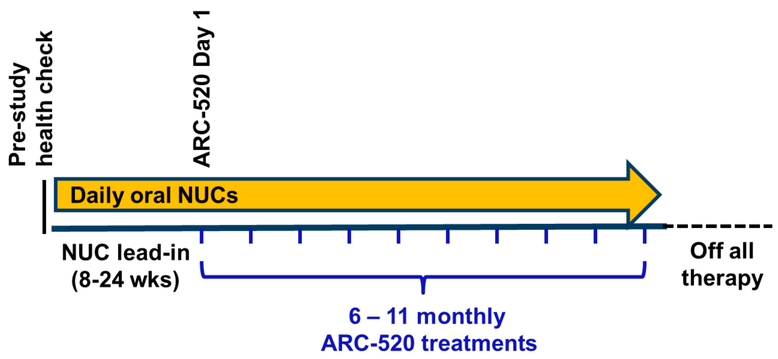
The HBV therapeutic ARC-520 was designed to decrease all cccDNA-derived viral transcripts and thus viral protein load via RNA interference (RNAi). ARC-520 consists of two cholesterol-conjugated siRNA molecules that target HBV sequences near DR1 plus a hepatocyte-targeted excipient for efficient delivery of the siRNA from the endosome to the cytoplasm where the RNAi machinery resides.



Study design:

Nine chimpanzees chronically infected with HBV for many years were included in the study. At start of study five chimps were HBeAg positive (HBeAg+) and four were HBeAg negative (HBeAg-). Deep sequencing and phylogenetic analyses indicated the HBV sequence was a chimpanzee variant of human HBV.

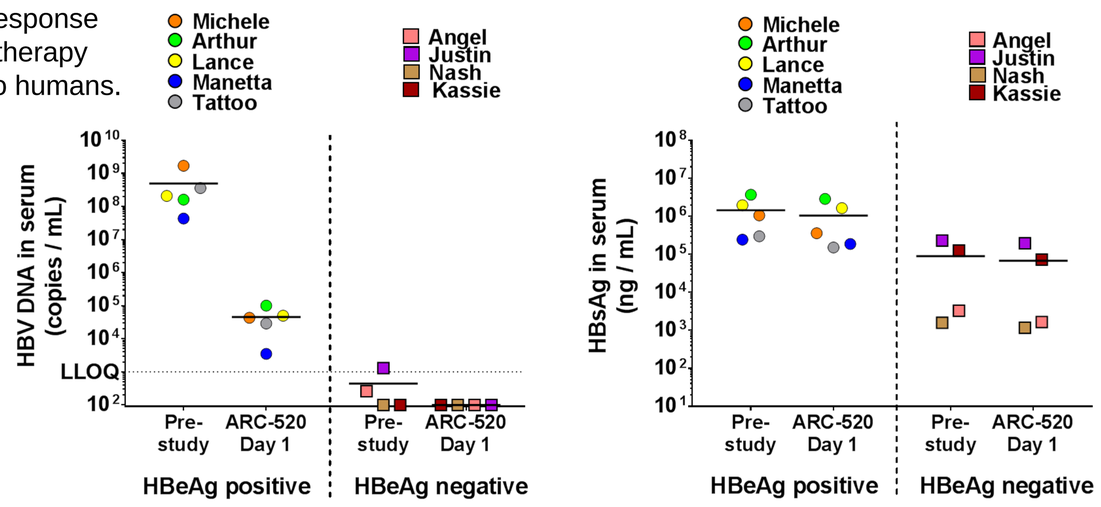
To reduce viral replication prior to treatment with ARC-520 Injection chimps were treated for 8-24 weeks with entecavir (ETV) or ETV+ tenofovir (TDF) in one case (chimp Michele). Following the lead-in period, animals were administered ARC-520 Injection intravenously at 4-week intervals. Dose levels were 2, 3, or 4 mg/kg ARC-520 Injection, along with maintenance doses of ETV or ETV+TDF.



NUC lead-in:

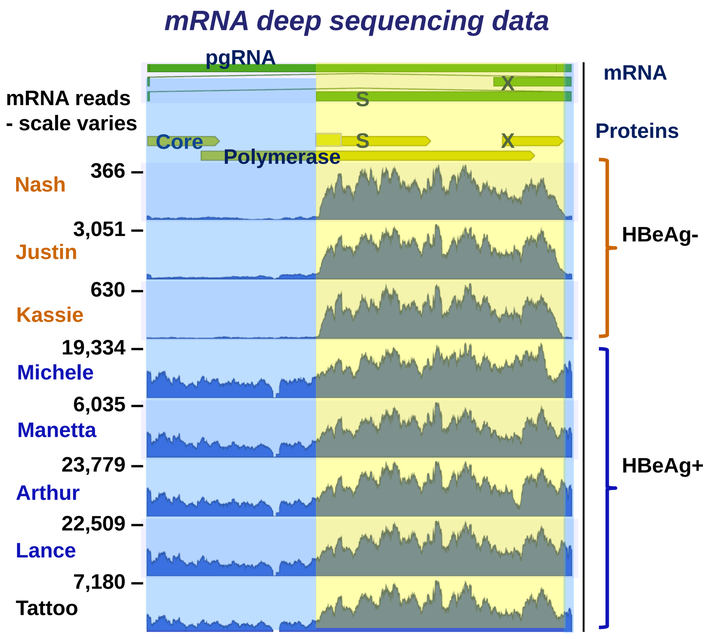
Serum HBV DNA decreased, but HBsAg unaffected by NUCs

Chimp response to NUC therapy similar to humans.



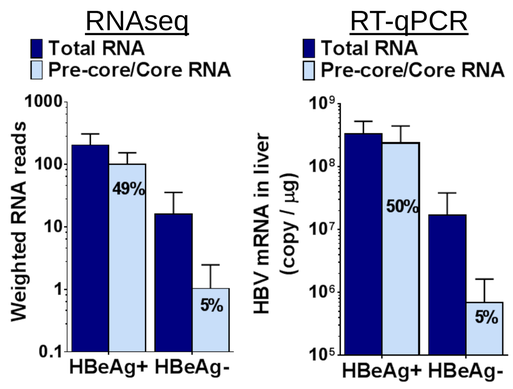
Less pre-core/core HBV RNA in liver of HBeAg- compared to HBeAg+

mRNA deep sequencing data

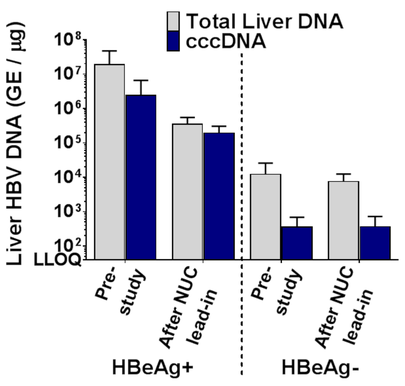


HBV RNA analyzed by deep sequencing and RT-qPCR gave similar results:

~5% of transcripts in HBeAg- chimps were pre-core/core/ pregenomic RNA length, whereas these comprised ~50% in HBeAg+ chimps.



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



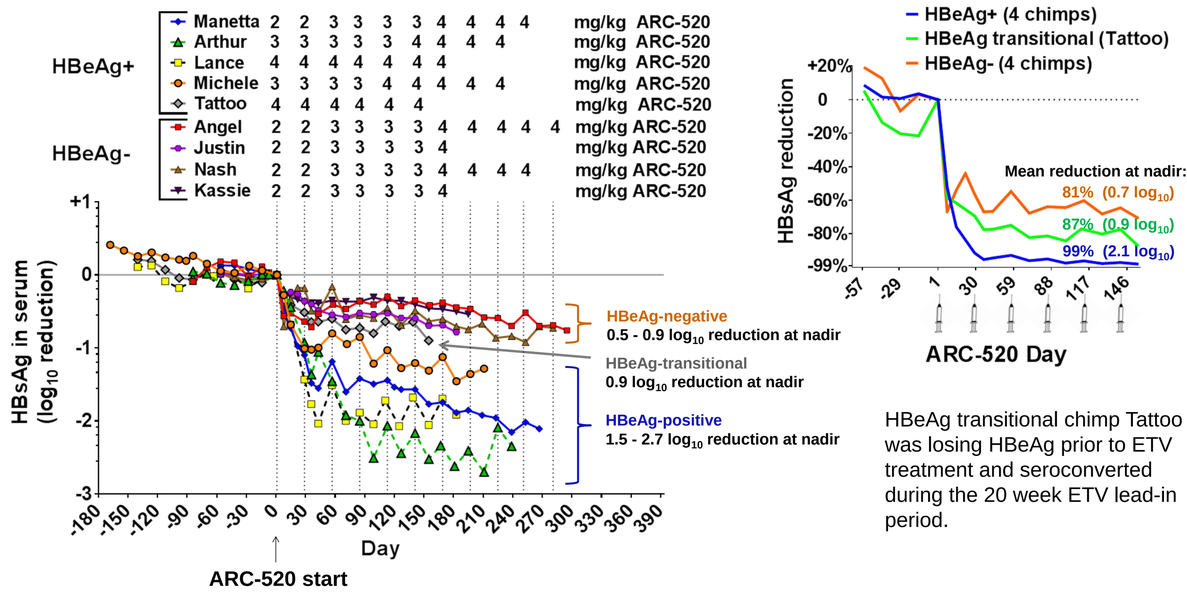
Liver biopsies at pre-study and after NUC lead-in:

- On NUCs, most of HBV DNA in liver of HBeAg+ chimps is cccDNA
- 500-fold less cccDNA in HBeAg- compared to HBeAg+
  - Only 5% of total HBV DNA in liver of HBeAg- was cccDNA
  - Liver DNA levels in HBeAg- were negligibly affected by NUCs

Conclusion: DNA profile in HBeAg- chimps is consistent with a high proportion of HBV DNA existing as integrated copies in the host genome.

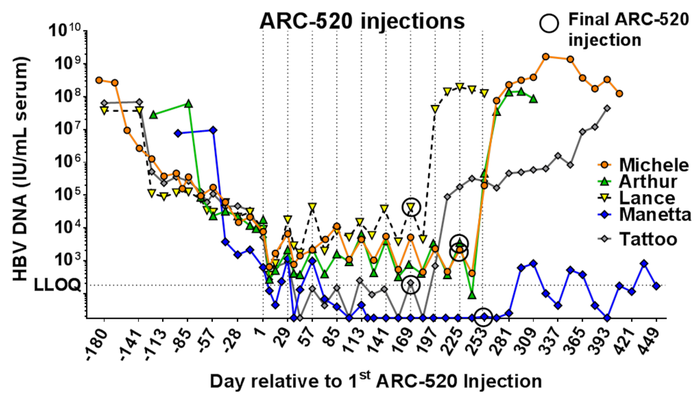
ARC-520 treatment:

Reduction in serum HBsAg with ARC-520



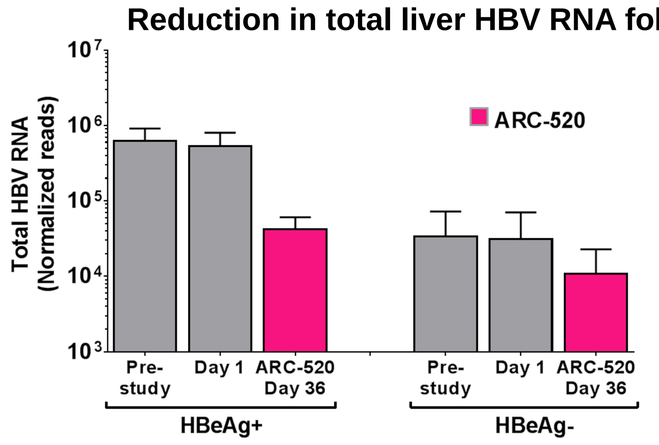
HBeAg transitional chimp Tattoo was losing HBeAg prior to ETV treatment and seroconverted during the 20 week ETV lead-in period.

Reduction in serum HBV DNA on NUC + ARC-520 treatment in HBeAg+ chimps



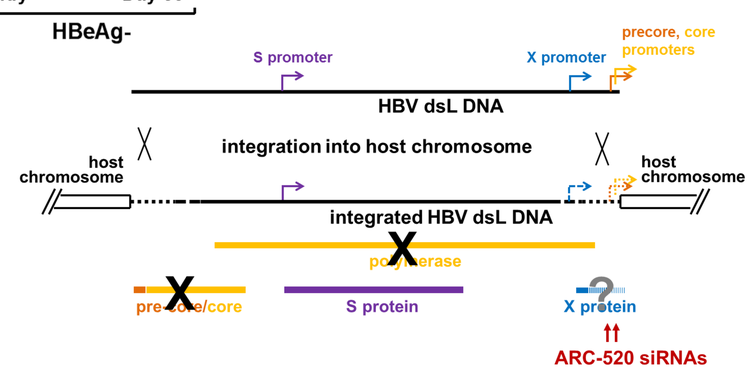
- DNA levels were stable prior to NUC treatment.
- NUCs reduced serum DNA prior to ARC-520 treatment.
- Further decreases in serum DNA following each ARC-520 injection.
- Sustained reduction of serum HBV DNA in Manetta 32 weeks after final ARC-520 injection.

Reduction in total liver HBV RNA following ARC-520 treatment



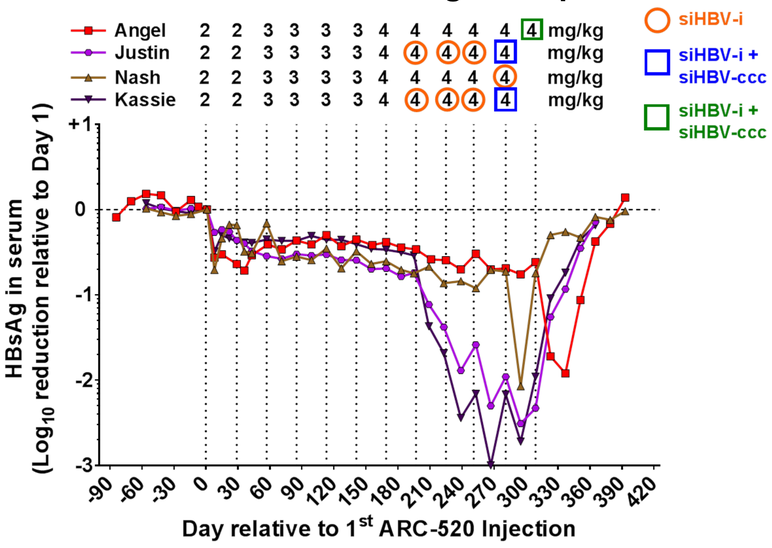
- At start of study, total HBV RNA in the liver was ~20-fold higher in HBeAg+ than in HBeAg- chimps.
- RNA levels were not changed by NUC treatment.
- ARC-520 mediated decrease in total HBV RNA in the liver was consistent with the degree of HBsAg reduction in HBeAg+ and HBeAg- chimps.

Process of HBV dsL DNA integration, deletion of ARC-520 siRNA target sites, and theoretical production of HBsAg



"siHBV-i" designed to target RNA derived from HBV integration products

- siRNA with target sequence outside of DR1-DR2 (siHBV-i) was designed to target HBV RNA expressed from integrated HBV DNA.
- siHBV-i was tested in all 4 HBeAg negative chimps.
- siHBV-i was also co-delivered with siRNA designed to target HBV RNA expressed from cccDNA (siHBV-ccc).



Conclusions

- Robust, sustained direct anti-viral effect on HBsAg production observed in all HBeAg positive and negative chimps during ARC-520 treatment. Manetta achieved SVR off-therapy.
  - HBeAg pos chimps displayed highest levels of HBsAg knockdown - **up to 2.7 log**
  - In HBeAg neg chimps, HBsAg knockdown was also substantial - **up to 0.9 log**
- ARC-520 was well tolerated after multiple doses up to 4 mg/kg ARC-520 (highest dose tested).
- ***Evidence indicates integrated HBV DNA is a significant source of total HBsAg, especially in HBeAg neg chimps.***