# Monthly dosing of ARC-520 in chronically hepatitis B virus infected chimpanzees produces rapid, deep and durable reductions in circulating viral antigens

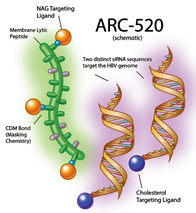
Christine I. Wooddell[1], Deborah Chavez[2], Jason E. Goetzmann[3], Ryan M. Peterson[1], Zhao Xu[1], Bernadette Guerra[2], Helen Lee[2], Lena Notvall-Elkey[2], Courtney Johnson[2], Julia O. Hegge[1], Robert G. Gish[4], Stephen A. Locarnini[5], Christopher R. Anzalone[1], Robert E. Lanford[2], David L. Lewis[1]

[1] Arrowhead Research Corporation, Madison, WI; [2] Texas Biomedical Research Institute, San Antonio, TX; [3] University of Louisiana, Lafayette, LA; [4] Stanford University Medical Center, San Diego, CA; [5] Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia

Arrowhead Research CORPORATION

## Background:

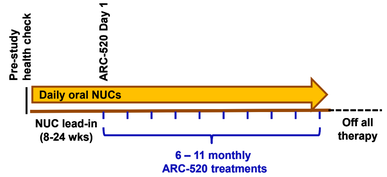
The HBV therapeutic ARC-520 was designed to decrease all cccDNA-derived viral transcripts and thus viral protein load via RNA interference (RNAi). ARC-520 consists of two cholesterol-conjugated RNAi triggers that target HBV sequences near DR1 plus a hepatocyte-targeted excipient for efficient delivery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




## Study design:

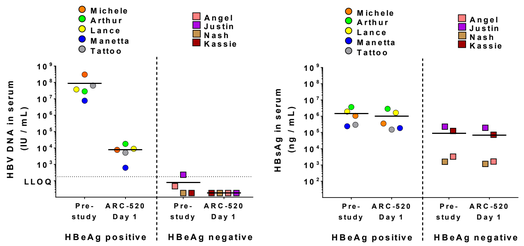
Nine chimpanzees (5 males, 4 females; 9-37 years-old) chronically infected with HBV were included in the study. Deep sequencing and phylogenetic analyses indicate the HBV sequence is a chimpanzee variant of human HBV. At start of study, five chimps were HBeAg positive (HBeAg+), baseline DNA 8-9 $\log_{10}$ IU/mL serum; and four were HBeAg negative (HBeAg-), baseline DNA ≤3 $\log_{10}$ IU/mL. HBsAg levels ranged from 250-3190 µg/mL in HBeAg+ chimps and from 1.2-200 µg/mL in HBeAg- chimps.

To reduce viral replication prior to treatment with ARC-520, chimps were treated for 8-24 weeks with entecavir (ETV) or in one case (chimp Michele) with ETV+ tenofovir (TDF). Following the NUC lead-in period, animals were administered ARC-520 intravenously at 4-week intervals (q4w). Dose levels were 2, 3, or 4 mg/kg ARC-520, along with maintenance doses of ETV or ETV+TDF.




## NUC lead-in:

### Serum HBV DNA decreased, but HBsAg unaffected by NUCs

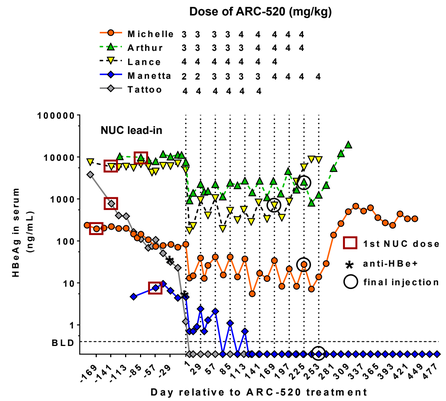



**Chimp response to NUC therapy similar to humans:**

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

## ARC-520 treatment:

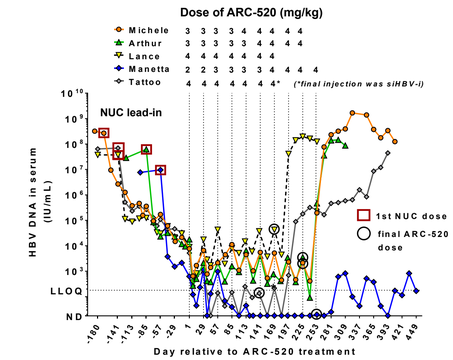
### HBeAg levels are decreased on NUC + ARC-520 treatment




- HBeAg levels changed only marginally during NUC lead-in phase. Steep drop observed after addition of ARC-520.
- Tattoo had decreasing levels of HBeAg prior to and during NUC lead-in. Anti-HBe detected 28 days prior to 1st ARC-520 dose. HBeAg lost following 1st ARC-520 dose and was sustained off-therapy.
- Manetta seroconverted for HBeAg during ARC-520 therapy. Anti-HBe antibodies detected on Day 1 of treatment with ARC-520. HBeAg lost after 5th ARC-520 dose and was sustained off-therapy.

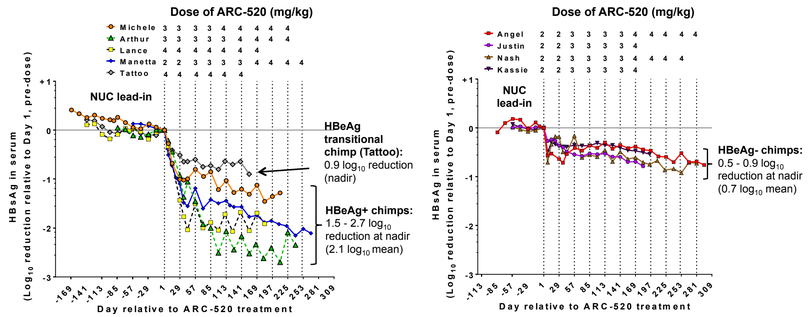
## ARC-520 treatment, cont'd:

### Serum HBV DNA levels on NUC + ARC-520 treatment in HBeAg positive chimps



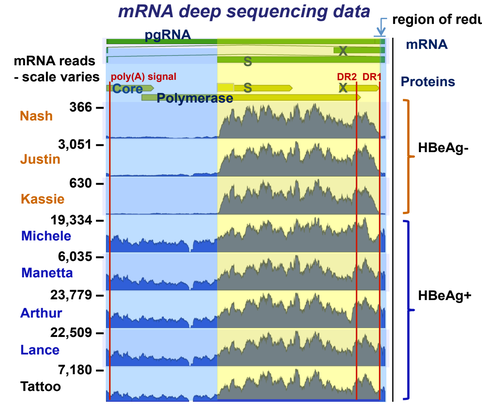

- DNA levels were stable prior to treatment with NUCs.
- NUCs reduced serum DNA prior to ARC-520 treatment.
- Further decreases in serum DNA followed each ARC-520 injection.
- Sustained reduction of serum HBV DNA in Manetta 32 weeks after final ARC-520 injection.

### HBsAg is strongly reduced in HBeAg positive chimps, less so in HBeAg negative chimps




### HBV transcript profiles differ between HBeAg- and HBeAg+ chimps

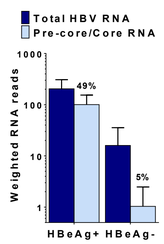
*mRNA deep sequencing data*




For replication of HBV, all viral transcripts terminate at a unique poly(A) signal downstream of HBV X gene ORF (UAUAAA motif at position 1789).

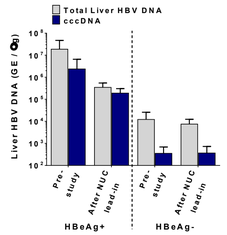
Direct repeats 1 and 2 (DR1 and DR2) both occur within HBV X gene region; integration events into host DNA often occur in or around the DR1 sequences of HBV, and the DR1 region is frequently located at the virus-host genome junction.

**Quantitation of deep sequencing data**




- Relatively less pre-core/core HBV RNA to total HBV RNA in liver of HBeAg- than HBeAg+ chimps.
- In HBeAg- chimps, HBsAg gene transcript reads are strongly reduced in a region just upstream and downstream of the DR1 site.

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




**Total DNA was isolated from needle biopsy liver tissue and HBV DNA was quantitated by method adapted from Bowden et al. (2004):**

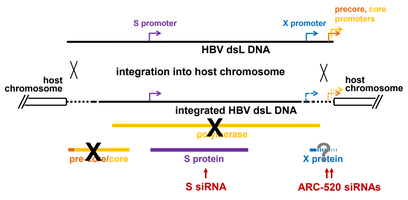
**Methods:**

- Copies of **total HBV DNA** (cccDNA + rcDNA + integrated HBV DNA) were measured by real time qPCR using primers in the core gene.
- Copies of **cccDNA** were measured by real time qPCR (same primers as total HBV DNA), after preferential digestion of rcDNA and linear (including chromosomal) DNA with Plasmid-Safe DNase.

**Results:**

- After NUC lead-in, most HBV DNA in liver of HBeAg+ chimps was cccDNA. Only ~5% of HBV DNA in liver of HBeAg- chimps was cccDNA.
- Liver HBV DNA levels in HBeAg- were negligibly affected by NUCs.

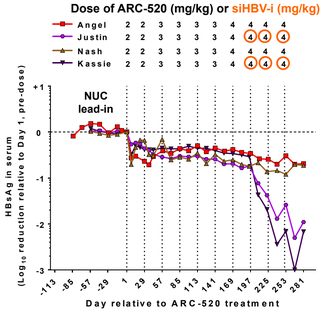
***DNA profile in HBeAg- chimps is consistent with a high proportion of HBV DNA existing as integrated copies in the host genome.***




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

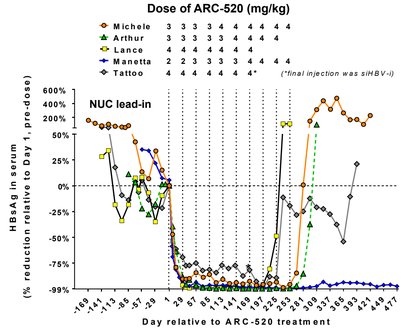
### "siHBV-i" targeting RNA from HBV integrants strongly reduces HBsAg in HBeAg- chimps

- 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 a deep reduction in HBsAg in HBeAg- chimps, similar to that observed using ARC-520 in HBeAg+ chimps.




### End of study HBsAg levels

- In three HBeAg+ chimps (7-9 ARC-520 injections) HBsAg levels rebounded to pre-study levels 10-12 weeks after final injection.
- In HBeAg transitional chimp Tattoo the HBsAg level was 25% (0.13 $\log_{10}$) reduced relative to pre-study at 32 weeks after 7th injection.
- In Manetta HBsAg was 98% (1.7 $\log_{10}$) reduced 32 weeks after 10th ARC-520 injection.




## Conclusions:

- Robust, sustained direct anti-viral effect on HBsAg production observed in all HBeAg positive and negative chimps during ARC-520 treatment. Manetta achieved a sustained virological response 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.***

University of Louisiana Lafayette

Texas Biomedical Research Institute

SNPRC Southwest National Primate Research Center