Sustained reduction of HBV DNA, RNA and proteins, and HBeAg seroconversion in a chronically HBV-infected chimpanzee treated with nucleoside analog/ARC-520 combination therapy

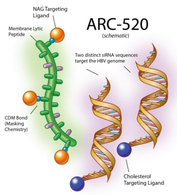
Christine I. Wooddell[1], Deborah Chavez[2], Jason E. Goetzmann[3], Ryan M. Peterson[1], Zhao Xu[1], Robert G. Gish[4,5], Stephen A. Locarnini[6], Christopher R. Anzalone[1], Robert E. Lanford[2] and David L. Lewis[1]

[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, Palo Alto, CA; [5] Hepatitis B Foundation, Doylestown, PA; [6] Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia

Arrowhead Research CORPORATION

Background:

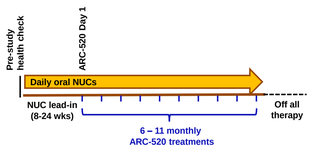
The HBV therapeutic ARC-520 was designed to decrease all cccDNA-derived viral transcripts and thus viral protein load via RNA interference (RNAi). ARC-520 consists of two cholesterol-conjugated RNAi triggers that target HBV sequences near DR1 plus a hepatocyte-targeted excipient for efficient delivery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

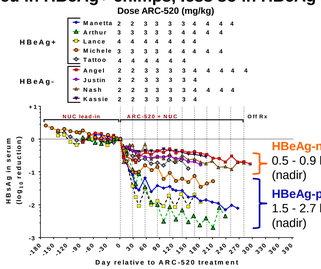
To reduce viral replication prior to treatment with ARC-520, chimps were treated for 8-24 weeks with entecavir (ETV) or in one case (chimp Michele) with ETV+ tenofovir (TDF). Following the NUC lead-in period, animals were administered ARC-520 intravenously at 4-week intervals (q4w). Dose levels were 2, 3, or 4 mg/kg ARC-520, along with maintenance doses of ETV or ETV+TDF. Chimps were monitored for up to 31 weeks off all treatment.



ARC-520 + NUC treatment:

Serum HBsAg is deeply reduced in HBeAg+ chimps, less so in HBeAg- chimps

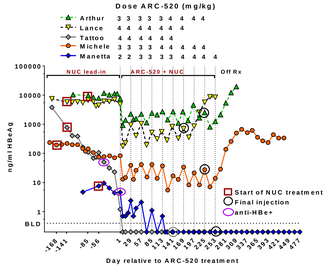
- NUC only therapy had minimal effect on serum HBsAg levels.
- HBsAg was reduced at nadir in HBeAg+ chimps by 2.1 $\log_{10}$ (avg.) and in HBeAg- chimps by 0.7 $\log_{10}$.
- Molecular biology approaches were used to elucidate the differences in HBV biology in the HBeAg+ vs. HBeAg- chimps. The HBeAg+ chimps had high levels of cccDNA in the liver, whereas almost all the HBV DNA in the liver of HBeAg- chimps (95%) appeared to be integrated.
- In this poster we evaluate response to ARC-520 therapy by measuring changes in HBV markers and in the serum cytokines associated with a T-cell response.



HBeAg-negative
0.5 - 0.9 $\log_{10}$ reduction (nadir)

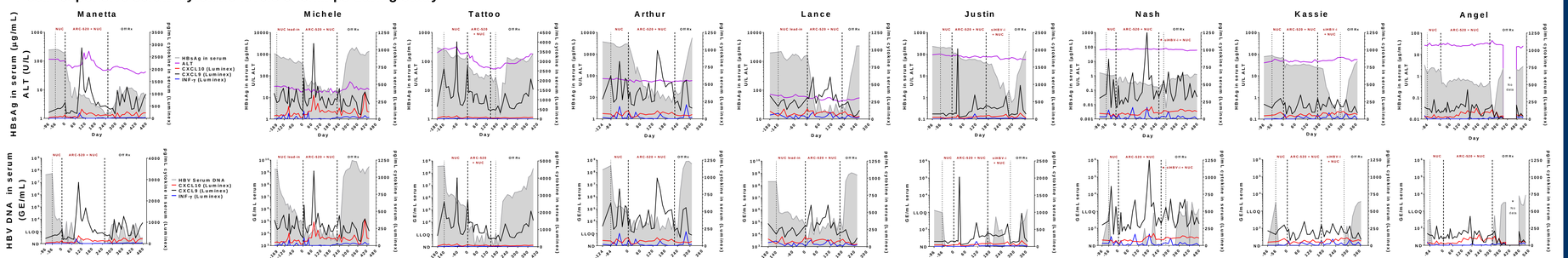
HBeAg-positive
1.5 - 2.7 $\log_{10}$ reduction (nadir)

Two HBeAg positive chimps, Manetta and Tattoo, seroconverted for HBeAg



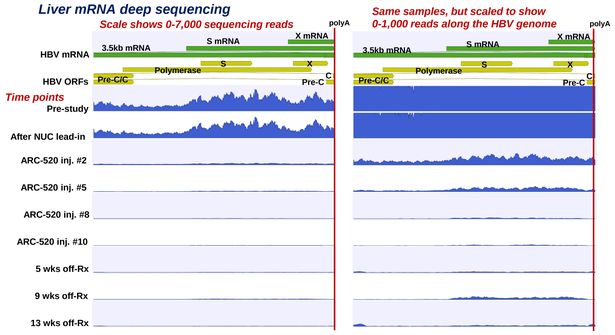
- Serum HBeAg levels changed only marginally during NUC lead-in phase.
- Tattoo had decreasing levels of HBeAg prior to and during NUC lead-in. Anti-HBe first detected 28 days prior to 1st ARC-520 injection. HBeAg lost following 1st ARC-520 dose and was sustained off-therapy.
- Manetta seroconverted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

T-cell responsive serum cytokine levels of chimps during study



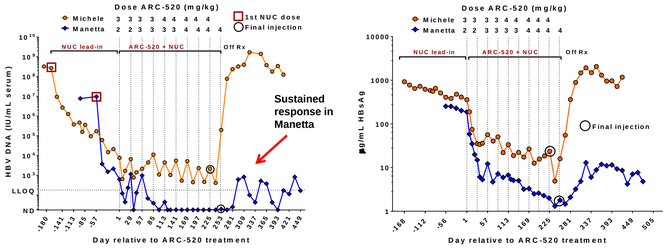
- 8 out of 9 chimps exhibited episodes of serum cytokine CXCL9 elevation. For 7 chimps these occurred during ARC-520 treatment and in one chimp (Tattoo) these coincided with seroclearance of HBeAg that began prior to study.
- In Manetta and Michele, IFN-gamma induction on ARC-520 treatment was followed by induction of chemokines CXCL9 and CXCL10. The beginning of this induction coincided with a time at which serum HBV DNA levels were low.
  - Peak levels of CXCL9 (Mig) were 2.6-fold higher in Manetta than Michele.
  - The sustained induction of CXCL9 in Manetta was associated with a therapeutic ALT flare (218 U/L) followed by HBeAg seroconversion.
  - In Manetta, off-treatment increases in HBV serum DNA were followed by CXCL9 and CXCL10 increases and subsequently decreased serum DNA and HBsAg.

Low liver HBV mRNA in Manetta off-treatment



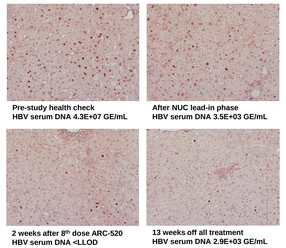
During ARC-520 treatment of Manetta: 91% reduction of total HBV mRNA after 2nd ARC-520 injection; 99.7% reduction after 10th injection. At 13 weeks off all treatment, total HBV mRNA was 99.3% reduced relative to pre-study.

Sustained virologic response in Manetta off-treatment

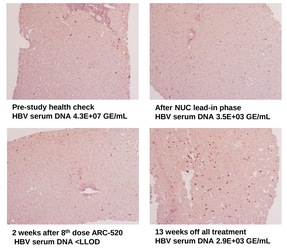


- Comparison of serum HBV DNA and HBsAg reduction in Manetta and Michele
  - End of study levels of HBsAg and HBV serum DNA were lower in Manetta than Michele. In Manetta HBV serum DNA was undetectable for 17 weeks while on ARC-520 + NUC treatment, but HBV DNA did not become undetectable in Michele.
  - Michele began the study with higher HBV DNA and HBsAg than Manetta, and Manetta received treatment longer. Both chimps experienced further declines upon repeat dosing.
- Manetta exhibited deep decreases in viral DNA, RNA and proteins on ARC-520 + NUC treatment and a sustained virologic response off treatment.
  - Effects of ARC-520 + NUC treatment:
    - Undetectable HBV serum DNA for 17 weeks
    - HBsAg level was reduced by 2.3 $\log_{10}$ fold at nadir, relative to pre-study, to a level of 1 µg/mL serum.
    - HBV mRNA in the liver was reduced by 99%.
    - The T-cell responsive cytokines INF-γ, CXCL9 and CXCL10 were induced during treatment, followed by an ALT flare and clearance of core antigen in the liver.
  - At 31 weeks off all treatment:
    - Final serum HBV DNA was 5 $\log_{10}$ fold lower than pre-study.
    - Final HBsAg was 1.7 $\log_{10}$ fold lower than pre-study.
    - Liver HBV RNA was 99% lower than pre-study.

HBcAg reduction in liver after flare in Manetta *(HBcAg immunostaining)*



SVR in Manetta coincided with hepatocyte turnover *(Ki67 immunostaining)*



SVR = sustained virologic response

Discussion and conclusions:

- All 9 chimpanzees responded to RNAi therapeutic ARC-520 with HBsAg reductions of 0.5 – 2.7 $\log_{10}$ at nadir, the greater reductions being in HBeAg+ chimps. Compared with the first dose response, HBsAg levels in all chimps decreased further with repeat dosing.
- 7 of 9 chimps, HBeAg positive and negative, exhibited indications of an immune response during ARC-520 treatment. HBV suppresses the immune system to allow chronic HBV infection. INF-γ is a key antiviral cytokine critical to innate and adaptive immune responses. INF-γ produced by NK and NKT cells induces the chemokines CXCL9 (Mig) and CXCL10 (IP-10) to mediate a T-cell response. Elevations of CXCL9 in these chimps are promising as a sign of immune system re-activation.
- Chimp Manetta exhibited deep decreases in HBV DNA, RNA and viral proteins during ARC-520 + NUC treatment, an on-treatment ALT flare, and a sustained virologic response 31 weeks off all treatment.

Special thanks to: University of Louisiana Lafayette; Texas Biomedical Research Institute; SNPRC Southwest National Primate Research Center