

# Kinetics of knockdown from RNAi therapeutic ARC-520 on HBV RNA, DNA and antigens in mice and chimpanzee

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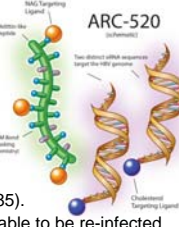
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## Background:

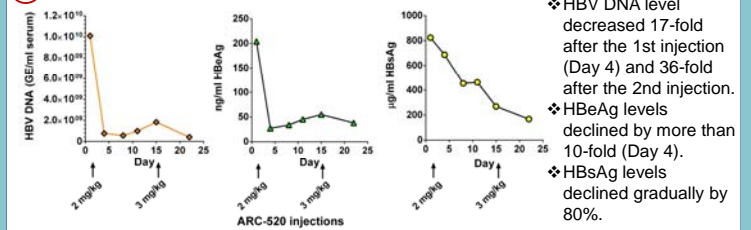
- Current therapies for HBV infection rarely reduce viral proteins implicated in sustainment of infection and progression of disease. We developed a small interfering RNA (siRNA)-based therapeutic named ARC-520 that is designed to decrease viral protein load via RNA interference (RNAi).
- ARC-520 is composed of 2 cholesterol-conjugated siRNAs and a hepatocyte-targeted membrane-lytic-peptide (NAG-MLP). When injected as ARC-520, cholesterol-siRNA is taken up by hepatocytes and released from endosomes by the action of NAG-MLP (also called ARC-520 Exo). Once in the cytoplasm, the siRNAs engage the RNAi machinery.
- The two siRNAs in ARC-520 together cover 99.6% of known genome sequences. They target sequences common to all the HBV transcripts (Wooddell et al., 2013, *Molecular Therapy* 21: 973-85).
- Mouse hepatocytes are able to replicate the human HBV but are unable to be re-infected. The chimpanzee is the only species other than human that is susceptible to infection with human HBV.
- Drug combination studies were initiated in anticipation of treating with ARC-520 patients who are presently on daily entecavir (ETV) treatment.

## Study design:

- Dose response, duration of effect, repeat dosing, and drug combination studies were conducted in mouse models of chronic HBV infection. These include transgenic HBV1.3.32 mice and transiently transfected NOD-SCID mice (the pHBV model).
- For dose response and duration of effect studies, mice were given a single iv injection of ARC-520. Drug activity was monitored by measuring HBsAg and HBeAg in serum by ELISA; HBV DNA levels in serum by qPCR; HBV RNA in the liver by Northern blotting and by RT-qPCR; HBV replicative intermediates in the liver by Southern blotting; and HBV core antigen in liver by immunohistochemistry.
- A proof-of-concept study was conducted in a single chimpanzee chronically infected with HBV genotype B for over 35 years. Chimpanzee 4x0139 is a large female (51 kg) with an exceptionally high viral titer ( $>10^{10}$  genomes/ml). ARC-520 activity was measured by monitoring HBsAg, HBeAg and HBV DNA in serum.
- For drug combination studies, pHBV model mice were treated with daily ETV by oral gavage, single iv injection of ARC-520, or both.

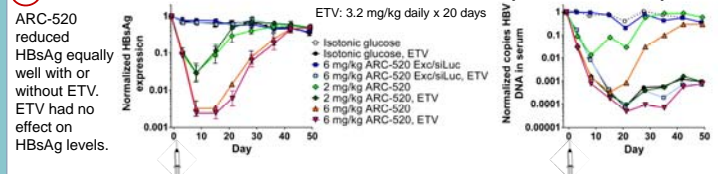


## 6 Chimpanzee treated with ARC-520



- HBV DNA level decreased 17-fold after the 1st injection (Day 4) and 36-fold after the 2nd injection.
- HBeAg levels declined by more than 10-fold (Day 4).
- HBsAg levels declined gradually by 80%.

## 7 Entecavir and ARC-520 in combination (mouse model)



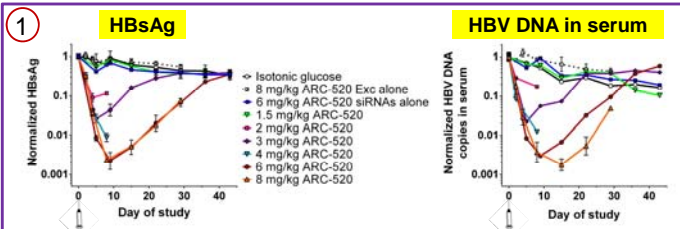
## 8 Drug combination index

ETV groups received daily ETV for 15 days; one ARC-520 injection given on the 10th day.  
Day 3 = 48 hr post ARC-520 injection.  
Day 6 = 5 days post-injection.  
Combination index values:  
0.1 – 0.3 ++++ Strong synergism  
0.3 – 0.7 +++ Synergism  
ARC-520 is synergistic with entecavir.

Treatment		Fold reduction of HBV serum DNA relative to vehicle control		Combination Index	
Entecavir (mg/kg)	ARC-520 (mg/kg)	Day 3	Day 6	Day 3	Day 6
0.001	0	78 ± 17	18 ± 7	N/A	N/A
0.01	0	548 ± 343	288 ± 104	N/A	N/A
0.1	0	533 ± 104	343 ± 122	N/A	N/A
0	2	10 ± 5	10 ± 6	N/A	N/A
0	3	41 ± 43	175 ± 191	N/A	N/A
0	4	72 ± 14	174 ± 72	N/A	N/A
0.001	2	557 ± 282	160 ± 56	0.277	0.451
0.01	3	1255 ± 186	1004 ± 487	0.259	0.414
0.1	4	3430 ± 2046	1628 ± 679	0.285	0.605

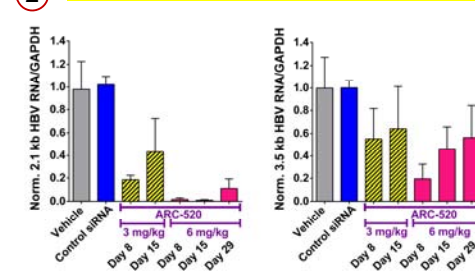
Combination index determined with CalcuSyn software.

## Dose and duration of effect in single injection studies



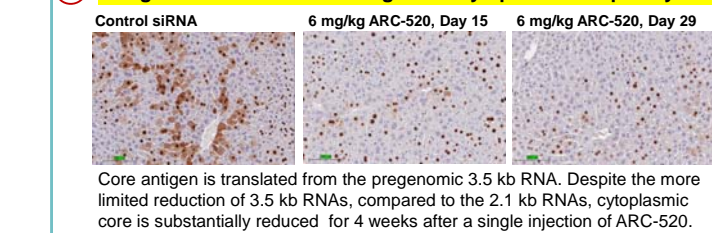
- Mice (pHBV model) were given a single IV injection of ARC-520. The magnitude of the HBsAg knockdown and HBV serum DNA reduction, as well as the duration of effect, were dose dependent.
- Control groups had no reduction of HBsAg or HBV DNA: vehicle (isotonic glucose); NAG-MLP alone (ARC-520 Exo); and ARC-520 siRNAs alone.

## 2 Northern blot analysis of HBV RNA in transgenic mouse liver



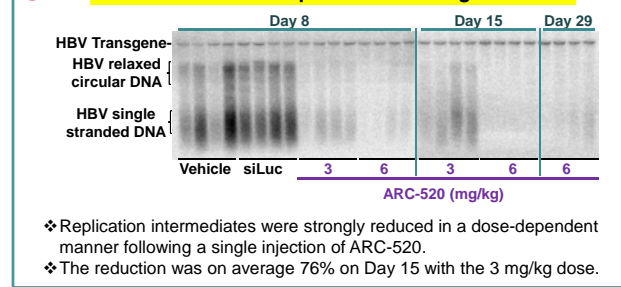
- The magnitude of reduction is greater for the 2.1 kb RNAs that encode HBsAg than for the 3.5 kb RNAs that:
  - serve as the pregenomic RNA and encode polymerase and core
  - encode HBeAg
- The duration of RNA reduction is greater for the 2.1 kb RNAs than for the 3.5 kb RNAs.
- Magnitude and duration of RNA knockdown are dose-dependent.

## 3 Long-term reduction of HBcAg in the cytoplasm of hepatocytes



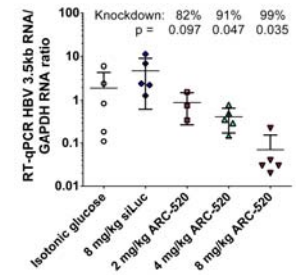
Core antigen is translated from the pregenomic 3.5 kb RNA. Despite the more limited reduction of 3.5 kb RNAs, compared to the 2.1 kb RNAs, cytoplasmic core is substantially reduced for 4 weeks after a single injection of ARC-520.

## 4 Reduction of HBV replication in transgenic mice



- Replication intermediates were strongly reduced in a dose-dependent manner following a single injection of ARC-520.
- The reduction was on average 76% on Day 15 with the 3 mg/kg dose.

## 5 Repeat injections for increased response



- Transgenic HBV mice were given 4 x q2w injections of ARC-520 and were evaluated one week after the last.
- Repeat dosing allowed for greater reductions in the 3.5 kb RNAs.
- The degree of knockdown was dose-dependent.

## Conclusions

- The RNAi therapeutic ARC-520 greatly reduces not only HBV replication but also the levels of HBV proteins in mouse models of chronic HBV infection with long duration of effect (>1 month).
- Repeat dosing (q2w) allows for greater suppression of virus and longer effect.
- ARC-520 therapy reduced HBV DNA, HBeAg and HBsAg in a chimpanzee chronically infected with a high viral titer. This chimpanzee study represents the first demonstration of efficient knockdown of HBV using systemic delivery of siRNA in a primate chronically infected with HBV.
- Drug combination studies demonstrate that entecavir and ARC-520 are synergistic for reduction of HBV DNA.
- The presence of entecavir does not inhibit the ability of ARC-520 to reduce viral antigens.
- Reduction of viral antigens is likely necessary to achieve HBsAg seroconversion and a functional cure of chronically infected HBV patients. ARC-520 shows promise as a treatment for chronic HBV infection.