ARC-520 RNAi therapeutic reduces HBV DNA, S and e antigen in a chimpanzee

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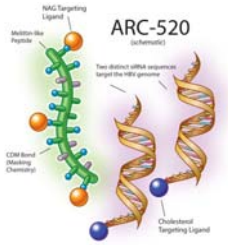
Background:

1. Therapeutic use of siRNA has not achieved the full potential of the technology due to poor targeting and cytoplasmic localization after systemic administration.
2. Dynamic PolyConjugate (DPC) technology provides targeted delivery of siRNA to liver hepatocytes. ARC-520 is a DPC therapeutic for treatment of HBV infection.
3. ARC-520 is composed of 2 cholesterol-conjugated siRNAs and a hepatocyte-targeted membrane-lytic-peptide (NAG-MLP). These are mixed prior to intravenous injection.
4. When injected as ARC-520, cholesterol-siRNA is taken up by hepatocytes and released from endosomes by the action of NAG-MLP. Once in the cytoplasm, the siRNAs engage the RNAi machinery.
5. The activity of this treatment for HBV was previously demonstrated in transgenic and transfected mouse models[1].
6. Here we demonstrated the efficacy of ARC-520 in a chronically infected chimpanzee, the only model for chronic human HBV infection.

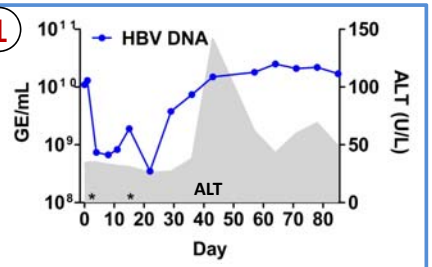


Design:

1. The two siRNA molecules in ARC-520 were selected from a panel of 140 HBV siRNAs using an *in vitro* system. Both sequences are highly conserved across HBV genotypes and together cover 99.6% of known genome sequences. They target sequences that are common to all the HBV RNA transcripts.[1]
2. For a proof-of-concept study, a single HBV chronically infected chimpanzee was selected.
3. Chimpanzee 4X0139 has been chronically infected with genotype B HBV for over 35 years, and is a large female chimpanzee (51 kg) with an exceptionally high HBV titer (> 10^{10} genomes per ml).
4. ARC-520 was injected on day 1 (2 mg/kg) and day 15 (3 mg/kg); asterisks in Figures 1-3.
5. Activity was monitored using serum levels of HBV DNA, HBeAg and HBsAg. Safety was monitored using CBC, blood chemistries, and serum levels of 39 cytokines and chemokines. Liver histology, viral antigens, and host transcripts were monitored in the liver.

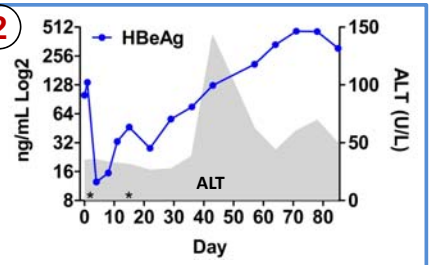
[1]Wooddell et al. Molecular Therapy 21:973-85, 2013.

1



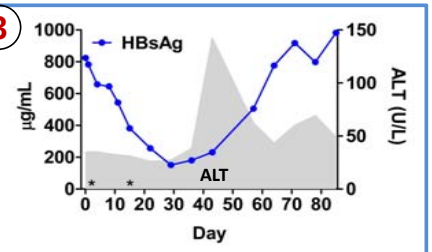
HBV DNA levels dropped by 17-fold by day 4, exhibited a 36-fold decline following the second dose, and returned to baseline by day 43.

2



HBeAg levels declined by more than one log by day 4 and returned to baseline by day 43.

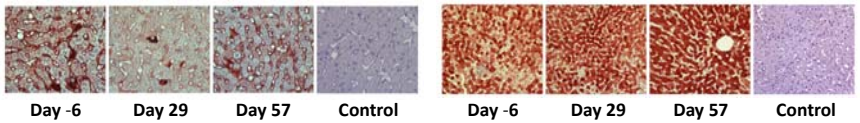
3



HBsAg levels declined gradually reaching a 5.2-fold reduction on day 29 and did not return to baseline until day 71. An increase in ALT was observed near the HBsAg nadir.

4

Immunohistochemical Staining of Liver Tissue

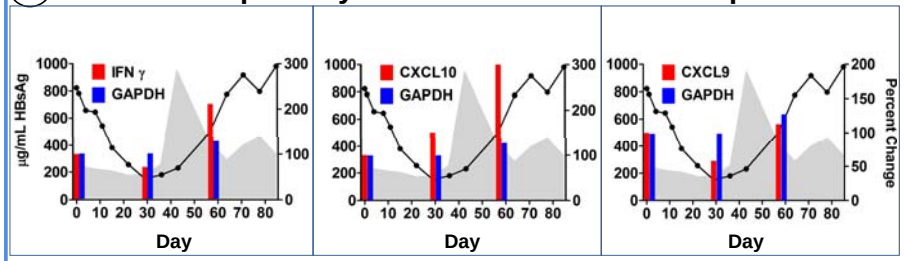


HBsAg has intense intercellular staining of secreted HBsAg prior to therapy and a marked reduction in staining on day 29, the time at which ELISA values for serum HBsAg were lowest.

HBcAg has intense intracellular staining prior to and throughout therapy. Most intracellular core protein is associated with highly stable nucleocapsids.

5

Intrahepatic Cytokine/Chemokine Transcripts



Induction of hepatic transcripts of cytokines and chemokines following ARC-520 dosing. A rise in ALT occurred on day 43 (shaded area), 4 weeks after the last dose of ARC-520 and after HBV DNA had returned to baseline. The ALT remained modestly elevated through day 85, the end of the study. The timing of the ALT rise suggested that it may be mechanistically related to the therapy and that it may involve an immunological event. Observed increases in key chemokine/cytokine mRNAs are also consistent with an immunological event. Total liver RNA was prepared from biopsies taken on days -6, 29 and 57 and transcripts for the T cell cytokine IFNγ and the IFNγ-induced chemokines CXCL9 (Mig) and CXCL10 (IP-10) were quantified by TaqMan RT-PCR. IFNγ was induced by 210% from baseline on day 57 during the ALT flare. At the same time point CXCL10 was induced by 310% from baseline, and CXCL9 was induced by 280% from the preceding biopsy. The values for the house keeping gene GAPDH are shown in the same panels.

Conclusions:

1. ARC-520 therapy demonstrated efficacy and safety in the chimpanzee model of HBV infection. Routine safety labs did not show signs of toxicity following ARC-520 dosing. This represents the first demonstration of efficient knockdown of HBV using systemic delivery of siRNA in a primate chronically infected with HBV.
2. Serum HBV DNA levels were reduced 36-fold and remained suppressed for 21 days after the final dose. Importantly, the viral antigens HBeAg and HBsAg were also suppressed. Reduction of viral antigens is likely necessary to achieve HBsAg seroconversion and a functional cure of chronically infected HBV in patients.
3. An ALT flare occurred 4 weeks after the last dose of ARC-520 suggestive of an immunological flare associated with de-repression of the adaptive immune response during antigen reduction.

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