Treatment of chronically HBV-infected chimpanzees with RNA interference therapeutic ARC-520 led to potent reduction of viral mRNA, DNA and proteins without observed drug resistance

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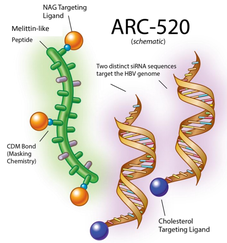
BACKGROUND AND AIMS

Worldwide up to 400 million people are chronically infected with HBV. Despite effective replication inhibitors (NUCs), functional cure is rare. We developed RNAi-based therapeutic ARC-520 to degrade viral transcripts, thereby reducing production of viral proteins that suppress the immune system and allow chronic infection. ARC-520, comprising cholesterol-conjugated RNAi triggers siHBV-74 and siHBV-77 and an excipient (ARC-EX1) that assists with endo/lysosomal escape of the RNAi triggers, demonstrated potent and durable knockdown in chronically HBV-infected chimpanzees and human patients. ARC-520 is currently in Phase 2b trials.



OBJECTIVES

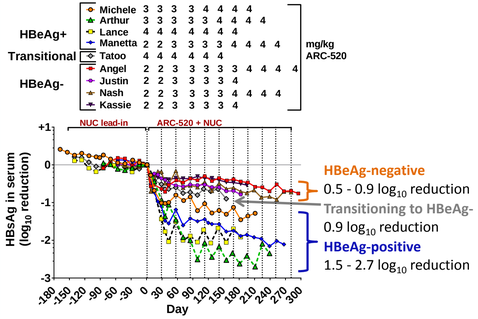
Assess whether HBV develops drug resistance upon multi-dose treatment with ARC-520 on a background of NUCs.

MATERIALS & METHODS

9 chimpanzees (9-37 years old, males and females) received NUCs for 8-24 weeks prior to ARC-520 dosing to suppress viral replication. They then received 6-11 monthly injections of ARC-520 (2-4 mg/kg) concurrent with NUC therapy. 5 chimps were initially HBeAg-positive (HBeAg+), baseline HBV DNA 8-9 $\log_{10}$ IU/mL serum, and 4 were HBeAg-negative (HBeAg-), ≤3 $\log_{10}$ IU/mL HBV DNA. Liver biopsies from 8 chimps were taken at baseline, completion of NUC lead-in period and periodically on study. Total RNA was isolated from the liver specimens. Parallel sequencing (RNA-seq) and computational analyses were performed to assess the HBV variants at each RNAi target site.

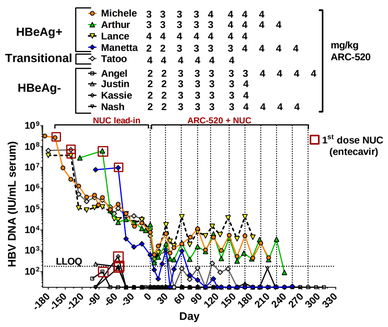
RESULTS

Figure 1a. HBsAg response to ARC-520



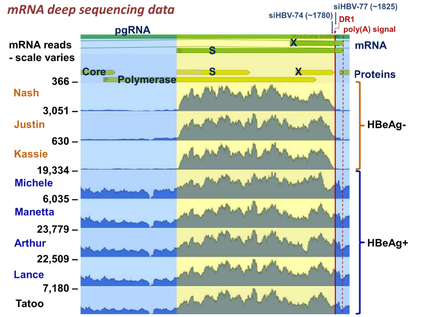
- HBsAg levels were deeply reduced after the initial ARC-520 dose and then decreased further upon repeat dosing.
- Greater reductions in HBsAg levels were observed in HBeAg+ chimps (1.7 ± 0.5 $\log_{10}$ reduction at nadir) than in HBeAg- chimps (0.7 ± 0.1 $\log_{10}$ reduction). Tatoo, who was in the process of HBeAg seroconversion prior to treatment, had an intermediate response.

Figure 1b. HBV serum DNA response



- 3-4 $\log_{10}$ reduction in HBV serum DNA during NUC lead-in.
- Additional ~1.5 $\log_{10}$ reduction with ARC-520.
- Response following each dose of ARC-520 suggests no emergence of resistance.

Figure 2. Distribution of HBV mRNA reads ***prior*** to ARC-520 treatment



- S transcripts make up a higher proportion of the total HBV transcripts in HBeAg- compared to HBeAg+ chimps.
- Very few transcripts contained sequence downstream of DR1 in HBeAg- chimps, consistent with a higher proportion of transcripts originating from integrated HBV in these chimps.

Table 1. Liver HBV mRNA deep sequencing reads containing ARC-520 target sites

Chimp name	HBeAg status	Time point of biopsy specimen (relative to ARC-520 Injections)	HBsAg (μg/mL) at time of biopsy		mRNA containing siHBV-74 target site		mRNA containing siHBV-77 target site(s)		Percentage of target sites containing dominant sequence	
				Total HBV mRNA reads	Number reads	Reduction following ARC-520 (%)	Number reads	Reduction following ARC-520 (%)	siHBV-74 target site	siHBV-77 target site
Michelle	HBeAg+	Pre-dose	358	498,566	4,511		4,763		97.8%	64.1%
		After 8th injection*	18.5	47,353	275	93.9%	292	93.9%	97.1%	61.3%
Arthur	HBeAg+	Pre-dose	2,866	795,621	5,471		11,111		98.5%	97.4%
		After 9th injection*	12.9	28,396	201	96.3%	370	96.7%	98.4%	98.3%
Lance	HBeAg+	Pre-dose	1,640	664,329	4,661		9,656		98.5%	97.8%
		After 7th injection*	19.9	80,865	427	90.8%	924	90.4%	98.1%	97.4%
Manetta	HBeAg+	Pre-dose	187	161,348	1,145		2,409		98.8%	98.4%
		After 10th injection*	2.3	589	4	99.7%	ND	ND	100.0%	NA
Tatoo	HBeAg+/-	Pre-dose	151	60,451	475		215		96.6%	95.1%
		After 6th injection*	19.1	18,045	57	88.0%	23	89.3%	100.0%	100.0%
Nash	HBeAg-	Pre-dose	1.2	5,815	87		28		98.8%	87.1%
		After 10th injection*	0.23	1,725	2	97.7%	4	85.7%	100.0%	100.0%
Justin	HBeAg-	Pre-dose	195	76,061	643		283		98.2%	95.7%
		After 2nd injection*	78	24,545	44	93.2%	18	93.6%	100.0%	90.0%
Kassie	HBeAg-	Pre-dose	72	12,255	60		15		96.7%	100.0%
		After 2nd injection*	30	5,670	2	96.7%	2	86.7%	100.0%	100.0%

*Last biopsy following an ARC-520 injection

- mRNA was isolated from liver biopsies taken after NUC lead-in and two weeks after ARC-520 injection (last injection when available), then deep sequenced at a depth of 40 million reads on the Illumina platform. Total mRNA reads were similar for all samples.
- HBeAg+ chimps had more mRNA target site reads for siHBV-77 than for siHBV-74. The siHBV-77 target site is redundant in the transcript that encodes HBeAg.
- HBeAg- chimps had fewer target sites for siHBV-77 than for siHBV-74, consistent with their limited production of the pregenomic RNA. This result is also consistent with integration occurring predominantly near the DR1 site, which would result in loss of the siHBV-77 target sites that coincide with DR1 more often than loss of the upstream siHBV-74 target site.
- mRNA reads containing the siHBV-74 target site were reduced with similar efficiency in HBeAg- (93.9% ± 3.8%) as HBeAg+ chimps (95.2% ± 3.2%).
- Prior to the ARC-520 injections, the dominant sequence of the siHBV-74 target site was more similar between animals (96.6% to 98.8%) than was the dominant sequence of the siHBV-77 target site (64.1% to 100%). The major variant of the siHBV-77 target site differs between chimpanzee and human HBV.
- The percentages of sites containing the dominant sequence in each animal did not vary significantly before and after ARC-520 treatment. Most of the minor variants had a frequency of <0.3%, the expected rate of sequencing error.

SUMMARY

All chimps responded to ARC-520 with deep reductions of HBsAg and HBV serum DNA. Following the deep initial reductions of HBsAg, levels further decreased with subsequent doses. HBV serum DNA decreased similarly after each ARC-520 dose. RNAseq alaysis revealed the distribution of siRNA target site sequences for each animal remained virtually unchanged from pre-ARC-520 treatment to the end of the treatment period. Together these date indicate that there was no development of drug resistance during the treatment period. Having two RNAi triggers in ARC-520 provides greater genome coverage (expected to be >99.6% in humans) and may also decrease the likelihood of developing drug resistance.

The HBeAg+ chimps responded with deeper HBsAg reduction (1.7 ± 0.5 $\log_{10}$) than the HBeAg- chimps (0.7 ± 0.1 $\log_{10}$), but mRNA containing the siRNA target sites was similarly reduced (>90%) in the HBeAg+ and HBeAg- chimps. These data, the observed distribution of mRNA reads along the HBV genome, and the reduced target sites just upstream of DR1 in the HBeAg- chimps are consistent with a significant number of the S mRNA transcripts being produced from integrated HBV genomes in these animals.

CONCLUSIONS

ARC-520 led to potent reduction of HBV mRNA, DNA and HBsAg with no observed drug resistance in chimpanzees chronically infected with HBV and treated concurrently with ARC-520 and NUCs for 6 to 11 months.

REFERENCE

Wooddell et al. (2013). Hepatocyte-targeted RNAi Therapeutics for the Treatment of Chronic Hepatitis B Virus Infection. Mol Ther 21: 973-985.

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