Differential reductions in viral antigens expressed from cccDNA vs integrated DNA in treatment naïve HBeAg positive and negative patients with chronic HBV after RNA interference therapy with ARC-520

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BACKGROUND

ARC-520 is a novel, short interfering RNA (siRNA)-containing, liver-targeted therapeutic for treatment of chronic hepatitis B patients (CHB) designed to reduce all cccDNA derived HBV transcripts via RNA interference. Viral proteins, in particular HBsAg, have been implicated in immune tolerance, sustained infection and disease progression. Therapies targeting viral proteins might allow host immune reconstitution, thereby promoting HBsAg seroclearance.

A study in HBV infected chimpanzees showed that in HBeAg negative chimps a significant portion of HBsAg production is derived from integrated DNA, which is not targeted by ARC-520 [1].

We previously reported safety and activity of ARC-520 in entecavir (ETV) experienced CHB and preliminary results in treatment-naïve CHB enrolled in a single dose phase 2a study [2]; here we report full safety and activity against multiple viral antigens and DNA in treatment-naïve CHB.

OBJECTIVES

Primary Study Objective:

Depth and duration of HBsAg reduction in response to a single dose or two doses (cohort 6) of ARC-520 in combination with entecavir

Additional Objectives:

Safety and tolerability of escalating single doses of ARC-520 co-administered with a fixed dose of entecavir

Multiple additional secondary and exploratory objectives

MATERIALS & METHODS

Table 1: Study cohorts and status

Cohort	Prior ETV	Pat Type	ARC-520 dose	Active / PBO	Baseline HBsAg mean (range)‡	Status
1	Yes**	HBeAg neg	1.0 mg/kg	6/2	3.4 (3.0-4.2)	Complete/Unblinded
2	Yes**	HBeAg neg	2.0 mg/kg	6/2	3.5 (3.2-4.3)	Complete/Unblinded
3	Yes**	HBeAg neg	3.0 mg/kg	6/2	3.6 (3.1-4.0)	Complete/Unblinded
4	Yes**	HBeAg neg	4.0 mg/kg	6/2	3.4 (3.2-4.0)	Complete/Unblinded
5	Yes**	HBeAg pos	4.0 mg/kg	6/2	3.6 (3.1-4.2)	Complete/Unblinded
6*	Yes**	HBeAg pos	2 x 2.0 mg/kg	6/0	3.3 (3.0-3.6)	Complete/Open label
7	No	HBeAg pos HBeAg neg	4.0 mg/kg	6/0 6/0	4.4 (3.1-4.9) 2.9 (0.8-3.6)	Complete/Open label

•* two doses two weeks apart; ** > 6 months; ‡ Log IU/mL for active subjects; PBO = normal saline

- Adult patients with HBeAg negative or positive chronic HBV, ALT/AST < 100 IU/mL and Fibroscan ≤ 8 at screening.
- Entecavir (ETV) experienced patients had HBsAg >1000 IU/mL (cohorts 1-4) or >500 IU/mL (cohorts 5-6) and HBV DNA < LLOQ at baseline.
- Treatment-naïve patients had no limits on HBsAg or HBV DNA at baseline, started daily oral ETV concomitantly with intravenous ARC-520 and continued ETV throughout the study.
- Viral DNA and antigen knockdown were measured over 85 days [quantitative HBsAg, HB core-related antigen (HBcrAg) in all, HBeAg in HBeAg-positive].

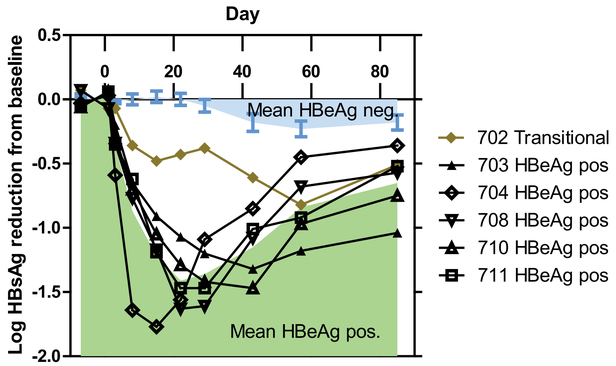
RESULTS

- Fifty-eight patients have been successfully dosed with 48 receiving drug and 10 receiving placebo. 20 females and 38 males were enrolled, all of Chinese ethnicity, with a mean age of 41 years (range of 23 to 59).
- There have been no serious AEs, no dose limiting toxicities, no discontinuations due to medication AEs, and a modest occurrence rate (8/58 patients = 14%) of AEs. All reported AEs were deemed unrelated to study drug by the PI.
- There was a low occurrence rate of abnormal laboratory tests, with no observed relationship to timing or dose of ARC-520 or placebo.

Table 2. Treatment emergent adverse events

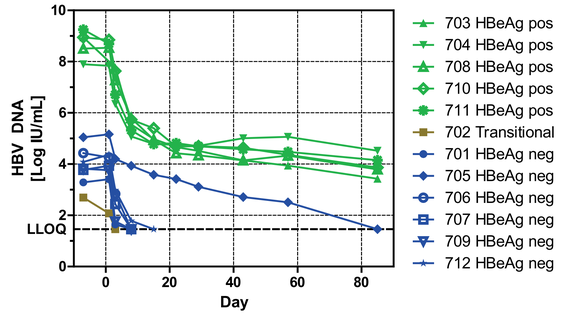
Adverse Evet	1 mg/kg n=6	2 mg/kg n=6	3 mg/kg n=6	4 mg/kg n=24	2 mg/kg x2; n=6	PBO n=10
All	1	5	1	2	1	0
Extravasation		1 mild				
Malaise		1 mod				
Influenza	1 mild					
Blood CK increase		1 mild				
Diabetes Mellitus		1 mild				
Pain in extremity			1 mild			
Presyncope		1 mod				
Headache				1 mild		
Dizziness				1 mild		
Fever					1 mild	

Figure 1: Reduction in HBsAg in treatment naïve CHB patients in response to a single dose of 4 mg/kg of ARC-520.



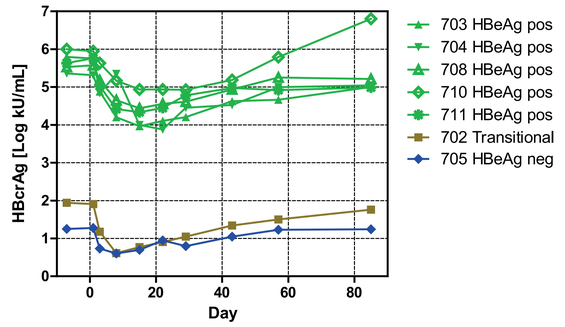
- Two distinct patterns of HBsAg reductions were seen in response to ARC-520.
- Five of six HBeAg positive, ETV naïve patients showed an immediate, direct antiviral effect. The maximum HBsAg reduction from baseline was 1.8 log, with a mean of 1.5 log. HBsAg was still reduced 85 days after a single dose or ARC-520.
- Five of six HBeAg negative, ETV naïve patients showed a delayed response several weeks after treatment. The maximum HBsAg reduction from baseline was 0.5 log, with a mean of 0.3 log.
- One transitional patient was HBeAg-positive at baseline and HBeAg negative at days 3 to 43 and showed an intermediate HBsAg response.

Figure 2: HBV DNA in treatment naïve CHB patients.



- All HBeAg negative and one transitional patient achieved serum HBV DNA below the limit of quantitation within a median of 15 days.
- Five of six HBeAg positive patients above LLOQ had a maximum DNA reduction of 5.5 log, with a mean reduction of 4.7 log.

Figure 3: HBcrAg in treatment naïve CHB patients after a single dose of 4 mg/kg ARC-520.



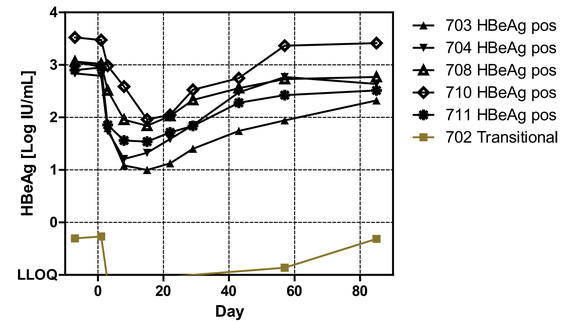
- Five of six HBeAg negative patients had HBcrAg below the limit of quantitation. One patient with measurable HBcrAg had a 0.7 log reduction from baseline.
- In HBeAg positive patients (including transitional), the maximum reduction in HBcrAg was 1.8 log, with a mean reduction of 1.4 log.

Table 3. Max reduction in viral antigens

				Log reduction from baseline Mean (max)		
Cohort	Dose [mg/kg]	HBeAg status	Prior ETV	HBsAg	HBcrAg	HBeAg
1	1	Neg	Y	-0.2 (-0.3)	-0.2 (-0.2)	N/A
2	2	Neg	Y	-0.2 (-0.3)	-0.5 (-0.5)	N/A
3	3	Neg	Y	-0.3 (-0.4)	-0.4 (-0.7)	N/A
4	4	Neg	Y	-0.4 (-0.5)	-0.9 (-1.1)	N/A
5	4	Pos	Y	-0.3 (-0.7)	-0.9 (-1.1)	-1.2 (-1.7)
6	2x2	Pos	Y	-0.5 (-1.0)	-0.7 (-1.2)	-0.7 (-1.1)
7	4	Pos	N	-1.5 (-1.8)†	-1.4 (-1.8)	-1.6 (-2.0)†
7	4	Neg	N	-0.3 (-0.5)	N/A (-0.7)	N/A

† Excluding transitional patient

Figure 4: HBeAg in treatment naïve CHB patients after a single dose of 4 mg/kg ARC-520.



- Five of six HBeAg positive patients above the LLOQ had a maximum HBeAg reduction of 2.0 log, with a mean reduction of 1.6 log.

CONCLUSIONS

1. ARC-520 was well tolerated.
2. ARC-520 + entecavir produced rapid DNA suppression consistent with their synergistic activity shown in preclinical studies.
3. ARC-520 effectively inhibited cccDNA-derived mRNA with viral protein reduction of up to 2.0 logs (99%) observed.
4. In HBeAg-positive, naïve CHB patients all viral antigens were effectively suppressed, while in HBeAg-negative patients less reduction in HBsAg was observed.
5. These findings are consistent with more cccDNA-driven antigen production in naïve HBeAg-positive and a higher fraction of HBsAg production from integrated DNA in HBeAg-negative patients.
6. These variations in viral protein knockdown are consistent with data from chimps [1] showing higher fractions of integrated DNA-derived viral mRNA in HBeAg-negative animals. Integration of HBV DNA results in deletions near the DR sites and viral mRNAs expressed from integrated DNA can lose their ARC-520 target sites.
7. Chronic ARC-520 studies aimed at producing HBsAg seroclearance are ongoing.

REFERENCES

[1] Wooddell, C. et al. (2015) Reductions in cccDNA under NUC and ARC-520 therapy in chimpanzees with chronic hepatitis B virus infection implicate integrated DNA in maintaining circulating HBsAg. Hepatology 62: 1(Suppl), 32.

[2] Yuen, M-F, et al. (2015) ARC-520 produces deep and durable knockdown of viral antigens and DNA in a phase II study in patients with chronic hepatitis B. Hepatology 62: 6 (Suppl), LB-9.