Predicting HBsAg clearance responses during ARC-520 RNA interference (RNAi) therapy based on HBsAg epitope profile analysis

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BACKGROUND

Functional cure of chronic hepatitis B requires HBsAg loss and seroconversion to anti-HBs antibody. ARC-520 RNAi drug therapy targets cccDNA derived mRNA in the liver, including the HBsAg transcript, to promote host immune recovery and HBsAg seroclearance. Few biomarkers can reliably predict this outcome. Mapping the HBsAg profile based on epitope availability or occupancy has identified a predictive HBsAg Clearance Profile (CP) associated with HBsAg clearance in antiviral therapy cohorts. The developing anti-HBs response, possibly due to immune recovery on therapy, can be detected by analysis of antigen/antibody complexes.

OBJECTIVES

To evaluate the association between HBsAg response on ARC-520 RNAi therapy and the development of an HBsAg CP predictive of seroclearance, and concomitant development of a co-existing anti-HBs response suggestive of immune recovery.

MATERIALS & METHODS

Study Cohort: Consisted of 40 ARC-520 study HBeAg-negative (n=32) and HBeAg-positive (n=8) patients (under code: 30 ARC-520; 10 placebo), from pre-treatment to day 85 [1]. All were entecavir suppressed prior to (mean 5 years) and during ARC-520 therapy.

Diagnostic Serology: Study samples were batch analysed for quantitative HBsAg (IU/mL) and (where applicable) for HBeAg (PE IU/mL) to end-point using the Roche Cobas or Diasorin Liason platforms respectively.

HBsAg Clearance Profile (CP): Analysis of HBsAg CPs was performed using a 19plex HBsAg epitope mapping assay [2,3], and results were related to HBsAg response on-treatment.

Complexed Anti-HBs Development: We have developed an EIA for detection of anti-HBs complexed with HBsAg, which is not able to be detected using current diagnostic assays. Briefly, HBsAg is captured by 2plex magnetic bead set conjugated with broadly specific anti-HBs mAbs, and concomitant patient-derived anti-HBs identified indirectly via the human IgG Fc domain detection.

RESULTS

ARC-520 Study: Details & Outcomes

- Adult patients (n=40) with chronic hepatitis B (HBeAg negative, n=32; HBeAg positive, n=8) were enrolled in ARC-520 cohorts 1-5 (active, n=30; placebo, n=10) dose escalations studies (1-4mg/kg)
- All patients were entecavir (ETV) experienced (>6mth, mean 5yr), continued during study
- At screening patients had HBsAg >1000 IU/mL, ALT/AST <100 IU/mL, and Fibroscan score <8

ARC-520 study details

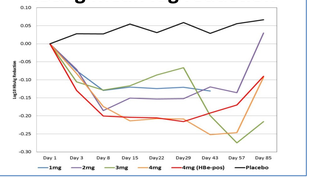
Cohort	HBeAg status	Dose (mg/kg)	ARC-520/ Placebo	Baseline HBsAg mean (range)‡
1	neg	1.0	6/2	3.4 (3.0-4.2)
2	neg	2.0	6/2	3.5 (3.2-4.3)
3	neg	3.0	6/2	3.6 (3.1-4.0)
4	neg	4.0	6/2	3.4 (3.2-4.0)
5	pos	4.0	6/2	3.6 (3.1-4.2)

- HBsAg responses were dose dependant in HBeAg-neg patients (cohorts 1-4)
- HBsAg responses were substantially enhanced in HBeAg-pos patients (cohort 5), and displayed a trend to occur more rapidly and persist longer
- Reduction of HBeAg load (in HBeAg-pos patients, cohort 5), achieved >1log early and was maintained to approximately week4

ARC-520 effect on viral antigen reduction

			Log reduction Max (mean)	
Cohort	Dose (mg/kg)	HBeAg	HBsAg	HBeAg
1	1	Neg	-0.3 (-0.2)	n/a
2	2	Neg	-0.3 (-0.2)	n/a
3	3	Neg	-0.4 (-0.3)	n/a
4	4	Neg	-0.5 (-0.4)	n/a
5	4	Pos	-0.7 (-0.3)	-1.7 (-1.2)

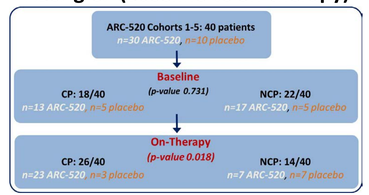
ARC-520 dose-dependant HBsAg mean log reduction



HBsAg CP Development on ARC-520 Therapy

- There was a significant association between the development of an HBsAg CP and ARC-520 therapy (p-value 0.018), specifically identified at:
  - Week 1 (p-value 0.038),
  - Enhanced at weeks 2 and 3 (p-values 0.019 & 0.003, respectively)
  - Late response association at week 6 (p-value 0.007)
- HBsAg CP preceded or coincided with an HBsAg response on ARC-520 therapy

HBsAg CP (Baseline vs on-therapy)



HBsAg CP detection on ARC-520 (all timepoints)

Cohorts 1-5	Baseline	Week1	Week2	Week3	Week4	Week6	Week8	Week12
ARC-520 (n=30)	13	11	12	16	15	14	5 (5/24 tested)	5
placebo (n=10)	5	0	0	0	2	0	1 (1/8 tested)	1
p-value	0.730	**0.038**	**0.019**	**0.003**	0.145	**0.007**	1.000	1.000

Complexed Anti-HBs Development on ARC-520 Therapy

- The development of detectable anti-HBs complexed with HBsAg coincided with both:
  - HBsAg response on ARC-520 therapy *(trend only, did not reach significance)*
  - HBsAg CP detection
- Development of complexed anti-HBs may represent:
  - Recovery of the immune response
  - Reduction of 'free' HBsAg to increase the ratio of complexed anti-HBs/HBsAg

CONCLUSIONS

HBsAg clearance and presumably the selective pressure of an effective anti-HBs response are key factors to achieve functional CHB cure. ARC-520 therapy reduced HBsAg load in a dose-dependent manner.

- Development of an HBsAg CP was predictive of HBsAg decline due to ARC-520 therapy, with a significant association escalating from week 1 (p-value 0.038) to weeks 2 and 3 (p-values 0.019 and 0.003 respectively), and preceded or coincided with the HBsAg decline.
- Detection of complexed anti-HBs, possibly reflective of immune recovery, coincided with HBsAg Clearance Profile.

REFERENCES

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3. Hyakumura M, Walsh R, et al. (2015) *J Virol* 89(22): 11312-22.