Infection with hepatitis B virus (HBV) may lead to acute or chronic hepatitis. HBsAg is the primary diagnostic target of serological diagnostic in HBV infection. Nucleic acid polymers (NAPs) clear HBsAg from the blood by blocking its absorption and antigen presentation. Clinical trials of the NAP REP 2139 were carried out to determine haplotypes in patients at different phases of REP 2139 therapy in the REP 102 protocol to better understand its outcome.

**METHODS**

1. Sequence analysis of a 314 kb haplotype was performed by deep sequencing targeted the major hydrophilic region fragment of HBV DNA.

2. Serum samples of 12 patients obtained throughout therapy and during follow-ups were used for analysis. Sample were selected up to the lowest possible HBsAg result (highlighted in green in Table 1) containing sufficient HBV DNA for sequencing analysis (see Table 1).

3. PCR products were directed and deep sequencing were performed by single or semi nested PCR of a HBsAg fragment of HBV DNA.

4. Deep sequencing targeted the major hydrophilic region (MHR) (including the "a"-determinant) of HBVAg.

5. Direct sequencing was performed using Sanger and analyzed with Geneious software.

6. NGS analysis was performed on Illumina® data from all samples.

**RESULTS**

- Of 12 patients treated (3A, 4A, 7A, 9A), 9 responders (with HBsAg reductions) and 3 non-responders (1A or less HBsAg reduction) were identified (Al-Mahtab et al., 2016, see Table 1).

- No mutations evolved in the "a"-determinant region during REP 2139 therapy in all 12 patients (Figure 1). Haplotype either decreased or remained unchanged during treatment (Figure 1).

- Among the 3 non-responders (Patient 1, 5, 10), a total of 4 mutations were observed occurring outside the "a"-determinant region which include G76C, L88P, V118G and P120T (Figure 2, Table 2).

- In the 9 responder patients, 24 different mutated positions were observed occurring outside the "a"-determinant region which include G76C, F85C, L88P, V118G, T189I (Figure 2, Table 2).

- The number of haplotypes remain stationary throughout the treatment for two of the non-responders, however, one non-responder (Patient 5) shows a dramatic increase following treatment.

- The number of haplotypes remained stationary throughout the treatment for two of the non-responders, however, one non-responder (Patient 5) shows a dramatic increase following treatment (Figure 1).

**CONCLUSIONS & PERSPECTIVE**

- Mutation of the "a"-determinant region of HBsAg or alteration in MHR quasispecies does not occur with REP 2139 therapy.

- Evolution of HBsAg variants escaping detection by HBsAg assays does not occur with chronic exposure to REP 2139.

- No relationship between haplotypes present within the MHR of HBsAg and response to REP 2139 therapy were observed.

- Treatment with REP 2139 does not appear to induce any selection pressure on the MHR.

- Circulating virus species persisting at later stages of REP 2139 treatment may not be recognized by an impaired host immune response.

**REFERENCES**

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**Figure 1 (A): MHR haplotype complexity during treatment in responders

Figure 1 (B): MHR haplotype complexity in non-responders