RAPID TURNOVER OF cccDNA

To maintain chronicity, the Hepatitis B Virus (HBV) relies on both the generation of and maintenance of cccDNA populations. Current strategies (i.e., Nuc therapy) are unable to clear virus from infected cells, due to the relative resistance of cccDNA to current therapies (including loss of detectable HBV antigen) while on Nuc therapy. Initial mathematical modeling, built on the premise that Nucs such as ADV and ETV effectively blocked new cccDNA formation, estimated that it would take approximately 14 years to clear intranuclear cccDNA from a chronically infected patient. We now know that Nucs do not completely block cccDNA formation and intranuclear cccDNA levels steadily increase with therapy. The current study, cccDNA biogenesis was revisited using a molecular genetic approach. By monitoring the emergence and disappearance of Nuc resistance in patient liver and serum samples as a genetic marker for cccDNA, we are able to evaluate the evolution of cccDNA populations over time. The current analysis has four requirements: 1) Diversity methods to establish distinct analysis of cccDNA, pgRNA and viral DNA populations from patient serum and liver biopsy samples; 2) Establish the relationship between genotypic sequence in pgRNA and cccDNA; 3) Measure the turnover rate of cccDNA pools; and 4) Determine if nucleotide substitutions of cccDNA exist.

**MODELS OF cccDNA BIOGENESIS**

**INTRODUCTION**

Rapid turnover of cccDNA in Chronic Hepatitis B Patients during Drug Resistance Emergence and Breakthrough

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**METHODS**

- Strategies: HBV DNA, pgRNA and cccDNA from longitudinal serum and paired biopsies of LAM or Tenofovir (TDF) therapy patients were evaluated. Nuc therapy was administered for up to 3 years and patients were enrolled on TDF alone or in combination with Nuc therapy.
- Seronegative HBV DNA and pgRNA Extraction, Amplification and Sequencing: HBV DNA and pgRNA were extracted from patient serum using Qiaxtract® HBV Virus DNA/DNA (Qiagen) or HBV DNA and pgRNA were isolated from liver biopsy samples using PureLink® Genomic DNA Kit (Invitrogen) and the percentage of each population was determined using population sequencing. Intranuclear cccDNA, HBV DNA and pgRNA Extraction: Amplification and Sequencing: intranuclear cccDNA was isolated (6) using the T5 exonuclease method, amplified by PCR and analyzed by population sequencing. Sequencing reactions (with a forward HBV DNA primer) determined that cccDNA was indeed present in liver biopsy samples.

**Rapid turnover of cccDNA**

- Data from serum and liver biopsy samples demonstrated that serum pgRNA correlated well with intranuclear RNA and cccDNA, which validated that serum pgRNA accurately reflects the genetic composition of cccDNA.
- The disappearance of resistant sequences was apparent from cccDNA in two patients who were lost to follow-up.

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**SUMMARY**

- Direct comparison of HBV DNA, RNA and cccDNA in nine patients established that serum pgRNA composition accurately reflects the intranuclear and cccDNA pools, allowing cccDNA biogenesis to be monitored by pgRNA composition and turnover.
- Biopsy results from two clinical studies provide little evidence for selection of resistant species of cccDNA (resistance emergence is the result of rapid turnover of cccDNA); or that HBV pgRNA are generated by only a subpopulation of active cccDNA molecules.
- All five TDF non-responders in Study NL15376 showed reversal of serum HBV DNA and pgRNA populations from NucR to WT in as few as 12 weeks when TDF selection pressure was withdrawn.
- In six HBV patients with virologic breakthrough on TDF therapy, pgRNA and cccDNA sequencing demonstrated rapid establishment of newly formed cccDNA molecules harboring NucR mutations.
- Turnover of WT cccDNA within a few weeks suggests that this cccDNA may decay faster than previously predicted.
- These data generated into an alternative model support the proposed “alternative” model of cccDNA biogenesis.
- This study suggests that cccDNA has a limited half-life, indicating that therapies which fully inhibit establishment of new cccDNA may lead to higher rates of cure for patients with CHB.