# Analysis of woodchuck hepatitis virus dynamics in the woodchuck-WHV infection model during acute versus chronic infection, before and after antiviral treatment

Md Deen Islam[1], DP Whiteside[2], L Al-Yasiri[1], JL Rothenburger[2], TI Michalak[3], S D'Souza[1], T Wilayat[1], A Chen[1], F van der Meer[2], G van Marle[1], CN Jenne[1], CS Coffin[1]
[1]Cumming School of Medicine, University of Calgary, AB, [2]Faculty of Veterinary Medicine, University of Calgary, AB, [3]Faculty of Medicine, Memorial University of Newfoundland, St. John's, NL
Email: mddeen.islam@ucalgary.ca

**Abstract:** The antiviral effects of Entecavir (ETV) against woodchuck hepatitis virus (WHV) and human hepatitis B virus (HBV) are well established. However, the dynamics of hepadnaviral diversity during disease progression and treatment are not well characterized. We aim to infect woodchucks with WHV followed by serial collection of blood and liver biopsy to assess pathogenesis and hepadnaviral quasispecies with or without ETV treatment. This study will inform the understanding of hepadnaviral reactivation risks after stopping ETV.

## Introduction

With 240 million chronic infections globally, HBV remains one of the major public health concerns (D'Souza et al., 2020; McGlynn et al., 2021). Most adults with acute hepatitis B infection (>95%) have self-limited acute hepatitis (SLAH) and only <5% progress to chronic infection with persistence of HBV surface antigen for >6 months (Liang et al., 2009). The woodchuck hepatitis virus (WHV) and HBV are members of the Hepadnaviridae family with similar disease pathogenesis (Menne et al., 2007). Eastern North American woodchucks (*Marmota monax*) with chronic woodchuck hepatitis virus (WHV surface antigen positive, WHsAg+) infection treated with nucleos(t)ide analogues (NUC, Entecavir) show suppressed viral replication and delayed liver disease progression (Colonno et al., 2001). Stopping therapy can lead to viral rebound in WHsAg+ woodchucks, similar to HBsAg+ chronic hepatitis B (CHB) patients (Colonno et al., 2001; Lai et al., 2020). In some CHB patients stopping NUC after 2-3 years of therapy can also lead to improved immune control and HBsAg Sero-clearance (Medas et al., 2021). However, the dynamics of hepadnaviral diversity during disease progression and ETV therapy are not well characterized.

We aim to investigate the dynamics of hepadnaviral quasispecies during acute, chronic and early ETV treatment before and after stopping therapy.

We compared adult woodchucks which were experimentally infected (inoculum = 0.5 ml x $10^6$ VGE/ml) with WHV (AH, n=4), chronically WHsAg+ hepatitis (CH, n=5) which were neonatally infected and treated daily until the viral load was suppressed till ~< 50 VGE/mL for at least 16 weeks, and the last group (n=2) were untreated CH animals. All 11 animals had serum, plasma/PBMC and liver biopsy collected for WHV replication markers and standard biochemical/histological/imaging analysis. To date, WHV X-gene diversity was assessed by Sanger; alignments/phylogenetic analyses were performed with MEGA11.

## Results:

The mean viral load of AH at week 1 post-infection (p.i.) was 3.0 x$10^6$ (range, 0.6-5.5 x$10^6$) vs 1.5 x$10^9$ (range, 0.5-2.6 x$10^9$) VGE/mL in CH (n=5) at baseline (a week before starting ETV), respectively. After 18 wks of ETV, WHV DNA declined by 7 logs (range, 6-9 log-fold), which rebounded at a median of 5 weeks (range, 2-11 weeks) to 3.5 x$10^4$ VGE/mL at the end of treatment (EOT) (n=3/5). During ETV treatment, alanine aminotransferase (ALT), declined from a median of 8 (range, 7-10) to 3 (range, 1-4) IU/ml at the EOT (n=3/5). Analysis of WHV X-gene diversity in AH vs CH did not show distinct clustering. *In the CH stop treatment group, clustering of WHV X-gene sequences was noted in early vs. late time-points under treatment, suggesting viral quasispecies change during treatment*. This observation will be further investigated with WHV whole genome sequence analysis. Other CH (n=2/5) animals are under continuous treatment for further observation.

## Significance:

Ongoing studies of peripheral and hepatic antiviral immune response in woodchucks during and following withdrawal will inform the understanding of reactivation risks in patients who stop NUC therapy.

## References

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