Targeting the hepatitis B virus covalently closed circular DNA genome using single domain antibodies

Simmone D'souza[1,2], Gerardo Balderas Figueroa[1], Maulik D Badmalia[1], Vanessa Meier-Stephenson[3], Carla S Coffin[2,4], and Trushar R Patel[1,2,3]

[1] *Alberta RNA Research and Training Institute, Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, T1K 3M4, Canada*
[2] *Department of Microbiology, Immunology and Infectious Diseases, Cumming School of Medicine, University of Calgary, Alberta, T2N 4N1, Canada*
[3] *DiscoveryLab, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Alberta, Canada*
[4] *Department of Medicine, Cumming School of Medicine, Calgary, AB, Canada*

Over 296 million people worldwide are chronically infected with Hepatitis B virus (HBV). HBV is the leading cause of hepatocellular carcinoma and liver failure, accounting for over 880,000 deaths annually. Chronic HBV infection is established in part through the virus' highly stable covalently closed circular DNA (cccDNA) persisting in the nuclei of infected hepatocytes. Currently approved antivirals against HBV do not lead to cure, as they fail to clear the persistent source of infection — the cccDNA.

Previously, we identified a highly guanine rich sequence in a key promoter region in the HBV pre-core/core promoter (PreC/C) which forms a DNA secondary structure known as a G-quadruplex (G4). This structure is comprised of planar arrangements of guanosines (G) that stack upon one another in a specific manner and functionally act as regulators of transcription. To disrupt HBV RNA transcription and impact cccDNA stability, we aim to target the G-quadruplex in the HBV cccDNA this promoter region using humanized single domain antibodies.

Using phage display technologies, we have identified 11 G-quadruplex binding single domain antibodies that can target the G4 present within the cccDNA. Using recombinant protein expression, we characterized the strongest binder (S10) and its interaction with a 22nt HBV pre-Core G-quadruplex forming oligo. We determined the binding affinity (K_D) between S10 and the target G4 was determined to be ~90 nM, which is 30x stronger for folded G4 versus unfolded oligos of the same sequence. To determine the effect of S10 on HBV replication, we have transduced the HepG2-NTCP-A3 cell line to express these sdAbs and are in the process of evaluating antiviral effects and target specificity for cccDNA.

The ability of S10 to discriminate between different sequences or secondary DNA structure provides an insight into how they can be exploited in future HBV therapeutic strategies.