TKM-HBV, a Novel RNA Interference Treatment for Chronic Hepatitis B, Rapidly Reduces Surface Antigen and other Viral Proteins in Both Intrahepatic and Peripheral Compartments

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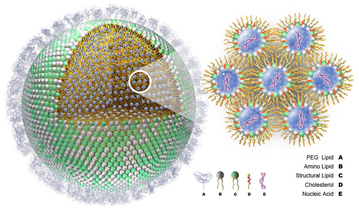
INTRODUCTION & AIMS

TKM-HBV is a novel RNA interference (RNAi) therapeutic for chronic HBV and currently in Phase 1 clinical development. It is designed to reduce the viral antigen load in chronically infected patients and allow the body to escape the virus-imposed state of immune repression.

Comprised of 3 oligonucleotide triggers encapsulated within a lipid nanoparticle (LNP) delivery system, TKM-HBV acts directly on all HBV RNAs (pregenomic RNA as well as viral mRNA) via nucleotide sequence-specific cleavage.

AIM: Show that TKM-HBV prevents the synthesis of viral proteins and reduces the overall antigen load in the body, both in the blood and hepatic compartments. This mode of drug action may present advantages over other approaches that seek to block viral protein secretion into the bloodstream thereby potentially causing intracellular build-up and ER stress.

RNAi DRUG TECHNOLOGY



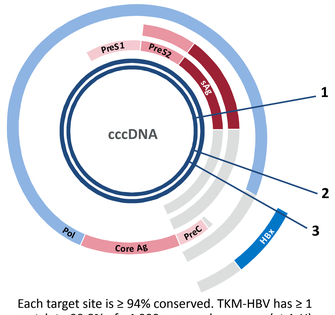
LNPs are composed of various lipids formulated in specific ratios in order to confer desired PKBD and PD properties.

LNPs protect nucleic acid drugs against nuclease degradation in the blood, and enable effective delivery of these macro-molecules to the target hepatocytes. To date, 9 LNP products have entered clinical trials with hundreds of patients treated, some with >1 year repeat-dosing duration. LNP enabled RNAi drugs have strong clinical validation.

RNAi TRIGGER DESIGN

Inclusion of 3 RNAi triggers allows for broad reductions of all viral antigens and pan-genotypic activity. Viral inhibition at multiple target sites reduces the risk of escape mutations.

Trigger 1 cleaves within the HBsAg coding region, thus TKM-HBV silences HBsAg even when it is expressed from Integrated DNA.



Each target site is ≥ 94% conserved. TKM-HBV has ≥ 1 match to 99.8% of >4,000 surveyed genomes (gt A-H).

RESULTS

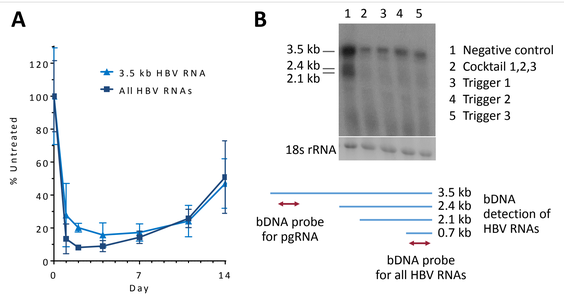


Figure 1. Cleavage of Multiple Viral mRNAs and pgRNA

A. A single dose of TKM-HBV mediates rapid reduction of all liver HBV RNAs in HDI mice (n=5F; mean ± SD). HBV RNAs were quantitated in terminal liver samples using an Affymetrix QuantiGene branched DNA (bDNA) assay. A slightly more moderate effect on the 3.5 kb RNA suggests this molecule is less accessible to RNAi machinery than other HBV RNAs. **B.** Trigger-specific reduction was confirmed via Northern blot analysis (^{32}P radiolabelled probe against the HBx region) of total RNA isolated from HepDE19 cells 24 h after treatment with TKM-HBV trigger cocktail, a negative control trigger, or individual triggers.

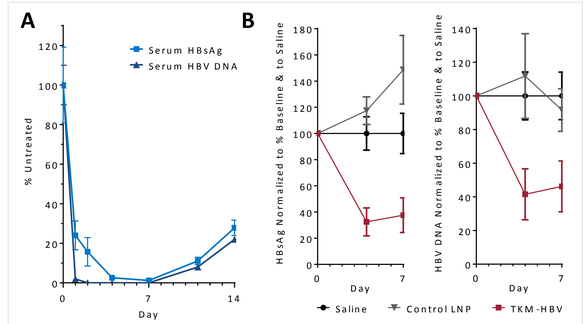


Figure 3. Rapid Reduction of Serum HBsAg & HBV DNA in HDI and Chimeric Mice. Animals were treated with a single dose of TKM-HBV on Day 0 (n=5F for HDI model, n=4-5 for PXB model). Data points in **A** (HDI mice) represent group mean ± SD of terminal samples. Data points in **B** (PXB mice) represent group mean ± SD of non-terminal samples. Serum HBV DNA measured by QPCR and HBsAg measured by Biorad GS HBsAg 3.0 EIA (HDI model) or Abbott Architect CLIA (PXB model).

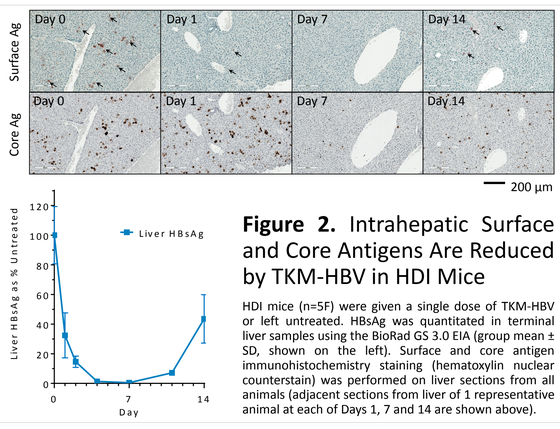


Figure 2. Intrahepatic Surface and Core Antigens Are Reduced by TKM-HBV in HDI Mice

HDI mice (n=5F) were given a single dose of TKM-HBV or left untreated. HBsAg was quantitated in terminal liver samples using the BioRad GS 3.0 EIA (group mean ± SD, shown on the left). Surface and core antigen immunohistochemistry staining (hematoxylin nuclear counterstain) was performed on liver sections from all animals (adjacent sections from liver of 1 representative animal at each of Days 1, 7 and 14 are shown above).

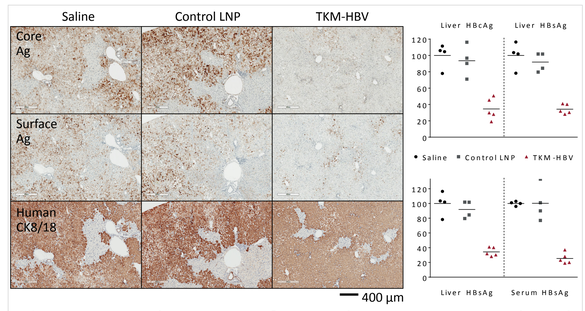
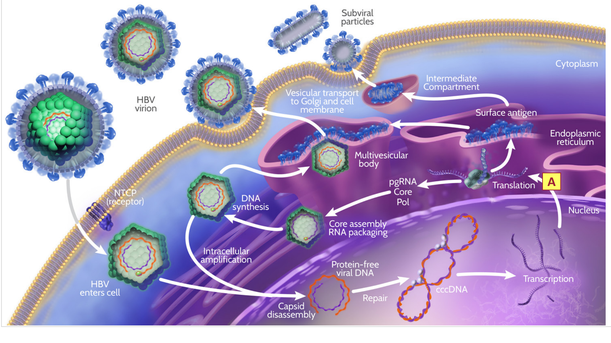


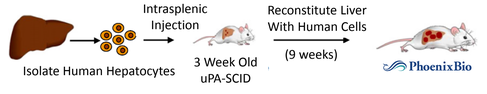
Figure 4. Intrahepatic Surface and Core Ags are Reduced by TKM-HBV in Chimeric Mice. At 4 days after 3 weekly doses of TKM-HBV, viral core and surface antigens were detected in terminal liver samples (n=4-5) using immunohistochemistry (IHC) staining. The signals were quantified using positive pixel counting (Aperio ImageScope) and compared to terminal serum HBsAg (Abbott Architect CLIA) expressed as % of Saline-treated group mean. Human CK8/18 signal indicates cells of human (vs. mouse) origin.

Targeting Different Nodes Of the Viral Life Cycle

- TKM-HBV blocks at point A



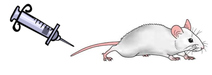
Hepato-Humanized PXB Chimeric Mouse Model of CHB



- > 70% human hepatocyte replacement index
- 8 week stabilized chronic infection of HBV genotype C
- Human-like viral titres and serum HBsAg levels
- Human cells can be infected and accumulate cccDNA whereas mouse cells cannot

Hydrodynamic Injection (HDI) Mouse Model of HBV

- Immunocompromised NOD.CB17-*Prkdc*^{scid}/J mice injected with plasmid encoding HBV genes
- Large volume injection forces plasmid into mouse liver cells
- Expression of viral RNAs, DNA & proteins from plasmid
- Infectious particles are formed & secreted into blood
- No cccDNA
- HBV genotype D



CONCLUSION

Lower serum HBsAg has been correlated with improved clinical outcome [1]. However, as the liver is a tolerizing environment, hepatocyte presentation of intracellular HBsAg to immune cells may also play a significant role in viral immune repression. Lower liver HBsAg has also been correlated with improved clinical outcome [2,3].

TKM-HBV rapidly and effectively removed viral antigens from both peripheral and intrahepatic compartments after a single treatment dose in HDI mice. These include surface and core proteins which are implicated in mediating the immune-repressed condition of chronic HBV infection.

- 92% reduction of liver total HBV RNA within 2 days (Fig 1).
- >98% reduction of serum HBV DNA within 1 day (Fig 3).
- Maximal reductions of intrahepatic (98%) and serum surface antigen (97%) were achieved at Day 4 whereas reduction of intrahepatic core antigen occurred more gradually (Fig 2,3).

In chronically HBV-infected hepato-humanized mice, equivalent 74-75% reductions of intracellular surface and core antigens were observed 4 days after 3 weekly doses of TKM-HBV (Fig 4).

REFERENCES

1. Sonneveld et al., Hepatology. 2013 Sep;58(3):872-80.
2. Su et al., J Gastroenterol. 2014 Feb;49(2):356-62.
3. Arends et al., J Viral Hepat. 2014 Dec;21(12):897-904.

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