Background and study design

ARC-520 was designed to decrease all cccDNA-derived viral transcripts via RNA interference (RNAi) and thus reduce all viral products - HBsAg, HBeAg, HBV replication products, core, polymerase and X protein – with the idea that reducing viral products may allow de-repression of the host immune system to control HBV infection. ARC-520 consists of two cholesterol-conjugated synthetic RNAi trigger molecules that target HBV sequences near DR1 plus a hepatocyte-targeted peptide (EX1) for efficient delivery of the RNAi triggers from the endosome to the cytoplasm where the RNAi machinery resides (Wooddell et al., 2013).

Following a NUC only lead-in of 8-24 weeks, 9 chronically HBV-infected chimpanzees were treated with daily NUCs plus monthly intravenous infusions of RNAi trigger(s) + EX1, then were taken off all treatment. All chimps received at least 6 doses of ARC-520. Some received an RNAi trigger (SHV75) designed to target transcripts from integrated HBV. Safety and efficacy were monitored with regular blood collection, and chimps received multiple liver biopsies, including after end of therapy. Liver mRNA was subjected to RNA-seq, differential gene expression (DEG) and Ingenuity Pathway Analysis to gain insight into animal responses.

Off-treatment serum HBV DNA

Off all treatment, HBV DNA and HBeAg rebounded to pre-study levels in all chimps except S-1. At 31 weeks off treatment, S-1 was HBeAg-negative, anti-HBeAg positive, ALT normal, and controlled HBV infection with HBV DNA 5 log_{10} lower and HBeAg 1.7 log_{10} lower than pre-study.

Minimal cytokine response of human whole blood exposed to ARC-520

ARC-520 RNAi triggers were fully chemically modified to prevent cytokine induction. Fresh human whole blood was exposed to ARC-520 at plasma equivalent exposures to intravenous doses of 2.12 mg/kg ARC-520 (20-120 µg/mL). The observed elevation of cytokine levels, typically by a few-fold, was quite small compared to the increase in cytokine levels by 2-3 orders of magnitude in positive controls (LPS and R848).

HBeAg-seroconverted chimpanzees

IFNγ, TNFα, CXCL9 and CXCL10 induced during NUC, peak prior to ALT flare, and when HBV was controlled off treatment.

HBeAg-positive chimpanzees

Periodic fluctuations of cytokines occurred during NUC lead-in, on NUC treatment, and off all treatment. TNFα higher during RNAi and during viral rebound, relative to the NUC lead-in. Minimal change in ALT.

HBeAg-negative chimpanzees

TNFα higher than IFNγ. Minimal changes in these cytokines and ALT. HBV DNA near LLQ.

Upstream Pathways Predicted to Lead to Expression of Canonical Pathways

Differential Gene Expression Analysis of Liver Biopsy mRNA (Ingenuity Pathway Analysis)

Canonical Pathways

Methods

NUC: Oral entecavir given daily in drink or fruit during lead-in and concomitant with RNAi treatment until 1 week after final RNAi dose.

Dosing: Monthly IV infusion of RNAi triggers (2-4 mg/kg ARC).

Cytokines (lumines panel of 25 human cytokines/chemokines) measured every 1-2 weeks throughout study. Among chimpanzees, none were consistently elevated during treatment. IFNγ, IFNα-responsive cytokines CXCL9 and CXCL10, and TNFα in serum were graphed along with HBsAg and alanine aminotransferase (ALT).

Differential gene expression (DGE) performed with RNA-seq data aligned to human genome; DEG relative to end of NUC lead-in; and expression analysis with Ingenuity Pathway Analysis (Qiagen).

Conclusions

The RNA therapeutic ARC-520 caused negligible cytokine response in human whole blood and healthy volunteers (Schluep et al., 2017). Chimpanzees chronically infected with HBV had periodic serum elevations of IFNγ, TNFα, CXCL9, and in some animals CXCL10, throughout the study. These elevations did not coincide with injection of RNAi treatment (half-life in plasma is <8 hrs). RNAi treatment appeared to generally reduce cytokine response but in some animals and at some times perhaps facilitated it, such as in chimps S-1 with repeatedly decreasing replication off treatment.

Pathway Analysis compares mRNA-seq gene expression data across time points to identify pathways that change by specified parameters, thereby allowing the generation of hypotheses. Dendritic cell maturation, PKCδ-signaling in T lymphocytes, role of NFAT in regulation of the immune response and IL-8 signaling. Common upstream regulators are LPS, IFNγ, and Vegf, among others.

REFERENCES

