SAT376

Decrease in the Burden of Integrated Hepatitis B Virus DNA in Chronic Hepatitis B Patients With Minimally Elevated Alanine Aminotransferase on Tenofovir Disoproxil Fumarate as Revealed by Long-Read DNA Sequencing

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Introduction

- Interchromosomal translocations near cancer driver genes have been shown to promote hepatocellular carcinoma (HCC)[1]
- van Buuren et al[2] showed that 31% (13/42) of chronic hepatitis B (CHB) patient samples in an immune-active population had ≥1 interchromosomal translocation
- A recent short-read RNA-sequencing (RNA-Seq) analysis demonstrated that treatment with tenofovir disoproxil fumarate (TDF), which blocks viral replication, leads to a reduction in transcriptionally active hepatitis B virus (HBV) integrations in patients with CHB and mild alanine aminotransferase (ALT) elevation[3]
- However, only a subset of transcribed HBV integrations can be captured by short-read RNA-Seq (ie, chimeric reads); thus long-read DNA sequencing (DNA-Seq) is an alternate methodology that can be used to directly interrogate HBV integration events and provide quantitative insight into the architecture of HBV integrations

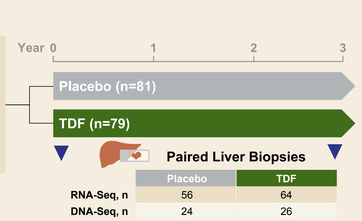
Objectives

- To evaluate the effect of TDF treatment on HBV integrations using targeted long-read DNA-Seq

Methods

Study Design

CHB patients (N=160)
- Serum viral load >2000 IU/mL
- Mild serum ALT elevation 1–2x ULN
- No clinical liver cirrhosis, decompensation, or HCC
- ETV added in 15 patients (n=13: placebo; n=2: TDF)

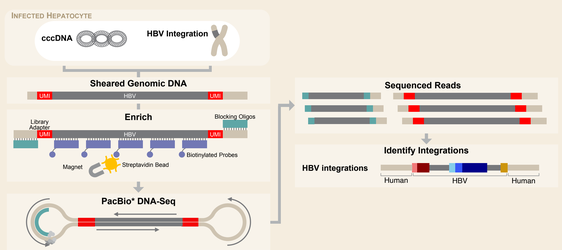


	Placebo	TDF
RNA-Seq, n	56	64
DNA-Seq, n	24	26

ETV, entecavir; ULN, upper limit of normal.

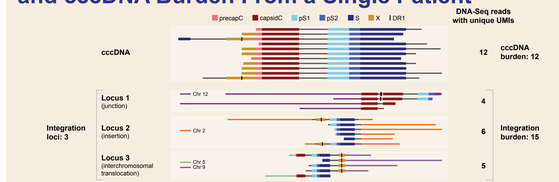
- Paired liver biopsy samples were obtained from a double-blind, placebo-controlled, randomized trial of TDF 300 mg qd po or placebo for 3 years in CHB patients (ClinicalTrials.gov NCT01522625)[1]
- Long-read DNA-Seq data from a subset of patients (excluding those on ETV) were analyzed using the ViraAL Integrations ANd Translocations (VALIANT) bioinformatics workflow[4]

VALIANT: a Method for Identifying Viral Integrations and Translocations



*Menlo Park, California, USA. cccDNA, covalently closed circular DNA; PCR, polymerase chain reaction; UMI, unique molecular identifier.

Examples of Integration Loci, Integration Burden, and cccDNA Burden From a Single Patient



- Normalization of integration loci, integration burden, and cccDNA burden were calculated using the geometric mean of UMI collapsed read counts for 20 human genes, reflective of library size

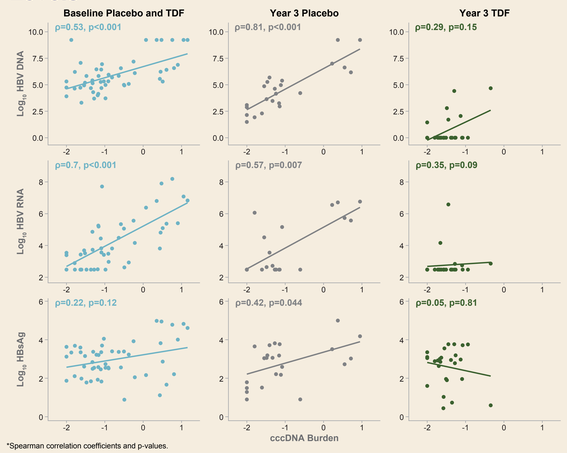
Results

Demographics for Patients With Long-Read DNA-Seq*

	Placebo: n=24	TDF: n=26	p-Value
Baseline characteristics before treatment			
Men	20 (83)	22 (85)	1.00
HBeAg+	8 (32)	3 (12)	0.09
Age, y	42 (37, 55)	47 (37, 53)	0.91
Serum ALT, U/L	56 (50, 66)	52 (48, 63)	0.37
Serum HBV DNA, $\log_{10}$ IU/mL	5.4 (4.9, 7.2)	5.6 (5.0, 6.5)	1.00
Serum HBsAg, $\log_{10}$ IU/mL	2.8 (2.1, 3.4)	3.3 (2.5, 3.6)	0.37
Serum HBV RNA, $\log_{10}$ IU/mL	3.5 (2.5, 5.4)	3.8 (3.4, 5.2)	0.67
Serum HBcrAg, $\log_{10}$ IU/mL	4.5 (3.0, 7.4)	5.1 (3.6, 6.1)	0.66
Characteristics after 3 y of treatment			
Serum ALT, U/L	37 (32, 50)	32 (23, 41)	0.054
Serum HBV DNA, $\log_{10}$ IU/mL	4.2 (3.0, 5.5)	1.5 (1.5, 1.5)‡	<0.001†
Serum HBsAg, $\log_{10}$ IU/mL	3.0 (2.0, 3.6)	2.9 (2.0, 3.2)	0.54
Serum HBV RNA, $\log_{10}$ IU/mL	2.6 (2.5, 5.6)	2.5 (2.5, 2.5)‡	0.019†
Serum HBcrAg, $\log_{10}$ IU/mL	4.0 (2.5, 6.2)	3.3 (2.7, 3.9)	0.34
Viral remission	0	20 (77)	<0.001

*Data are n (%) or median (interquartile range [IQR]); p-values determined from Fisher exact test for categorical variables and Wilcoxon rank-sum test for continuous variables; †Assay value was at lower limit of detection. HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

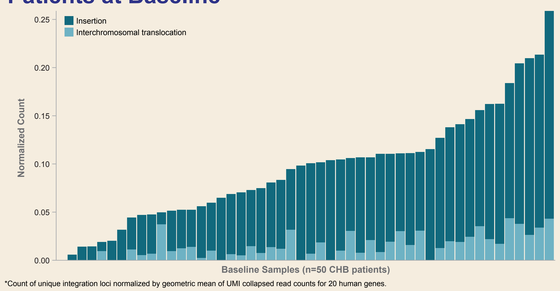
Correlations of HBV Viral Parameters With cccDNA Levels*



*Spearman correlation coefficients and p-values.

- cccDNA burden strongly correlated with HBV DNA and RNA levels at baseline
- The lack of correlation between cccDNA burden and viral load at Year 3 in the TDF arm is likely due to effective viral suppression

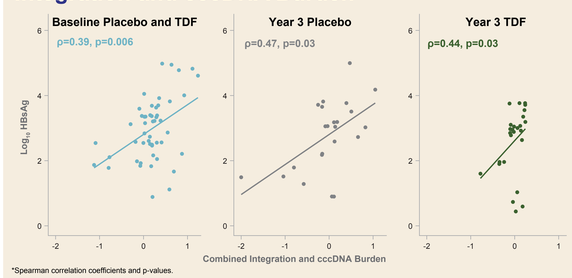
High Number of Insertions and Translocations in Patients at Baseline*



*Count of unique integration loci normalized by geometric mean of UMI collapsed read counts for 20 human genes.

- High prevalence of integrations was observed at baseline in patients with mild disease
  - 98% (n=49/50) had ≥1 insertion and 80% (n=40/50) had ≥1 interchromosomal translocation

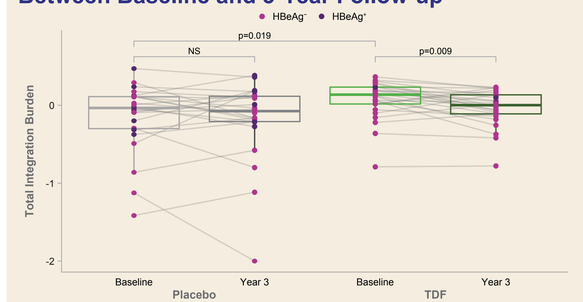
Correlations of Serum HBsAg With Combined Integration and cccDNA Burden*



*Spearman correlation coefficients and p-values.

- Consistent with the literature, the combination of cccDNA and integrated HBV DNA correlated significantly with HBsAg levels at baseline and Year 3 for both placebo- and TDF-treated patients
- HBsAg levels were not significantly correlated with total integration burdens at any time point, except in TDF-treated patients at Year 3, likely due to HBsAg being produced predominantly by integrated HBV DNA in virally suppressed patients
  - Baseline: ρ=0.26, p=0.06; Year 3 placebo: ρ=0.35, p=0.10; Year 3 TDF: ρ=0.49, p=0.012

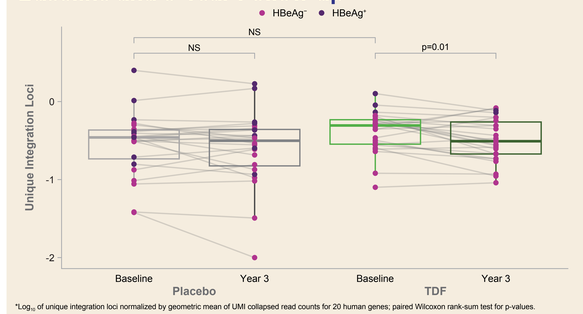
Treatment Effect on Total Integration Burden Between Baseline and 3-Year Follow-up*



*$\log_{10}$ of unique integration loci normalized by geometric mean of UMI collapsed read counts for 20 human genes; paired Wilcoxon rank-sum test for p-values. NS, not significant.

- Long-read DNA-Seq analysis showed decreases in total integration burden in TDF-treated patients, but not in those receiving placebo
  - Higher integration burden in TDF-treated patients at baseline may be due to greater proportion of those who are HBeAg−

Treatment Effect on Unique Integration Loci Between Baseline and 3-Year Follow-up*



*$\log_{10}$ of unique integration loci normalized by geometric mean of UMI collapsed read counts for 20 human genes; paired Wilcoxon rank-sum test for p-values.

- In addition to decreasing total integration burden, long-read DNA-Seq showed that TDF treatment decreased distinct HBV integration loci, suggesting a decrease in the overall number of HBV-integrated cells
  - This confirms previous findings from short-read RNA-Seq
- The decrease in cccDNA burden (p<0.001) indicates that the number of HBV-infected cells also decreased

Conclusions

- Targeted long-read DNA-Seq analysis revealed pervasive integrated HBV DNA in CHB patients with minimally elevated ALT, with 80% having evidence of HBV-related interchromosomal translocation at baseline
- TDF treatment significantly reduced HBV integration burden in these CHB patients
  - The declines in integration loci, integration burden, and cccDNA burden in virally suppressed patients indicate that the numbers of HBV-infected and -integrated cells were likely decreased
- These findings suggest that CHB patients with minimally elevated ALT may benefit from nucleos(t)ide treatment to reduce the integration burden of HBV, which is commonly associated with HCC

References: **1.** Péneau C, et al. Gut 2021;71:616-26; **2.** van Buuren N, et al. JHEP Rep 2021;4:100388; **3.** Hsu YC, et al. Gastroenterology 2022;162:1160-70.e1; **4.** Soulette C, et al. EASL 2021, abstr 654.

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