Intrahepatic virology for which you need core biopsies

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Disclosures

- Sanofi provided funding to JHU to study HBV using scLCM

The Burden of Viral Hepatitis

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Plasma measurements do not fully uncover intrahepatic replication

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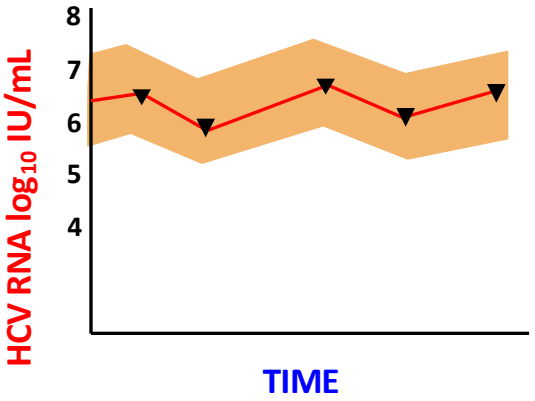
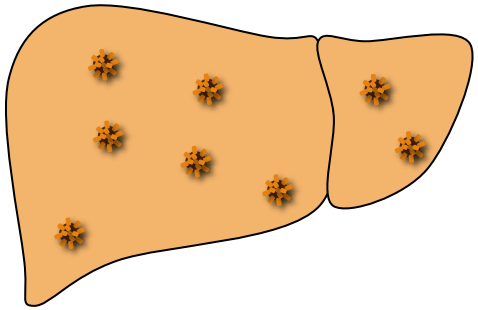
Plasma measurements do not fully uncover intrahepatic replication

How can we translate viral dynamics to a more tangible understanding of replication in an organ?

How do plasma quantities relate to the intrahepatic viral burden?





Hypothesis: plasma HCV RNA levels reflect the number of infected hepatocytes, and thus the time required for turnover of those hepatocytes during treatment

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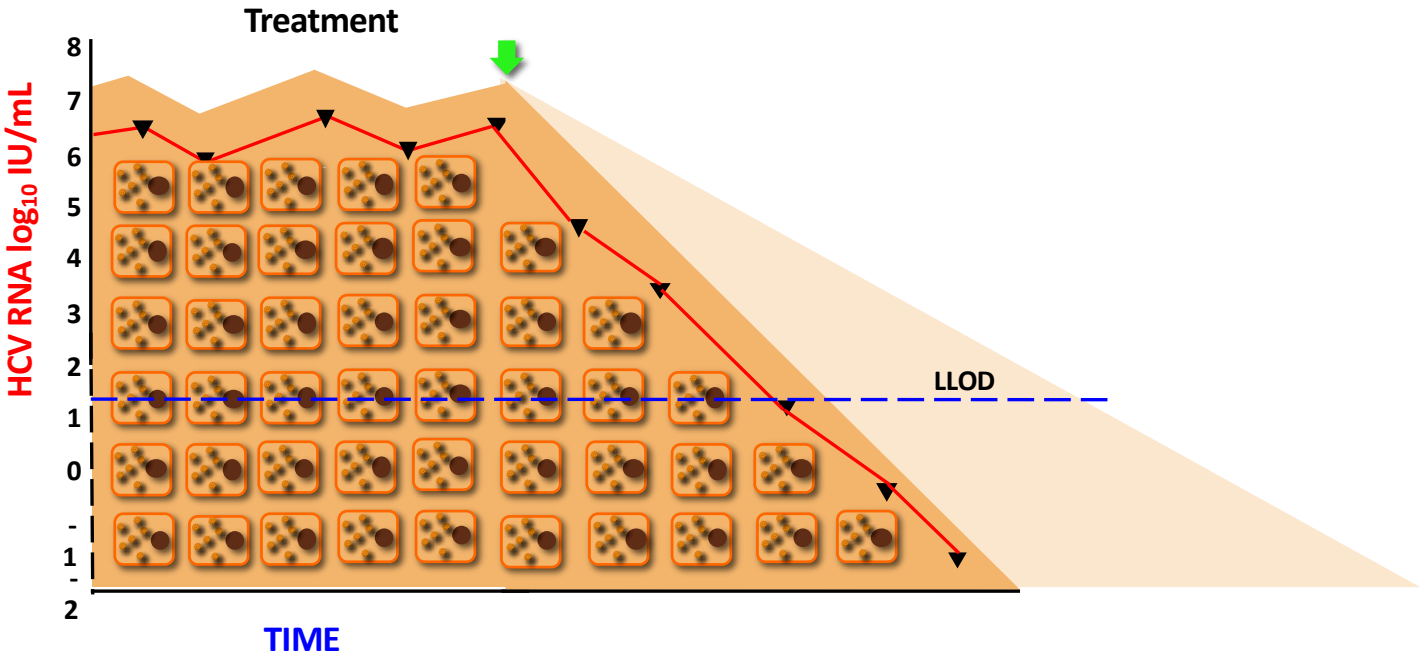
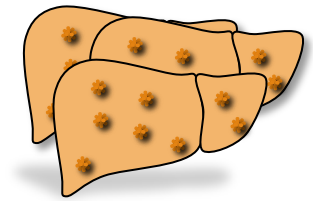
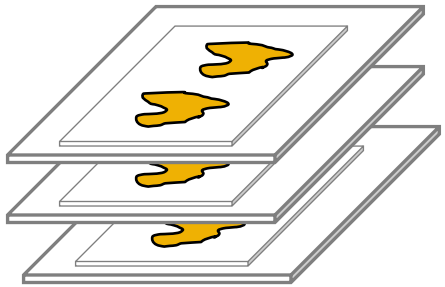


SINGLE CELL LASER CAPTURE MICRODISSECTION (scLCM)

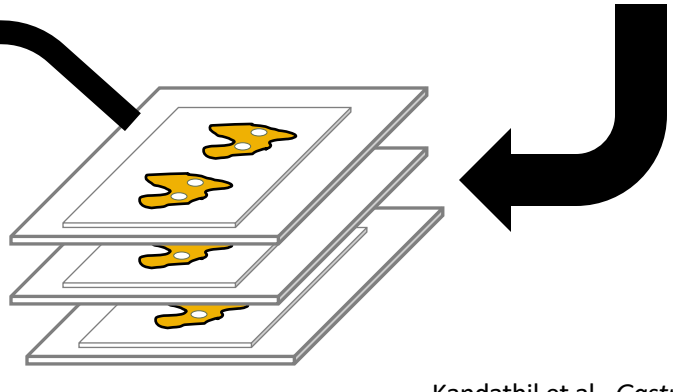


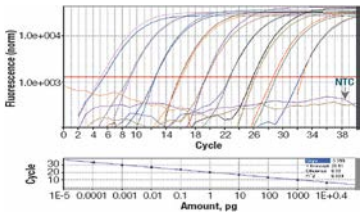
Archived Snap-Frozen Liver Tissue



10 µm Cryosections & Hematoxylin Staining

Laser Capture Microdissection

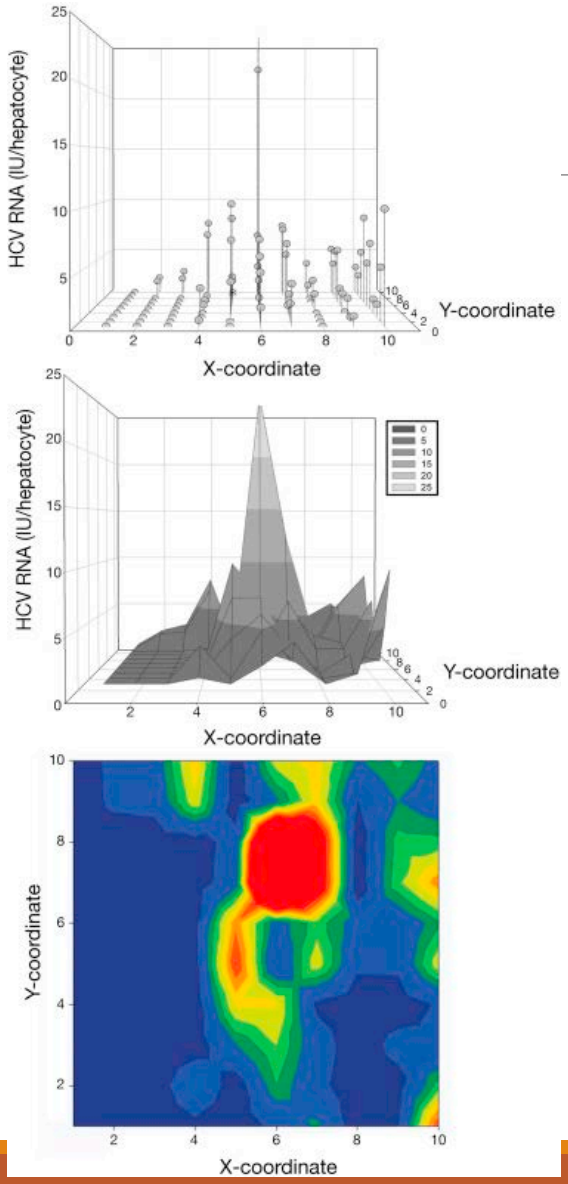




qPCR

Kandathil et al., *Gastroenterology,* 2013
Graw et al., *PLoS Comp Bio,* 2014
Balagopal et al., *JID,* 2020

Viral Landscape/ Viroscape



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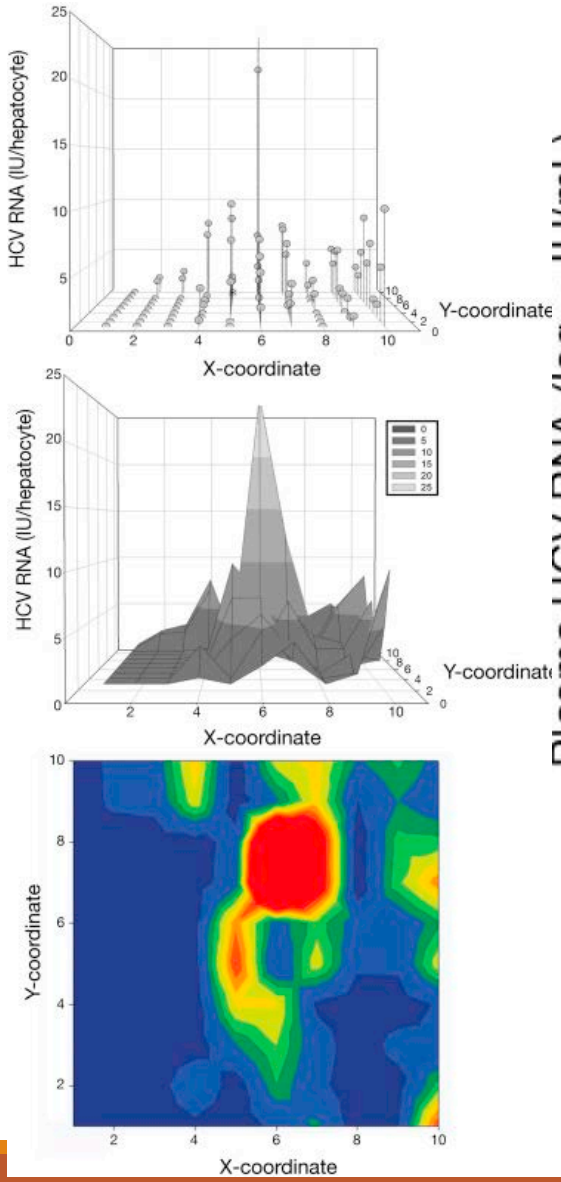

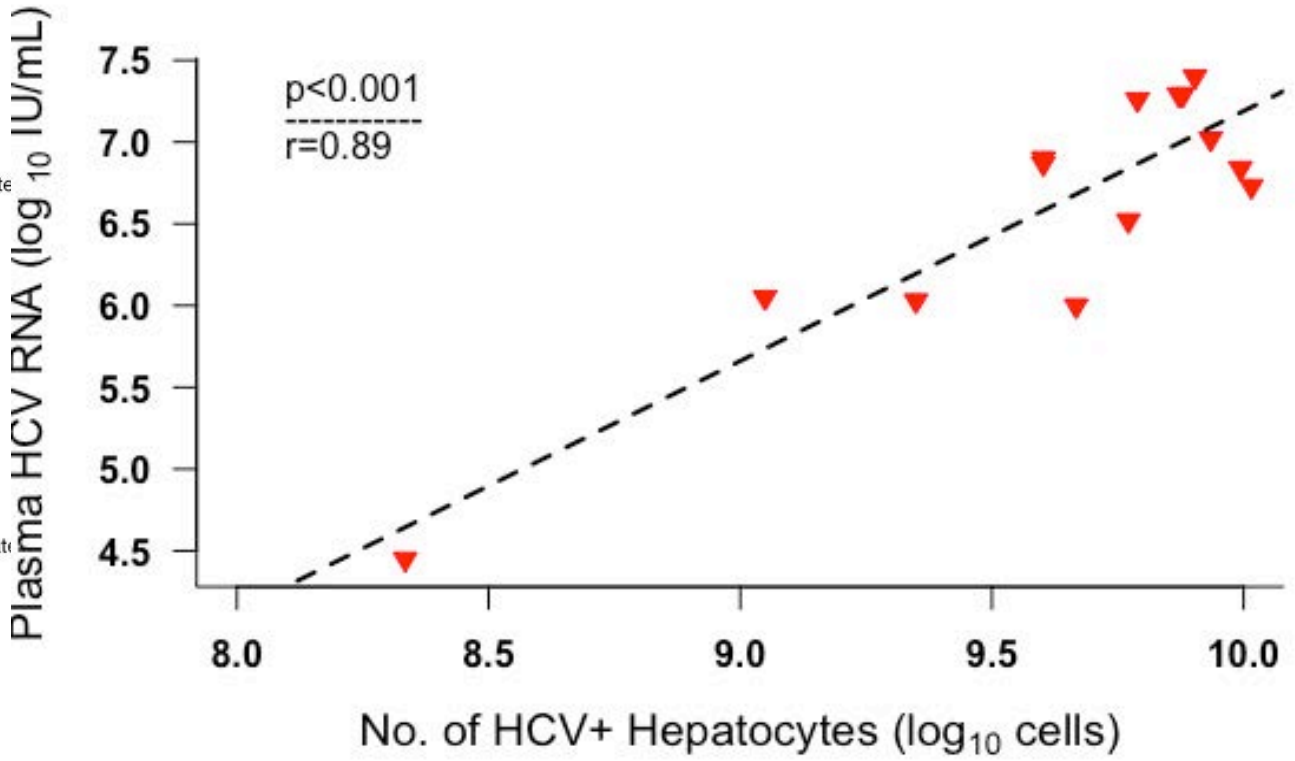

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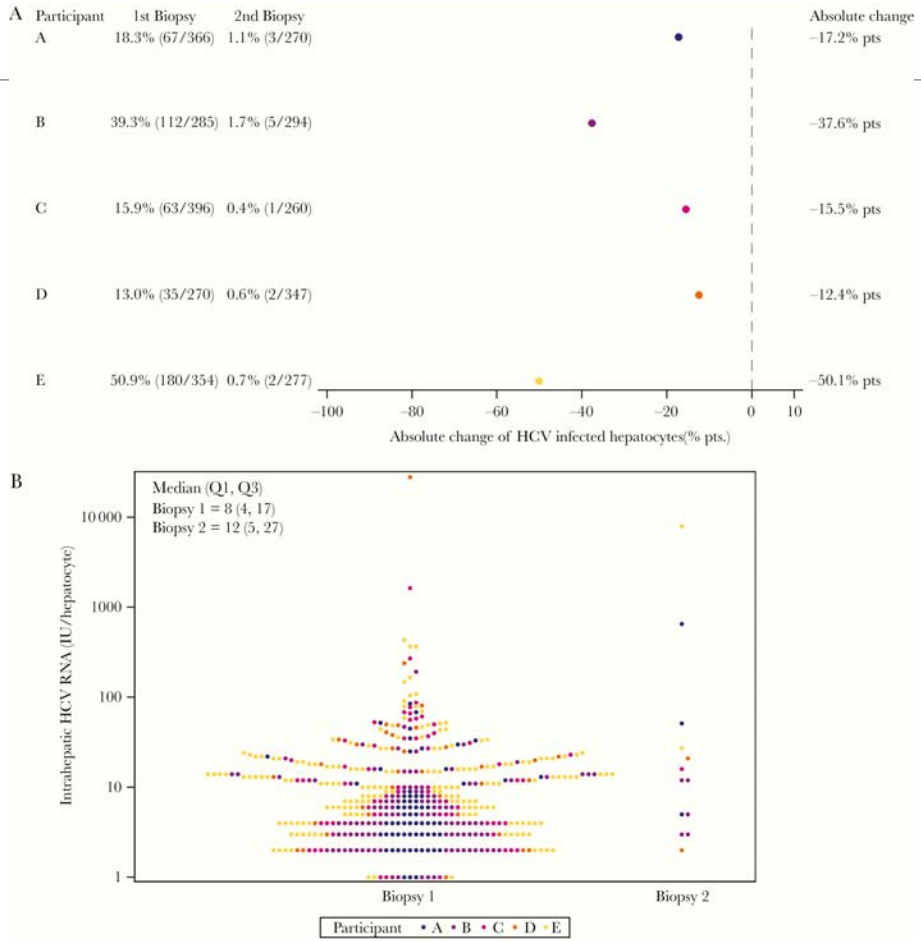
Intrahepatic burden of HCV before and during direct-acting antivirals



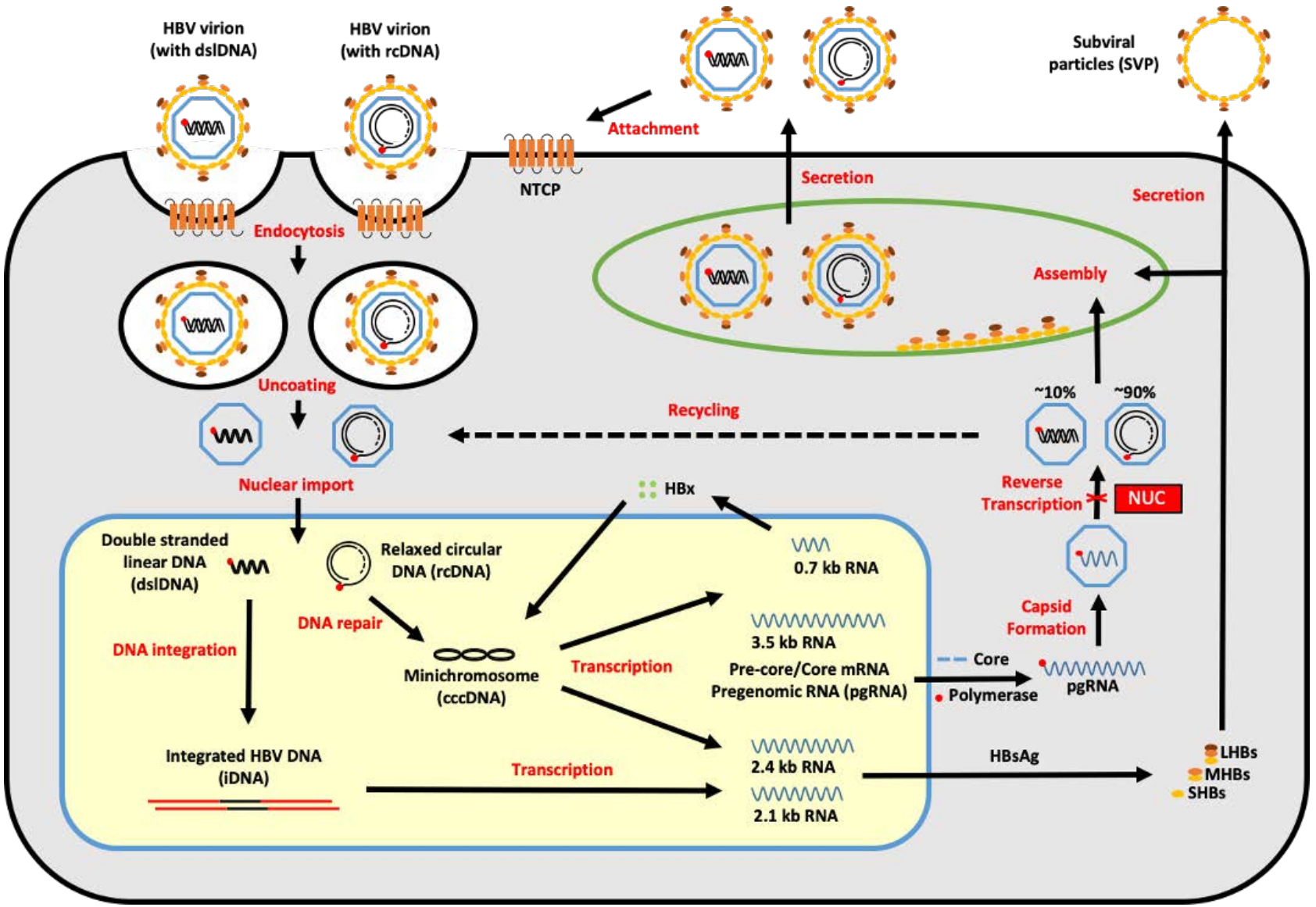
J Infect Dis, Volume 222, Issue 4, 15 August 2020, Pages 601–610, https://doi.org/10.1093/infdis/jiaa126

Can we apply this strategy to Hepatitis B?

scLCM HBV Team

- **Chloe L. Thio**
- Hyon (John) Hwang
- Tanner Grudda
- Jeff Quinn
- Yasmeen Saad
- Michael Murphy
- Katie Ward
- Bill Osburn
- Richard Sterling (HBRN)
- Ruy M. Ribeiro
- Alan S. Perelson
- Mark S. Sulkowski

HBV replication cycle



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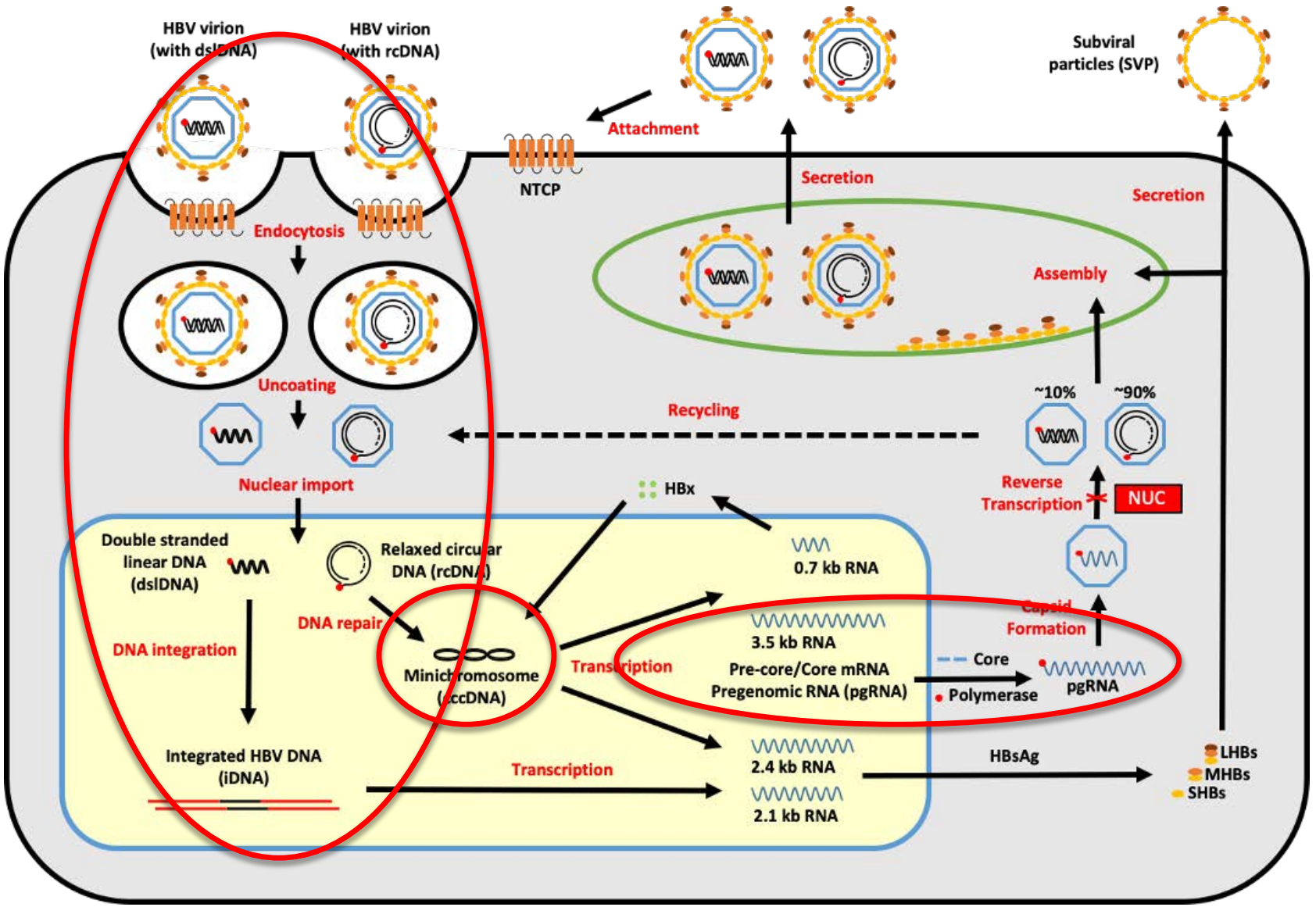
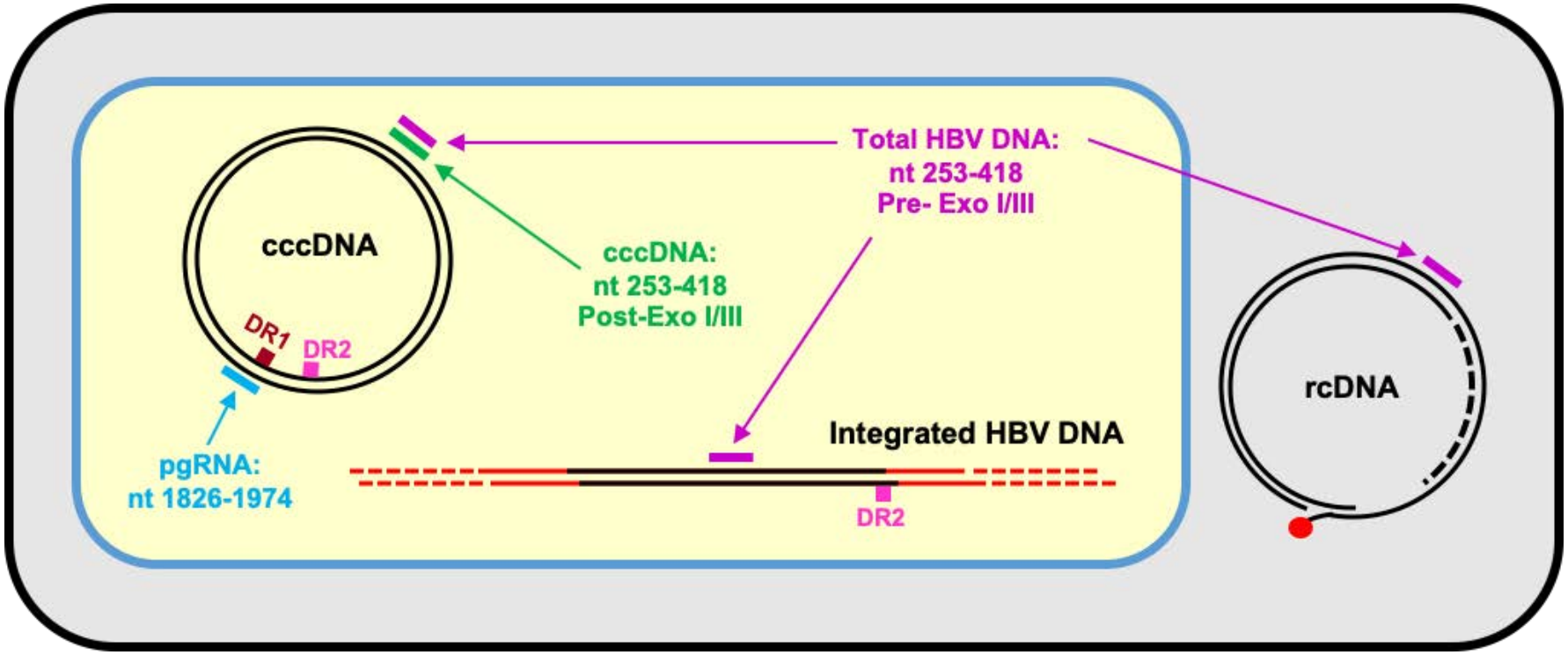
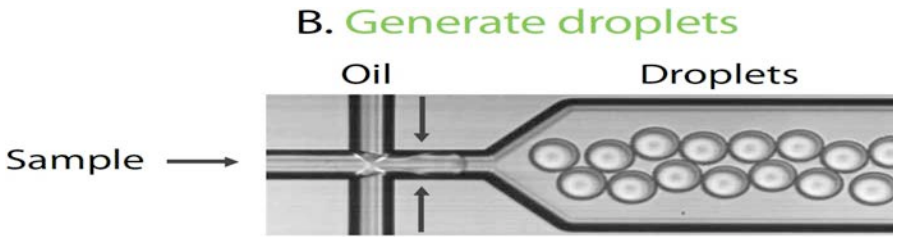
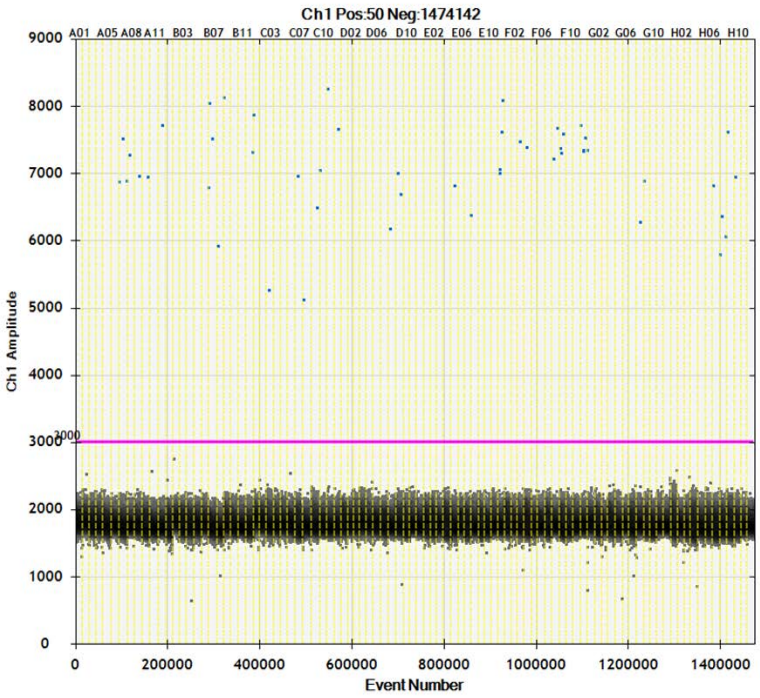


HBV molecular targets



Droplet digital PCR (ddPCR)



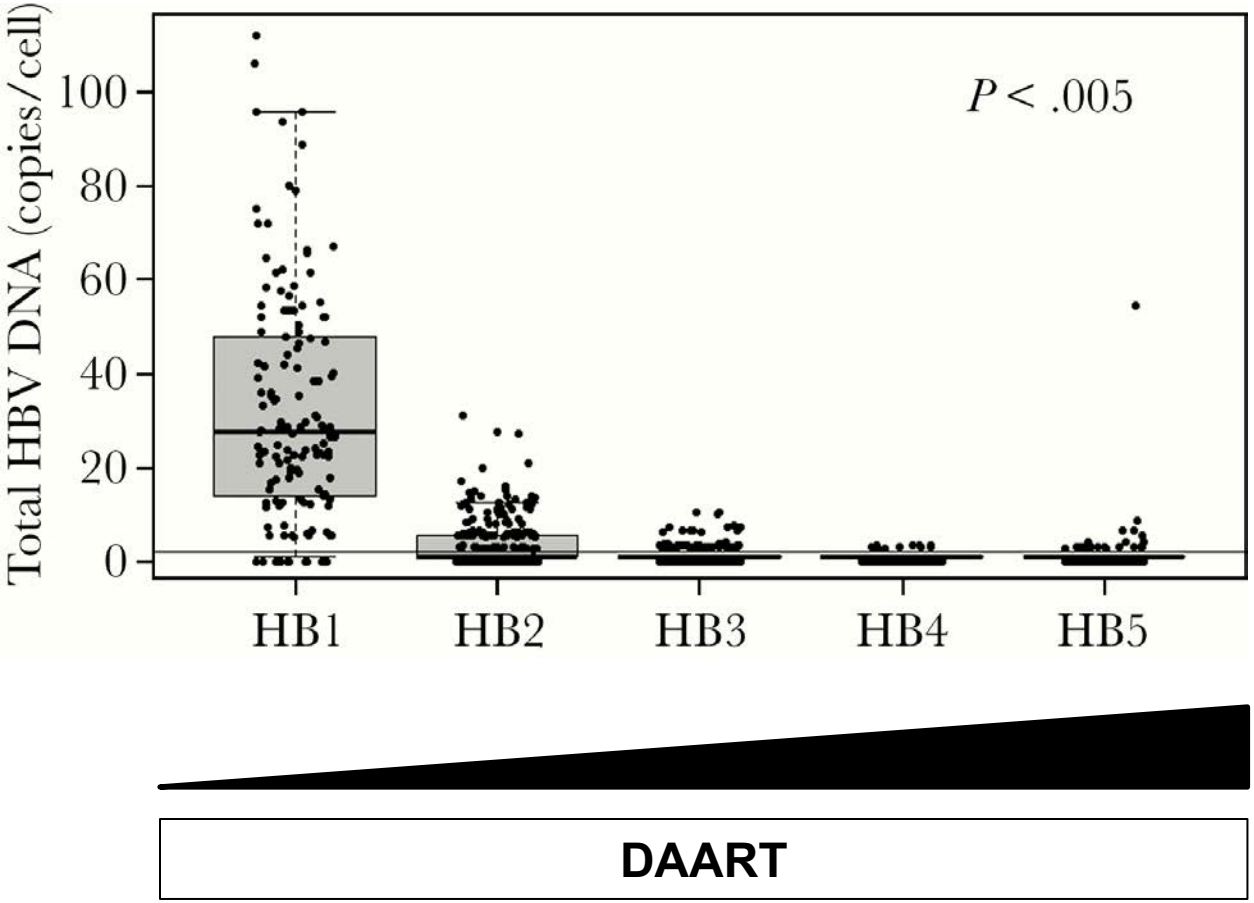


- 20,000 droplets with individual PCR reactions
- Fraction of positive droplets analyzed using Poisson statistics to determine concentration in original sample
  - Positive control: 7SL
  - Negative control: PEN membrane
  - Previously published primers

Total HBV DNA – LLOD 2-3 cp/cell
cccDNA – LLOD 2-3 cp/cell
pgRNA – LLOD 5-6 cp/cell

Laras et al. Hepatology. 2006 Sep;44(3):694-702.
Werle-Lapostolle et al. Gastroenterology. 2004 Jun;126(7):1750-8
Mu et al. Biotechnol Lett. 2015 Oct;37(10):2063-73

Total intracellular hepatitis B virus (HBV) DNA in five HIV/HBV co-infected persons receiving dually-active antiretroviral therapy



J Infect Dis, Volume 221, Issue 9, 1 May 2020, Pages 1462–1469, https://doi.org/10.1093/infdis/jiz607

Can we use these tools to characterize HBV longitudinally?

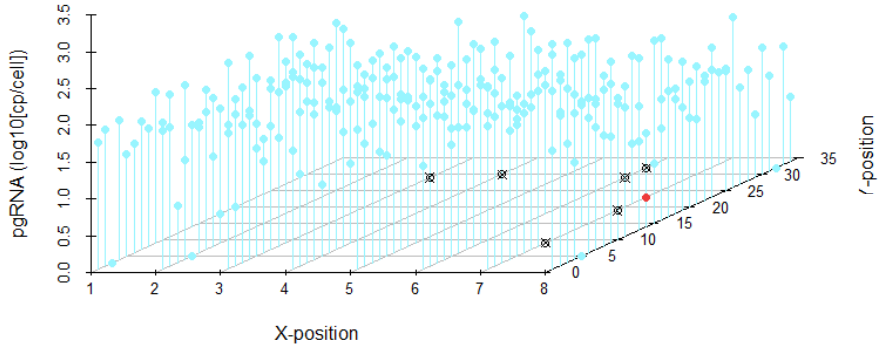
Participant characteristics

	HB6	HB2	HB7	HB3	HB4
Age, yrs	53	46	47	28	51
CD4+ T cell count, cells/ul	655	153	299	390	399
HIV RNA (cp/ml)	<20	241-358	UD	54	UD
HBV DNA (log_{10}IU/ml) at first biopsy	8.48 (~2 weeks prior to biopsy)	8.56 (~2 weeks prior to biopsy)	4.15 (~3 weeks prior to biopsy)	4.55 – 5.66 (~6 months prior to biopsy)	1.60
HBV DNA (log_{10}IU/ml) at second biopsy	1.63 (~2 weeks prior to biopsy)	UD (~2 weeks prior to biopsy)	2.89 (~3 weeks prior to biopsy)	2.93 (~2 weeks prior to biopsy)	UD (~3 weeks prior to biopsy)
Antiretroviral therapy (ART) (HBV-active nucleos(t)ides are in bold)	**TDF/FTC**/DTG/RPV/ DRV/r	ABC/DTG/DRVr/**ETV**	**TDF/FTC**/ATV/r	**TDF/FTC**/ATV/r	**TDF/FTC**/DRV/r/RAL
Duration of HBV-active ART	2 weeks	Stopped TDF 11 mos before bx	3.5 years but intermittent compliance	4 years but intermittent compliance. Restarted ART 7 mos before bx	7 years (adherent ~1 year)
Interval between biopsies, yrs	3.6	3.6	3.7	2.7	3.6

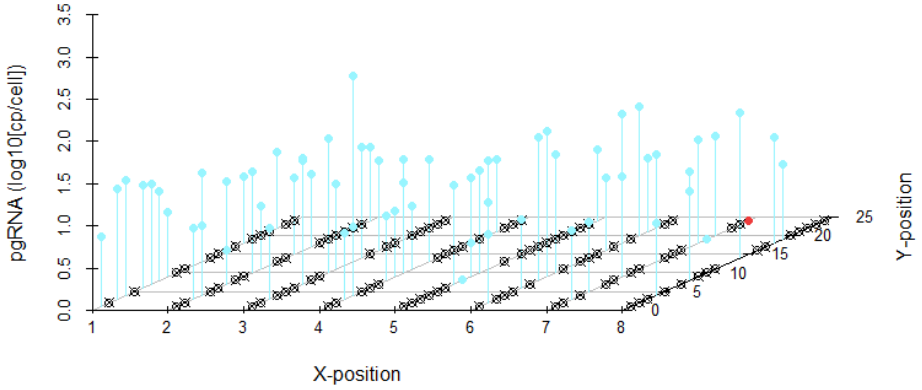
All participants were male, African-American, and HBeAg+. UD – undetectable; TDF – tenofovir disoproxil fumarate; FTC – emtricitabine; DTG – dolutegravir; RPV – rilpivirine; ABC – abacavir; DRV/r – darunavir boosted with ritonavir; ARV/r – atazanavir boosted with ritonavir; RAL - raltegravir

Changes in the viroscape with time

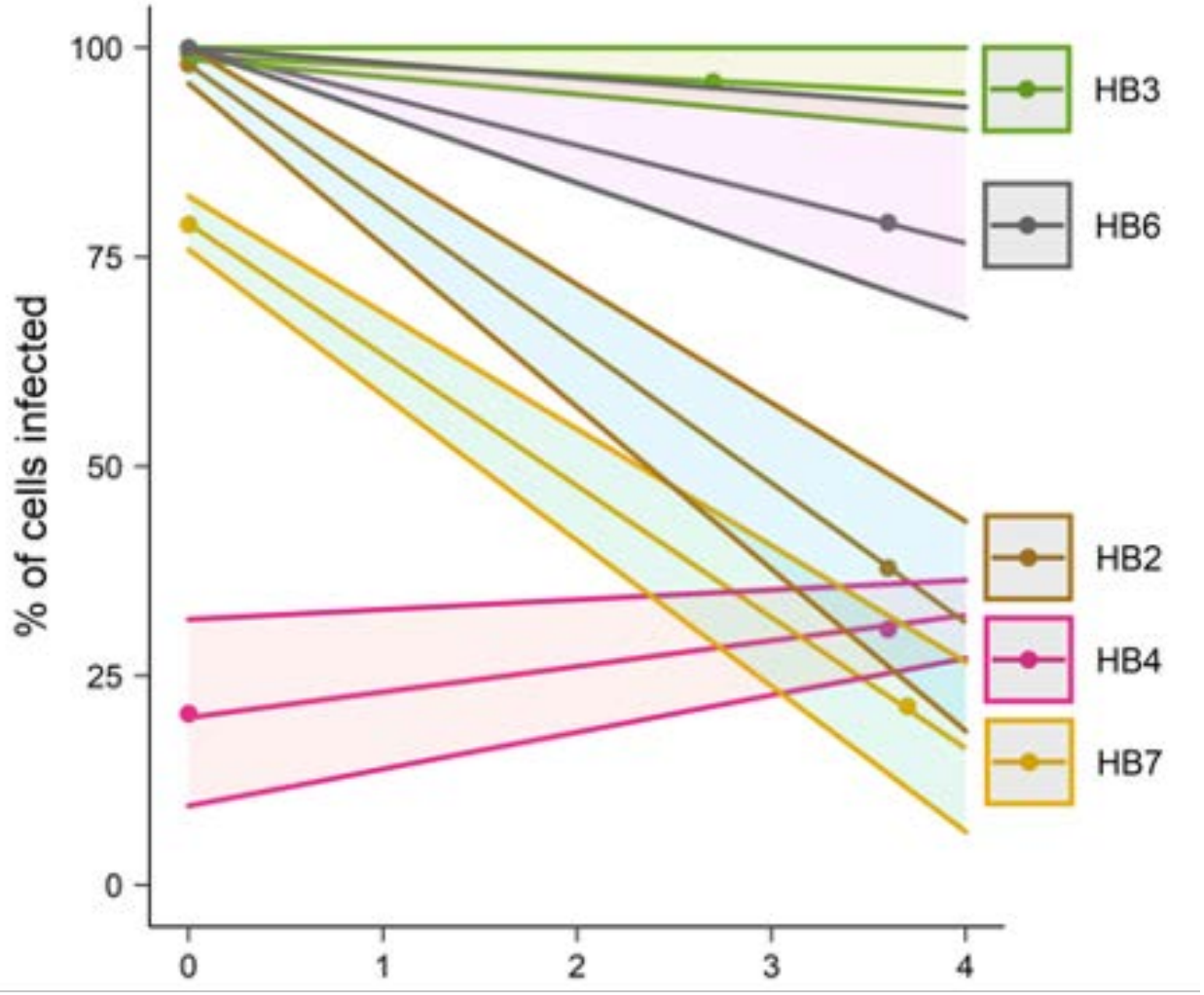
HB2 Biopsy 1



HB2 Biopsy 2



Change in proportion of infected hepatocytes between biopsies



Years of treatment with NUCs

Balagopal, *JCI Insight,* 2020 (accepted)

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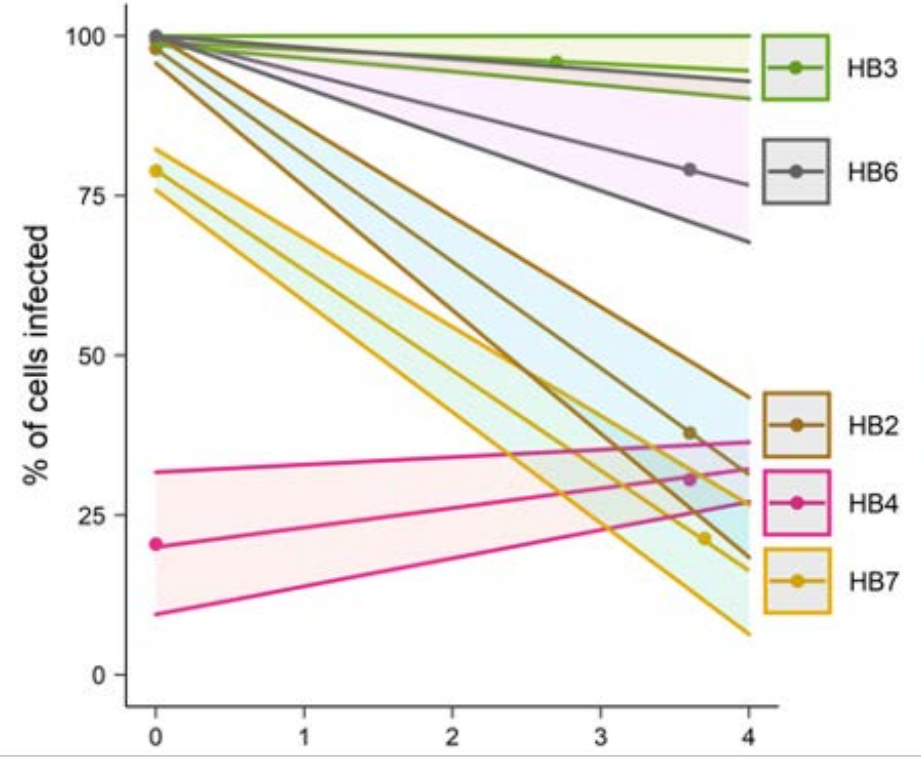

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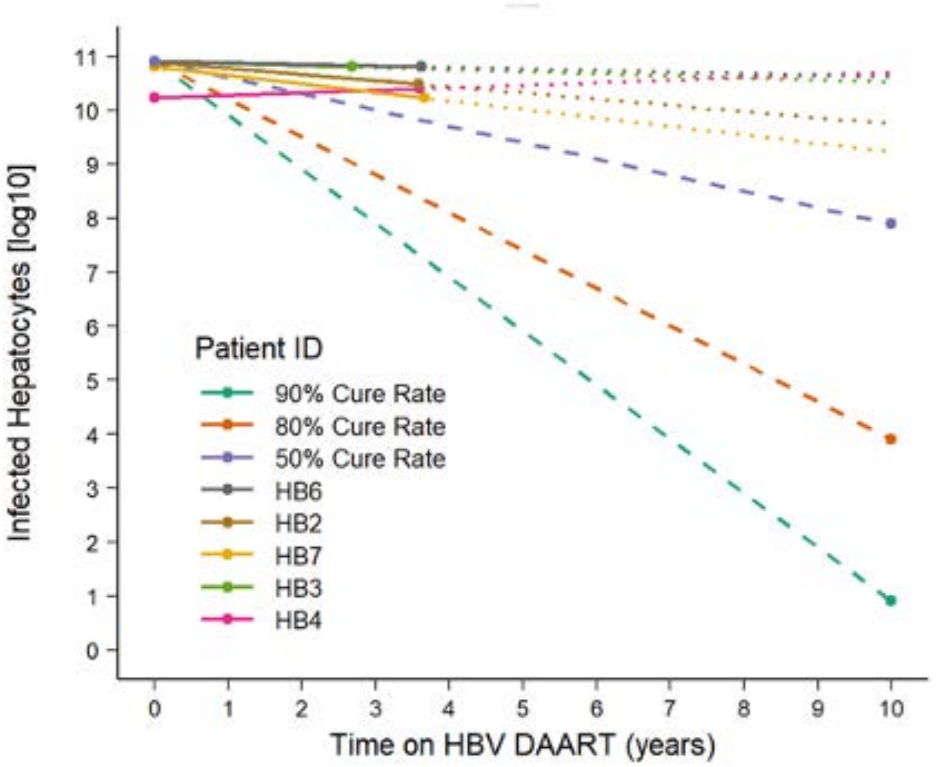

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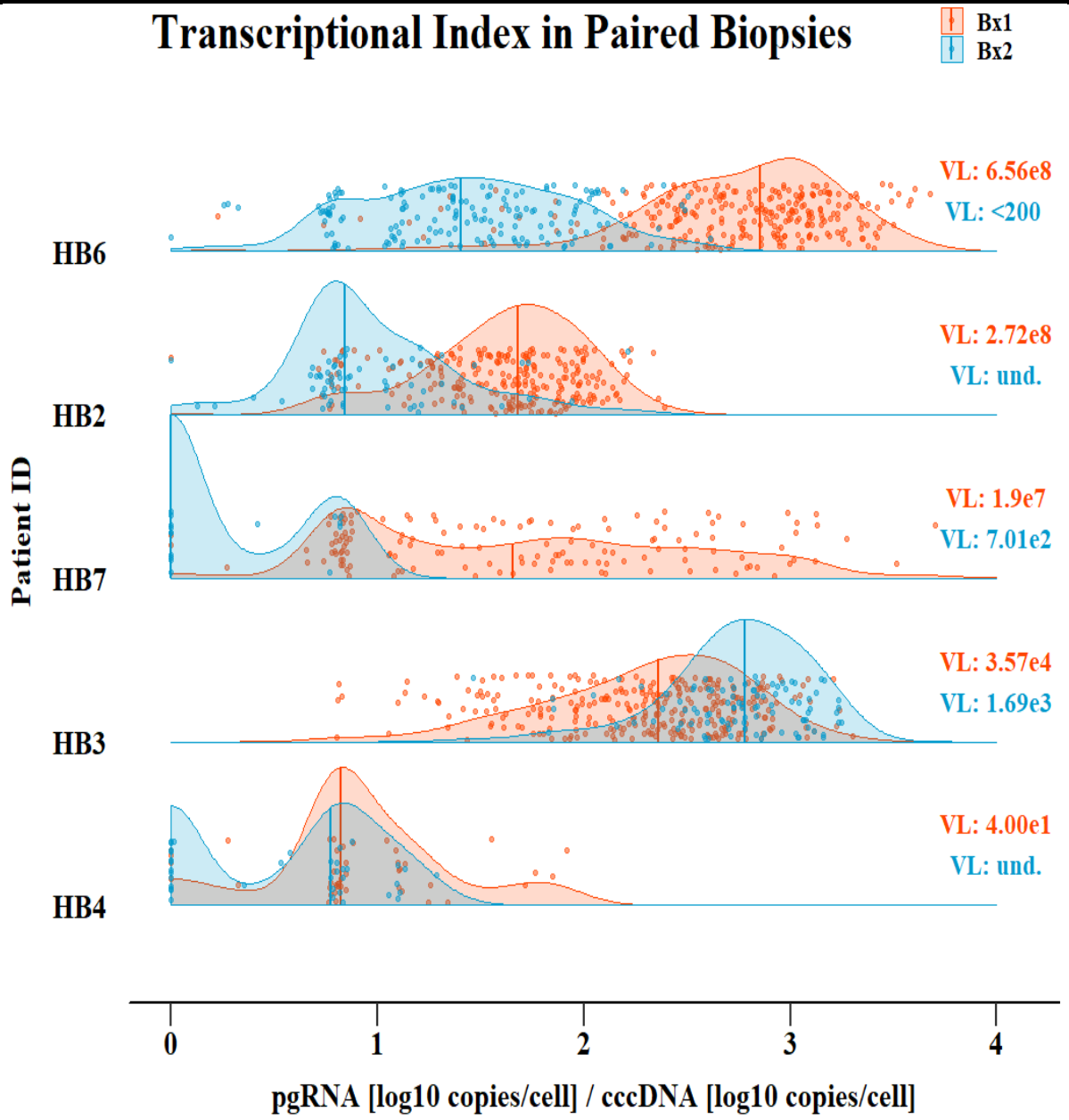
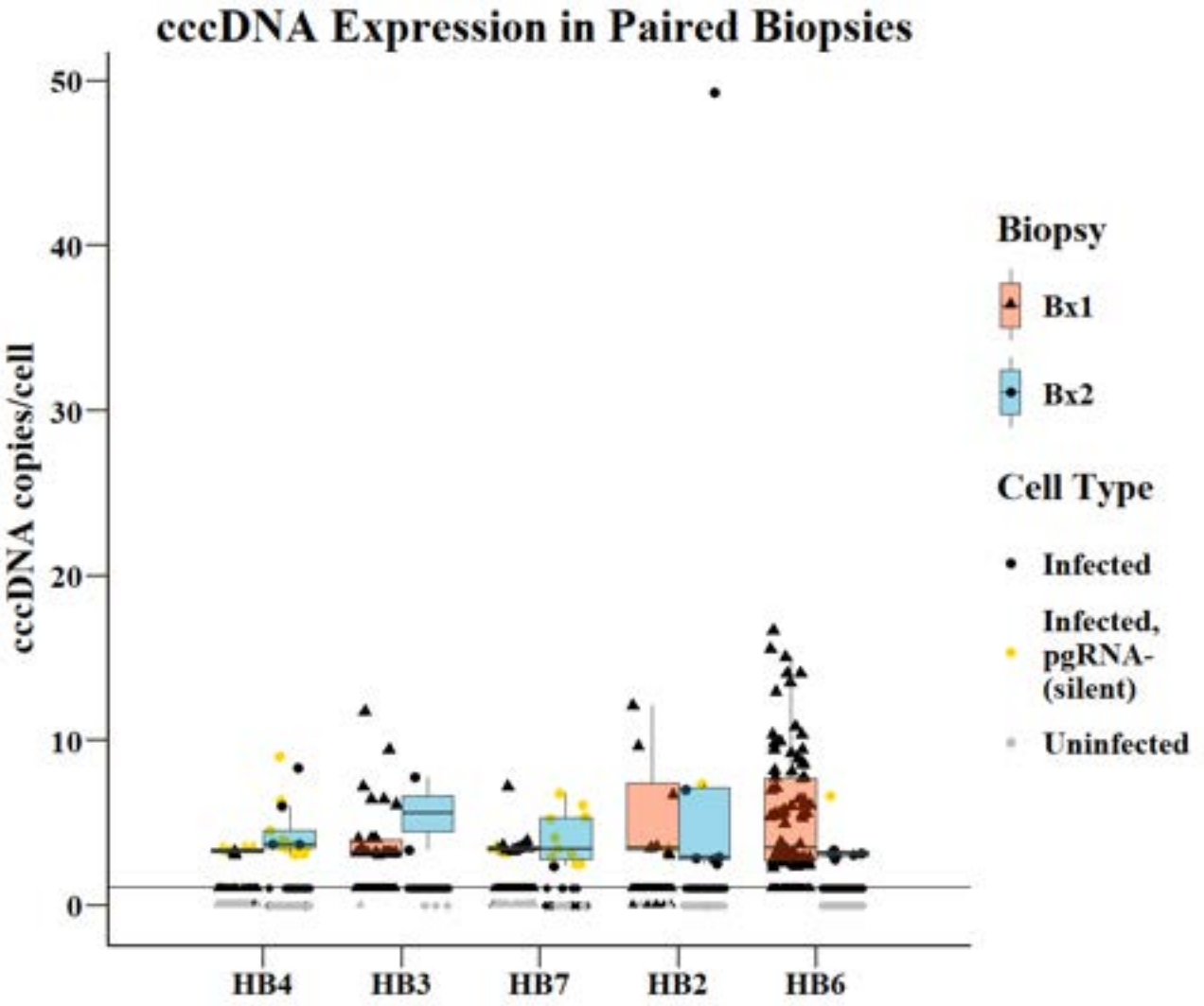
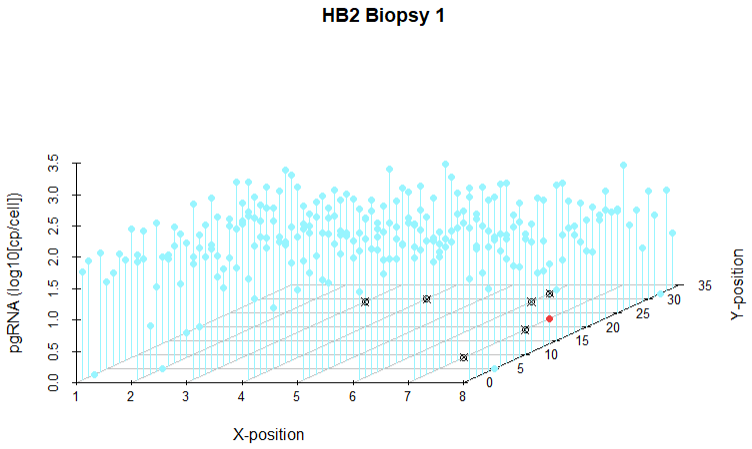
Transcription normalized to cccDNA decreases with NUCs

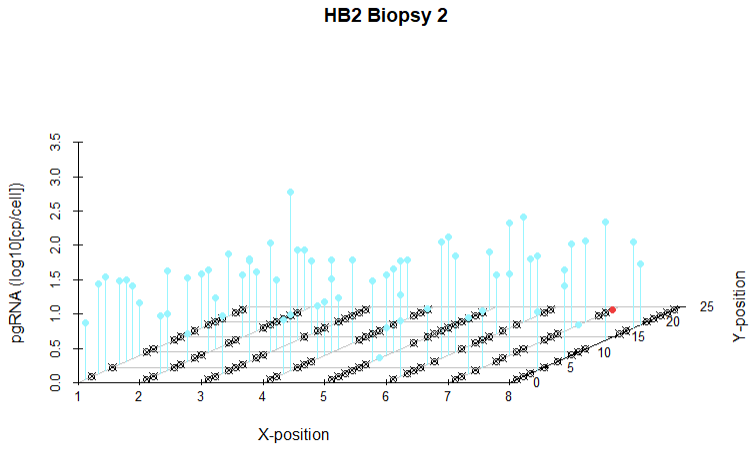


cccDNA stable between biopsies



Changes in the viroscape with time





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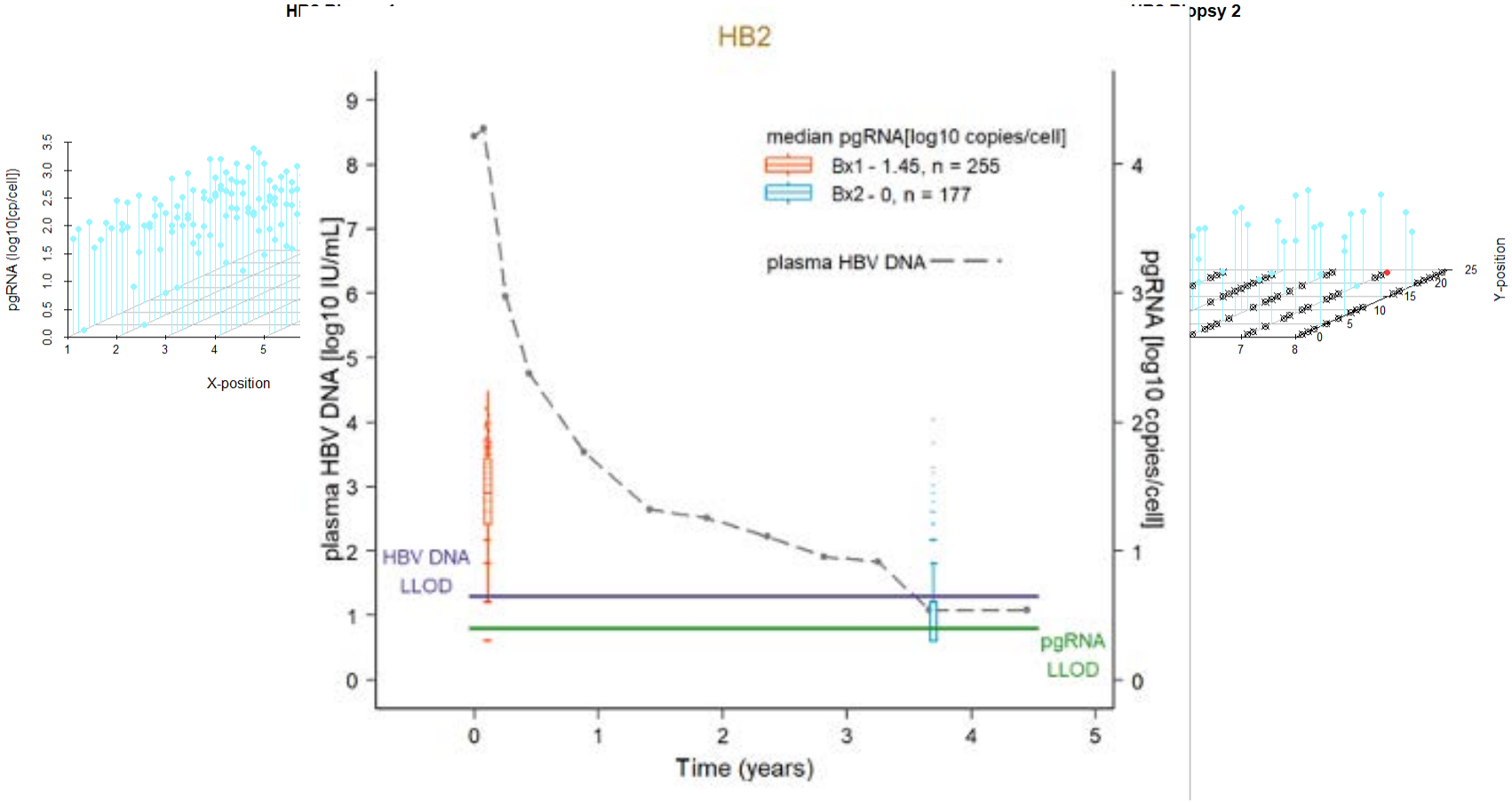
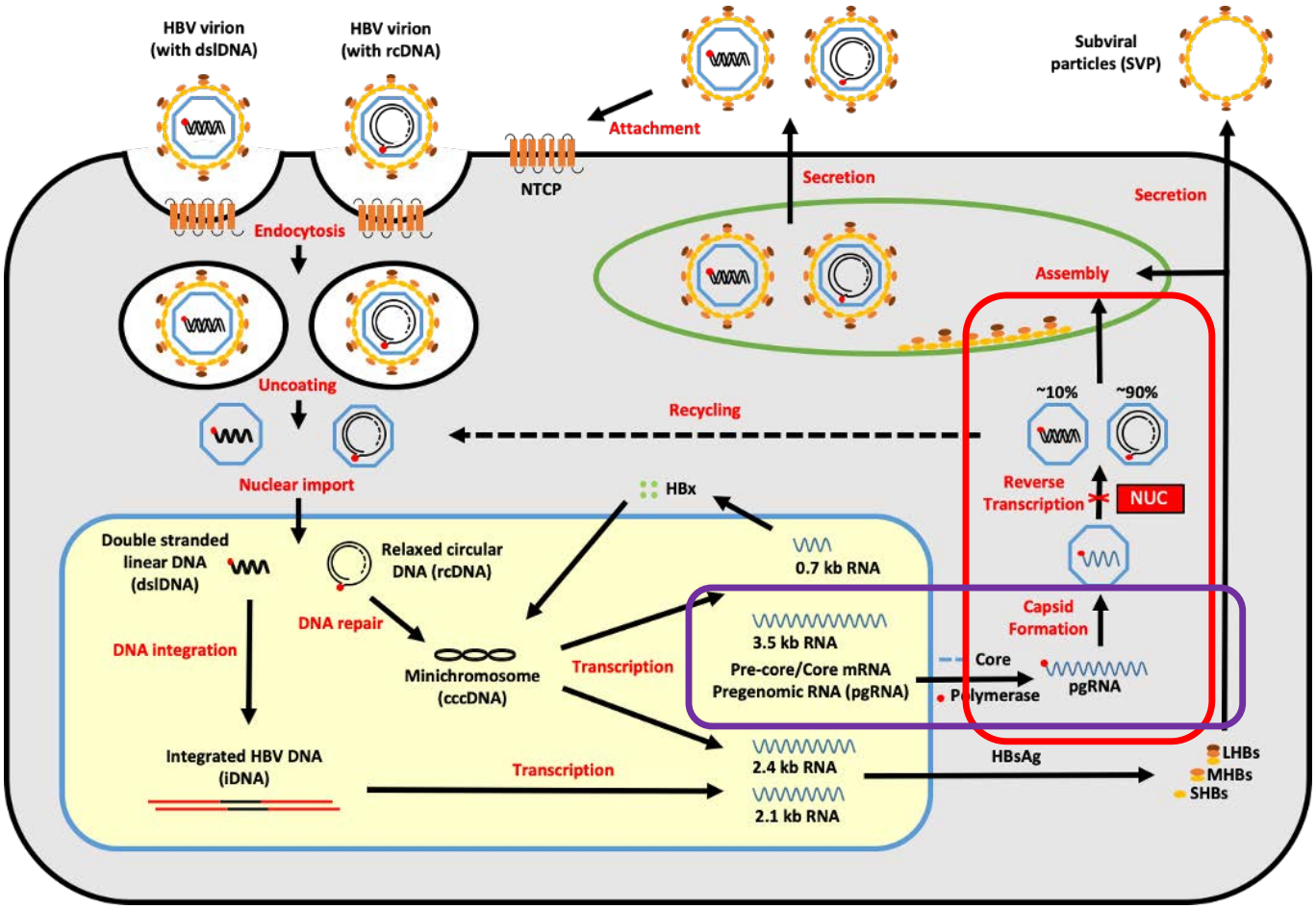


HBV replication cycle



Viroscapes and Immunohistochemistry show similar findings of diminished HBV gene expression

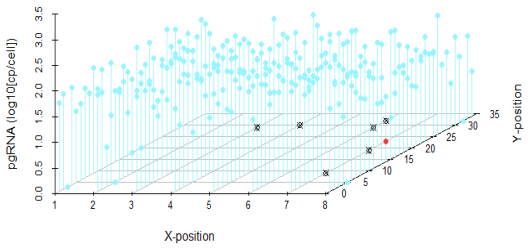
HB2

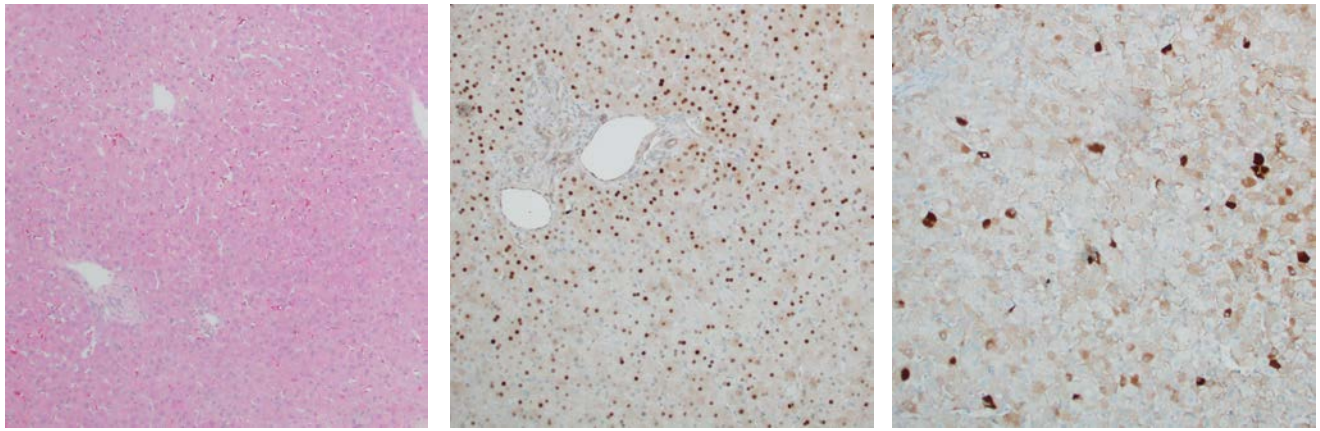
H&E

anti-HBcAg

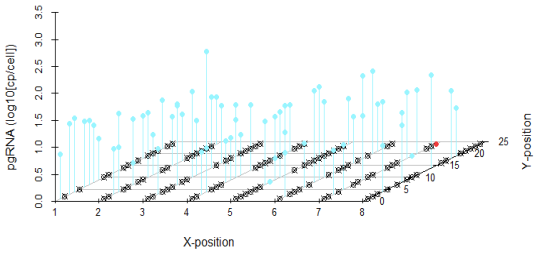
anti-HBsAg

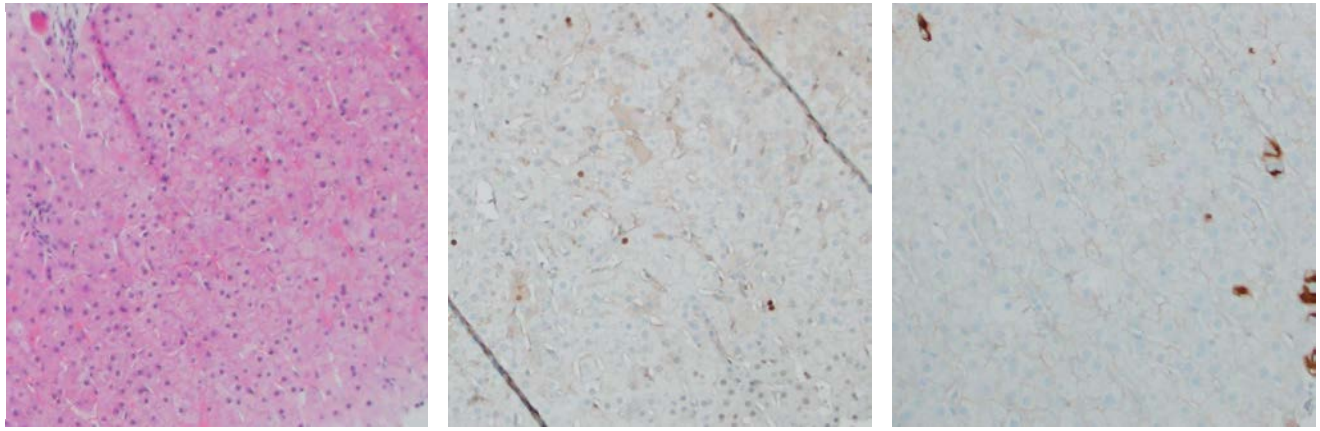
Biopsy 1





Biopsy 2





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HB2

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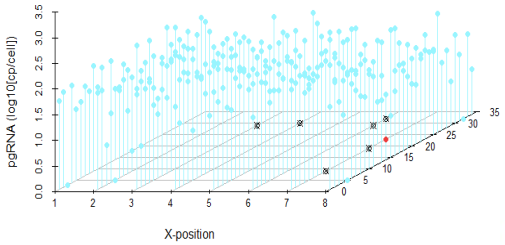

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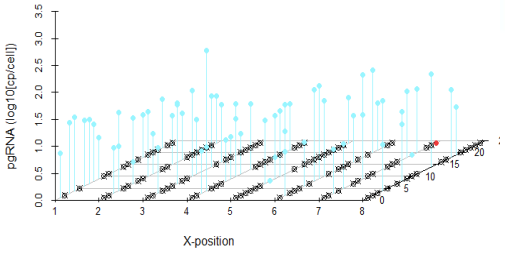

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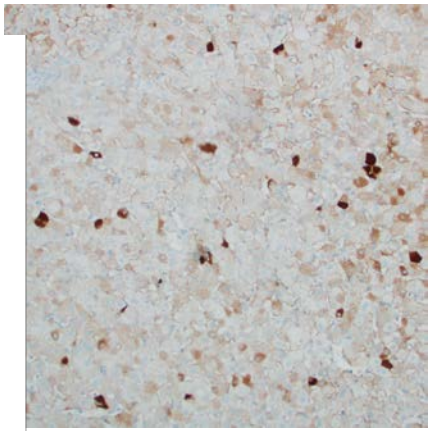

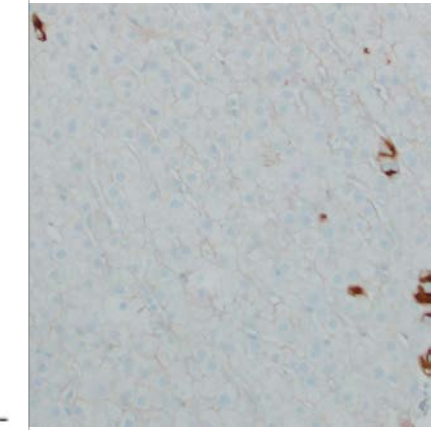

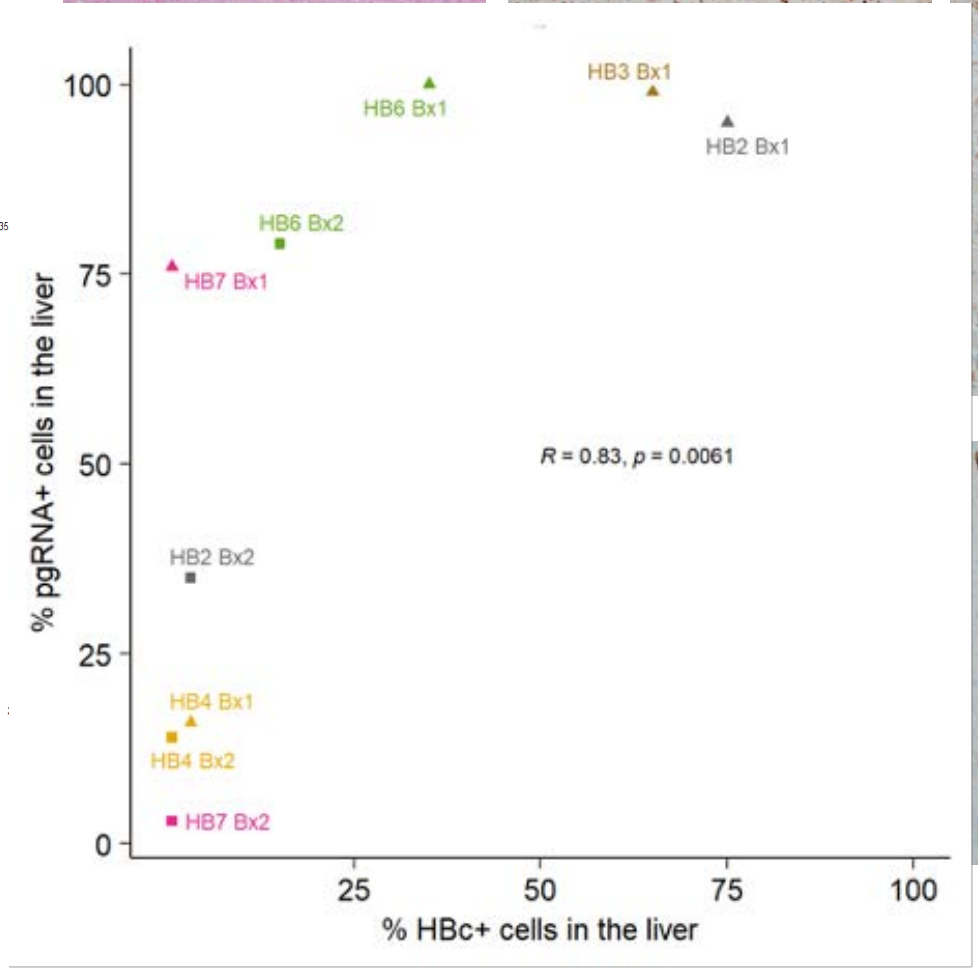

Summary

- Hepatocytes remain infected with long-term treatment
- There is heterogeneous HBV infection and transcription, especially during treatment
- Transcriptionally regulation of HBV during NUCs
  - Proportion increases with treatment duration
  - Possible reservoir for reactivation
  - Difficult to eliminate with immunotherapy
  - Mechanism unclear
- Liver biopsies and in situ analysis are an important approach for understanding the HBV lifecycle
  - Especially during therapy (NUCs or emerging therapy)

Thank you

STUDY Participants
STUDY Coordinators

- **Center for Viral Hepatitis Research (JHU)**
- Chloe L. Thio
- Mark S. Sulkowski
- David L. Thomas
- Richard Sterling
- Abraham J. Kandathil
- John Hwang
- Jeffrey Quinn
- Jaiprasath Sachithanandham
- Hyon (John) Hwang
- Tanner Grudda
- Ken Bowden
- Katie Ward

Johns Hopkins CRS

Yuka Manabe
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Ilene Wiggins
Denise Wright
Jamilla Howard

UPR CRS

Jorge Santana

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