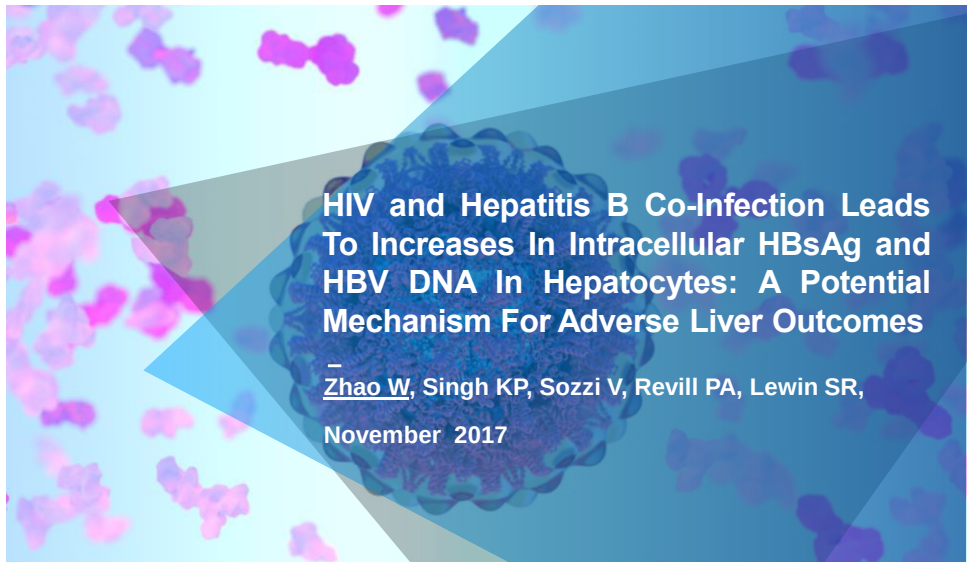


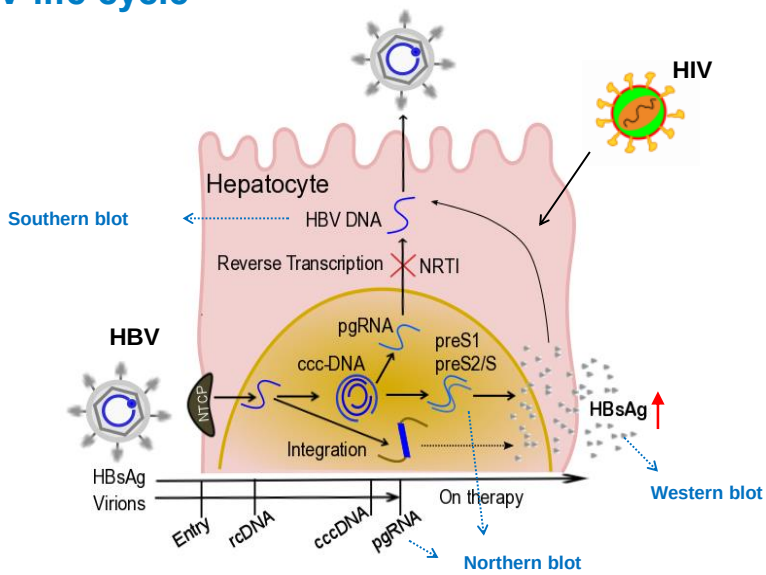
Doherty Institute
A joint venture between The University of Melbourne and The Royal Melbourne Hospital

HIV-HBV co-infection

- **5-20% of HIV infected individuals are co-infected with HBV.** [1]
- **HIV infection has a significant impact on the natural history of chronic HBV infection compared to HBV mono-infection.** [2]
  - Increased levels of HBV DNA
  - Accelerated progression of liver disease
  - Increased liver-associated mortality
- **Despite effective antiretroviral therapy (ART), which controls HIV and HBV replication, life expectancy of HIV-HBV co-infected individuals remains reduced and liver morbidity and mortality remain accelerated.** [1, 3]
  - The mechanism is poorly understood.

1, Singh et al., AIDS 2017; 2, Thio et al. Lancet;, 2010 3, Klein et al., CID 2016

HBV life cycle



Cao et al., AIDS 1992; Housset et al., 1993; Singh et al., AIDS 2017; Iser et al., JVI 2010

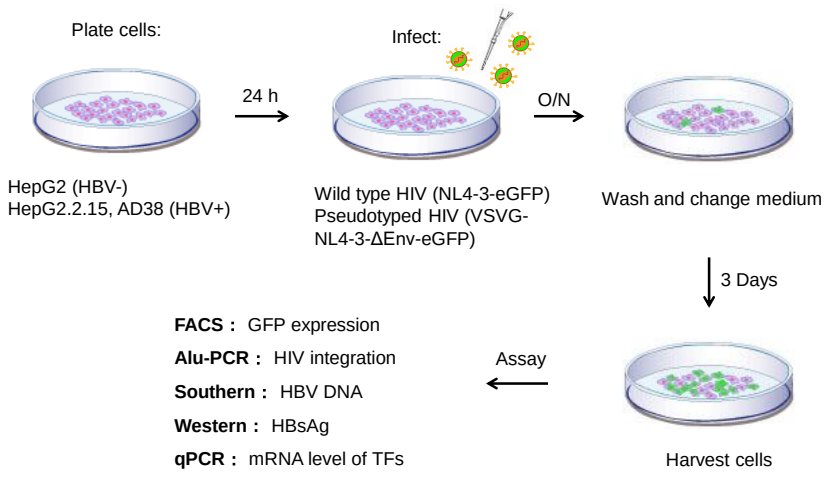
Hypothesis

HIV infected hepatocytes persist in the liver in HIV-HBV co-infected patients on HBV-active ART and ongoing production of HIV RNA drives increased production of HBsAg, hepatocyte apoptosis and adverse liver outcomes.

Aim

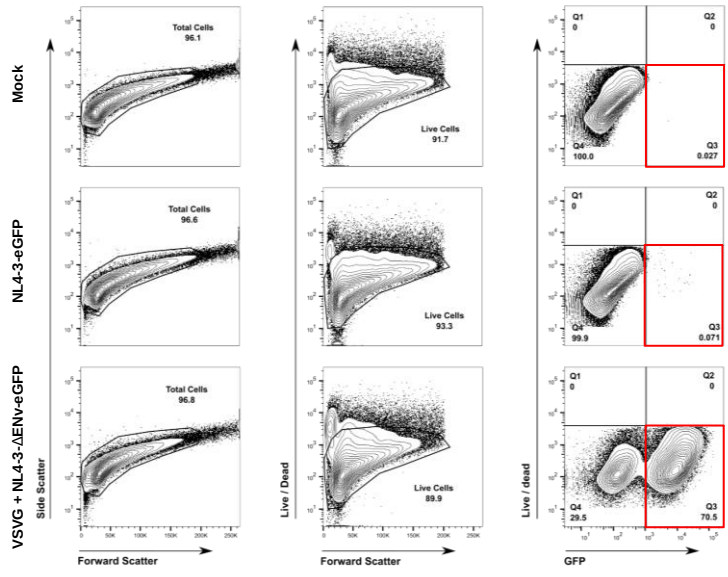
- Establish an in vitro HIV-HBV co-infection model that mimics treated HIV-HBV co-infection
- Identify which step of the HIV life cycle has an impact on HBV replication
- Identify the transcription factors (TFs) that drive the increase in HBsAg

Methods

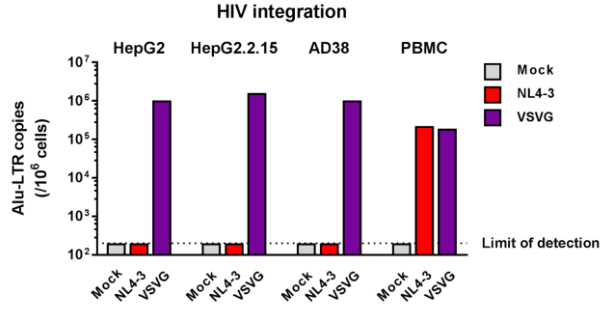


GFP: Green Fluorescent Protein

Only VSV pseudotyped virus led to HIV production in hepatocytes



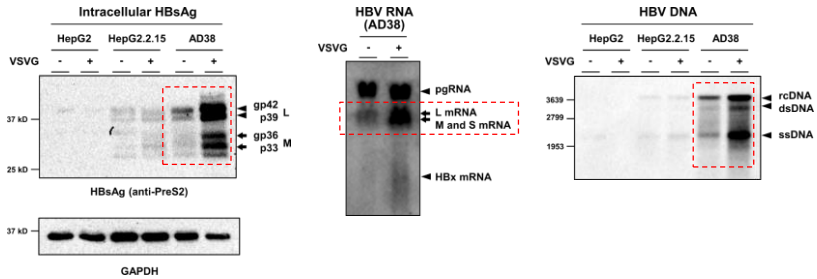
Only VSV pseudotyped virus led to HIV integration in hepatocytes



HepG2- hepatocyte cell line
HepG2.2.15 – HBV transfected cell line (HepG2-derived)
AD38 – HBV transfected cell line (HepG2-derived)

n=1

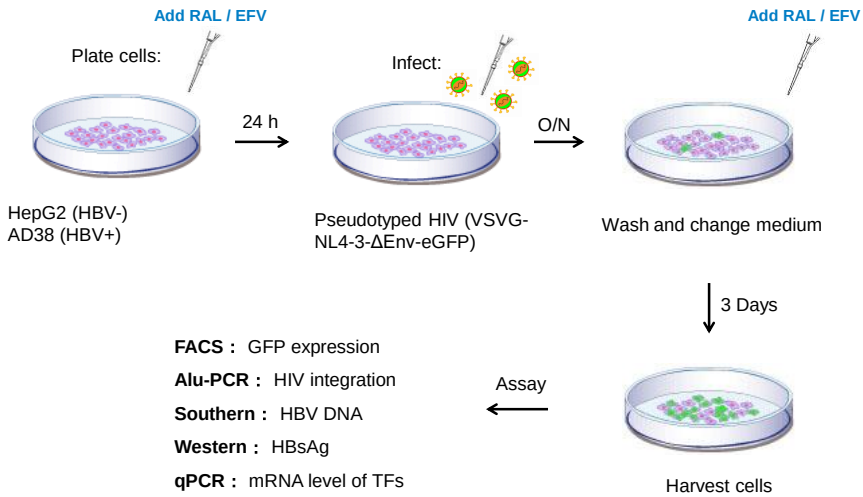
VSV pseudotyped HIV infection led to increase in HBs intracellular protein / mRNA and HBV DNA



HepG2- hepatocyte cell line
HepG2.2.15 – HBV transfected cell line (HepG2-derived)
AD38 – HBV transfected cell line (HepG2-derived)

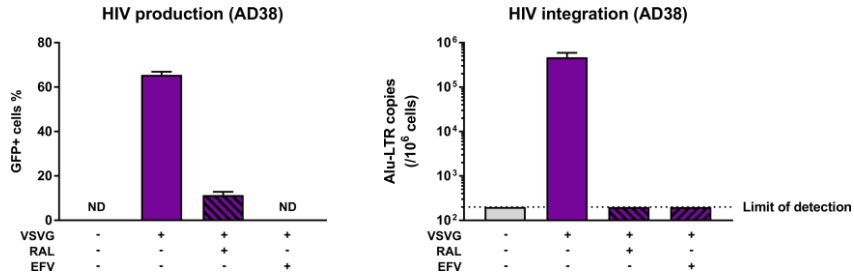
Representative of 2-3 experiments

Methods



RAL = raltegravir (integrase inhibitor); EFV = efavirenz (non-nucleoside reverse transcriptase inhibitor)

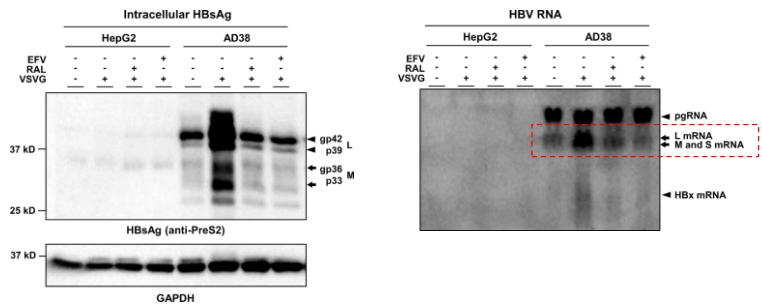
VSV pseudotyped HIV virus infection was inhibited by RAL / EFV



ND = not detected
RAL = raltegravir (integrase inhibitor); EFV = efavirenz (non-nucleoside reverse transcriptase inhibitor)
AD38 – HBV transfected cell line (HepG2-derived)

n = 3, mean + SEM

VSV pseudotyped HIV infection led to increase in HBs intracellular protein / mRNA

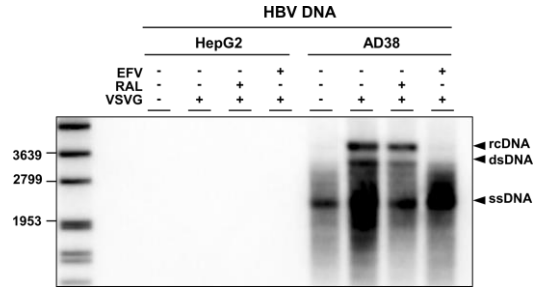


- **The increase in HBsAg intracellular protein or mRNA was rescued by RAL or EFV**

HepG2- hepatocyte cell line
AD38 – HBV transfected cell line (HepG2-derived)

Representative of n = 2

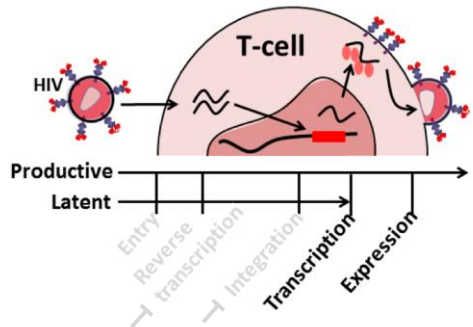
VSV pseudotyped HIV infection led to an increase in HBV DNA



- **The increase in HBV DNA was reduced by RAL or EFV.**

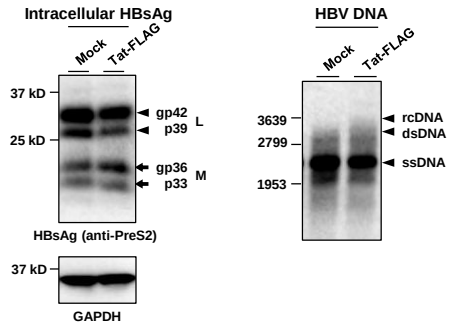
HepG2- hepatocyte cell line
AD38 – HBV transfected cell line (HepG2-derived)

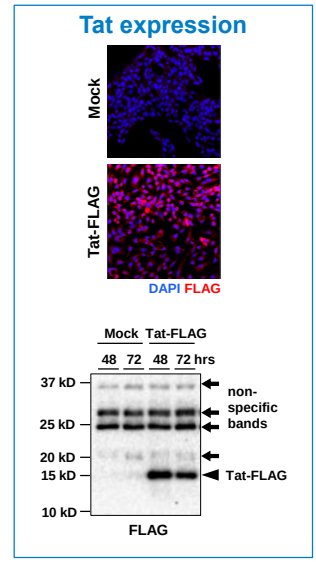
Representative of n = 2



Events downstream of HIV integration lead to the increases in intracellular HBsAg, HBs RNA and all forms of HBV DNA.

HIV Tat did not affect intracellular HBsAg expression or HBV DNA in AD38



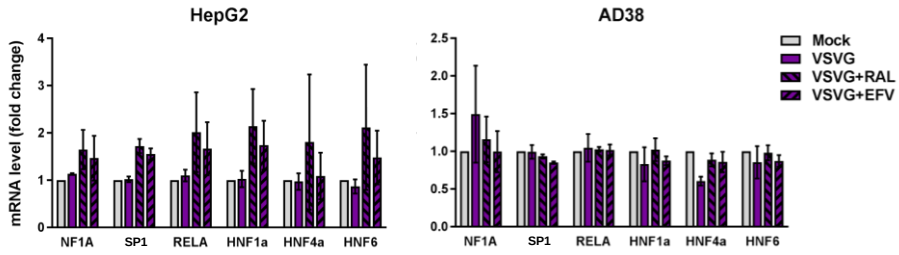


Transcription factors involved in hepatitis B virus transcription

Factor	Binding site	Effect on viral enhancers/promoters	Ref.
HNF1α	PreS1	Activation	[60]
	Enh II	Activation (interaction with hB1F)	[61]
	Enh II	Suppression (mutant HBV core promoter)	[50]
	Enh II	Activation	[62]
	Enh II	Activation (mutant HBV core promoter)	[49]
HNF3β	Enh I	Activation (interaction with STAT3)	[48]
	Enh I	Suppression (HepG2)/ Activation (SK-Hep1)	[68]
	Enh II	Suppression	[67]
	Enh II	Activation	[66]
HNF4α	EnhII/PreS1	Activation	[73]
	Enh II	Activation	[130]
HNF6	PreS2	Suppression	[78]
C/EBP	Enh I	Suppression	[83]
	Enh II	Activation	[81]
	Enh II	Activation	[46]
	Enh II	Activation	[80]
FXR/RXR	Enh II	Activation	[99]
HLF	Enh II	Activation	[6]
NF1	PreS2	Activation	[101]
	Enh I	Suppression	[102]
SP1	Enh II	Activation	[104,105]
	PreS1	Activation	[106]
	PreS2	Activation	[107]

Kim et al., 2016, World J Gastroenterol

No significant changes in gene expression of nuclear factor in the liver upon VSV pseudotyped HIV infection



NF1A: nuclear factor 1A
SP1: specificity protein 1
RELA: REL-associated protein A (NF-κB subunit)
HNF: hepatocyte nuclear factor

HepG2- hepatocyte cell line
AD38 – HBV transfected cell line (HepG2-derived)

n = 2, SEM

Summary

- HIV co-infection of HBV-expressing hepatocytes led to increases in:
  - Intracellular HBs protein and HBs mRNA levels
  - HBV DNA
- Addition of the HIV reverse transcriptase inhibitor or integrase inhibitor
  - Abolished HIV production or integration
  - Rescued the increase in HBsAg and HBV DNA)
- HIV Tat is not involved in the impact of HIV infection on HBV replication.

We conclude that there is a significant effect of co-infection with HIV on the HBV life cycle through an increased synthesis of HBV DNA. This effect is after HIV integration which means this can potentially persist on ART.

Future work

- Modify HIV-HBV co-infection model: HepG2-NTCP cells, primary liver cells and liver organoids.
- Assess antivirals that block HIV and HBV replication (tenofovir) or only HBV replication (telbiviudine).
- Target the HIV life cycle post integration (mutate HIV accessory proteins, RNAi).
- Use a non-biased assessment of the transcription factors binding to the HBsAg promoter region of cccDNA (ChIP and RNAseq).

Acknowledgments

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