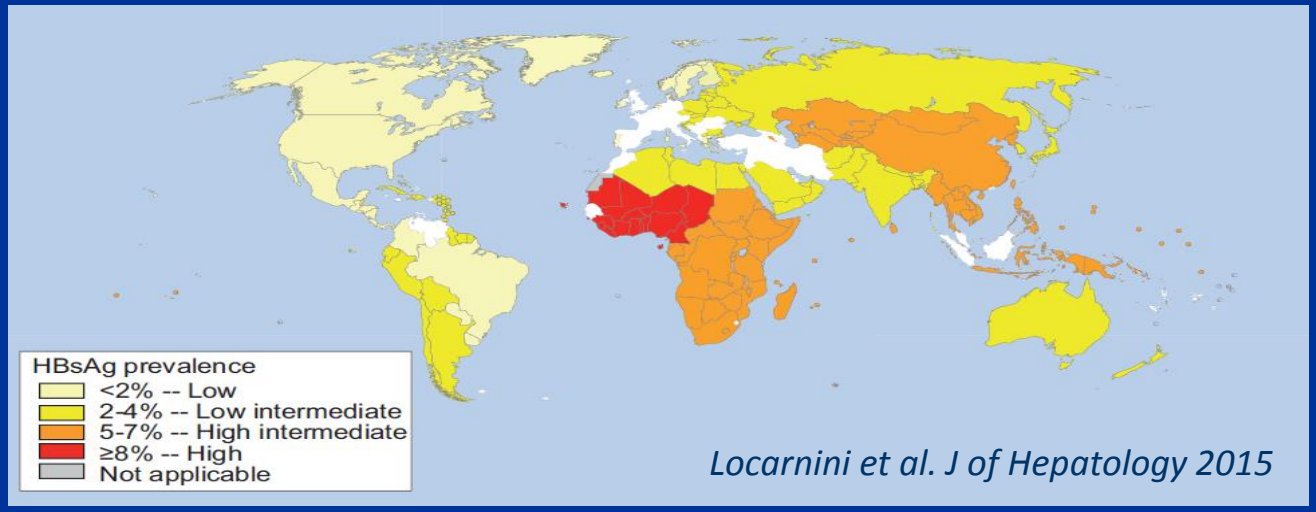
The Precore Antigen evades Interferon response in Hepatitis B

Zina Valaydon

HBV Burden of Disease



Locarnini et al. J of Hepatology 2015

240 million chronic HBV

Cirrhosis and hepatocellular carcinoma: 1 million deaths/ year

> 90% vertically transmitted HBV becomes chronic

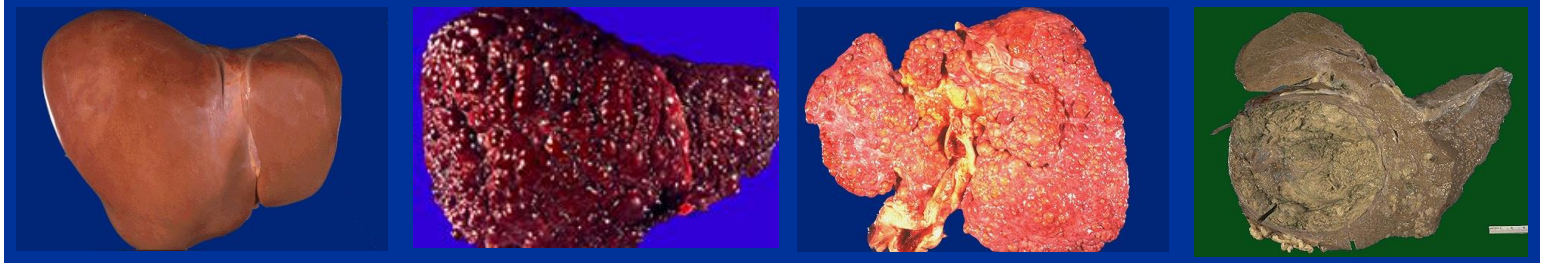
Prevalence in Australia is rising

Cowie et al. MJA 2015

Liver Disease Progression

Time 20-30 years

Normal | Cirrhosis | Cirrhosis | HCC



HBV → **CHRONIC HEPATITIS B** → **CIRRHOSIS ESLD** → **HEPATOCELLULAR CARCINOMA**

PRIMARY PREVENTION

Vaccination

CURRENT TREATMENTS

Peg-IFN-α

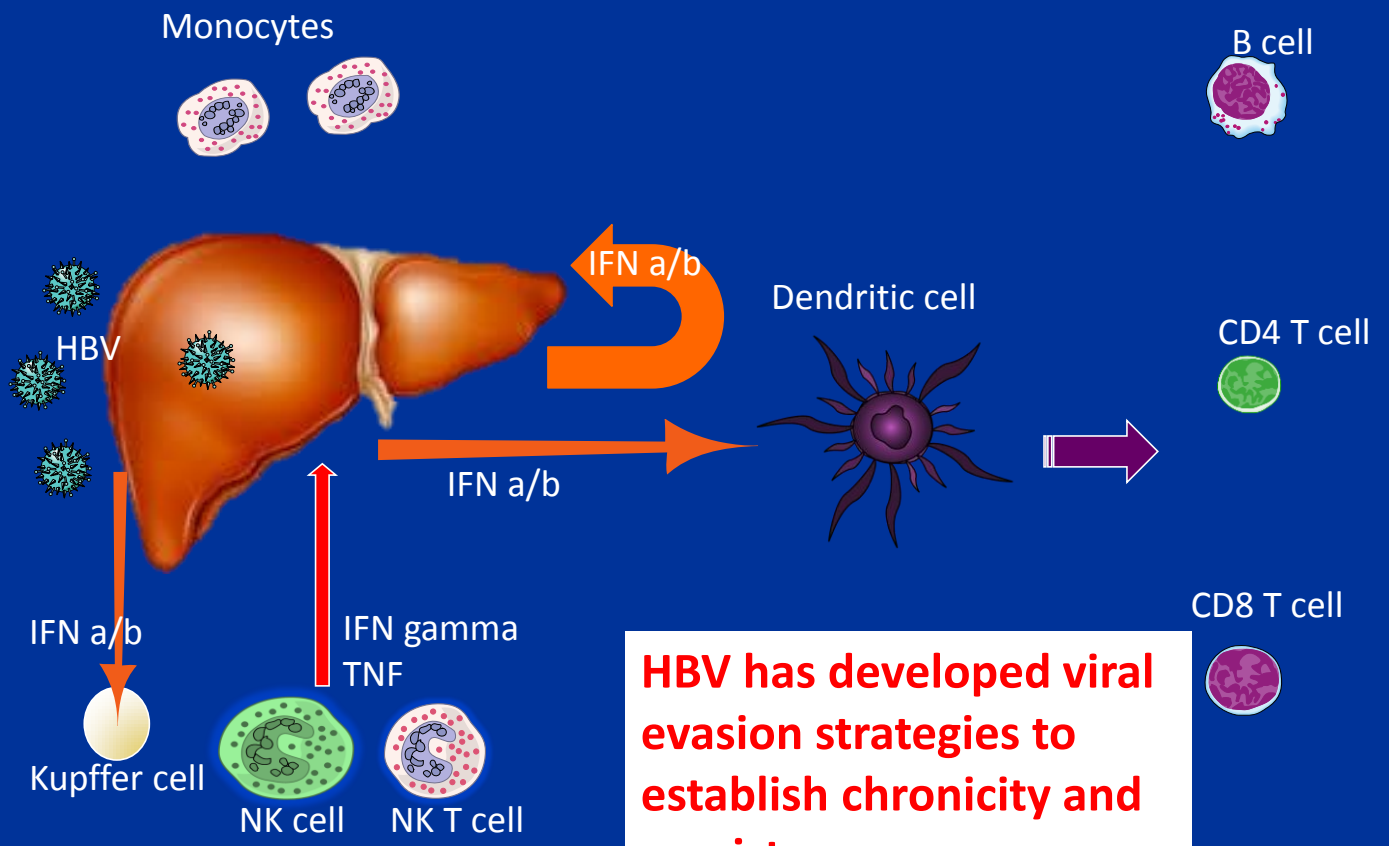
Antivirals

TDF, ETV

CHB is INCURABLE

Novel immunotherapy approaches require a deeper understanding of the host-viral interaction

HBV is an immune mediated disease



HBV has developed viral evasion strategies to establish chronicity and persistence

Interferon in HBV

- Interferon is a key player in the innate defense against viral infections
  - Hepatitis C, HIV
- HBV is highly susceptible to interferon *in vitro*
- Evades immune response *in vivo*
- Viral evasion strategy to establish chronicity

? How does HBV evade interferon

?Structural protein

The Precore antigen: HBeAg

Not required for infectivity or viral replication

?Immunomodulator ? Tolerogen

<u>Necessary for chronicity</u>

HBeAg negative HBV does not become chronic

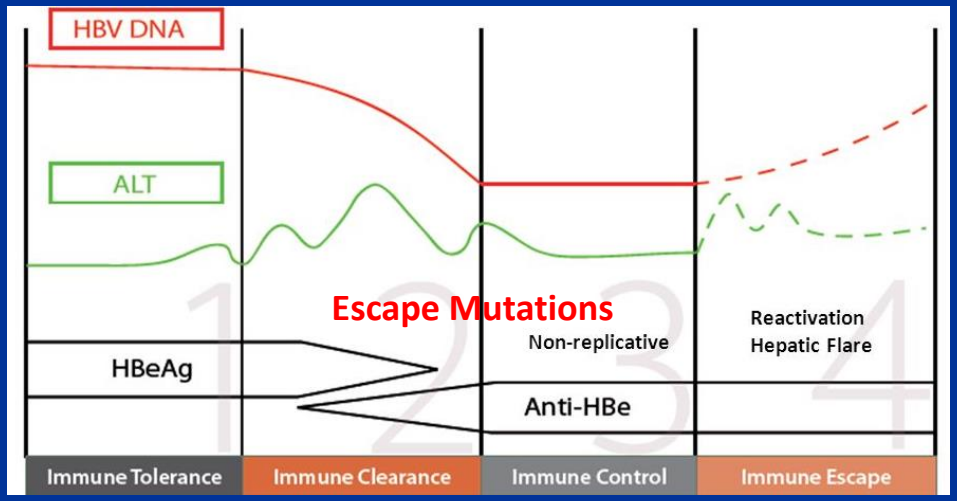
Evades Toll-like receptor

Tolerises T cells

Response to interferon treatment is poor in HBeAg positive patients

Visvanathan et al. Hepatology 2007
Milich et al. Hepatology 2003

HBeAg in the natural history of HBV infection

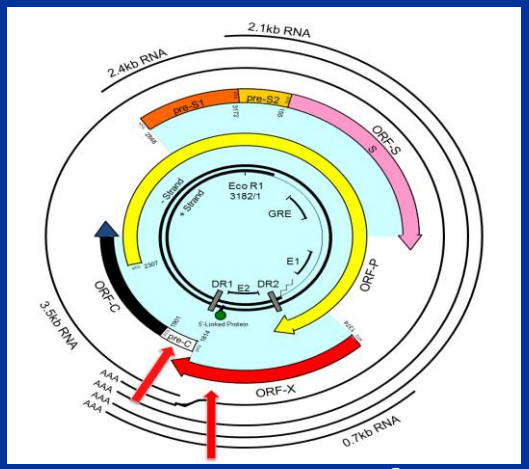


HBeAg is only present in early phases of disease
Escape mutations are common

Basal core promoter and precore variants reduce/abolish HBeAg

PC Δ=G1896A

Translational stop codon
no HBeAg translated



BCP Δ = A1762T/G1764A

Relative reduction in transcription
Reduced HBeAg

BCP and PC mutations are independently associated with advanced fibrosis

Valaydon et al. EASL 2016

Hypothesis

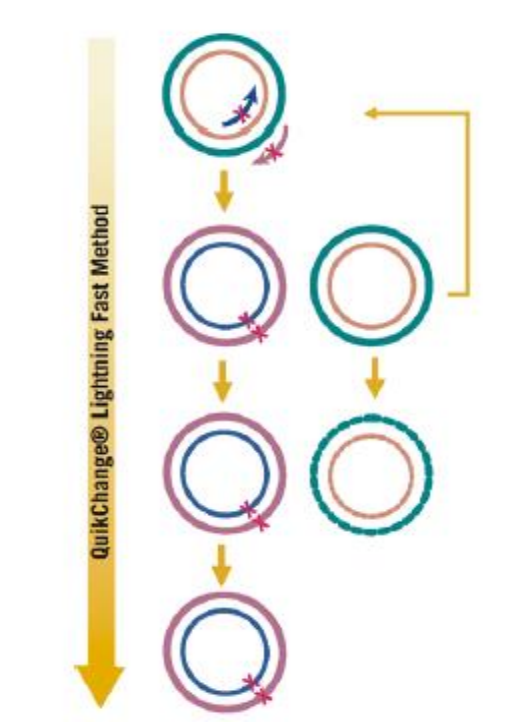
HBeAg has a significant immunomodulatory effect in early HBV infection

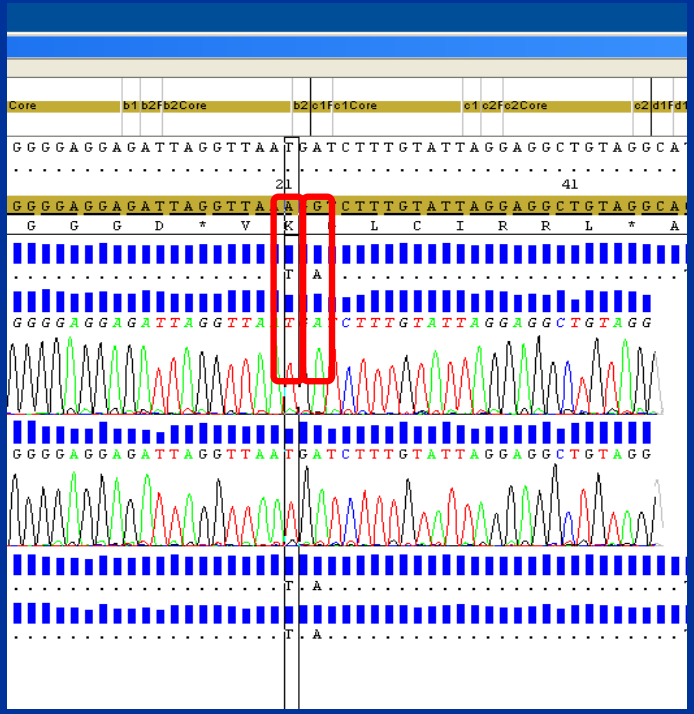
Aims

To characterize and compare the viral kinetics of HBeAg- negative variants vs wild type HBV using a mouse model of HBV infection

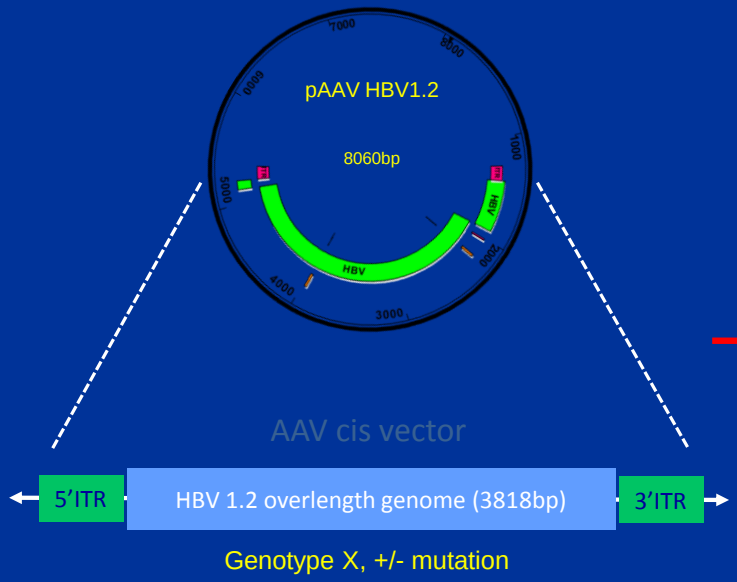
Examine effect of HBeAg on immune mediators of HBV using a mouse model of HBV infection

Mutagenesis





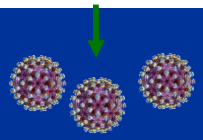
Hydrodynamic injection (HDI) of HBV-DNA to induce HBV infection in mice



Hydrodynamic injection

10µg DNA

Immuno competent C57/BL6 Mouse



HBV

Ebert, Pellegrini et al. PNAS 2015

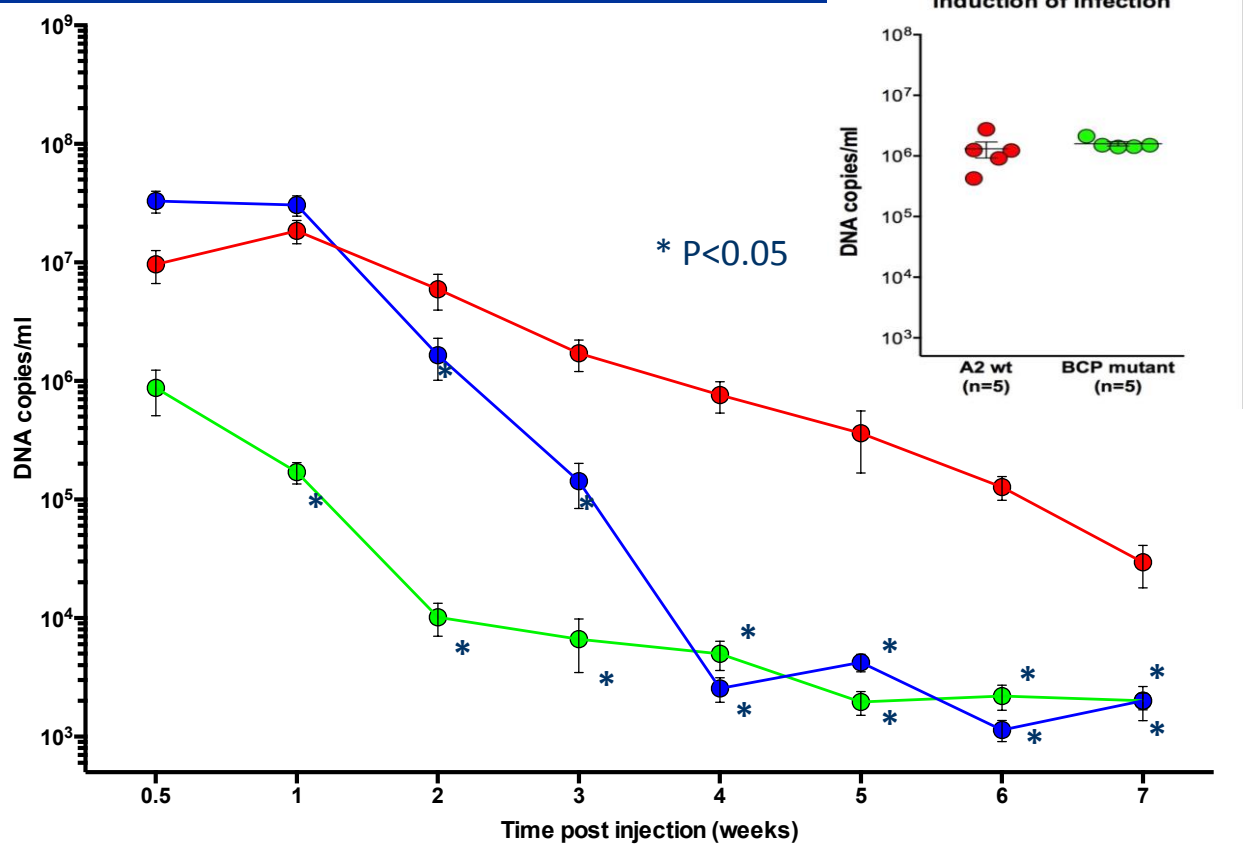
BCP and PC vs WT

- Hydrodynamic injection of immuno competent C57BL/6

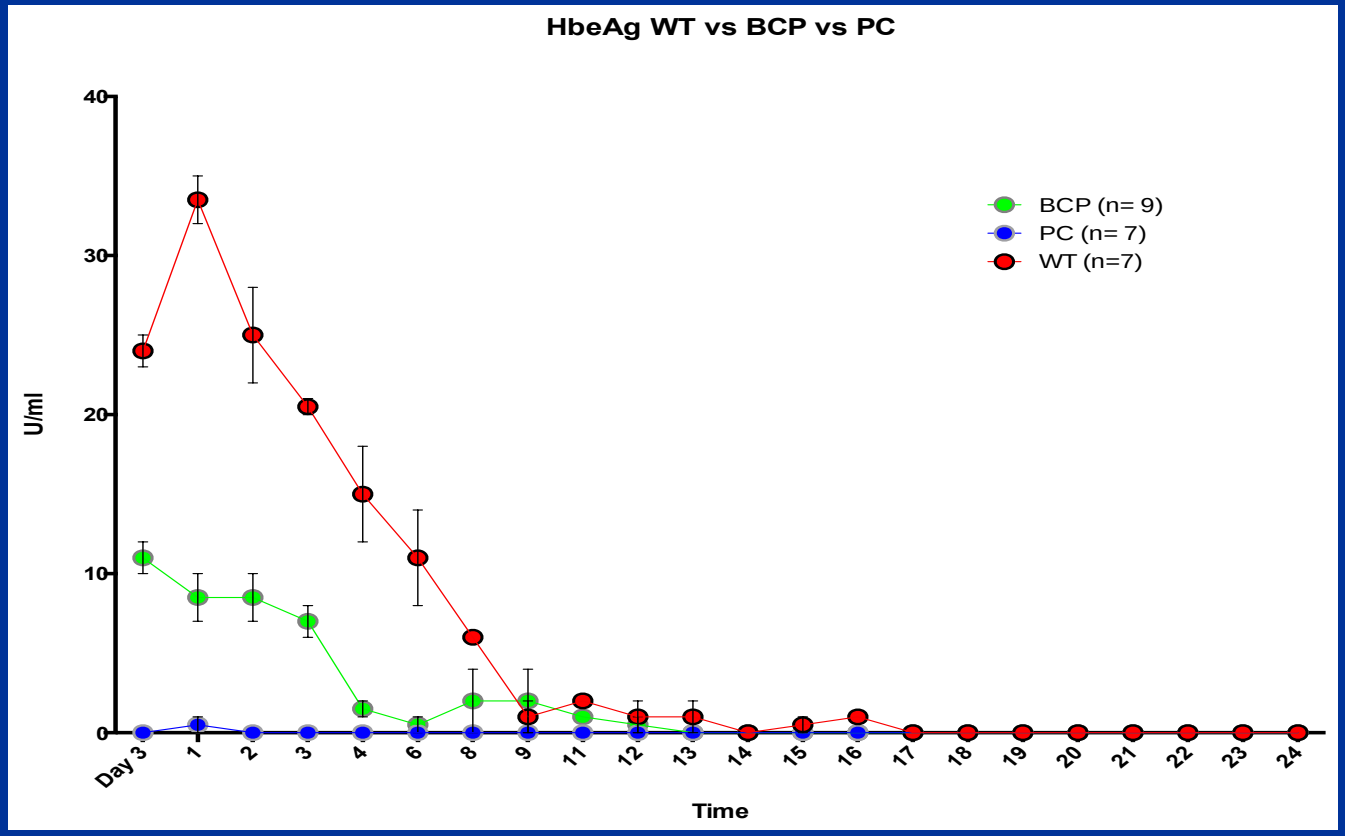
1.2 mer HBV A2 WILD TYPE	**VS**	**1.2 mer HBV A2 PC**	**VS**	**1.2 mer HBV A2 BCP**

- HBV DNA measured by qPCR
- Serology measured by ELISA
- Terminal bleeds for ALT/ AST levels

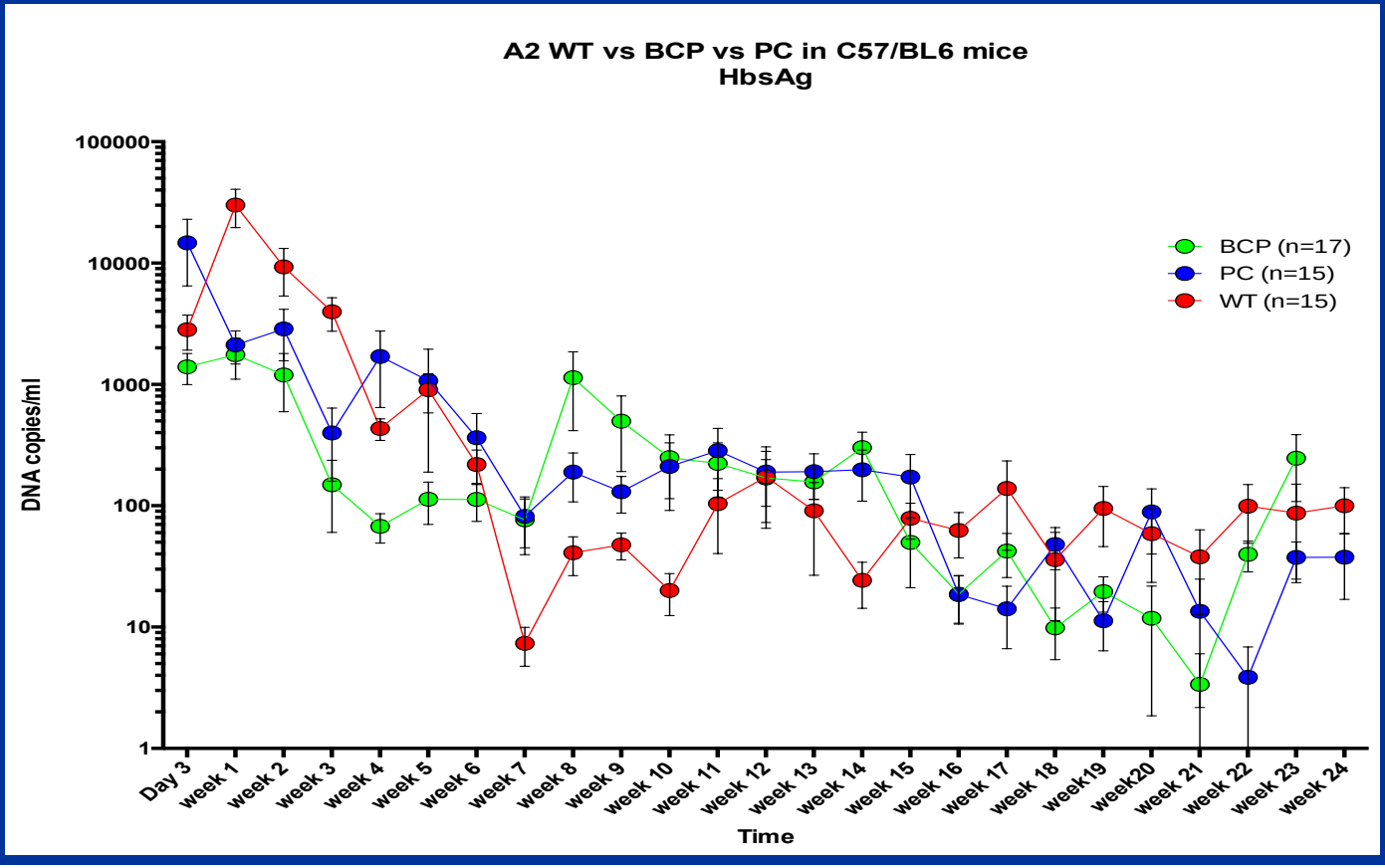
Rapid early viral suppression in PC and BCP mutants



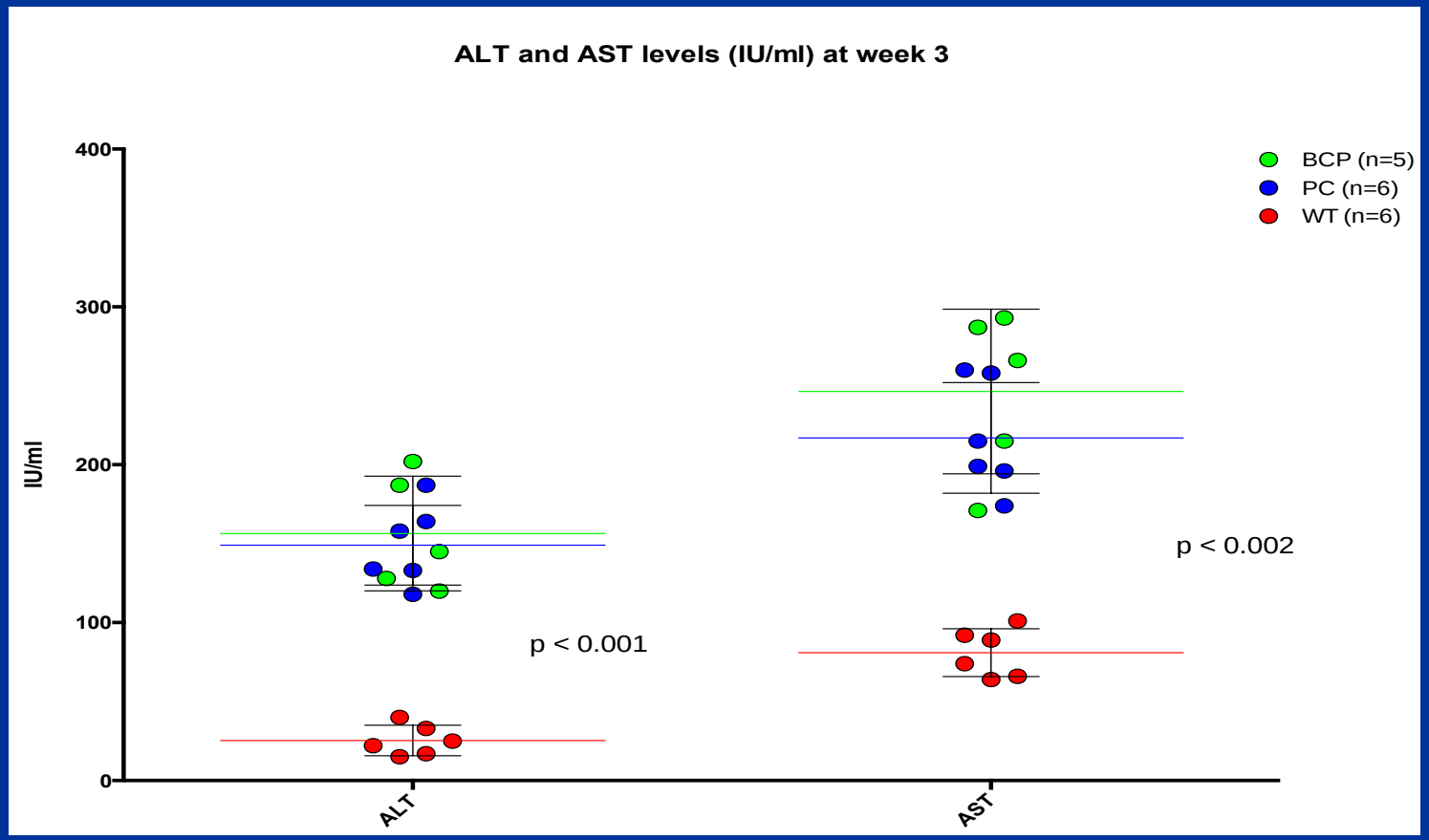
HBeAg was reduced in BCP mutants and absent in PC mutants



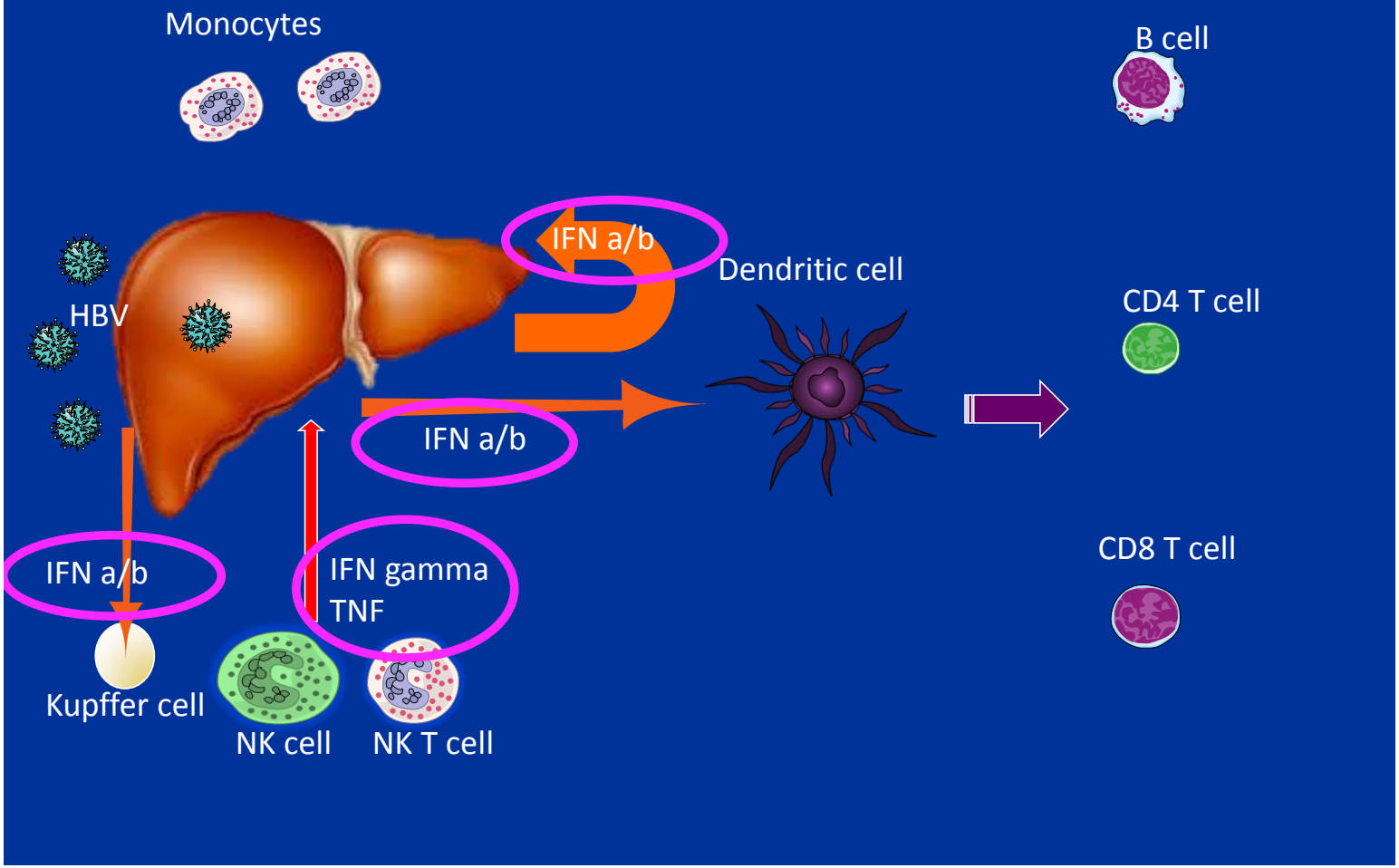
No difference in HBsAg levels between mutants and WT



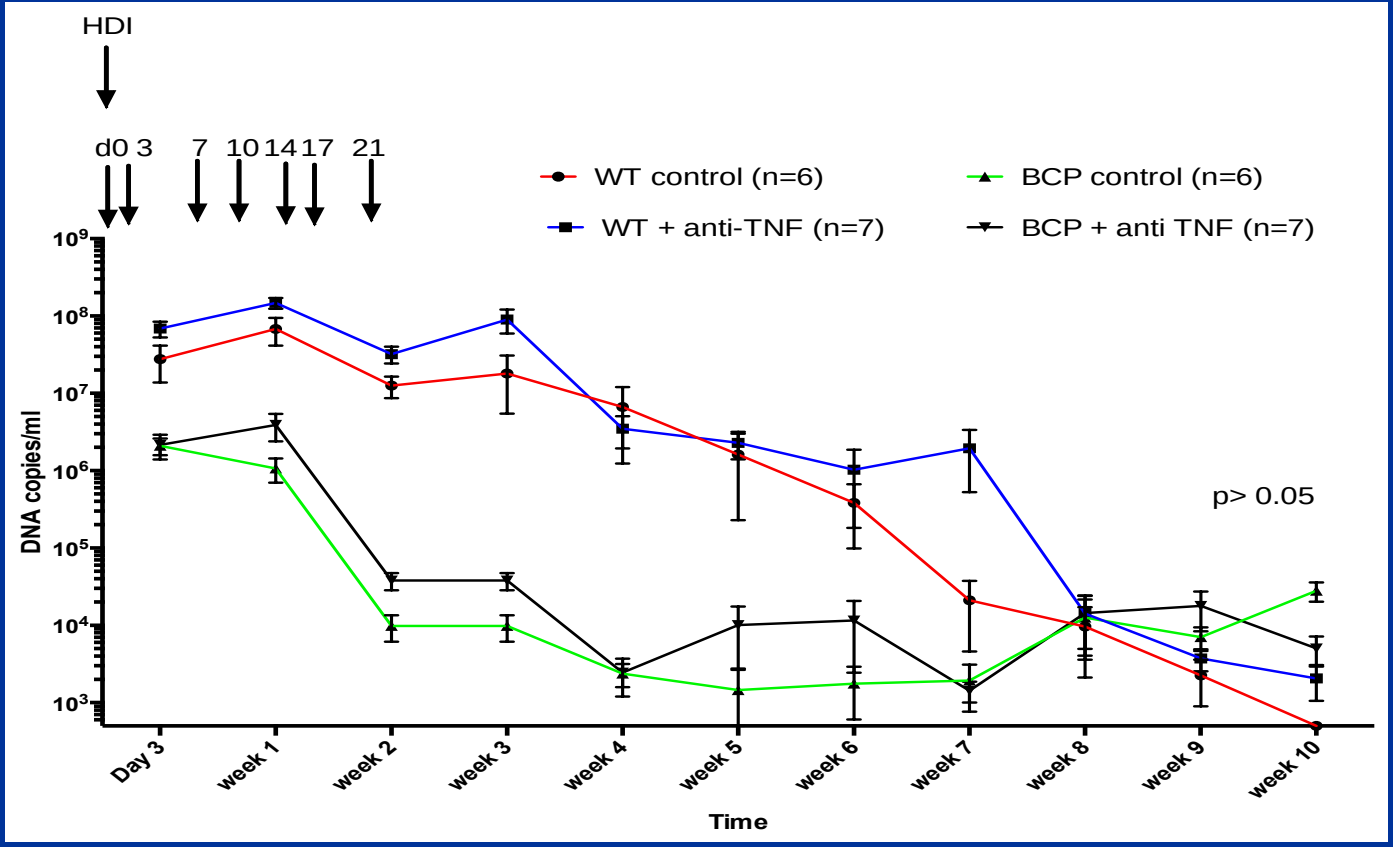
Levels of ALT and AST were significantly increased in BCP and PC mutants



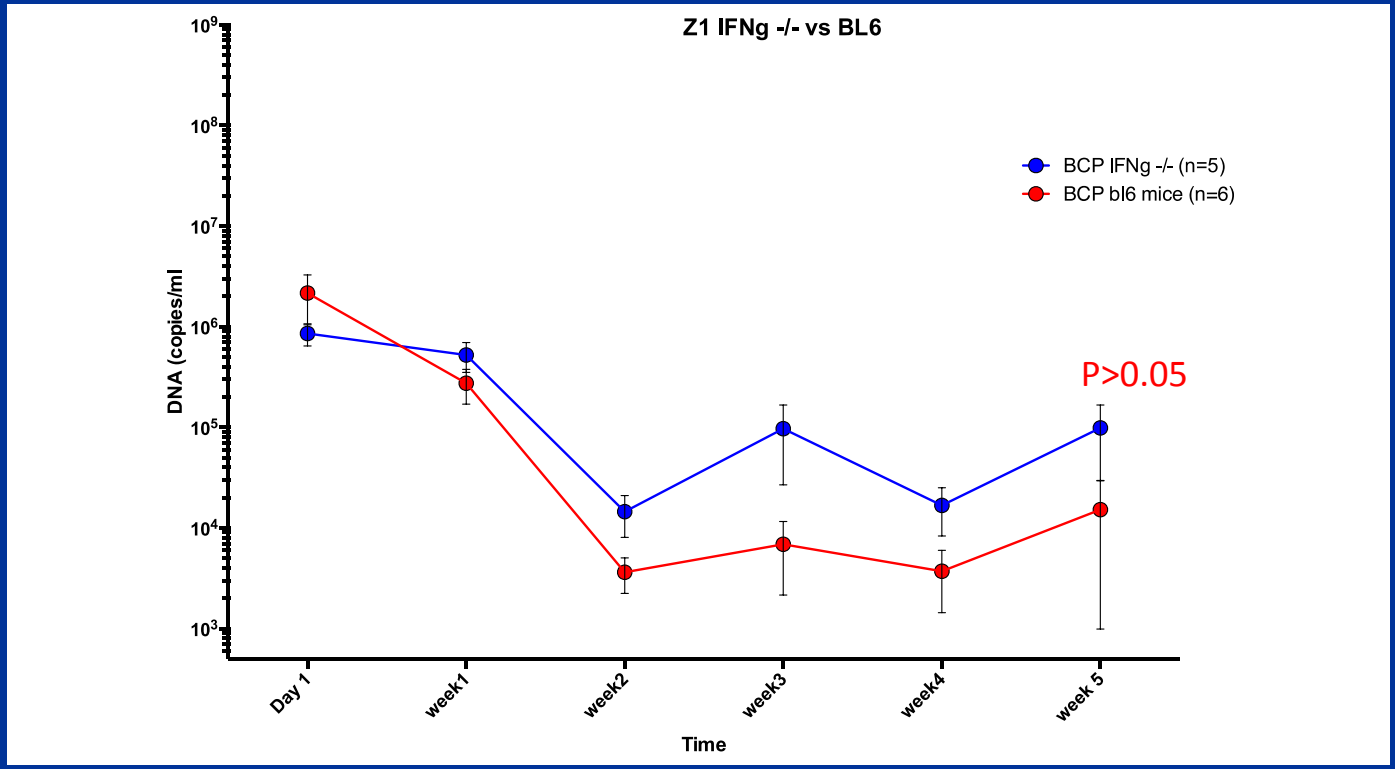
The interferon response in HBV



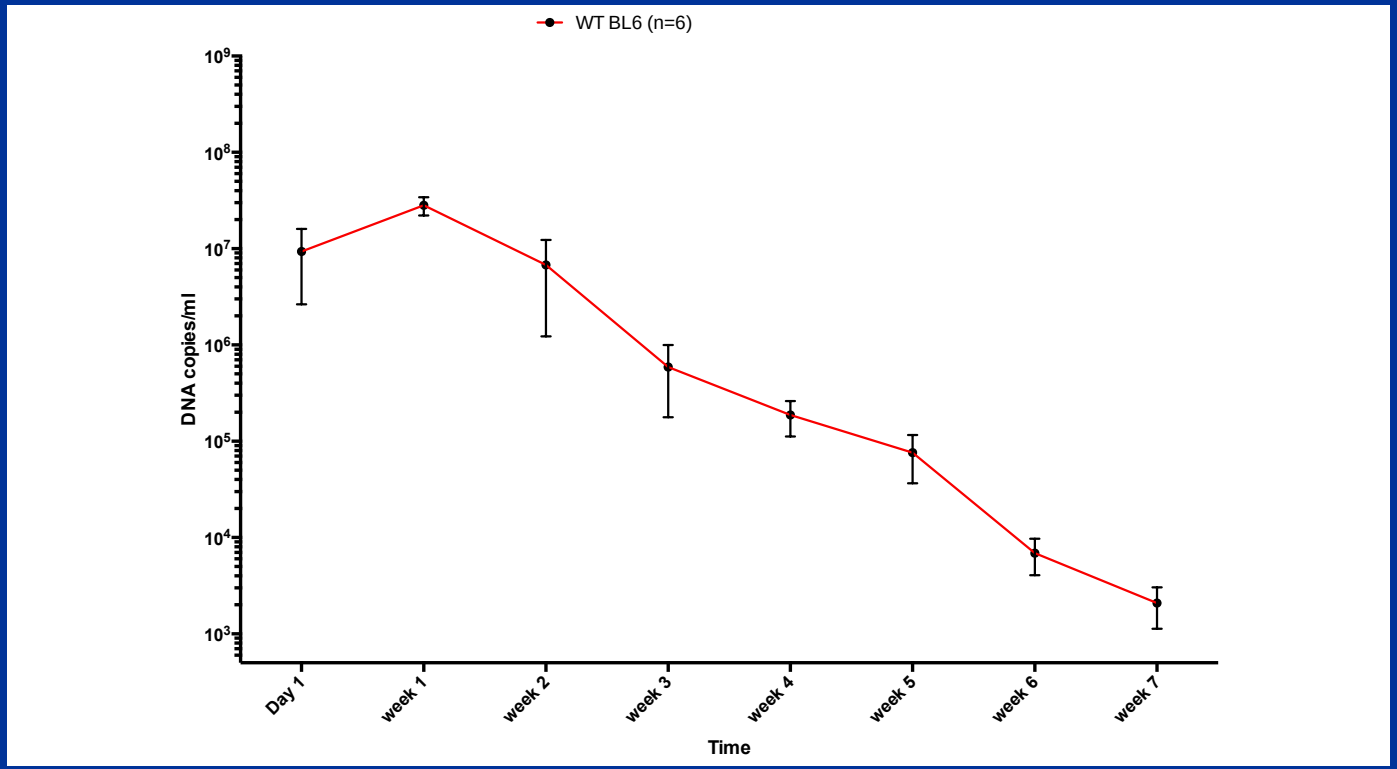
TNF was not a key mediator in viral suppression in mutants



IFN gamma was not a key mediator in viral suppression in mutants



IFN alpha is a key mediator in viral suppression in mutants



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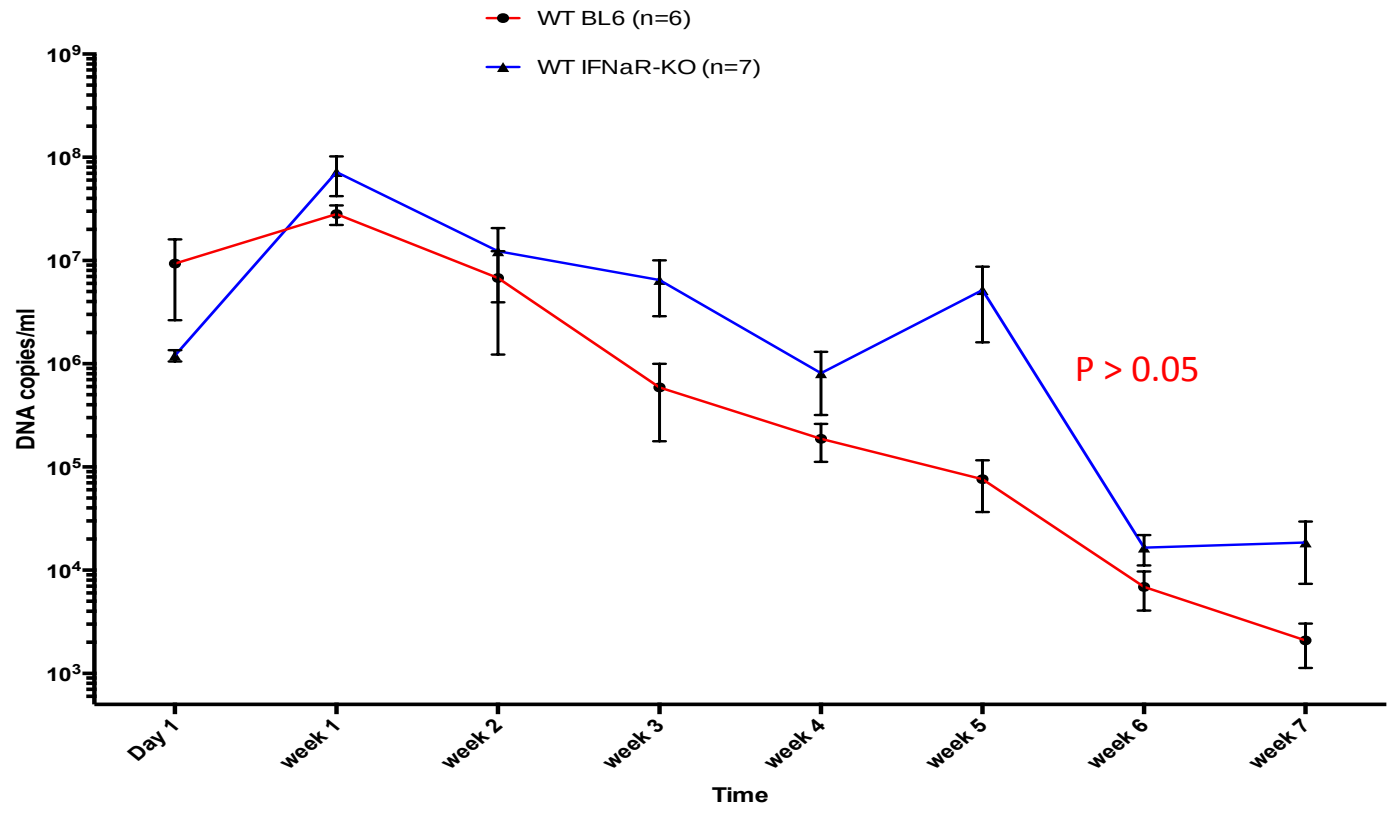

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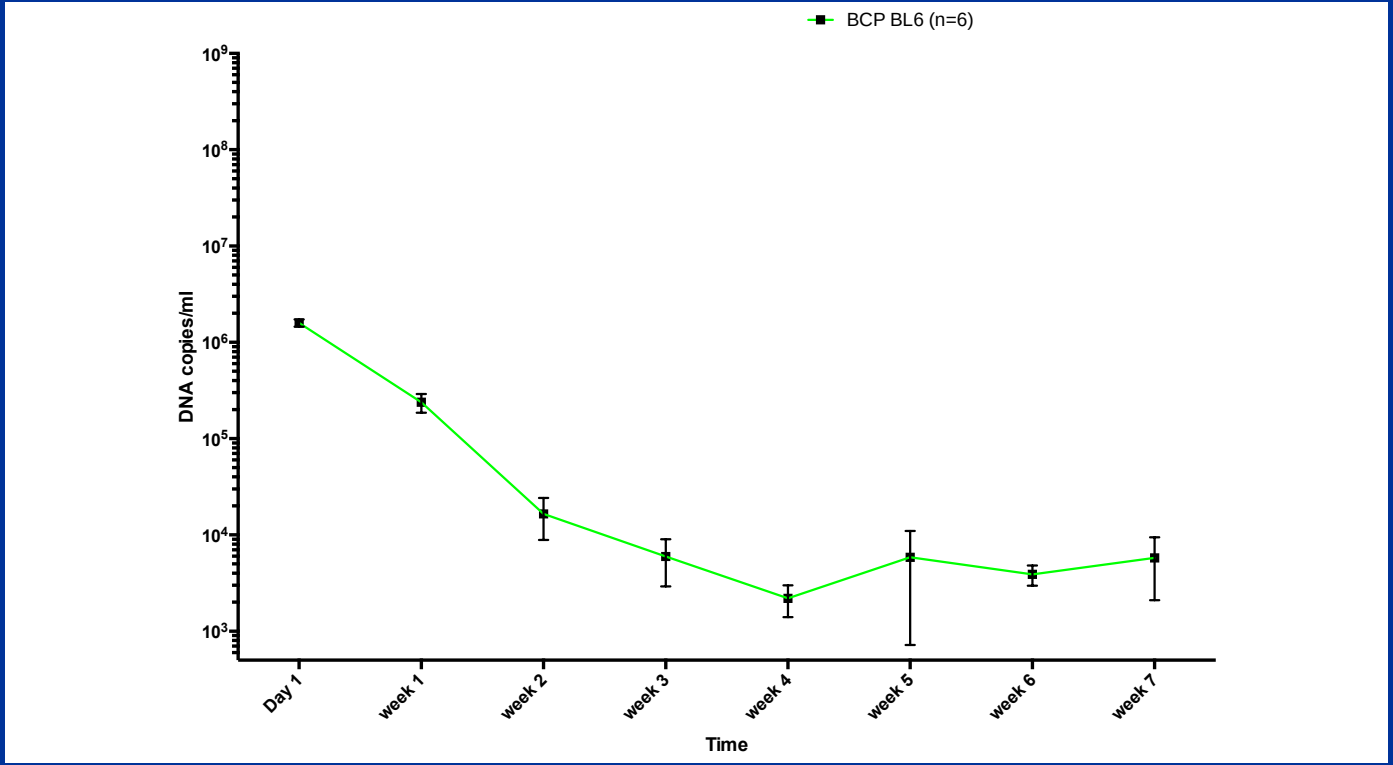

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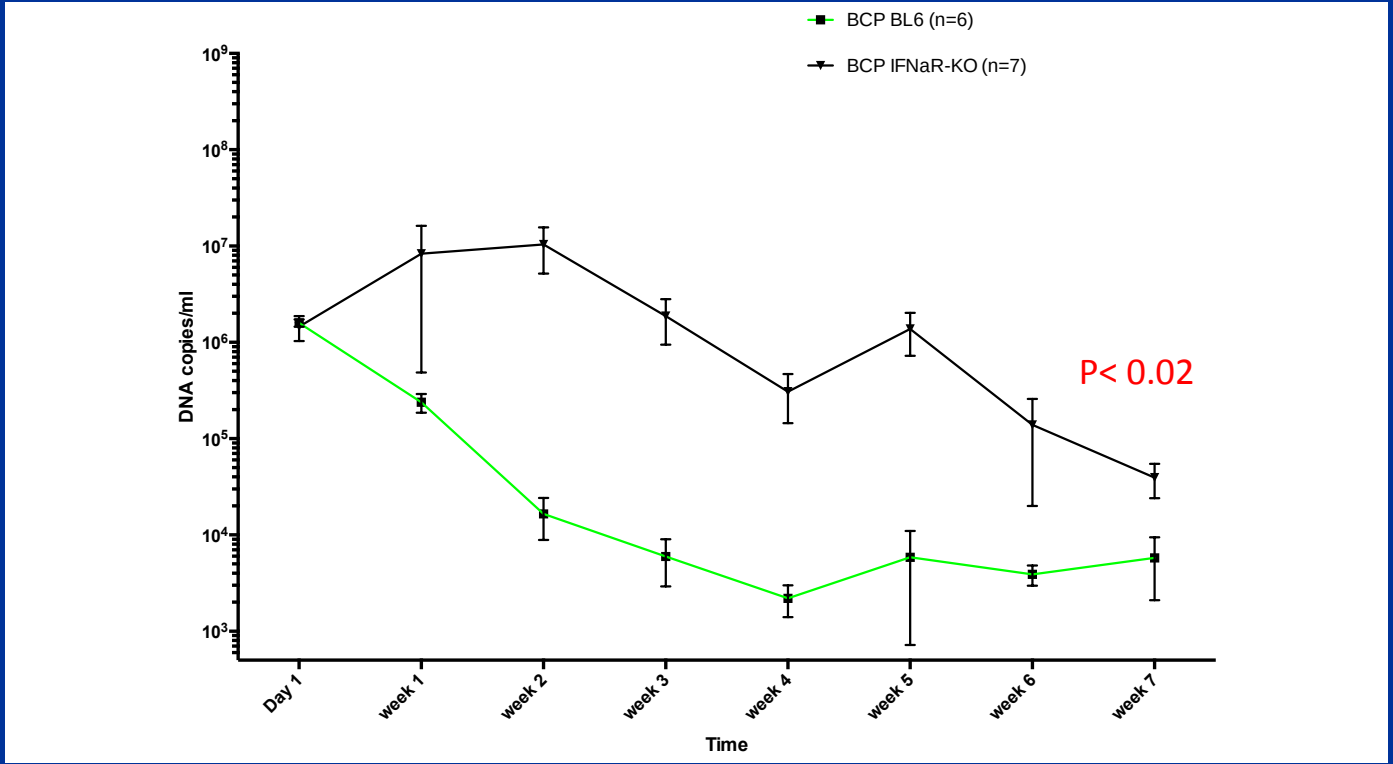

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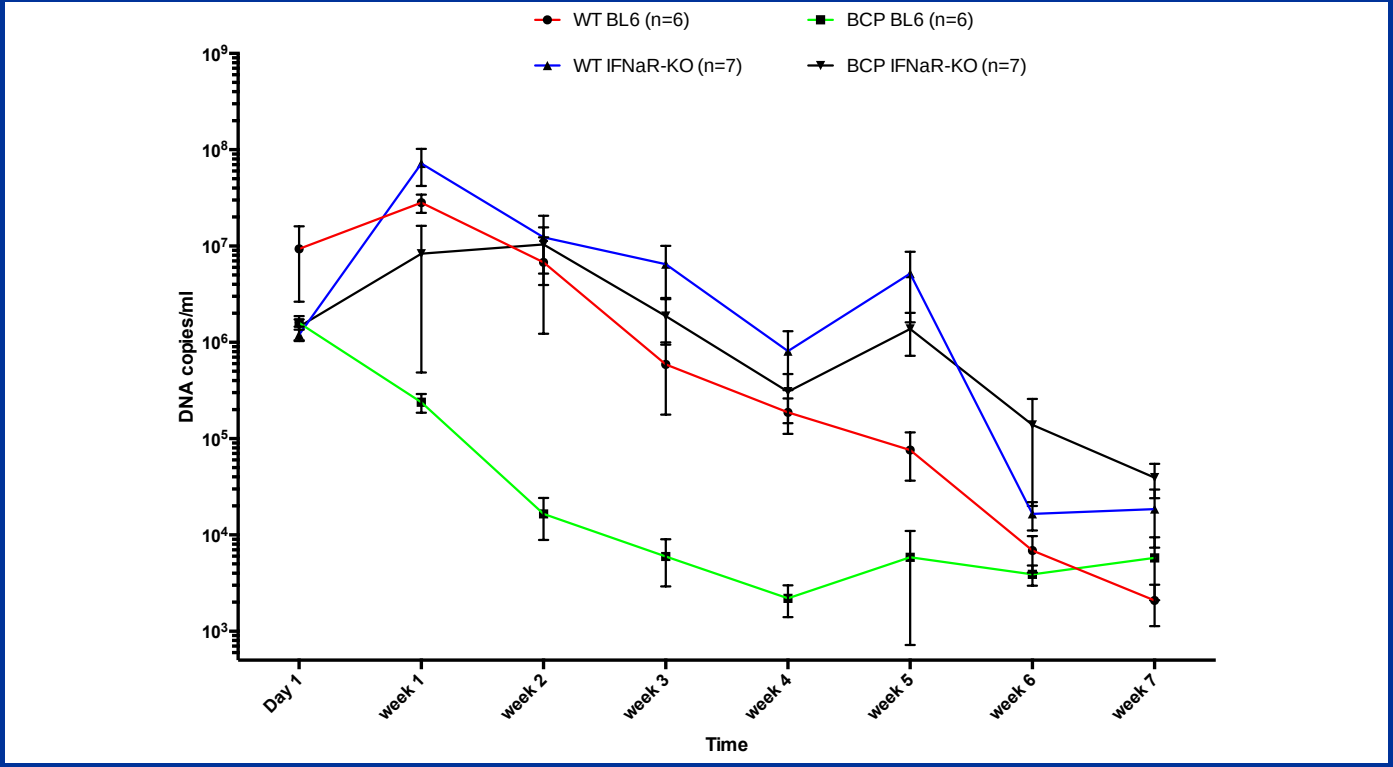

Conclusions

- New small animal model to study the immuno pathogenesis of HBV
- Major differences in the viral kinetics of HBeAg negative mutants
- Mediated by Type 1 Interferon
- HBeAg may be an interferon resistance protein allowing immune evasion
- Significant therapeutic implications
  - Neutralisation of HBeAg may improve rate sof viral clearance with interferon treatment

Future Directions

- In vivo testing of anti-Hbe neutralising antibodies to improve viral suppression and clearance
- Complementation studies
  - Mix of WT and mutant strains
- Liver transcriptome for further elucidation of the interferon pathway
- Proteomics to investigate associated proteins

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Does the preclinical mouse model recapitulate HBV replicative lifecycle in human hepatocytes ?