

# ***Fine needle aspirates in Hepatitis B***

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***HBV Forum:  
Intrahepatic Panel***



***Liver Sampling in Hepatitis B:  
A vital “telescope onto the battlefield”***

***Why?***


***When?***

***How?***

# ***Compartmentalised intrahepatic virology and immunology***

- *Histological assessment, exclusion of other pathology*
- *Compartmentalised viral reservoirs: cccDNA & integrated DNA*
- ***What about immune responses?***
- *-can blood monitoring adequately represent the hepatic immune landscape - the site of host/pathogen interaction?*

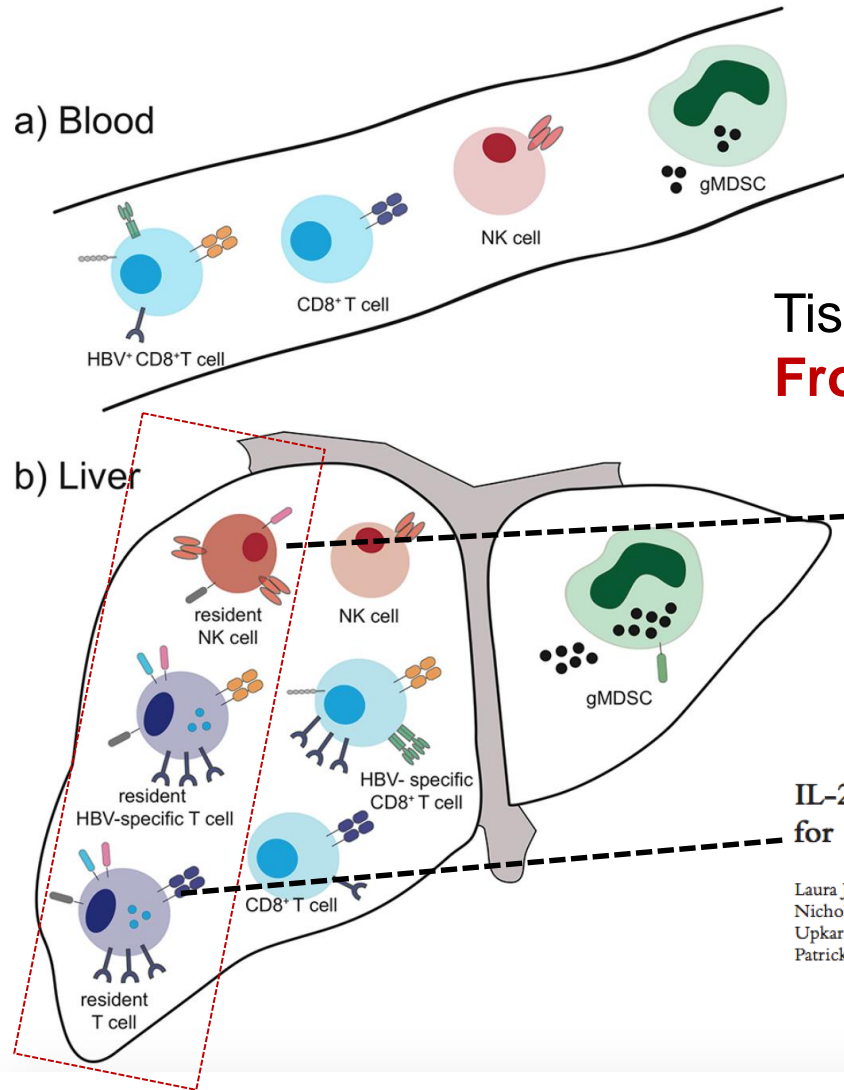
Recent advances in basic science

Liver sampling: a vital window into HBV pathogenesis on the path to functional cure 

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*Gut* 2018 Jan 13

# Unique immune responses are sequestered in the liver



Tissue-resident lymphocytes:  
**Frontline pathogen defence**

**OPEN** CXCR6 marks a novel subset of  
T-bet<sup>lo</sup>Eomes<sup>hi</sup> natural killer cells  
residing in human liver

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IL-2<sup>high</sup> tissue-resident T cells in the human liver: Sentinels  
for hepatotropic infection

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# ***WHEN is liver sampling needed in CHB?***

- ***Basic research into HBV pathogenesis***
- ***New insights into fundamental aspects of tissue-specific immunology***
  - discovery novel cell types & unique adaptations
- ***Optimising HBV functional cure strategies***
  - antiviral approaches: indirect intrahepatic immune reconstitution?
  - immunomodulators: liver-targeted mechanism of action?
  - early phase studies: understanding how to tailor therapy or select optimal combos
- ***Identifying peripheral biomarkers reflecting intrahepatic virological/immunological changes***
  - for larger scale drug trial immune monitoring using blood samples

# ***HOW to approach liver sampling in Hepatitis B***

- ***Liver biopsy remains gold standard***
  - *Optimise research samples when clinically indicated*
  - *Paired biopsies to assess some new therapies?*
- ***Need in situ immunostaining for spatial relationships***  
***e.g. between infected hepatocytes and immune cells***
  - *Make use of historical paraffin-embedded / frozen tissue blocks*
  - *Apply new multiplexed immunostaining*
- ***Unbiased comprehensive analyses e.g. scRNA-seq***
- ***Hypothesis-driven focused studies tailored to the drug target***  
*e.g. HBV-specific immunity, cccDNA*

# **Can fine needle aspirates provide an alternative to biopsies in HBV?**

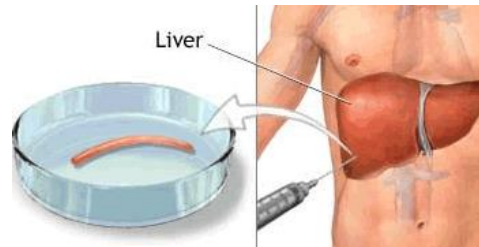
**Upkar Gill**

Patrick Kennedy  
Barts & the London



*Diagnostic liver biopsies being replaced by non-invasive fibrosis tests*

*Can fine needle aspirates (FNA) be used for longitudinal monitoring of novel HBV therapies?*



*FNA pioneered in HBV by Janssen group 2005*

*Used for repetitive longitudinal therapy monitoring in HCV Pembroke Gut 2014*

***Can multiparameter flow cytometry of FNA samples probe compartmentalised liver immunity?***

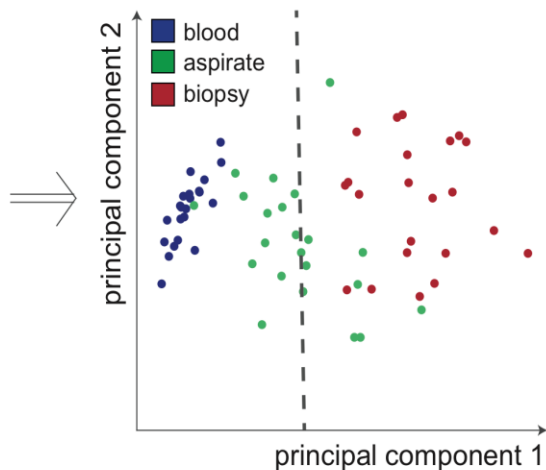
*Gill, Pallett et al, Gut 2018*

# FNA reflect immune composition of biopsies

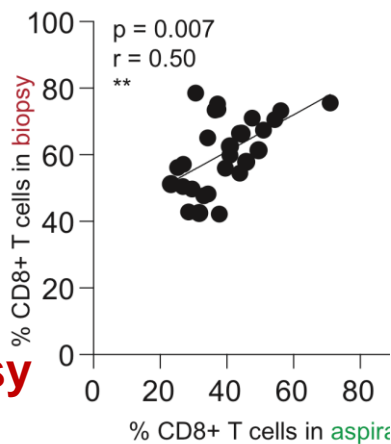
Upkar Gill



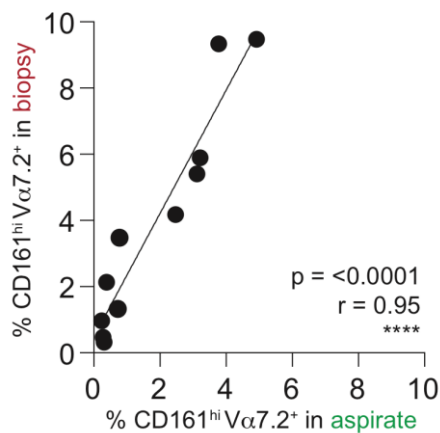
Laura Pallett



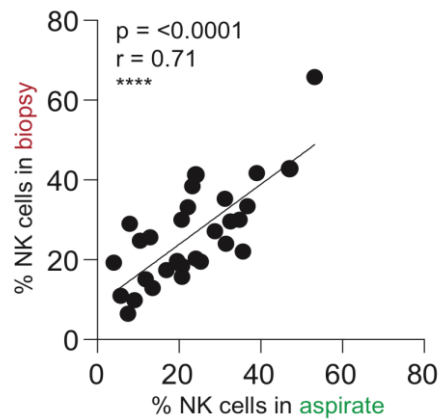
## CD8 T cells



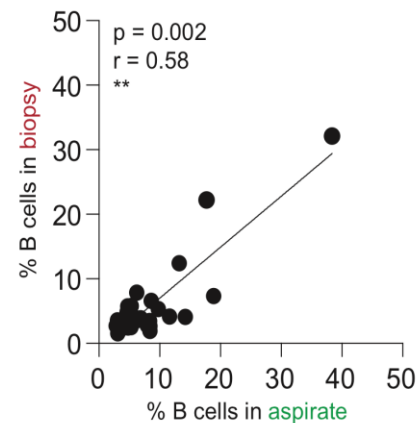
## MAIT cells



## NK cells



## B cells



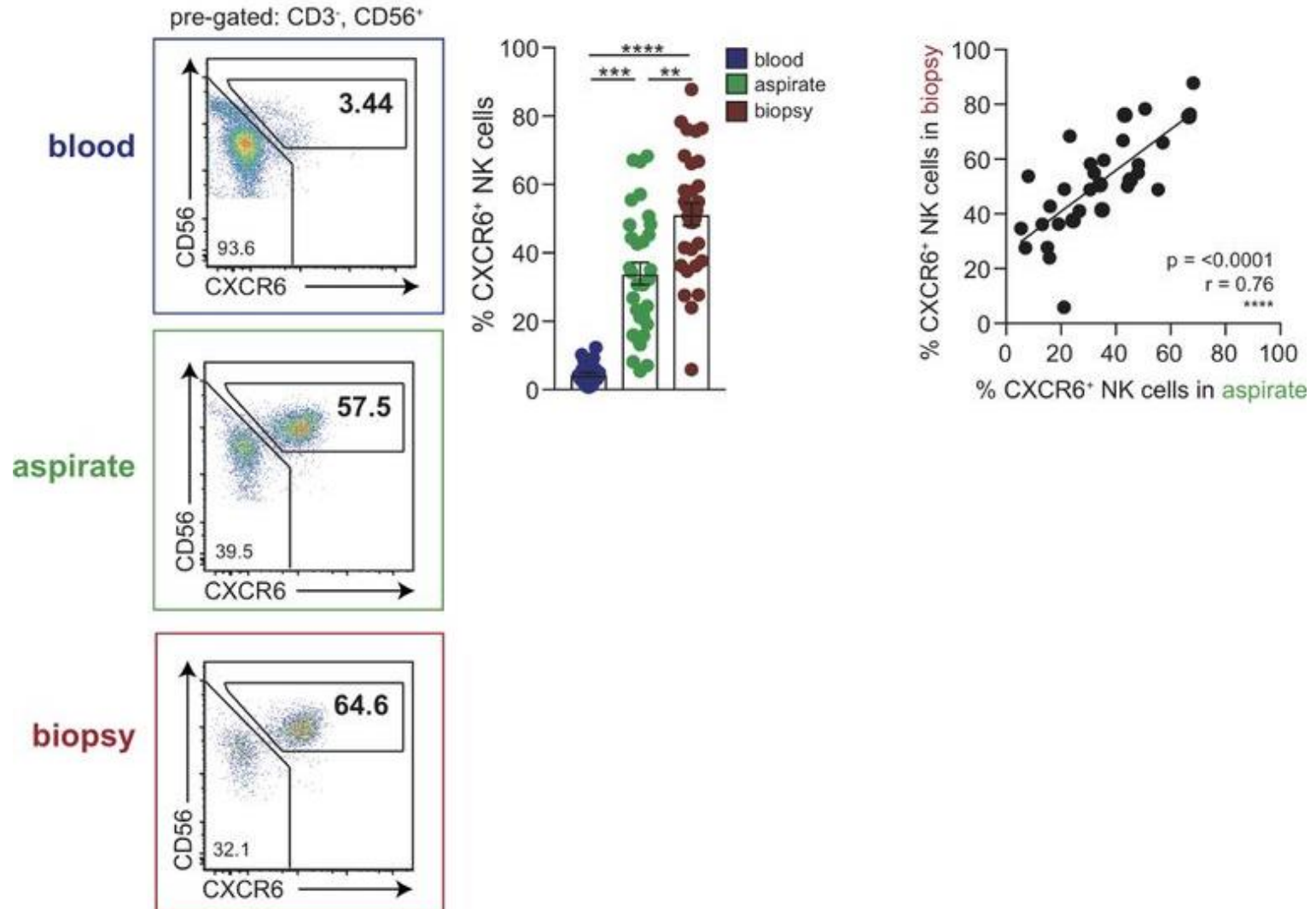
Biopsy



FNA

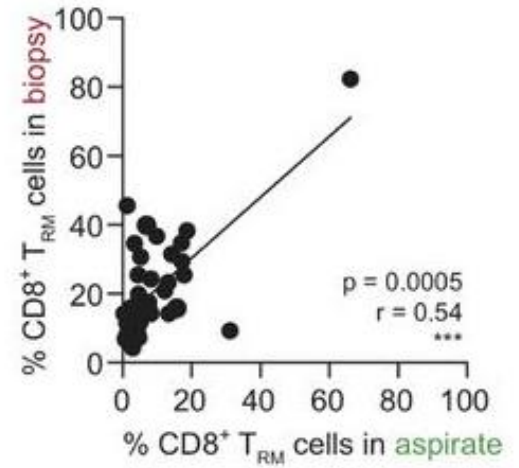
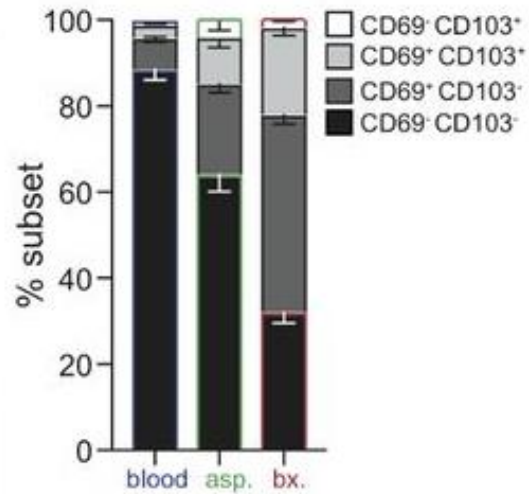
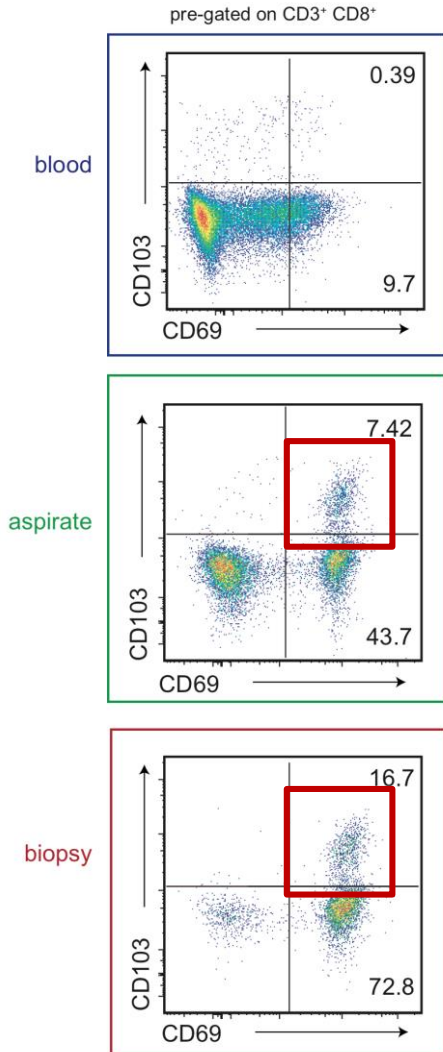


# FNA sample liver-resident NK cells



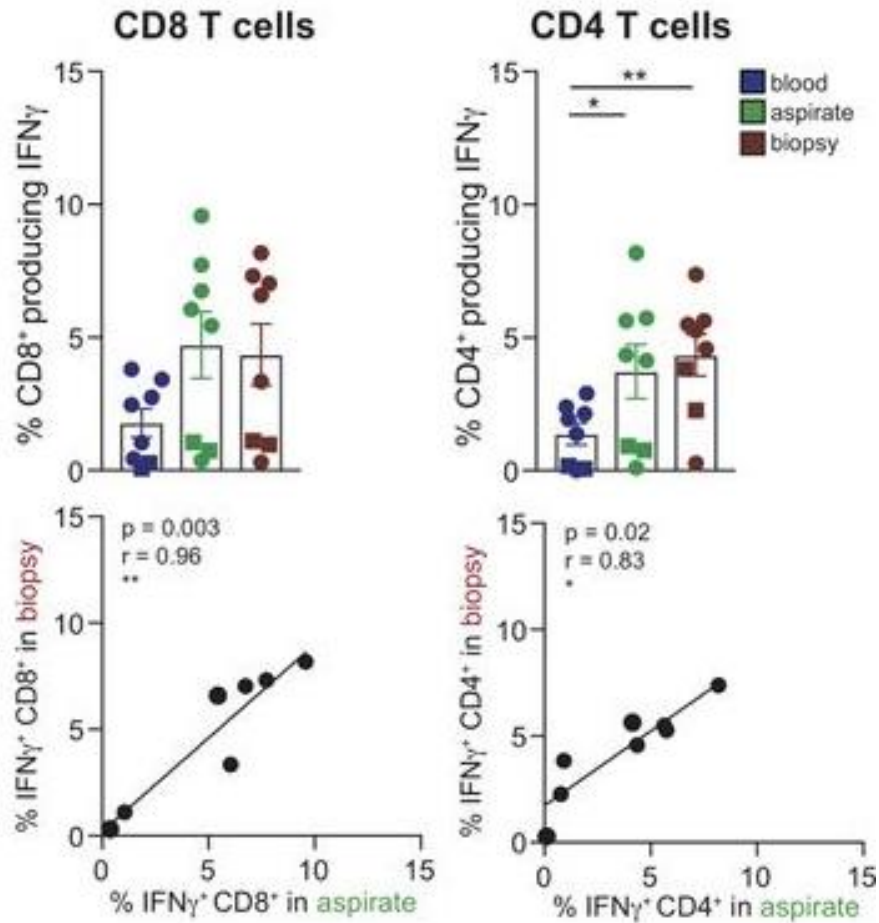
# FNA sample liver-resident CD8 T cells

## Liver-resident CD69+CD103+ T cells



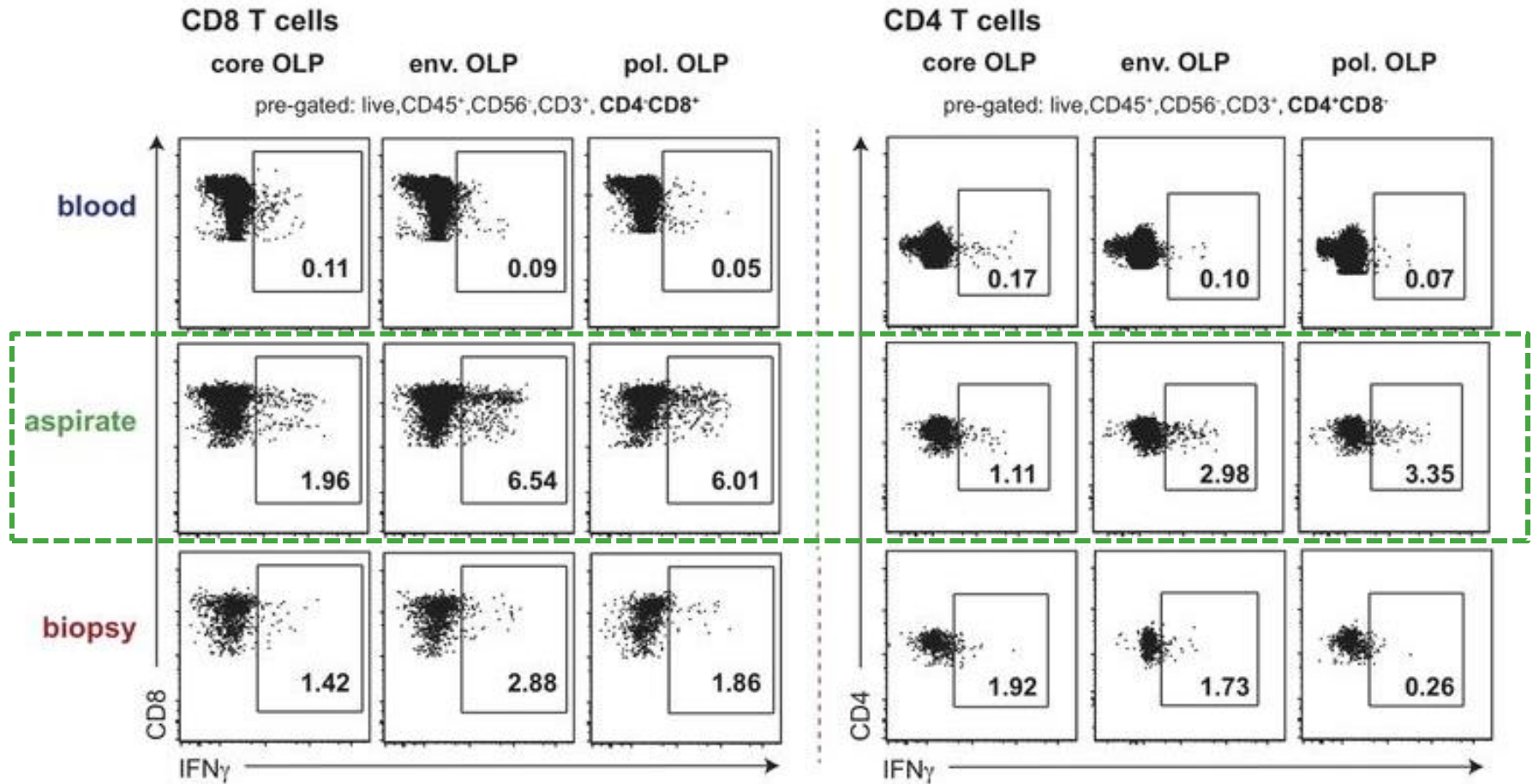
# FNA sample functional HBV-specific T cells

ICS for IFN- $\gamma$  with OLP

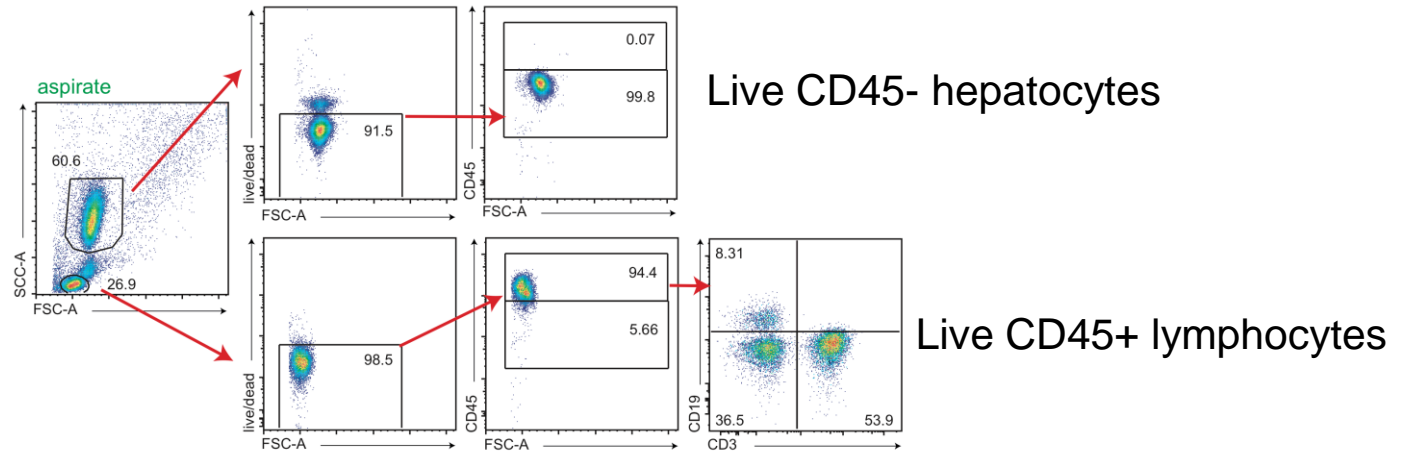


# *FNA can dissect the specificity of intrahepatic HBV-specific T cell reconstitution on antivirals*

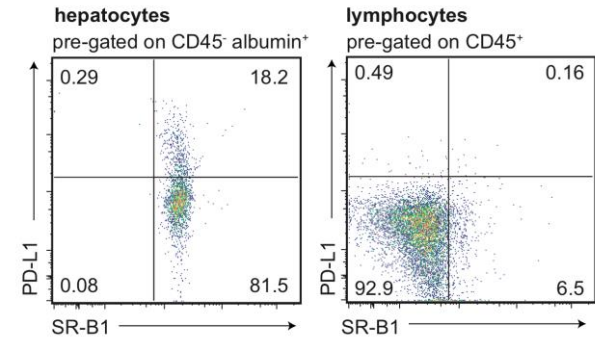
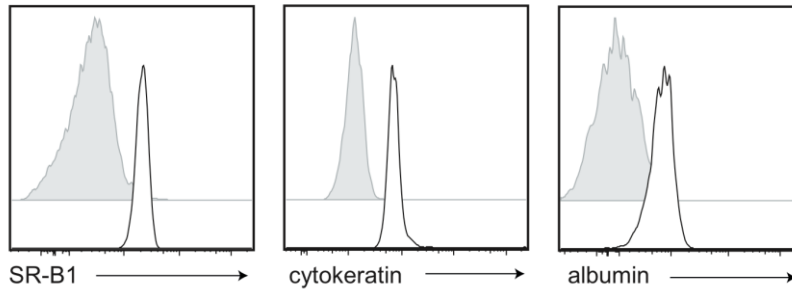
## ICS for IFN- $\gamma$ with OLP



# FNA for simultaneous analysis of lymphocytes and hepatocytes



small FSC, CD45<sup>+</sup> lymphocytes  
 large FSC, CD45<sup>-</sup> hepatocytes



**PD-L1+**  
hepatocytes

# ***Fine needle aspirates for HBV functional cure trials***

## ***Fine needle aspirates:***

- *sufficient cells for 3 flow cytometry panels (16-30 parameters each)*
- *simultaneous analysis of leukocytes & hepatocytes*  
*e.g. PD1 T cells /PD-L1 hepatocytes; HBV-specific T cells/HBsAg+hepatocytes*
- *proportional representation of all intrahepatic leukocytes including MAITs, myeloid cells, DCs*
- *sample tissue-resident T and NK cells*
- *enriched for functional HBV-specific T cells*
- *can be scored for "liver-like" sampling for standardization*
- *well-tolerated for repeated sampling*

*Gill et al, Gut Apr 2018*

*Gill, Pallett et al, Gut Nov 2018*

# Acknowledgements

*Division of Infection and Immunity, UCL*

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