Is there a role for tissue sampling and machine learning in studies of novel HBV therapies?

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Disclosures

- Research: Abbvie, Eiger, Enanta, Gilead, Janssen, Roche, Wako/Fujifilm

- Consulting: Abbvie, Antios, Arbutus, Enanta, Finch, Gilead, GSK

Outline

- **Tissue sampling**
  - Core biopsies
  - Fine needle aspiration biopsy (FNAB)
- **Machine Learning**
  - Where it's been used
  - How it could be useful

Liver biopsy

1. Confirm/discover mechanism(s) of action
2. Assess adequacy of target engagement/MOA
3. Assessment of cccDNA and/or integrated HBV DNA
4. ALT flares
   - Distinguishing the ‘good’ from the ‘bad’

Not about pathology (at least mostly)

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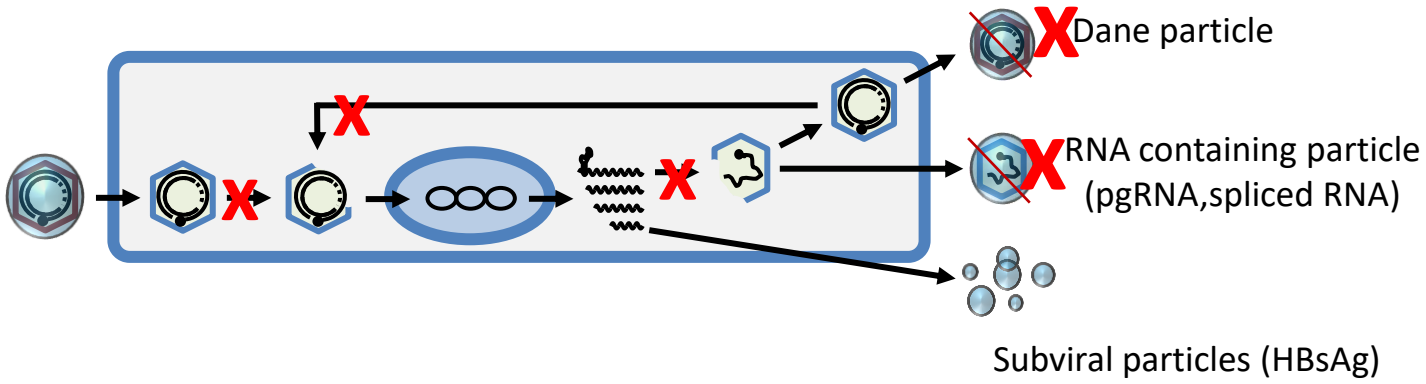
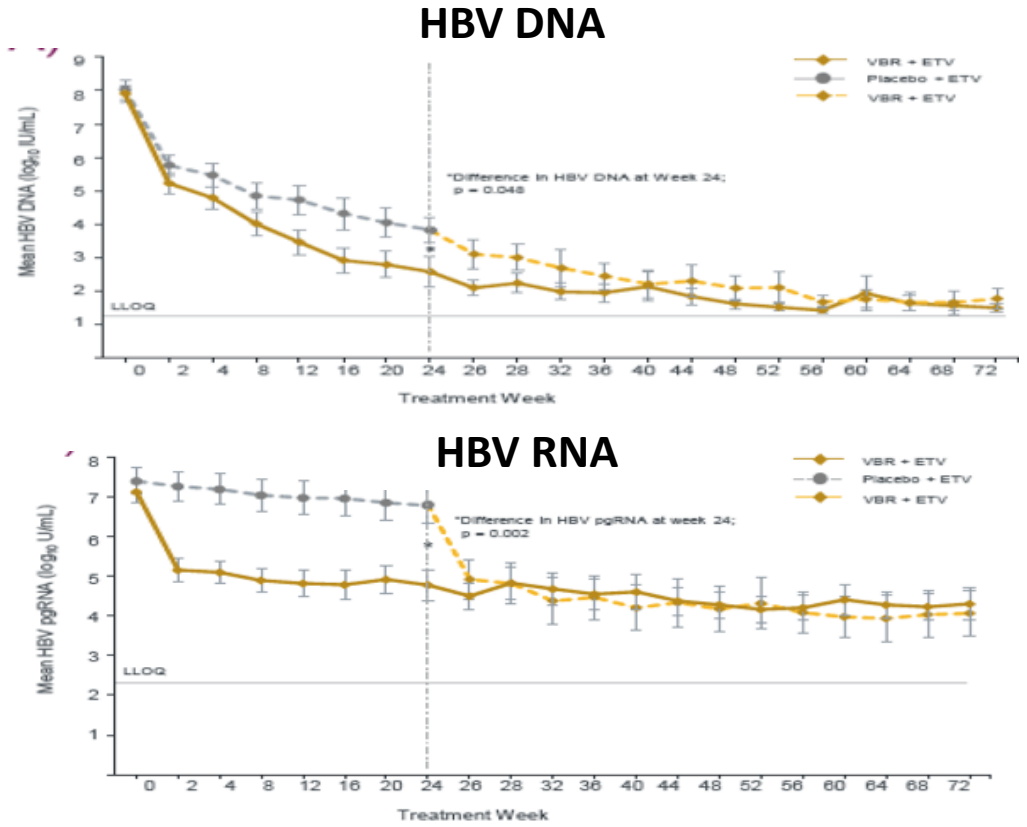
Capsid Assembly Modulators (CAMs)

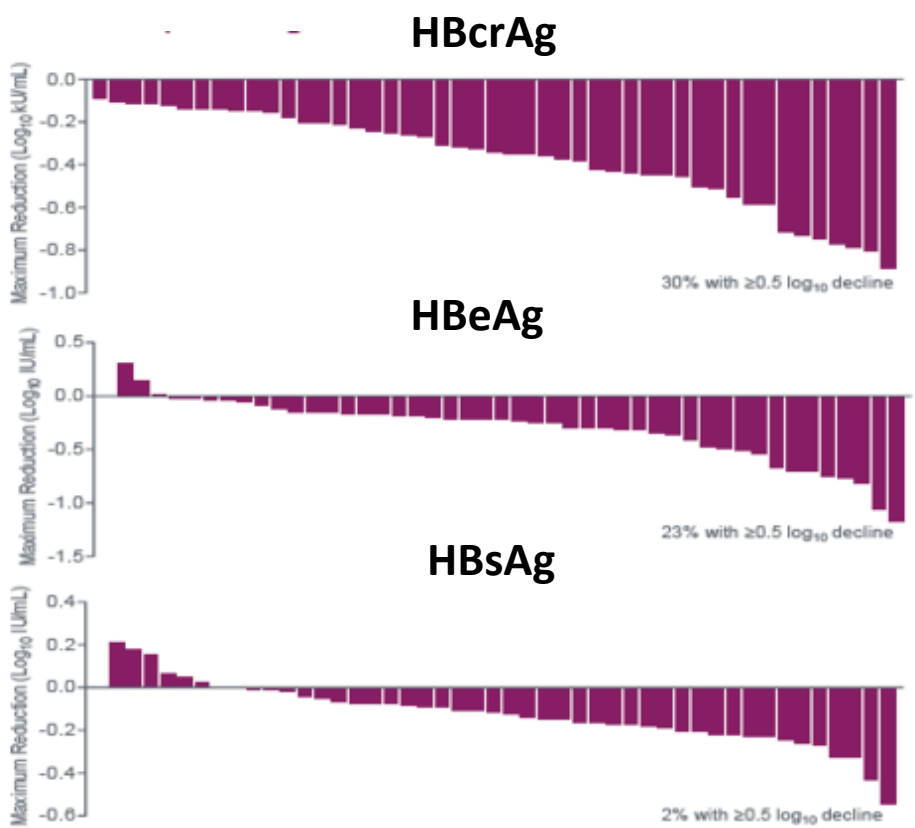


- Clarifying the mechanism(s) of action
  1. **Prevent encapsidation → well shown with HBV RNA decline**
  2. **Prevent formation/replenishment of cccDNA → *harder to demonstrate…but arguably more important***

Capsid Assembly Modulators (CAMs)

Vebicorvir (731) + ETV PO OD in Treatment naïve or nuc-suppressed non-cirrhotic HBeAg+ CHB

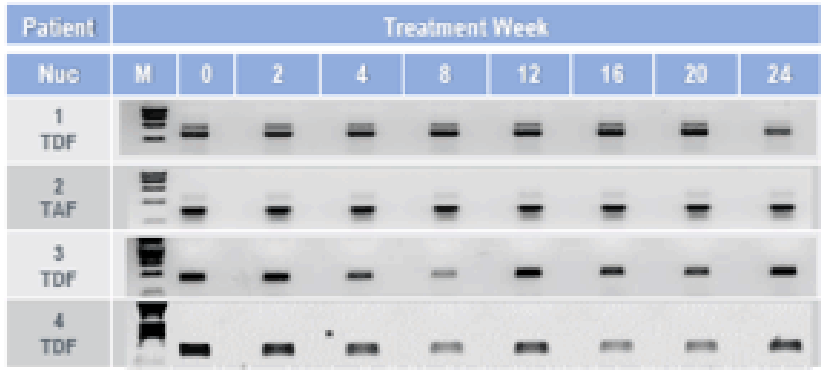




- Clearly shows inhibition of encapsidation…***deeper block of replication than NA alone***
- But very limited decline in antigen levels…is it really affecting cccDNA?

‘Shutting off replication’

Nuc Monotherapy



Residual viremia not eliminated by Nuc

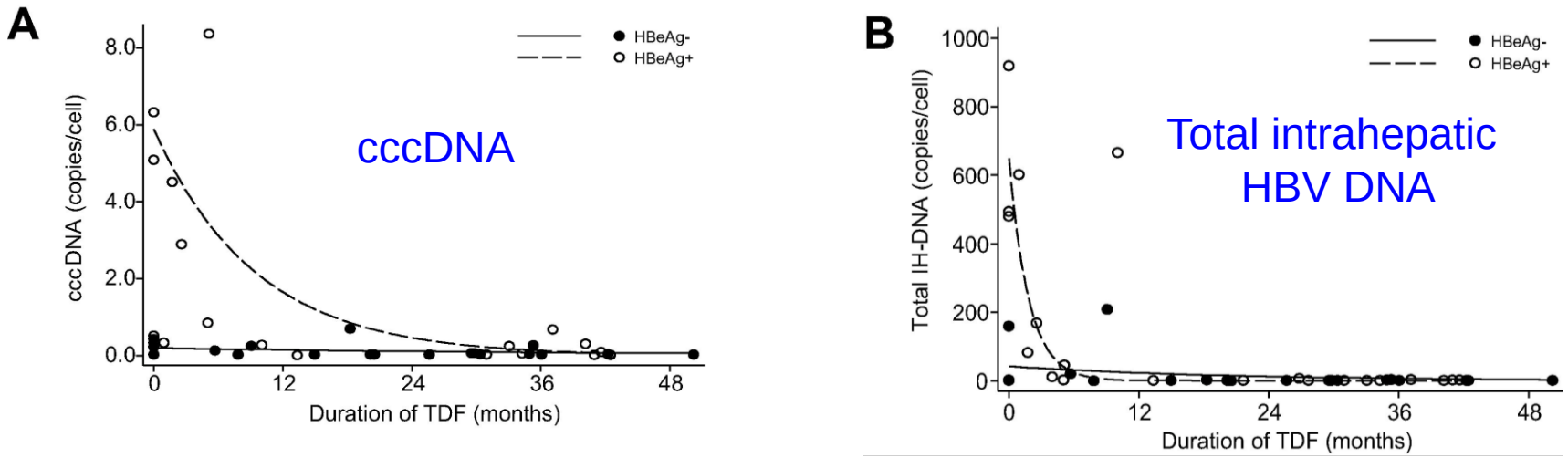
731 Combo Therapy

Residual viremia decline below detection (2-5 IU/mL)

- Low level replication despite NA
- Add CAM and lower level of replication…block 100%? Unclear…
- ***Need to go to the liver to see if there is still ongoing replication…***

The ‘leak’

Intrahepatic HBV DNA during long-term TDF therapy in HIV/HBV co-infection



- Very slow decline and persistence of cccDNA long-term + detectable intrahepatic non-cccDNA support ***ongoing replication*** despite ‘complete suppression’ **ie the leak!**
- cccDNA replenishment - re-circulation + de novo infection
- ***Need this study done with patients on CAMs + NA***

Boyd J Hep 2016

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Liver biopsy

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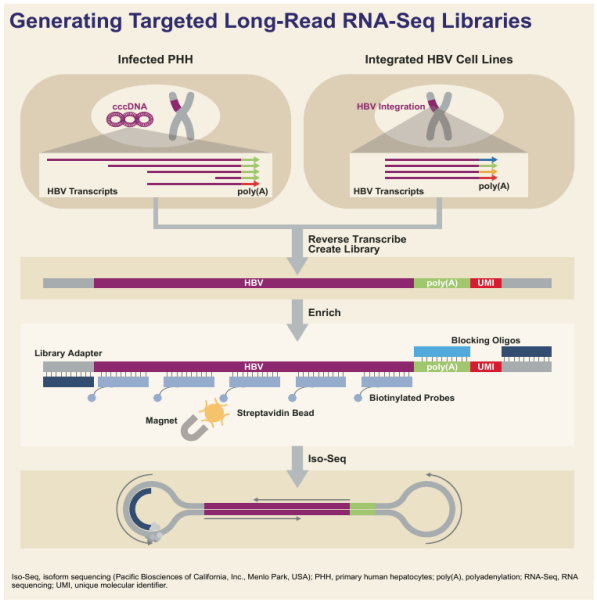
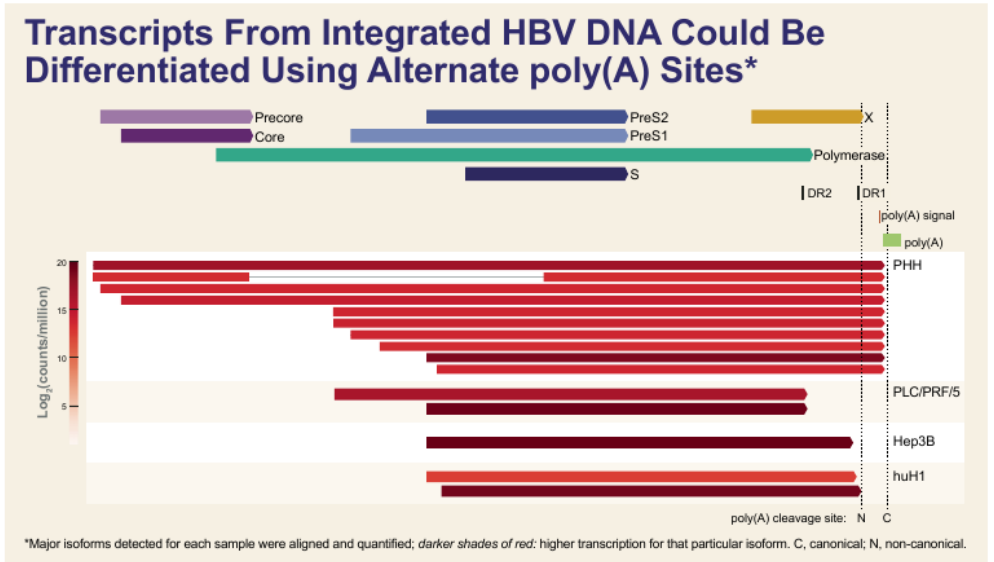
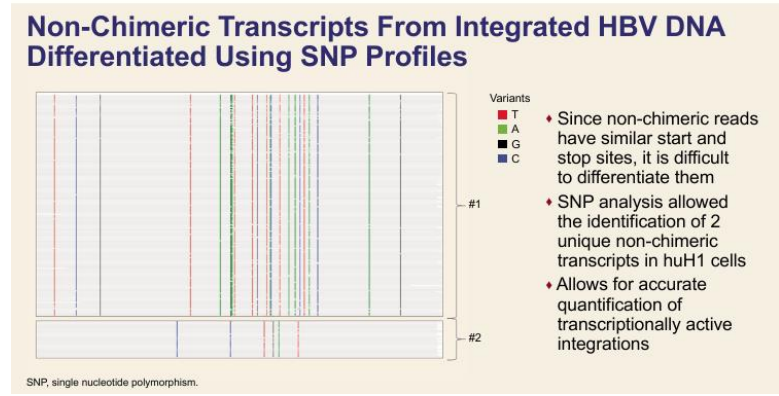
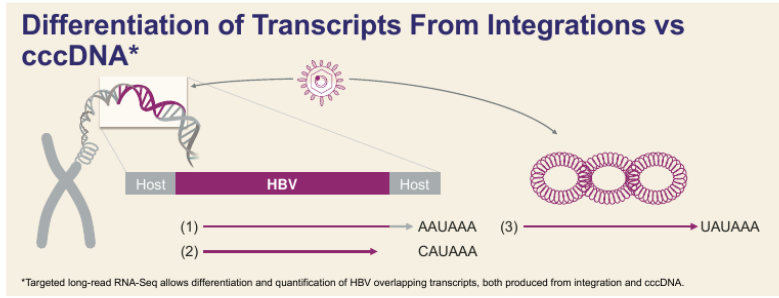
An important question: Integrated vs cccDNA-derived HBsAg



- We combined target enrichment for HBV with Iso-Seq to generate high-coverage, long-read sequencing data consisting of full-length HBV transcripts
- We applied this method to infected PHHs and HBV hepatocellular carcinoma integrated cell lines (Hep3B, huH1, and PLC/PRF/5)







- HBV transcript types found:
  - AAUAAA (host) and CAUAAA (non-canonical) poly(A) sites found on integrated transcripts
  - UAUAAA (canonical) poly(A) site found on cccDNA transcripts

- Long read HBV RNA transcripts in **PHH and integrated cell lines**
- **Integrated** – some chimeric ie up to 1000 bp host…easy
- But non-chimeric also can be identified as from integrated DNA…
  - From **cccDNA 3' – canonical UAUAAA poly(A) tail**
  - From **integrated DNA – AAUAAA (host) and CAUAAA non-canonical non-host**

Might allow to identify & quantify cccDNA vs integrated DNA-derived sAg

But need liver tissue – biopsy!

van Buuren et al EASL 2020

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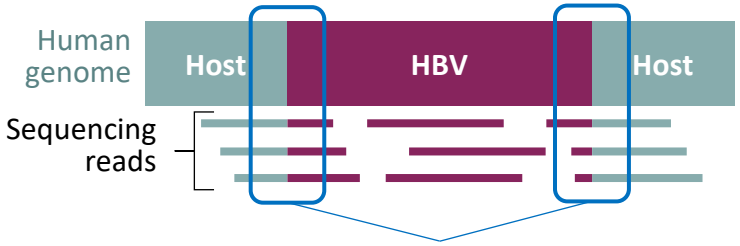
Effect of therapy on integration events

TDF vs placebo x 3 years – paired liver biopsies (n=66) – HBV integrants

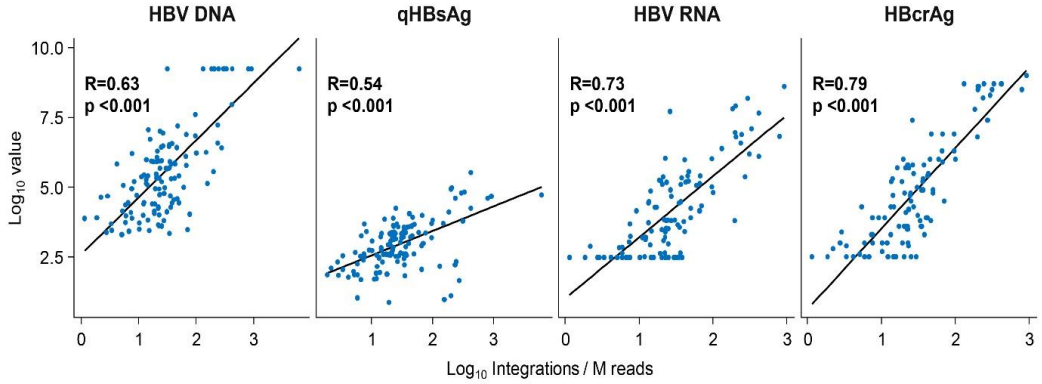
Align reads to human and HBV genomes **Split chimeric reads**



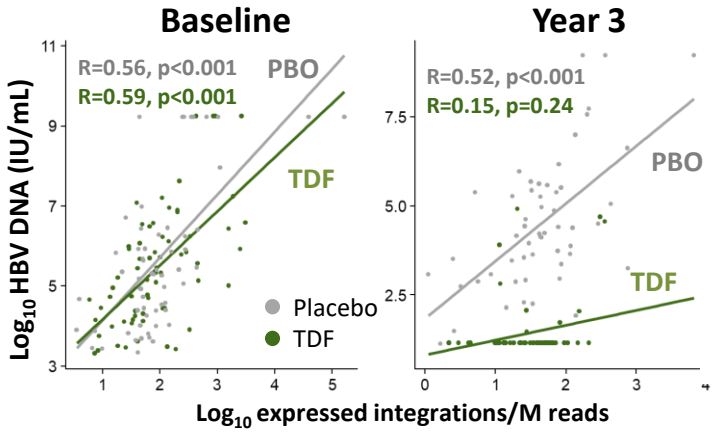
Chimeric reads: Evidence of viral integration

Cluster breakpoints by human and HBV coordinates and strand

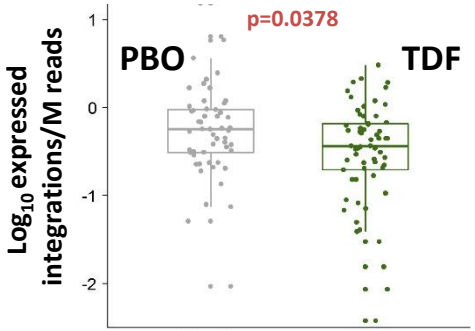
Catalog of expressed integration loci
(units Log_{10} expressed integrations per million reads)



Loss of correlation upon TDF-mediated HBV suppression; reduction of iMr* at Year 3



Fold change in integrations



-0.258 (±0.139) -0.516 (±0.143)

- **Similar result with LAM (different method)**
- ***Useful data for other agents…only with biopsies***

Hsu Y-C, et al. AASLD 2020. Oral #16, Chow AASLD 2020 Oral 22

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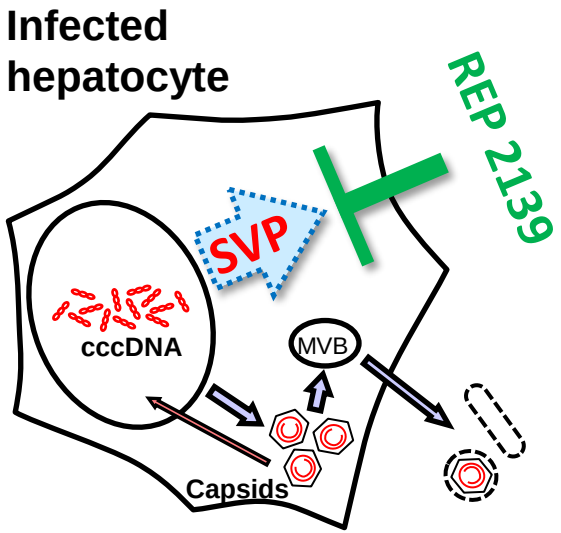
Liver biopsy

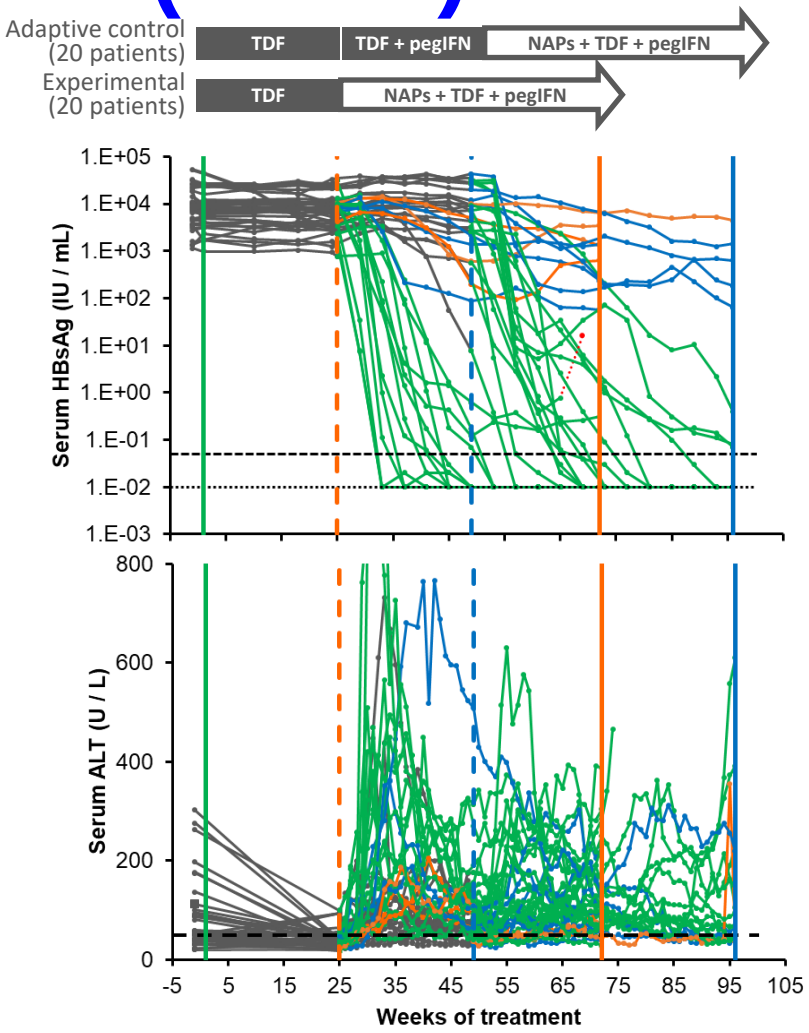
1. Confirm/discover mechanism(s) of action
2. Assess adequacy of target engagement/MOA
3. Assessment of cccDNA and/or integrated HBV DNA
4. **ALT flares**
   - **Distinguishing the 'good' from the 'bad'**

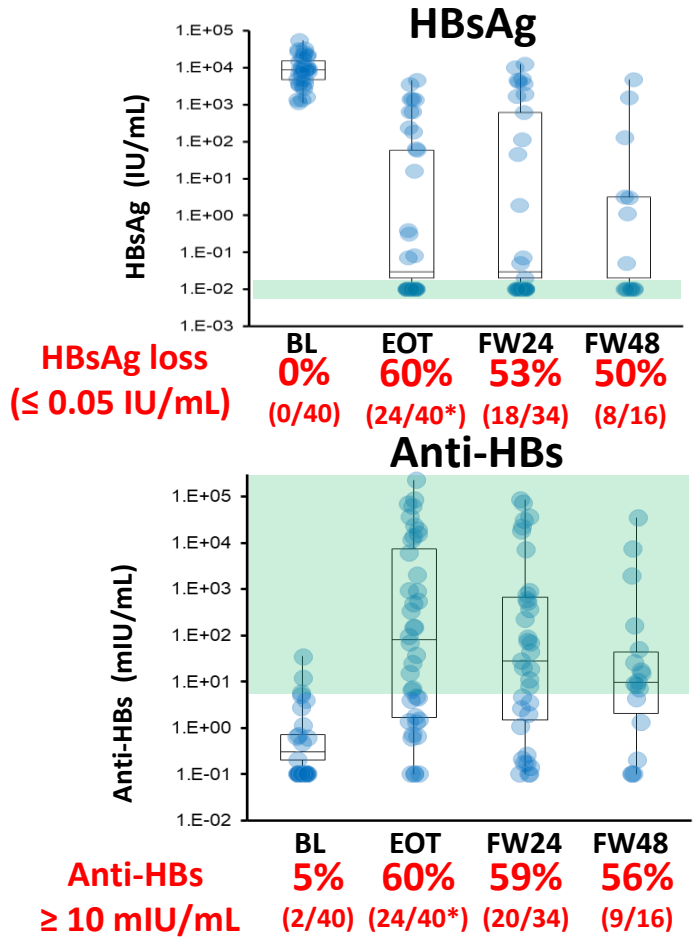
Not about pathology (at least mostly)

Nucleic Acid Polymers (NAPs)

- NAPs block assembly/release of **subviral particles**
- Aim to restore immune response → viral control







- ALT flares coincident with HBsAg decline → **immune restoration leading to flare and control?**
- Maybe…**need to prove it** with immunological studies → **in the blood (PBMCs), in the liver (FNAB)**

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Liver Fine-needle Aspirates (FNA) for Longitudinal Liver Sampling

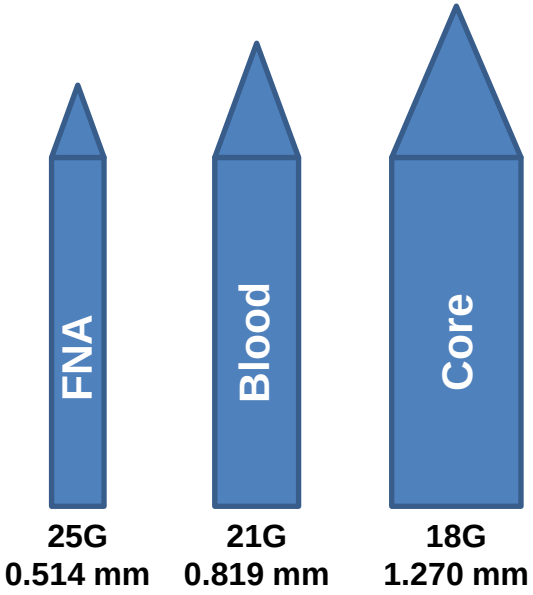
Regular sampling permitted by needle size

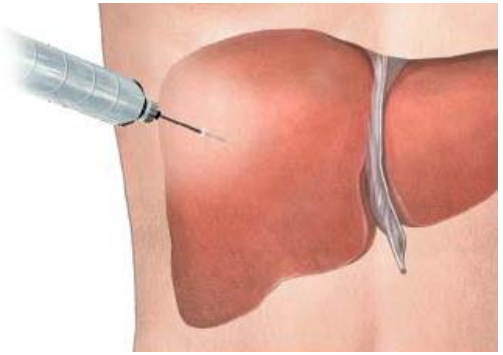
Smaller than blood draw needle

- Minimal risk
- Minimal pain
- Minimal risk of bleeding

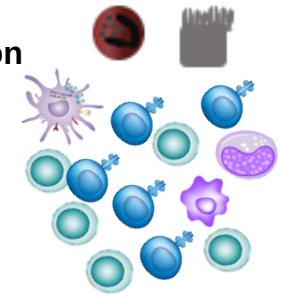
Caveats

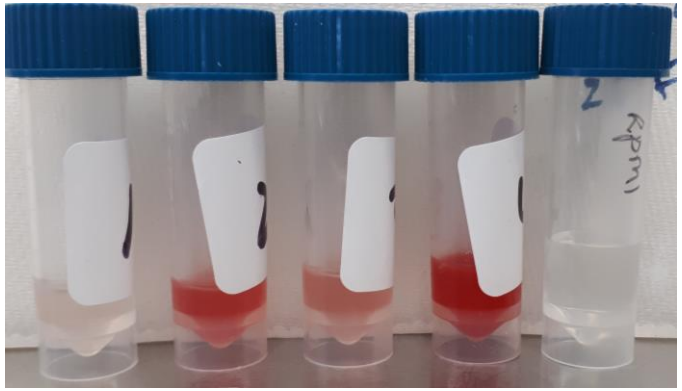
- Low cell numbers
- No liver architecture
- Hepatocyte recovery variable
- Blood contamination





Single cell suspension
50 k cells
90% Leukocytes
5-10% hepatocytes





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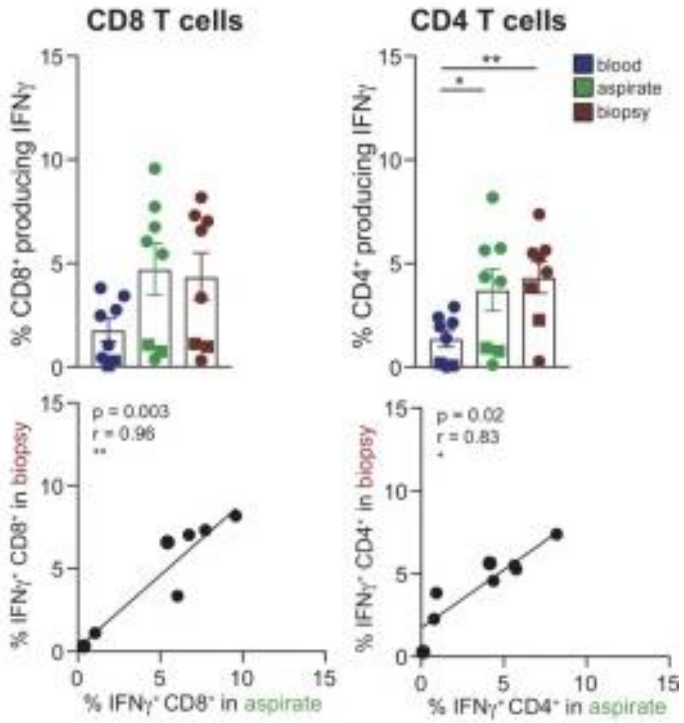
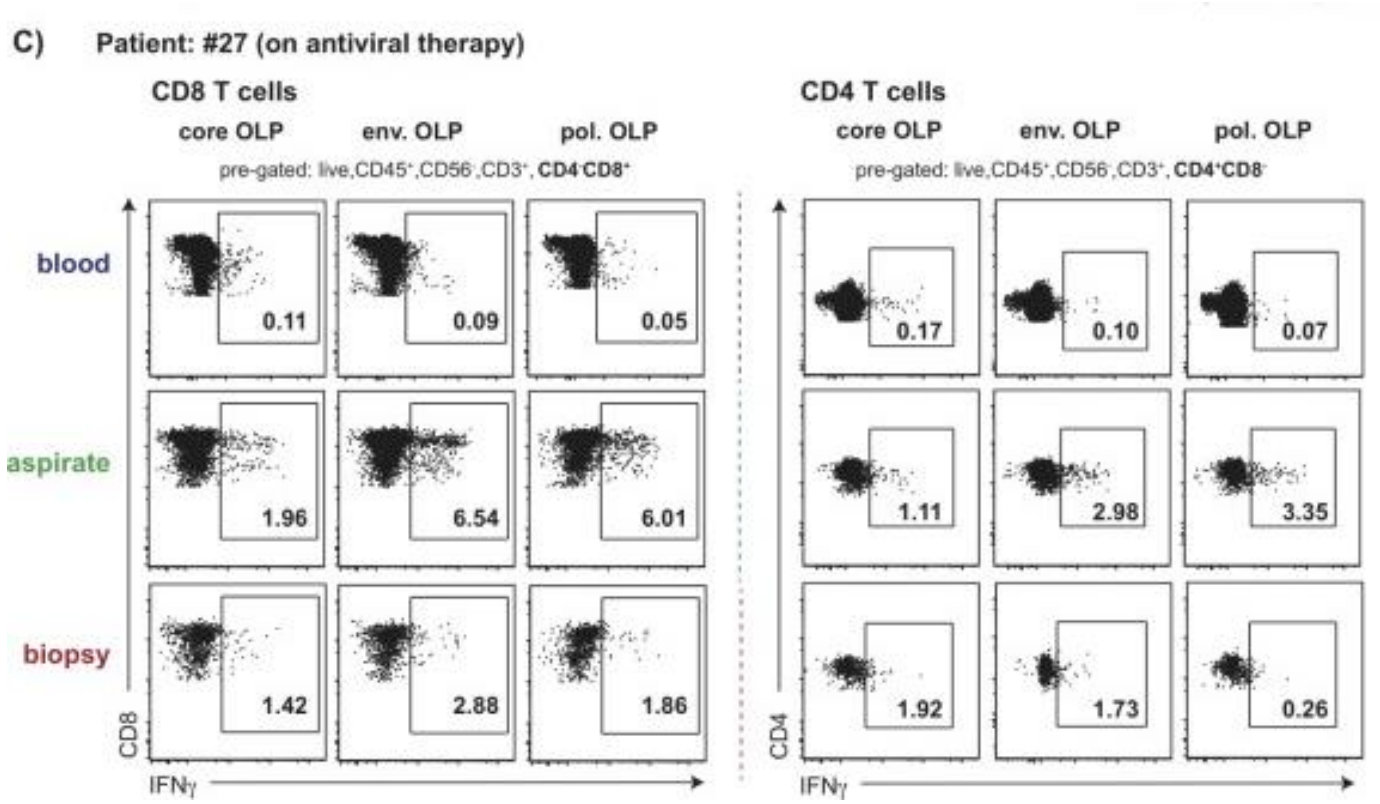
FNAB representative of core biopsy





Similar immune cell populations with ability to assess frequency & function

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Utility of FNAB

Advantages

- Safe & non-invasive (relatively)…serial sampling
- Excellent sampling of intrahepatic immune compartment

Limitations

- Limited number and quality of hepatocytes…getting better
  - No hepatic architecture
  - Infected vs uninfected cells…possible but more challenging
  - Are the hepatocytes you aspirate representative?

Still useful for same things as core biopsy but with some caveats

1. Confirm/discover mechanism(s) of action
2. Assess adequacy of target engagement/MOA
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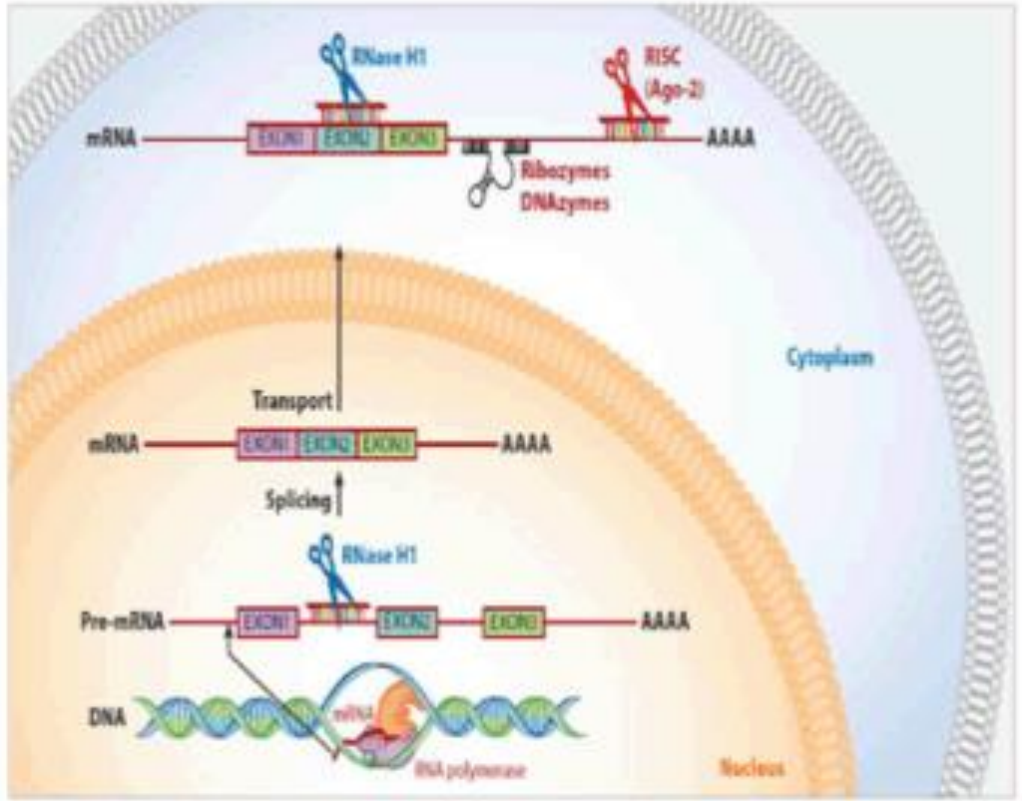
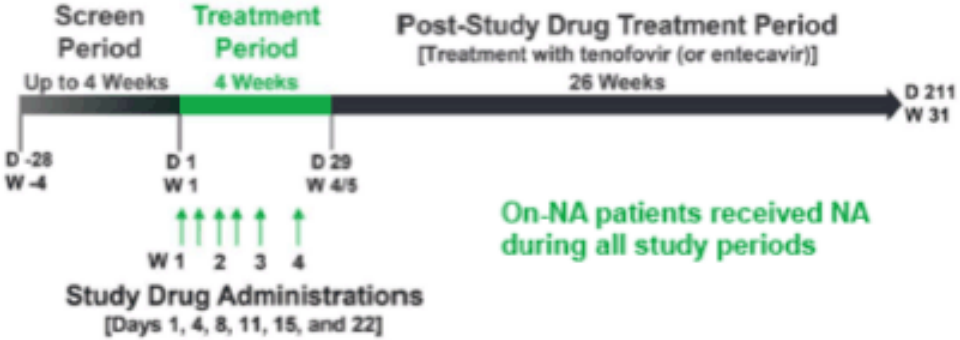
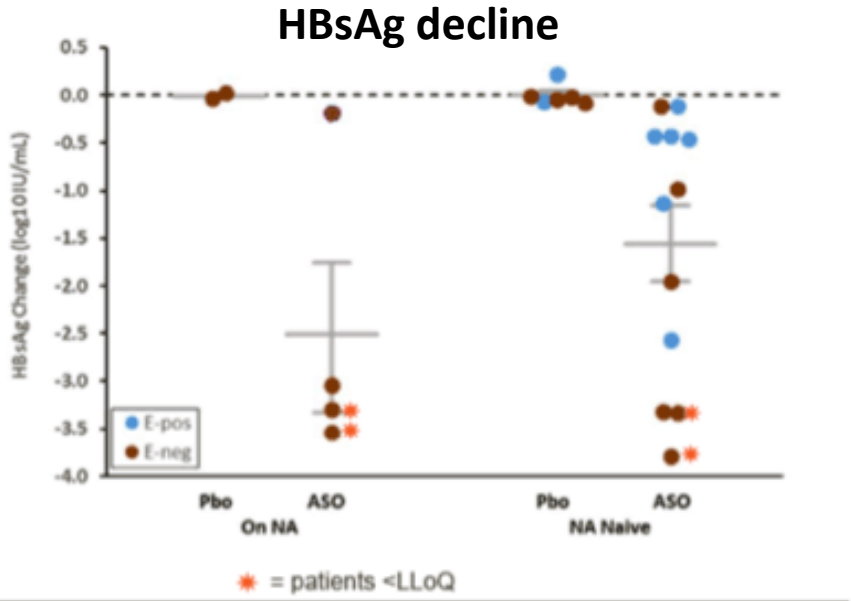
Antisense oligonucleotide – GSK 836

- Similar concept to RNAi
- ASO binds HBV RNA species and degraded by RNase H rather than Ago







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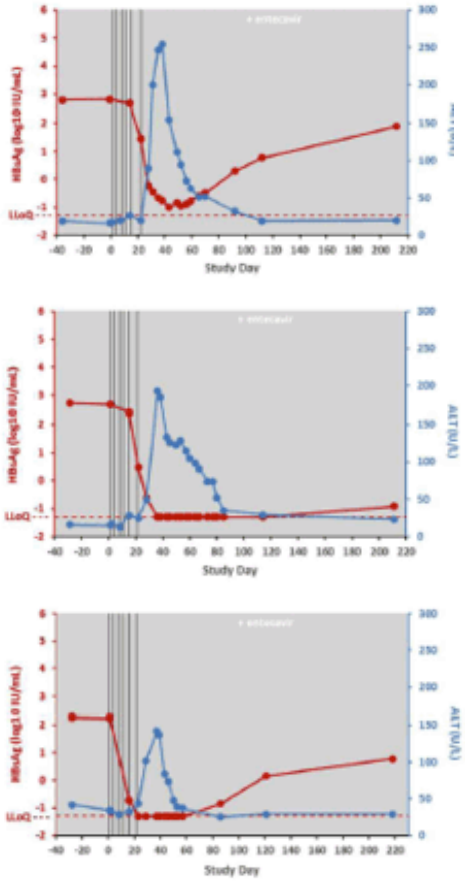
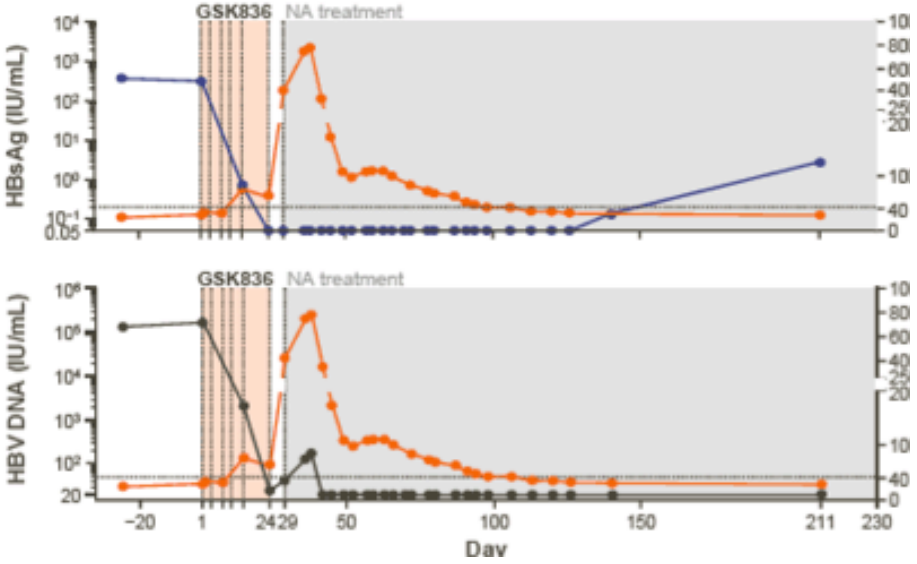
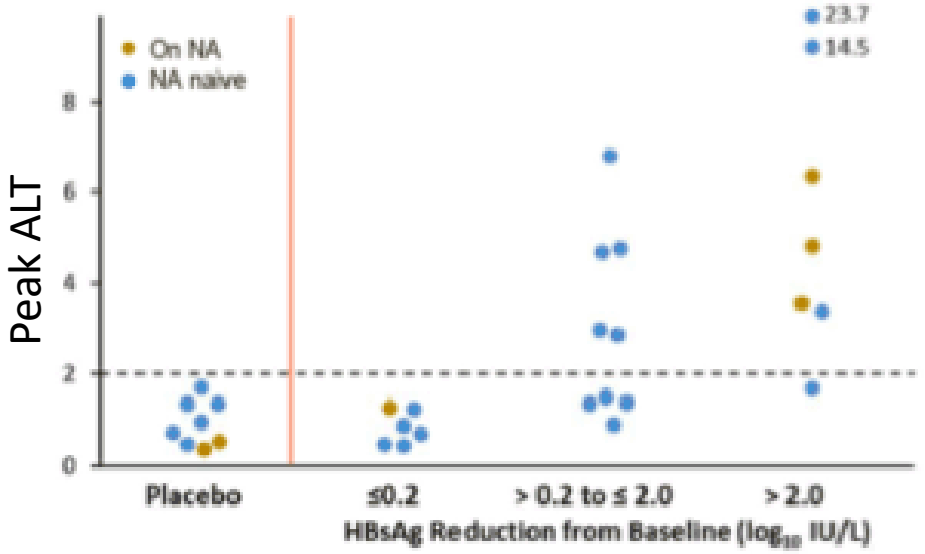
HBsAg decline associated with ALT flares

Nuc suppressed patients



Nuc-naïve patients



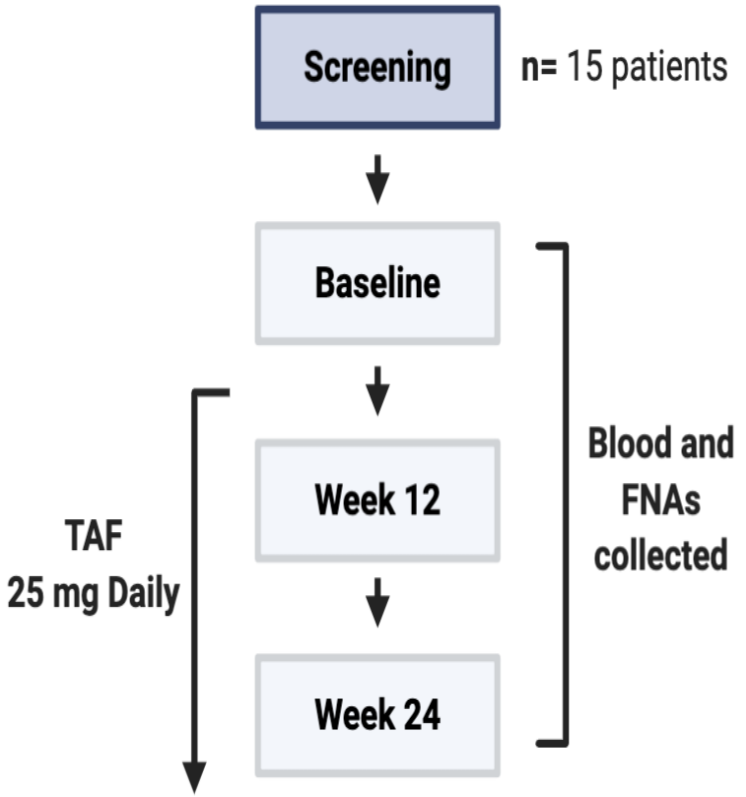


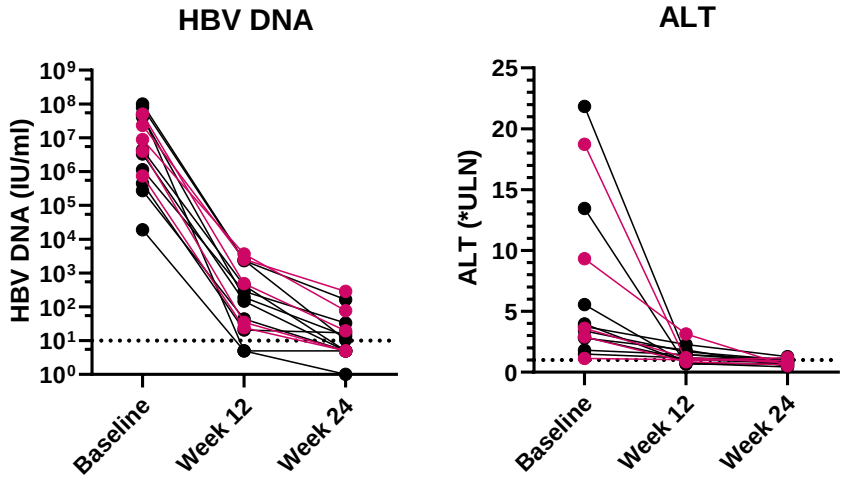
HBsAg reduction

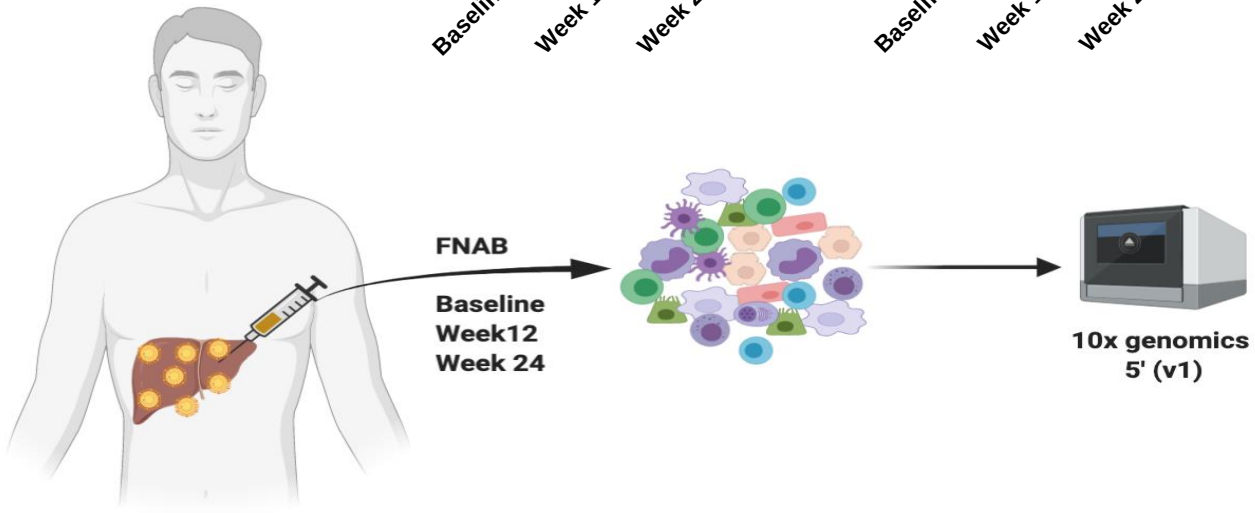
- What is the mechanism?
- Are these immune restoration, toxicity or something else entirely?
- Non-GalNac targeting more effective – active in non-parenchymal cells?
- *Serial FNAB may be able to answer these and other questions…*

Longitudinal Clinical Study to Investigate Changes in Intrahepatic Immune Activation

Hepatitis B patients with liver inflammation







Gehring *In Preparation*

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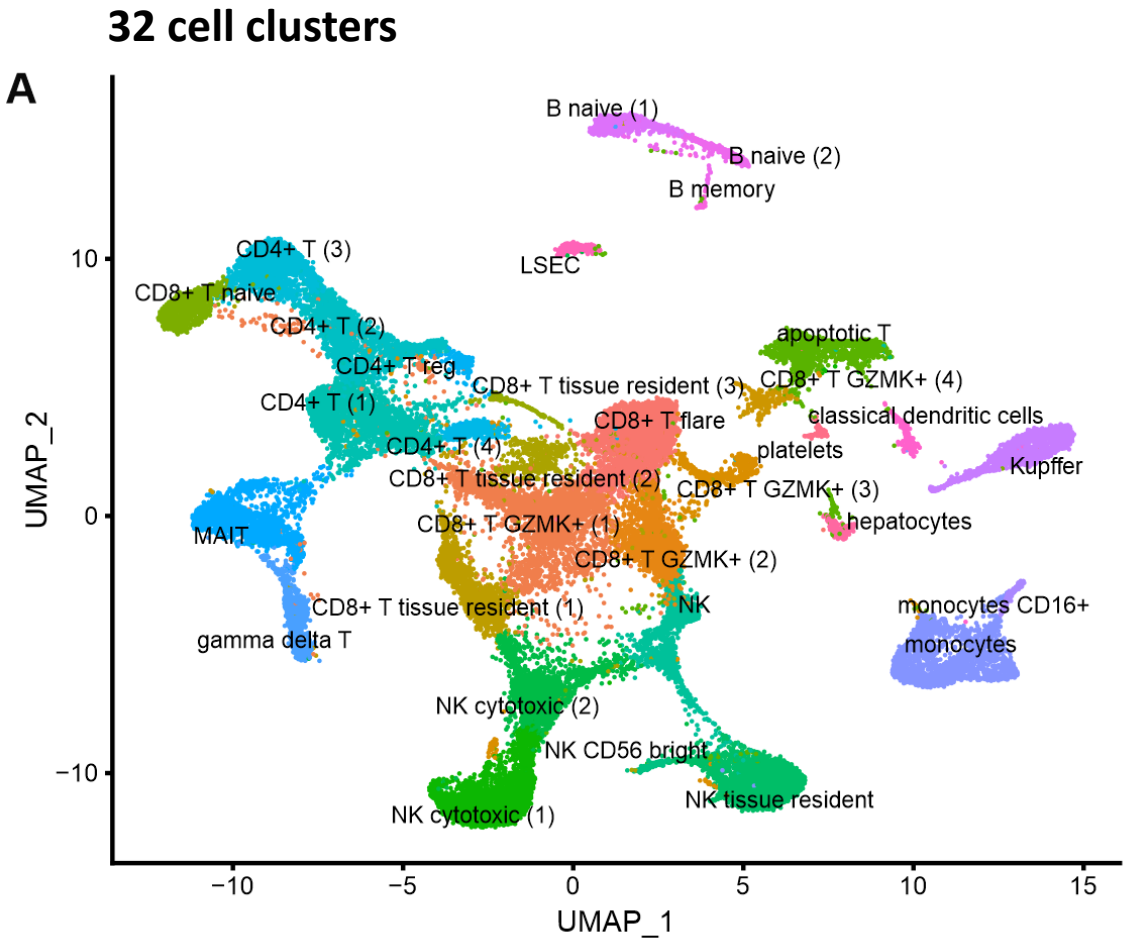
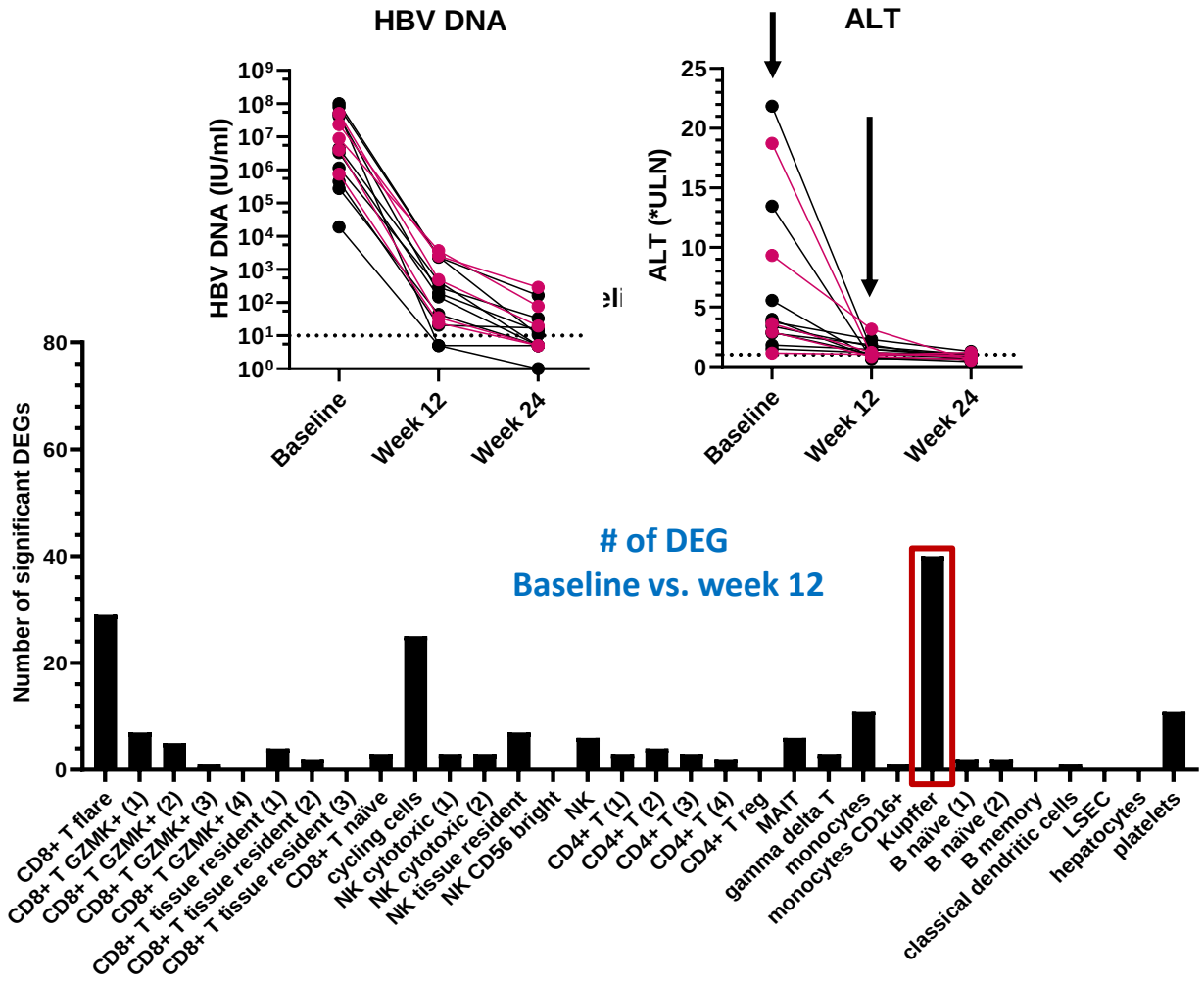
The power of scRNAseq: cell-type specific changes to understand pathogenesis





Gehring *In Preparation*

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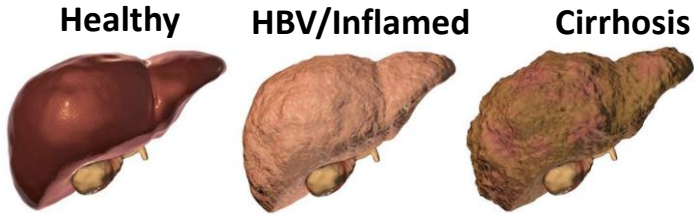
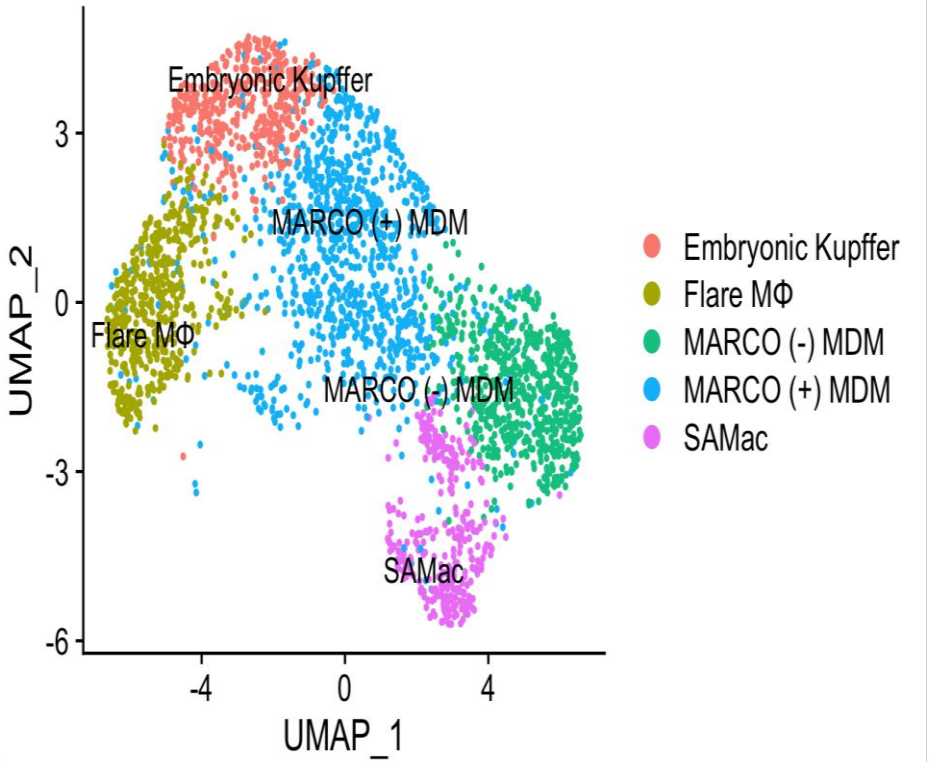
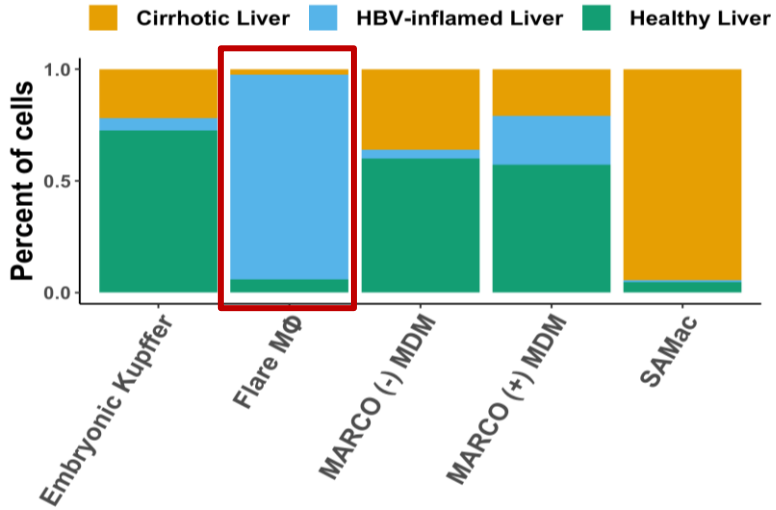
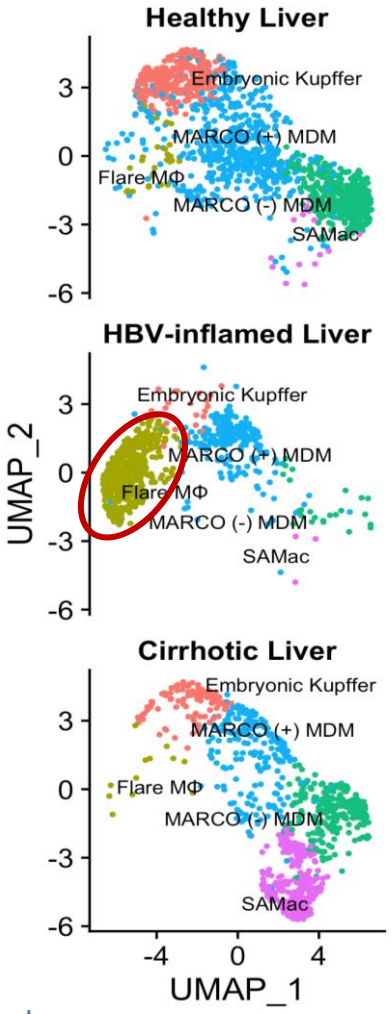
Population of Unique Inflammatory Macrophages in the Inflamed liver

Macrophage composition between the HBV infected, healthy and cirrhotic livers









Gehring *In Preparation*

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Machine Learning

- Applying iterative unbiased processes to learn from data to improve prediction models
- Multiple approaches – with pros and cons relevant to data type or question asked
- Utilities:
  - **Risk prediction** – outcomes, treatment response, biomarker discovery
  - **Pathogenesis** – identify unrecognized ‘connections’/relevant factors, interactions

Increasingly being evaluated in hepatology

HEPATOLOGY

AASLD

REVIEWS | HEPATOLOGY, VOL. 71, NO. 3, 2020

Applying Machine Learning in Liver Disease and Transplantation: A Comprehensive Review

Ashley Spann [1], Angeline Yasodhara,[2] Justin Kang,[3] Kymberly Watt,[4] Bo Wang,[2] Anna Goldenberg,[2] and Mamatha Bhat[3,5]

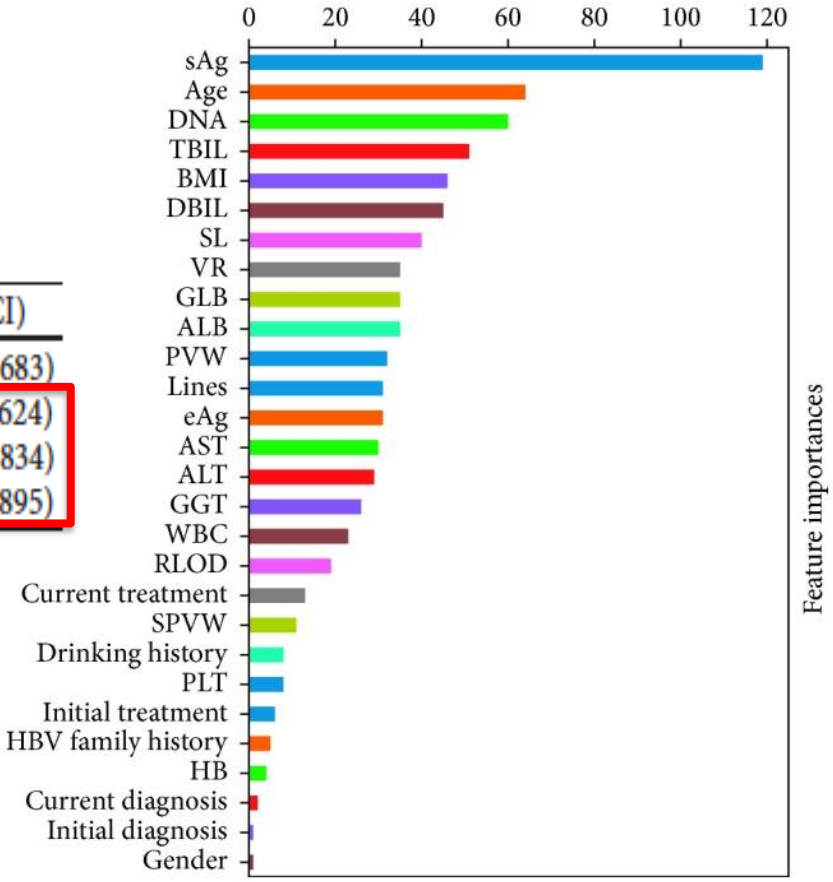
- Fibrosis prediction
- HCC prediction
- Treatment response
- Clinical outcomes – graft/patient survival

Helpful but we were actually not so bad

2,235 Chinese patients CHB → 106 with HBsAg loss

Table 3: Summary of predictive performance of each model.

Model	TP	FN	TN	FP	Precision	Sensitivity	*F*-score	AUC (95% CI)
Logistic regression	0	35	636	0	1.00	0.95	0.97	0.680 (0.677, 0.683)
Decision tree	4	31	627	9	0.97	0.94	0.95	0.619 (0.614, 0.624)
Random forest	4	31	635	1	0.99	0.95	0.97	0.829 (0.824, 0.834)
Extreme gradient boosting	9	26	632	4	0.98	0.96	0.97	0.891 (0.889, 0.895)



- Most important factors previously recognized
- But potentially other 'novel things to explore'

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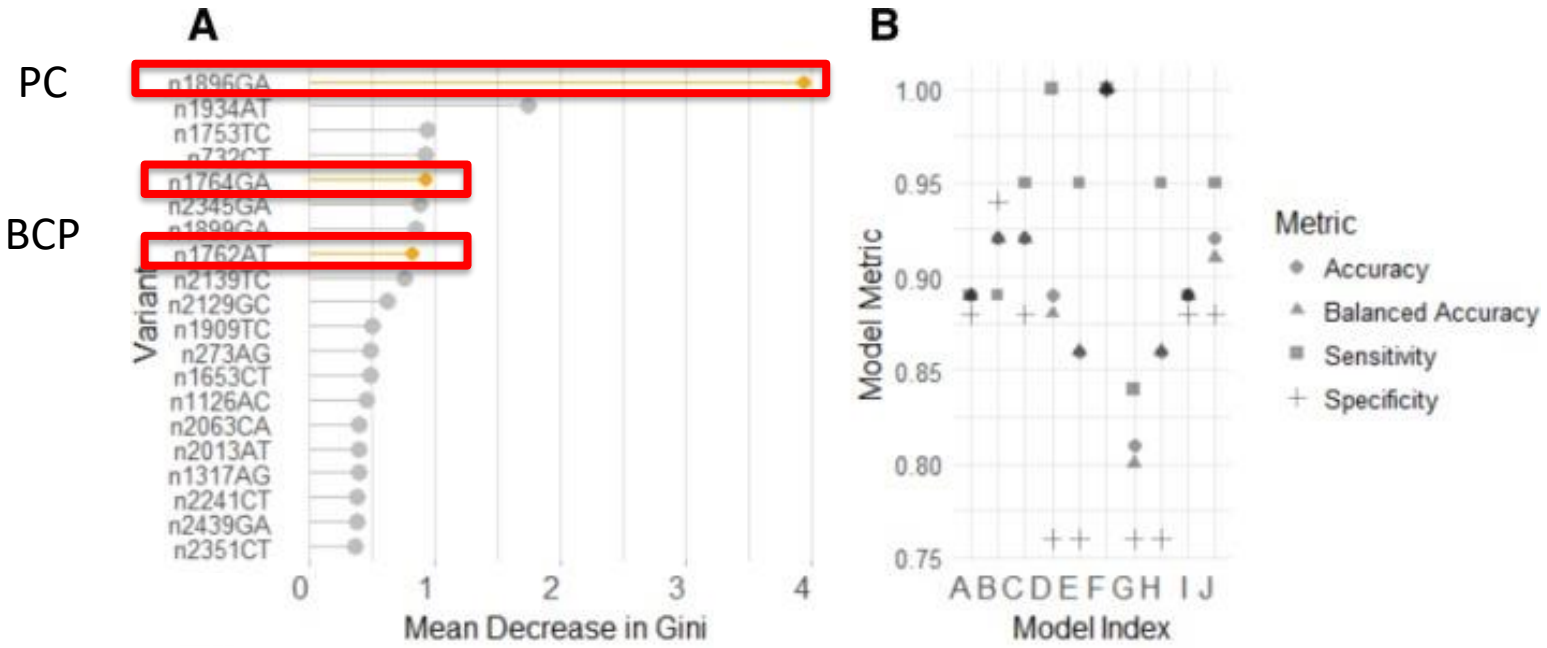
Sequencing data

Untreated HBV – predictors of HBeAg seroconversion in 182 European CHB & 207 Chinese using deep sequencing



- Most relevant variants associated with HBeAg loss → **PC & BCP**
- But also discovered related variants not previously recognized

How can machine learning be used with new HBV therapies?

- **Endpoints**
  - Combining factors may more accurately predict outcomes
- **Response prediction**
  - Many biomarkers with lots of inter-relatedness…what is most important?
  - Possibly using info from liver biopsy!
- **Mechanism(s) of action**
  - CIBERSORT and similar approaches to big data from scRNAseq or other big data → uncover new targets and novel mechanisms of action
  - Requires tissue!
  - With this in mind…may guide rationale and possibly unexpected combinations

- Early days, to date of limited true utility…
- But could be useful down the road…lots of potential

Summary – is there a role for tissue sampling & machine learning in studies of novel HBV therapies

- Yes, and very possibly
- Biopsy & FNAB useful tools to understand
  - MOA/adequacy of target engagement
  - Assessment of cccDNA
  - ALT flares
- Small sub-studies can be VERY impactful
- Combined with machine learning – tissue sampling may uncover unexpected clues to pathogenesis and novel therapeutic targets and/or combinations