Expanding Immune Monitoring in HBV Trials
- Part I -

Adam J. Gehring, Ph.D.

Biology Lead
Toronto Centre for Liver Disease
University Health Network (UHN)

Assistant Professor
Department of Immunology
University of Toronto

TORONTO CENTRE FOR LIVER DISEASE

UNIVERSITY OF TORONTO

UHN Toronto General Toronto Western Princess Margaret Toronto Rehab

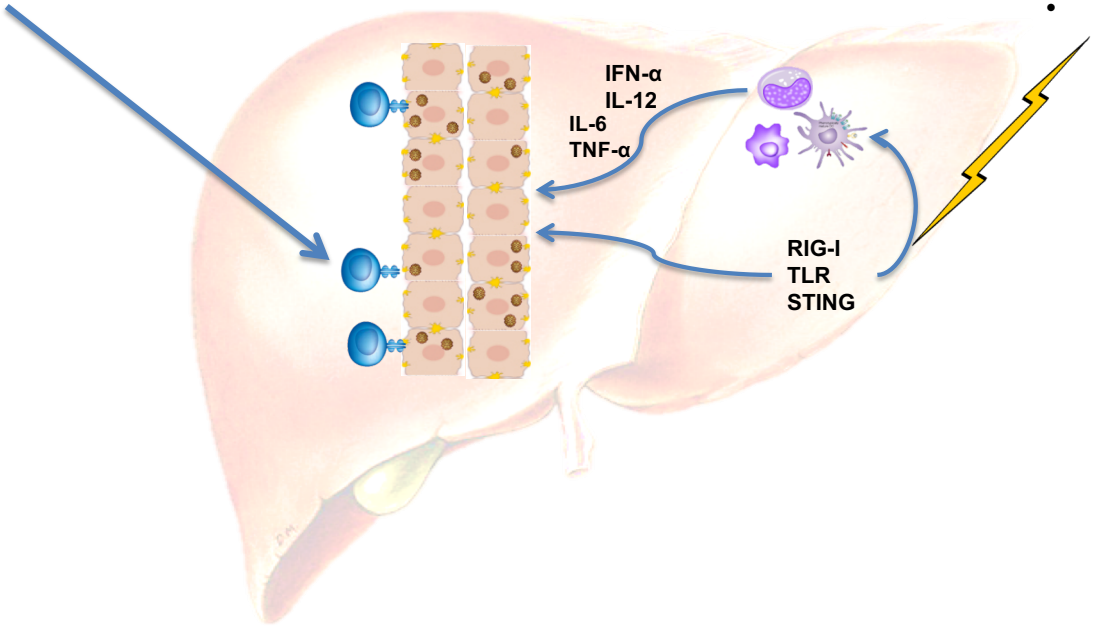
Immunotherapeutic Strategies

Improve T cell activation

- **Vaccination**: Shift Antigen Presentation
- **Checkpoint blockade**: Block inhibitory signals

Innate stimulation

- **Cytokine production**
  - Antiviral
  - T cell co-stimulation
  - alter liver environment



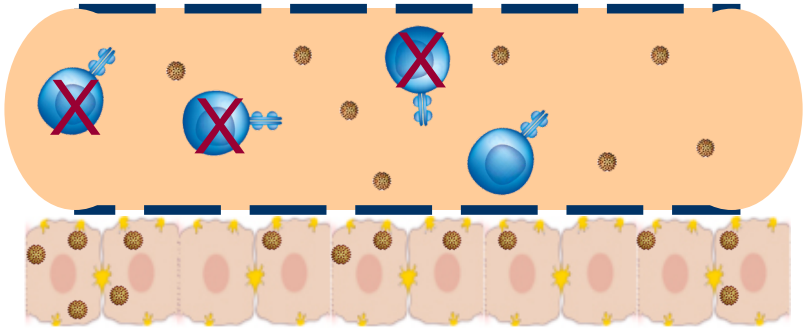
Adaptive Immunotherapy

Mechanisms of T cell dysfunction

1. **HBV-specific T cells are prone to apoptosis**
   a. Lopes, J Clin Invest 2008; 118: 1835–1845.

2. **Co-express inhibitory receptors – PD-1, CTLA-4, Tim-3**
   a. Nebbia, PLoS ONE 2012; 7: e47648.
   b. Boni, J Virol 2007; 81: 4215–4225.
   c. Schurich Hepatology. 2011; 53: 1494–1503
   d. Bengsch, J. Hepatol. 2014 Dec;61(6):1212–9
   e. Fisicaro Gastroenterology. 2010 Feb;138(2):682–93

3. **Metabolic Dysfunction**
   a. Fisicaro Nat Med. 2017 Feb 6;23(3):327–36.



Therapeutic vaccines
- Increase magnitude of HBV-specific T cells
- Co-stimulation

Checkpoint Blockade
- Restore function & improve target recognition
- Increase magnitude ???

Antigen reduction
- Effect on immunity???

Goal: Increase adaptive anti-HBV immunity

How much increase is possible?

How much increase is needed?
Magnitude? Function? Both?

Will vaccines provide sufficient coverage for genotypes?

Will there be Anti-HBs B cell recovery/stimulation?

Will Ag reduction restore T cell response? Combinations?

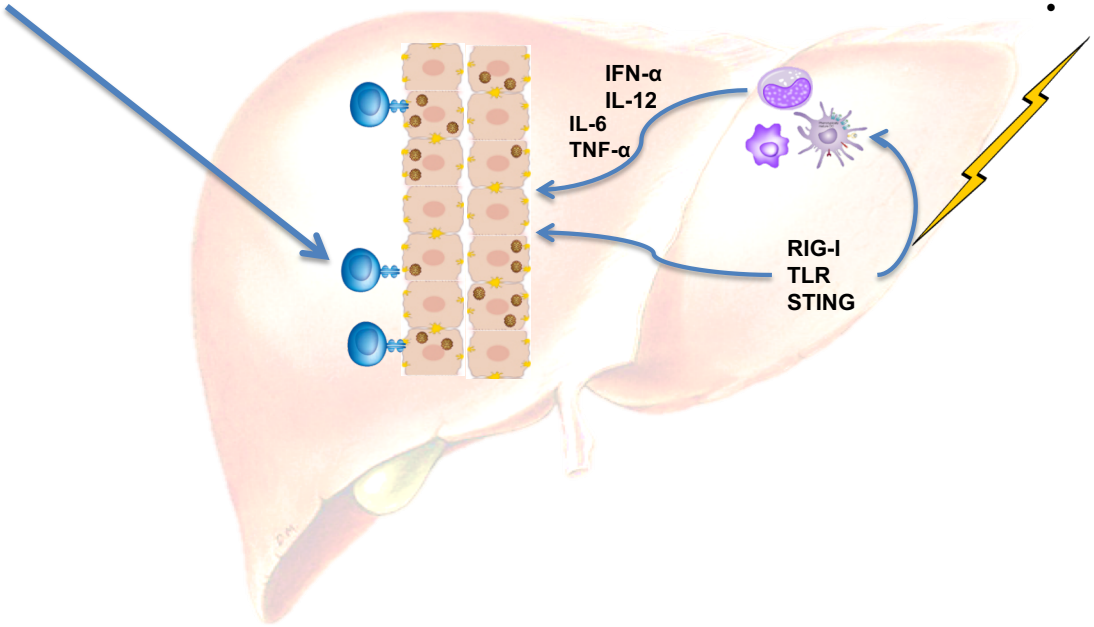
Immunotherapeutic Strategies

Improve T cell activation

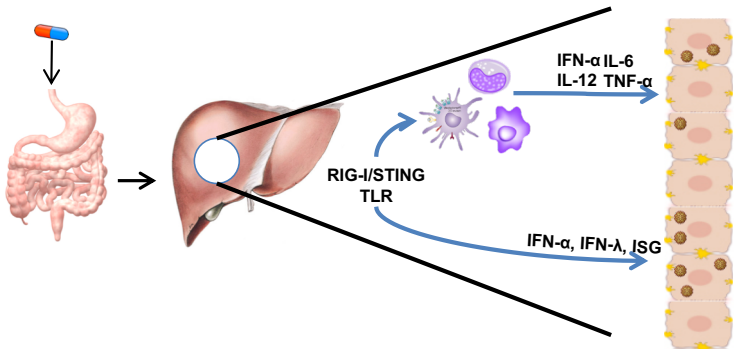
- **Vaccination**: Shift Antigen Presentation
- **Checkpoint blockade**: Block inhibitory signals

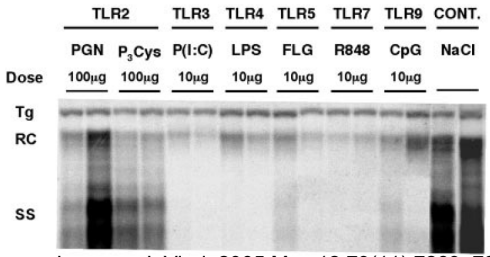
Innate stimulation

- **Cytokine production**
  - Antiviral
  - T cell co-stimulation
  - alter liver environment

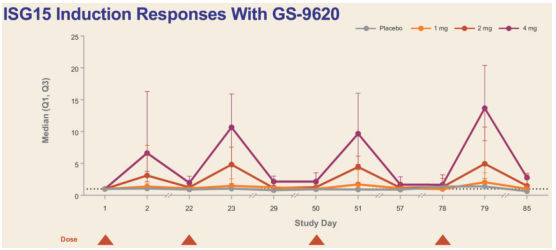


Innate Immunotherapy

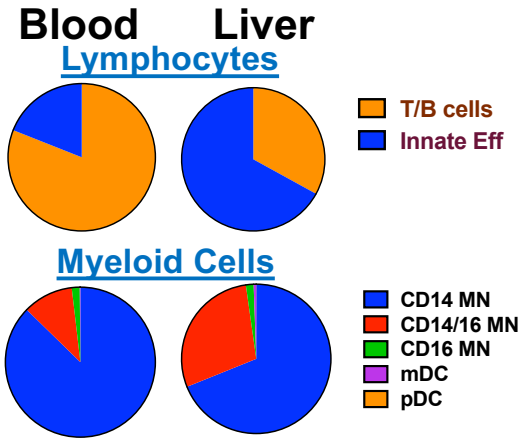




Isogawa J. Virol. 2005 May 12;79(11):7269–72.



Janssen et. al. J. Hepatol. 2017 Nov 2.



Innate immune activation works in mice

- Highly dependent on IFN-α
- Evidence for innate effector (NKT) activation

Bioavailability in humans

- Oral delivery GS-9620 showed ISG15 induction

Mechanism of action in patients?

Response in liver vs. data from blood?

Direct effect…IFN-α/IFN-λ?

Indirect effect…NK/MAIT/γδ T cells?

- What population?
- What effector function?

Separate efficacy from toxicity?

Immune Biomarkers & Immune Monitoring

- If we are going to target immunity we need to be able to measure it

- Most drugs have an immunological component
  - Vaccines & Checkpoint blockade = T cells
  - Innate immuno-modulators = cytokines & lymphocyte activation???
  - siRNA/HBsAg inhibitors/nucleic acid polymers = reduce antigen > restore immunity???
  - Capsid inhibitors = more core in hepatocytes > better T cell target???

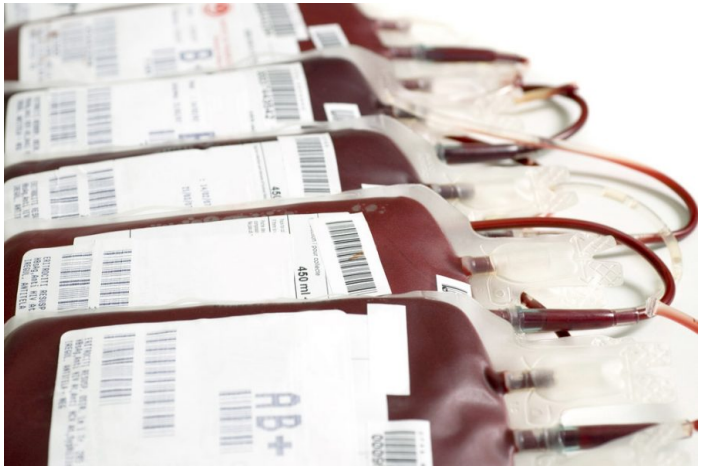
- Immunology relegated to later trials

- Immunology has to be incorporated into early stage trials

Immune Biomarkers & Immune Monitoring

Immunology needs to be more efficient

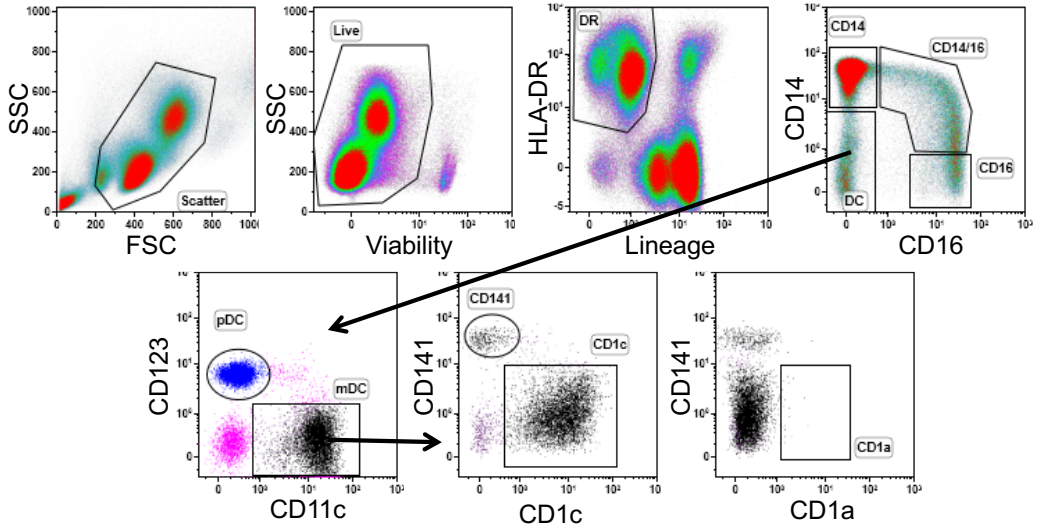
- New drugs have 12/24w Tx window
- 550 ml blood/8w = 65 ml/w
  - Baseline visits and early pK studies use 200ml+
    - limits the critical baseline sample for immunology
  - Remaining blood used for pK, safety, new virological biomarkers



Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

Myeloid cells (10^6 cells)



Myeloid Cells = 7* populations + phenotype

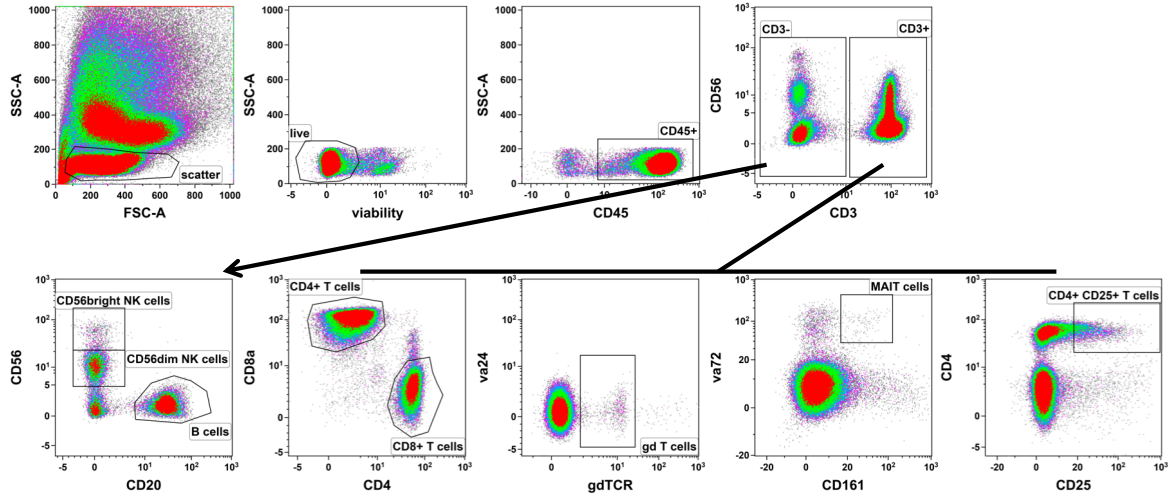
1. **CD14+ MN**
2. **CD14/16+ MN**
3. **CD16+ MN**
4. **pDC**
5. **CD141+ mDC**
6. **CD1c+ mDC**
7. ***Neutrophils** (whole blood)

Ph: CD80, CD86, CD40, CD83, PD-L1

Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

Lymphocytes (10^6 cells)



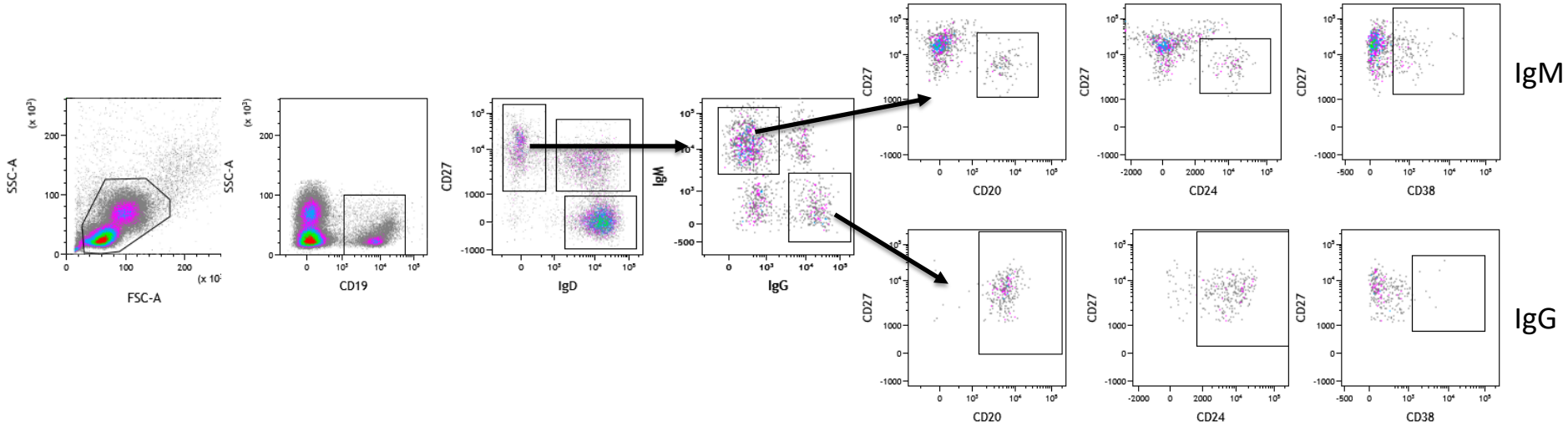
Lymphocyte = 8 populations

1. CD56^{bright} NK
2. CD56^{dim} NK
3. B cells
4. CD4+ T cells
5. CD8+ T cells
6. gd T cells
7. MAIT cells
8. CD4+/CD25+/CD127-+ T_{reg} cells

Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

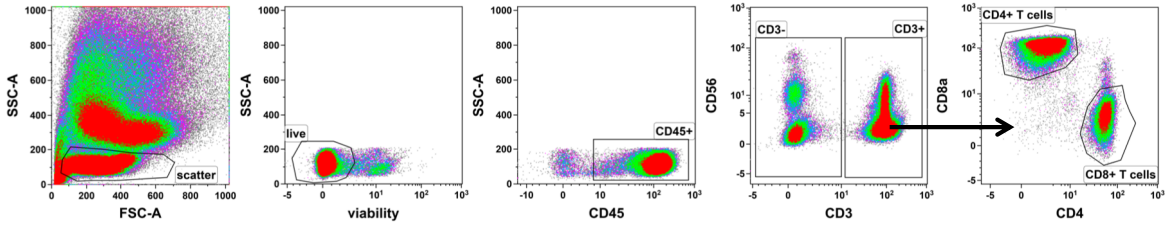
NK/B cells
(10^6 cells)



Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

T cell Inhibitory/activation (10^6 cells)



CD4/CD8Phenotype

- +/- Tetramer
- CD38
- HLA-DR
- CD127
- 2B4
- PD-1
- CD160
- CD57
- KLRG1

Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

Myeloid cells (10^6 cells)

Lymphocytes (10^6 cells)

NK/B cells (10^6 cells)

T cell Inhibitory/activation (10^6 cells)

Sorting

Nanostring

RNAseq

Single-cell RNAseq

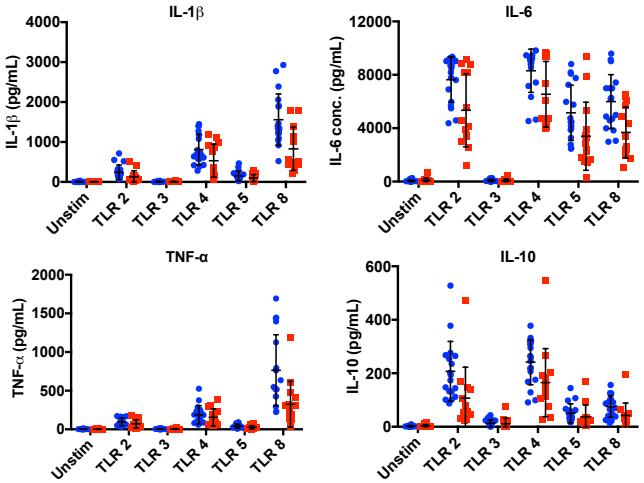
Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC

Function (2x10^6)

General Immune function

- TLR-2
- TLR-3
- TLR-4
- TLR-5
- TLR-7
- TLR-8
- RIG-I
- STING
- CD3/CD28

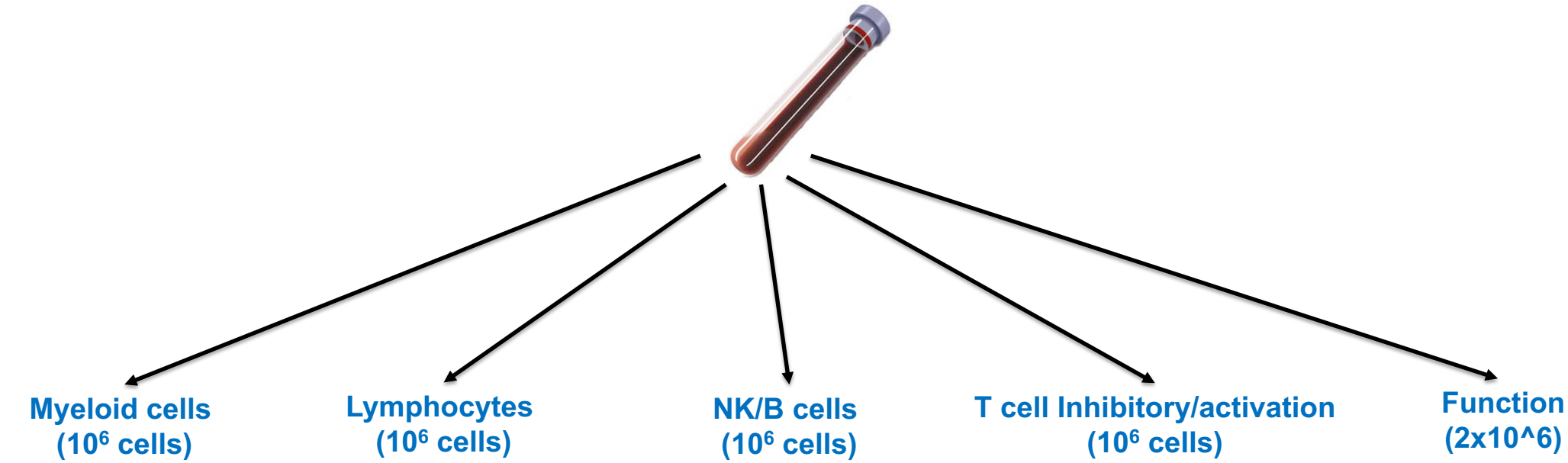


Multi-plex analysis

- customized panels
- Comprehensive Panels (>40 cytokines)

Efficient Immuno-Monitoring for Clinical Trials
- 1 Tube -

10 ml tube of whole blood = 8 – 12x10^6 PBMC



1. **Flow cytometry with option to sort populations**
2. **Cytof for comprehensive analysis in single sample**

Efficient Immuno-Monitoring for Clinical Trials
- 2 Tubes -

2nd - 10 ml tube of whole blood = 16 – 24x10^6 PBMC

Not routinely performed

- Reagents & technical expertise
- <10% detection in standard elispot

We have adapted elispot assay

- optimized: culture, stimulation, numbers

Total Ex vivo HBV-specific T cell Response (6x10^6 cells)

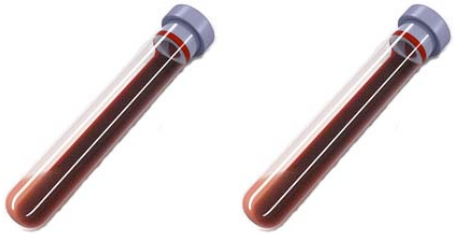
>70% +patients
n=20/28

unpublished data

HBsAg-specific Memory B cells (2x10^6 cells)

unpublished data

Feasibility



Broad immune monitoring with minimal demand for blood

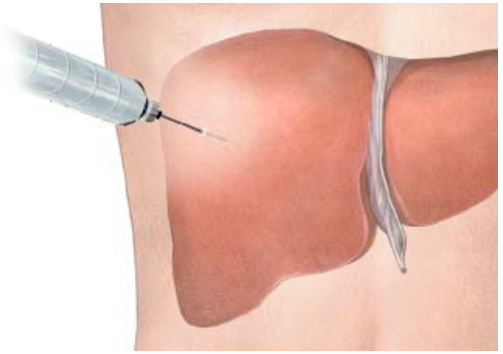
- **Complete profile of the immune populations in the peripheral blood**
- **Now possible to measure ex vivo HBV-specific T cell immunity using elispot in >70% patients**
- **Measuring HBs-specific B cell frequency now possible**

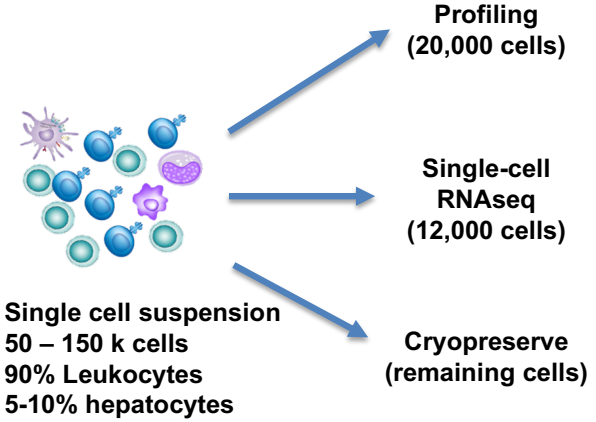
Efficient Immuno-Profiling for Clinical Trials
- The Liver -

Core biopsy - desirable but challenging

- Good cell numbers
- 3-dimensional architecture
- Immunology + virology assays

Safety and technology limit justification

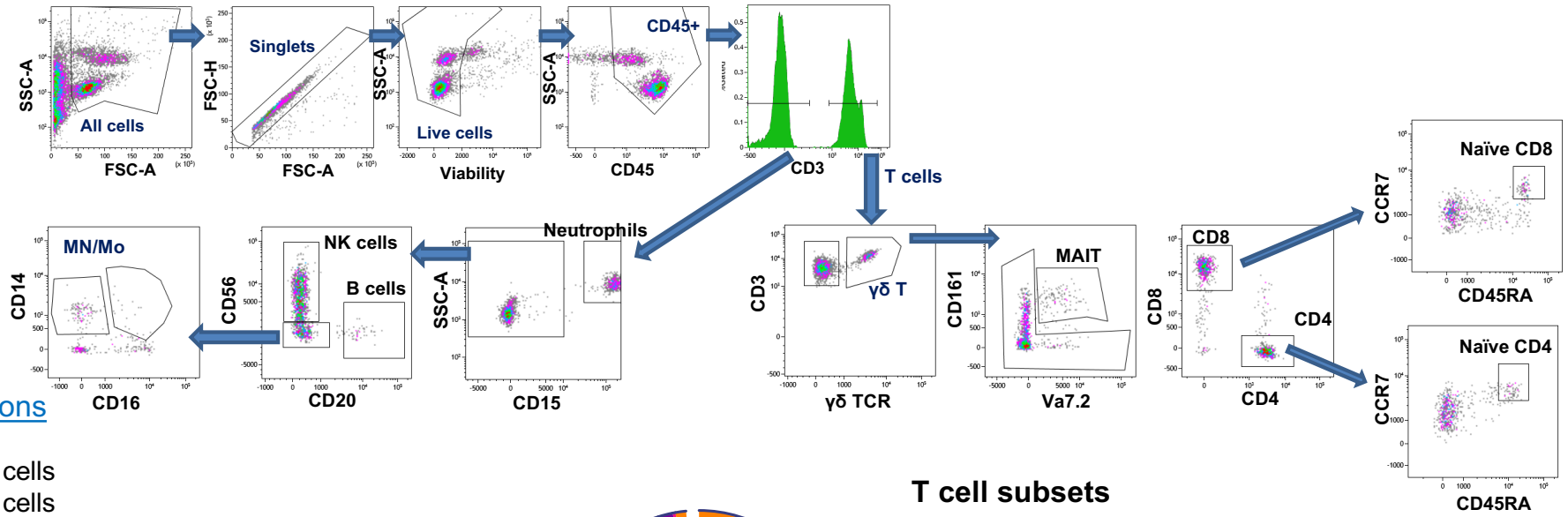




Fine Needle Aspirate Biopsy (FNAB)

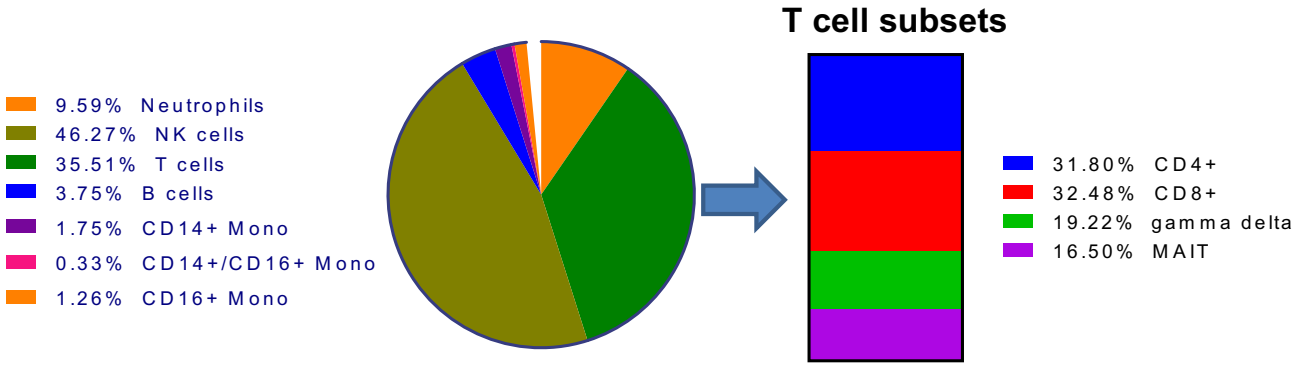
- performed in clinic on regular visit
- 25 gauge spinal needle
- regular longitudinal sampling
    - little as 1 w between FNAB
- From liver to experiment in <1h
- Caveats - No architecture and few hepatocytes

Efficient Immuno-Profiling for Clinical Trials
- The Liver -



Cell Populations

1. Neutrophils
2. CD56hi NK cells
3. CD56lo NK cells
4. B cells
5. CD14 MN
6. CD14/16 MN
7. CD16 MN
8. γδ T cells
9. MAIT Cells
10. CD8 T cells
11. Naïve CD8 T cells
12. CD4 T cells
13. Naïve CD4 T cells



Efficient Immuno-Profiling for Clinical Trials
- The Liver -

10x Genomics 3' single-cell RNAseq

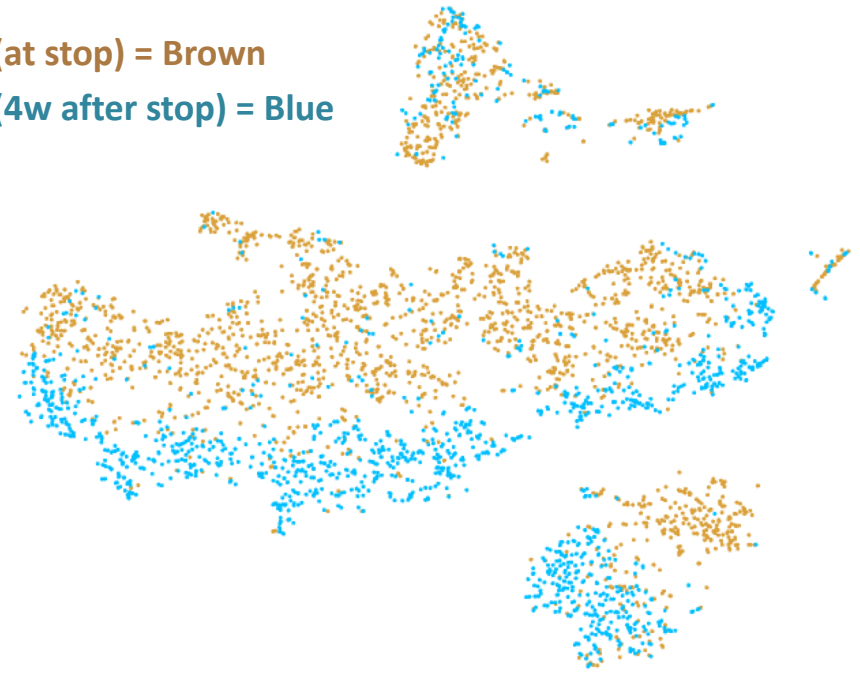
Input = 12,000 total cells (RBC depleted)
2,000 – 2,500 cells/sample

Longitudinal Sampling

- Patient stopping Tenofovir
- RNAseq on 2,200 total leukocytes & hepatocytes
- Non-overlapping transcriptional profiles

T0 (at stop) = Brown

T4 (4w after stop) = Blue



Combined Immune monitoring

1. **Peripheral blood data – Profile and HBV immunity**
2. **FNAB sub-study**
   a. **See populations by flow cytometry**
   b. **Measure change in function by RNAseq**

Comprehensive picture of immunological response to therapeutic intervention

Summary

Need strategic immune monitoring to identify biomarkers to assess impact

- **Need to know if immune drugs are working in vivo**
  - **Are hypothesized immune cells activated?**
  - **Does immune activation impact virological parameters?**

- **It is possible to do this with minimal blood.**
  - **ID points where can we get larger samples for deeper analysis, i.e. Leukapheresis**

- **Immune monitoring in early stage trials is valuable**
  - **Even data from negative trials will be informative immunologically**

- **Not restricted to immune-targeting drugs – siRNA, Capsid inihibtors, NAPs, etc…**

Toronto Center for Liver Disease

Gehring lab members

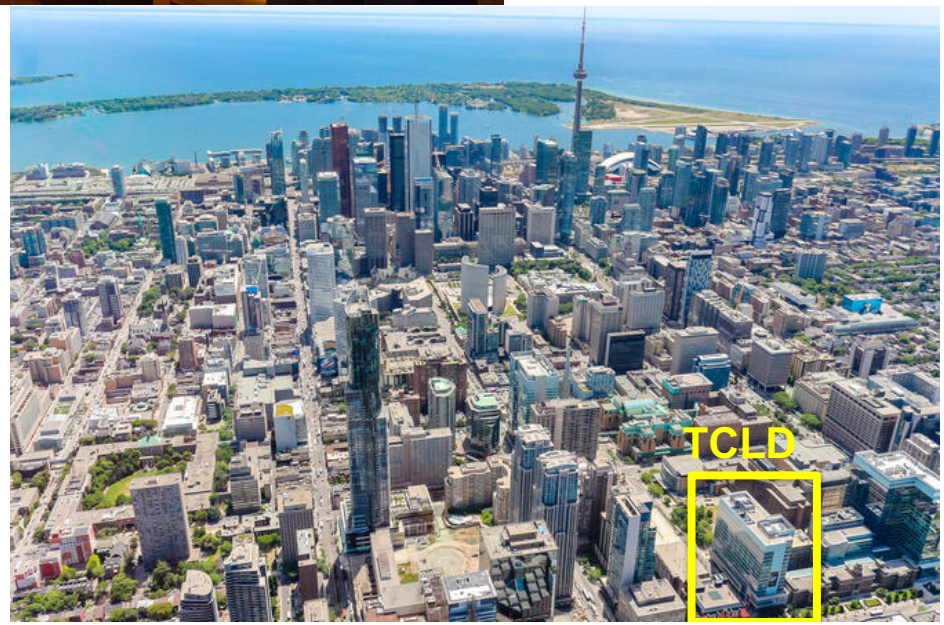
Deeqa Mahamed
Aman Mehrotra
Conan Chua
Adrian Kuipery
Michelle Yang
Sonya Kim
Steve Lee
Alexandra Johnson-Valiente

Clinical Collaborators

Harry Janssen
David Wong
Jordan Feld
Scott Fung

Coordinators

Danie La
Jenny Chen



Princess Margaret Genomics Centre

Neil Winegarden
Nick Khuu