Flu immunology for the non-immunologist

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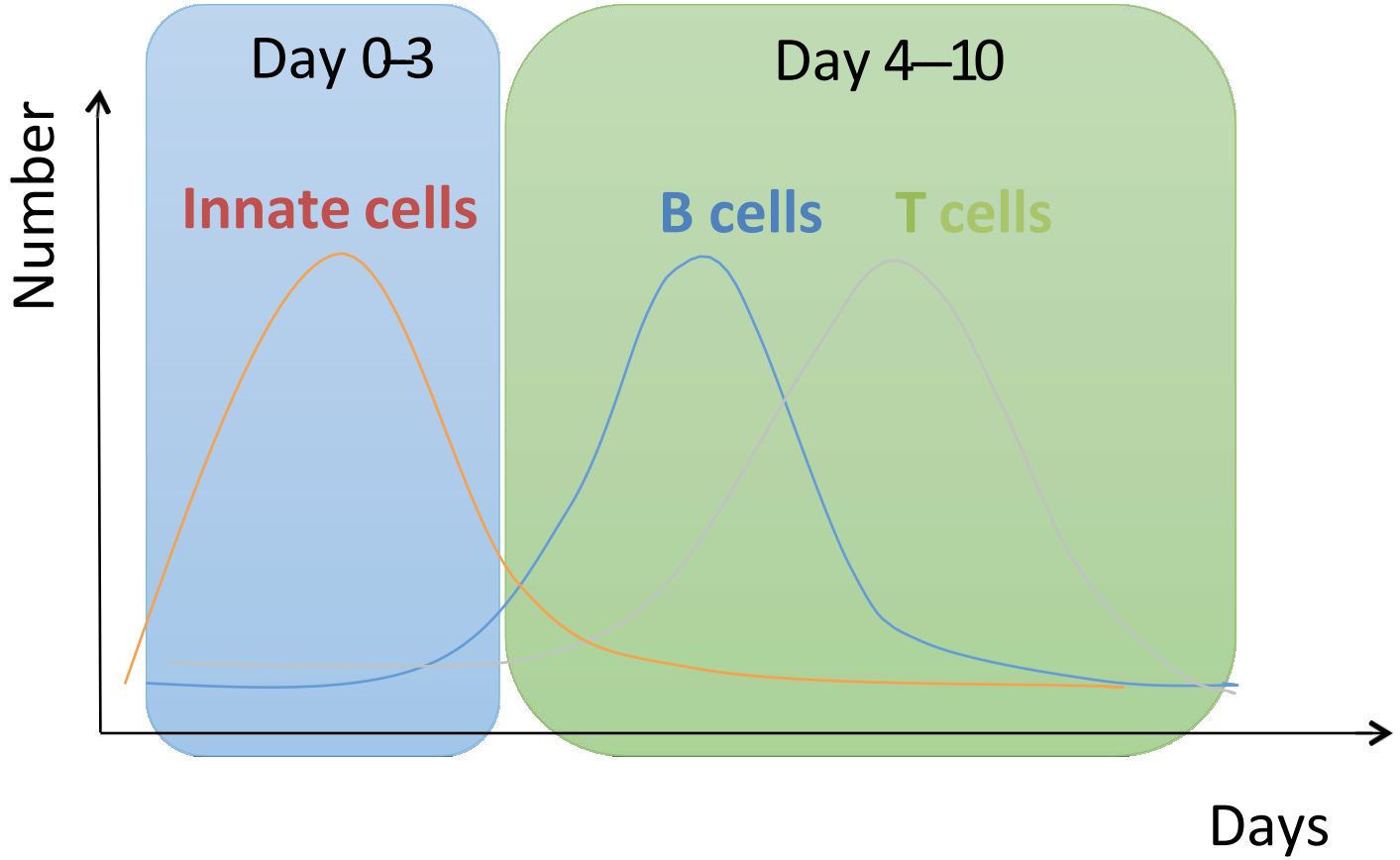
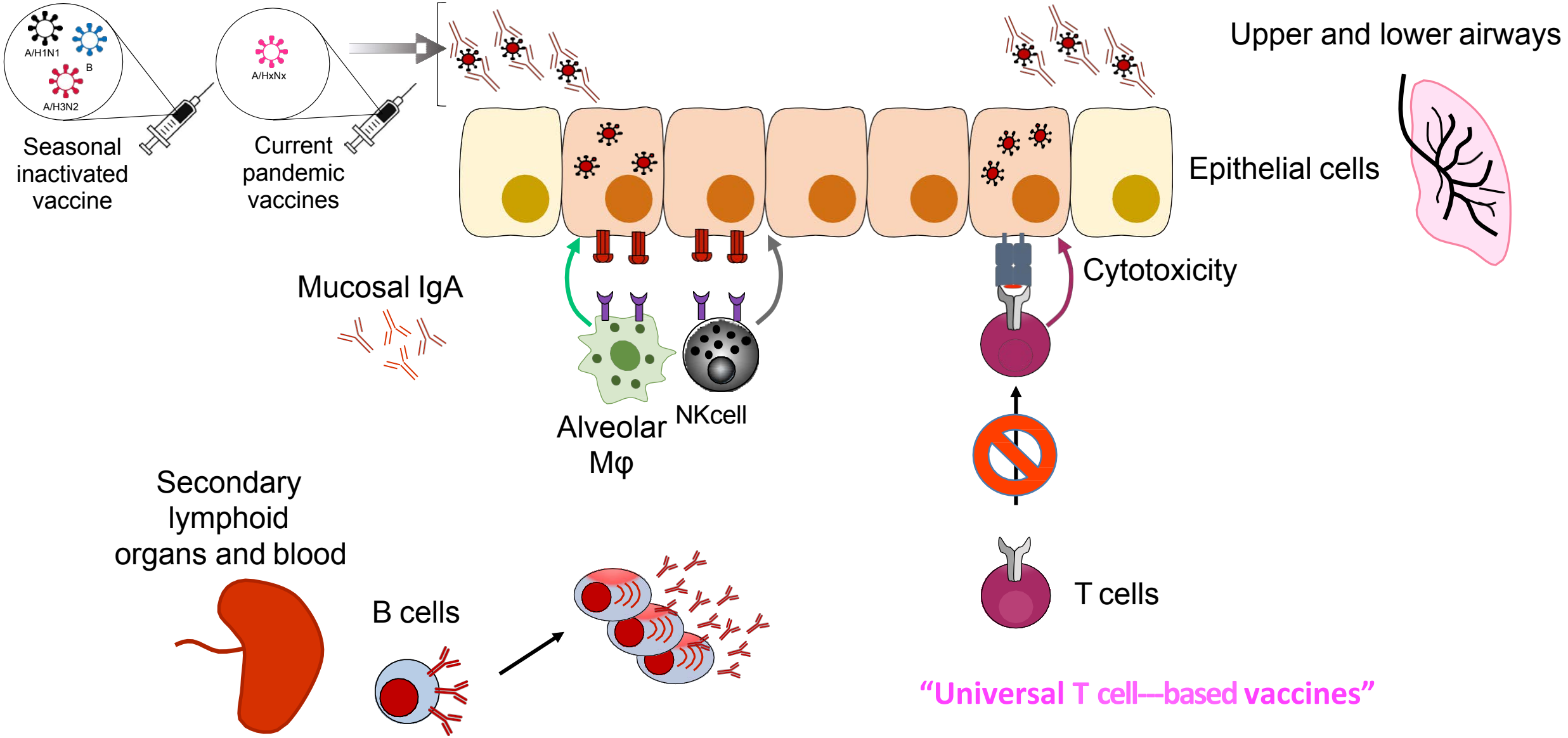
THE UNIVERSITY OF MELBOURNE

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

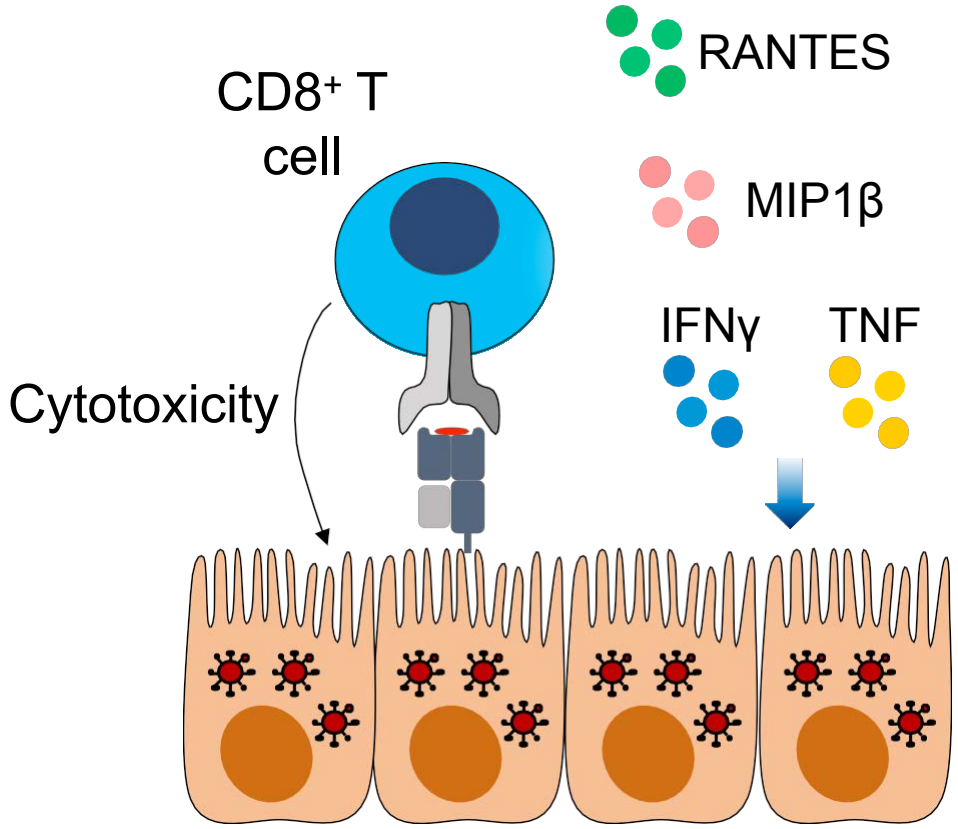
Immune response to influenza virus infection



Immune protection against influenza virus infection



CD8+ T cells kill virally-infected cells



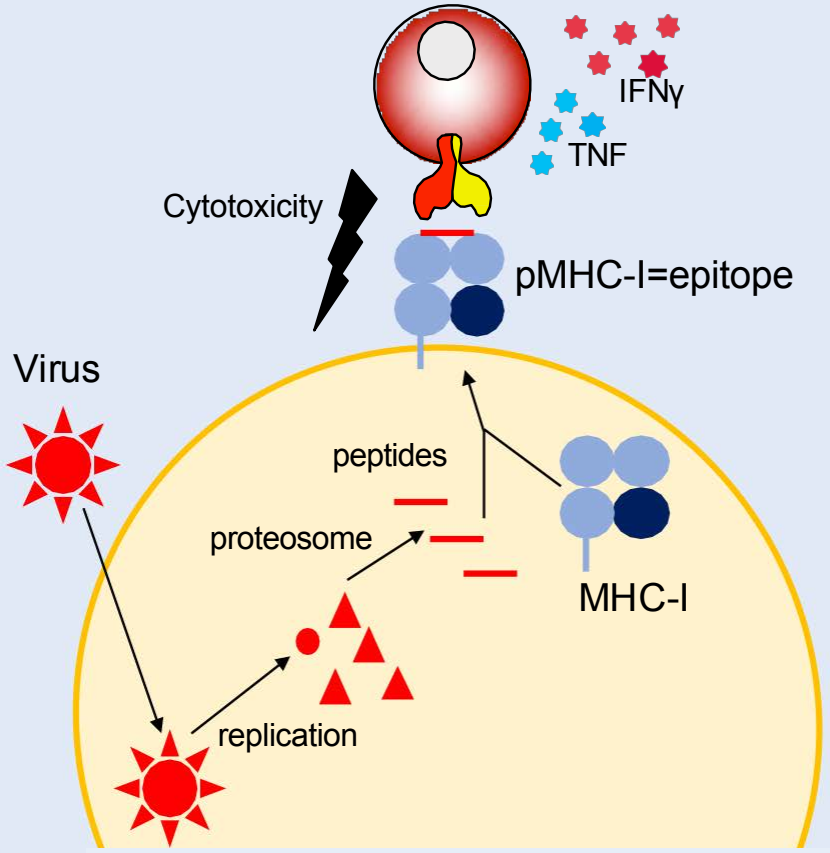
CD8+ T cells recognize peptides presented by MHC-I

CD8+ T cells recognize peptides presented by MHC-I

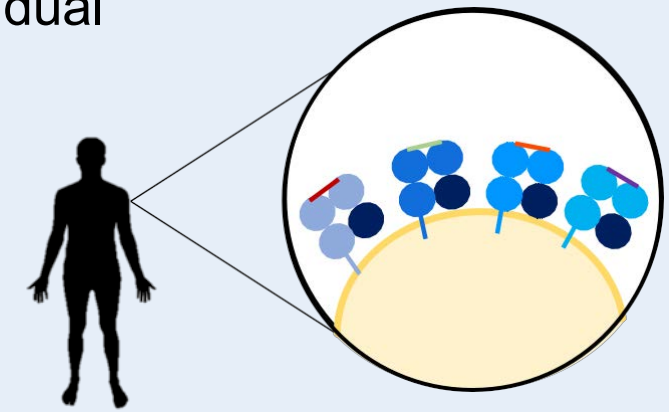
MHC-I = HLA→→many different alleles

HLA-A
HLA-B
HLA-C

A*01:01 B*07:02
A*02:01 B*08:01
A*03:01 B*18:01
A*24:02 B*27:02
etc etc

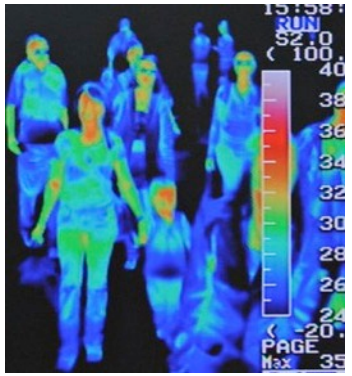


→→Up to 2 different HLA-A and 2 HLA-B alleles per individual

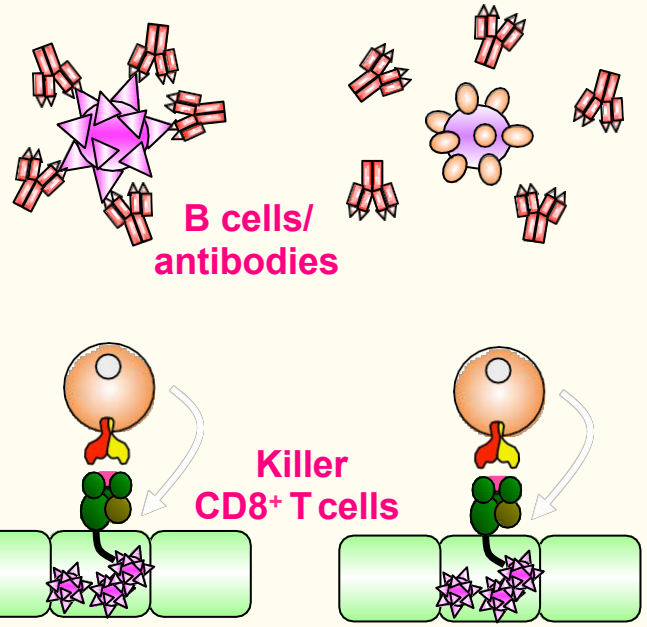


→→Differential frequency of HLA alleles across ethnic groups

CD8+ T cells provide broad immunity against influenza viruses

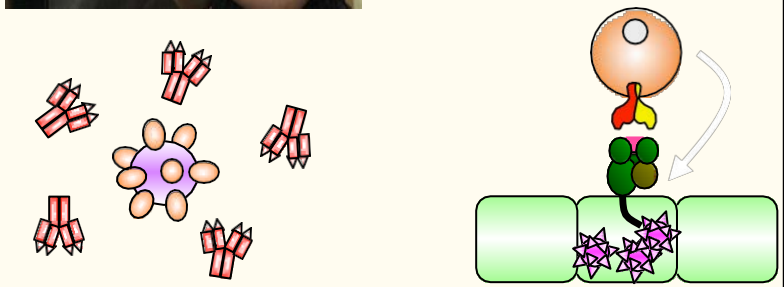


•Seasonal annual epidemics



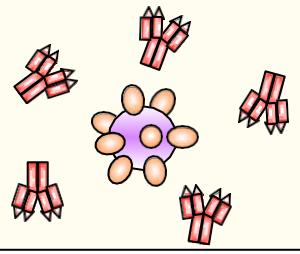
• Influenza pandemics

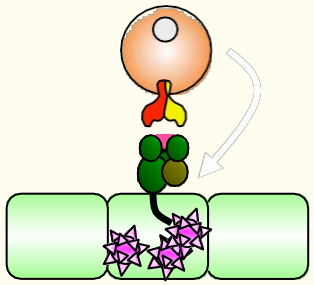
H1N1-1918
H2N2-1957
H3N2-1968
H1N1-2009



•Influenza in animals

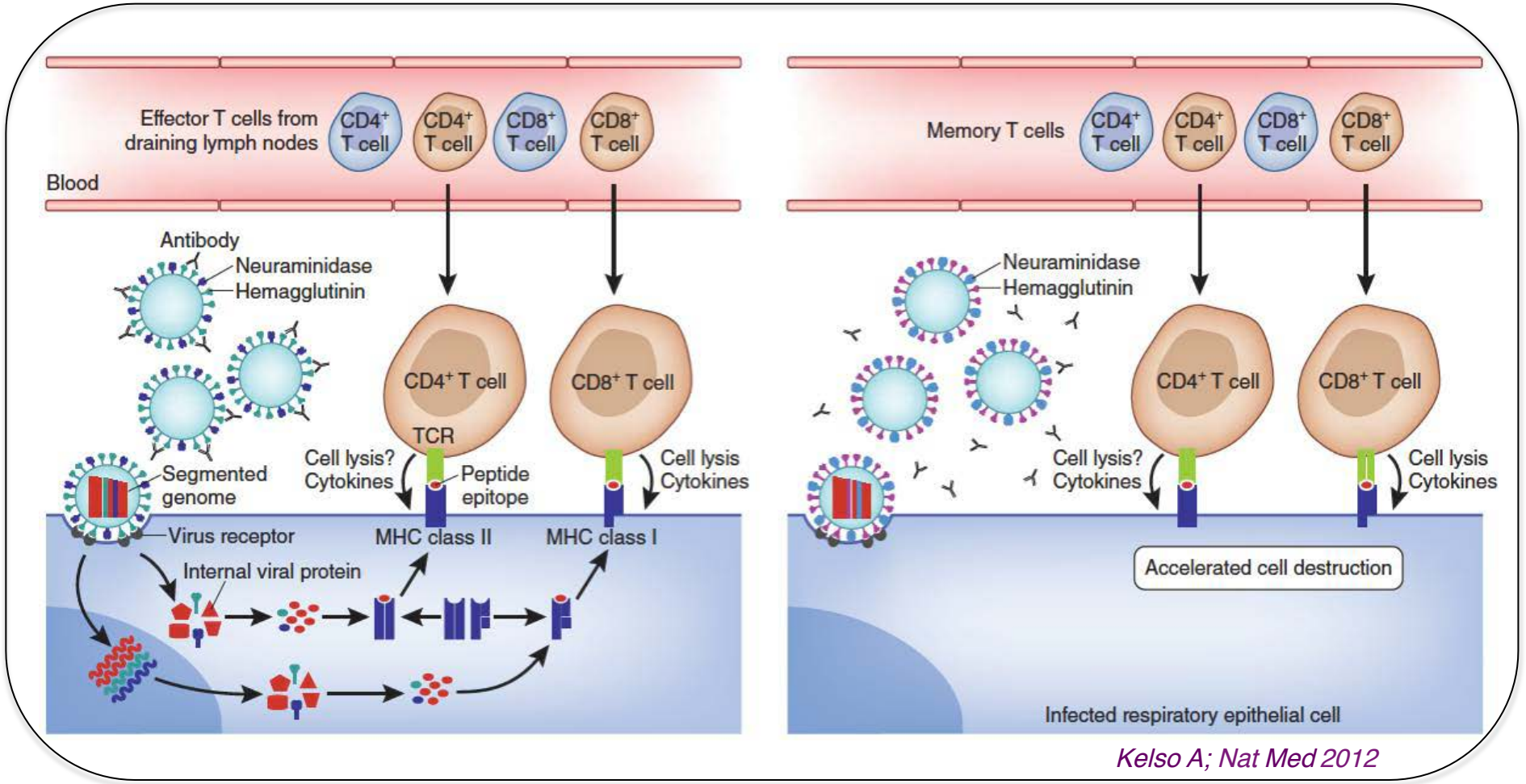
H5N1
H7N9





CD8+ T cells provide broad immunity against IAVs

Primary infection with seasonal strain **Challenge with a novel strain (pandemic potential)**



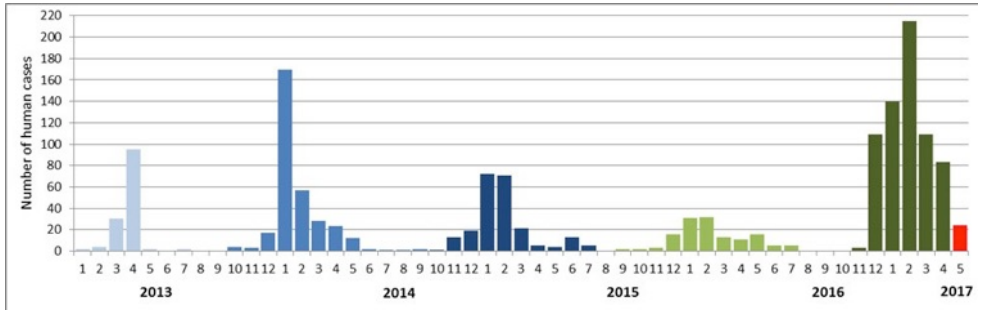
Kelso A; Nat Med 2012

In the absence of pre-existing antibodies, memory T cells can provide heterologous immunity and reduce the severity of influenza disease

McMichael A et al, NEJM 1983; Epstein SL, JID 2006; Kreijtz JH et al, JVI 2008; Lee et al, JCI 2008; Wilkinson et al, Nat Med 2011; Sridhar et al, Nat Med 2013; Wang Z et al, Nat Comm 2015;

A novel influenza strain capable of infecting humans: A/H7N9

- April 1st WHO compiled a report that during Feb-Mar 2013 China's CDC reported three fatal human infections with a novel avian-origin reassortant A(H7N9)
- ≈ 1489 cases/588 deaths, **mortality rate of 40%**



CONCERNS:

1) Lack of neutralizing Abs

2) Natural reservoir for the virus, but mild disease in birds

3) Possibility to acquire human-human transmission

→→need to understand and control A/H7N9

Shanghai Public Health Clinical Centre

-> a first class teaching and research infectious diseases hospital affiliated to Fudan University

Shanghai Institute for Emerging and Re-emerging Infectious Diseases

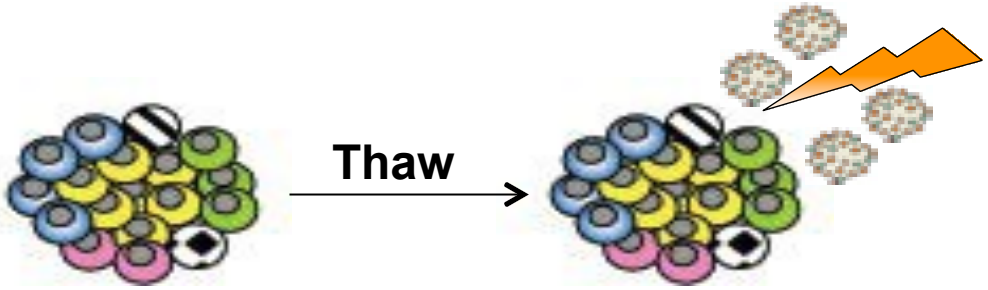
Dr Zhongfang Wang

Sergio Quinones Parra

Dr Liyen Loh

Prof Jianqing Xu

Ex-vivo functional assessment of the cellular immunity against H7N9 influenza virus

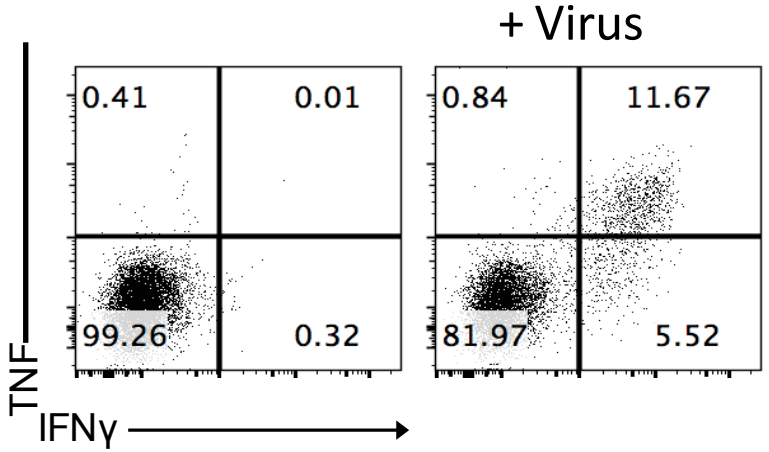


Peripheral blood mononuclear cells (frozen)
- contain CD8+ T cells

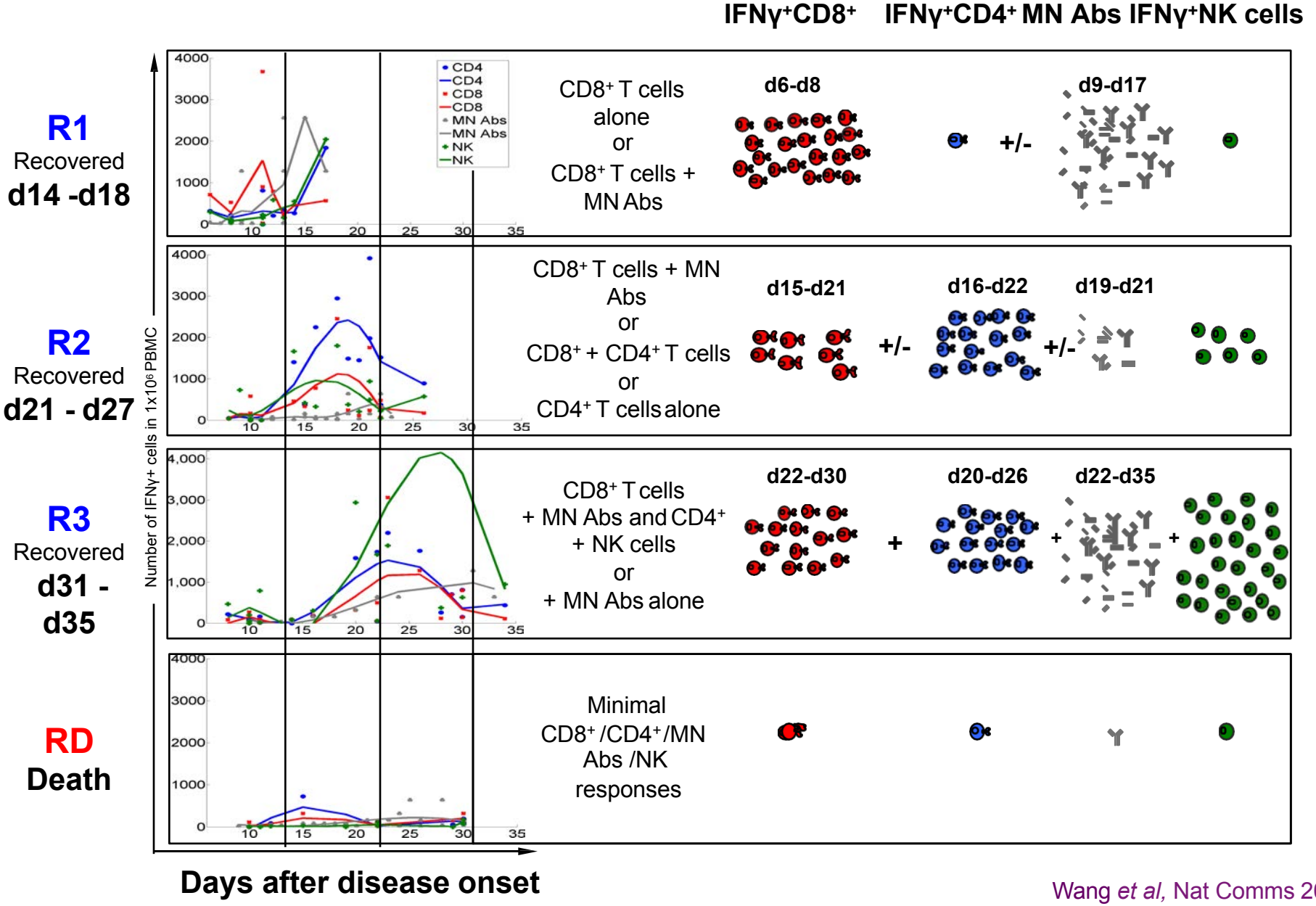
IAV Infection

18 hrs

Measure CD8+ T cell response by flow cytometry



Rapid recovery is associated with early CD8+ T cell responses



Wang *et al*, Nat Comms 2015

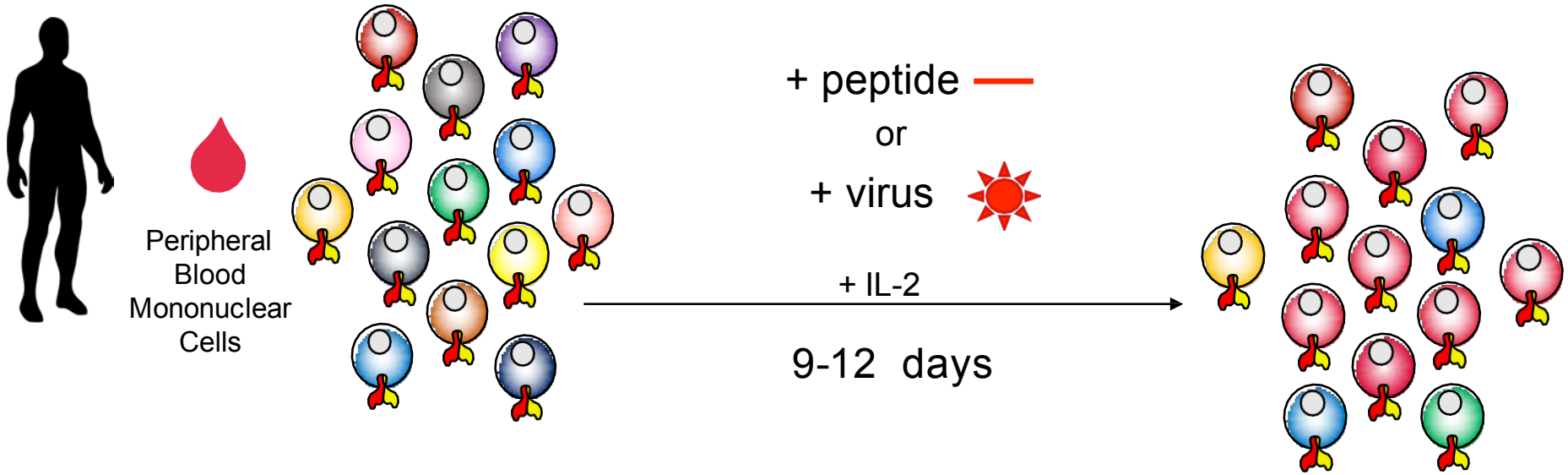
Study Aim

- To dissect cross-strain protective $CD8^+$ T cell immunity across distinct influenza strains and different HLAs/ethnicities
  - Identification of novel epitopes (IAV & IBV)

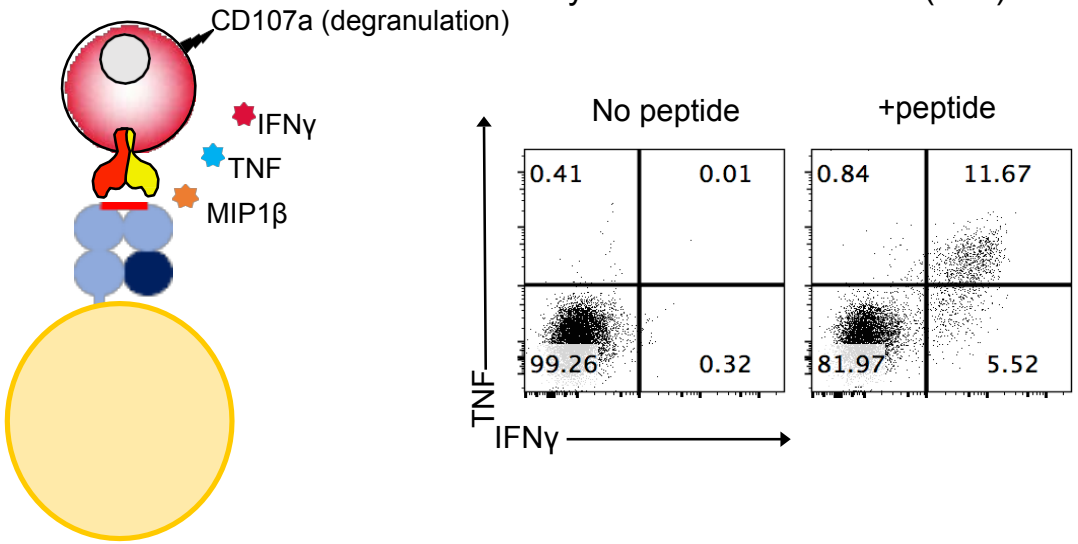
→→To identify novel influenza $CD8^+$ T cell epitopes for universal immunity

Detecting antigen-specific CD8⁺ T cell responses *in vitro*

Expansion of antigen-specific *memory* CD8⁺ T cells



Cytokine measurement (ICS)



T cell responses measured by cytokine production

CD8+ T cell responses to IAV are well characterized

→→195 potential CD8+ T cell epitopes restricted by 24 different HLA alleles

Immunomic Analysis of the Repertoire of T-Cell Specificities for Influenza A Virus in Humans[v†‡]

Erika Assarsson,[1] Huynh-Hoa Bui,[1] John Sidney,[1] Qing Zhang,[1] Jean Glenn,[1] Carla Oseroff,[1] Innocent N. Mbawuike,[2] Jeff Alexander,[3] Mark J. Newman,[3] Howard Grey,[1] and Alessandro Sette[1]*

Nucleoprotein of influenza A virus is a major target of immunodominant CD8þ T-cell responses

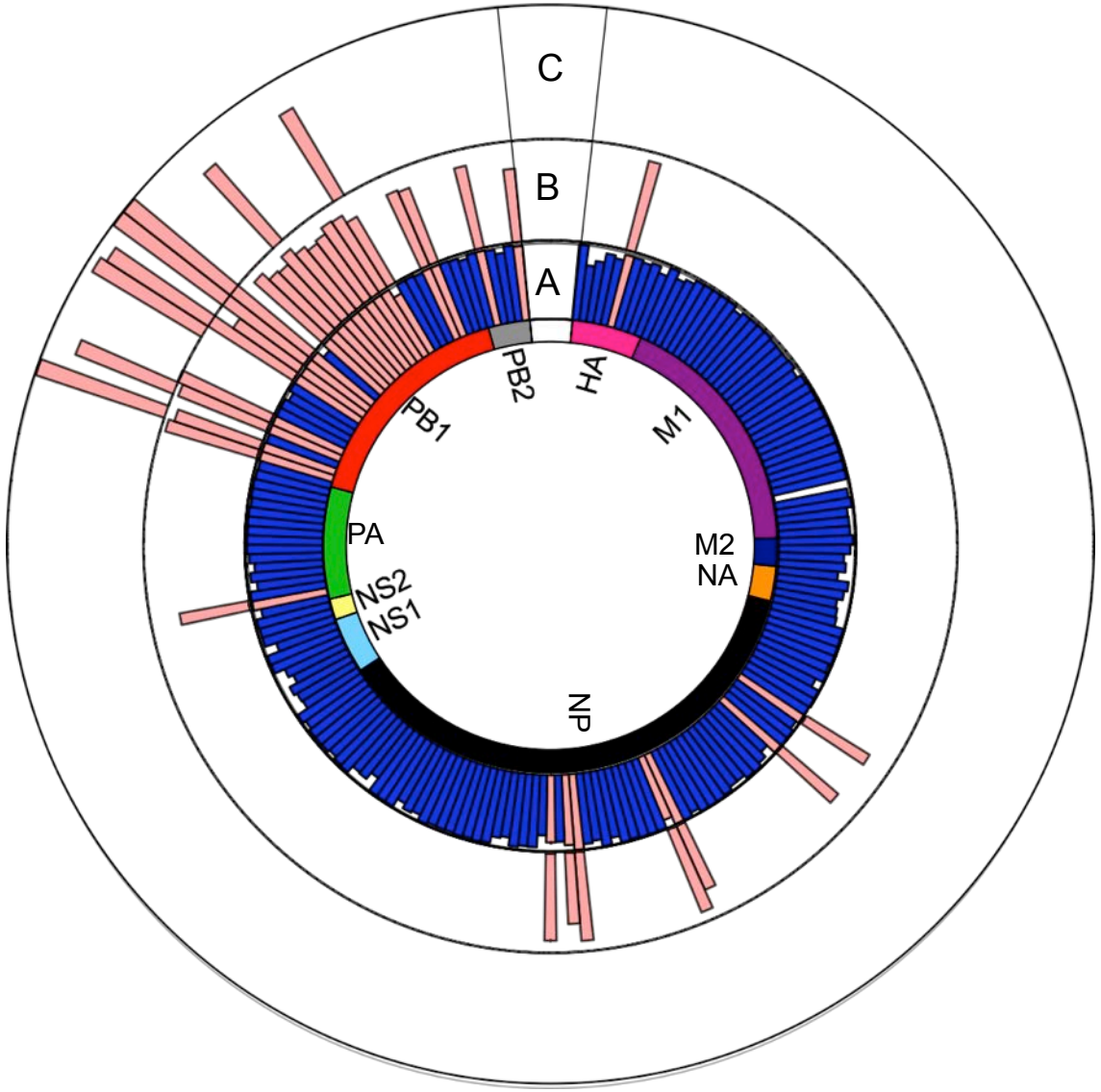
Emma Grant[1,5], Chao Wu[2,3,5], Kok-Fei Chan[3], Sidonia Eckle[1], Mandvi Bharadwaj[1], Quan Ming Zou[2], Katherine Kedzierska[1] and Weisan Chen[3,4]

Identification of broad binding class I HLA supertype epitopes to provide universal coverage of influenza A virus

Jeff Alexander [a,*], Pamuk Bilsel [a], Marie-France del Guercio [a], Aleksandra Marinkovic-Petrovic [a], Scott Southwood [a], Stephani Stewart [a], Glenn Ishioka [a], Maya F. Kotturi [b], Jason Botten [c], John Sidney [b], Mark Newman [a], Alessandro Sette [b]

Marios Koutsakos

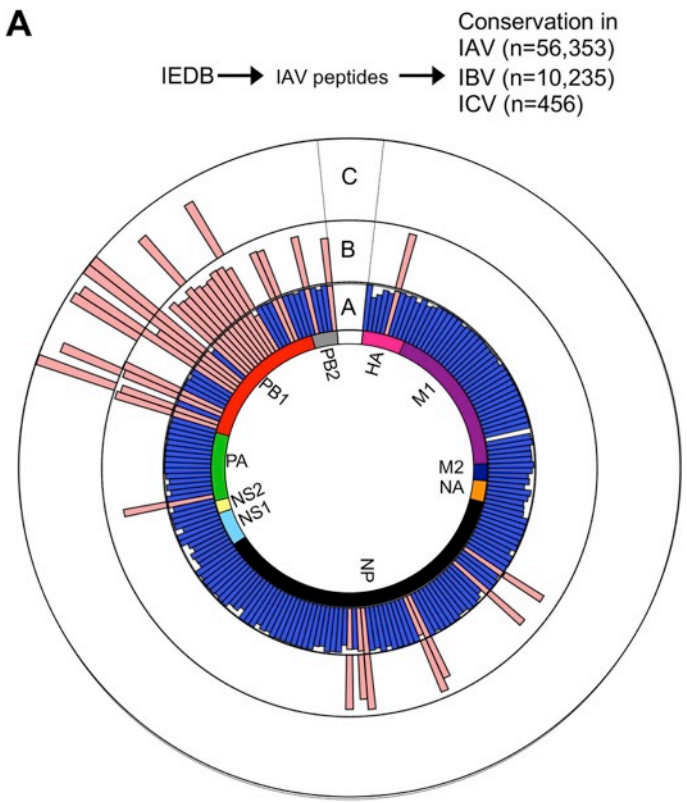
Identification of peptides conserved across IAV, IBV and ICV

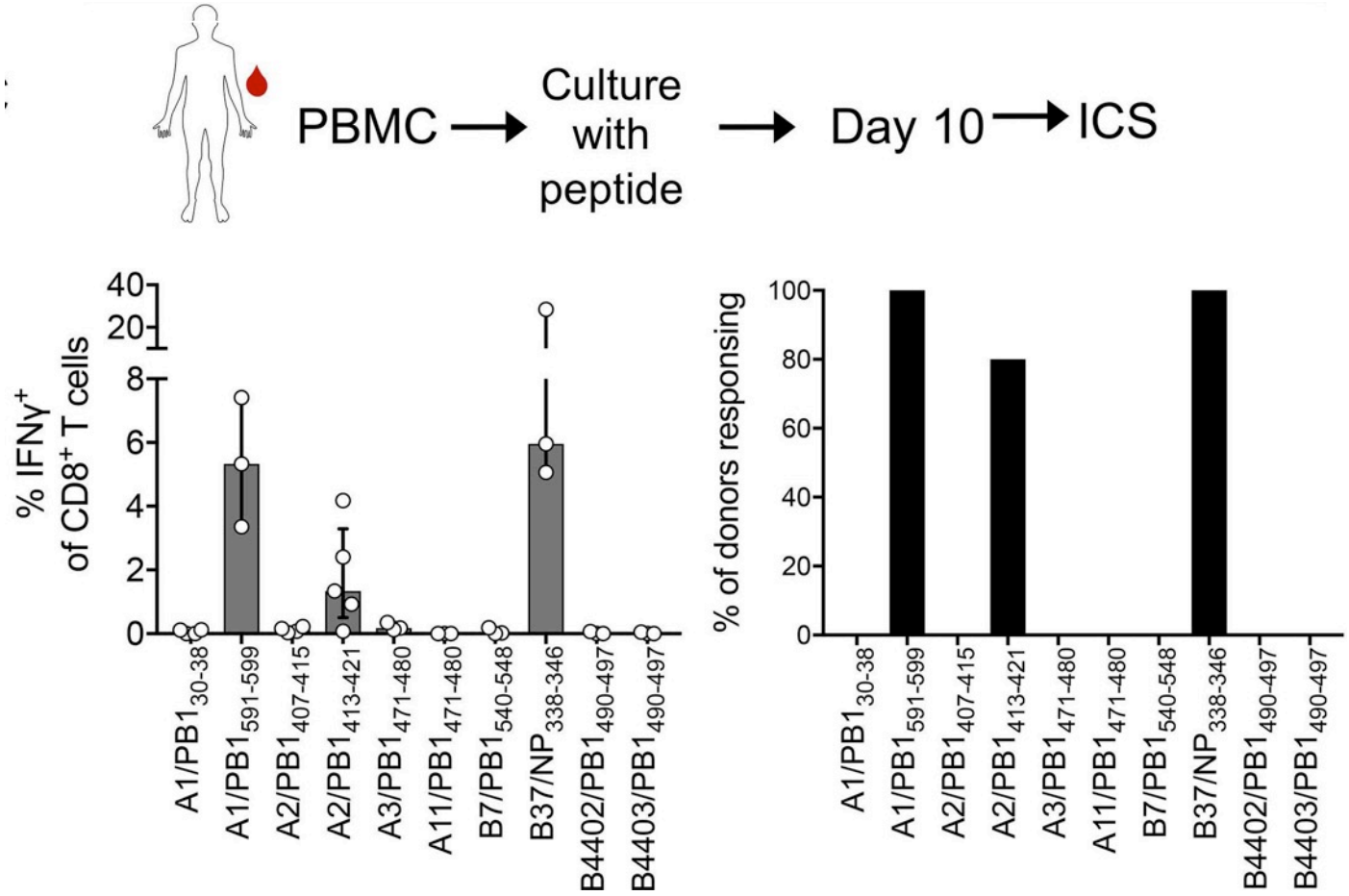


with Sneha Sant, Dr. Dhanasekaran Vijaykrishna and Don Teng

Koutsakos M *et al*, in press

Functional validation of peptides conserved across IAV, IBV and ICV





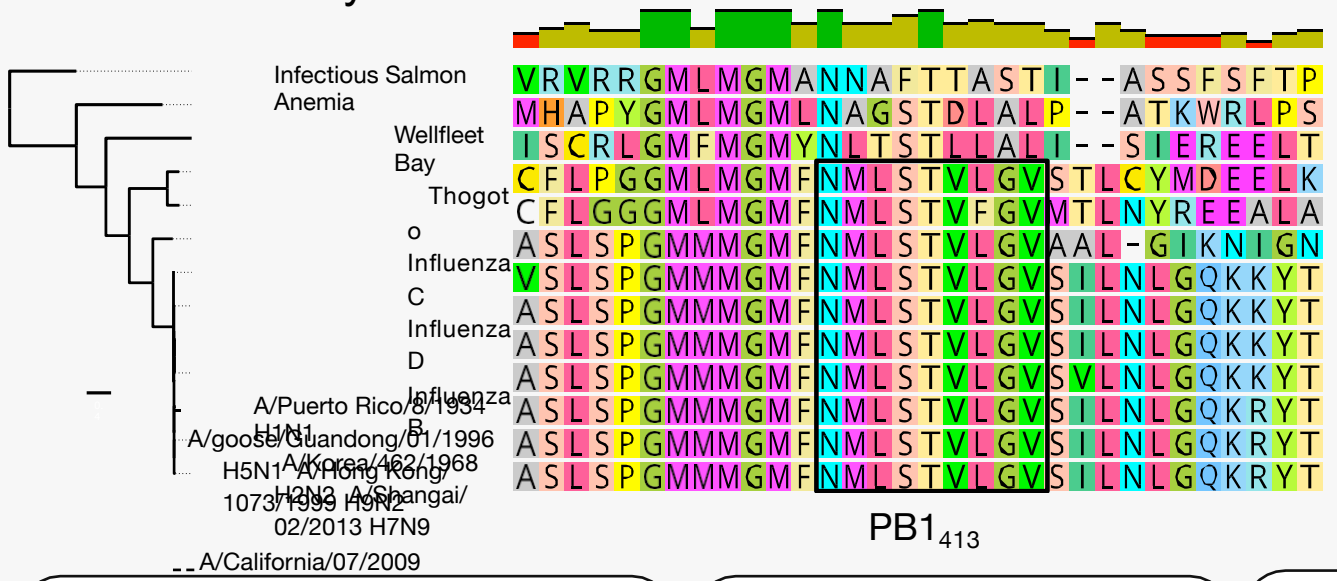
Conserved epitopes

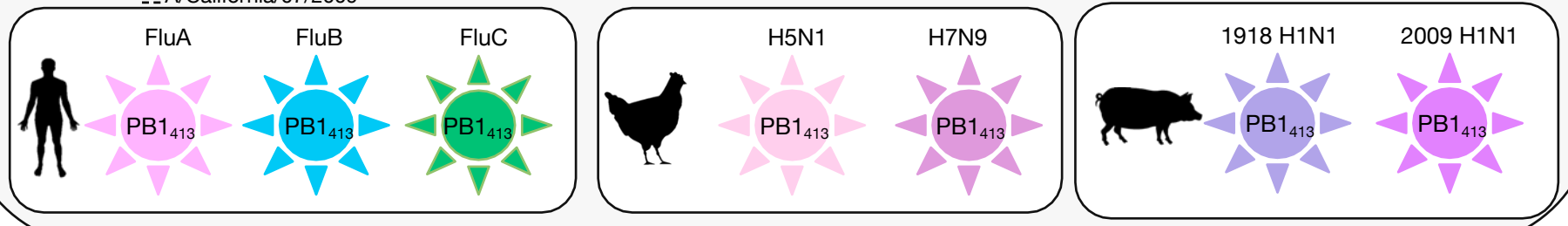
Conserved epitopes

Koutsakos M *et al*, in press

A2/PB1$_{413\text{-}421}$: a promising vaccine target

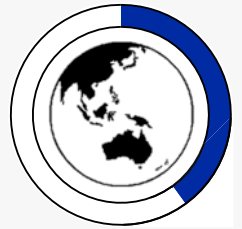
➢ Universally conserved in all influenza viruses





➢ Presented by HLA-A2, one of the most common HLA alleles

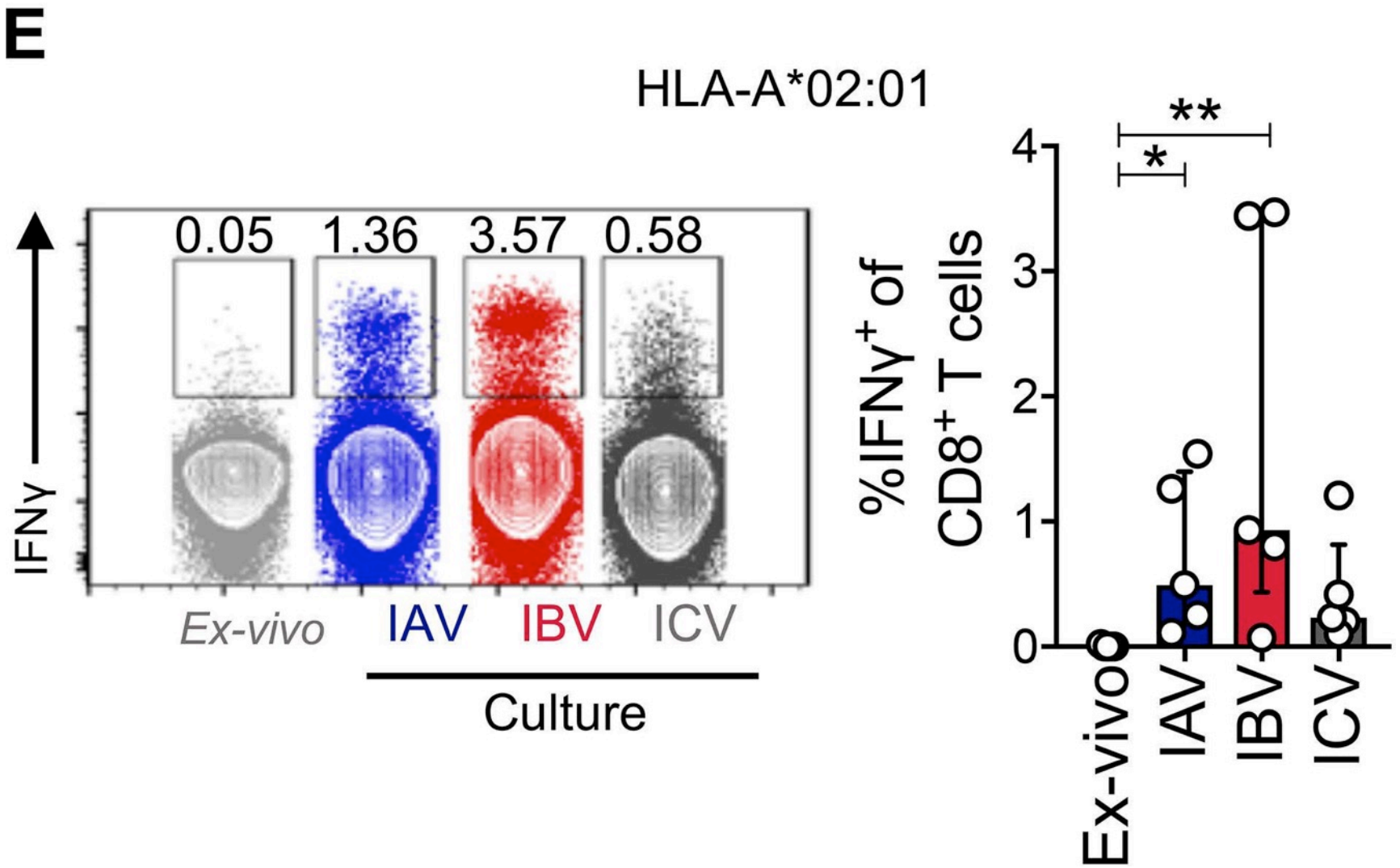
➢ ~40% worldwide



39.8%

Calculated by the IEDB population coverage tool

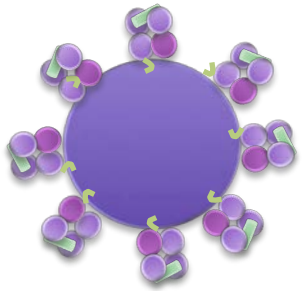
CD8+ T cell cross-reactivity across IAV, IBV and ICV in HLA-A*02:01+ humans



What IBV-derived CD8+ T cell epitopes?

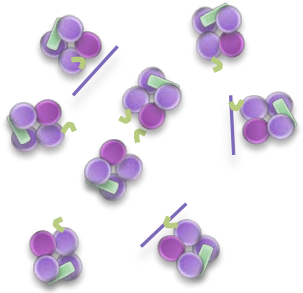
Koutsakos M *et al*, in press

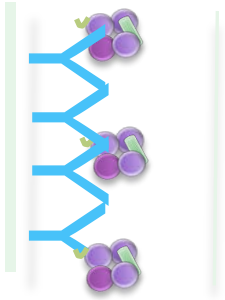
Identification of novel HLA-I epitopes for influenza: mass spectrometry



10^9 cells: CIR-HLA-A2 cell line – infected or uninfected

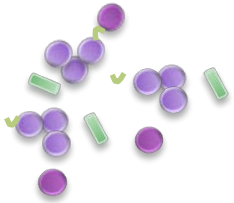
Lyse cells





Affinity purify with Antibody specific for HLA class I (w6/32)

Elute with Acetic Acid



Subject to LC/MS/MS
AB SCIEX TripleTOF™ 5600 System

Identification of HLA bound ligands

- Search against human proteome
- Search against human + IAV proteome

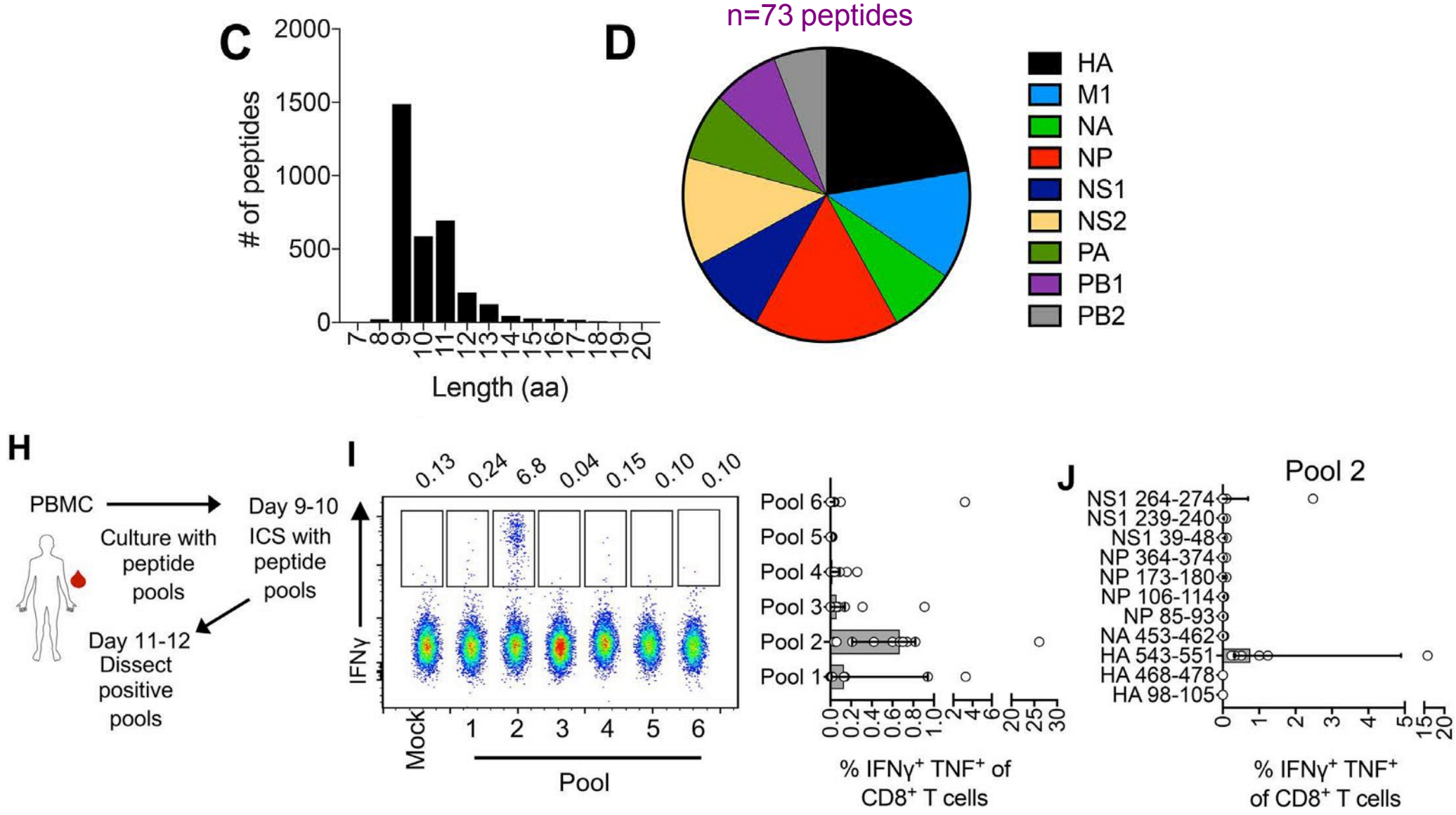
Samples

C1R-Parentals – Uninfected
C1R-Parentals – IAV/IBV infected
C1R-A2– Uninfected
C1R-A2 – IAV/IBV Infected

Look for peptides exclusive to the C1R-A2 IAV/IBV infected cell line

Dr Patricia Illing, Dr Nicole Mifsud, Prof Tony Purcell

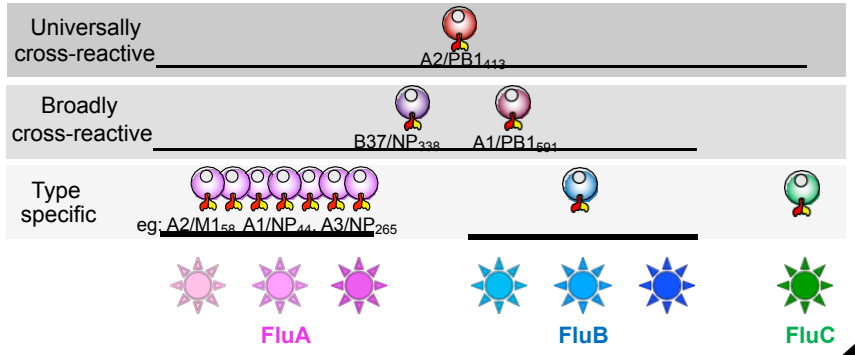
Discovery of new epitopes targeted by CD8+ T cells



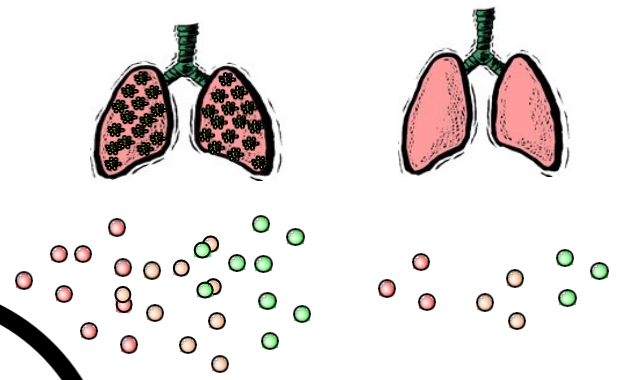
Koutsakos M *et al*, in press

Designing a flu vaccine that does not require annual reformulation

Breadth

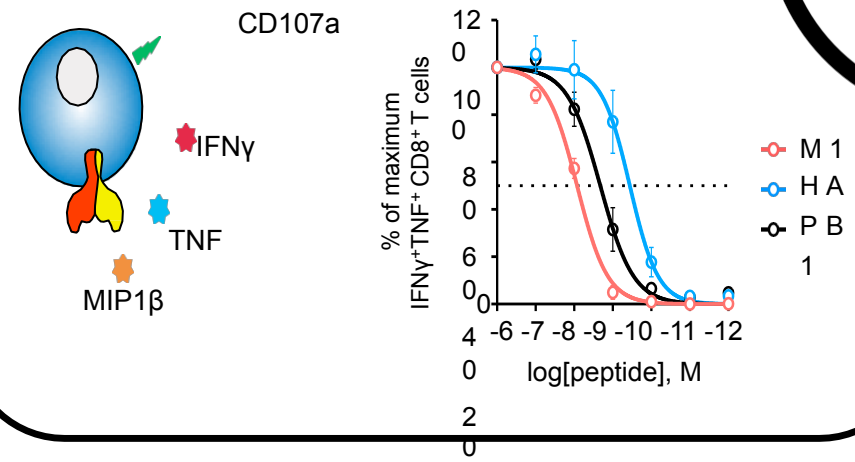


Protection

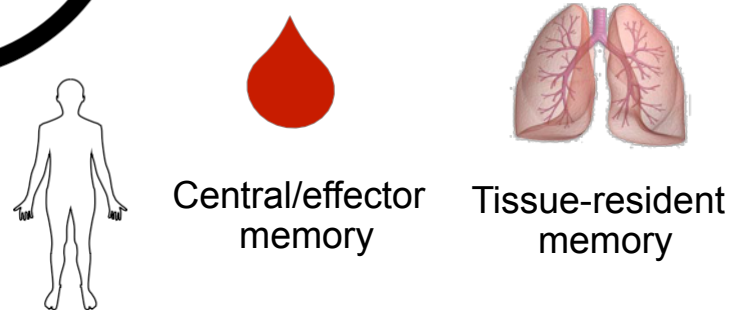


What makes a good T cell vaccine?

Quality



Location



-> T cell-targeted flu vaccine to provide broadly-cross-reactive and protective immunity to unpredicted influenza viruses

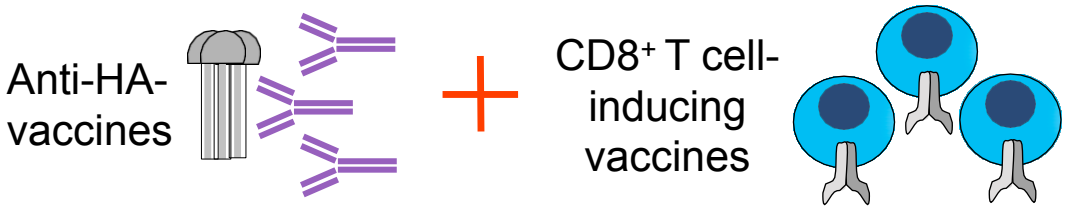
Current advances and hurdles for T cell-based vaccines

- $CD8^+$ T cell epitopes from this study would cover ~3.5 billion people ($HLA\text{-}A2^+$ population)
  - Certain ethnic groups would not be covered

- Broadly cross-reactive epitopes can be derived from the highly-variable surface glycoproteins
  - not only from highly conserved internal proteins

- Novel vaccine formulations are needed to boost $CD8^+$ T cells against influenza viruses
  - Provide longer protection beyond 1 year
  - Broad immunity against antigenically diverse strains

- Immune correlates of protection required for universal protection is still not answered
  - Combination of T cells and B cells?



Acknowledgements: Kedzierska Lab

Shanghai Public Health Clinical Centre, Fudan University
Prof Jianqing Xu and the Team

WHO Influenza Centre
A/Prof Aeron Hurt
Prof Ian Barr

Alfred Hospital
Prof Allen Cheng
A/Prof Tom Kotsimbos

Monash University
Prof Jamie Rossjohn
Dr Stephanie Gras
Prof Anthony Purcell
Dr Nicole Mifsud
Dr Patricia Illing

St Jude Research Children's Hospital, Memphis
A/Prof Paul Thomas
Dr Jeremy Crawford
Dr Emma Allen