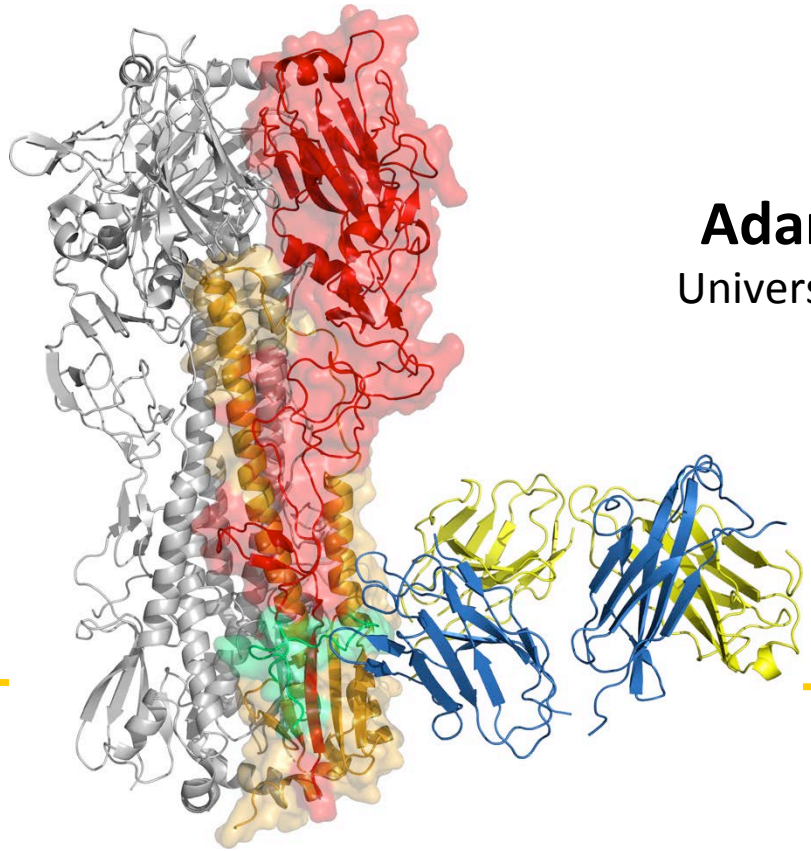
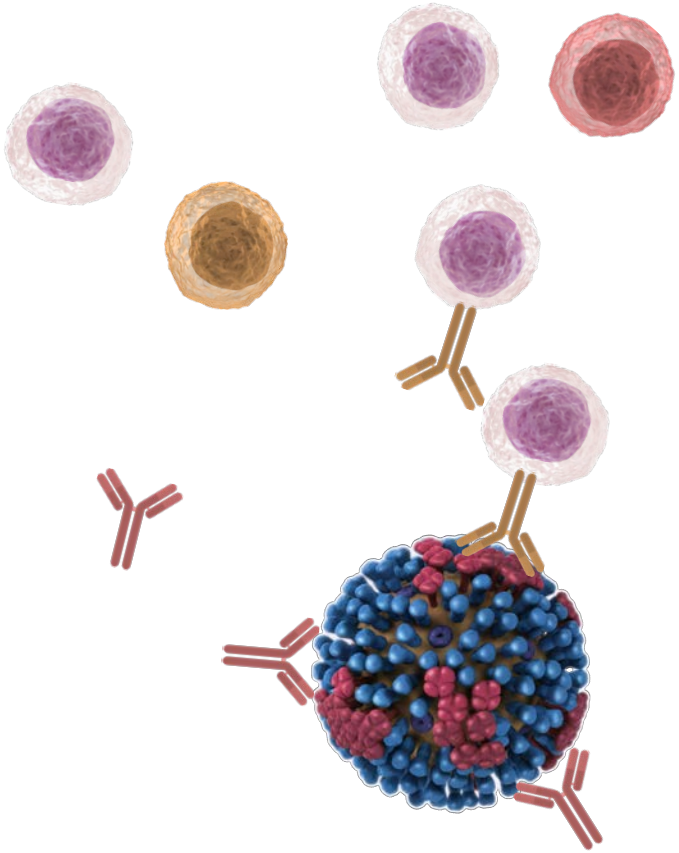
Universal vaccines and monoclonal antibodies



Adam Wheatley
University of Melbourne



Vaccine protection against influenza

Vaccines for epidemic influenza (seasonal)

- Trivalent or quadrivalent formulations
- Inactivated viral preparations mostly HA (IIV3/4)
- Live attenuated? (LAIV3/4)
- **Limitations:**
  - Protection limited to closely matched strains – narrow
  - Variable year to year effectiveness
  - Necessitates influenza surveillance and frequent vaccine reformulation

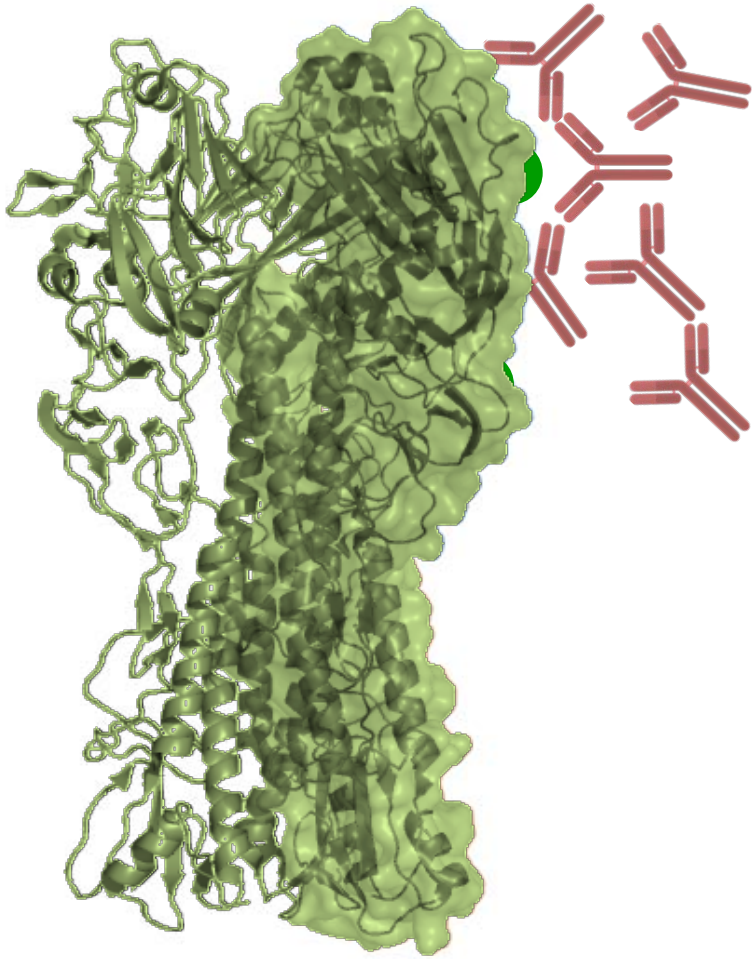
Vaccines for pandemic influenza

- Monovalent formulations against H5N1 or H7N9 strains
- **Limitations:**
  - Long lead times for production → stockpiling
  - Unclear if emergent viruses will match vaccine stocks

So why do current influenza vaccines provide such narrow protection?

The viral hemagglutinin (HA) – a shifting target for antibody

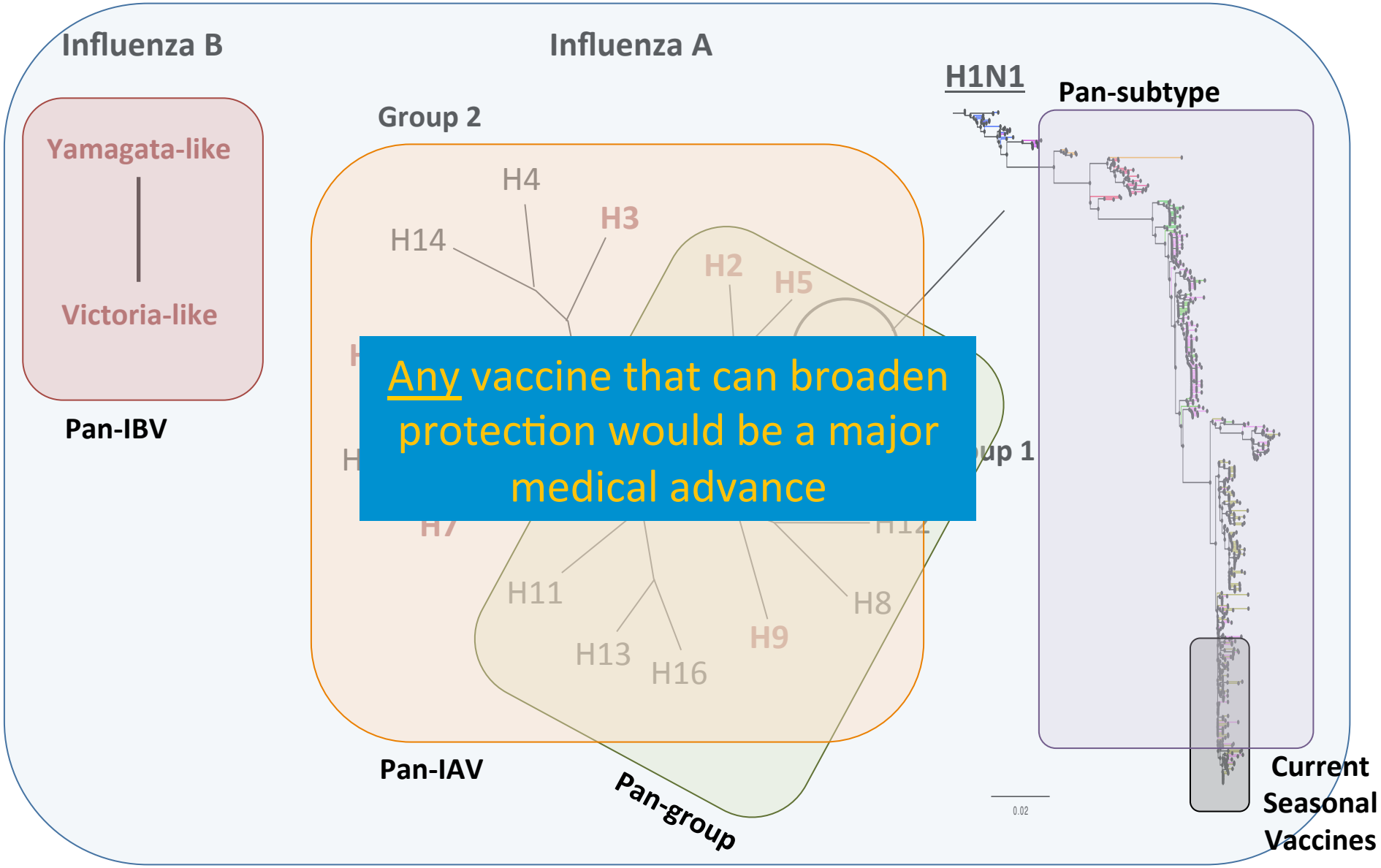
- Immunity preventing acquisition of influenza is primarily mediated by antibody to the major surface proteins - hemagglutinin (HA) and neuraminidase (NA)



Gamblin et al 2004; PDB ID:1RUZ

- Protective antibodies elicited by infection or vaccination target variable sites surrounding the RBD → blocks viral entry
- Antigenic changes driven by immune pressure leads to a progressive loss of immune recognition (**antigenic drift**)
  - Loss of vaccine effectiveness
- Genetic recombination with environmental viruses (IAV) (**antigenic shift)** and complete loss of immune recognition
  - Loss of vaccine effectiveness

Extending influenza vaccine coverage



UNIVERSAL VACCINE

Diverse strategies to elicit universal influenza immunity

Common Goal

- Immune responses (vaccines) that better account for antigenic diversity
- Simultaneous protection from both circulating seasonal (antigenic drift) and emerging/pandemic (antigenic shift) influenza strains

1. Cross-reactive T cells

- Antiviral CD4 or CD8 responses targeted to highly conserved viral epitopes
- Limit pathogenesis, viral replication and transmission

2. Antibody responses to conserved viral proteins

- Deriving immunogens from viral proteins with comparatively higher sequence conservation
  - NP, M2, NA

3. Cross-reactive antibody responses to HA

- Identification of highly conserved protective/neutralizing epitopes in HA
- Focusing the humoral responses towards protective neutralising epitopes

A strategy for HA-directed universal vaccines

1. Identify cross-reactive HA epitopes recognised by human antibody/B cells
   - Map epitopes
   - Define the prevalence and ontogeny
   - Define protective breadth, potency and mechanism

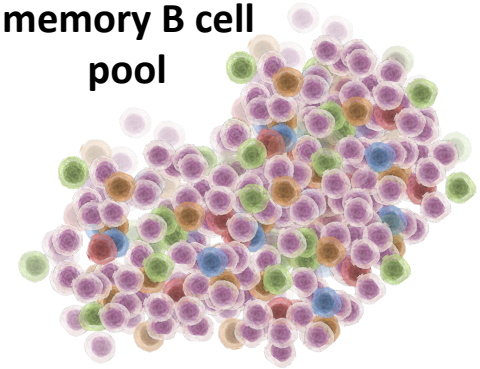
2. Reprogram vaccine immunity to target these epitopes
   - Maximise humoral responses targeting cross-reactive epitopes
   - Minimise humoral responses targeting canonical strain-specific epitopes

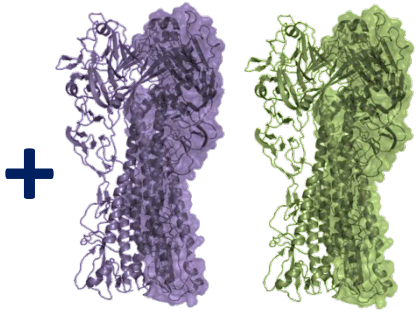
Interrogation of memory B cell pools using recombinant HA probes

Serum antibody

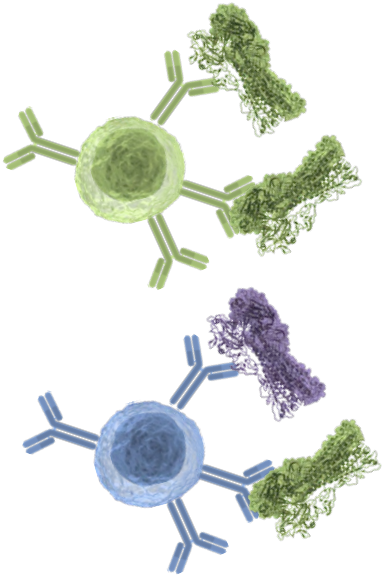
- HI assay – indirect neutralisation at the RBD
- Direct virus neutralisation assays
- Antibody effector functions (ADCC, ADCP)

Long lived memory B cell pool





Fluorescent rHA tetramers

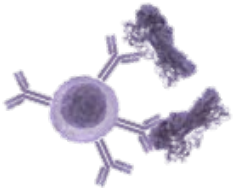


Single specificity

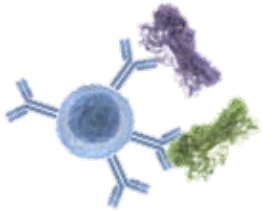
Cross-reactive

HA probes – Whittle et al. (2013) *J Virol* 88(8):4047-57

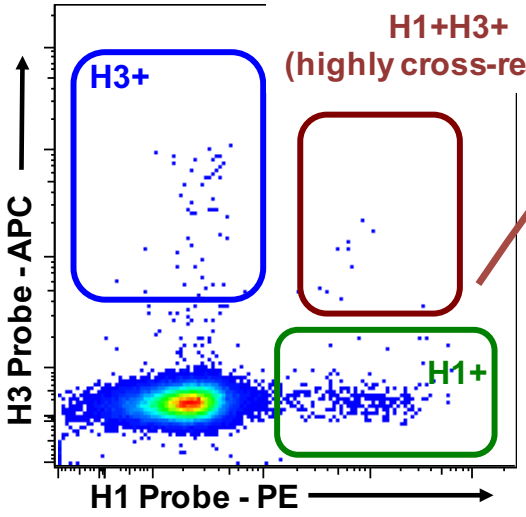
Characterising frequency, phenotype and specificity of HA probe+ B cells



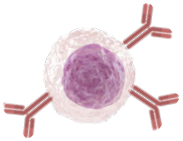
Single specificity



Cross-reactive

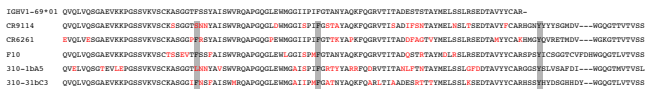


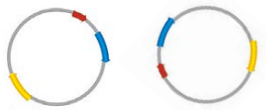
Single specificity



B cell

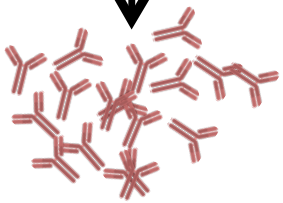
BCR sequences





Heavy Light

Expression plasmids

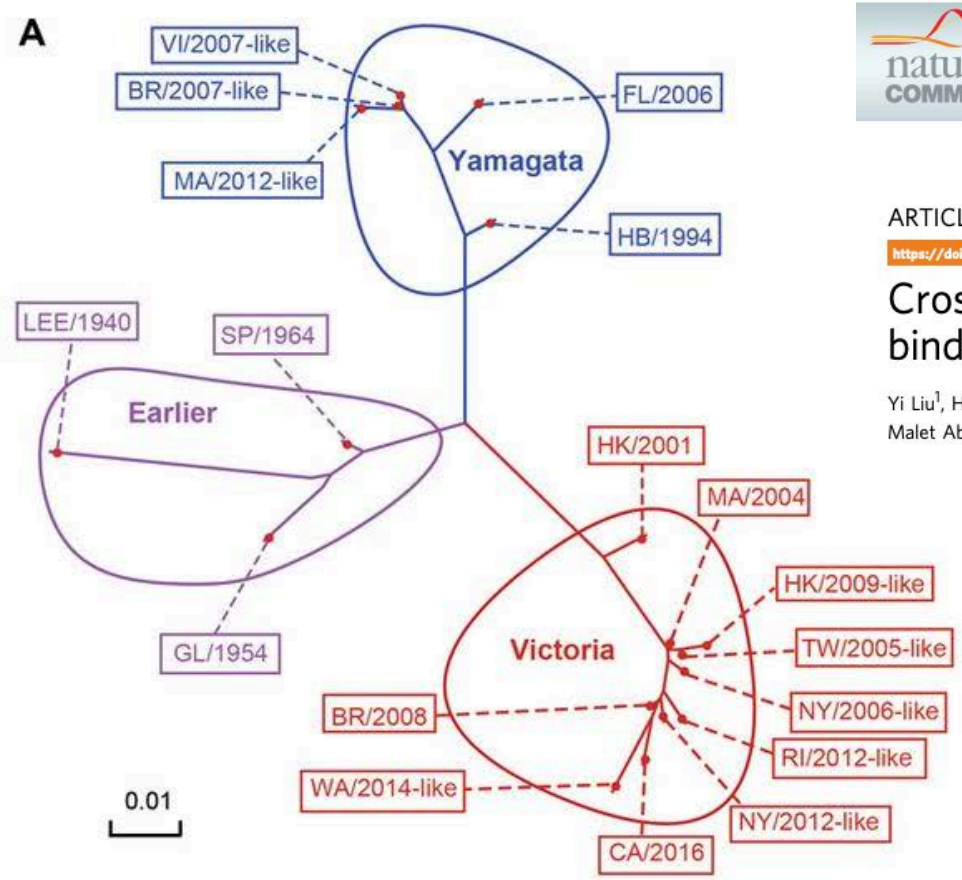


Recombinant mAbs

- Single B cells sorted by flow cytometry
- Generate cDNA
- Multiplex PCR to recover immunoglobulin genes
- Sanger or deep sequencing
- Cloned into mammalian expression vectors
  - Recombinant antibodies
  - Functional characterisation

HA probes – Whittle et al. (2013) *J Virol* 88(8):4047-57

Cross-reactive recognition of influenza B by human antibody



nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-018-08165-y OPEN

Cross-lineage protection by human antibodies binding the influenza B hemagglutinin

Yi Liu[1], Hyon-Xhi Tan[1], Marios Koutsakos[1], Sinthujan Jegaskanda[1], Robyn Esterbauer[1], Danielle Tilmanis[2], Malet Aban[2], Katherine Kedzierska[1], Aeron C. Hurt[1,2], Stephen J. Kent[1,3,4] & Adam K. Wheatley[1]

Questions?

- How much cross-reactivity between the two lineages?
- Is the IBV stem a target?
- IIV3 vs IIV4?

Shen et al. (2017)

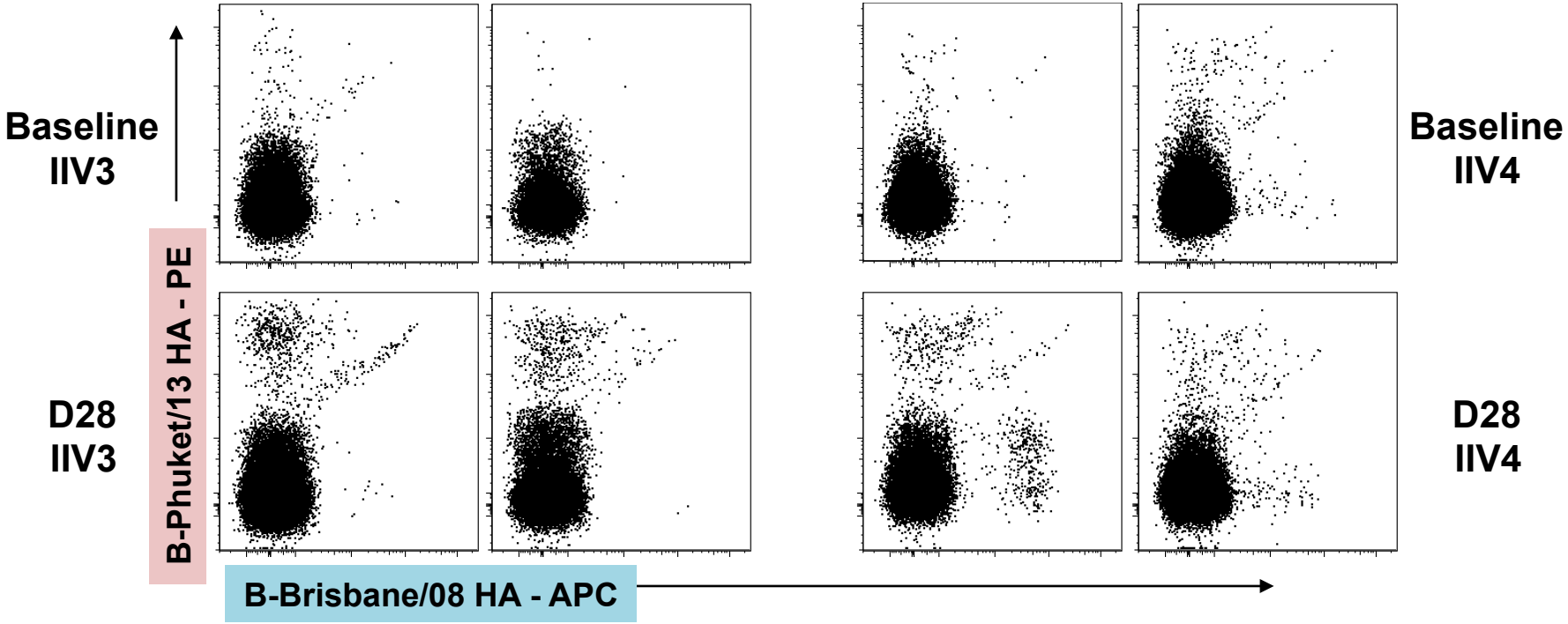
Resolving HA-specific B cell populations in IIV3 and IIV4 cohorts

- Recruited 29 subjects (25-56 years old)
- Immunised with 2015 Afluria® (BioCSL)

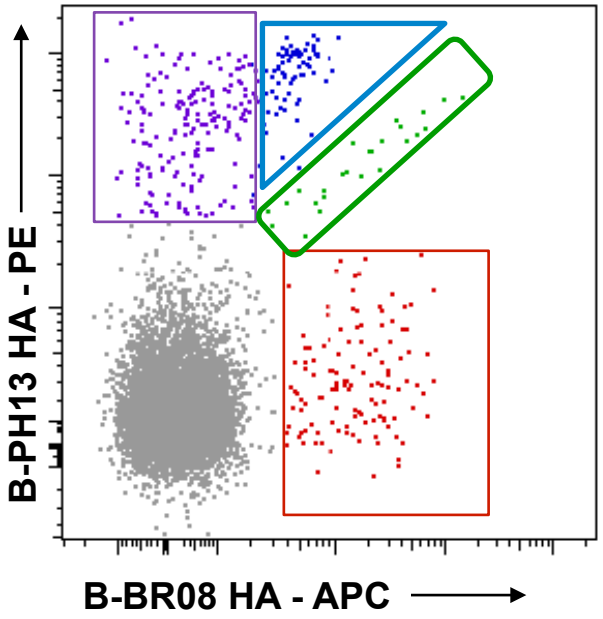
A (H1N1): an A/California/4/2009 (H1N1)pdm09 like virus
A (H3N2): an A/Switzerland/9715293/2013 (H3N2) like virus
B: a **B/Phuket/3073/2013** like virus.

- Recruited 20 subjects (25-56 years old)
- Immunised with 2016 Fluquadri® (Sanofi)

A (H1N1): an A/California/4/2009 (H1N1)pdm09 like virus
A (H3N2): an A/Hong Kong/4801/2014 (H3N2) like virus
B: a **B/Brisbane/60/2008** like virus
B: a **B/Phuket/3073/2013** like virus.



Seasonal vaccines drive the expansion of IBV cross-reactive B cells

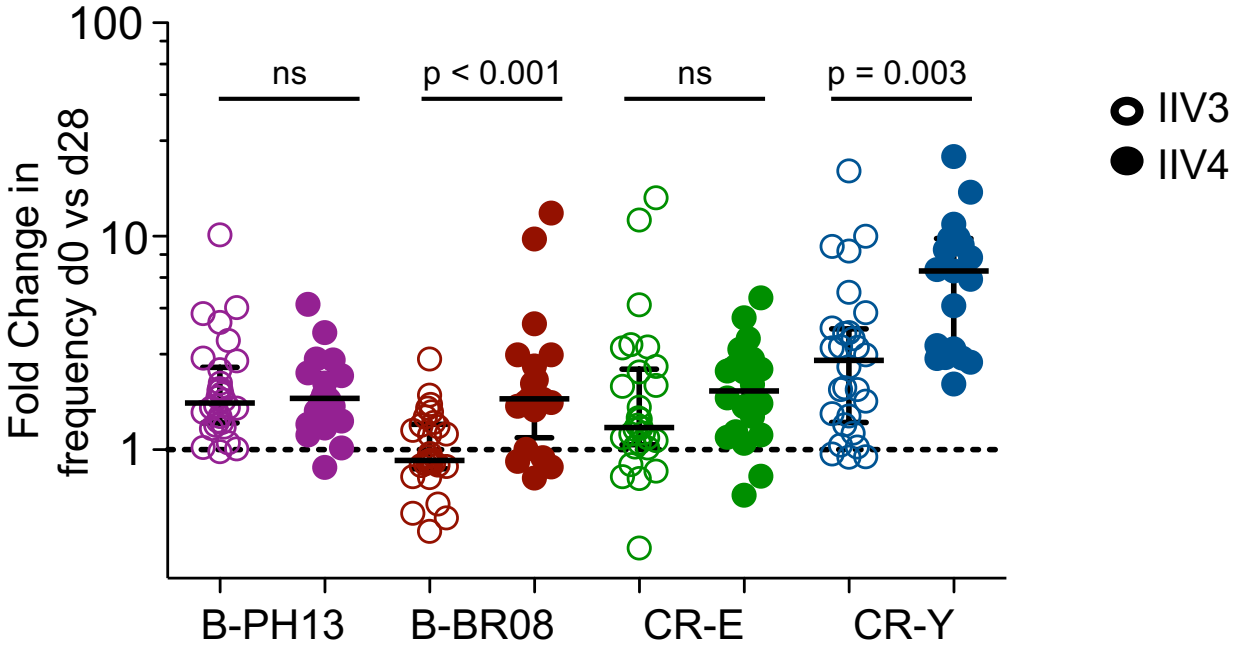


B-Phu13-specific (Yamagata)

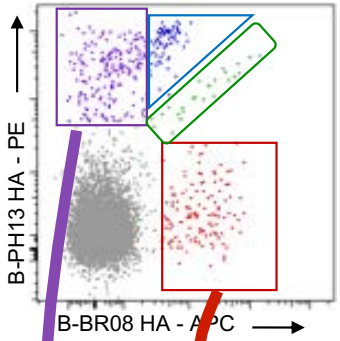
Cross-reactive, Yamagata preference (CR-Y)

Cross-reactive, equivalent (CR-E)

B-Bri08-specific (Victoria)



“Strain-specific” B cells actually recognise pan-lineage HA



B-Phu13-specific (Yamagata)
Cross-reactive, Yamagata preference (CR-Y)
Cross-reactive, equivalent (CR-E)
B-Bri08-specific (Victoria)

B-Bri08+

B-Phu13+

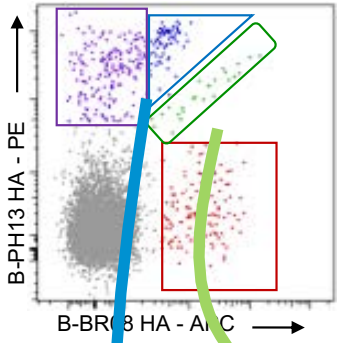
Sort Population	mAb	ELISA											HIA			FRA		
		Yamagata			Victoria				HA1		Stem							
		PH/13	FL/06	YAM/88	BR/08	MA/04	VIC/87	LEE/40	PH-HA1	BR-HA1	PH-SS	BR-SS	PH/13	BR/08	LEE/40	PH/13	BR/08	LEE/40
	K77-1C06																	
	W85-1E03																	
	K77-2D11																	
	W85-2C03																	
	R95-1D08																	
	K77-2E02																	
	W85-2D09																	
	R95-1D10																	
	R95-1E06																	
	R95-1E12																	
	W85-1B01																	
	R95-1E05																	
	K77-1G12																	
	W85-1B01																	
	W85-1C08																	
	K77-1H02																	
	W85-1C09																	
	W85-1D01																	
	W85-2B04																	

ELISA
- EC_{50} <0.01 µg/ml
- EC_{50} 0.01-0.1 µg/ml
- EC_{50} 0.1-1 µg/ml
- EC_{50} >1 µg/ml
- Weak binding

HIA
- >10240
- 1280-5120
- 160-640
- 10-80
- <10

FRA
- >1280
- 1280
- 160-640
- 10-80
- <10

Highly cross-reactive B cells bind the IBV HA head and stem



B-Phu13-specific (Yamagata)
Cross-reactive, Yamagata preference (CR-Y)
Cross-reactive, equivalent (CR-E)
B-Bri08-specific (Victoria)

Cross-reactive equivalent (CR-E)

Cross-reactive Yamagata (CR-Y)

Sort Population	mAb	ELISA										HIA			FRA		
		Yamagata			Victoria				HA1		Stem						
		PH/13	FL/06	YAM/88	BR/08	MA/04	VIC/87	LEE/40	PH-HA1	BR-HA1	PH-SS	BR-SS	PH/13	BR/08	LEE/40	PH/13	BR/08	LEE/40
	W85-3F06																	
	K77-1C02																	
	R95-1H09																	
	K77-1G03																	
	R95-1B11																	
	K77-1G12																	
	R95-1C01																	
	R95-1D05																	
	K77-2B06																	
	K77-2D11																	
	R95-1E03																	
	R95-1E05																	
	K77-2H12																	
	R95-1E07																	
	R95-1F04																	
	W85-1A07																	
	R95-1H08																	
	K77-1H05																	
	W85-3E10																	
	R95-1H09																	
	K77-2C08																	
	W85-3G03																	
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	K77-2E07																	
	R95-2A08																	
	R95-2C02																	
	R95-2G10																	

ELISA
- EC_{50} <0.01 µg/ml
- EC_{50} 0.01-0.1 µg/ml
- EC_{50} 0.1-1 µg/ml
- EC_{50} >1 µg/ml
- Weak binding

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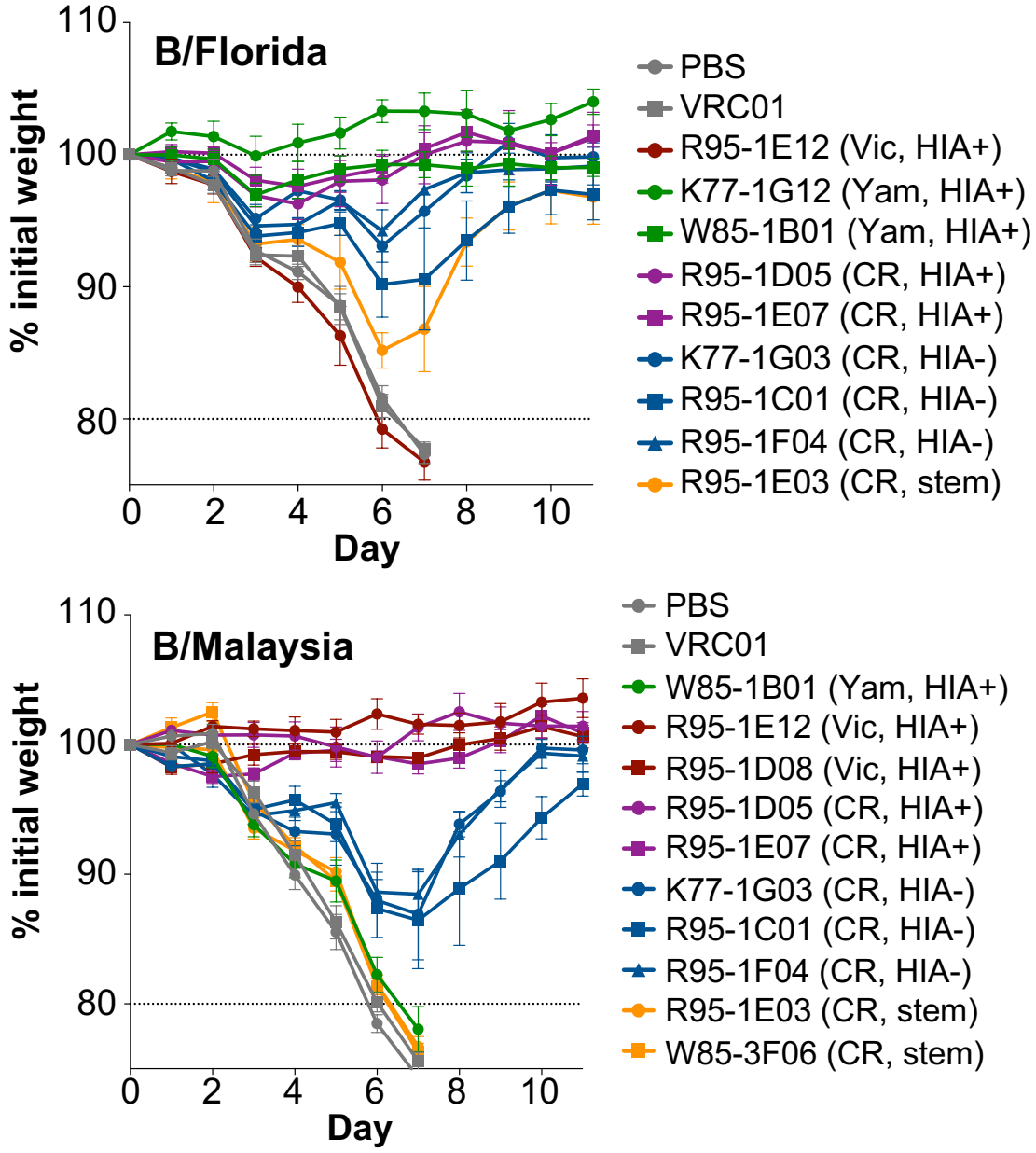
- >10240
- 1280-5120
- 160-640
- 10-80
- <10

FRA
- >1280
- 1280
- 160-640
- 10-80
- <10

Passive protection by human mAbs in IBV challenged mice



Summary

- IBV ”strain-specific” memory B cells recognise pan-lineage HA (>25y antigenic drift)
  - Contrasts with IAV

- Cross-lineage B cells are common in seasonal vaccine recipients
  - CR-E B cells – often pan-IBV recognition
  - CR-Y B cells - more patchy

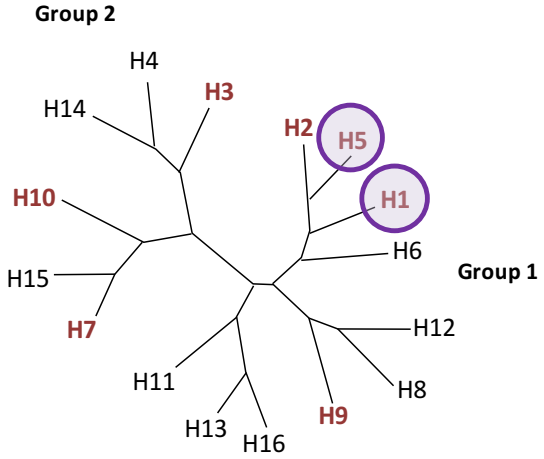
- Monoclonal antibodies derived from cross-lineage B cells
  - Protect mice in passive infusion studies
  - Can recognise HAI+ epitopes in the HA head
  - Often require Fc function for protection
  - Stem mAbs are poorly protective

- Both IIV3 and IIV4 drive expansion of IBV cross-reactive B cells

- **Cross-reactive B cell interrogation and isolation of monoclonal antibody lineages**
  - **Potential to inform vaccine design**
  - **Identify novel immunotherapeutics**

What about IAV?

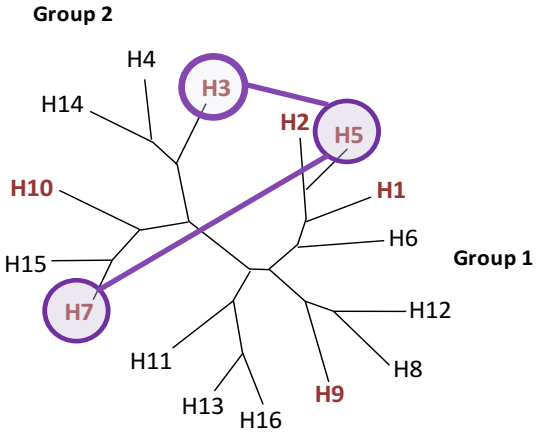
inter-group1



- Mostly stem
- Frequent
- Neutralising
- VH1-69 derived
- Age-associated increase in frequencies
- Inducible by immunisation (H5, pH1)

Whittle et al. (2014) *J Virology*
Wheatley et al. (2015) *J Immunol*

group1/2

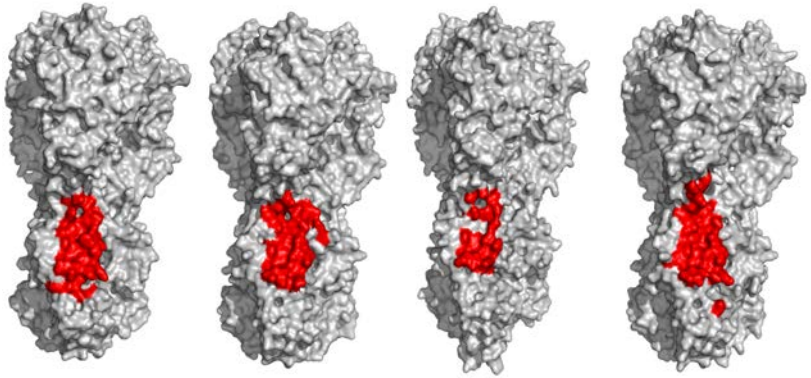


- Mostly stem
- Infrequent
- Neutralising
- Found in common ”classes” between donors
- Inducible by immunisation (H5, H7)

Joyce et al. (2016) *Cell*
Andrews et al. (2017) *Sci Immunol*

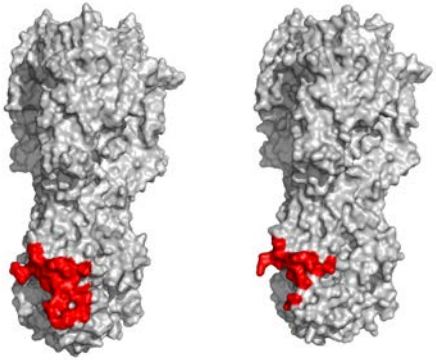
For IAV - the HA stem as conserved “supersite” of antibody recognition

Pan-Group1 (mostly VH1-69)



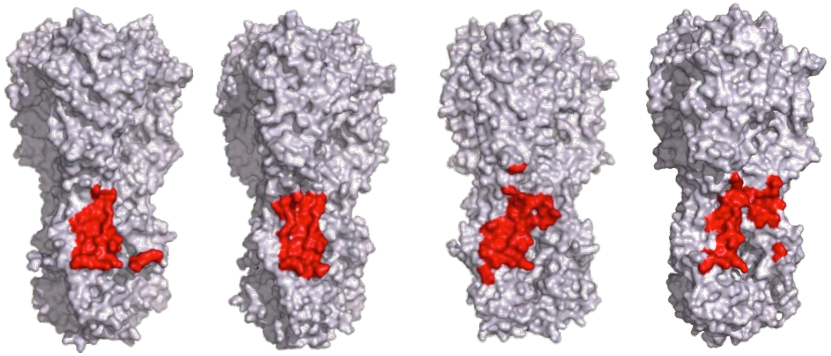
C179 CR6261 F10 3.1

Pan-Group2

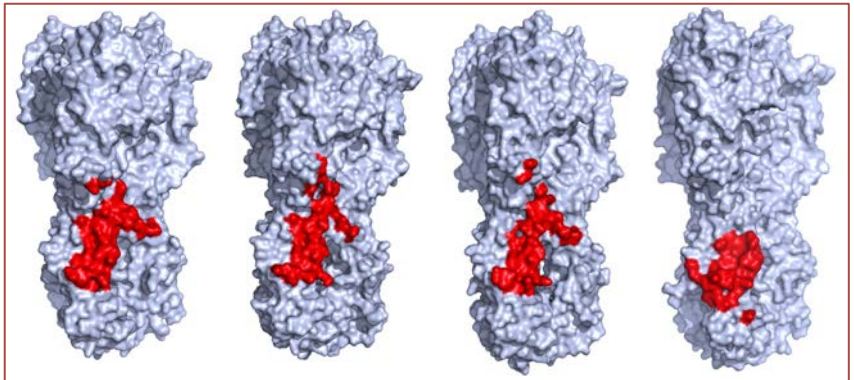


CR8020 CR8043

Inter-group



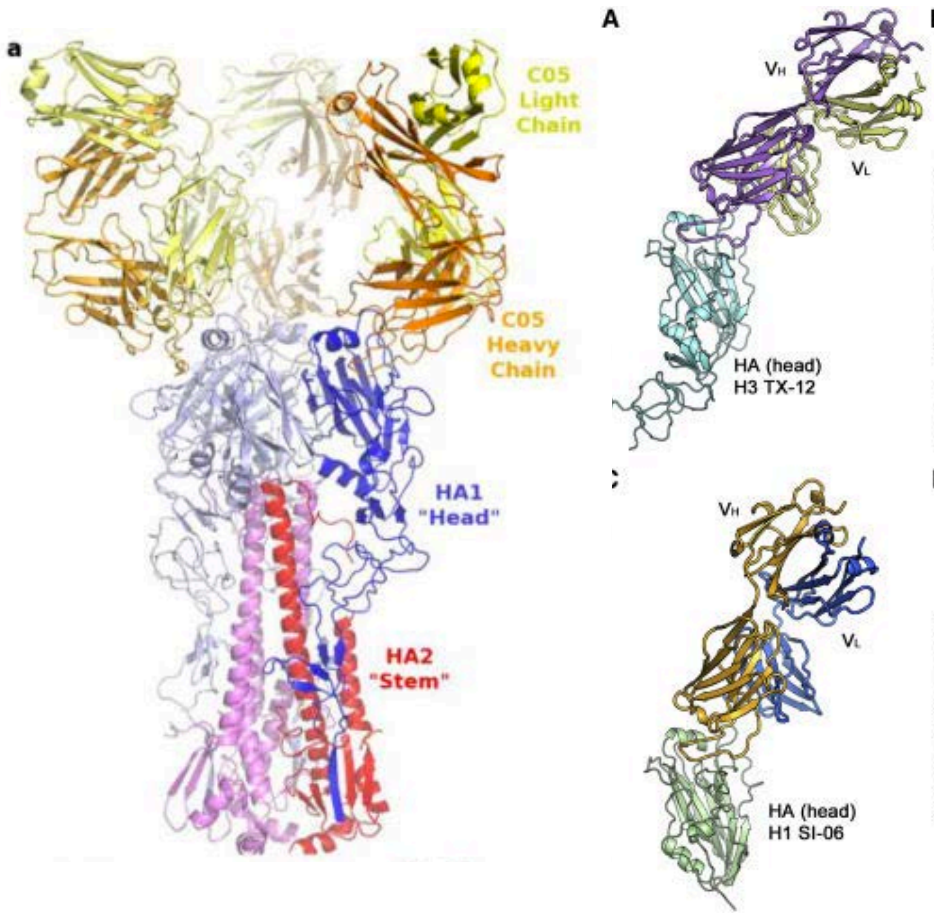
FI6v3 CR9114 39.29 CT149



31.a.83 16.a.26 16.g.07 56.a.09

Cross-reactive, non-stem epitopes?

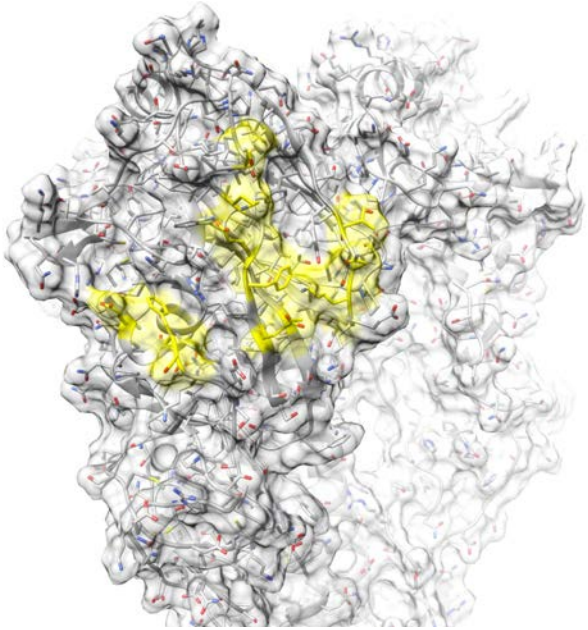
mAbs that mimic the SA receptor



Ekiert et al. Nature. 2012

McCathey et al. Immunity. 2018

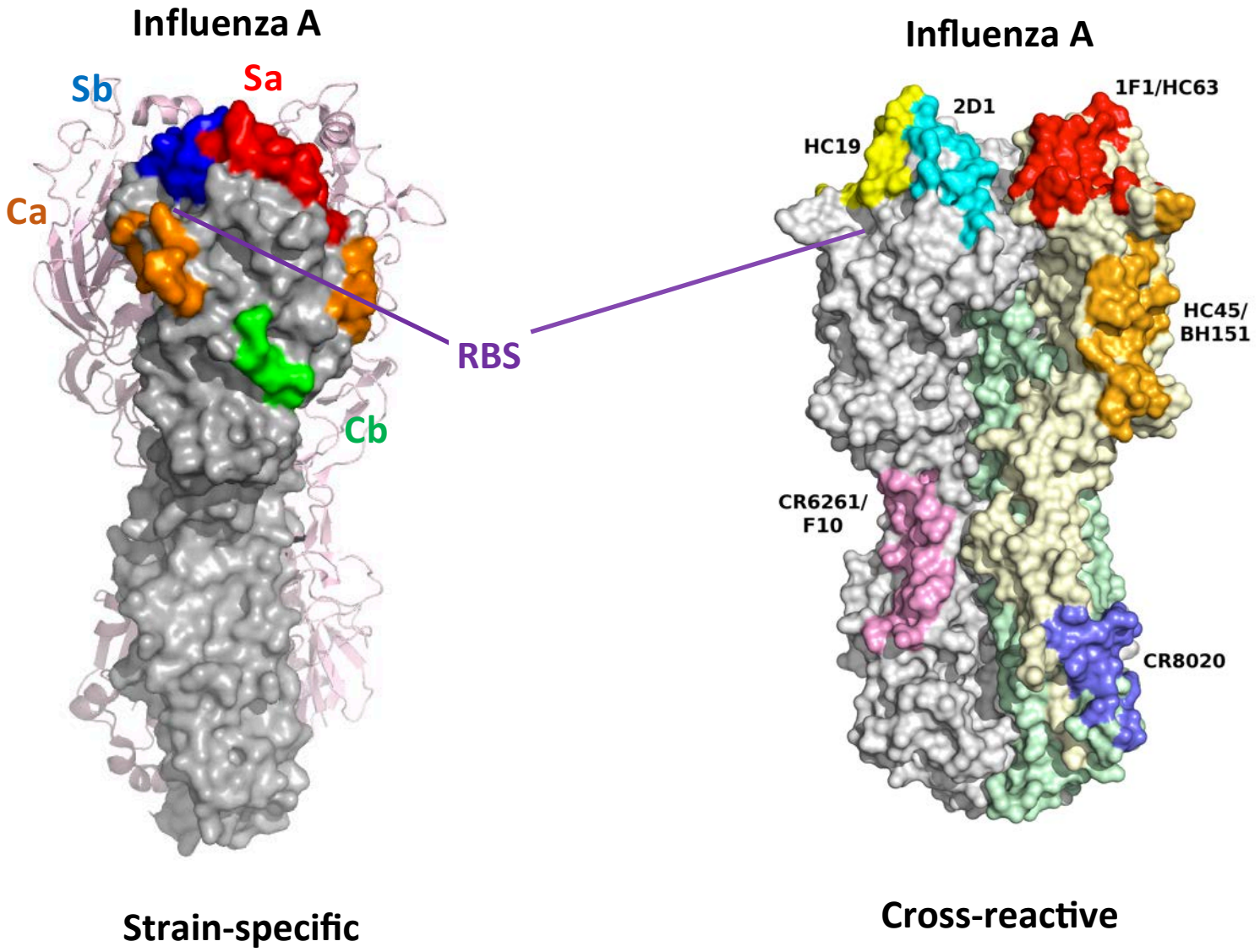
Alternative sites in the HA head



mAb 446D1
Pan-H1 activity

Kanekiyo et al. Nat Immunol. *In Press*

Moving on from classical epitopes to new targets (IAV)

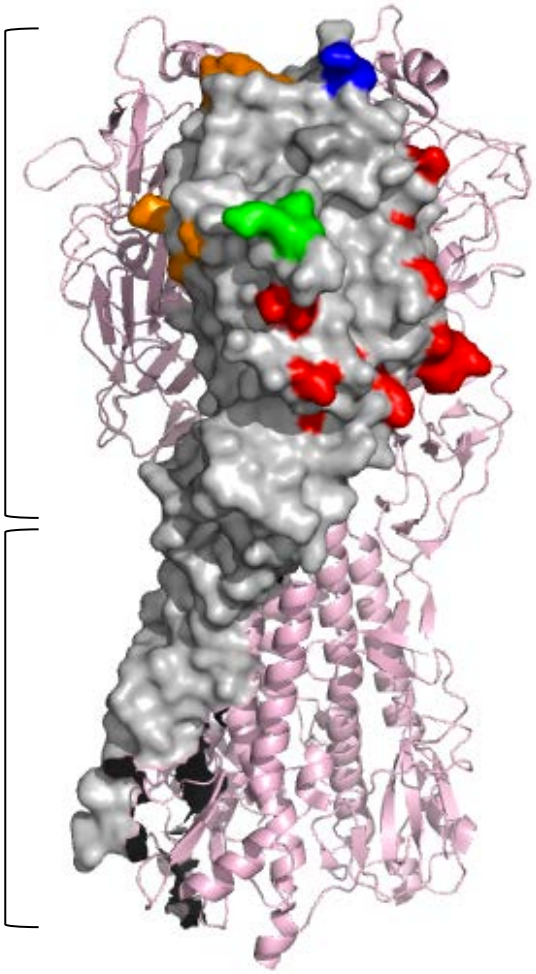


Moving on from classical epitopes to new targets (IBV)

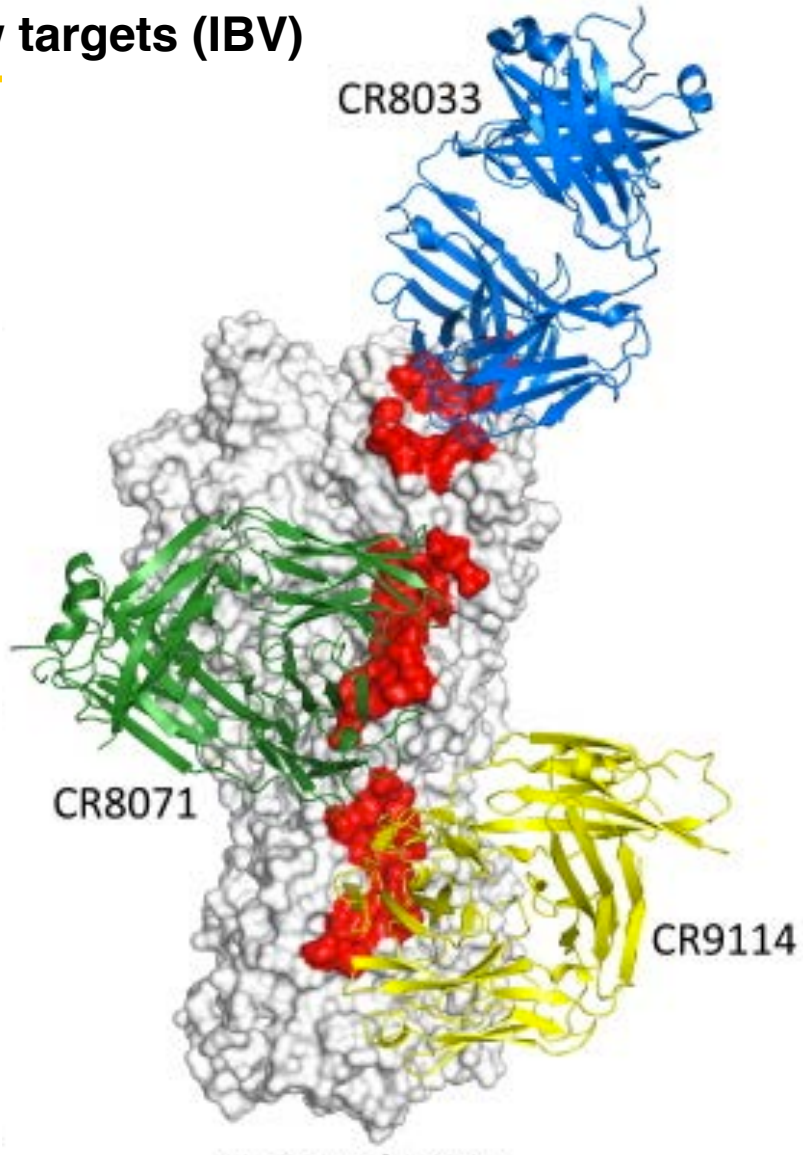
Influenza B

HA1 (head)

HA2 (stem)



Strain-specific



Cross-reactive

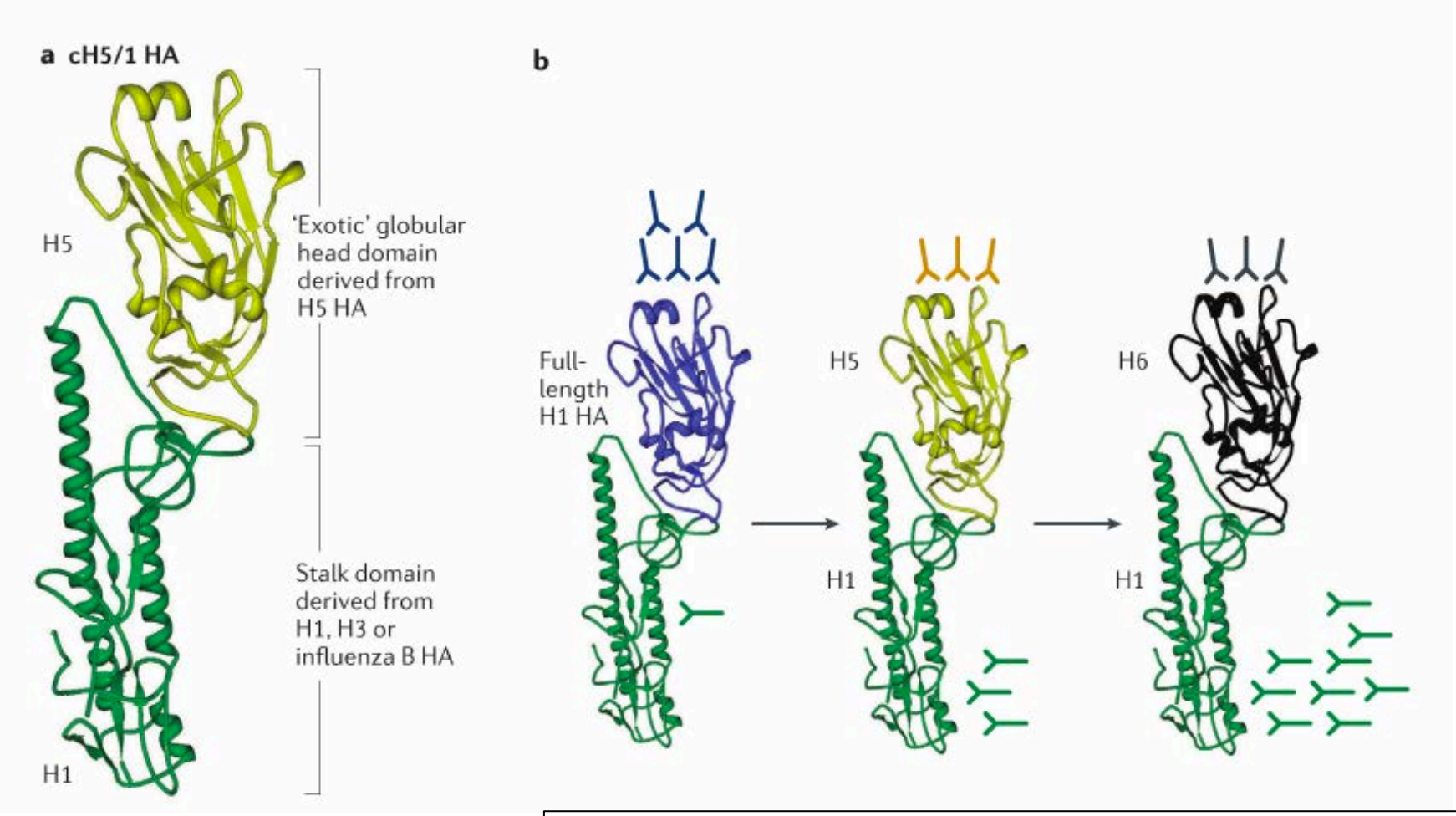
A strategy for HA-directed universal vaccines

1. Identify cross-reactive HA epitopes recognised by human antibody/B cells
   - Map epitopes
   - Define the prevalence and ontogeny
   - Define protective breadth, potency and mechanism

2. Reprogram vaccine immunity to target broadly protective epitopes
   - Maximise humoral responses targeting cross-reactive epitopes
   - Minimise humoral responses targeting canonical strain-specific epitopes

Vaccination with chimeric HA

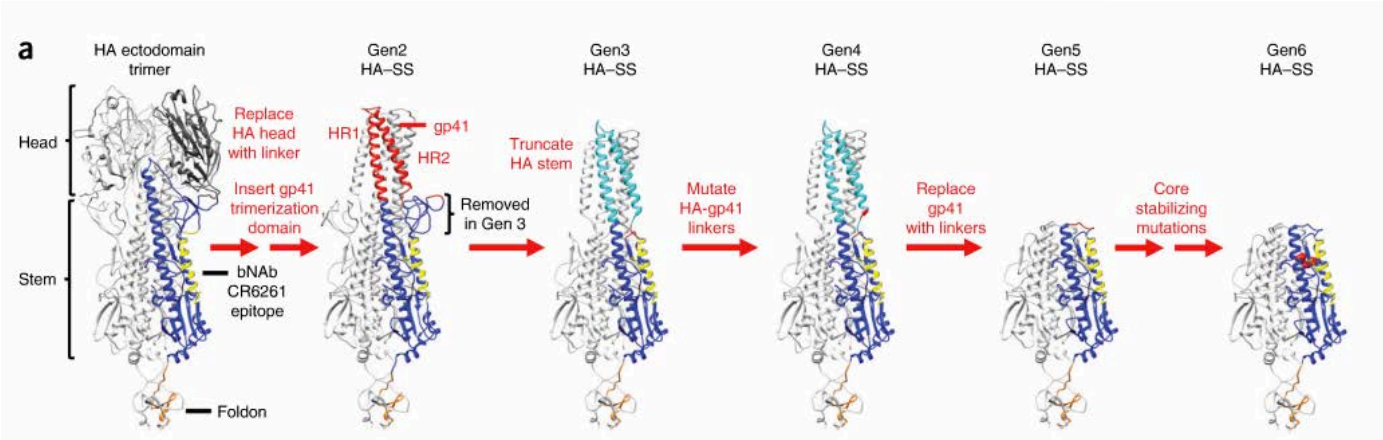
- Strategies that maximise exposure of the HA stem by sequential vaccination with chimeric HA molecules bearing a common HA2 domain
- →Major advantage – could utilise existing vaccine production pathways



Krammer and Palese (2015) *Nat Rev Drug Discov* 14:167-182

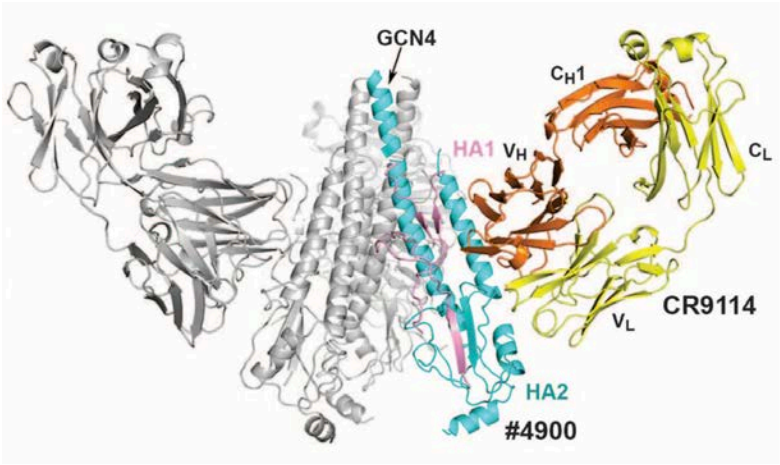
Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection

Hadi M Yassine[1,6], Jeffrey C Boyington[1,6], Patrick M McTamney[1,5,6], Chih-Jen Wei[1,5,6], Masaru Kanekiyo[1], Wing-Pui Kong[1], John R Gallagher[2], Lingshu Wang[1], Yi Zhang[1], M Gordon Joyce[1], Daniel Lingwood[1,5], Syed M Moin[1], Hanne Andersen[3], Yoshinobu Okuno[4], Srinivas S Rao[1,5], Audray K Harris[2], Peter D Kwong[1], John R Mascola[1], Gary J Nabel[1,5] & Barney S Graham[1]



A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen

Antonietta Impagliazzo,[1*†] Fin Milder,[1‡§] Harmjan Kuipers,[1‡§] Michelle V. Wagner,[2‡||] Xueyong Zhu,[3‡] Ryan M. B. Hoffman,[3‡] Ruud van Meersbergen,[1§] Jeroen Huizingh,[1§] Patrick Wanningen,[1§] Johan Verspuij,[1§] Martijn de Man,[1§] Zhaoqing Ding,[2||] Adrian Apetri,[1†] Başak Kükrer,[1†] Eveline Sneekes-Vriese,[1] Danuta Tomkiewicz,[1†] Nick S. Laursen,[3¶] Peter S. Lee,[3] Anna Zakrzewska,[1§] Liesbeth Dekking,[1§] Jeroen Tolboom,[1§] Lisanne Tettero,[1§] Sander van Meerten,[1§] Wenli Yu,[3] Wouter Koudstaal,[1†] Jaap Goudsmit,[1†] Andrew B. Ward,[3] Wim Meijberg,[1§] Ian A. Wilson,[3*] Katarina Radošević[1#]



Yassine et al. (2015) *Nat Med* 21(9):1065-70

Impagliazzo et al. (2015) *Science* 349(6254):1301-6

Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection

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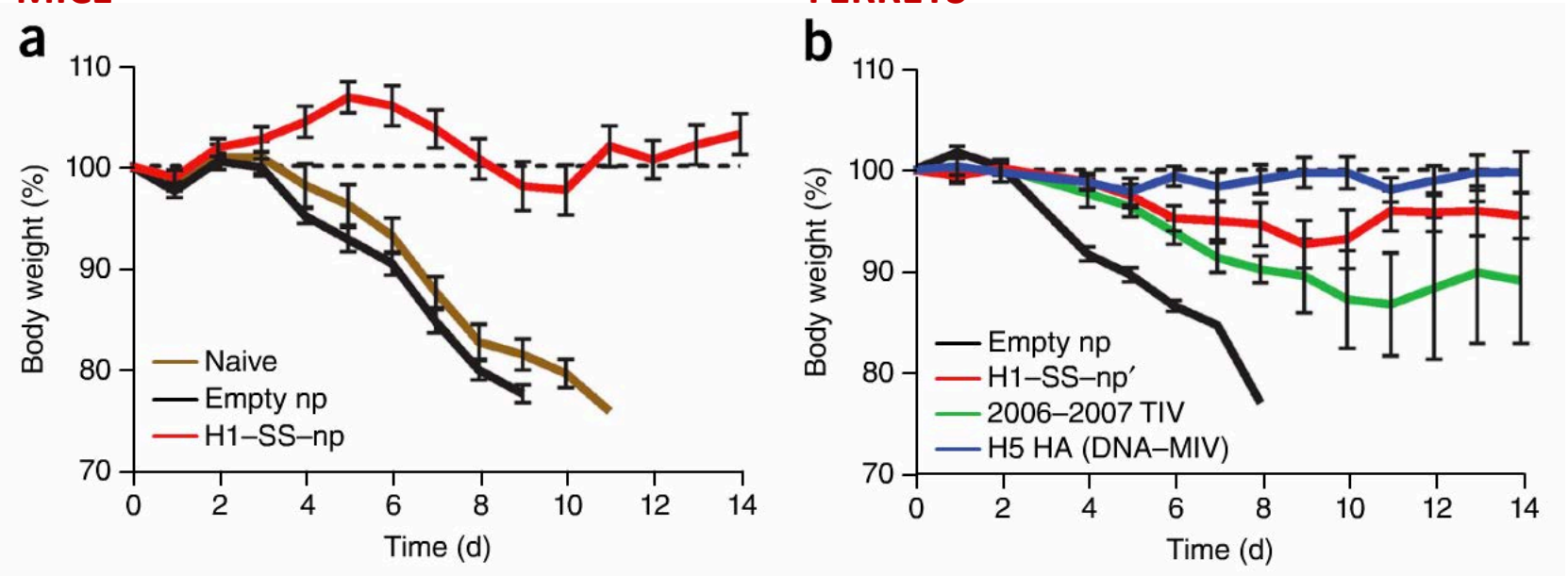
MICE **FERRETS**



- Vaccination with “stabilised stem” nanoparticles derived from seasonal H1N1 (A/New Caledonia/20/99) protected against lethal H5N1 (VN) challenge in mice and ferrets

Yassine et al. (2015) *Nat Med* 21(9):1065-70

Summary – Human cross-reactive HA epitopes

- Humans make subdominant but highly cross-reactive humoral responses to influenza
- Cross-reactive memory B cells recognise epitopes in both the head (HA1) and stem (HA2) domain
- Can mediate a range of effector functions – ADCC etc.
- To date, the broadest neutralising antibodies all bind in the HA stem
- Epitopes and B cell lineages remarkably conserved between antibodies/subjects
  - Convergent structures
  - Convergent genetic elements
- Breadth can extend to:
  - IAV - diverse Group1 and Group2 viruses
  - IBV – both antigenic lineages

Summary – HA-based UIV approaches

- Both chimeric HA and headless stem only constructs
  - Commencing clinical testing

- **Question - Translatability of animal immunisation models to humans**
  - Clarify the influence of pre-existing baseline immunity in humans
  - Development of animal models to better account for influenza immunity

- **Question - Can dominant HAI+ antibody responses be avoided/subverted?**

- **Question – Is HA stem antibody at all protective?**
  - Acquisition
  - Severity

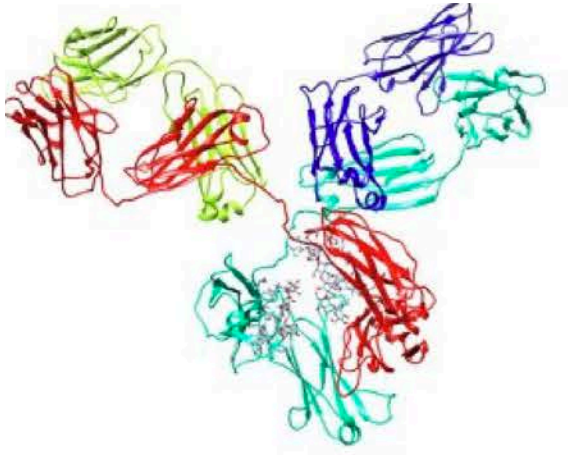
The case for monoclonal antibody therapeutics/prophylactics

There is a need for novel agents for:

- clinical treatment of severe influenza infection

- pandemic prophylaxis

What about mAbs?



- **Advantages**
  - Rapid activity
  - High tolerability
  - Low/no resistance
  - Long biological half-life (advantage for prophylaxis)

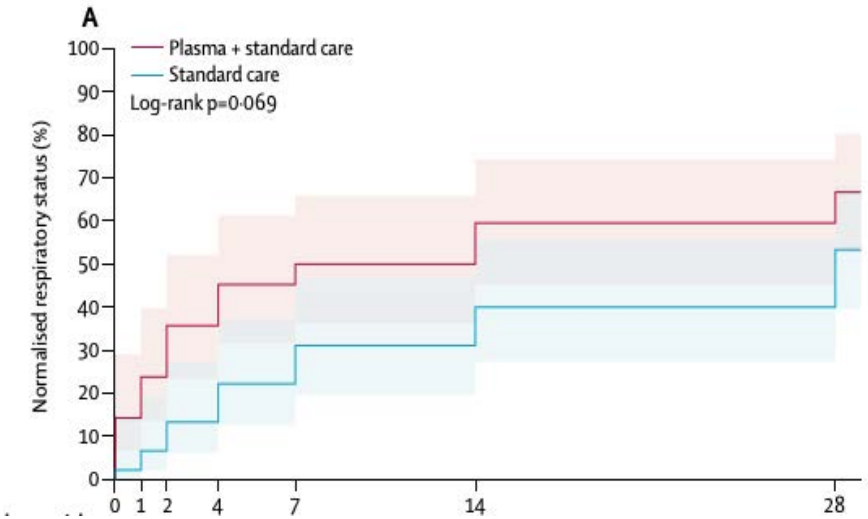
- **Disadvantages**
  - Cost (getting lower but still high)
  - Need to account for viral diversity

Anti-influenza mAbs – are they actually protective?

- 1918 influenza pandemic – treatment with plasma, serum or blood
  - Reduced case-fatality rate – (16% vs 37%) – Luke et al. 2006 *Ann Intern Med*

- Immune plasma for the treatment of severe influenza
  - Some potential clinical benefit
  - Beigal et al. 2017 *Lancet Respir Med*

- INSIGHT FLU005
  - Assessing flu-IVIG for clinical benefit

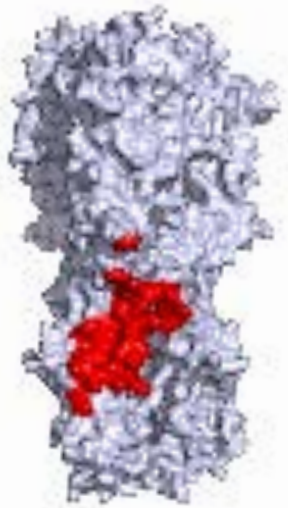


- Human monoclonal antibodies – extensively characterised in mice and ferrets
  - Effective treatment – Up to 3 days post-infection
  - Prophylactic protection against seasonal and high path avian viruses
  - Generally utilise Fc-FcR interactions (ADCC, ADP) for protection

Anti-influenza mAbs – human clinical trials

MEDI8852 (Medimmune) – Group1/Group2 IAV stem
- reduced symptoms during natural infection
- Ali et al. 2018 *AAC*

MHAA4549A (Genentech) - Group1/Group2 IAV stem
- Reduction in viral burden during H3N2 challenge
- McBride et al. 2017 *AAC*



MHAA4549A

VIS410 (Visterra) - Group1/Group2 IAV stem
- Reduction in viral shedding during pH1N1 challenge : Sloan et al. Options IX

CR9114/CR6261/CR8020 (Crucell) - Group1 and/or Group2 IAV stem
- Trials pulled or not reported

TCN-032 (Theraclone) - anti-M2e
- lower symptom scores and shedding during H3N2 challenge : Ramos et al. 201

Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin

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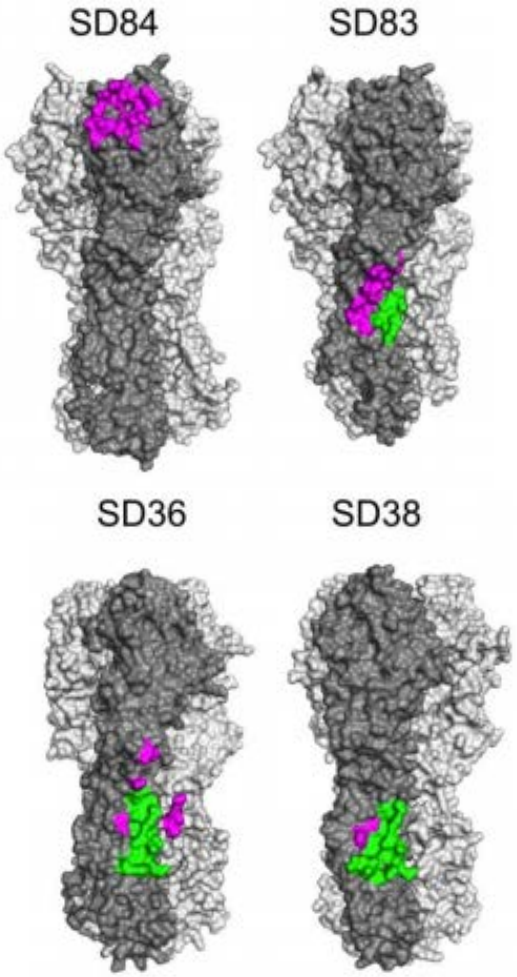

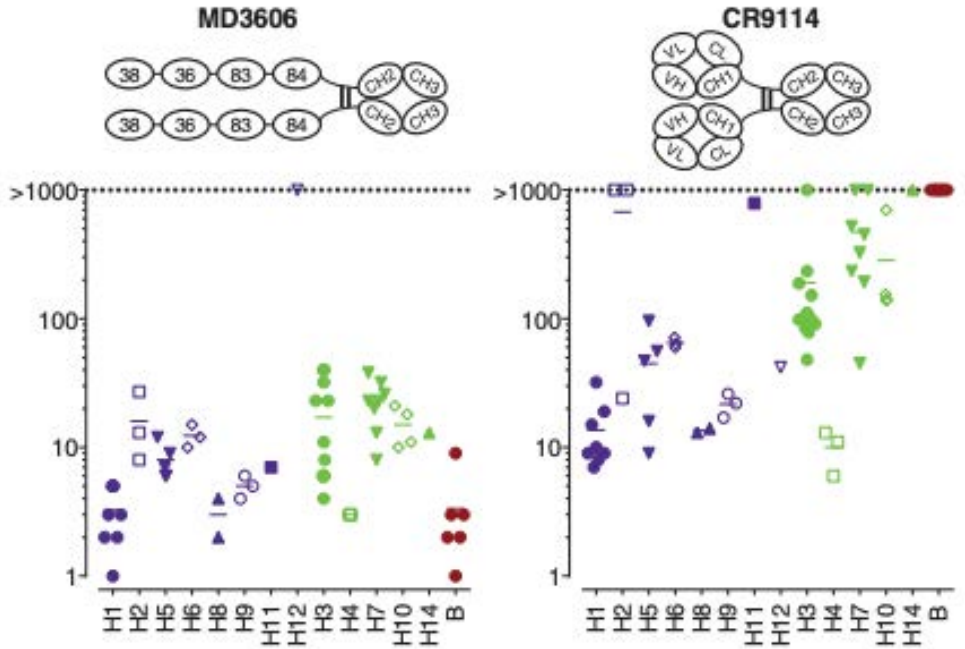

Laursen et al. (2018) *Science*

Summary – anti-influenza mAbs

- Animal trials consistently show human mAbs have promise

- Evidence of clinical benefit for mAb treatment in humans still sparse
  - Natural infection - some symptom alleviation
  - Experimental challenge models - some reductions in viral burden
  - Hampered by poor reporting
  - Larger trials are ongoing

- No demonstration of prophylactic effect to date in humans

- New mAbs or antibody-engineering technologies
  - Opportunity for using potent neutralising antibodies while maintaining effective breadth
  - Opportunity to increase biological half-lives, respiratory tract delivery

Acknowledgments

Stephen Kent
Hyon-Xhi Tan
Jen Juno
Yi Liu
Hannah Kelly
Julius Wong
Wenbo Jiang
Robyn Esterbauer
Vinca Alcantara
Sinthujan Jegaskanda
Katherine Kedzierska
Oanh Nguyen
Marios Koutsakos
Kanta Subbarao
Aeron Hurt
Danni, Malet
Adrian McDermott
Rick Koup
Sandeep Narpala
Madhu Prabhakaran
Sarah Andrews
Barney Graham
Masaru Kanekiyo
M Gordon Joyce
Hadi Yassine

Clinical trial teams and participants

Funding: