Norovirus Diversity and Immune-Driven Evolution: Mechanisms of Protection and Implications for Vaccine Design

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Ralph Baric

Lindesmith and Baric 2015

Outline

Norovirus GII.4 diversity and evolution

- host susceptibility
- breadth and longevity of protective immunity

Norovirus vaccine candidate

- cross-strain protection

Norovirus Disease Burden

Public Health Interest

Major cause of epidemic viral gastroenteritis, worldwide

~200,000 deaths/yr (Infants, young children, elderly)

Most important cause of viral gastroenteritis/infants-children (Rotavirus vaccine)

Chronic Infection/yrs (Immunosuppressed-Transplant Recipients)

Traveler's Diarrhea

Food and Waterborne Pathogen, Very Stable in Environment

Global Pandemic to Endemic Disease Cycles

What are the fundamental properties of NoV evolution, immunity and immunogenicity and how will these factor effect vaccine design?

HBGAs-Introduction

Large, complex, multivalent carbohydrates (CHO) bound to proteins or lipids.

Found on the surface of mucosal cells and free in mucosal secretions.

Expression is tissue dependent

Host genetics mediate expression patterns of gylcosyltransferases

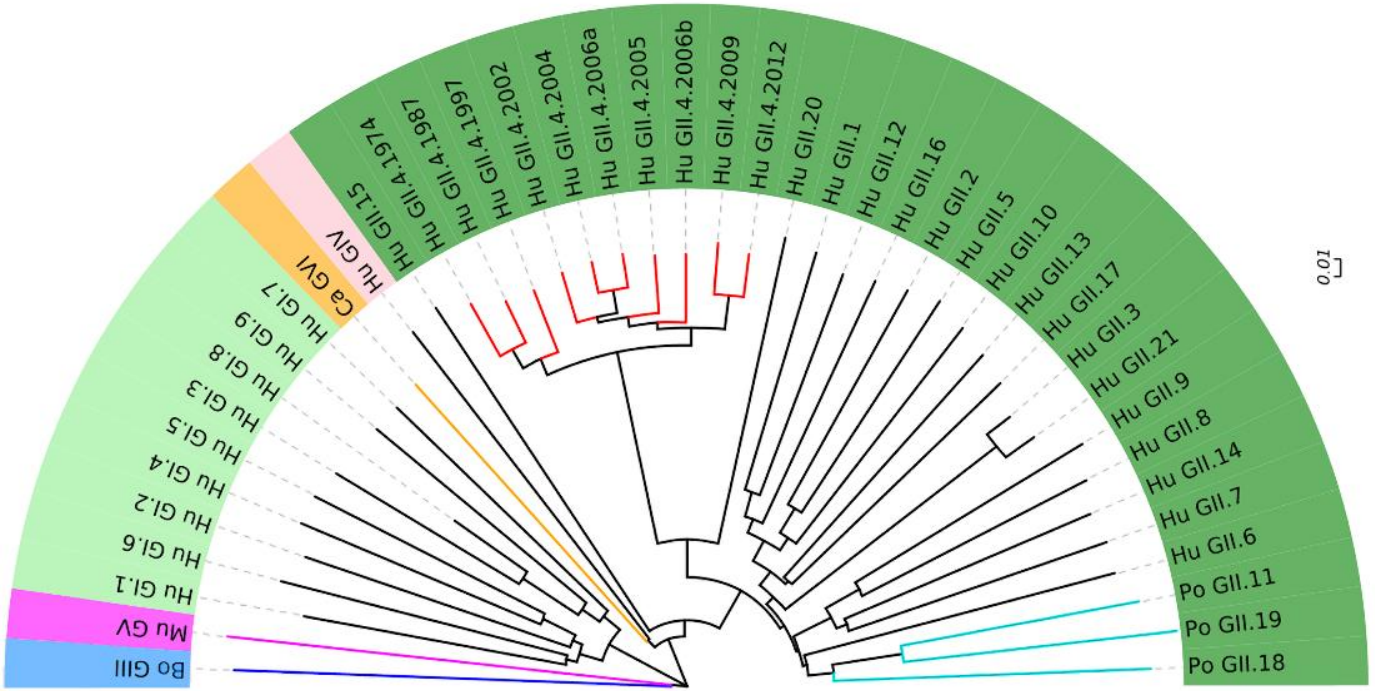
Likley norovirus receptor or co-receptor

Secretor phenotype and blood type are associated with specific NoV strain infection.

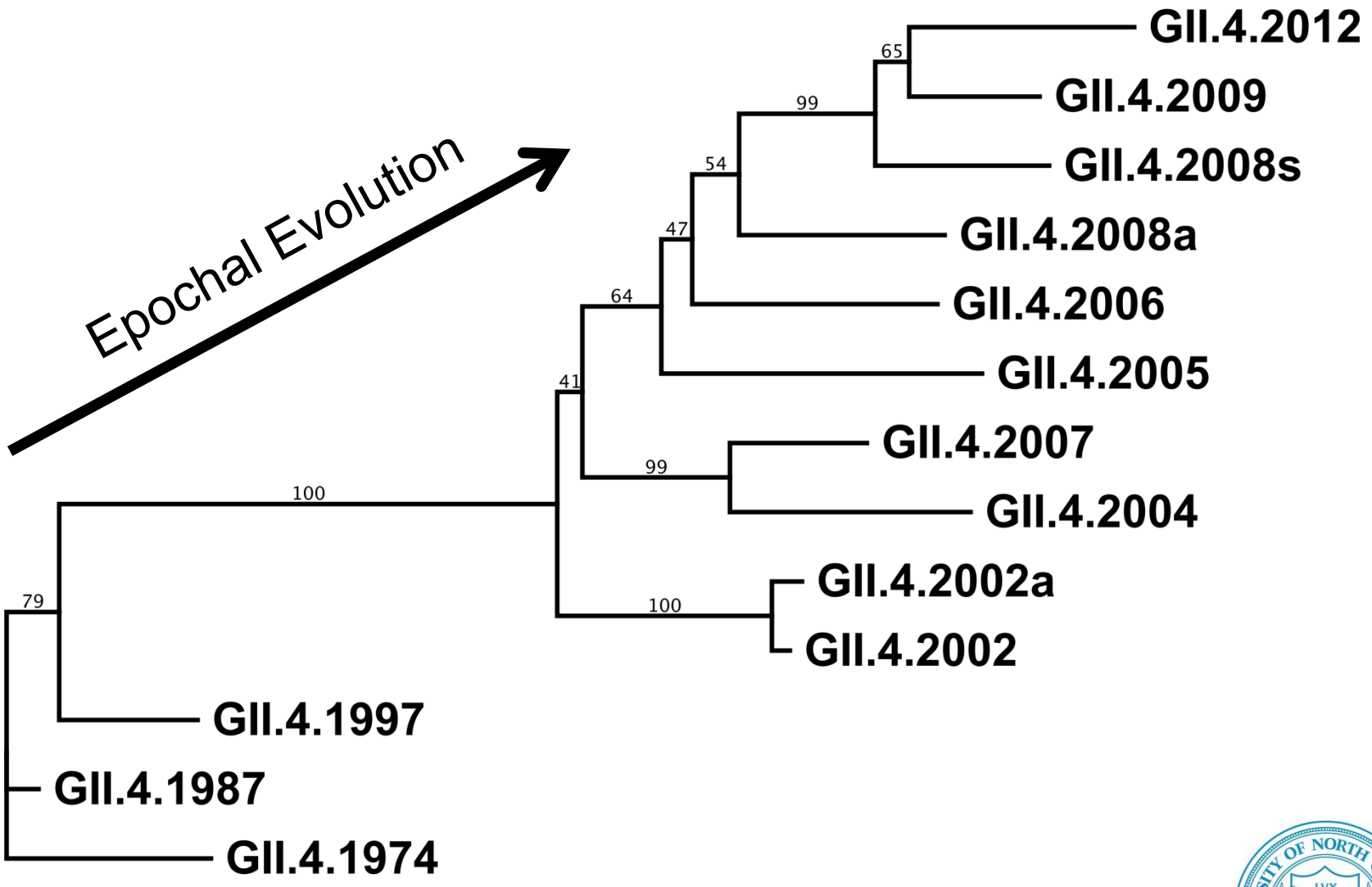
Host genetics mediate NoV strain susceptibility.

Human Norovirus Introduction

- Challenges to successful vaccine design
  - No validated small animal or cell culture system (VLPs)
  - Unclear effect of host genetics and pre-exposure history
  - Strain diversity: >40 genotypes infect humans
  - Antigenic Drift within the pandemic GII.4 genotype : 70-80% of outbreaks



GII.4 Capsid is changing over time.



Bayesian tree of GII.4 ORF2 capsid sequences

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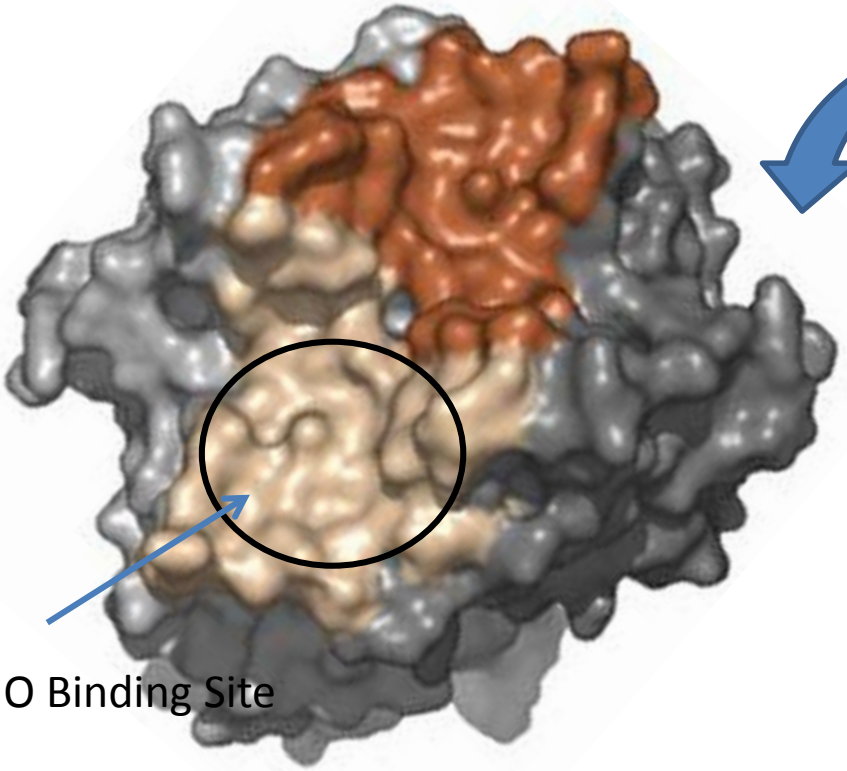
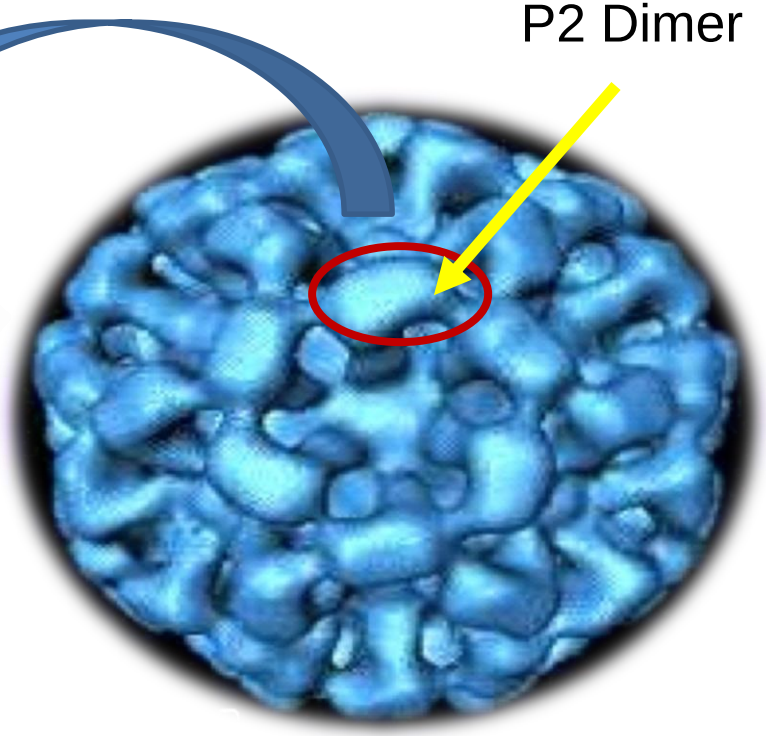
GII.4 Change Occurring in the P2 subdomain

Dimer of P2 domain



CHO Binding Site

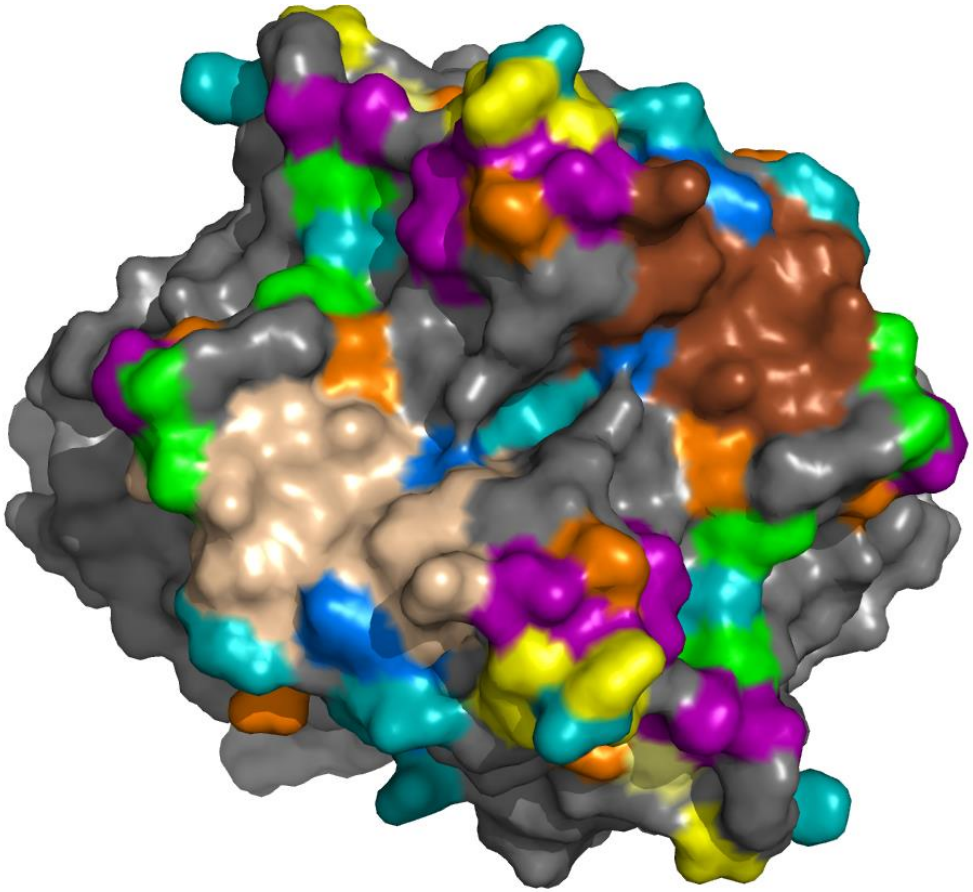
P2 Dimer



Norovirus VLP, Prasad et al. 1999. Science

Lindesmith and Baric 2015

GII.4 P2 Subdomain Amino Acid Changes



Color change=residue change over time
Binding pocket conserved
Ridge around pocket variable
P2 Change Affects HBGA and Ab binding affinity

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GII.4 HBGA Binding

		Se- Le+		Se+ Le-		Se+ Le+		Se+ A/B +	
VLP	**Cluster**	**LeA**	**LeX**	**H1**	**H3**	**LeB**	**LeY**	**A**	**B**
GII.4.1987	Camberwell								
GII.4.1997	Grimsby								
GII.4.2002a	Farmington Hills								
GII.4.2002	Farmington Hills								
GII.4.2006	Minerva								
GII.4.2009	New Orleans								
GII.4.2012	Sydney								

Breadth of binding greater than non-GII.4 strains.
Profiles vary between GII.4 strains, especially by affinity

Lindesmith and Baric 2015

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Breadth of binding greater than non-GII.4 strains.
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Microvariation in the P2 domain modulates HBGA binding in vitro and potentially population susceptibility in vivo.

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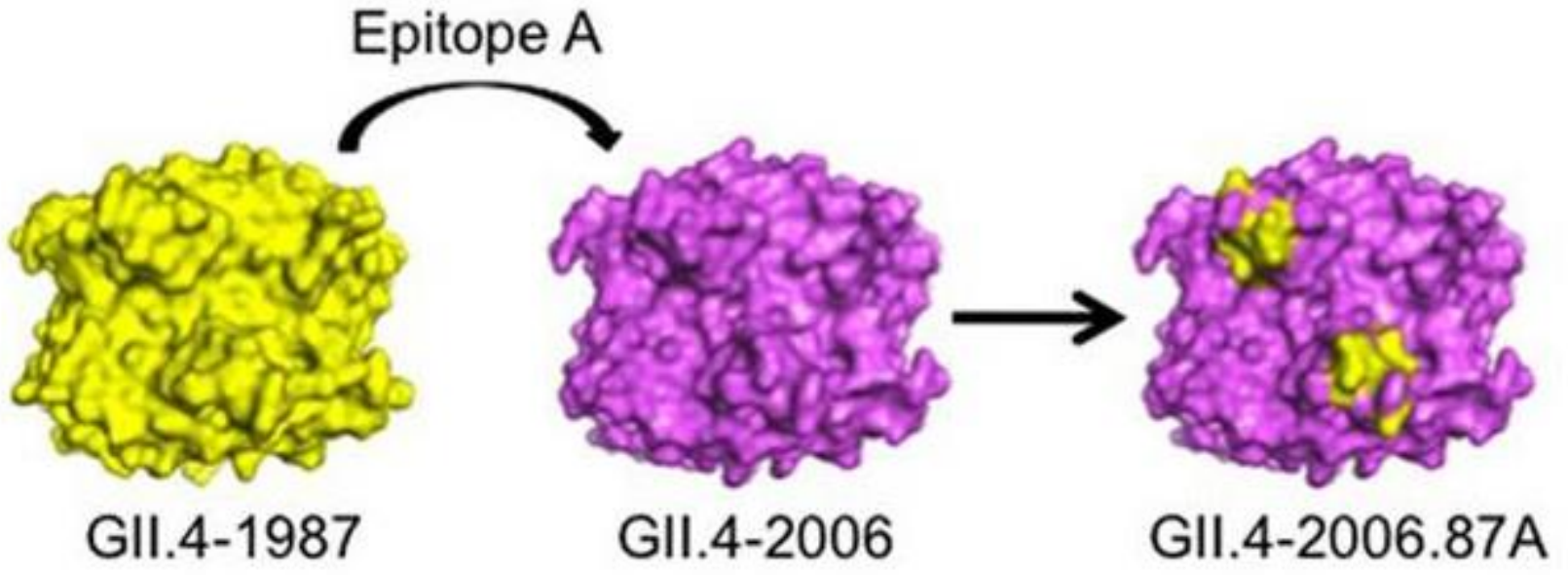
Blockade-Epitope Chimeric VLP



Does transplanted sequence change Ab reactivity?

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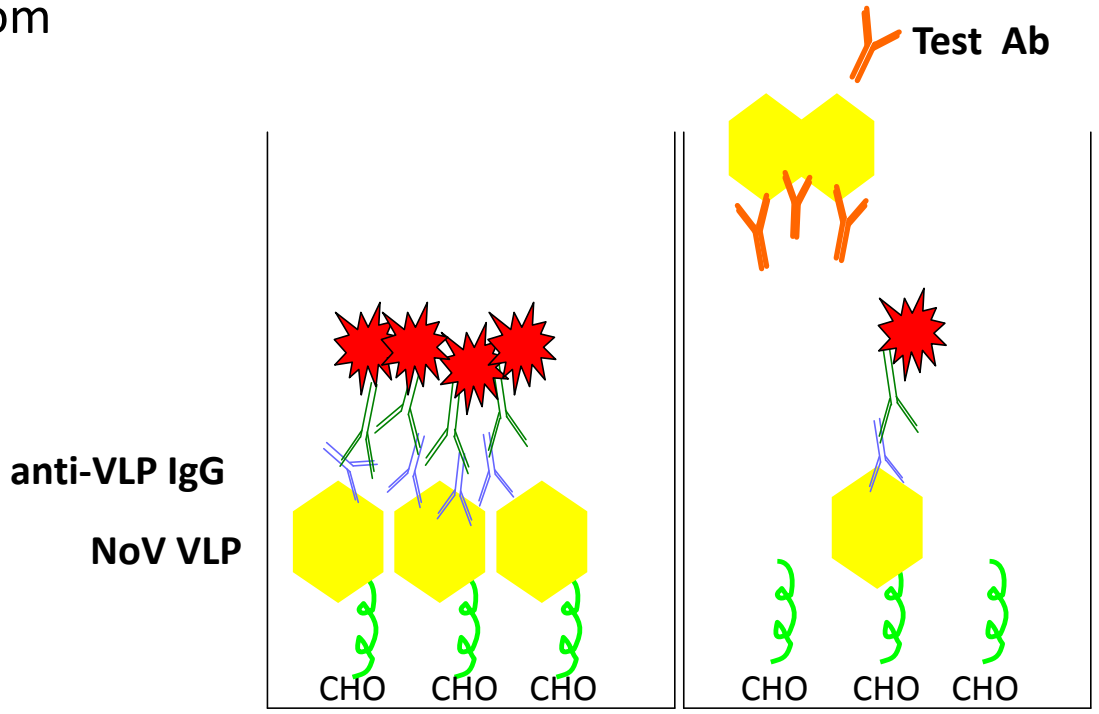
VLP-Carbohydrate Ab "Blockade" Assay

Surrogate neutralization assay

Correlate to protection from infection in humans



100% Control Binding

% Control Binding (Blockade Potential) =

(OD Ab present / OD Ab absence) x 100

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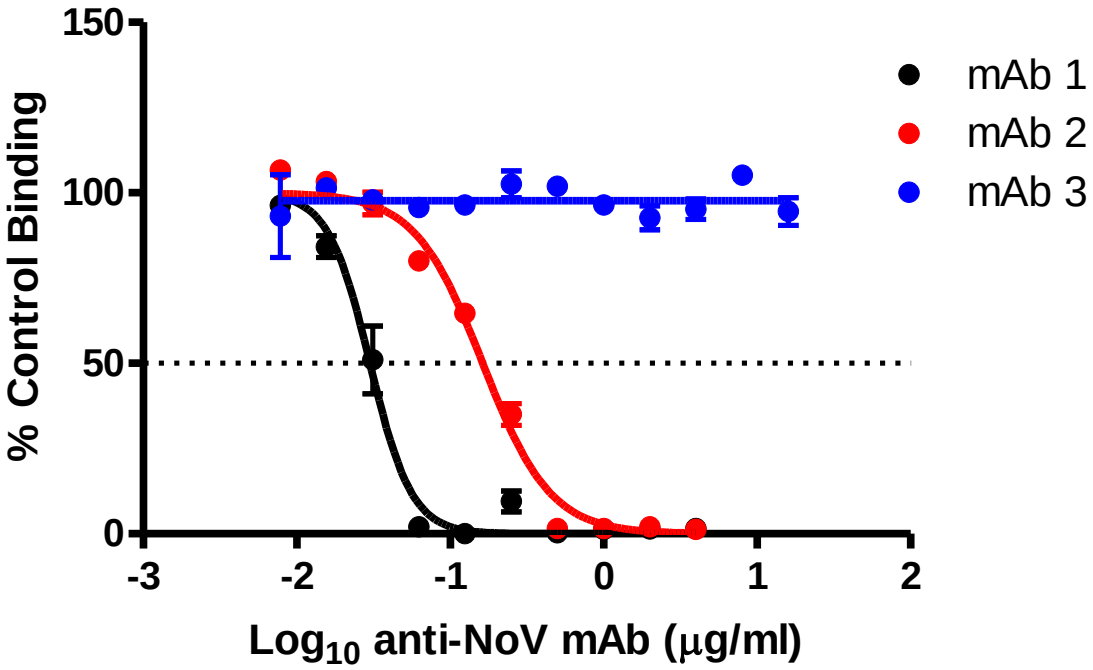
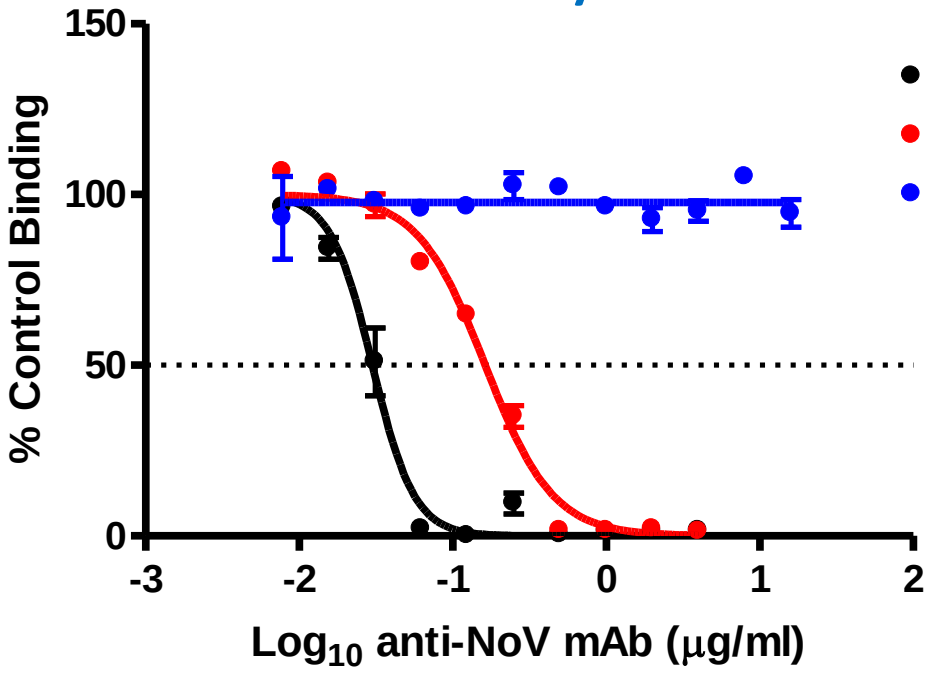
VLP-Carbohydrate "Blockade" Assay



% Control Binding data are fit with Sigmoidal curves and an EC50 calculated.

Lindesmith and Baric 2015

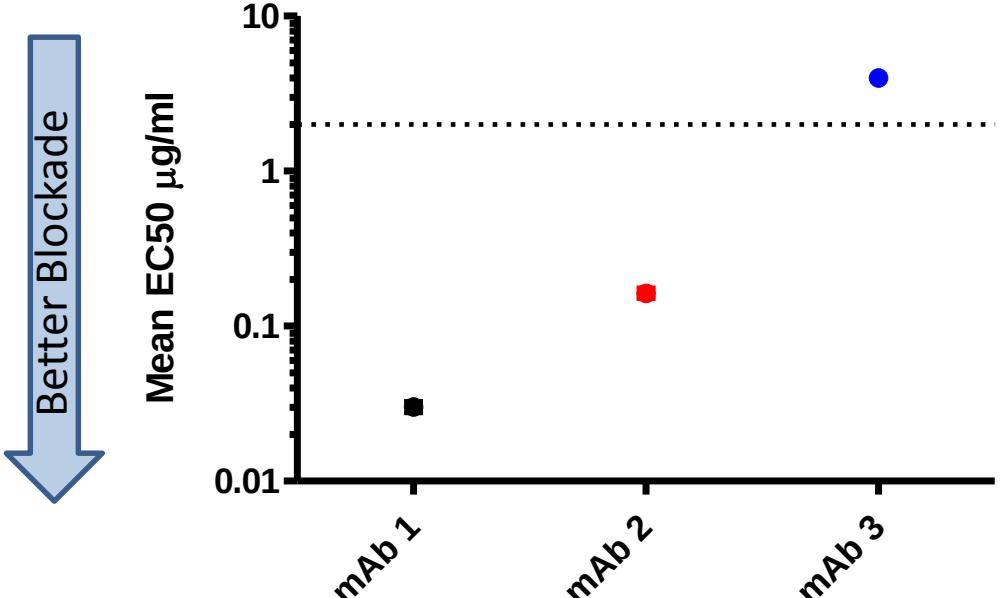
VLP-Carbohydrate "Blockade" Assay



% Control Binding data are fit with Sigmoidal curves and an EC50 calculated.

"Blockade" Ab if block ≥50%

No blockade assigned
EC50=2X ULD



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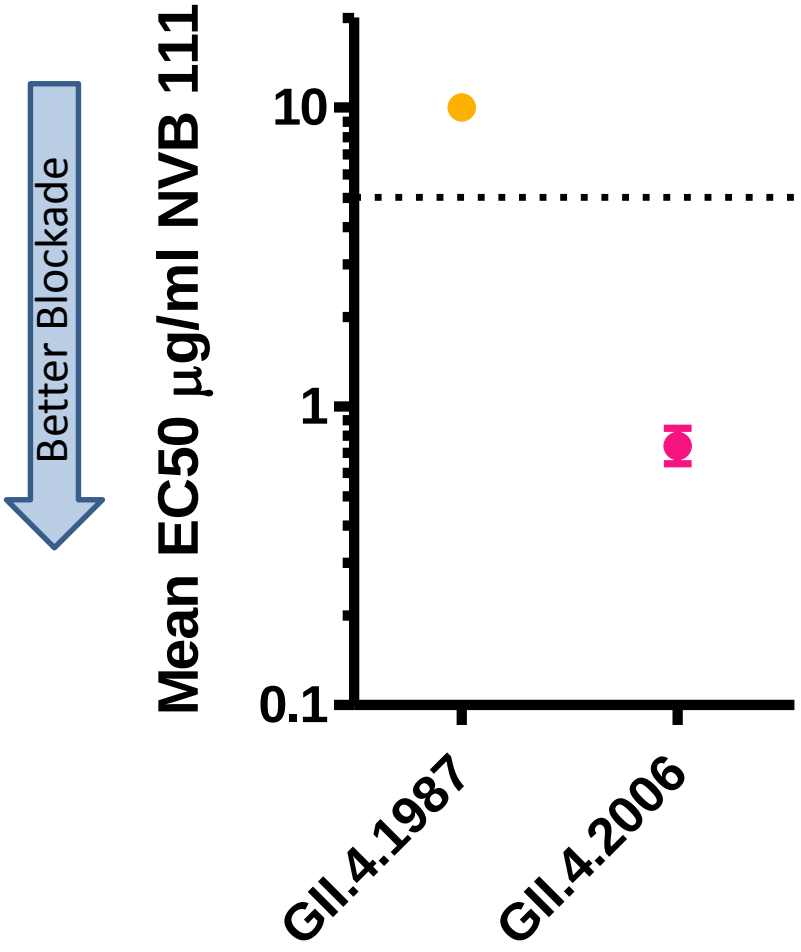
Epitope A is an Evolving Blockade Epitope



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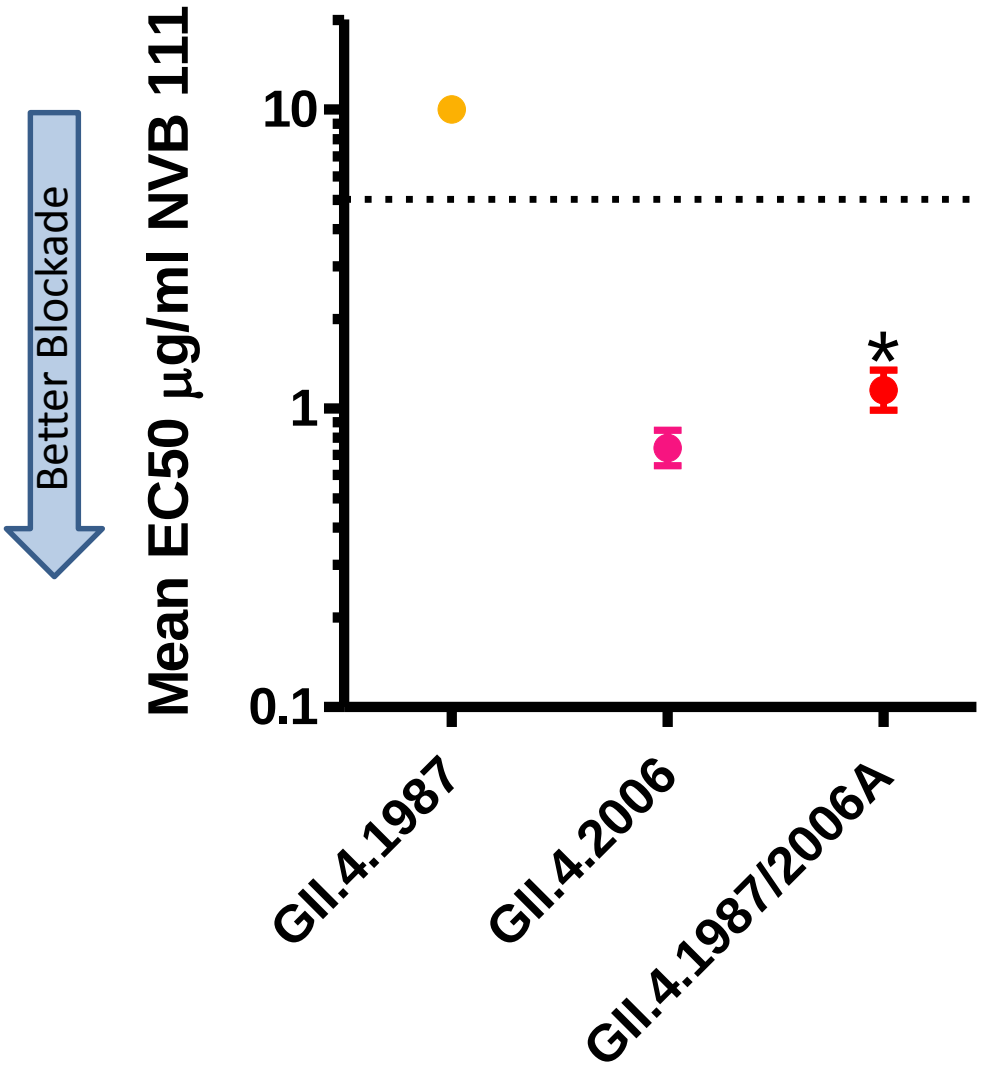

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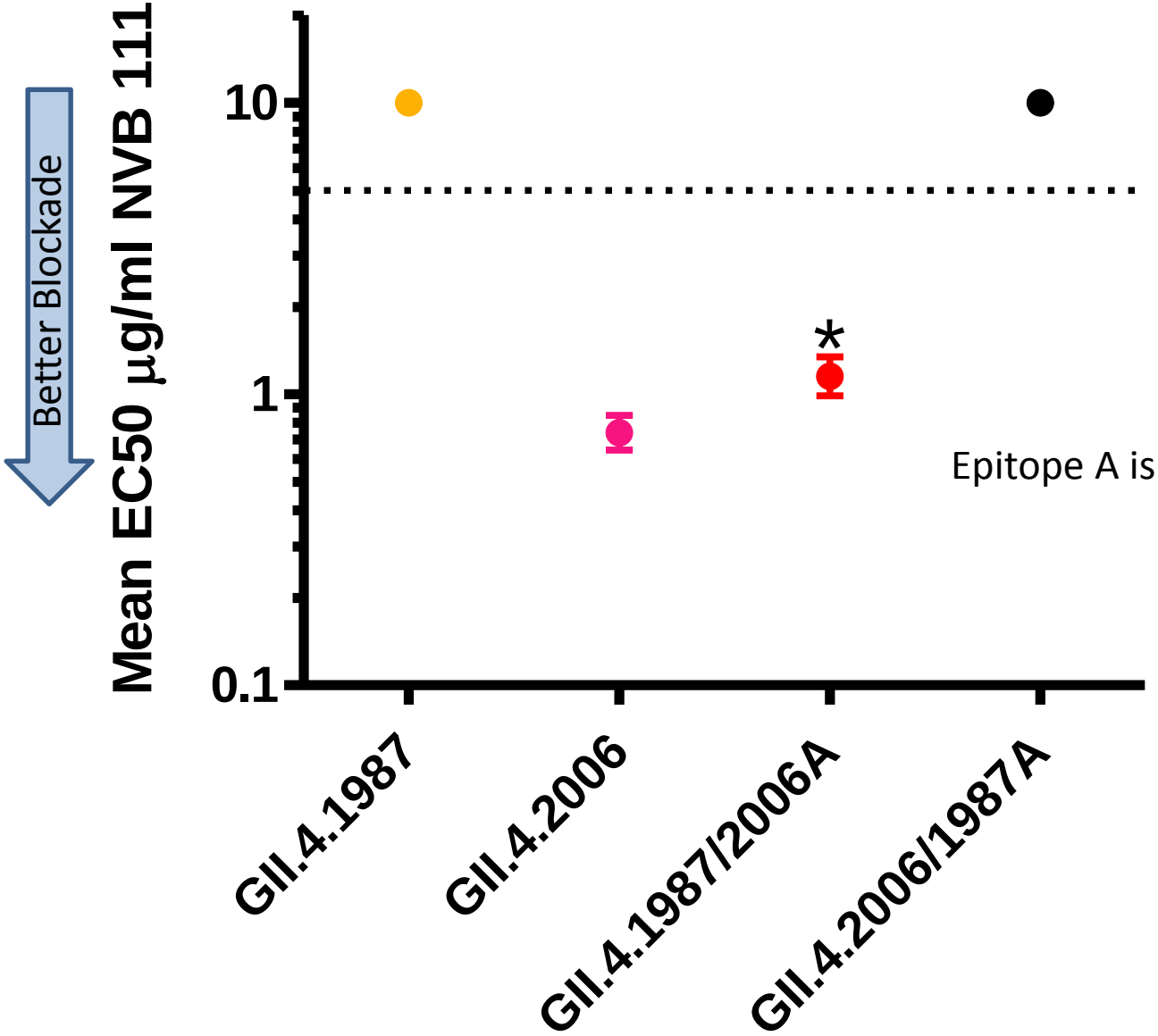

Epitope A is an evolving blockade epitope

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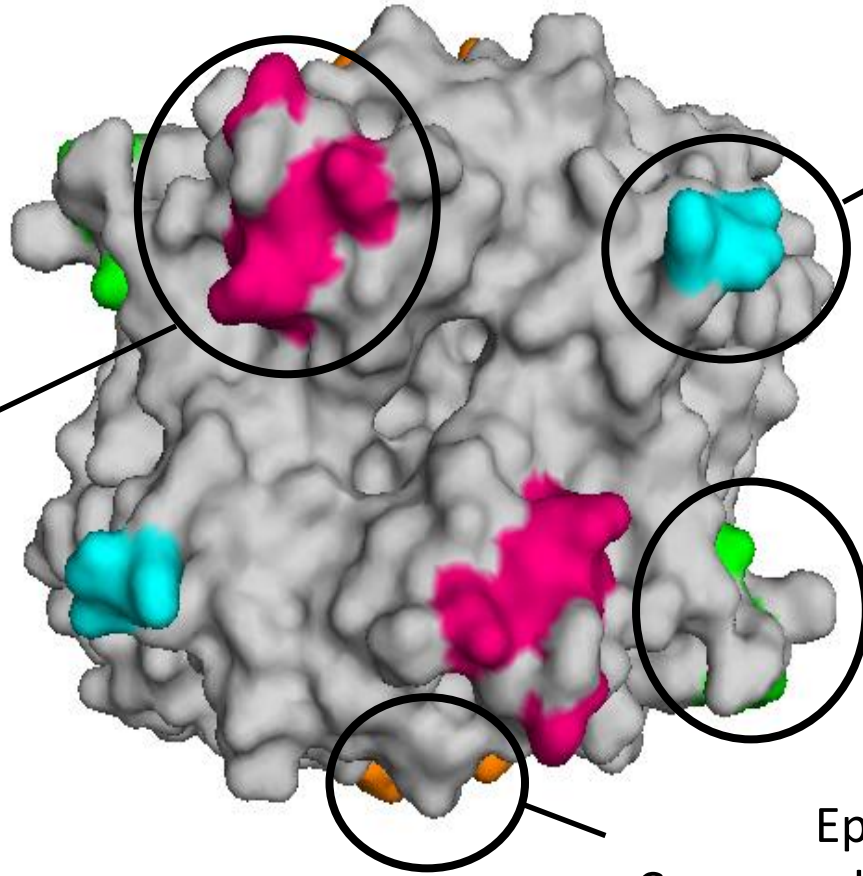
GII.4 NoV Blockade Epitopes



Epitope A
Hypervariable
40-50% serum
response

Epitope D
Modulates HBGA
Binding Affinity

Epitope E

Epitope F
Conserved GII.4 Blockade
epitope

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How GII.4 Evolution Impacts Vaccine Design?

Conclusion: Sequence changes in the P2 subdomain alter GII.4 HBGA binding and antigenicity.

Good news:

Know where to watch for sequence changes

Know to how evaluate these changes for potential escape variants (vaccine reformulation)

Challenge:

Design a vaccine that elicits an immune response broad enough to accommodate GII.4 evolution.

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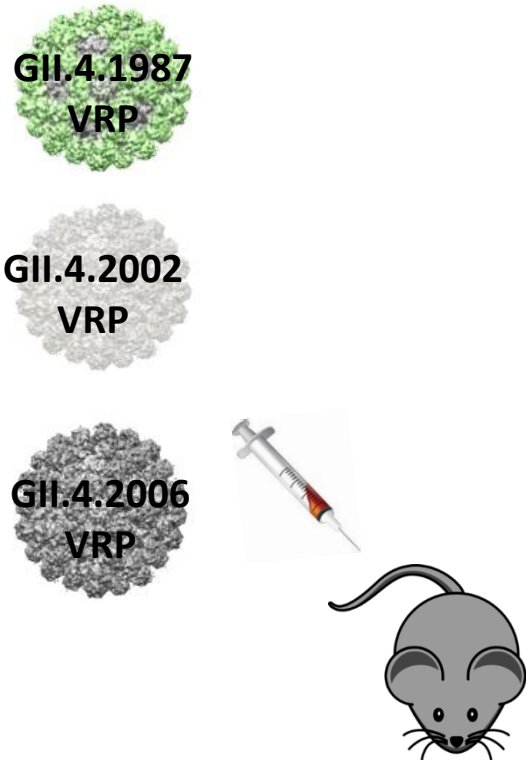
Multivalent Norovirus Vaccine Approaches

Multiple strains VRP simultaneously



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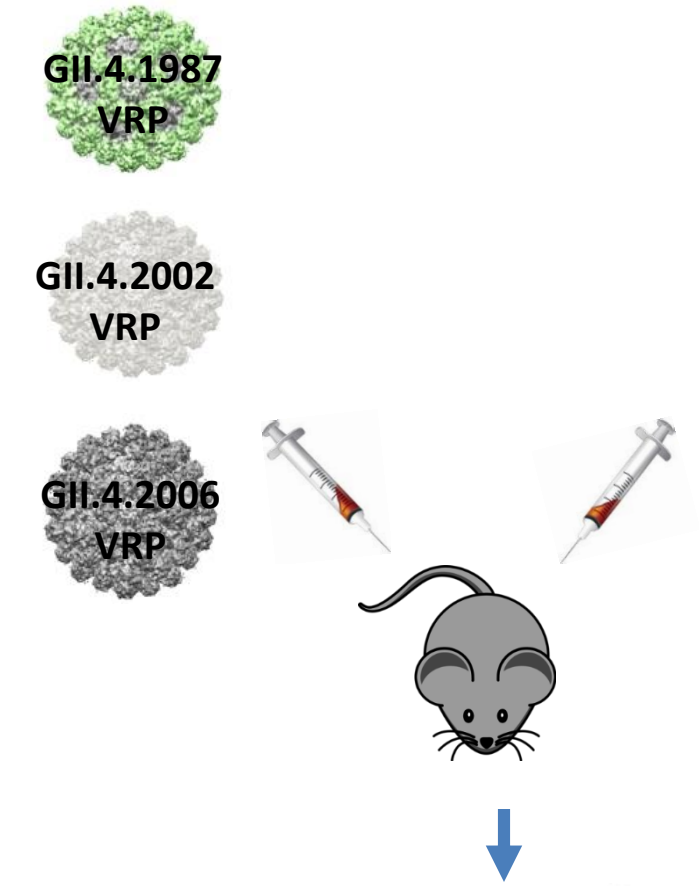
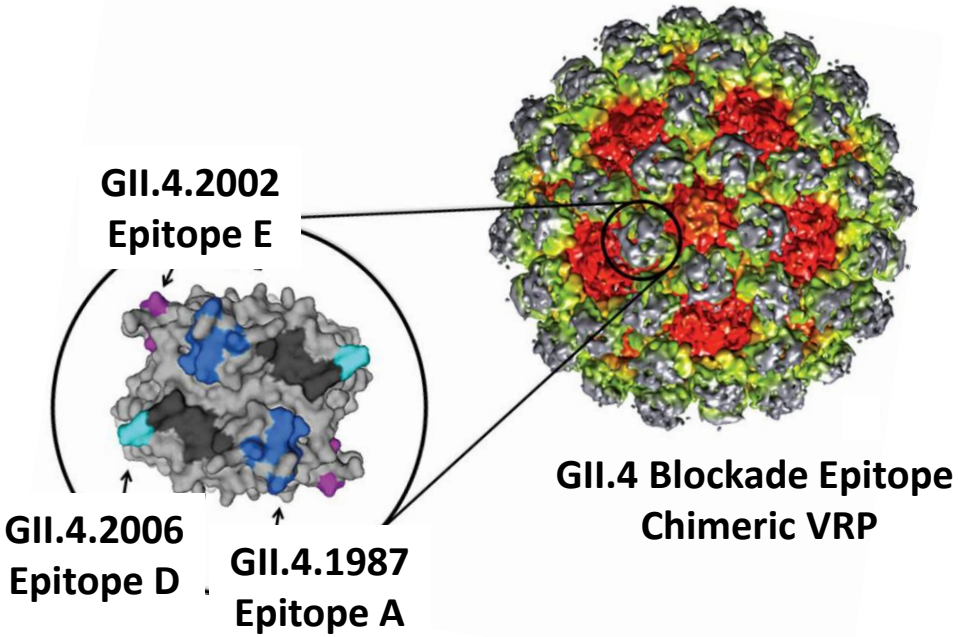


Multiple strain epitopes within a singe strain VRP



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Multivalent immunization results in blockade Ab cross-reactive with GII.4 strains not included in the vaccine.

What happens in people with pre-exposure history?

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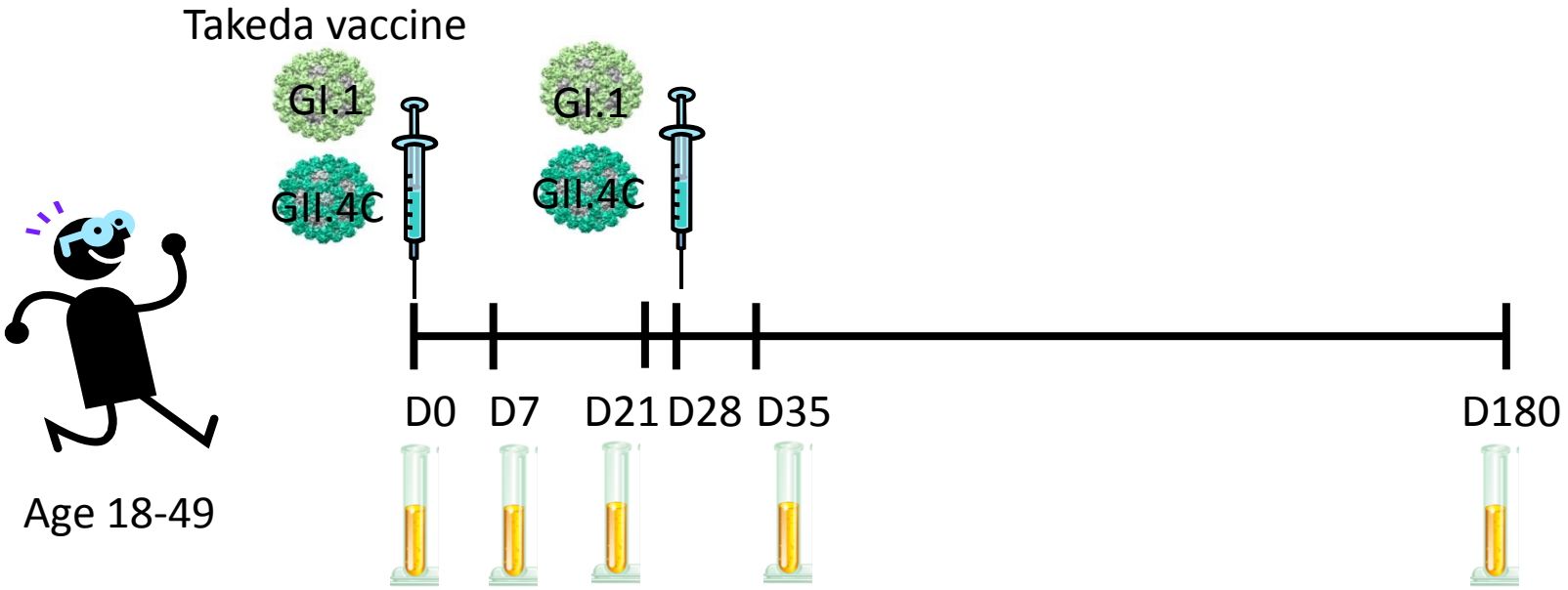
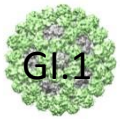
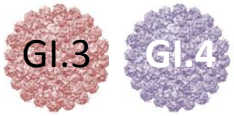
Evaluating cross-strain immune responses to NoV VLP Vaccine



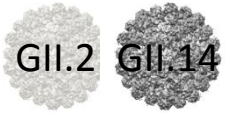


Homotypic VLP Antibody



GI VLPs

GII.4 VLPs

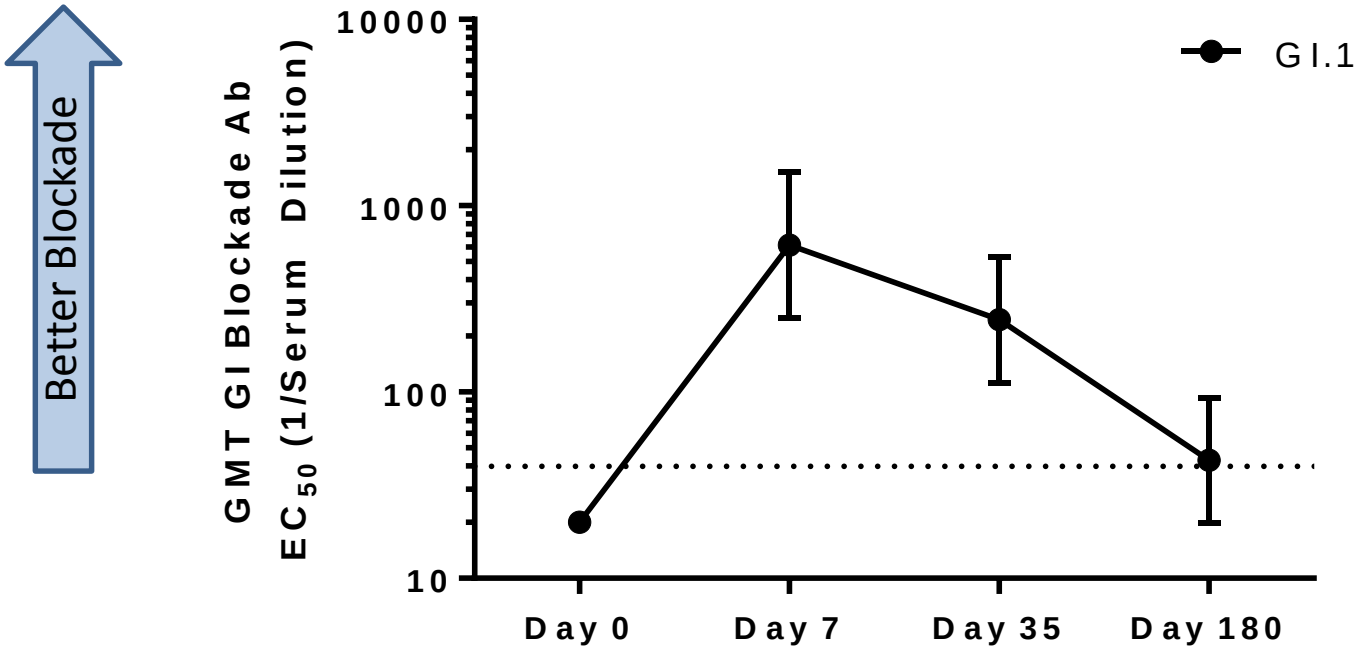


GII VLPs

Heterotypic VLP Antibody

Ag-limiting conditions

GI VLP Blockade Ab Response



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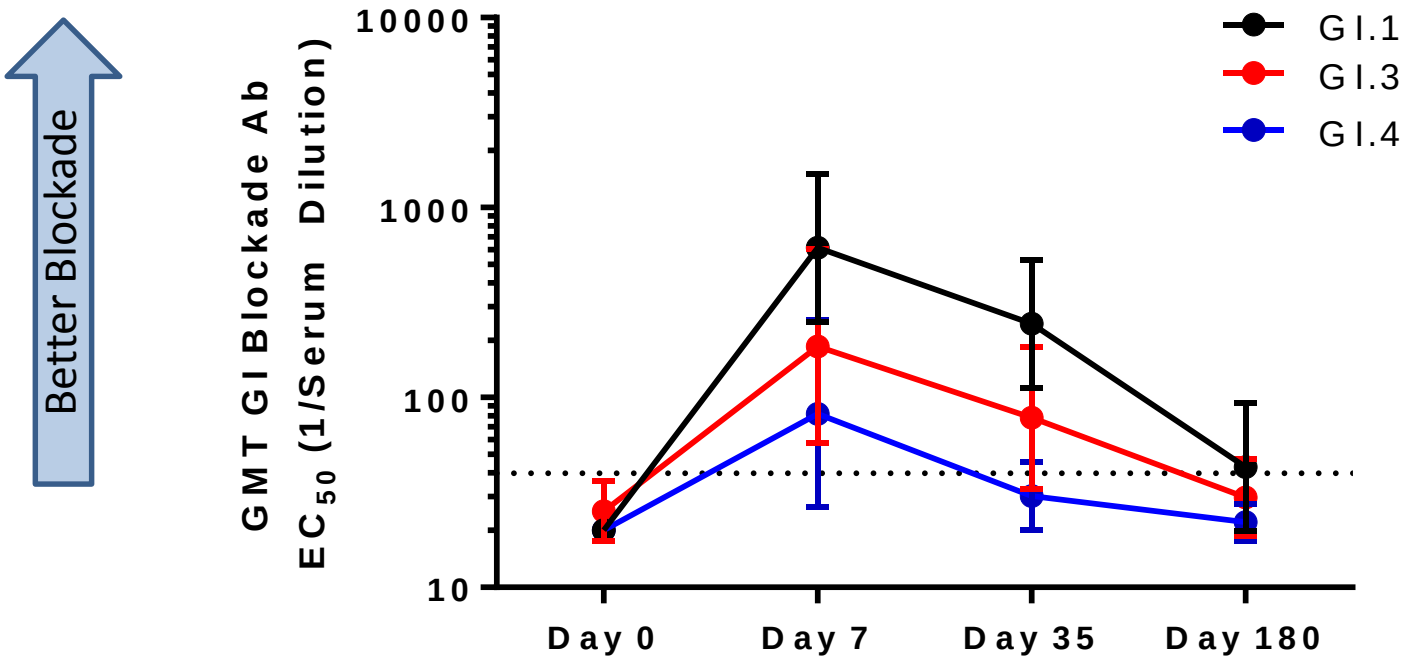
GI VLP Blockade Ab Response



GI Blockade Ab Response

-Most potent response to GI.1

-Moderate cross-reactivity with other GI VLPs

-Suggests GI.1-specific Ab

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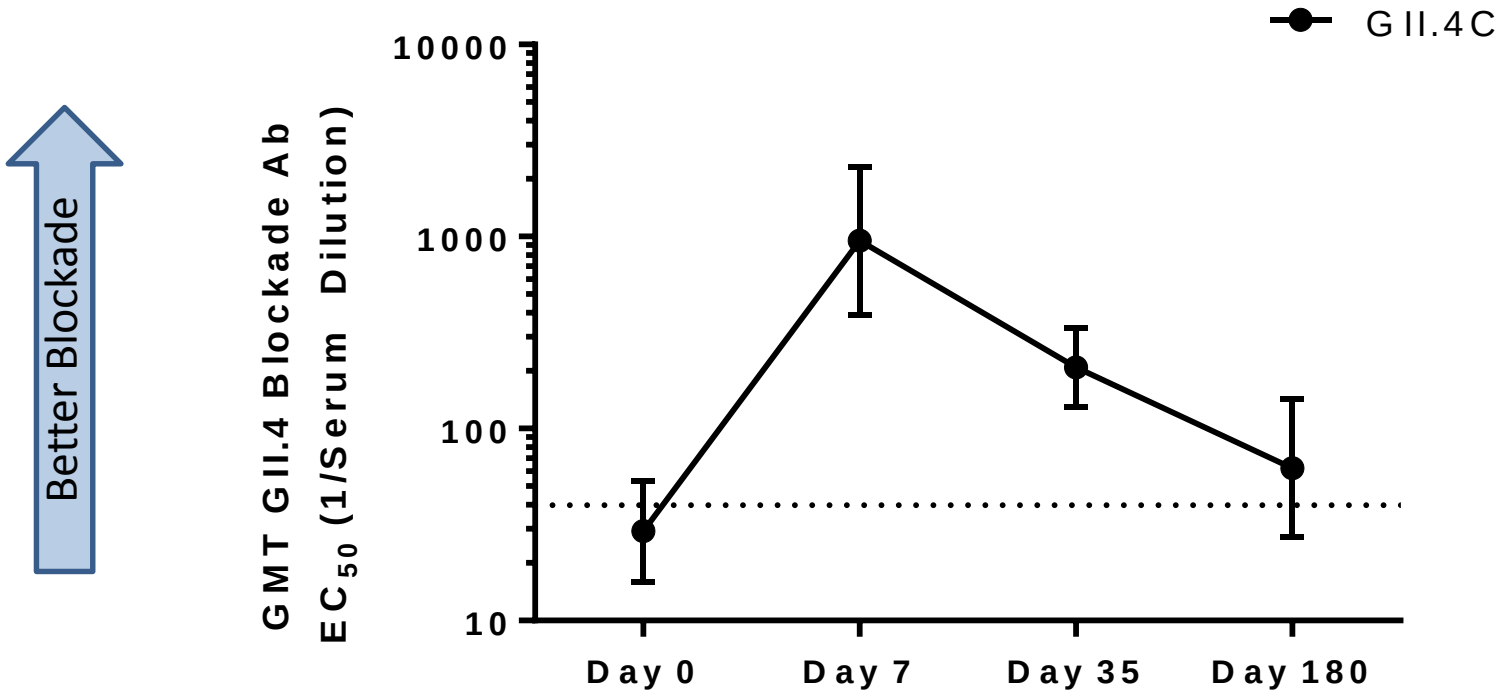
GII.4 VLP Blockade Ab Response



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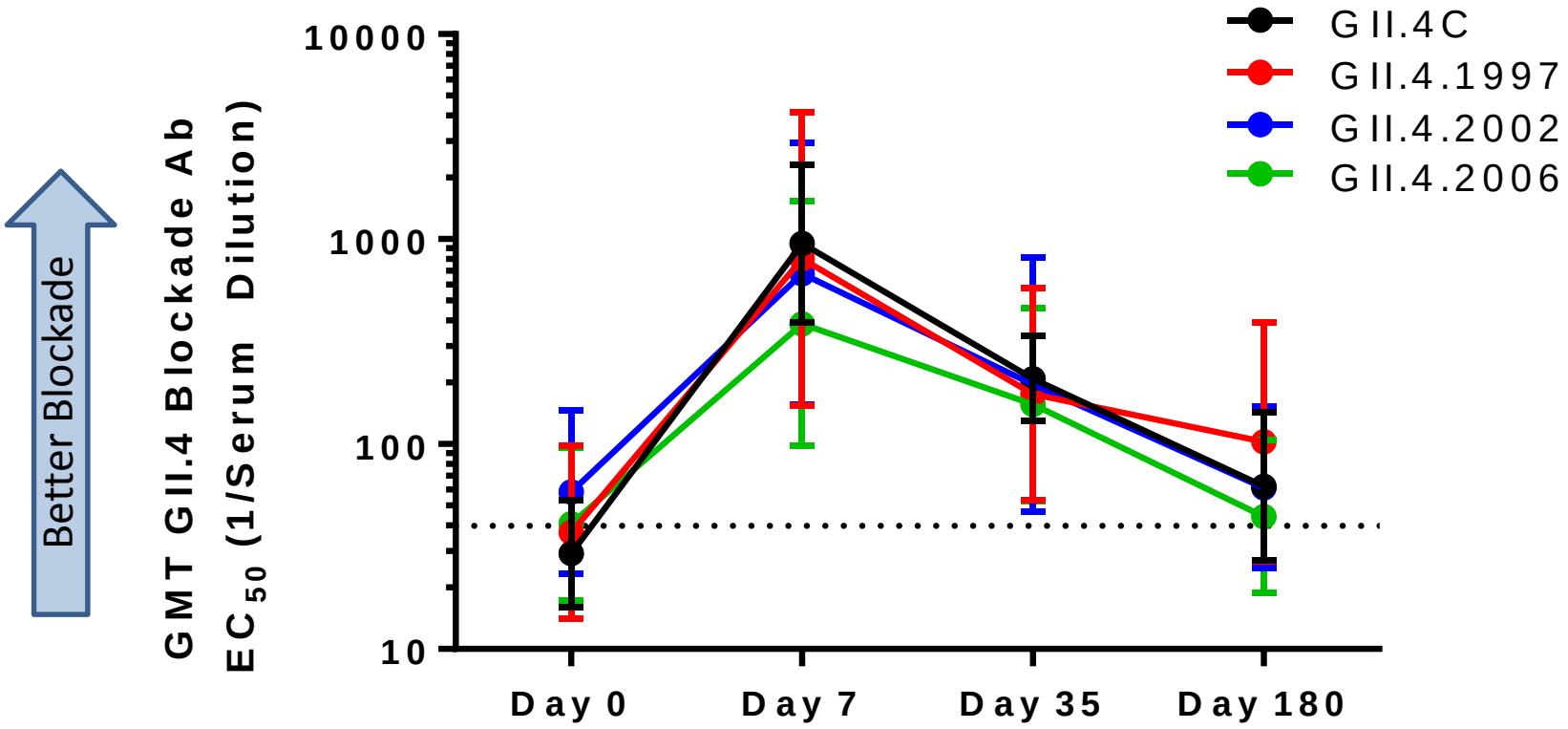
GII.4 VLP Blockade Ab Response



GII.4 Blockade Ab Response

-Potency relatively consistent between strains

-Suggests cross-reactive memory Ab response

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Can vaccination induce a cross-blockade Ab response to novel GII.4 strain VLPs?

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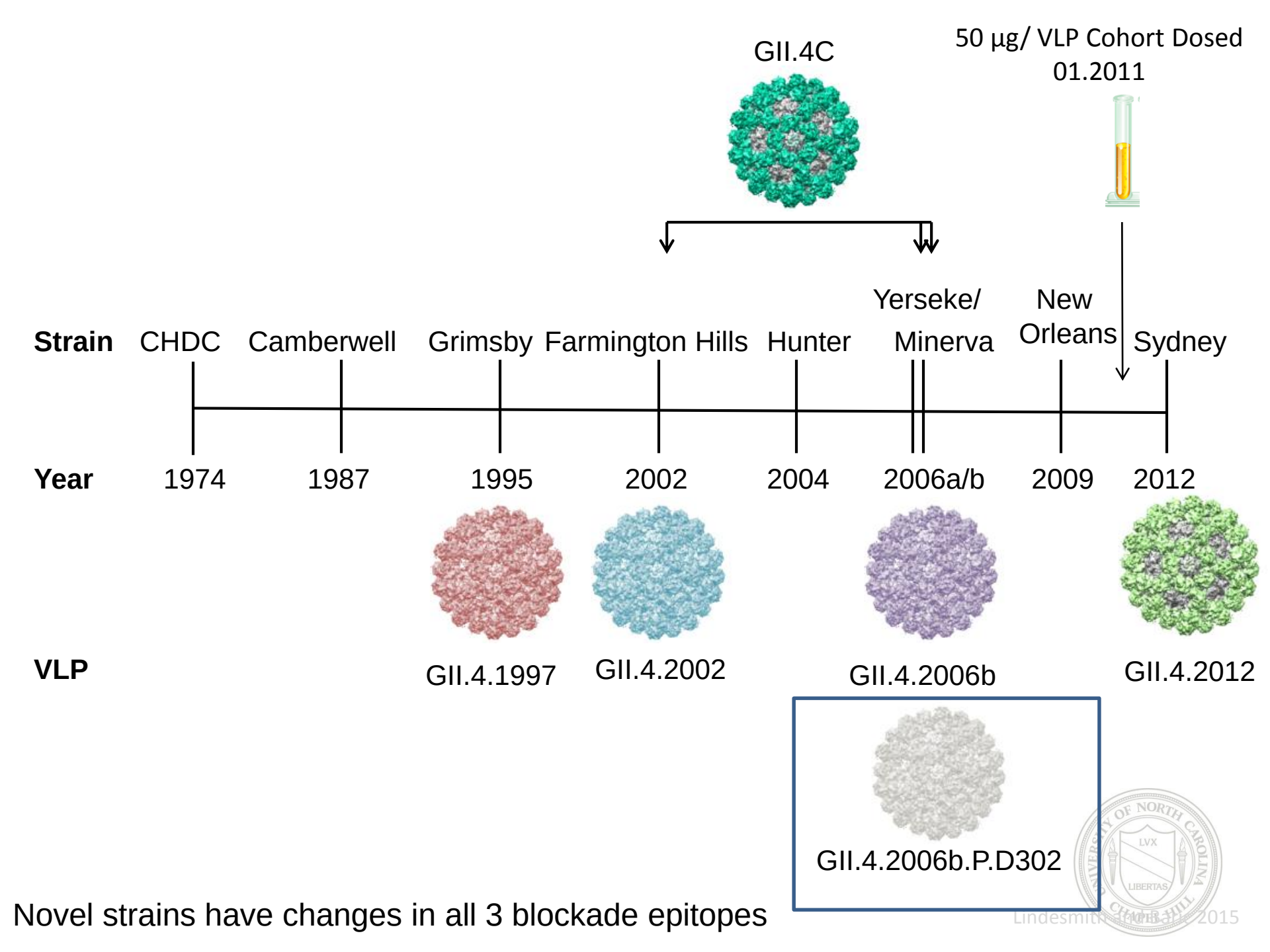
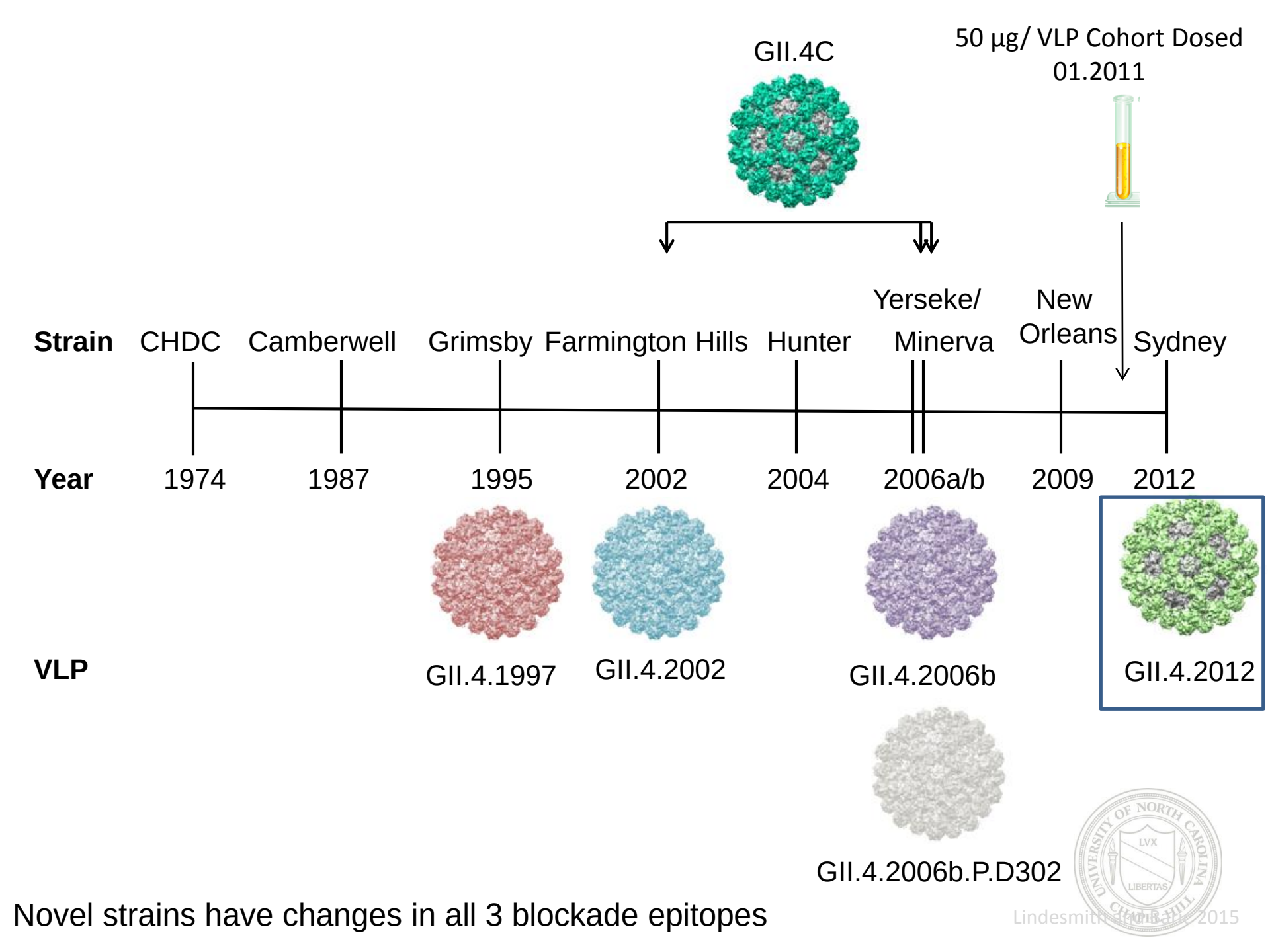


Novel strains have changes in all 3 blockade epitopes



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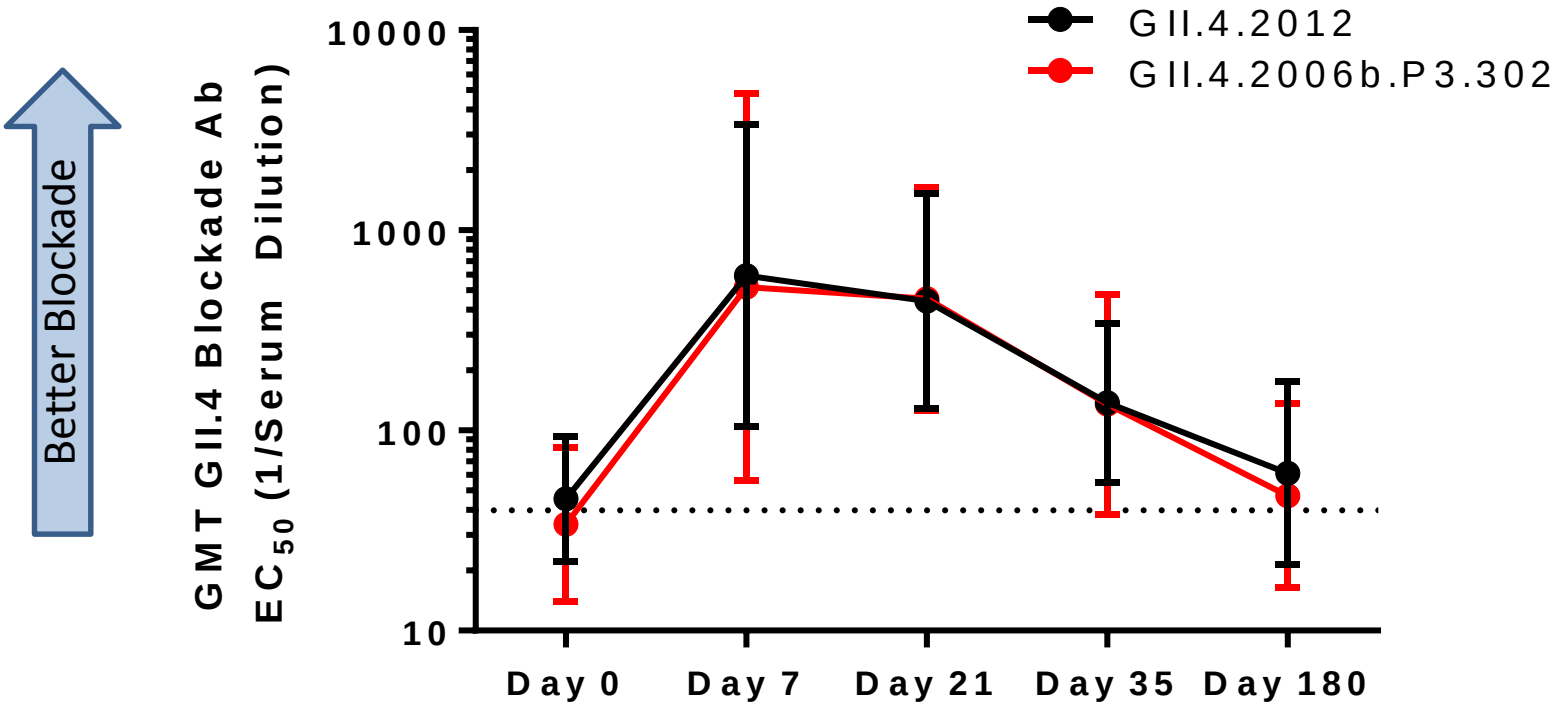
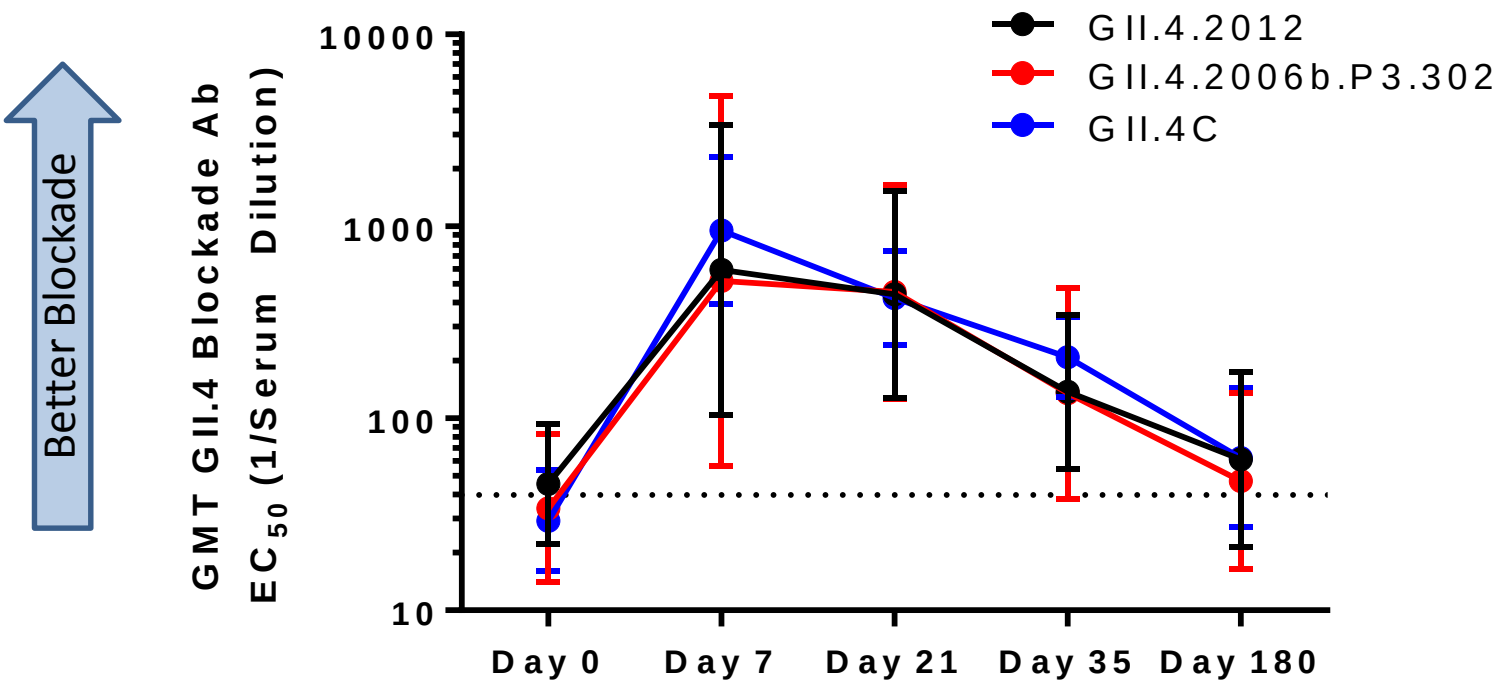
Novel GII.4 VLP Response



Novel GII.4 VLP Response



Novel GII.4 Blockade Ab Response

-Similar potency to each other and the other GII.4s

-Suggests cross-reactive memory Ab response

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What is the driving mechanism of the cross-blockade Ab response following vaccination?

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Antigenic Cartography

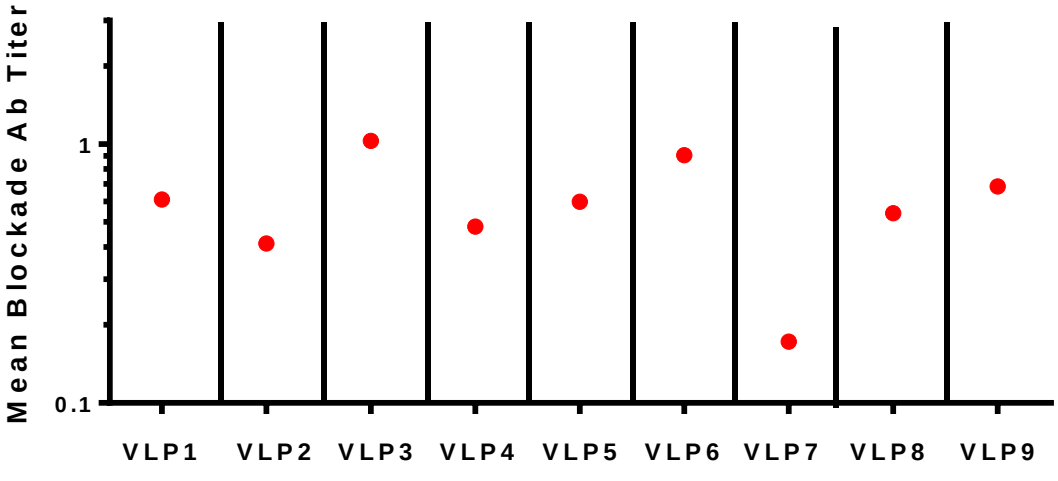
Bioinformatics approach to visualizing the antigenic relation among multiple antigens and sera.

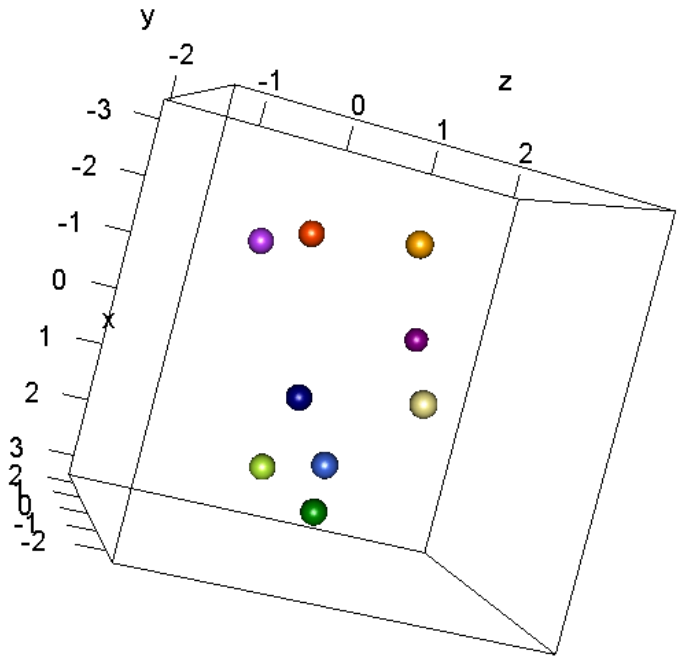
Dr. Marty Ferris

Lindesmith and Baric 2015

Antigenic Cartography

Bioinformatics approach to visualizing the antigenic relation among multiple antigens and sera.





Dr. Marty Ferris

Antigen map3D
Multi Dimensional
Scaling

Each ball represents one VLP.

Difference in titer ≈ 3D distance

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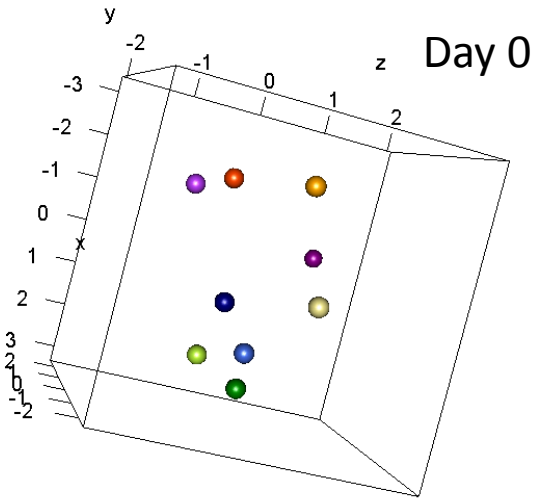
Antigenic Cartography: Blockade Ab



Day 0:

1. Titers are low but VLPs are distinct.

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A

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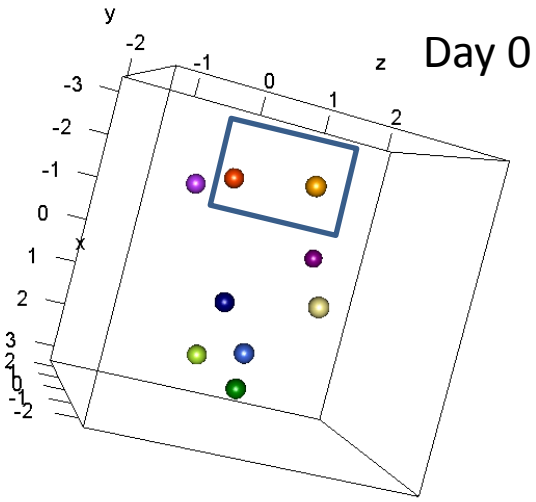

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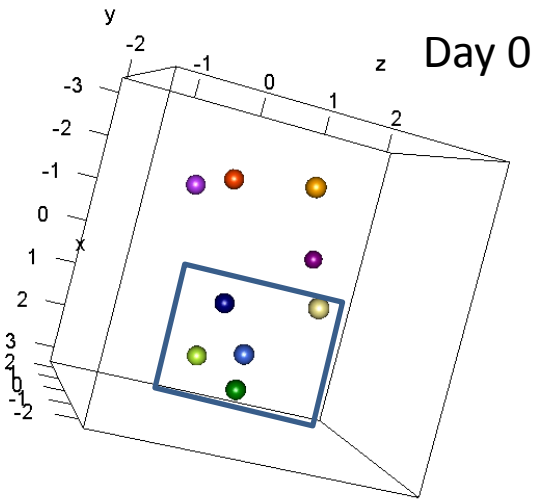

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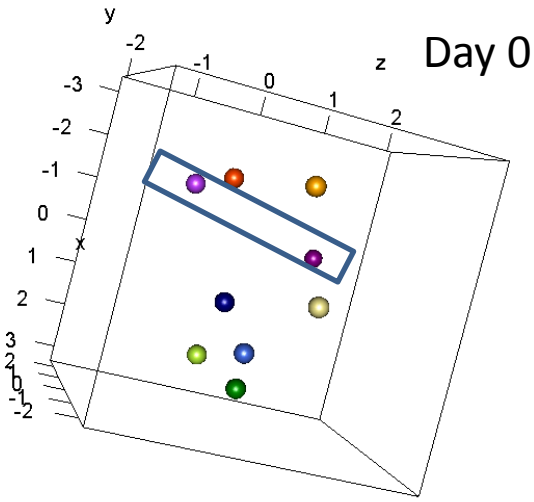

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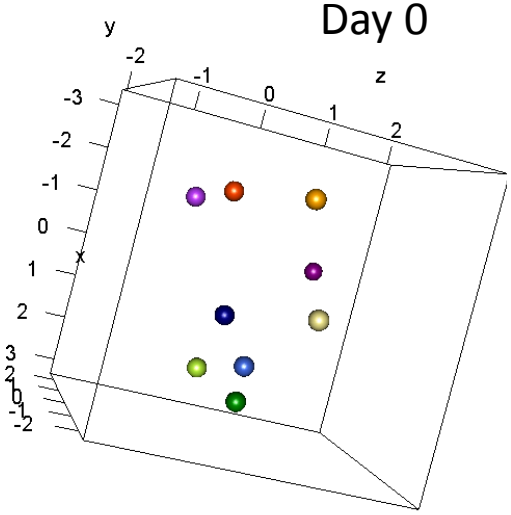
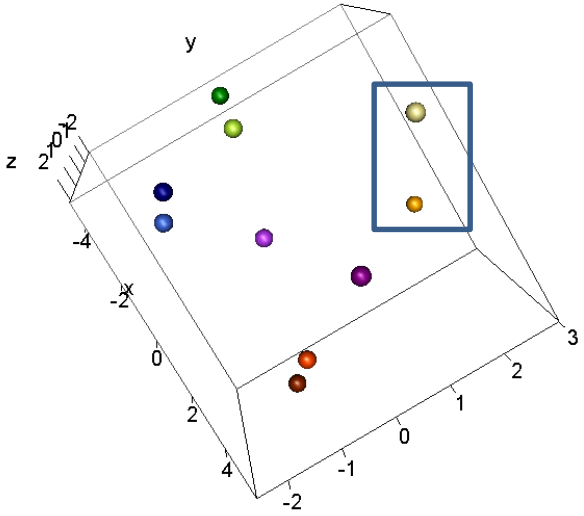


Day 7



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1. Titers are low and VLPs are distinct.

Day 7:

1. Highest responses to vaccine components.

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Antigenic Cartography: Blockade Ab

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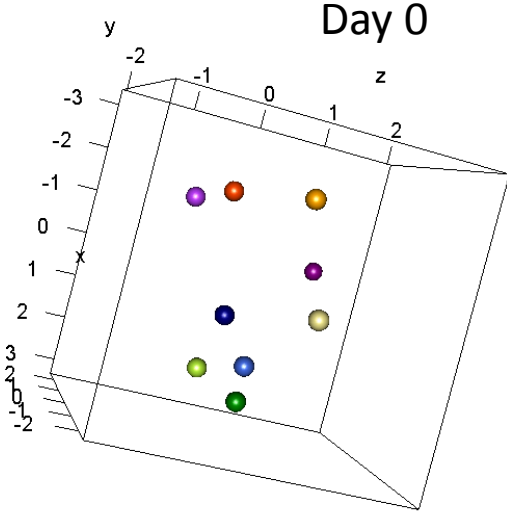

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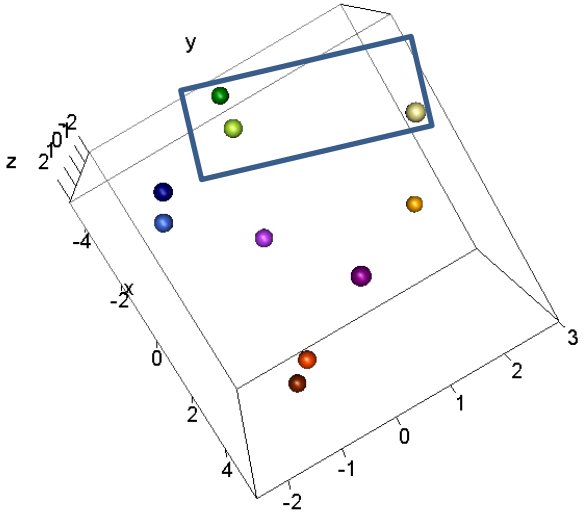

Day 0:

1. Titers are low and VLPs are distinct.

Day 7:

1. Highest responses to vaccine components.

2. Early GII.4 cluster and are most close to GII.4C: cross-reactive Ab response.

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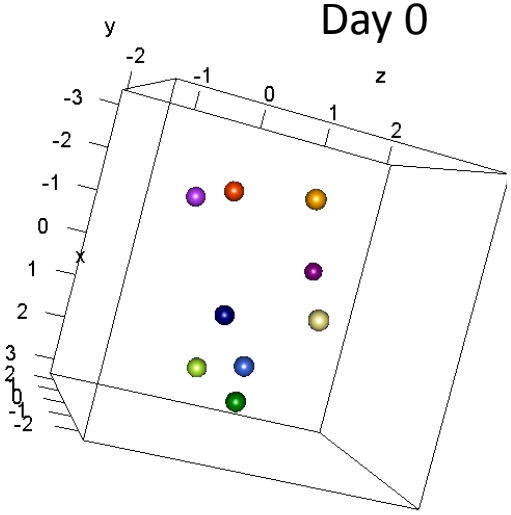
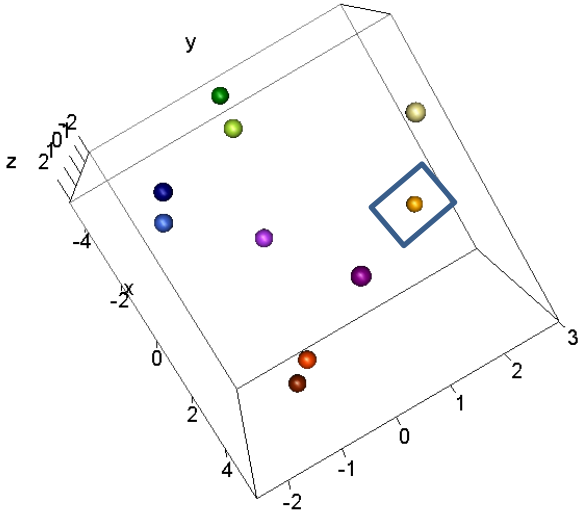
Antigenic Cartography: Blockade Ab

Day 0



Day 7



Day 0:

1. Titers are low and VLPs are distinct.

Day 7:

1. Highest responses to vaccine components.
2. Early GII.4 cluster and are most close to GII.4C: memory response to multiple strains.
3. GI.1 distinct from other GI VLPs: secondary Ab response to GI.1.

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Antigenic Cartography: Blockade Ab

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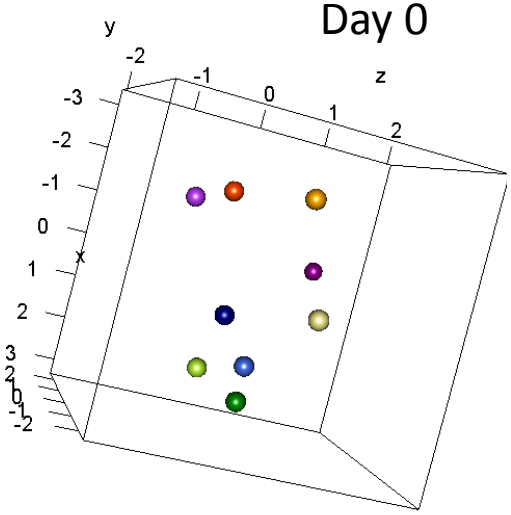
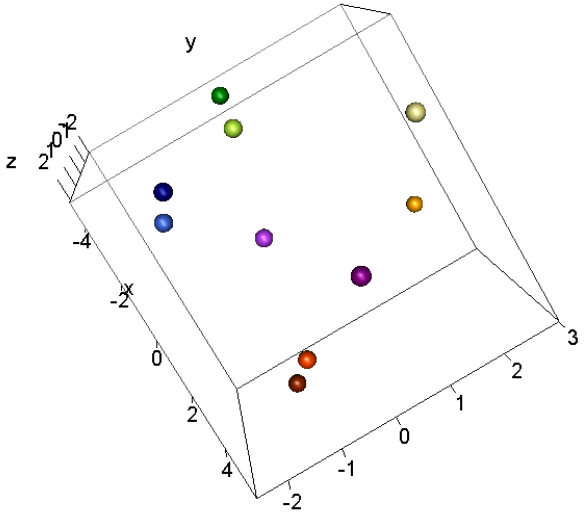
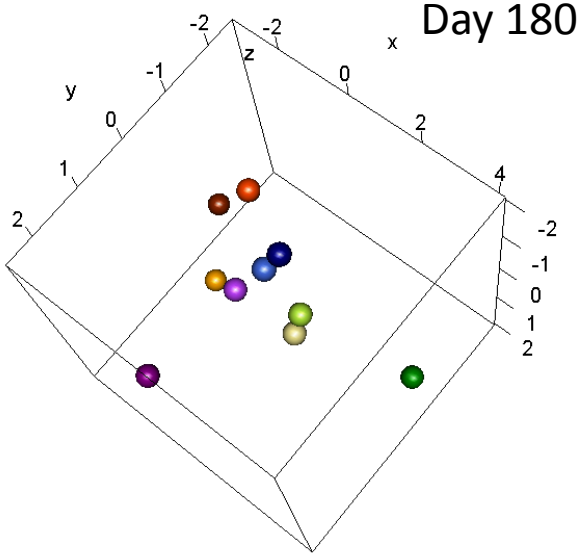


Day 7



Day 180



Day 0:

1. Titers are low and VLPs are distinct.

Day 7:

1. Highest responses to vaccine components.
2. Early GII.4 cluster and are most close to GII.4C: memory response to multiple strains.
3. GI.1 distinct from other GI VLPs: secondary Ab response to GI.1.

Day 180:

1. All of the titer have decreased, distances between VLPs is less.

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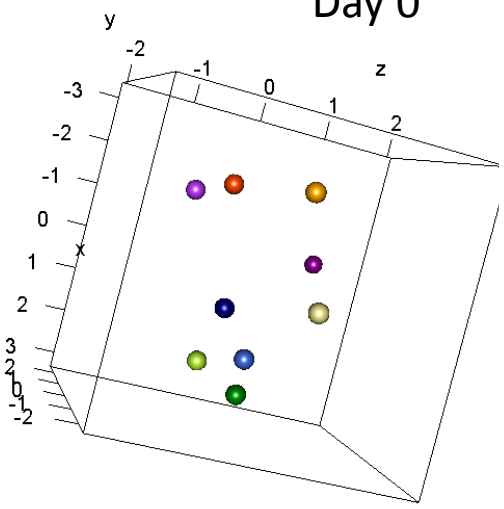
Antigenic Cartography: Blockade Ab

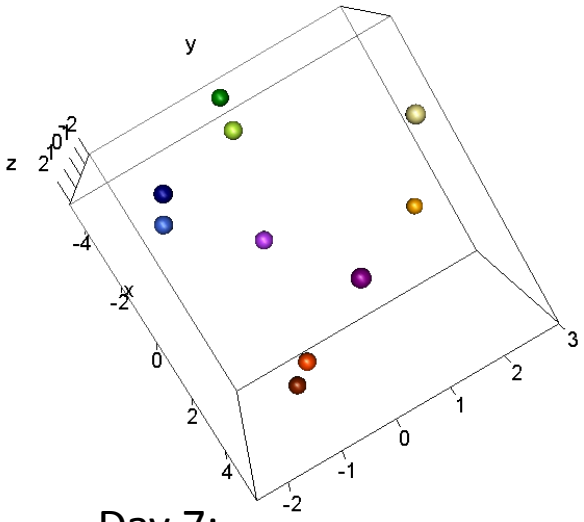
Day 0



Day 0:

1. Titers are but VLPs are distinct.

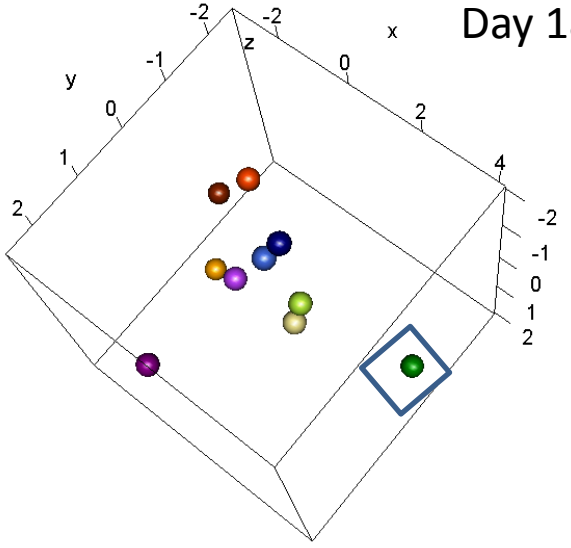
Day 7



Day 7:

1. Highest responses to vaccine components.
2. Early GII.4 cluster and are most close to GII.4C: memory response to multiple strains.
3. GI.1 distinct from other GI VLPs: secondary Ab response to GI.1.

Day 18



Day 180:

1. All of the titer have decreased, distances between VLPs is less.
2. GII.4.1997 Ab is predominant response suggesting original GII.4 strain exposure continues to be boosted and may shape subsequent GII.4 Ab responses.

Biochemical assay validation of bioinformatics cartography data

GII.4C vaccine response is cross-reactive Ab
GII.4.1997 responses predominate?

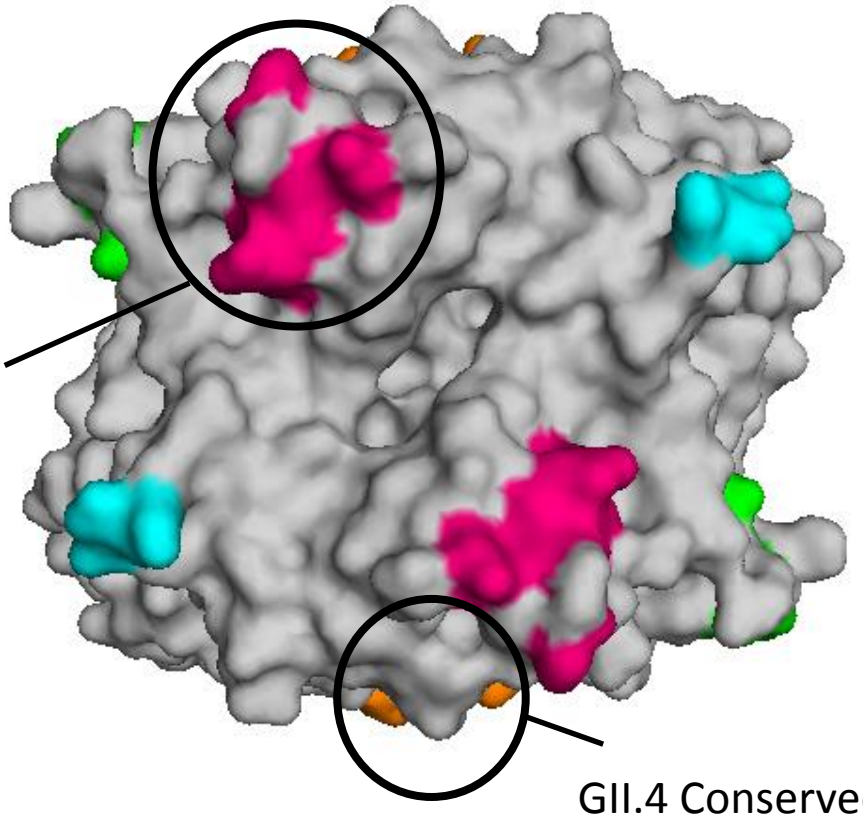
Epitope-Specific Blockade of Binding (BOB) Assay

Lindesmith and Baric 2015

Epitope Blockade of Binding (BOB) Assay

Does serum block binding of epitope-specific mAbs?

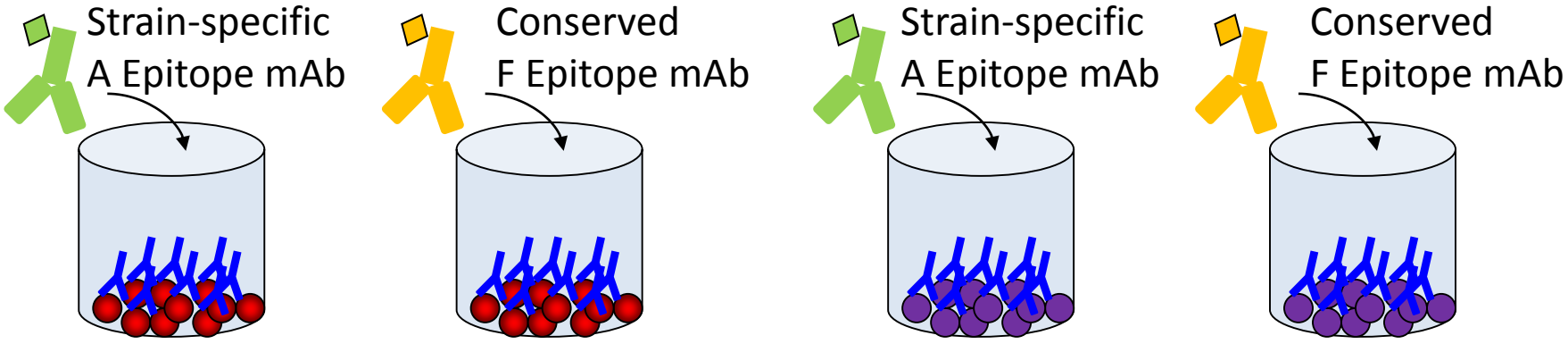
Stain-specific blockade epitope Changes with each significant strain emergence
Epitope A



GII.4 Conserved Blockade Epitope
Epitope F

Is cross blockade the result of Ab to conserved blockade epitope or multiple strain-specific epitopes?

Does serum have Abs to the hypervariable A or conserved F epitopes?



GII.4.1997 VLP

GII.4.2006b VLP

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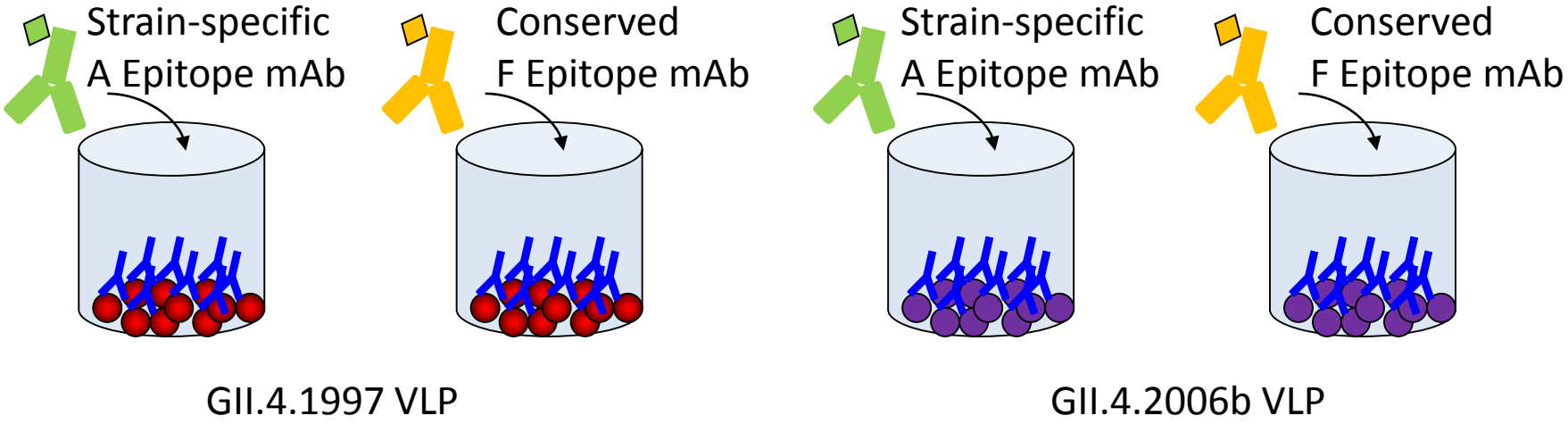
Does serum have Abs to the hypervariable A or conserved F epitopes?



Day 0

No sera blockade of GII.4.1997 Epitope A or F

No sera blockade of GII.4.2006b Epitope A or F

No/Low Abs to either blockade epitope in either strain.

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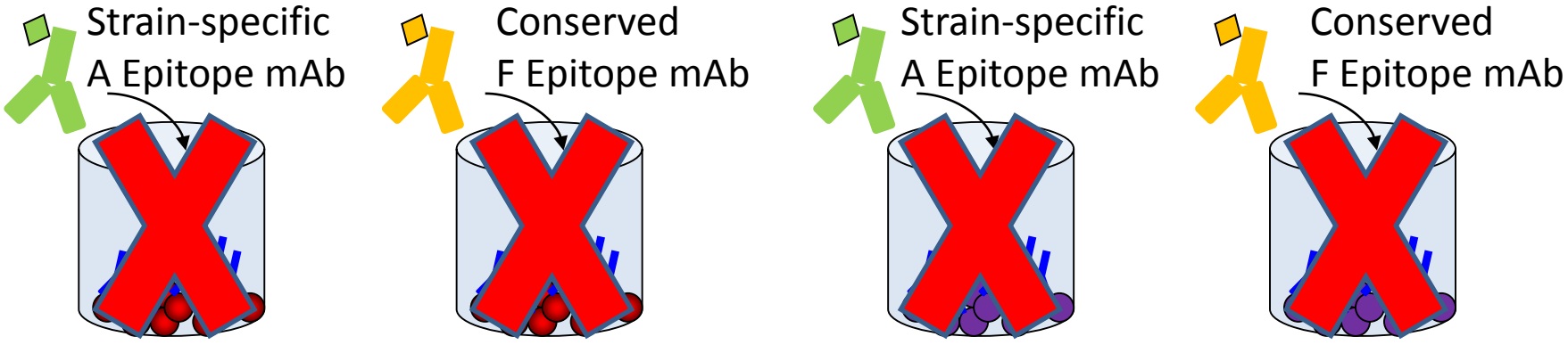
Does serum have Abs to the hypervariable A or conserved F epitopes?



Day 0

No sera blockade of GII.4.1997 Epitope A or F
No sera blockade of GII.4.2006b Epitope A or F
No/Low Abs to either blockade epitope in either strain.

Day 7

Peak sera blockade of GII.4.1997 Epitope A > F
Peak sera blockade of GII.4.2006b Epitope A > F
Abs to conserved blockade epitope & strain-specific epitopes in multiple GII.4 strains.

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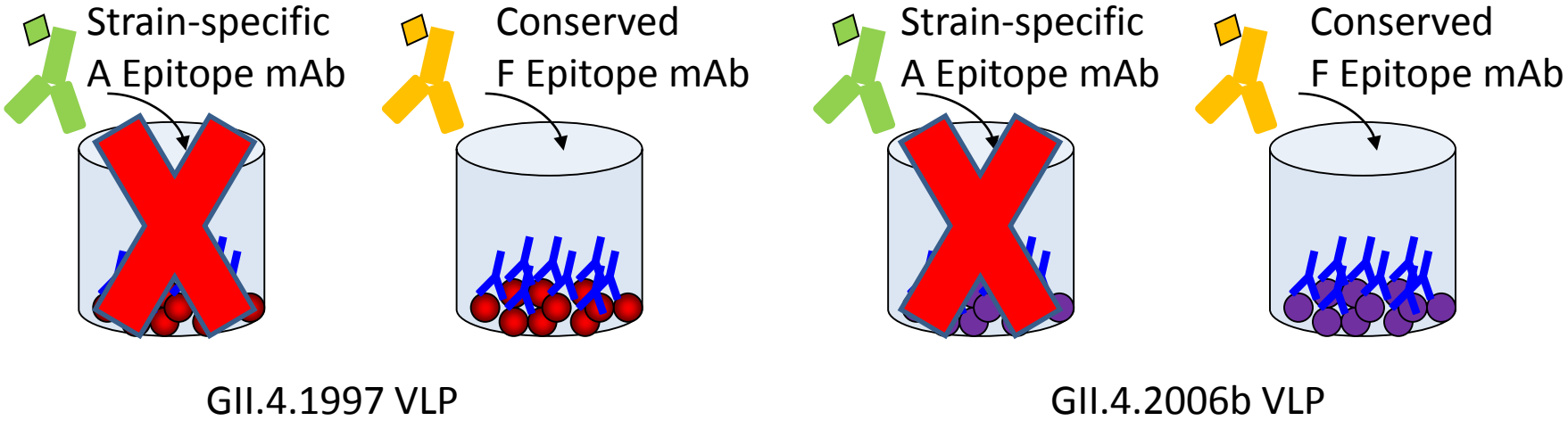
Does serum have Abs to the hypervariable A or conserved F epitopes?



Day 0
No sera blockade of GII.4.1997 Epitope A or F
No sera blockade of GII.4.2006b Epitope A or F
No/Low Ab to either blockade epitope in either strain.

Day 7
Peak sera blockade of GII.4.1997 Epitope A > F
Peak sera blockade of GII.4.2006b Epitope A > F
Ab to conserved blockade epitope & strain-specific epitopes in multiple GII.4 strains.

Day 35
Sera blockade of GII.4.1997 Epitope A only
Sera blockade of GII.4.2006b Epitope A only
Ab to only strain-specific epitopes in multiple GII.4 strains.

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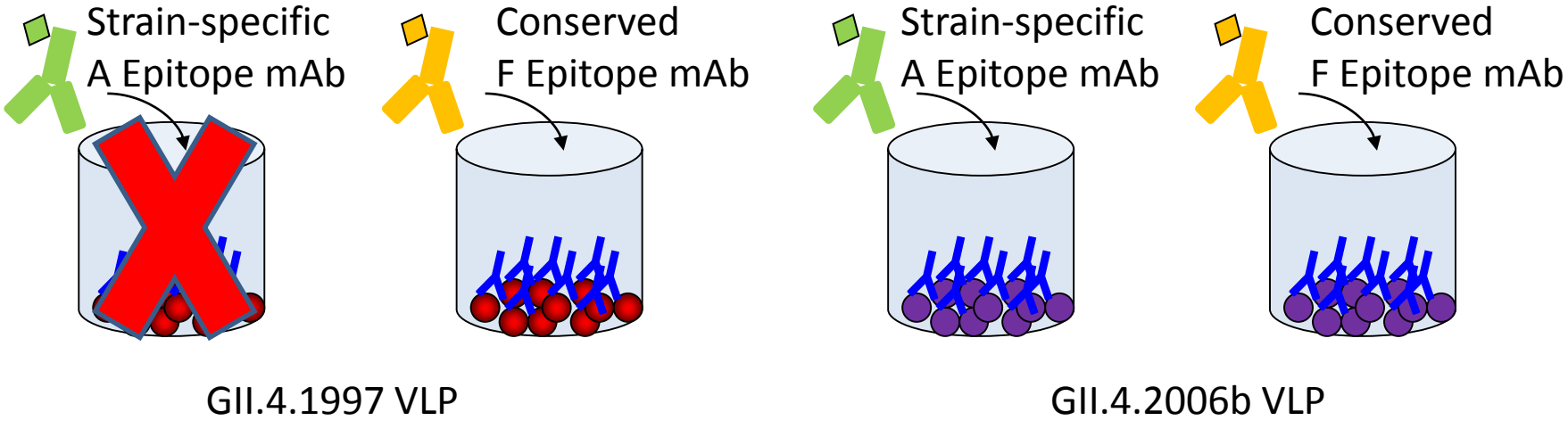
Does serum have Abs to the hypervariable A or conserved F epitopes?



Day 0
No sera blockade of GII.4.1997 Epitope A or F
No sera blockade of GII.4.2006b Epitope A or F
No/Low Ab to either blockade epitope to either strain.

Day 7
Peak sera blockade of GII.4.1997 Epitope A > F
Peak sera blockade of GII.4.2006b Epitope A > F
Ab to conserved blockade epitope & strain-specific epitopes in multiple GII.4 strains.

Day 35
Sera blockade of GII.4.1997 Epitope A only
Sera blockade of GII.4.2006b Epitope A only
Ab to only strain-specific epitopes in multiple GII.4 strains.

Day 180
Sera blockade of GII.4.1997 Epitope A only
Ab to only GII.4.1997-specific

How can the vaccine induce a response to hypervariable epitope A in Multiple Strains?

Explain the BOB data:

1. Not really epitope A, steric hindrance
   Other mabs or pAb don't block binding of epitope A mabs.

2. Rare class of cross-reactive epitope A Abs that are expressed post - vaccination due to the novel antigen that GII.4C represents.
   Chimeric GII.4C VLP unique epitope A, then vaccination could preferentially select for clones that recognize a conserved set of residues that are either part of epitope A or structurally near epitope A.

3. Epitope A has a linear, conserved stretch of amino acids
   Cross-GII.4 EIA binding with select mabs.

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Conclusions

GII.4 evolution impacts HBGA binding (host range) and antigenicity (breadth and duration of protective immunity).

A multivalent vaccination approach expands the blockade Ab response.

Questions:

Blockade Ab titers associated with protection for GII.4 (other) strains?

Impact of blockade Ab titer “ceiling” (lack of boost after second dose)?

How to test vaccine efficacy in the context of universal exposure?

Highest priority for progress?

Human challenge studies

Lindesmith and Baric 2015

Acknowledgements

Ralph Baric Lab

Ralph Baric

Norovirus

Lisa Lindesmith

Michael Mallory

Excel Swan

Dengue Virus

Emily Gallichotte

Stephanie Marcett

Jessica Plante

Jesica Swanstrom

Doug Widman

Ellen Young

Coronavirus

Anne Beall

Adam Cockrell

Rachel Graham

Lisa Gralinski

Steven Hartman

Cara Jenson

Jacob Kocher

Sara Leist

Vineet Menachery

Kayla Peck

Alexandra Schaefer

Trevor Scobey

Timothy Sheahan

Amy Sims

Ande West

Boyd Yount

Antigenic Cartography

Martin Ferris-UNC

Takeda Vaccines Inc.

Conducted the study

Frank Baehner

Robert Bargatze

Jennifer Ferreira

Robert Goodwin

Paul Mendelman

Charles Richardson

NIAID Funding

R01AI056351

R56A15-0756

U19 AI109761 CETR