Efficacy of a Trivalent Subunit Antigen Vaccine in Prevention of Genital Herpes

HSV Epidemiology

HSV-1

- **5 billion infected worldwide: 60% in high income countries, 90% in low income countries**
- **HSV-1 more common than HSV-2 as cause of 1st time genital herpes**
- **200 million cases of genital herpes caused by HSV-1**

HSV-2

- **½ billion infected worldwide: 11% seroprevalence 15-49 yr worldwide, 30% in Africa (higher in sub-Saharan Africa), 14% USA**
- **Ratio of infection female to male: 60:40**

Rationale for an HSV-2 vaccine

- **Some individuals get very ill**
- **Many worry about transmitting, including to newborn**
- **3-4-fold higher risk of acquiring and transmitting HIV that is not reduced by acyclovir**
- **In settings with high HSV-2 prevalence, 25-50% of HIV infections are attributable to HSV-2**

Concerns

- **Animal models have not been used well to predict likelihood of success in humans**

Latest HSV-2 subunit vaccine trial

GSK/NIH gD2 subunit vaccine

- **Design:** **> 8000 HSV-1, -2 seronegative women, age 18-30 years**

  gD2 or hep A as control, 3 doses at 0, 1, 6 months
- **1° endpoint:** **HSV-1 or HSV-2 genital herpes disease**
- **Result:** **Vaccine was efficacious against HSV-1 genital disease (57%) but not HSV-2**
- **Comment:** **Neutralizing antibody titers were low (peaked at 1:29) and did not persist**

Belshe et al NEJM 2012

Why is developing an HSV-2 vaccine difficult?

- **HSV-1 and -2 encode many proteins that inhibit innate and acquired immunity**
- **2 immune evasion molecules are expressed on the virus envelop and at the surface of infected cells, making them potential targets for blocking antibodies**

  gC - inhibits complement activation

  gE - inhibits antibody activities

Subunit antigen vaccine

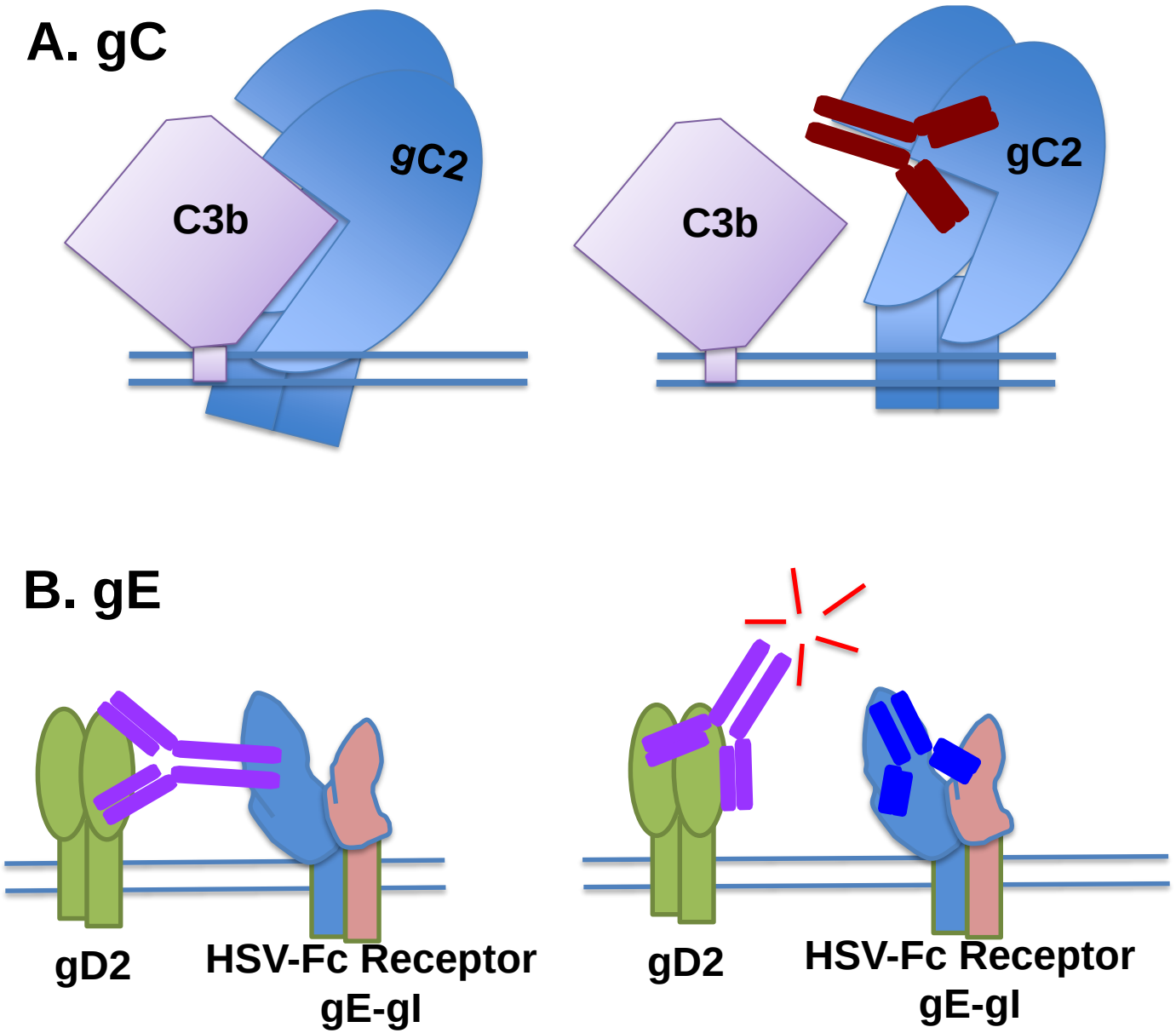
Baculovirus subunit antigens - almost the entire ectodomains:

- **gD2 – block entry**
- **gC2 – block immune evasion from complement**
- **gE2 – block immune evasion from antibody**

Adjuvants:

- **CpG – good B and T cell agonist**
- **Alum – good B cell agonist**

Immunization with gC and gE generates antibodies that block C3b and IgG Fc binding



gC2/gD2/gE2 as HSV-2 prophylactic vaccine

Goals:

Prevent acute disease
Prevent recurrent disease
Prevent risk of transmission to partner

The perfect result:

Acute disease: 0 days
Recurrent disease: 0 days
Genital shedding of HSV-2 virus: 0 days

gC2/gD2/gE2 as HSV-2 prophylactic vaccine

Goals:

Prevent acute disease
Prevent recurrent disease
Prevent risk of transmission to partner

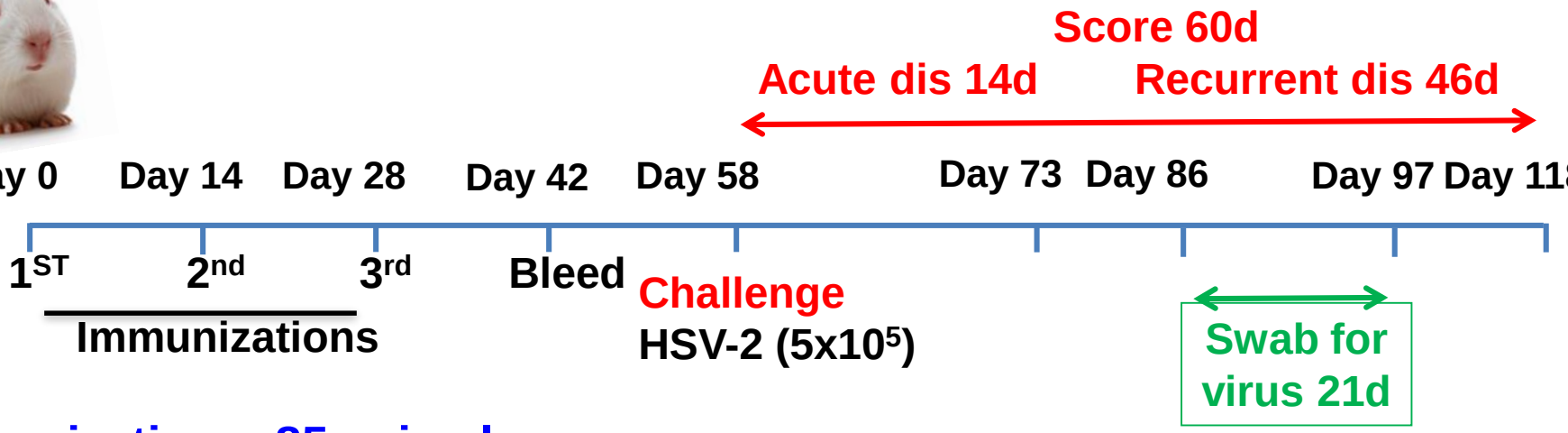
The perfect result:

Acute disease: 0 days
Recurrent disease: 0 days
Genital shedding of HSV-2 virus: 0 days

Our result:

Acute disease: 0.3% days
Recurrent disease: 1% days
Genital shedding of infectious virus during recurrent phase of infection: 0.2% of days

Prevention studies in guinea pigs

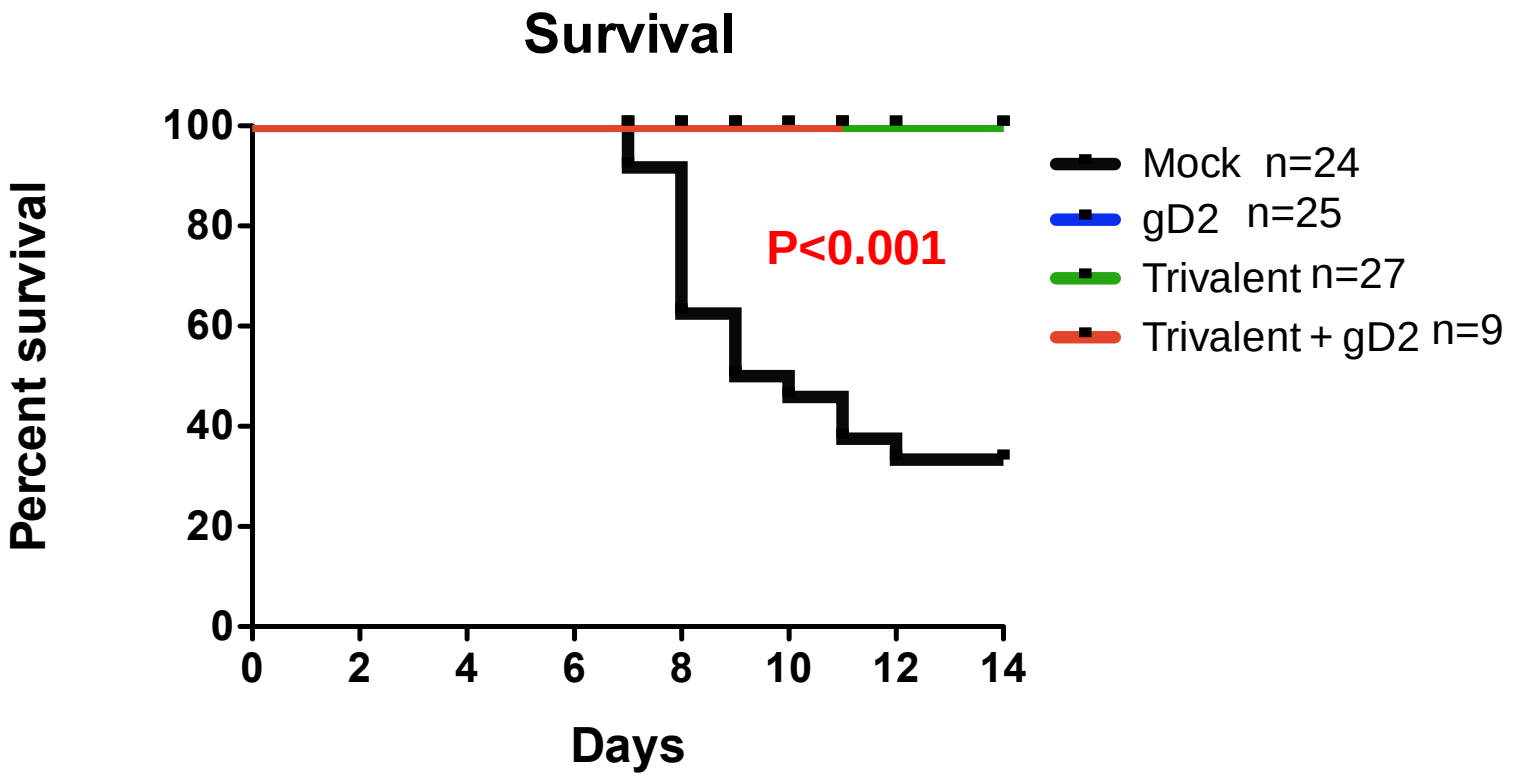


Immunizations: 85 animals

1. Mock (adjuvant alone) – 3 doses
2. gD2 with CpG/alum – 3 doses
3. gC2/gD2/gE2 antigen with CpG/alum adjuvants 3 doses
4. gC2/gD2/gE2 with CpG/alum + 4th dose with gD2 CpG/alum

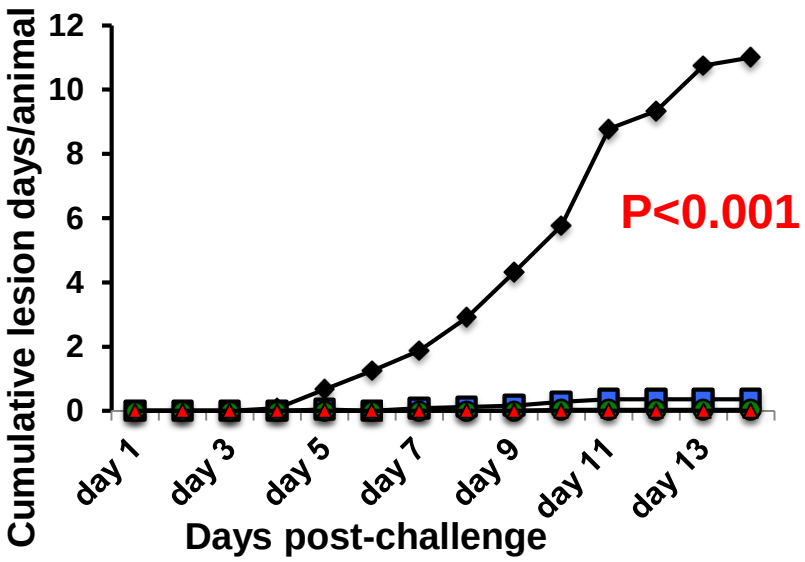
Awasthi et al PLOS Path 2017

Survival and other disease events in guinea pigs

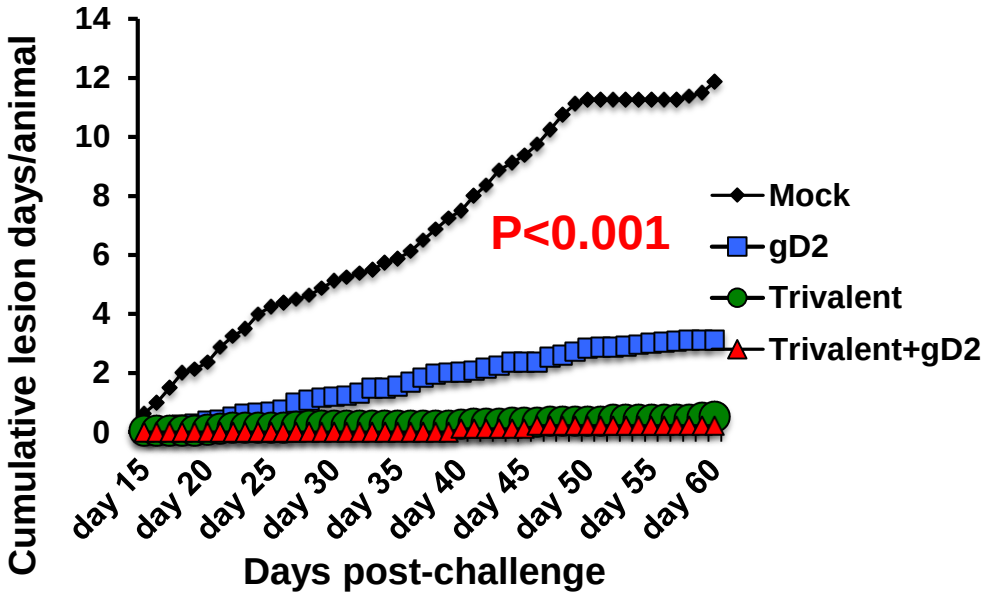


Guinea pigs: genital disease

Days acute genital lesions



Days recurrent genital lesions



Days with acute lesions

Group	Days
Mock (n=24)	88/250 (35.2%)
gD2 (n=25)	9/350 (2.6%)
Trivalent (n=27)	1/378 (0.3%)
Trivalent + gD2 (n=9)	0/126 (0%)

** (gD2 (n=25) vs Trivalent (n=27))

Days with recurrent lesions

Group	Days
Mock (n=8)	85/340 (25%)
gD2 (n=25)	77/1087 (7.1%)
Trivalent (n=27)	14/1158 (1.2%)
Trivalent + gD2 (n=9)	2/351 (0.6%)

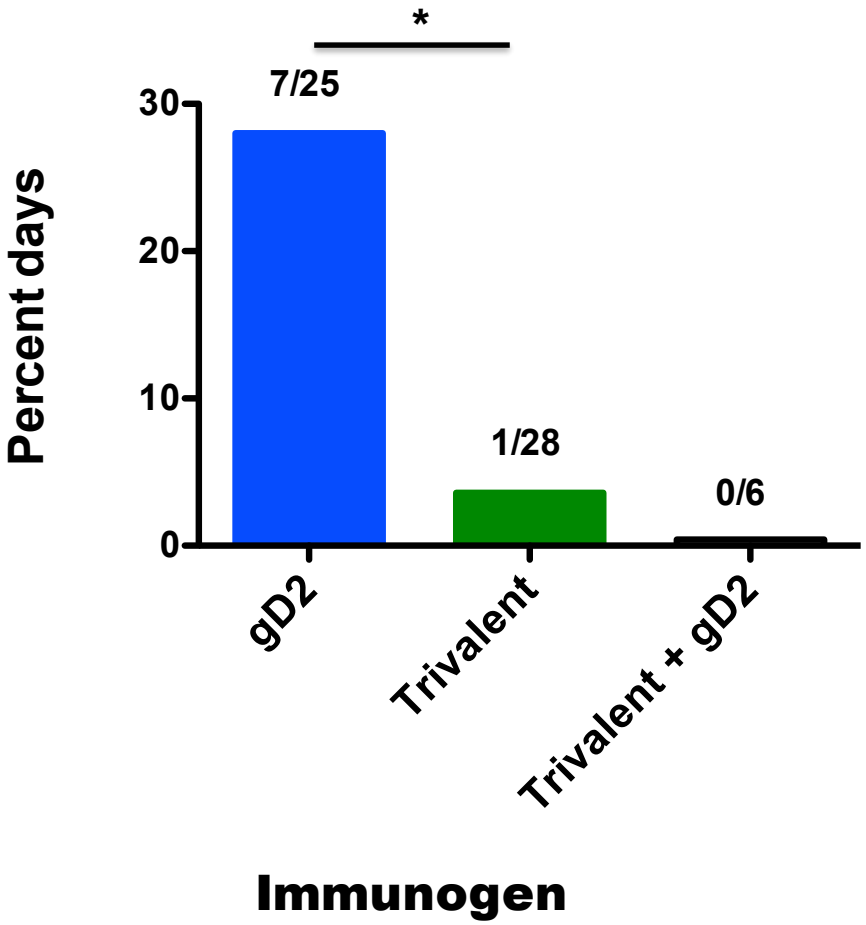
*** (gD2 (n=25) vs Trivalent (n=27))

*** (Mock (n=8) vs Trivalent + gD2 (n=9))

Vaccine efficacy based on days with lesions:

gD2 = 80%, trivalent = 97%, trivalent + gD2 = 99%

Vaginal shedding of replication competent virus



Conclusion guinea pig studies

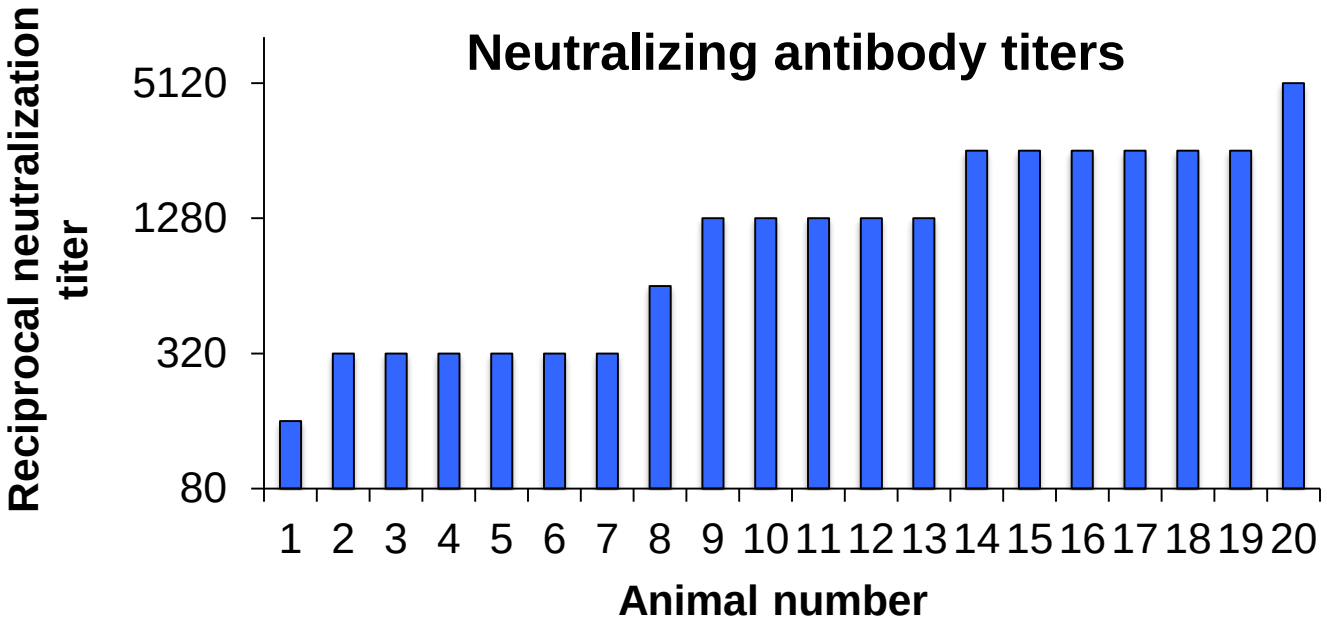
A trivalent vaccine that includes strategies to prevent HSV-2 immune evasion is a promising candidate for human trials.

Next steps

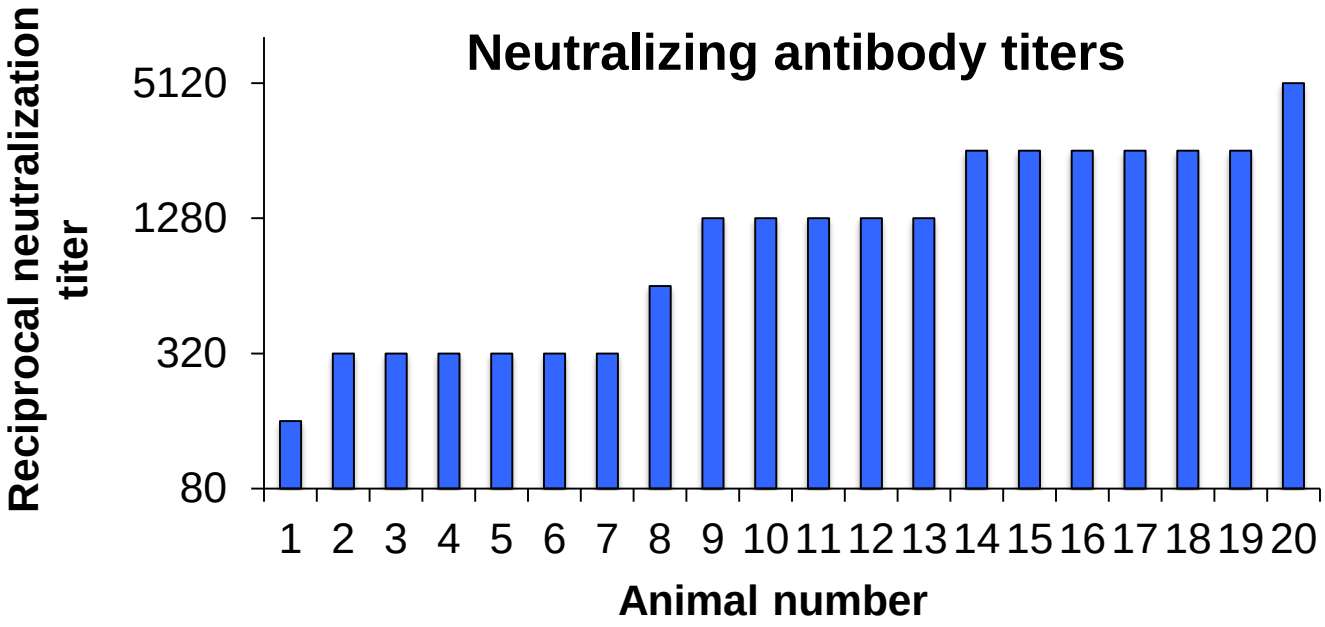
Find a sponsor for phase I/II human trials

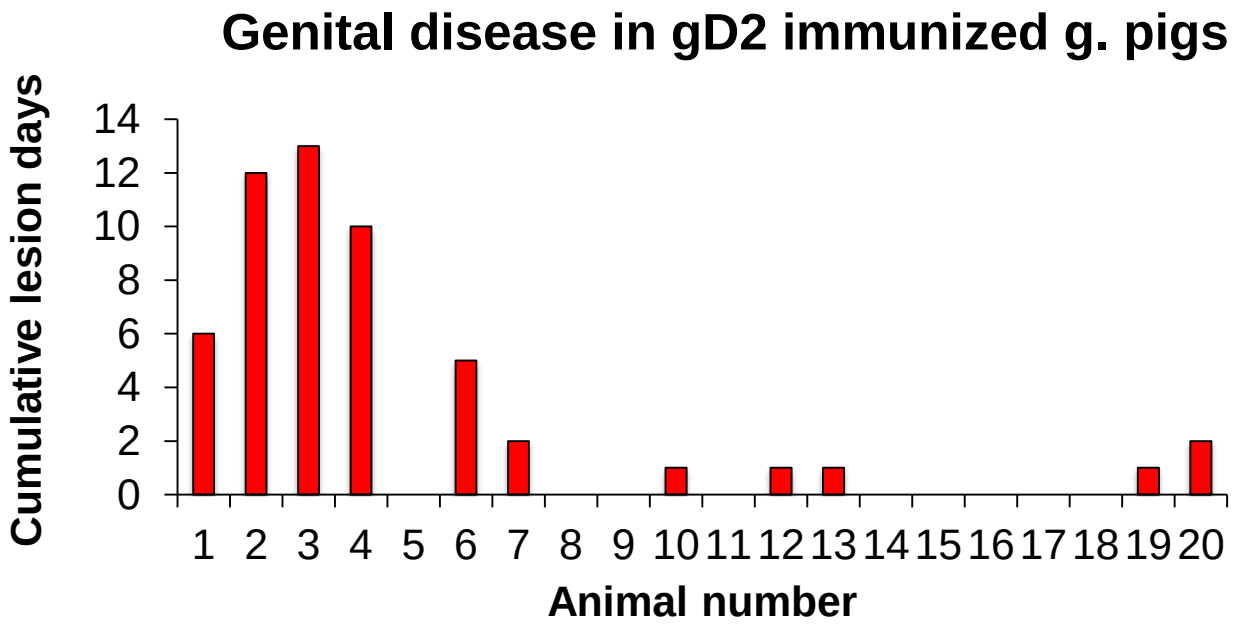
Use guinea pig model to determine immune correlates of protection to improve predictive power of animal studies for human trials

Antibody correlates of protection against gD2

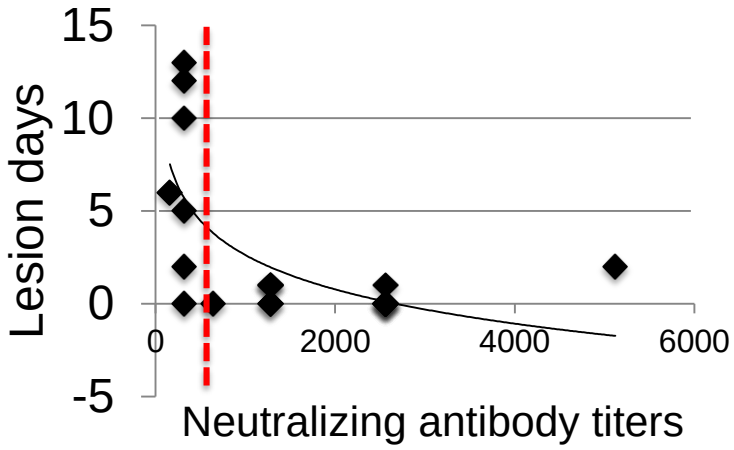


Antibody correlates of protection against gD2





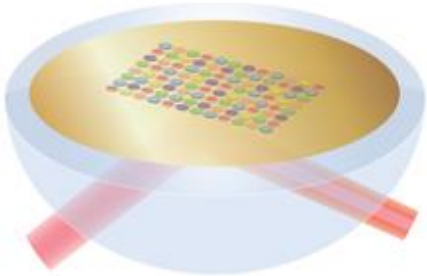
Neutralizing antibody response correlates with protection from HSV-2 disease

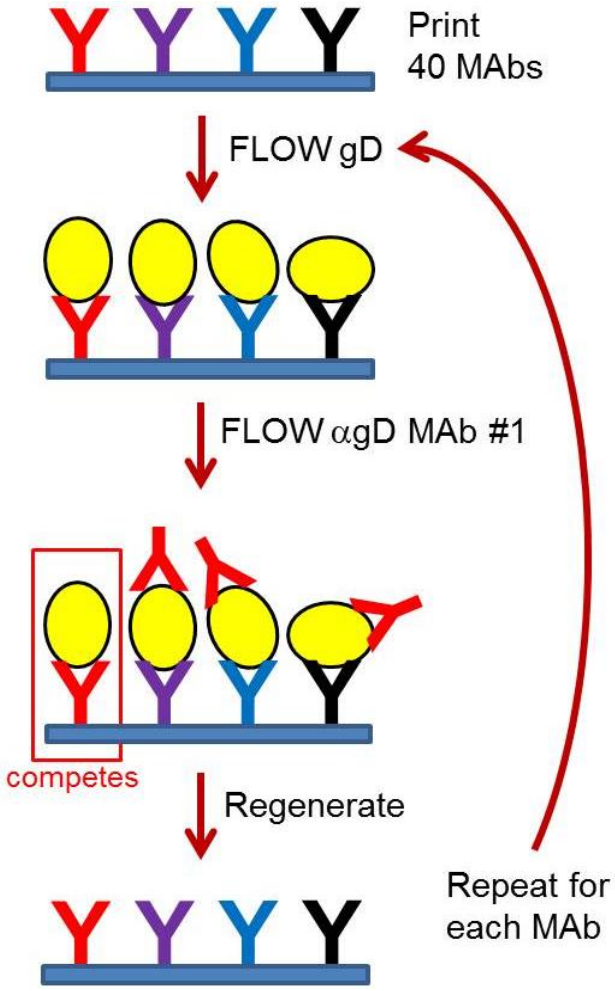


r = -0.57
p<0.01

Threshold neutralizing antibody titer: ≥1:320 correlates with strong protection

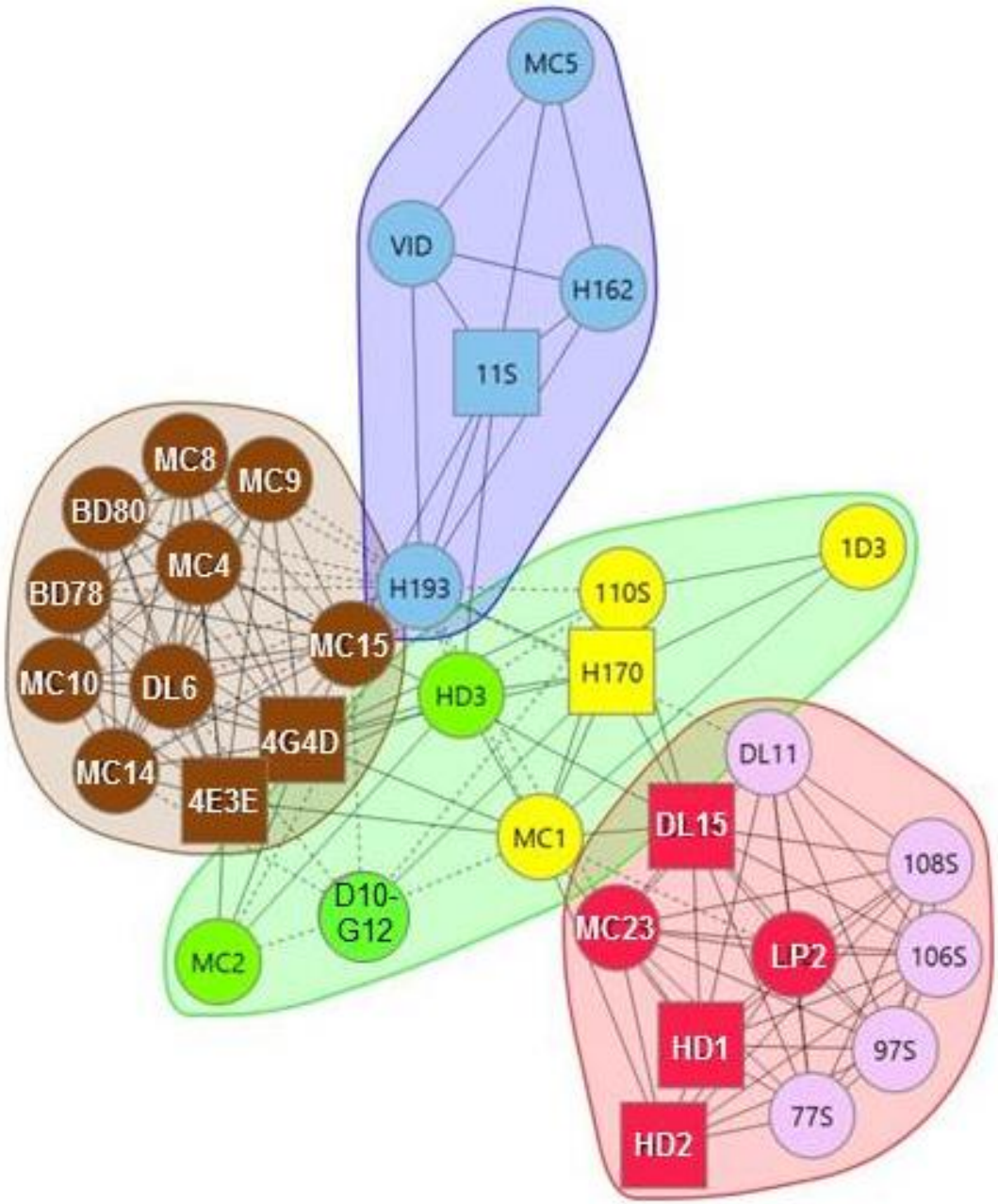
Measuring epitope-specific antibody responses



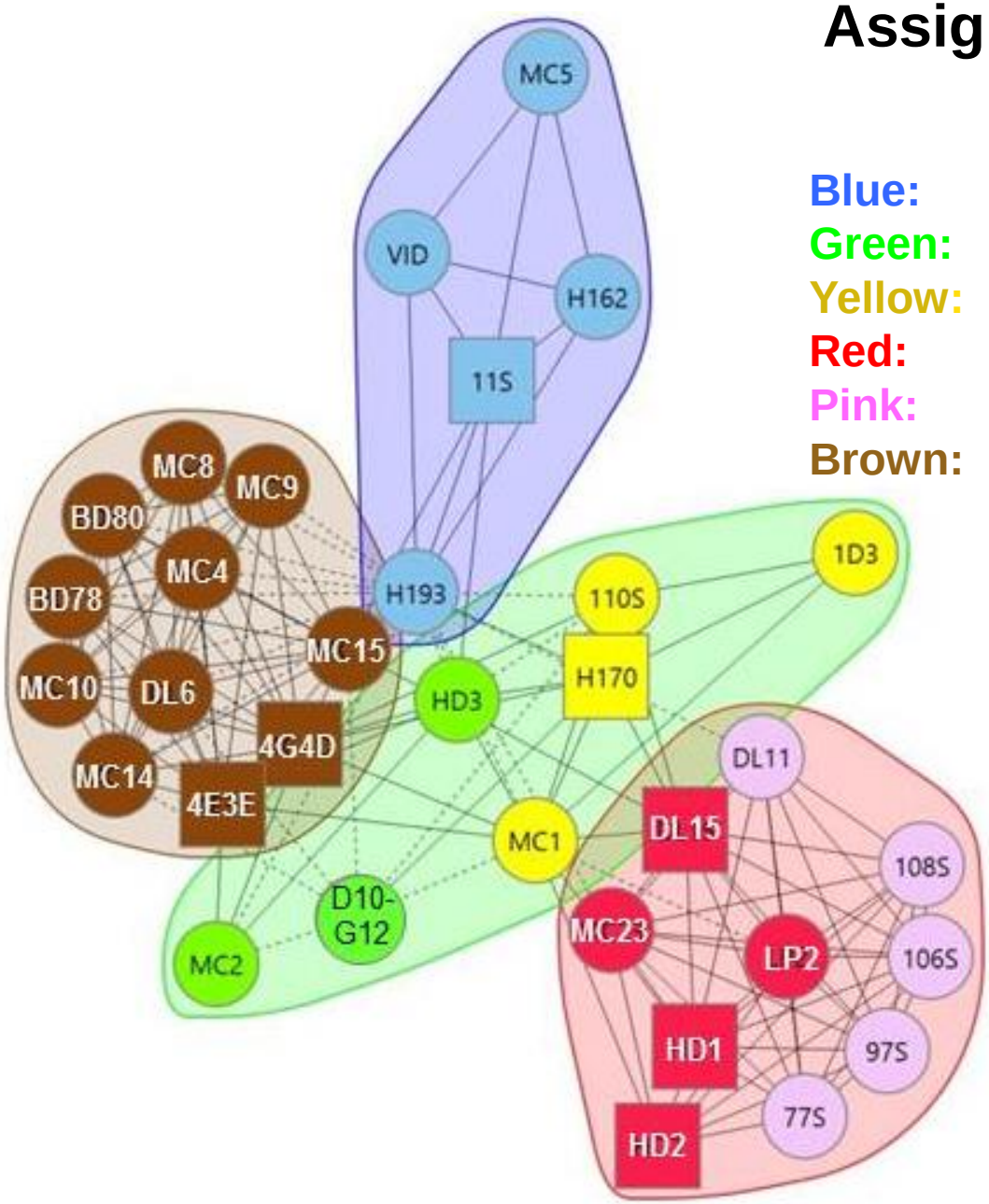


Carterra high throughput biosensor imager (left) and sensor chip with printed MAb (right).

Group MAb into communities and sub communities



Group MAb into communities and sub communities



Assign functions to MAb by in vitro assays

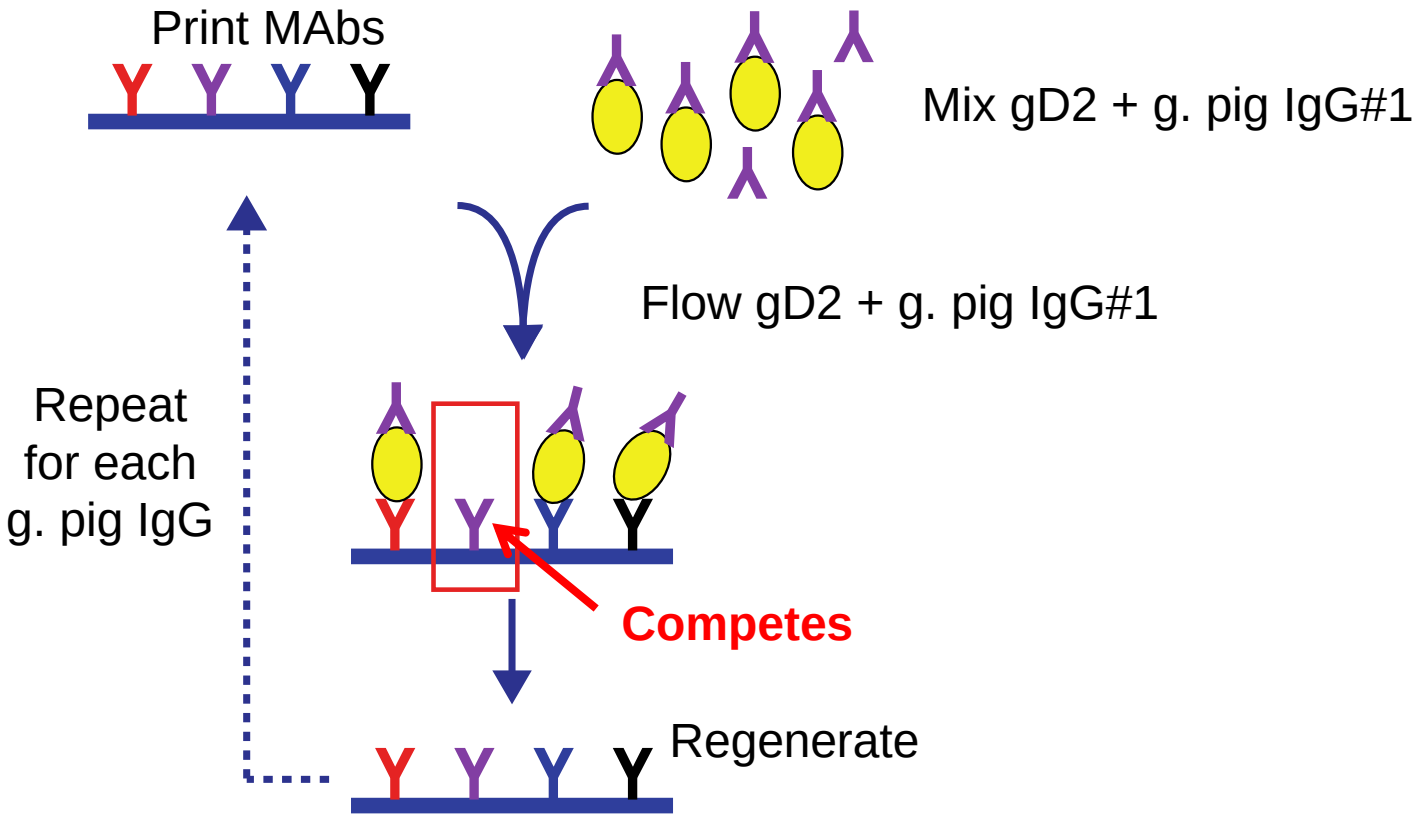
Blue: Blocks gD interaction with gH/gL
Green: Blocks gD interaction with gH/gL
Yellow: Blocks HVEM binding
Red: Blocks nectin-1 binding
Pink: Blocks nectin-1 & HVEM binding
Brown: Blocks cell-to-cell spread

Passive transfer in mice to assess whether MAb to crucial epitopes protect

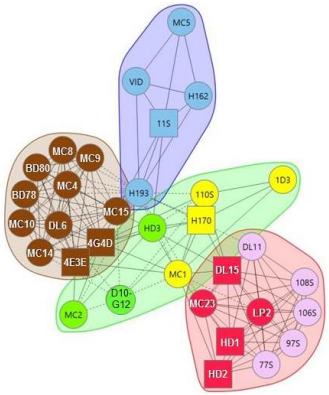
Community	MAb prototype	Function blocked	Survival (n=5)
Pink	DL11	Entry via nectin-1 & HVEM	100%
Blue	MC5	gD interaction with gH/gL	80%
Yellow	1D3	Entry via HVEM	80%
Green	MC2	gD interaction with gH/gL	60%
Brown	DL6	Cell-to-cell spread	60%
Red	MC23	Entry via nectin-1	40%
None	MC16	Binds non-crucial gD2 epitope	0%
None	Nonimmune IgG	None	0%

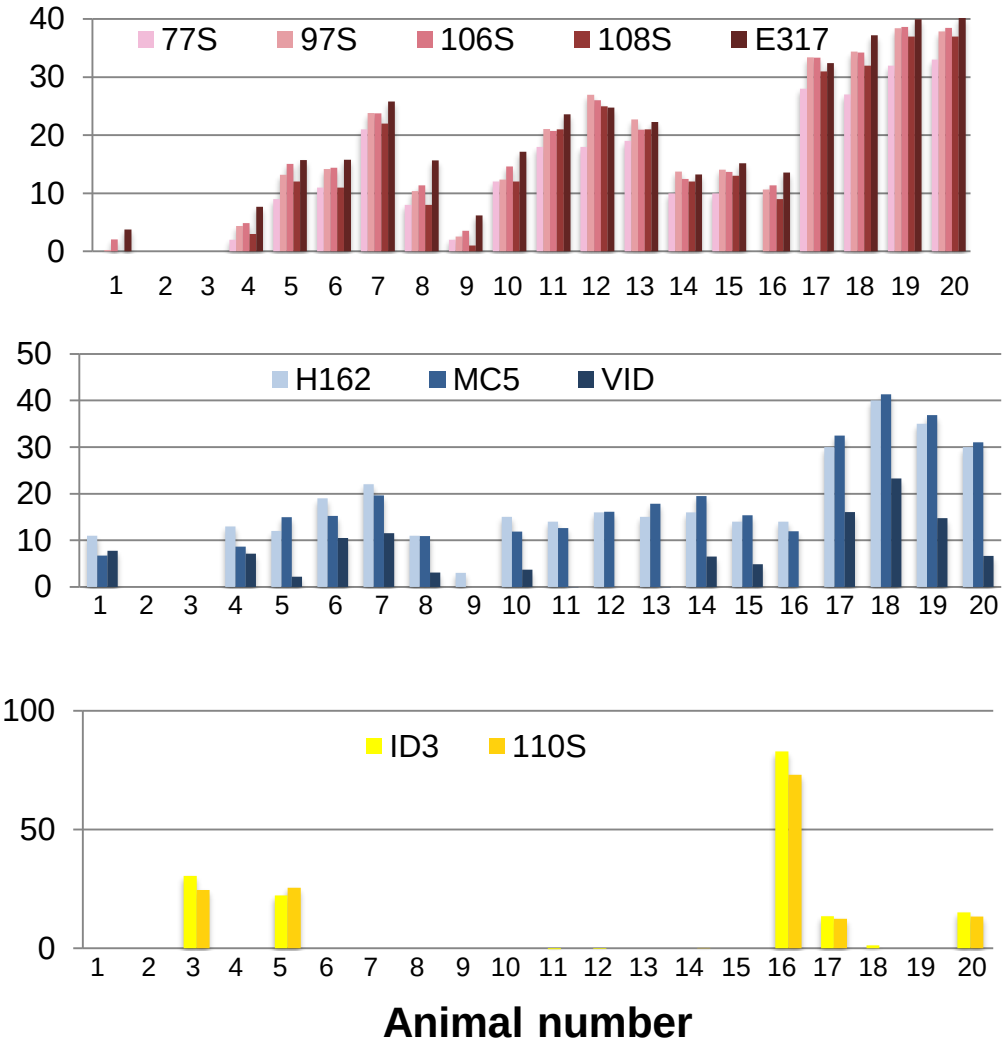
All MAb that block crucial gD functions *in vitro* protected *in vivo*, but some are more protective than others

Biosensor-based guinea pig immune IgG competition assay



Define epitope-specific antibody responses in immunized guinea pig IgG





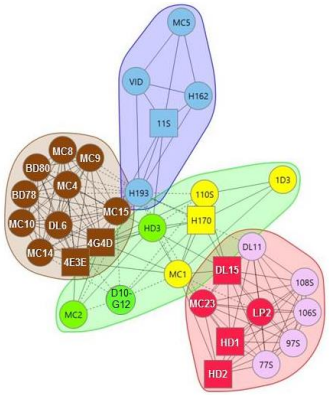
Pink: nectin-1 & HVEM
100% protection in mice

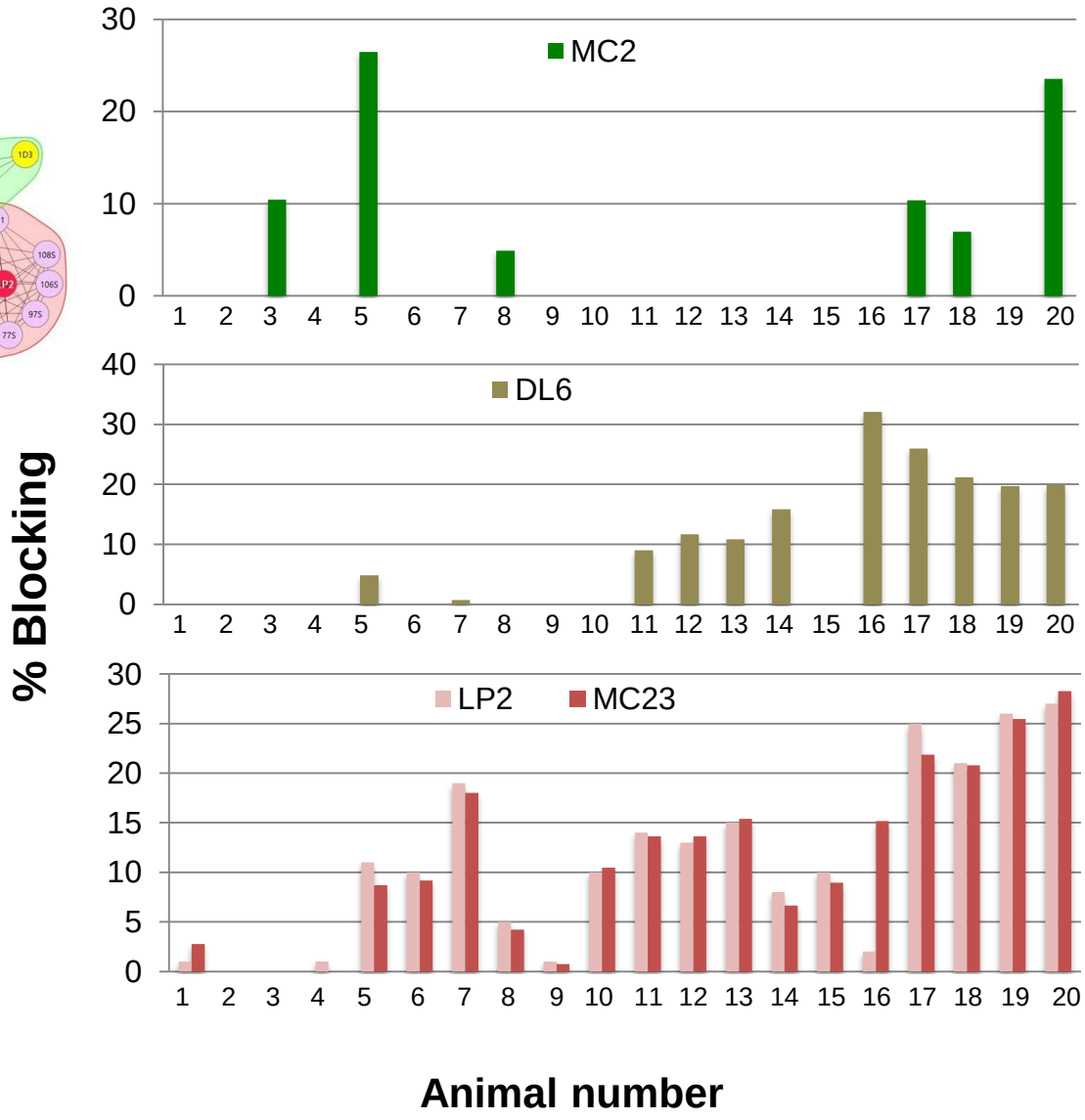
Blue: gD-gH/gL
80% protection in mice

Yellow: HVEM
80% protection in mice

Most animals produce antibodies to epitopes recognized by pink and blue but not yellow communities

Define epitope-specific antibody responses in immunized guinea pig IgG





Green: gD - gH/gL
60% protection in mice

Brown: Cell - cell spread
60% protection in mice

Red: nectin-1
40% protection in mice

Few animals produce antibodies to green and brown communities

Comparison of epitope-specific responses in gD2 immunized guinea pigs and humans

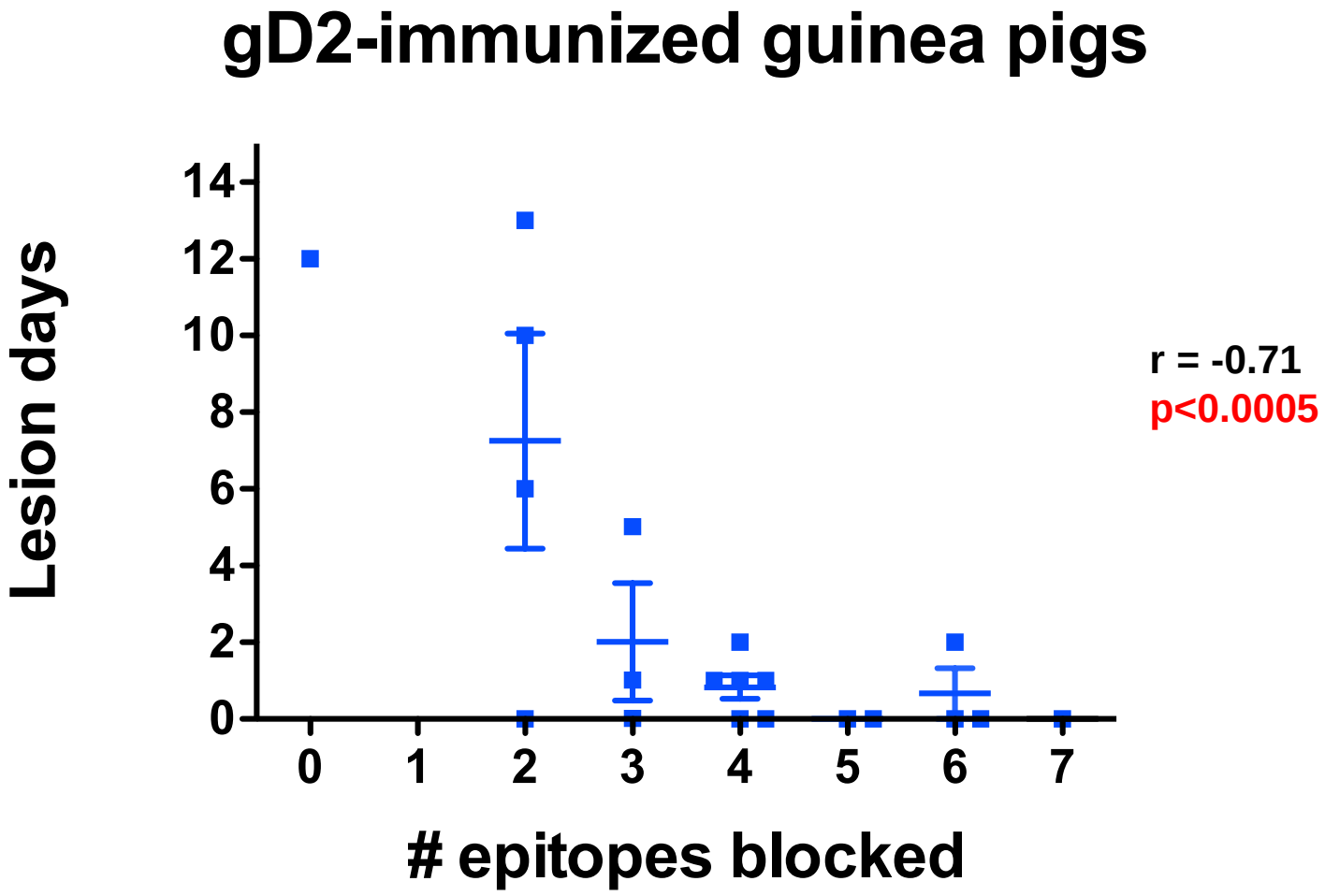
Table. gD2 immunization of humans

MAb	Community	Function of gD2 epitope	% protection in mice	Humans[1]
DL11 or 77S	Pink	Binds to nectin-1 & HVEM receptors	100%	29/29 (100%)
MC5	Blue	Interacts with gH/gL	80%	28/29 (97%)
1D3	Yellow	Binds to HVEM receptor	80%	0/29 (0%)
MC2	Green	Interacts with gH/gL	60%	28/29 (97%)
DL6	Brown	Promotes cell-to-cell spread	60%	0/29 (0%)
MC23	Red	Binds to nectin-1 receptor	40%	28/29 (97%)

[1] Whitbeck et al. J Virol 2014

Future goal: Improve antibody responses to yellow and brown communities, which were also weak immunogens in guinea pigs

Correlation between epitopes blocked and genital lesions



Conclusions

Protection correlates strongly with the number of crucial gD2 epitopes blocked

gD2 immunization in humans failed to produce antibodies to some epitopes that are highly protective

Future directions

Develop strategies to produce antibodies to all crucial gD2 epitopes

Perform similar studies with gC2 and gE2

Defining epitope-specific immune correlates of protection will improve the accuracy of animal models in predicting outcomes of human trials

Acknowledgments

Friedman lab

Sita Awasthi
Carolyn Shaw
Lauren Hook
Fushan Wang

Cohen / Eisenberg lab

Tina Cairns

Carterra, Inc.

Ben Brooks
Noah Ditto

Funding

NIH