How Would We License and Use a CMV Vaccine?

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Why a CMV Vaccine?

To prevent congenital infection

To prevent CMV infection in transplant recipients

- **-Seronegative solid organ transplant recipients at high risk of primary infection**
- **-Seropositive bone marrow transplant patients at high risk of reactivation**

Role of CMV in atherosclerosis and restenosis ?

Role of CMV in immunosenescence and cognitive function?

US National Academy of Sciences highest priority

Live CMV Vaccines in Development

Attenuated strain (Towne)	**Med Coll VA**
Recombinants with wild virus (Towne-Toledo)	**Medimmune**
Replication-defective virus	**Merck**
Alphavirus Replicon, VLP and RNA **Vectored: pox, adeno, LCMV**	**Novartis** **Sanofi Pasteur, City of Hope** **Queensland Inst. , Paxvax,** **Hookipa**

Non-Living CMV Vaccines in Development

Recombinant gB glycoprotein with adjuvant (2)	Sanofi Pasteur, GSK
DNA plasmids	Vical, Inovio
Self-Replicating RNA	Novartis
Peptides	City of Hope
Dense bodies	Vaccine Project Management (Germany)
Virus-like particles and soluble ntamers	Variations Bio, Redbiotech, Humabs

Lessons Learned from Prior CMV Vaccine Trials and Other Studies in Humans

- Neutralizing responses to gB can be elicited by a variety of vaccine approaches
- gB, if adjuvanted, is a protective antigen against CMV infection in seronegative women and solid organ transplant recipients.
- Importance of antibodies that neutralize entry into epithelial cells?
- Live attenuated virus also protected immunosuppressed solid organ transplant recipients against severe disease, and volunteers against low dose challenge.
- CTL cell responses to pp65 can reduce replication of CMV in hematogenous stem cell recipients

Chief Unanswered Questions About Prevention of CMV

- **Importance of cellular immune response in maternal-fetal transmission?**

- **Can maternal-fetal infection in seropositive women be prevented by boosting antibody or CMI?**

- **Can protective immune responses be prolonged?**

Probable First Targets for CMV Vaccination

Girls 11-13 yrs. of age
(association with HPV, TdAcP, MCV4)

Seronegative women of child-bearing age

Seronegative solid organ transplant recipients

Seropositive hematogenous stem cell recipients

How to Demonstrate Efficacy of a CMV Vaccine

- Artificial challenge with low passage virus
- Prevent infection of women whose children are in day care
- Prevent infection of children entered in day care
- Prevent disease or infection in solid organ or stem cell transplant recipients
- Cohort study in pre-pregnant women to prevent later fetal infection
- Prevention of fetal disease

Challenge Studies

Endpoint: Infection

Advantages: Small numbers of subjects
Quick answer

Disadvantages:
-Need challenge virus of defined virulence and dose
-Can results be extrapolated
to natural infection?
to other CMV strains?
-Ethical issues

Vaccination of Non-Pregnant Women Whose Children are in Day Care

Endpoint:	**Infection of women**
Advantages	**"Real-life" challenge** **High exposure** **Easy to define whether virus came from child**
Disadvantages	**Ethical issue about other means of prevention** **Doesn't test protection of fetus**

Vaccination of Children Attending Day Care

Endpoint:	**Infection of children**
Advantages	**Decreases circulation of CMV**
	Protects their mothers
Disadvantages	**Will immunity last until child-bearing age?**

Vaccination of Women Intending Pregnancy

Endpoint:	**Infection of women**
Advantages	**Real test of public health value of vaccination**
	If not infected, can't transmit to fetus
	Answer available with minimum specimens
Disadvantage	**Doesn't measure protection of fetus**

Vaccination of Women Intending Pregnancy with Follow-up

Endpoint: Infection of fetus

Advantages:

- Can demonstrate prevention of transmission to fetus
- Demonstrates real public health value

Disadvantages:

- Long study duration

Diagnosis of Neonatal Infection

(Sensitivity)

PCR – urine (100%) blood (100%)

Virus – urine (100%), saliva (100%), blood (28%)

IgM antibody (71%)

Antigen – blood (43%)

Vaccination in Solid Organ Transplantation (Recipients)

Possible Endpoint:

Viral Load

Use of Antivirals

Graft rejection

Vaccination in Bone Marrow Transplantation (Recipients ± Donors)

Possible Endpoint:

Return of CTL
Viral Load
Use of Antivirals
Disease

Speculative Targets for CMV Vaccination

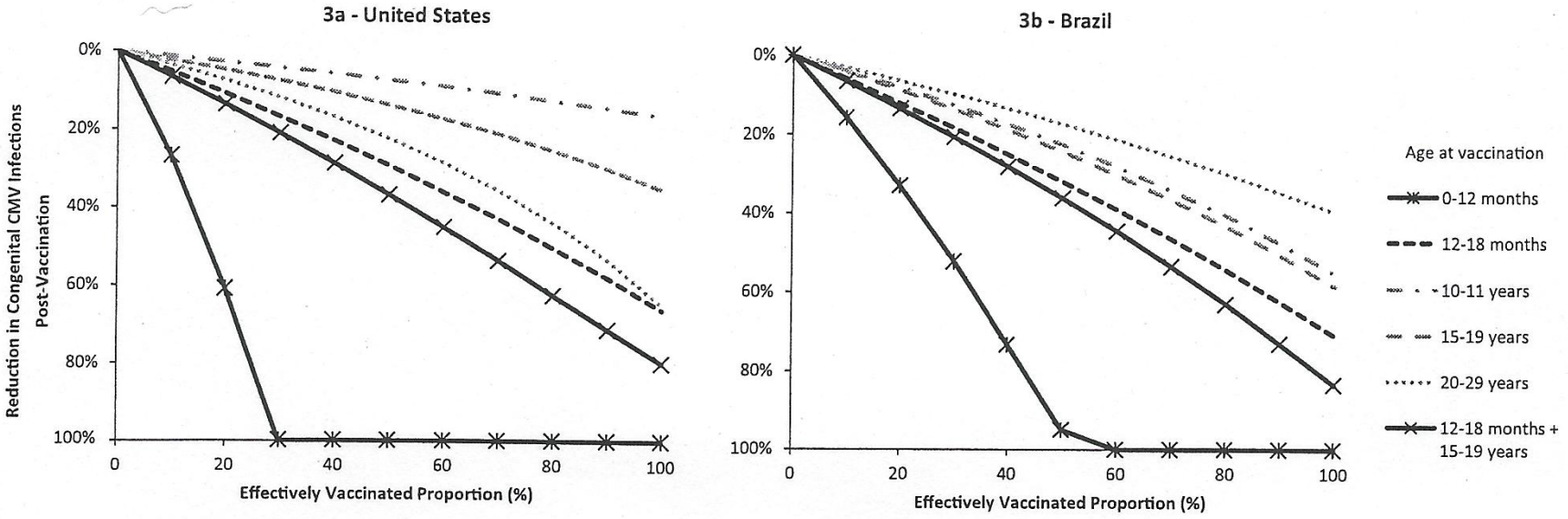
All infants, to reduce viral circulation

Seropositive recipients of bone marrow transplant

Prospective cardiac bypass patients
(to prevent atherosclerosis)

All elderly (to prevent immunosenescense)

Overall reduction in the annual number of cCMV infections at equilibrium by proportion of individuals effectively vaccinated by age at vaccination, assuming age-specific duration of infectiousness, 20-year duration of latency, and a vaccine with 5-year duration of protection, United States and Brazil.



Lanzieri TM, et al. Vaccine 32, 2014,

Demonstration of VE: Examples of Required Number of Primary Endpoint Cases

True (unknown) Vaccine Efficacy	Power	Total Cases	Critical Split	
			Vaccine	Placebo
50%	80%	209	≤75	≥134
	85%	234	≤85	≥149
	90%	277	≤102	≥175
75%	80%	33	≤8	≥25
	86%	39	≤10	≥29
	91%	44	≤12	≥32
80%	80%	24	≤5	≥19
	85%	27	≤6	≥21
	91%	33	≤8	≥25

Trials to Demonstrate Efficacy of a CMV Vaccine

Vaccinated Population	Endpoint	Incidence in Placebo Group (%)	No. of Subjects* 1 yr follow-up	2 yr follow-up
Mother of children in day care	Infection	25†	368	184
Pre-pregnant women	Fetal Infection	1	9,190	4,595
Pre-pregnant women	Fetal Infection	5‡	1838	919
Pre-pregnant women	Fetal disease	0.1†	91.900	45,950

Plotkin S. Ped Inf DisJ 1999

* Assuming vaccine efficacy is 80%, confidence limit ~ 50%, $\propto = 0.05$, $ß = 0.8$
Number is total of vaccine and placebo groups.
† Conservative estimate. ‡ High risk adolescents in lower socioeconomic group

Demonstration of VE: Number of Subjects (*CMV Seronegative*) Required to Accrue Required Primary Endpoint Cases (*cCMV Infection*)

Attrition (per year)	Pregnancy Rate (per year)	Infection Rate† (per year)	Transmission Rate‡ (per year)	Probability of becoming a case (per year)	Total Subjects to Enroll and Followed at the Indicated Duration to Acquire the Required Cases		
					2 years	3 years	4 years
Required cases = 44 [Power = 91% when VE = 75%]							
15%	10%	1%	30%	0.0003	126,864	91,246	73,670
			40%	0.0004	95,153	68,440	55,260
		2%	30%	0.0006	63,441	45,635	36,849
			40%	0.0008	47,585	34,232	27,644
	15%	1%	30%	0.0005	84,582	60,839	49,123
			40%	0.0006	63,441	45,635	36,849
		2%	30%	0.0009	42,300	30,432	24,576
			40%	0.0012	31,729	22,830	18,439

† Among CMV seronegative women; ‡ Among CMV seronegative women with primary infection.

Conclusions

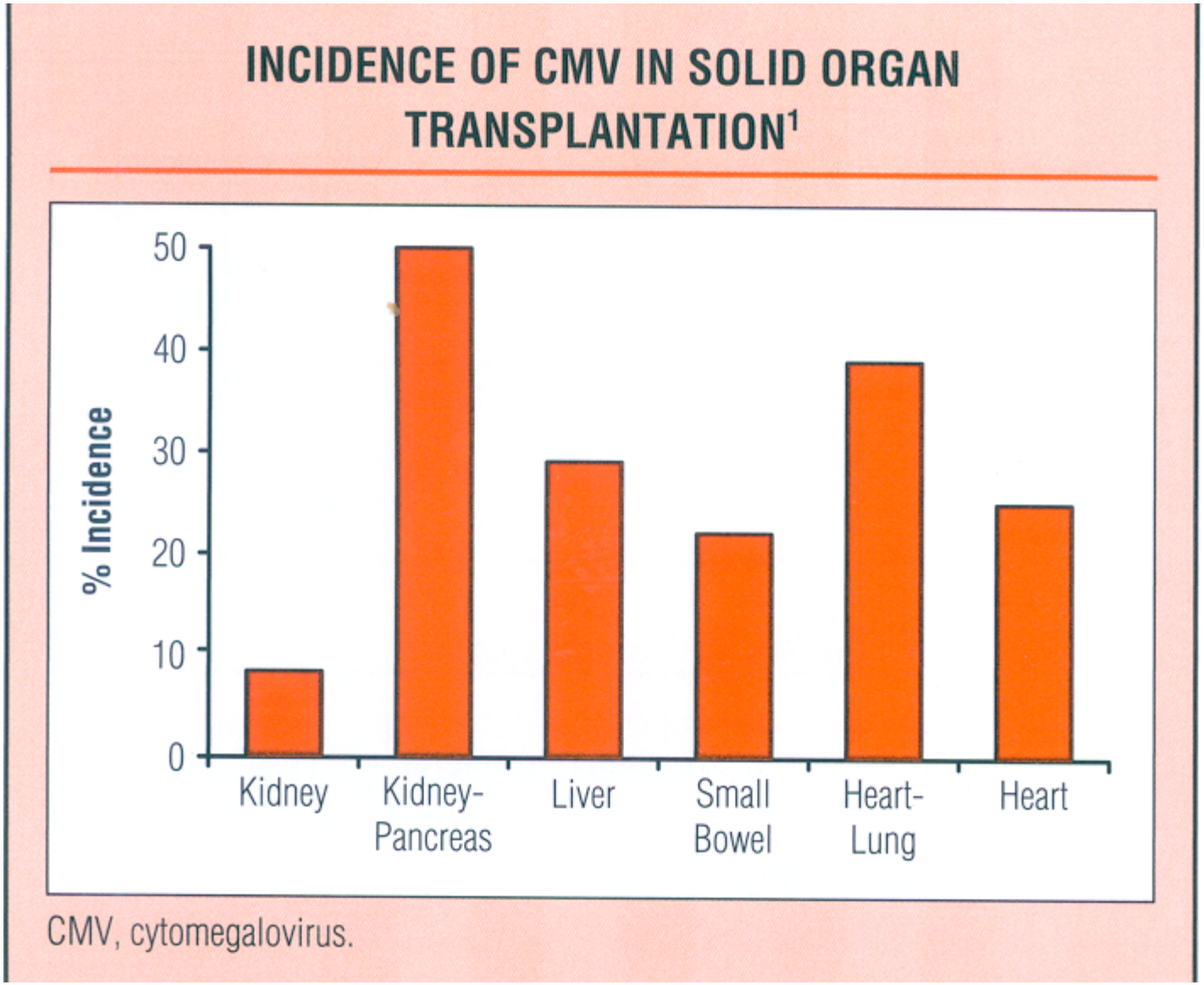
The components of a CMV vaccine are still being defined, but the candidates are known

Licensure of a CMV Vaccine is feasible, but:

- the choice of target populations is not yet defined.

- at the minimum they will include seronegative women and transplant recipients

Back Up



Clinical Courier 24(18):1 (May 2006)

Situations in which Antibodies Protect Against CMV Disease

- Newborns exposed to WBC carrying CMV
- Solid organ transplant recipients given passive antibodies
- Bone Marrow transplant recipients given passive antibodies (equivocal)
- Animal models (guinea pigs, mice)
- Protection of placenta by maternal antibodies
- Protection of fetus by infused antibodies ?

Neutralizing titers to CMV in adults After Natural Infection or Towne vaccine

Group	No. of Virus	No. of Doses/ Subject	Subjects	Reciprocal Antibodies Geometric Mean Neutralizing Titer
Females	Wild-type	0	15	488 (256-2048)
Males	Towne	1	23	270 (128-1024)
Males	Towne	2	43	402 (128-2048)
Males	Towne	3	12	512 (256-1024)

Adler S, et al. PIDJ 17:200-206, 1998

Situations in which Cellular Immunity Protects Against CMV Disease

Recovery of CD8 T cells after solid organ transplant

Recovery of CD8 T cells after bone marrow transplant

Infusion of CD8-CMV specific T cells after transplant

Closure of chronic CMV infection

Reduction of HIV by antivirals – recovery of CD4 T cells

Viral Antigens that might be Included in a Vaccine

For Antibodies

- **gB**
- **gH/gL**
- **gH/gL/UL128-131**

For CTL (% Positive in Seropositives)

- **pp65 (92%)**
- **IE1 (76%)**
- **gB (33%)**
- **pp150 (30%)**

Gyulai et al.

Studies of T cell Responses by K. Frueh, L. Picker, et al.

- pp65 T cell responses do not prevent reinfection but reduce viral dissemination during primary infection

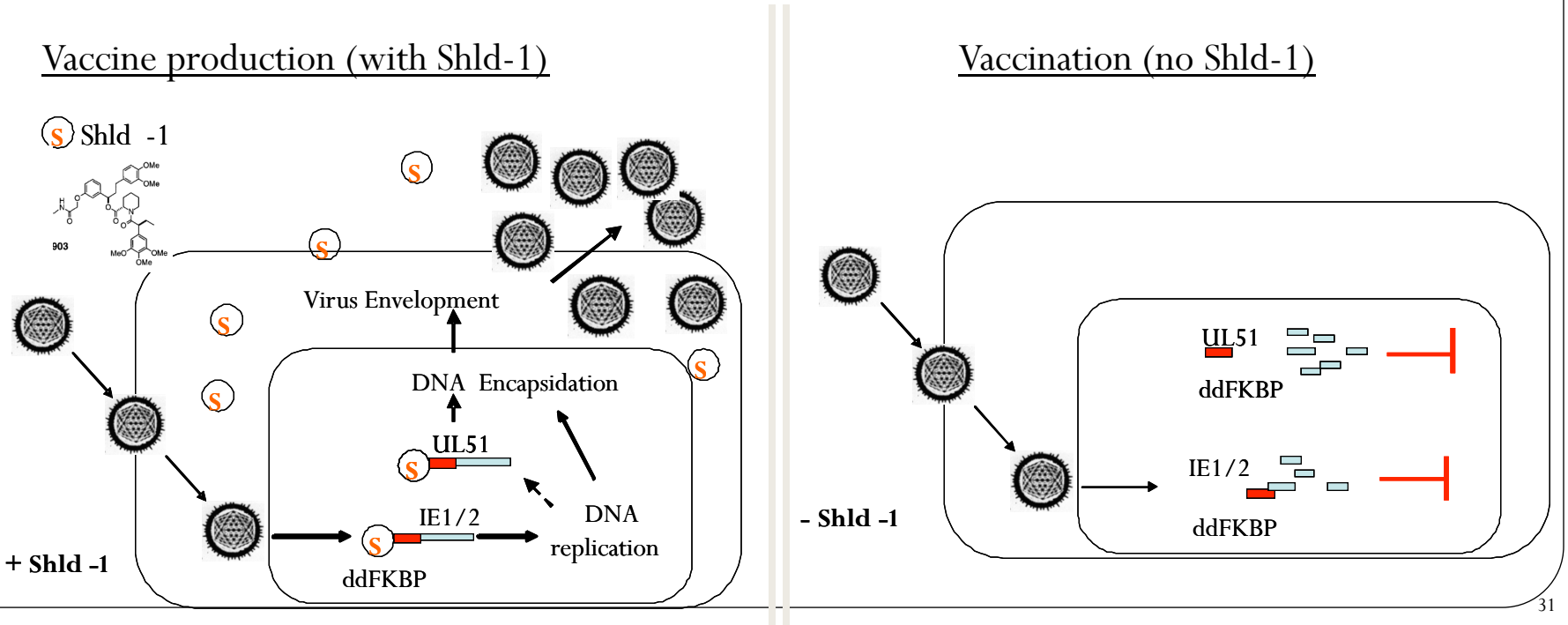
- Reinfection is mediated by the action of US2-11, which inhibits HLA-mediated host responses.

Doses of Subcutaneous CMV Challenge Required to Infect or Cause Disease in 50% of Different Groups

	Infection	Disease
Seronegatives	< 10 PFU	< 10 PFU
Natural seropositives	≈ 500 PFU	1000 PFU
Vaccinees	100 PFU	>100 PFU

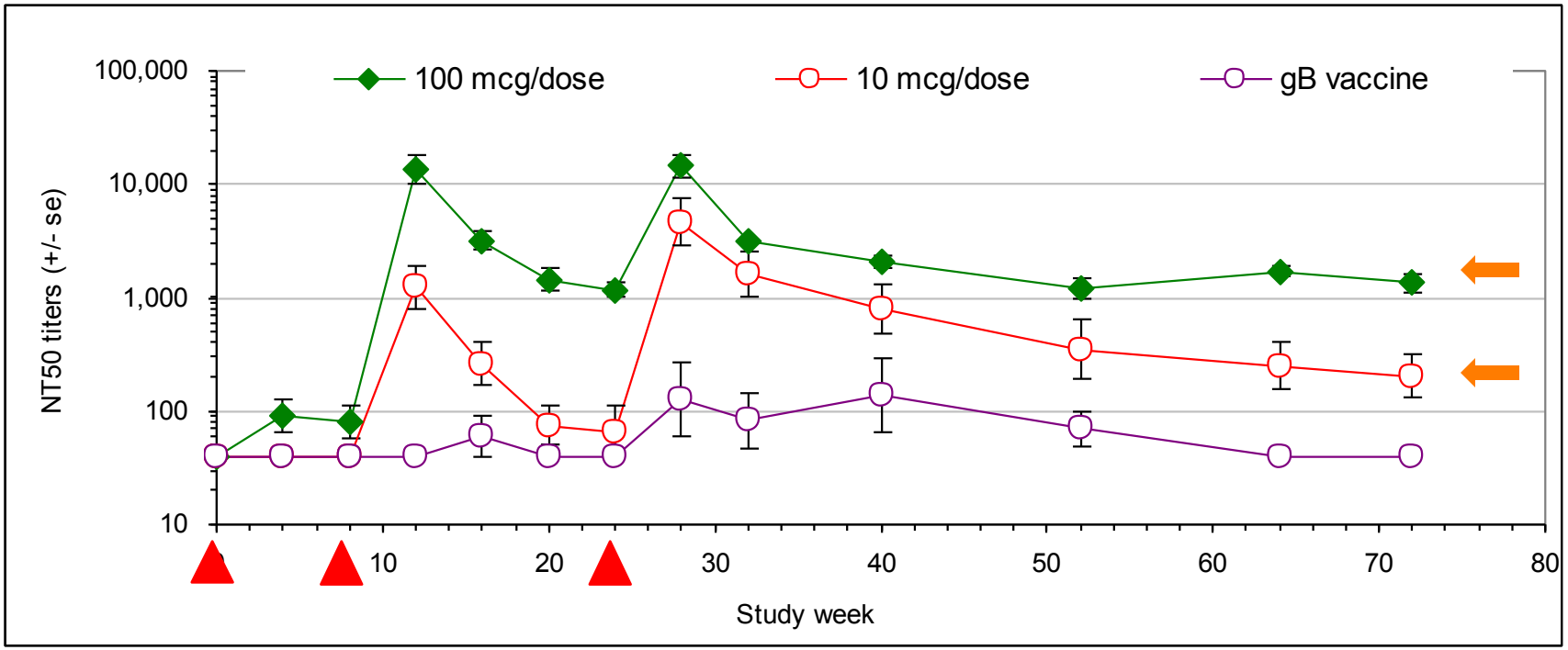
Merck CMV vaccine concept is based on

- inclusion of pentameric glycoprotein H (gH) complex
- T-cells that may contribute to (1) protective immunity and (2) durability of vaccine-induced protection
- UL51 and IE1/2 are fused to ddFKBP, which renders the CMV proteins unstable and therefore prevents replication, whereas the addition of Shld-1 stablilizes the ddFKBP and therefore permits replication.



31

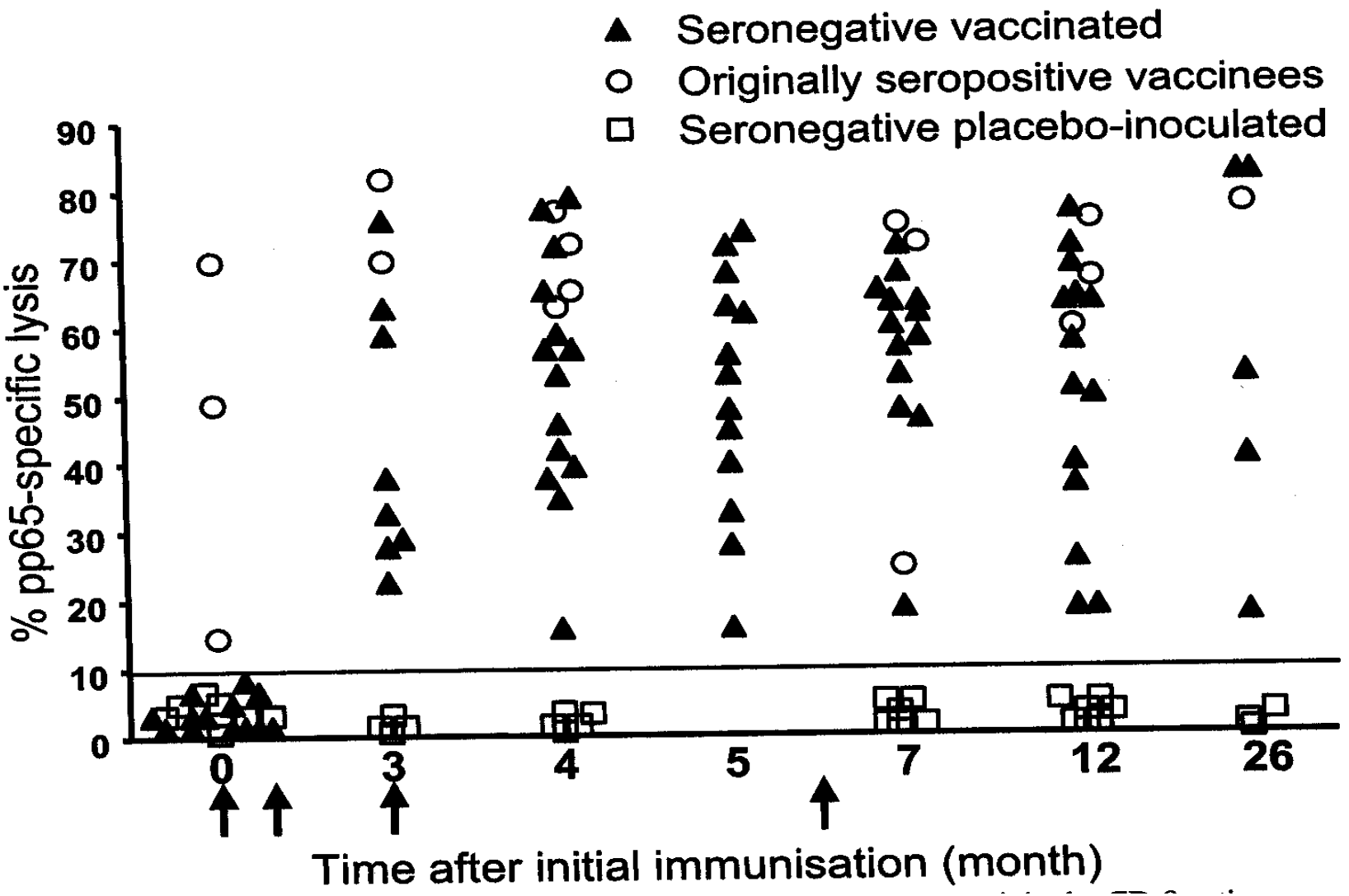
Merck CMV vaccine elicits neutralizing Abs in rhesus monkeys



- Vaccine was administrated at 100 or 10 μg/dose in rhesus macaques (n=5).
- Neutralizing Abs against viral epithelial entry are measured at the indicated time points.
- Recombinant gB vaccine with an oil-in-water emulsion adjuvant

T-cell responses to multiple viral antigens were demonstrated in ELISPOT assay (Data not shown)

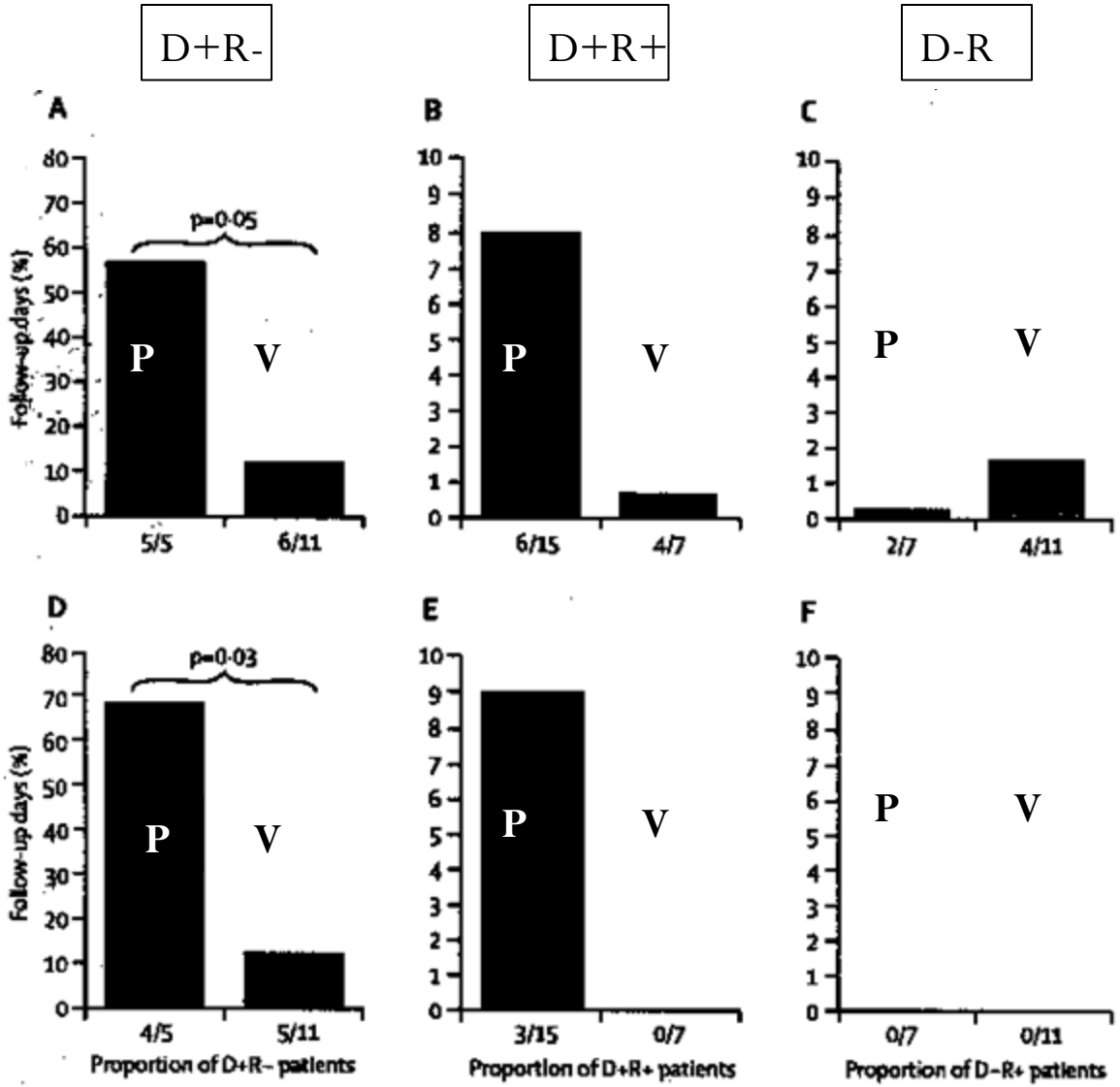
CTL Induction by Canarypox-pp65



Gonczol, Plotkin Exp. Opin. Biol. Ther. 2001, 1(3):405.

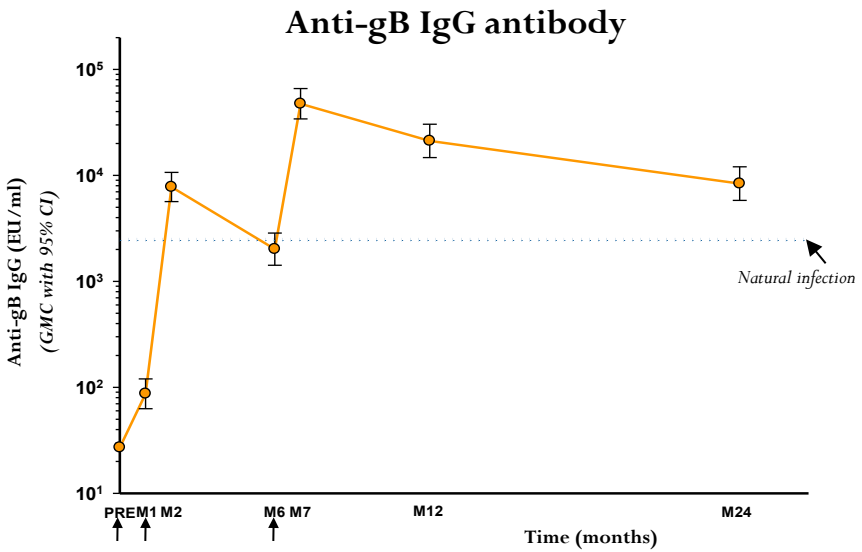
Sanofi-Pasteur gB/MF59 in Kidney or Liver Transplant Patients

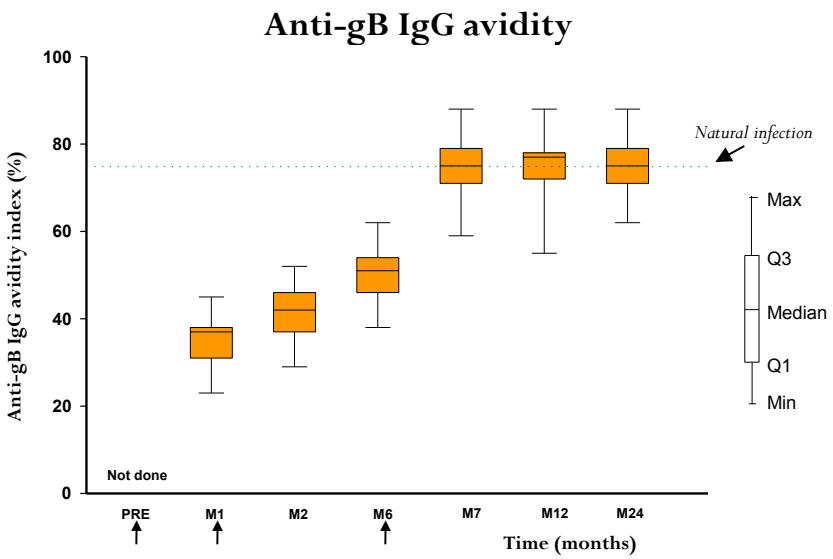
Proportion of days that patients in the three subgroups at risk of CMV infection

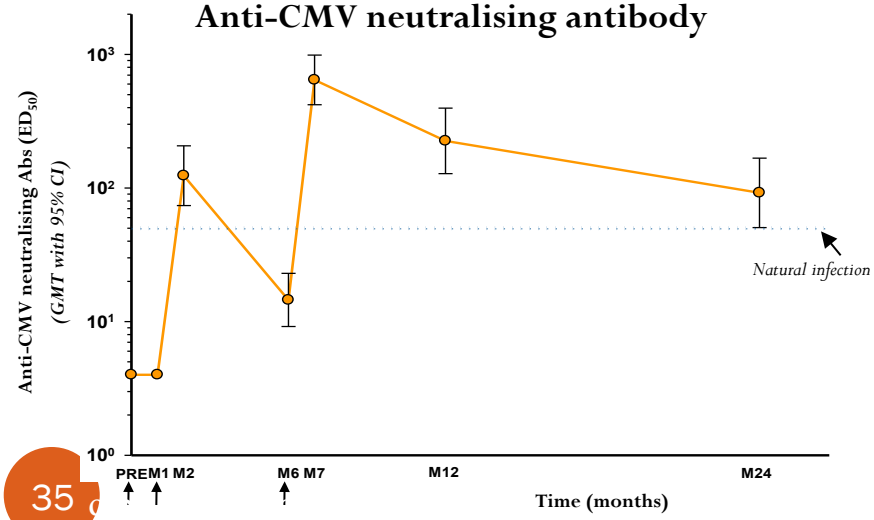


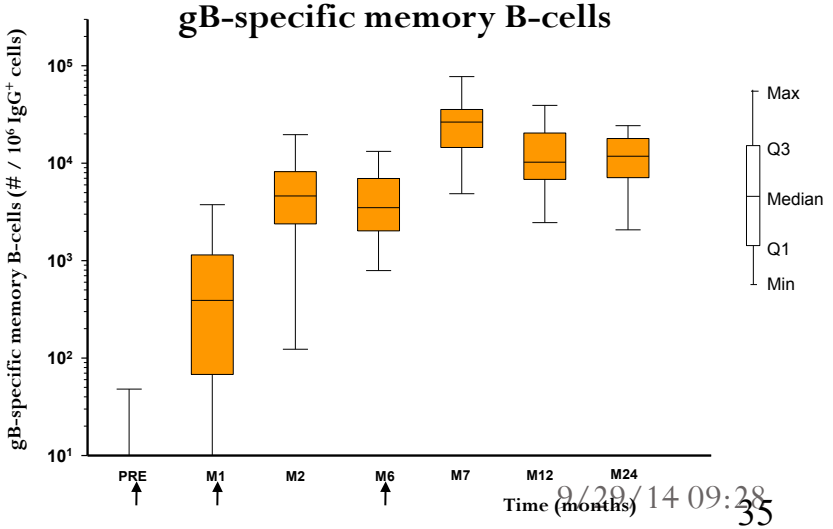
Griffiths PD, et al. Lancet 2001,377:1256

Antibody and memory B-cell responses To GSK 15 mcgx 3 gB/AS01 (A. Marchant et al, 2011)









Natural infection level of immunity is defined by testing sera from 39 healthy subjects with the same assays

Vical CMV DNA Vaccine

Bivalent – DNA for gB and pp65
Poloxamer adjuvant (nanoparticle)

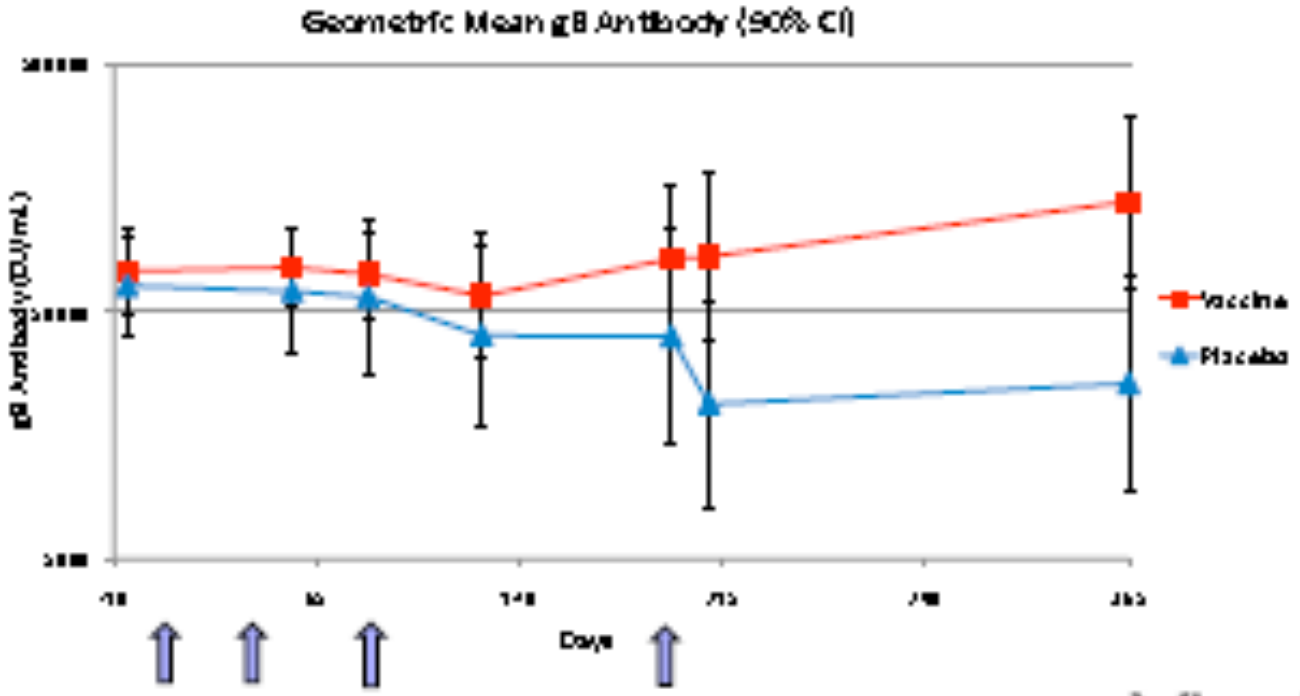
After 5 mg dose x3 or 4

In Seropositive Bone Marrow Transplant recipients

- ↓ viral load
- ↓ antiviral therapy

Antibody Responses to gB

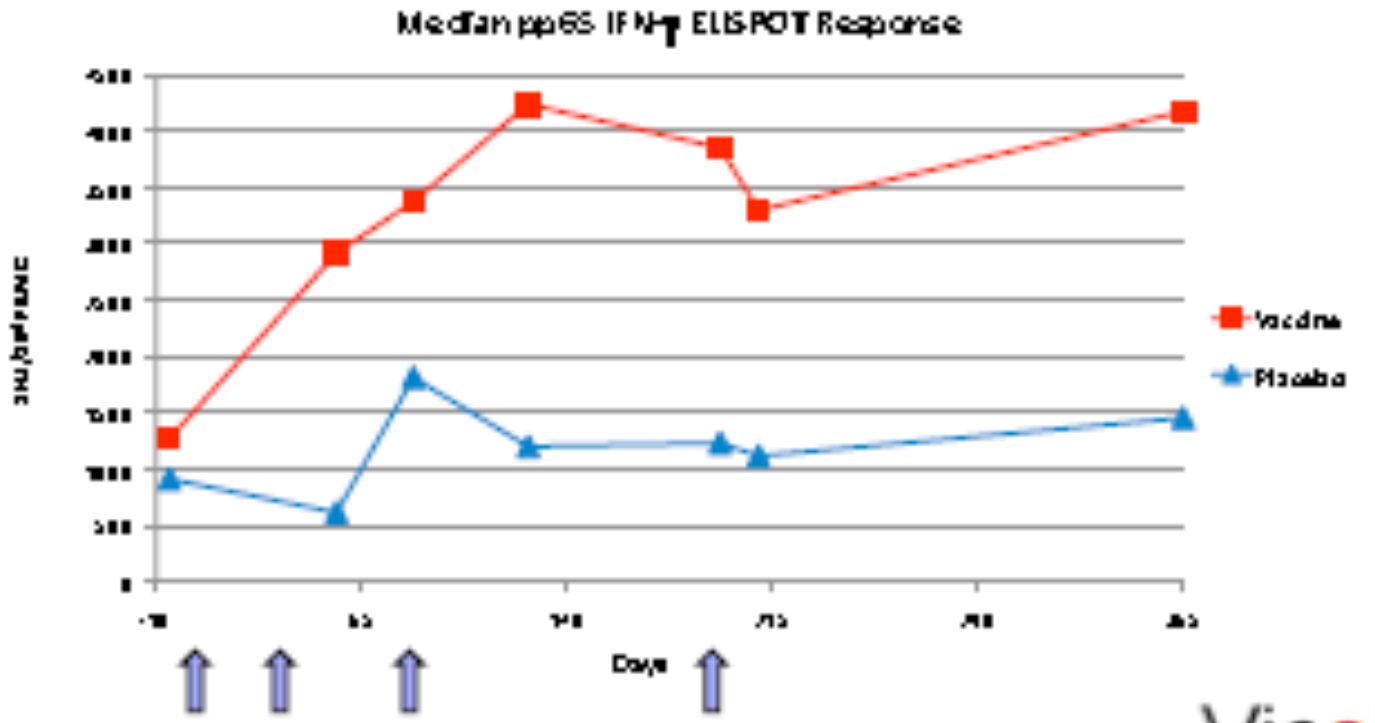
Up to 1 Year



Vical

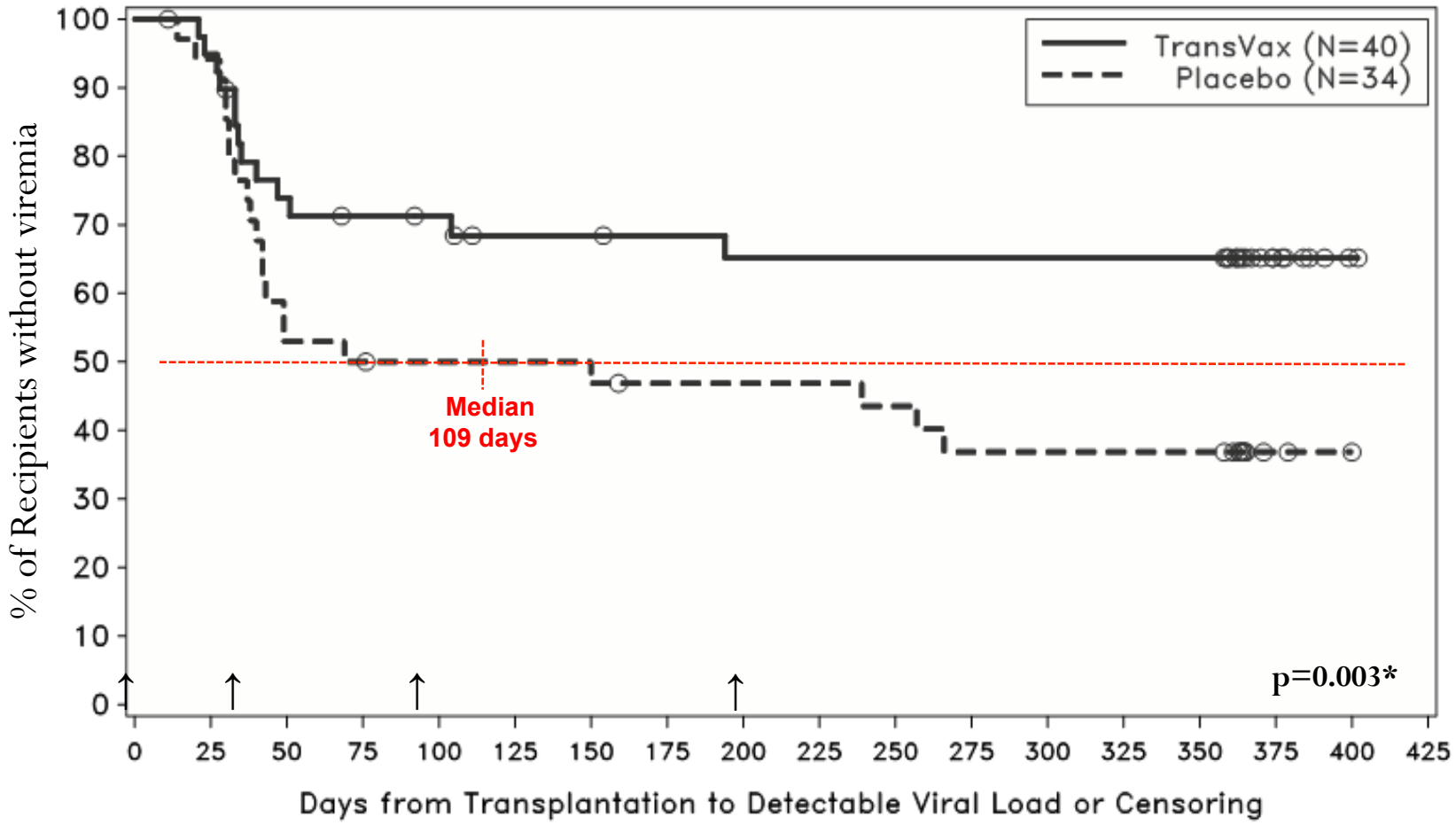
T-cell Responses to pp65

Up to 1 Year



Vical

% Subjects with ≥ 500 CMV copies/ml

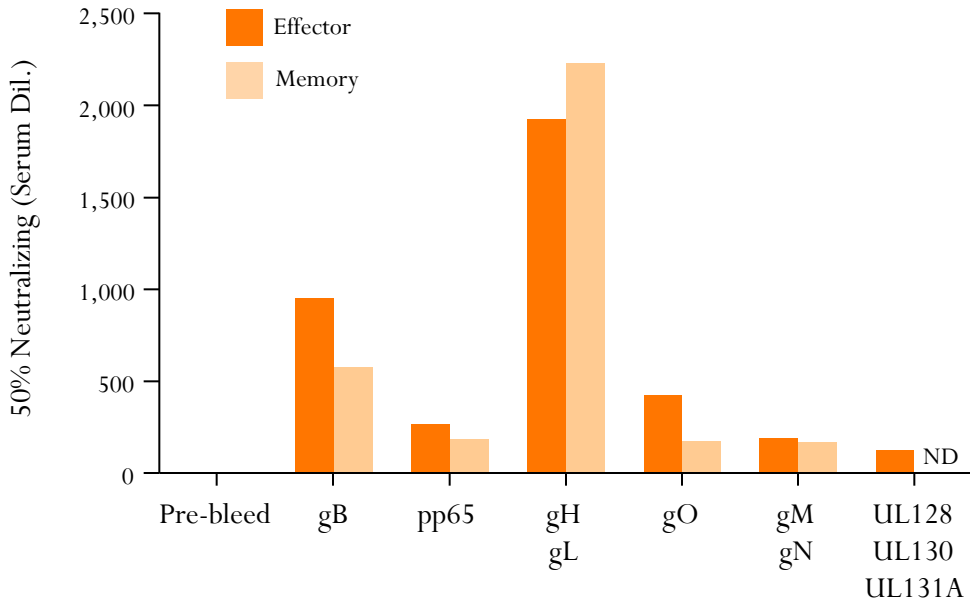


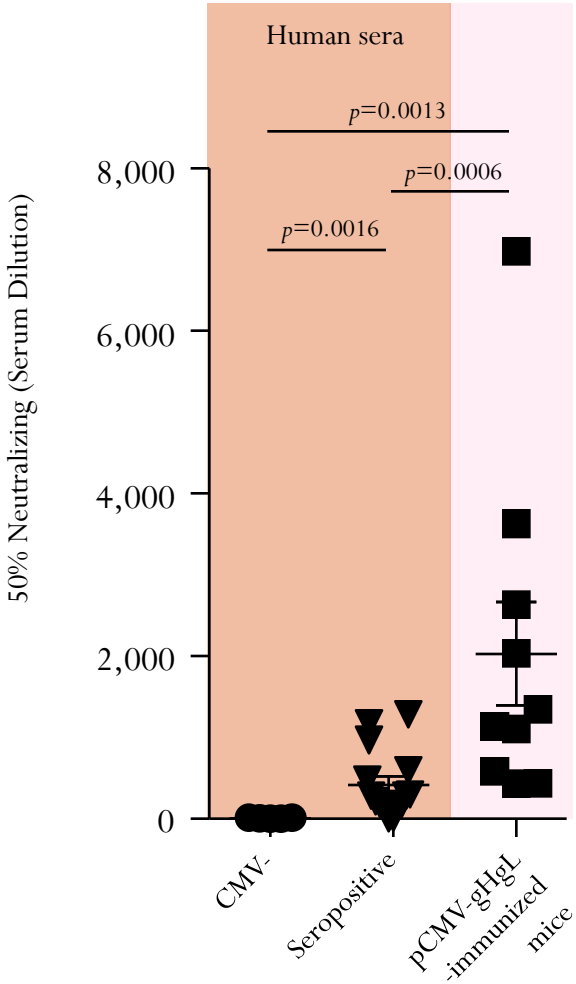
** p-value from a log-rank test with stratification by site; Plotted circles represent censored data; Viral load determined by a central lab PCR assay*

39

Development of a surface expressed CMV gH/gL optimized DNA vaccine induces potent neutralizing antibody responses (Inovio)

HCMV Towne neutralization (LE-HFF assay) assessed with vaccinated mouse serum post immunization (effector) & 6 months post final immunization (memory)

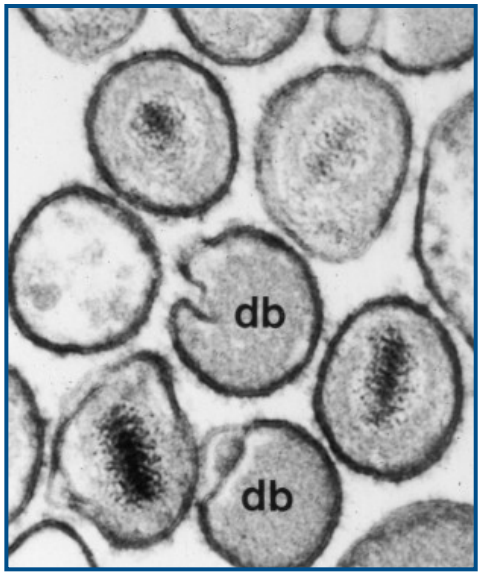


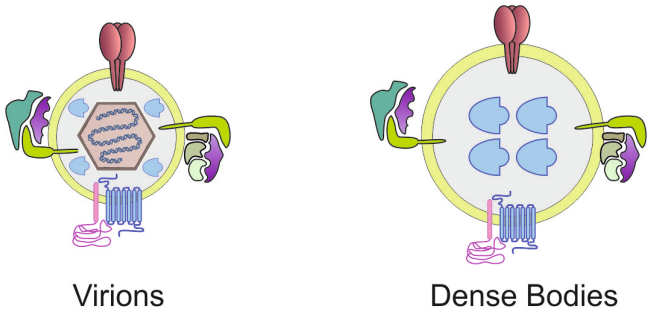


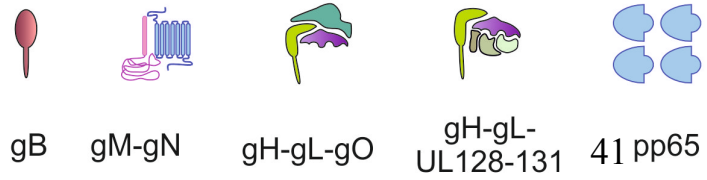
-Animals were immunized with the constructs above
-and assayed for neutralization activity.

Shedlock, Sardesai, Awasthi, Weiner *et al.* – Ms in preparation

- Non-infectious (no DNA, no capsid)
- Released in large amounts from infected cells
- Easily purified
- Envelope contains viral glycoproteins in their natural configuration (neutralizing antibodies) [1,4]
- Major constituents: Tegument proteins (cellular immune response) [1,4]
- Efficent targeting of antigen presenting cells
- Amenable to „antigenetic engineering“ [2,3,4]







1 Pepperl et al., **J.Virol. 74**, (2000) 6132-6146.
2 Pepperl-Klindworth, S. et al., **Gene Ther. 10**, (2003), 278-284.
3 Mersseman, V. et al., 2008. **J.Gen.Virol. 89,** (2008), 369-379.
4 Becke et al., **Vaccine 28**, 2010, 6191-6198