Human Cytomegalovirus Vaccines

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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**

US National Academy of Sciences highest priority

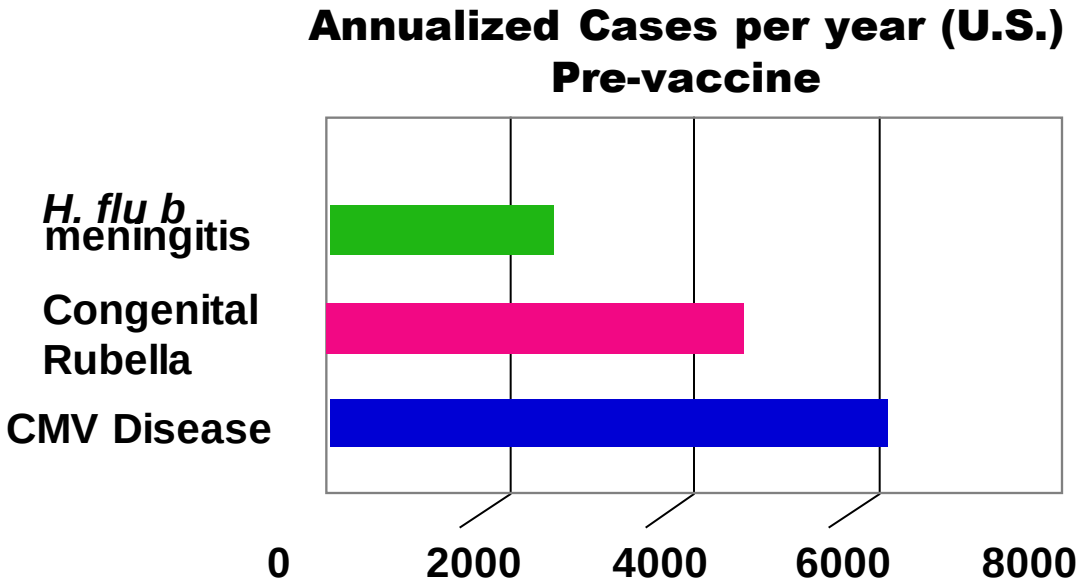
Congenital CMV

- 0.5 – 2% of all pregnancies complicated by CMV infection

- After primary infection:
    1. 10% symptomatic infection at birth, causes microcephaly, encephalitis, retinitis, hepatosplenomegaly, purpura
    2. 90% asymptomatic at birth, but about 20% will have deafness or neurologic sequelae
    3. 100% of infected fetuses will excrete CMV at birth in saliva and urine, 80% in blood

- Reinfection occurs in 1-2% of seropositive women

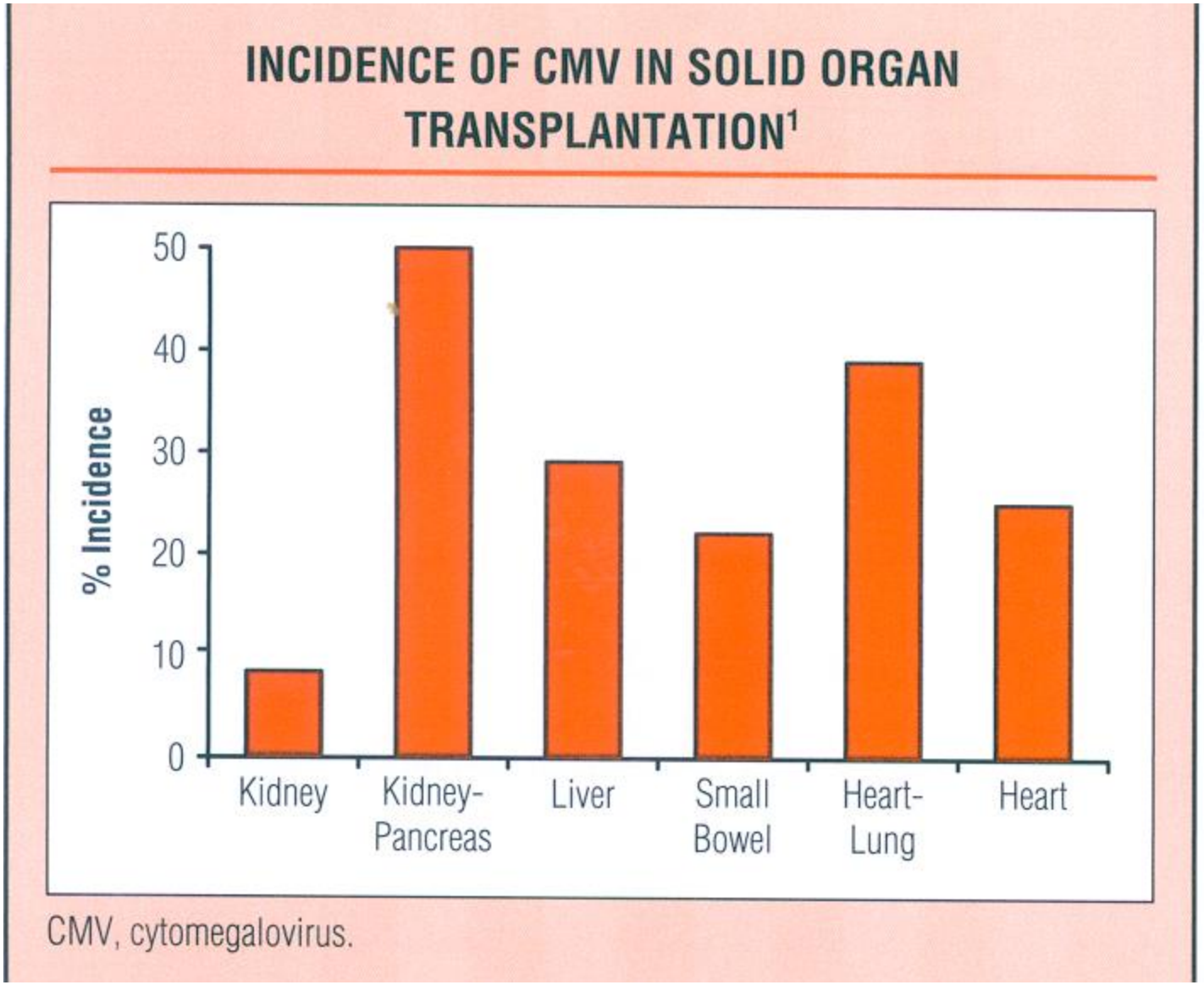
Infectious Causes of Neurologic Damage in Infancy



Congenital CMV Infection in the United States, per Year (1)

Live Births	**4,000,000**
Seronegative women	2,000,000
Transmitters – 0.7%	14,000
Sequelae	889
Asymptomatic at birth	12,222
Sequelae	1,650
TOTAL Sequelae (serious)	**2,539**

Seropositive women	**2,000**
Transmitters 1.0%	20,000
Sequelae (mild)	3,000 ?



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

In Hematopoietic Stem Cell Transplants:

CMV reactivation in seropositive recipients occurs in 70-80%.

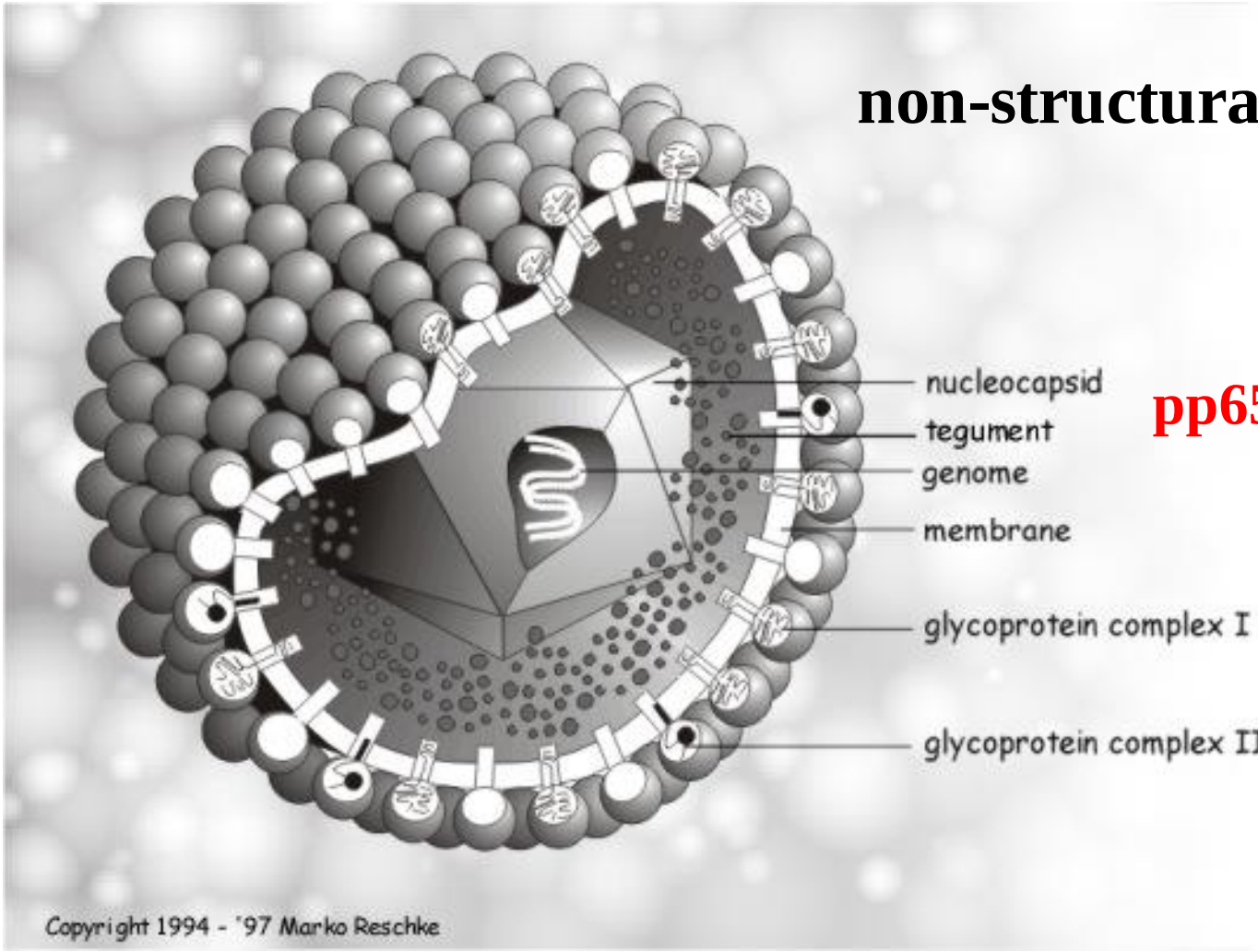
In the absence of antivirals CMV disease occurr in 20-35%.

Nichols W, J Clin Virol, 2000

Medical Conditions Suspected of Being Caused by CMV Aside from Congenital Infection and Post-Transplant Diseases

- Atherosclerosis
- Glioblastoma
- Immunosenescence
- Deterioration while in Intensive Care
- Growth retardation

Cytomegalovirus



non-structural protein: IE1

pp65, pp150

gB

gH/gL/ UL128-131

Targets of Neutralizing Antibody Against CMV

gB

gH/gL/UL128/IL130/UL131

Targets of Cytotoxic T cells Against CMV

pp65

IE1

pp150

Likely Protective Immune Correlates

- Neutralizing antibodies against:
  - Fibroblast entry mediated by gB
  - Epithelial cell entry mediated
    - by pentameric gH/gL/UL128-131 complex
    - *but not mutually exclusive*

- CD4+ T cells providing help
  - mediated by Tfh cells for B cells

- CD8+ T cells providing CTL:
  - mediated principally by pp65 tegument protein and IE1 non-structural protein

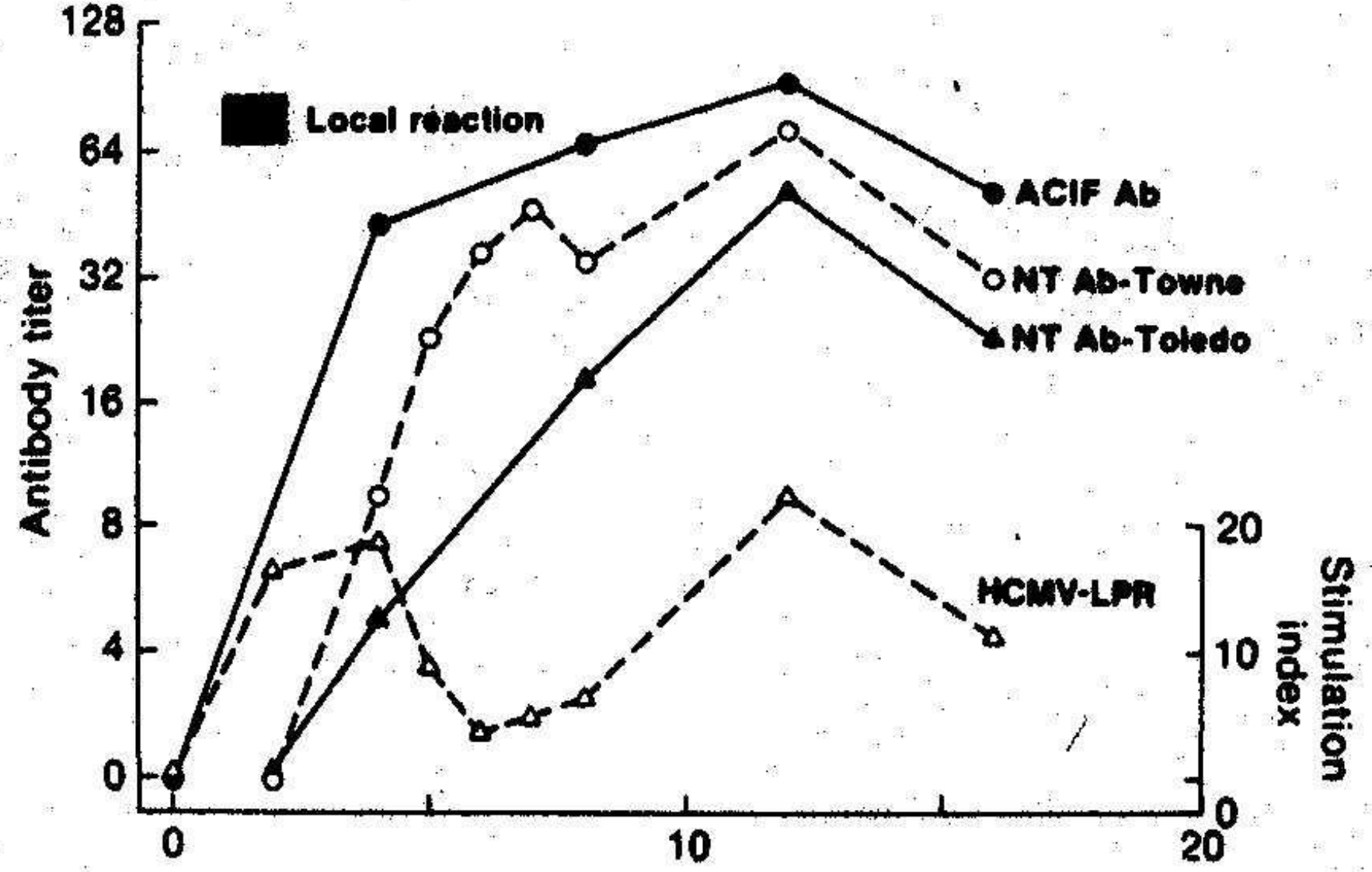
Live CMV Vaccines in Development

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

Some Genes in CMV UL/b[1] Region of Low Passage Viruses Missing in Towne

Codes for

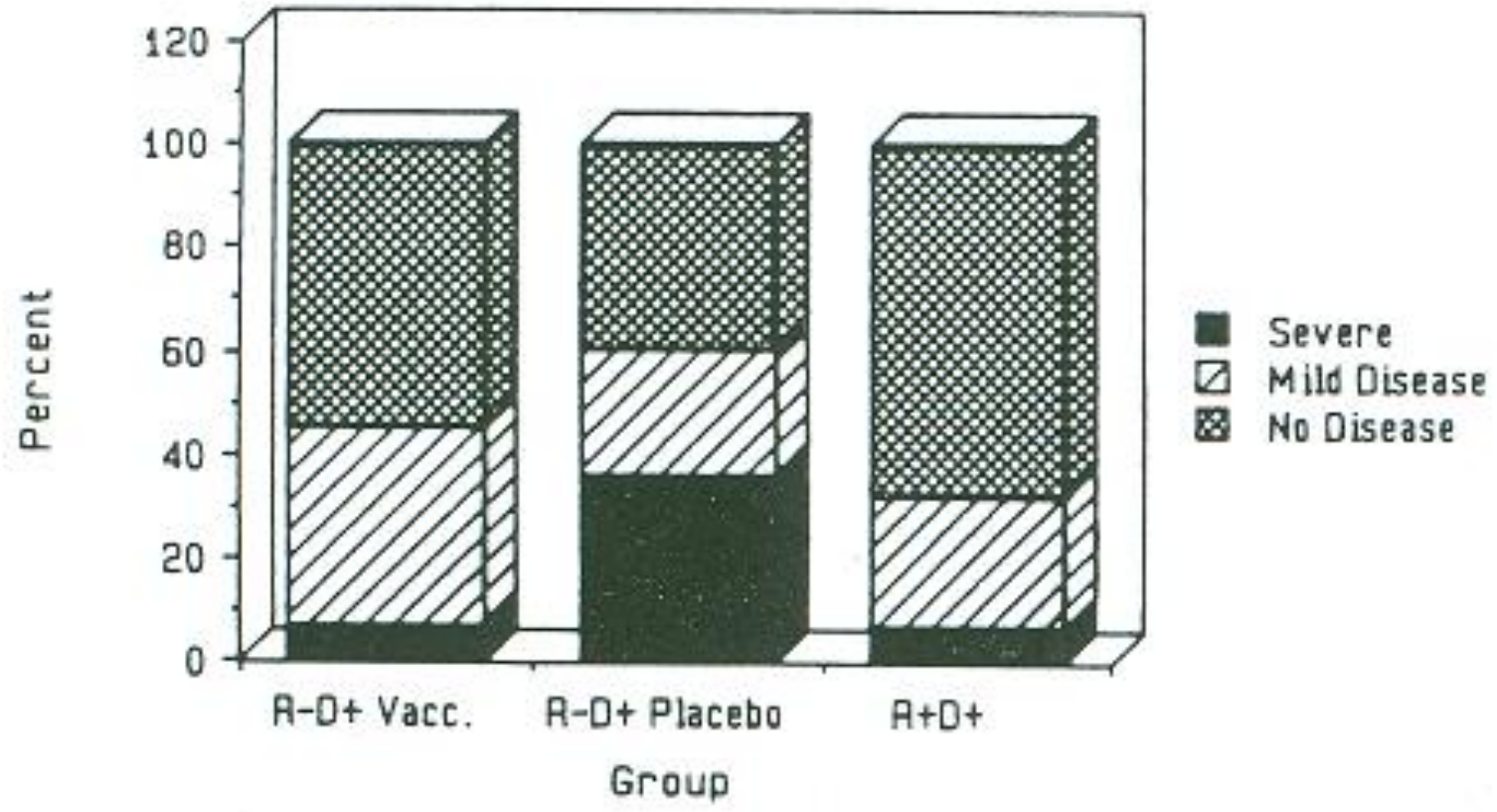
UL146	**Alpha Chemokine that activates PMNs**
UL147	**Alpha Chemokine**
UL144	**TNR receptor homolog**
UL128-131	**Endothelial cell tropism**
UL138	**Latency**


128
64
32
16
8
4
0
Antibody titer
Local reaction
ACIF Ab
NT Ab-Towne
NT Ab-Toledo
HCMV-LPR
20
10
0
Stimulation index
0
10
20

Various Results of SQ Inoculation of Towne Attenuated CMV

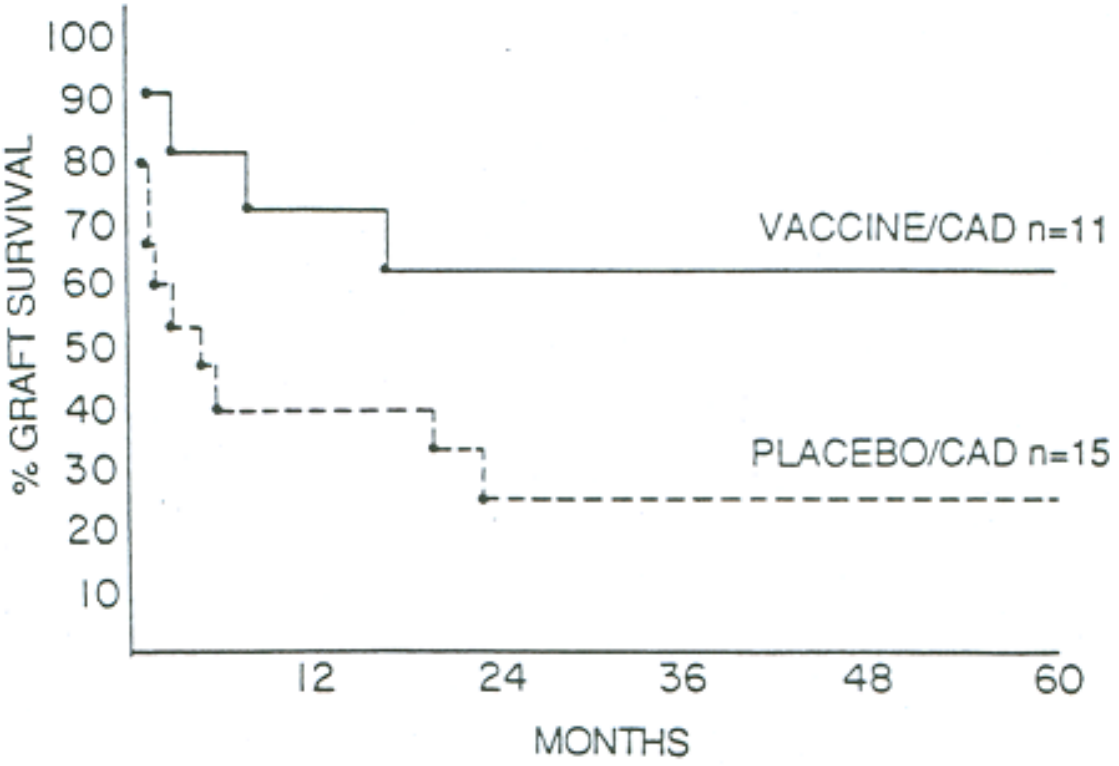
- Local reaction at 7-14 days, no systemic reaction
- No virus excretion in throat or urine
- No evidence for latency in immuno-suppressed vaccinees
- Neutralizing titers similar to prior natural infection
- CMV-specific CD4+ T cell proliferation in all vaccinees
- CTL induced in most vaccinees, short lived
- Protected volunteers against low dose challenge but not high dose challenge
- Conversion of skin test with CMV antigens to positivity
- However, did not prevent transmission of CMV from toddlers to vaccinated mothers.

Outcome of Exposure to Transplanted Kidney from a CMV-seropositive donor (D+) in Renal Transplant Recipients.



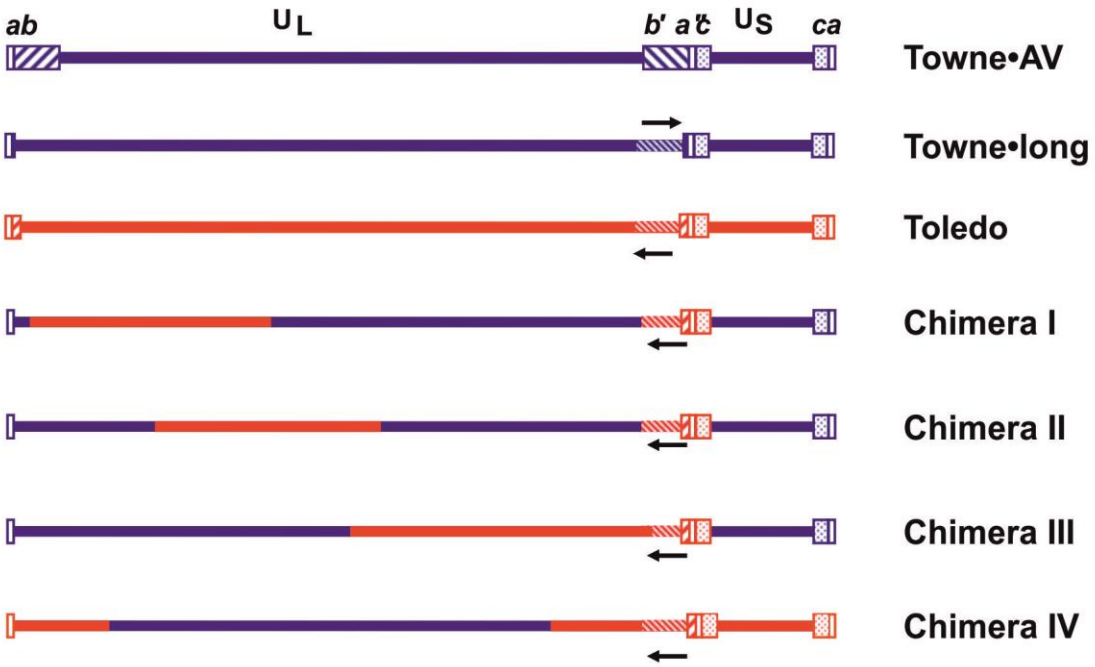
Plotkin SA, et al. Rev Inf. Dis 1990;12, Suppl 7, S827-S838

Cadaveric (CAD) renal Allograft Actuarial Survival For R-D+ Group



Plotkin SA, et al. Rev Inf. Dis 1990;12, Suppl 7, S827-S838

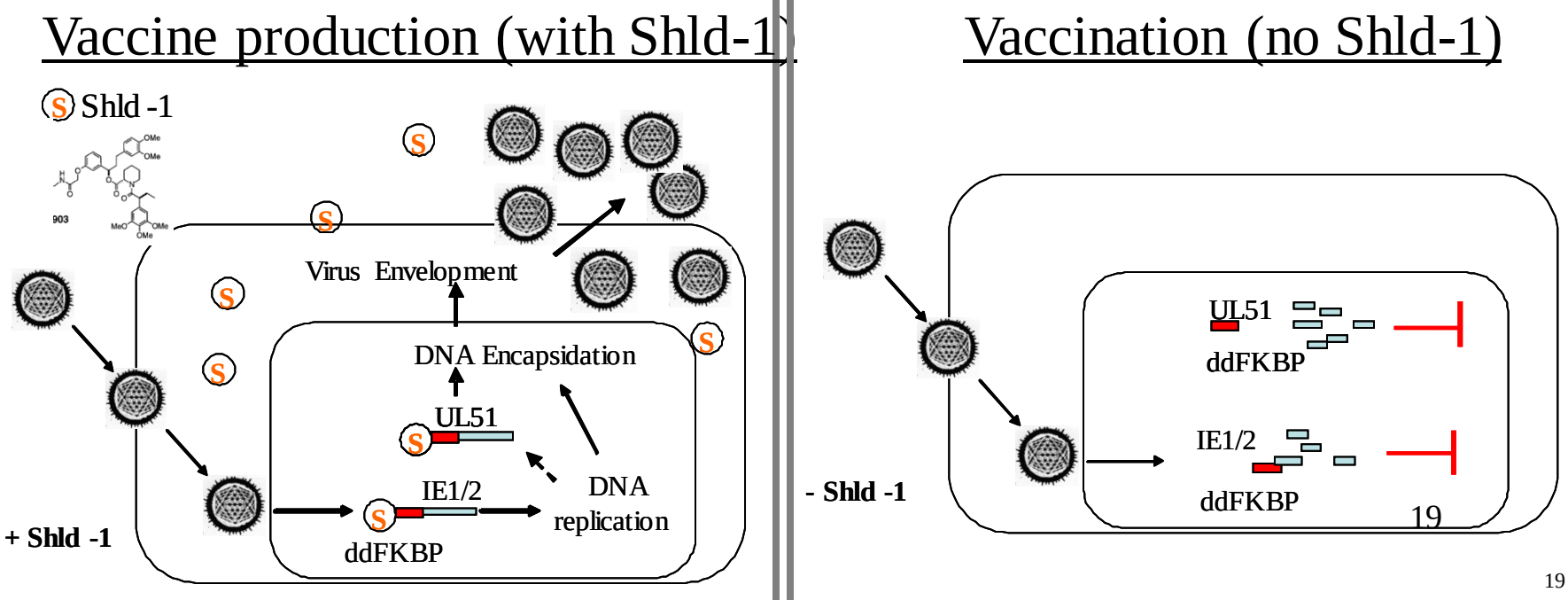
Towne-Toledo Recombinants (MedImmune)



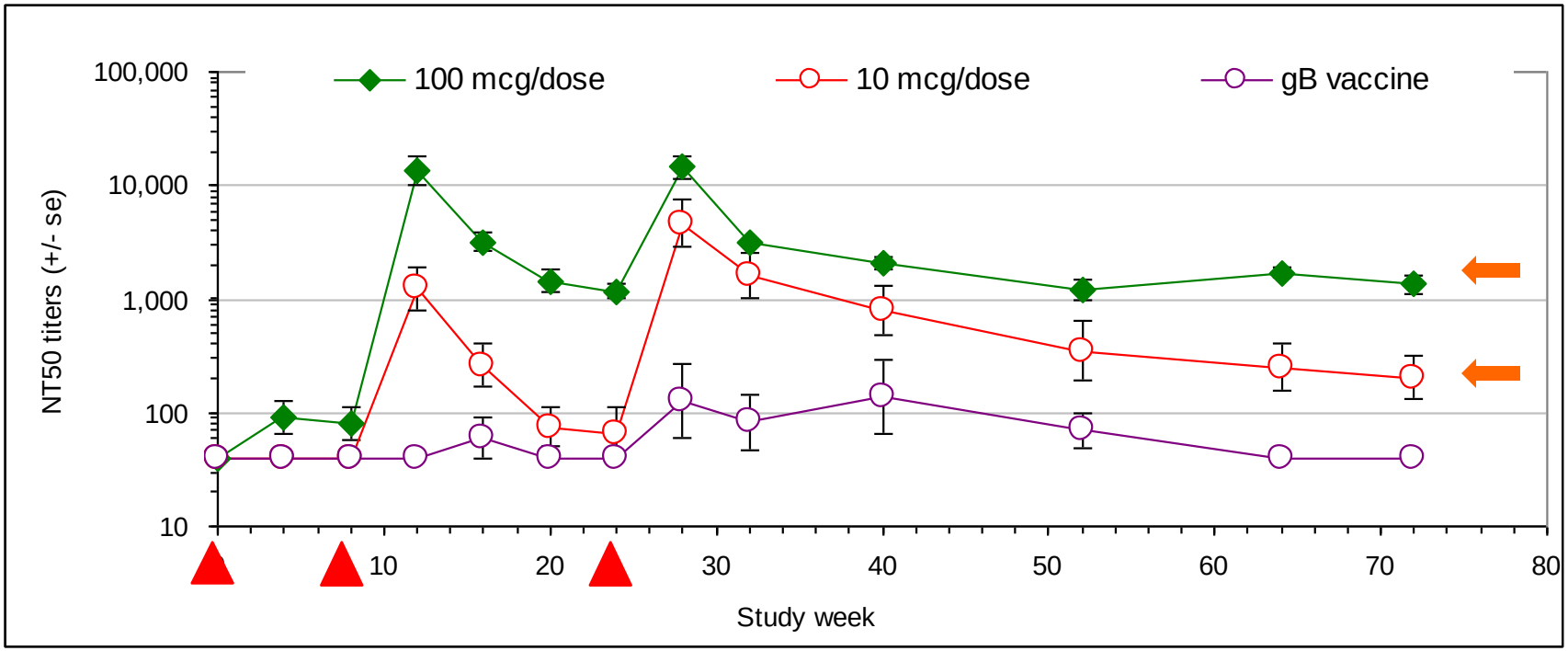
In seropositive subjects no reactions but no evidence of infection by virus detection or serology. Tests in seronegatives just beginning.

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.



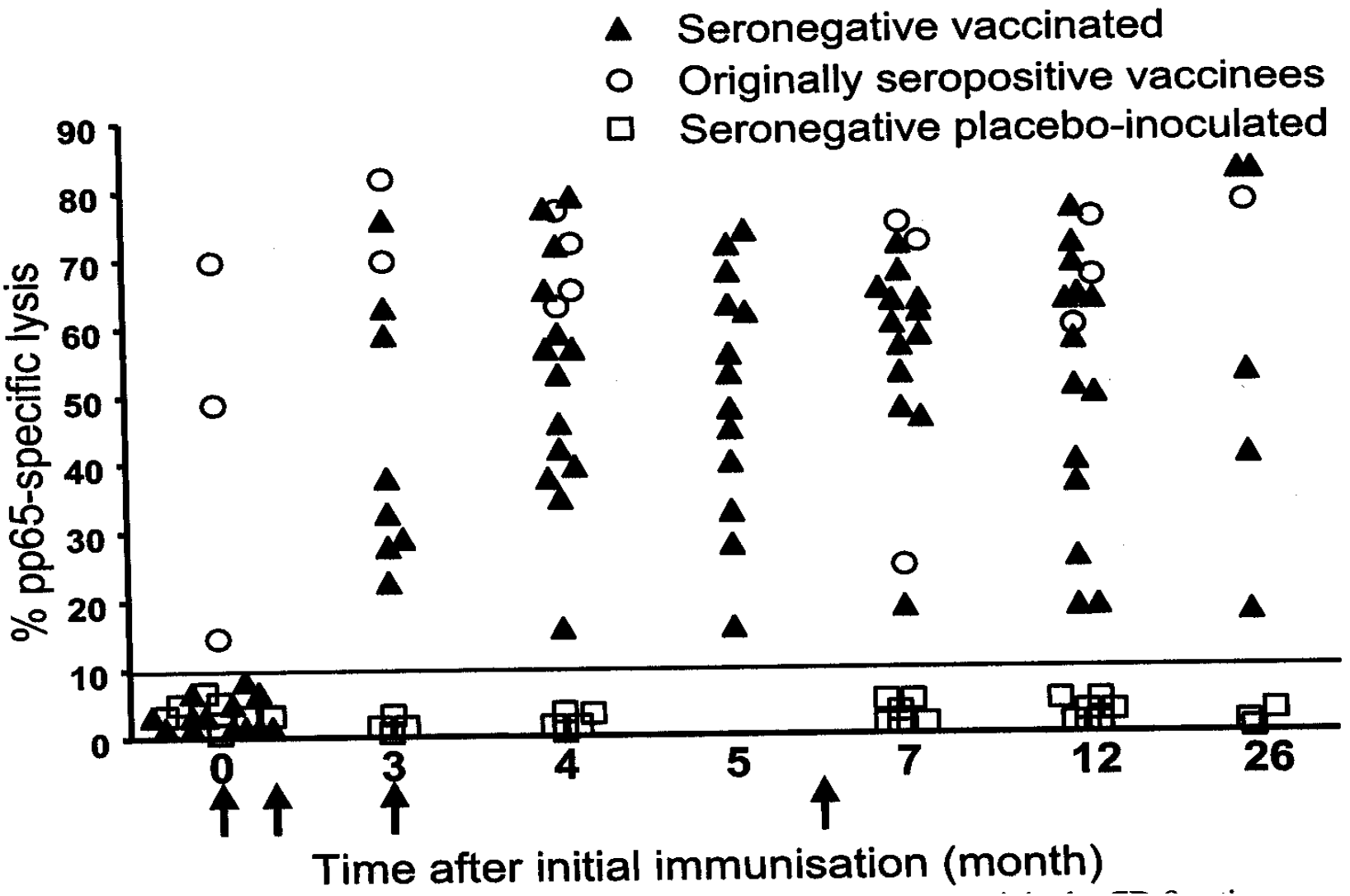
Merck CMV vaccine elicits neutralizing Abs in rhesus monkeys



- 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.

Non-Living CMV Vaccines in Development

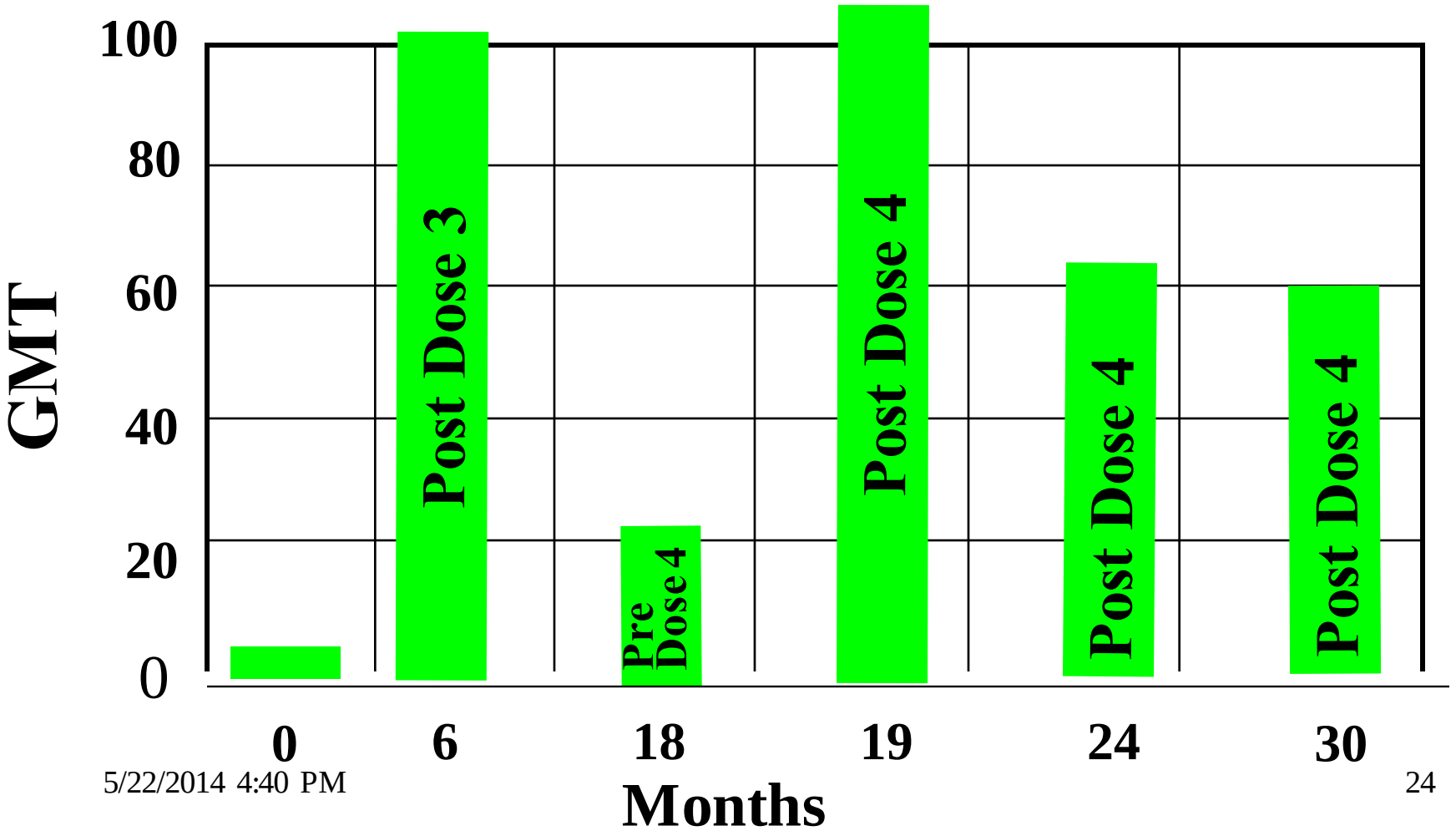
Recombinant gB glycoprotein with adjuvant (2)	**Sanofi Pasteur, GSK**
DNA plasmids	**Vical, Inovio**
Peptides	**City of Hope**
Dense bodies	**Vaccine Project Management (Germany)**
Virus-like particles	**Variations Bio, Redbiotech**

CMV gB Vaccine: Adjuvant

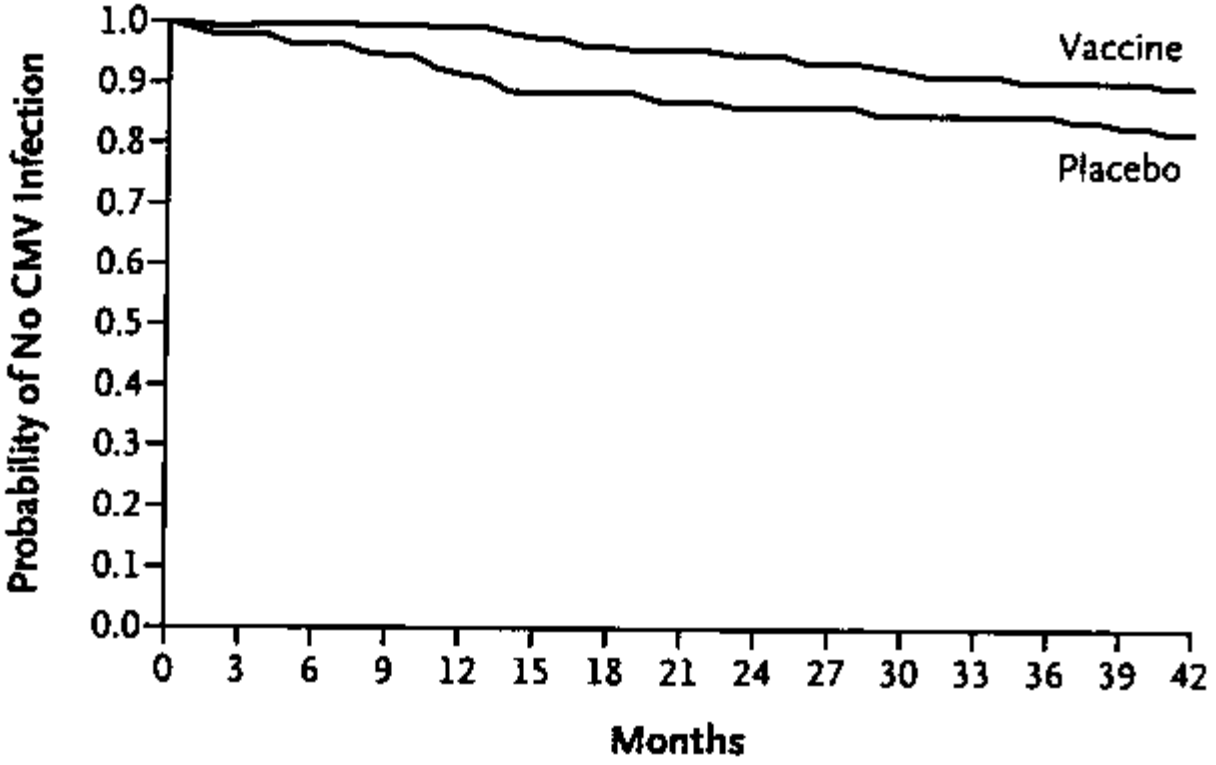
- MF59: proprietary (Chiron) oil in water emulsion
- Composed of squalene, sorbitan trioleate and polysorbate 50
- Gives a stable emulsion at room temperature

- Forms 150 nm particle with gB on surface

Subunit CMV Glycoprotein B with MF59 Adjuvant in Adults (Chiron)



Kaplan-Meier Estimates of Probability of Remaining Free of CMV Infection

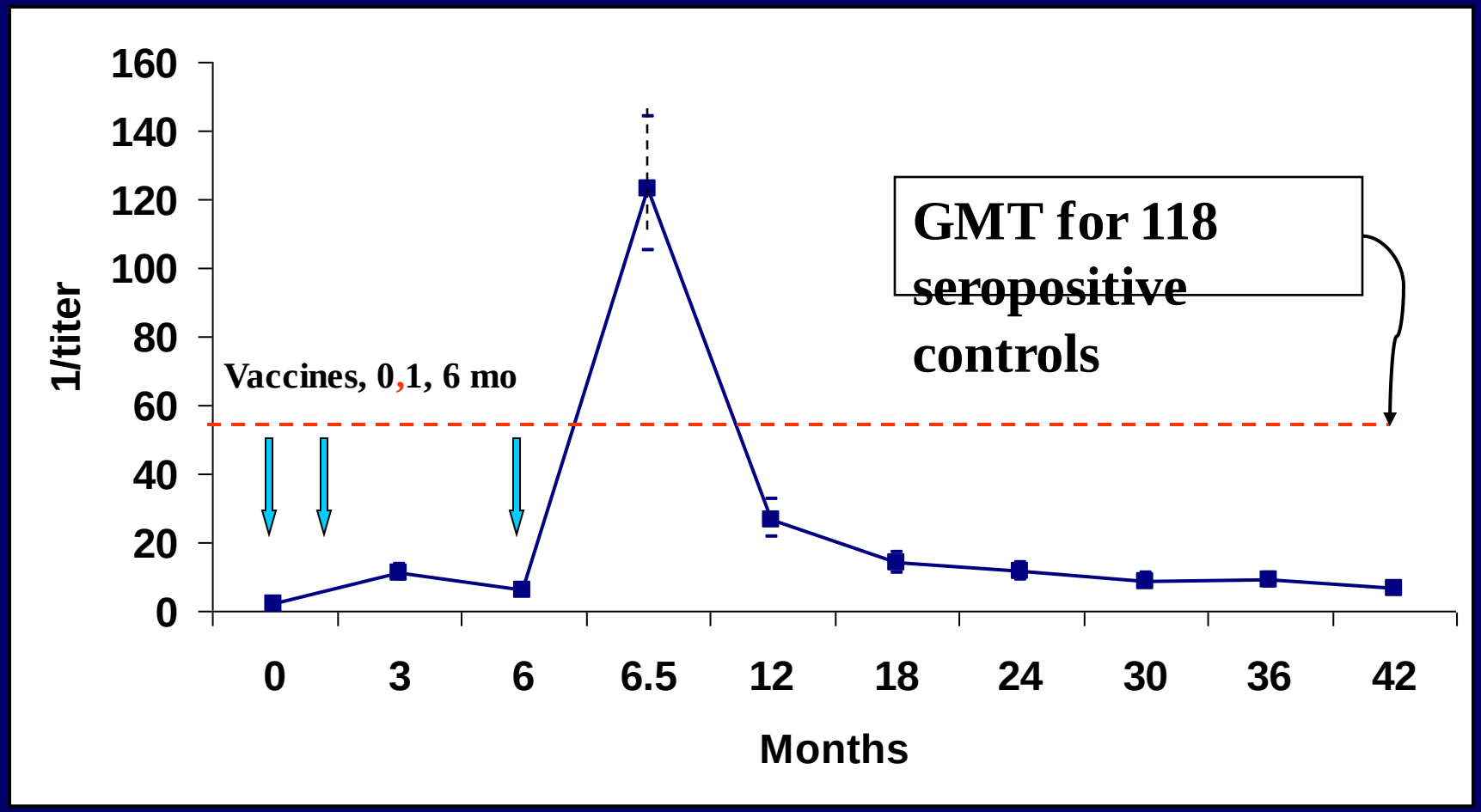


No. at Risk														
Vaccine	225	213	211	204	195	178	160	154	145	136	127	116	112	98	88
Placebo	216	193	185	178	169	153	141	128	121	114	108	104	97	87	75

Pass RF. NEJM 360:1191-9, 2009

Neutralizing antibody to gB: 136 recipients of 3 injections of CMV gB vaccine, GMT and 95% C.I

N from 92 to 136



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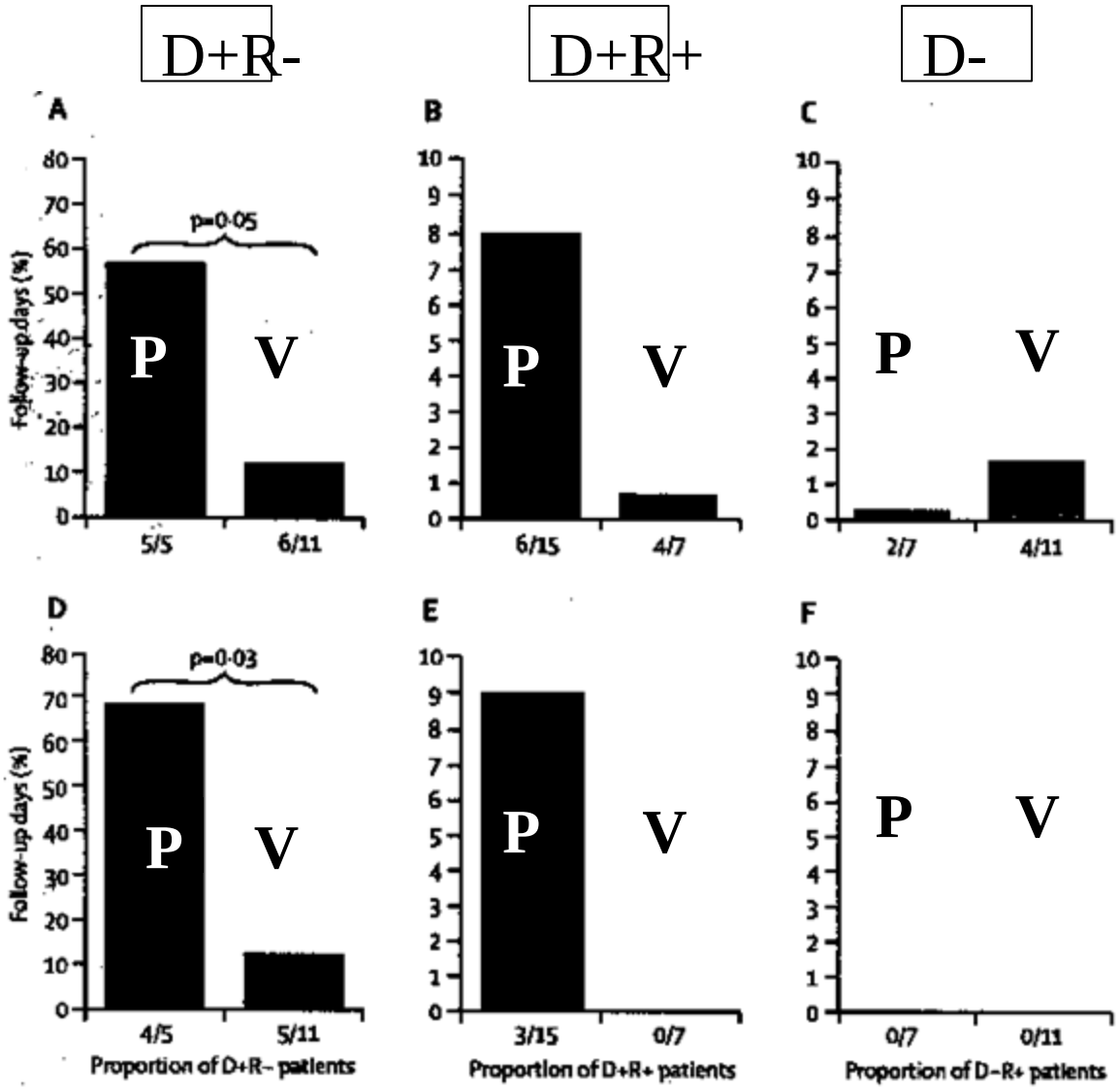
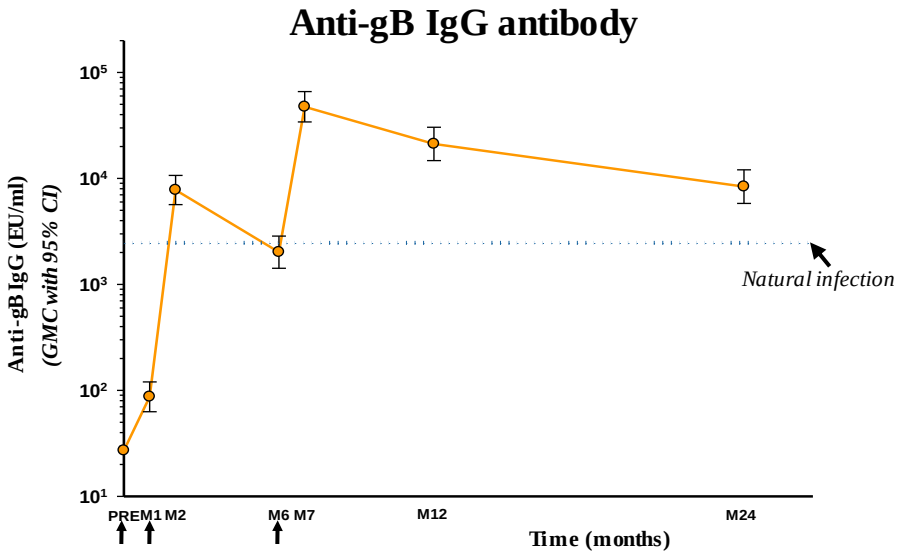
Sanofi-Pasteur gB/MF59 in Kidney or Liver Transplant Patients

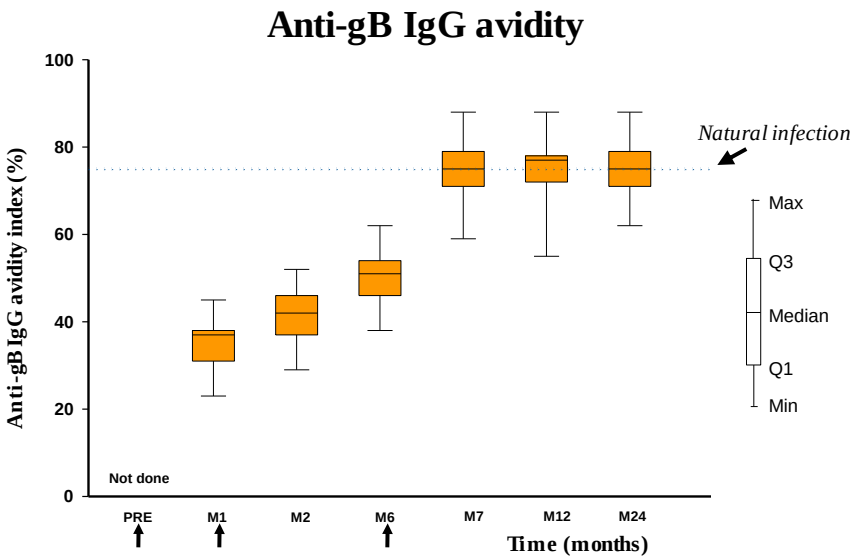
Proportion of days that patients in the three subgroups at risk of CMV infection

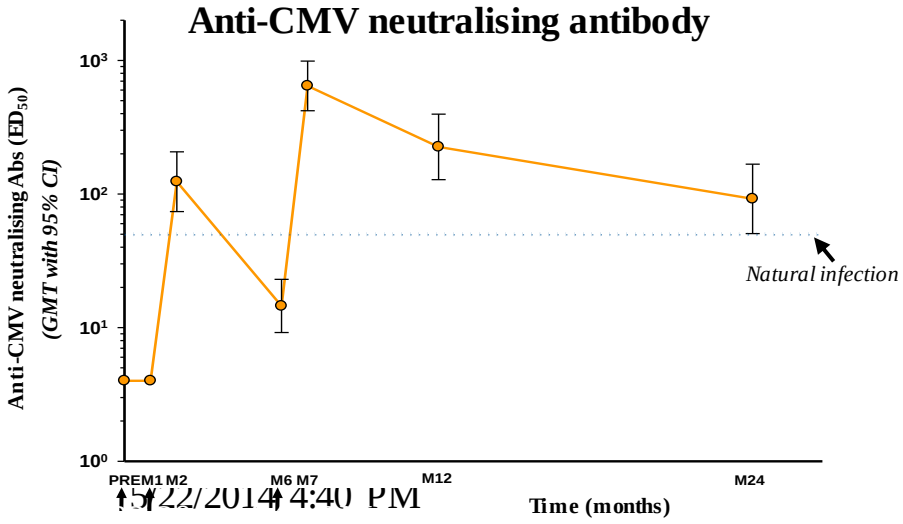


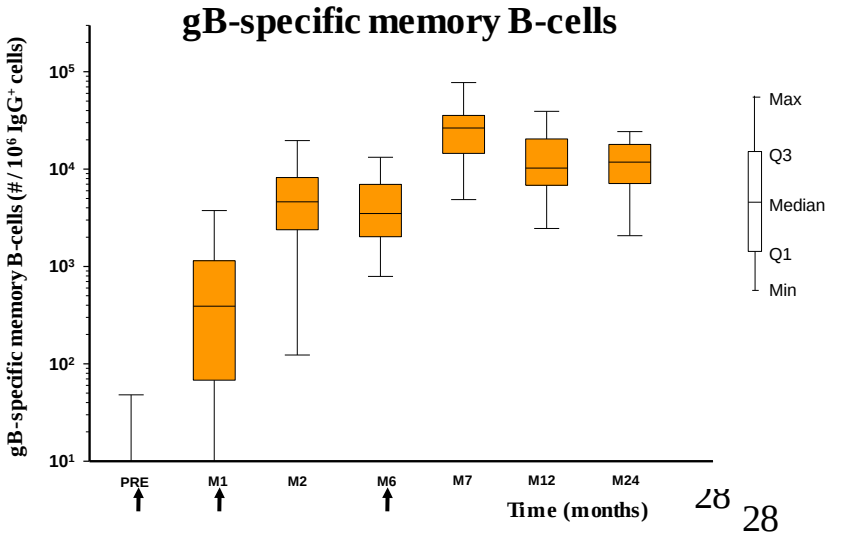
Griffiths PD, et al. Lancet 2001,377:1256

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

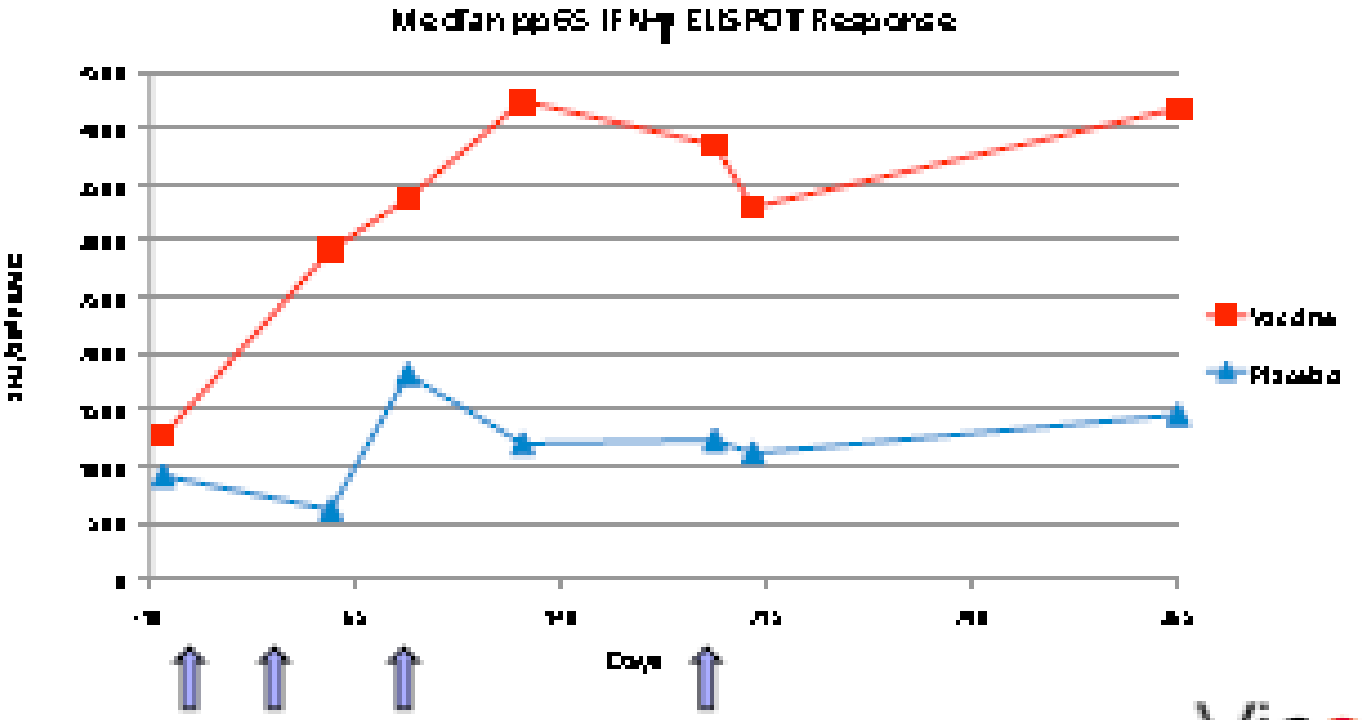
Bivalent – DNA for gB and pp65
Poloxamer adjuvant (nanoparticle)

After 5 mg dose x3 or 4 in Seropositive Bone Marrow Transplant recipients

- **viral load**
- **antiviral therapy**

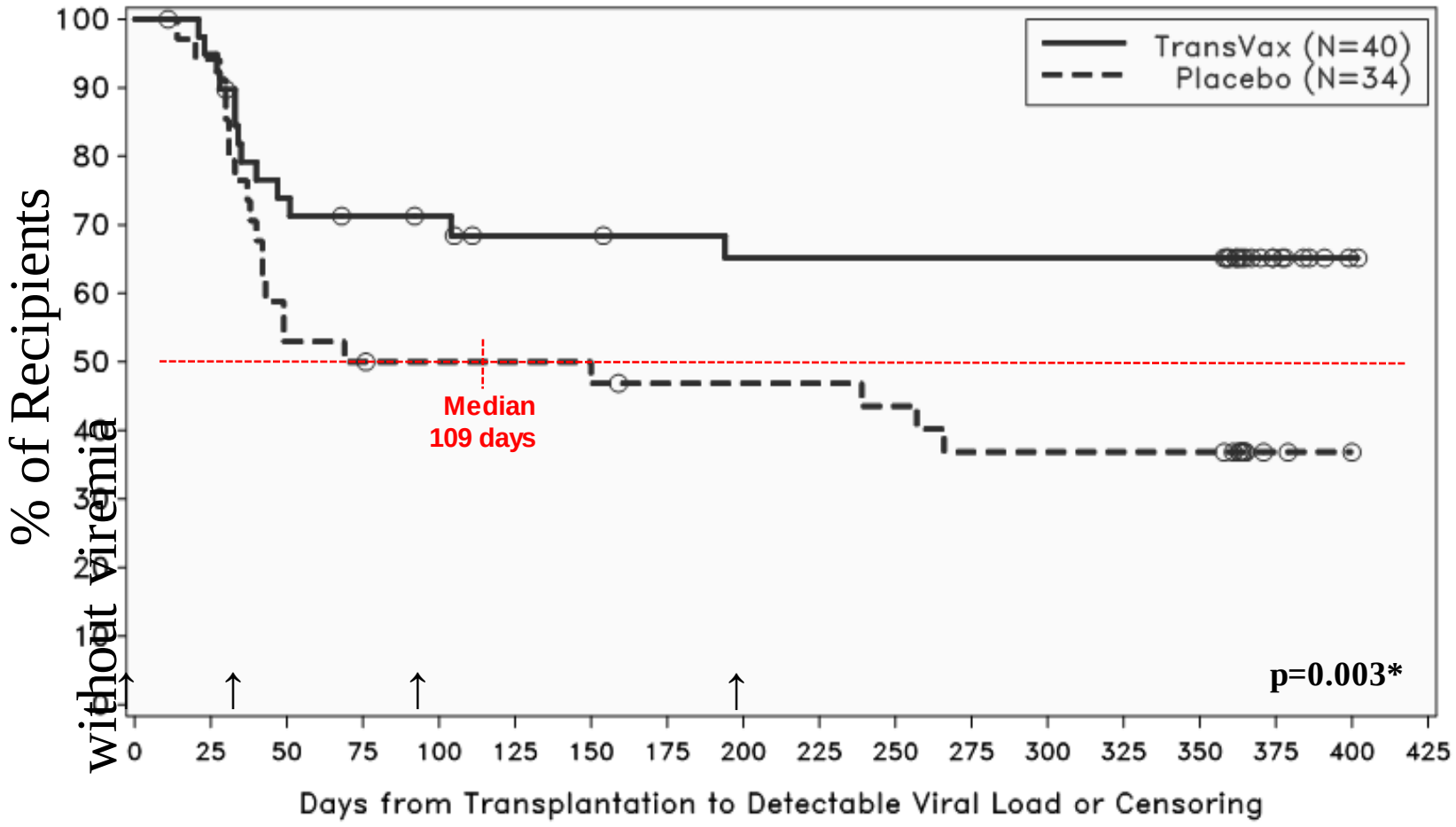
T-cell Responses to pp65

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*

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Current Issue:

How to produce the gH/gL/UL128-131

pentameric complex and use it to vaccinate?

Replicate defective particles ?

Virus-like particle ?

Soluble complex ?

Chief Unanswered Questions About Prevention of CMV

- **Importance of antibodies against gH/gL/UL128-131 that neutralize entry into epithelial cells?**
- **Importance of cellular immune responses in maternal-fetal transmission?**
- **Can maternal-fetal infection in seropositive women be prevented by boosting antibody or CMI?**

Probable 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 with seropositive donors

Possible Targets for CMV Vaccination

Female infants, if immunity long-lasting (like rubella)

Seropositive solid organ transplant recipients (if vaccine induces booster to CMI)

Bone marrow transplant recipients after transplant

Speculative Targets for CMV Vaccination

All infants, to reduce viral circulation

Seronegative bone marrow transplant donors to seropositive recipients

Prospective cardiac bypass patients (to prevent atherosclerosis)

All elderly (to prevent immunosenescense)

How to Demonstrate Efficacy of a CMV Vaccine (1)

- 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 and hematoposetic stem cell transplant recipients
- Cohort study in pre-pregnant women to prevent later fetal infection
- Prevention of fetal disease

How to Demonstrate Efficacy of a CMV Vaccine (2)

- **Vaccination of Seropositive Bone Marrow Transplant Recipients**

- **Vaccination of Seronegative Solid Organ Transplant Recipients who receive an organ from a seropositive donor**

Possible Endpoint:

Return of CTL
Viral Load
Use of Antivirals
Disease

On the Positive Side:

- **Protection has been demonstrated clinically with known antigens, both against acquisition and viral load.**
- **Clinical Trials to show prevention of acquisition by women are feasible.**
- **For phase III prevention of transmission to fetus will be the end point, measured by presence of CMV in the newborn.**
- **Clinical trials in transplant recipients to show reduced disease and antiviral use are feasible.**
- **Many candidate vaccines are available.**