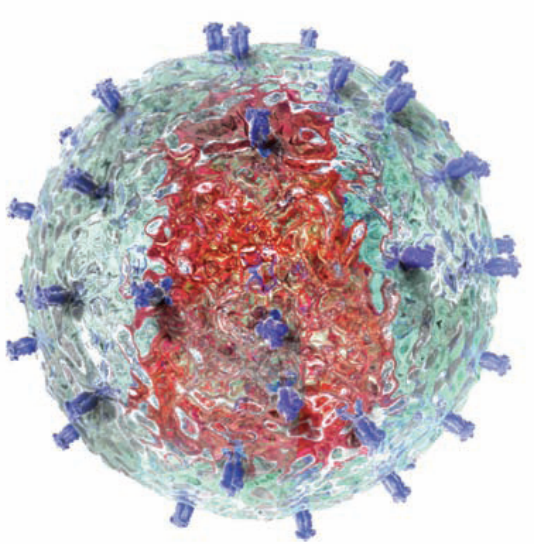


The AIDS pandemic: History, present, and prospects for AIDS vaccines

George N. Pavlakis

National Cancer Institute, USA

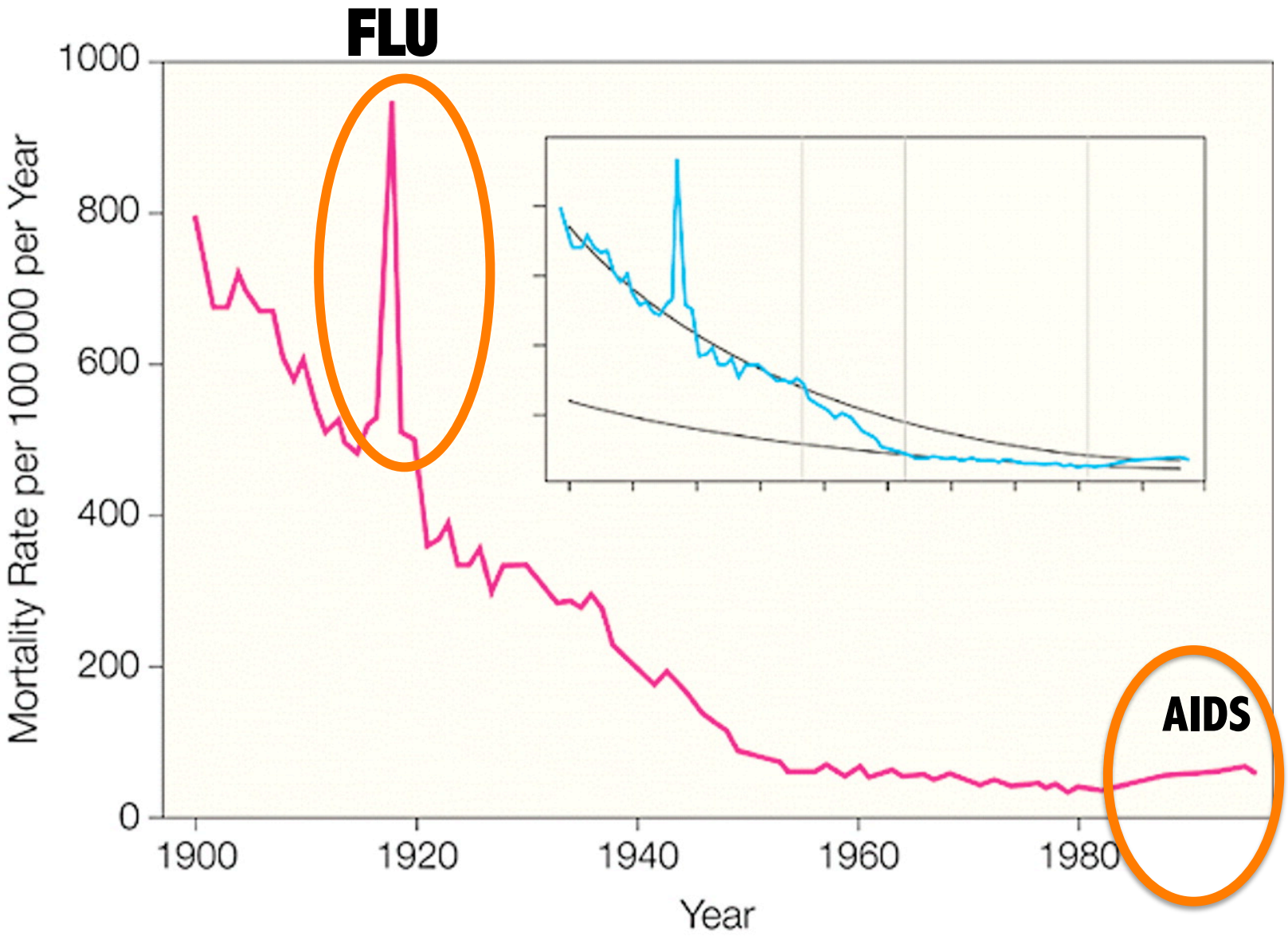
AIDS at 30: 1981-2011

- June 4, 1981
- 5 healthy men from LA, with PCP
- **Centers for Disease Control (CDC). Pneumocystis pneumonia—Los Angeles.** MMWR Morb Mortal Wkly Rep. 1981;30:250-2
- July 4, 1981
- 26 additional young men, all gay, from SF, with KS
- **Centers for Disease Control (CDC). Kaposi's sarcoma and Pneumocystis** pneumonia among homosexual men—New York City and California. MMWR Morb Mortal Wkly Rep. 1981;30:305-8
- GRID, a new disease?

AIDS: One Of The Greatest Pandemics In Human History

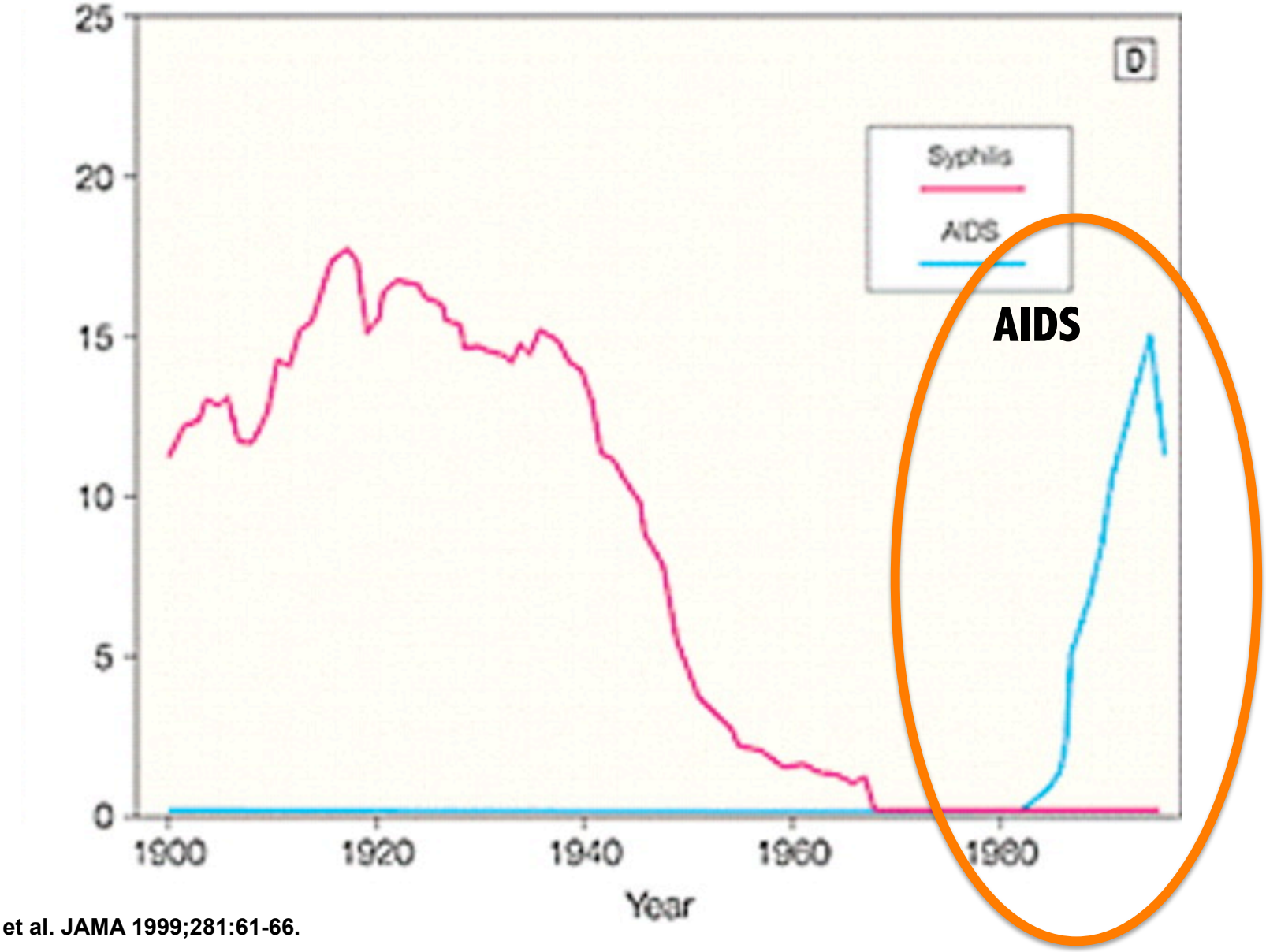
- Continues to expand world wide
- Absence of a curative therapy
- Sexually transmitted disease
- No vaccine
- Growing apathy and acceptance after >25 years of headlines

Crude Infectious Disease Mortality Rate in the United States from 1900 Through 1996



Armstrong, G. L. et al. JAMA 1999;281:61-66.

Crude Mortality Rates for Syphilis And AIDS



Armstrong, G. L. et al. JAMA 1999;281:61-66.

GLOBAL REPORT

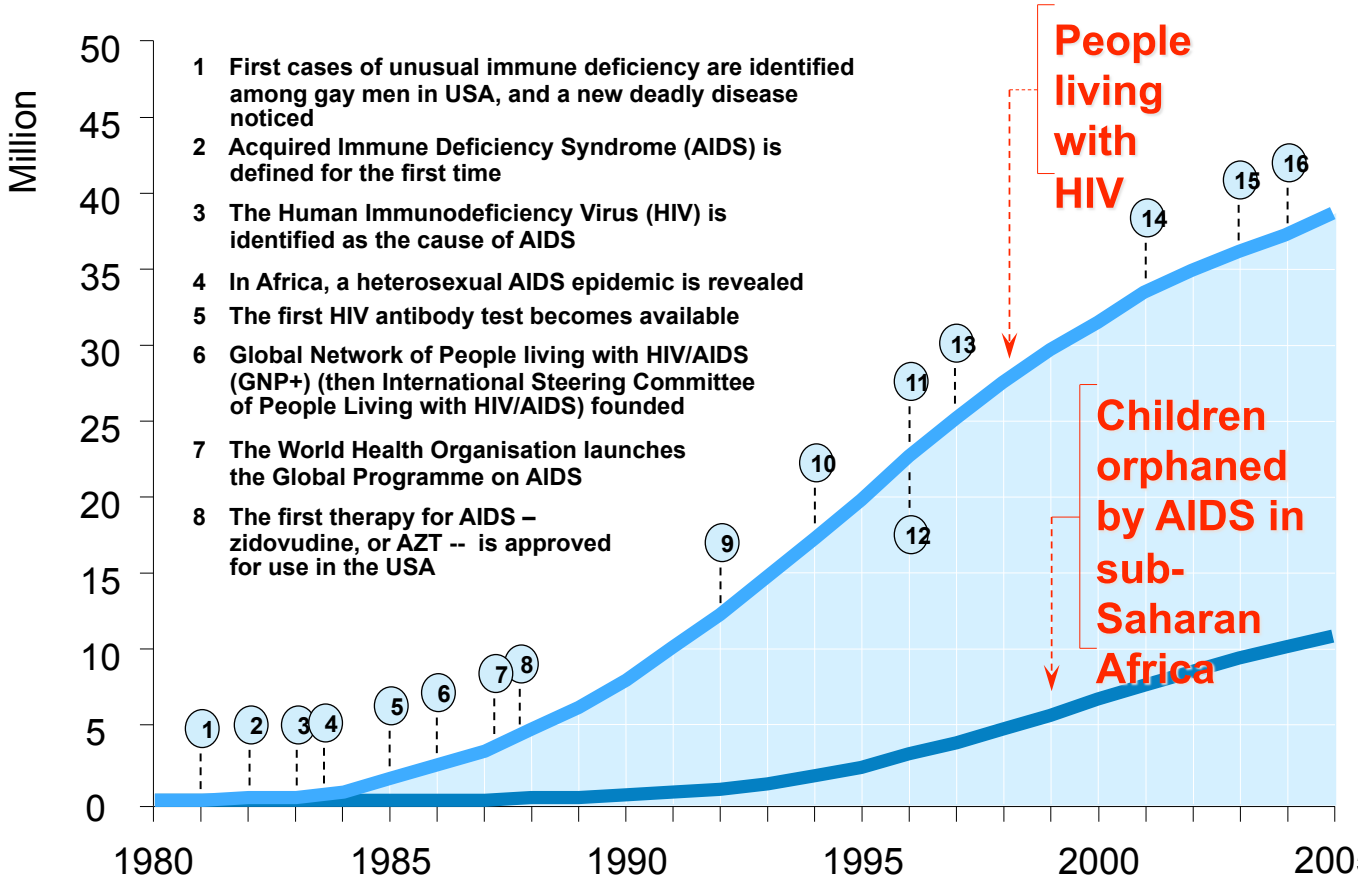
Global estimates for adults and children | 2009

People living with HIV	33.3 million [31.4 million – 35.3 million]
New HIV infections in 2009	2.6 million [2.3 million – 2.8 million]
Deaths due to AIDS in 2009	1.8 million [1.6 million – 2.1 million]

World Health Organization

UNAIDS

25 years of AIDS



1 First cases of unusual immune deficiency are identified among gay men in USA, and a new deadly disease noticed

2 Acquired Immune Deficiency Syndrome (AIDS) is defined for the first time

3 The Human Immunodeficiency Virus (HIV) is identified as the cause of AIDS

4 In Africa, a heterosexual AIDS epidemic is revealed

5 The first HIV antibody test becomes available

6 Global Network of People living with HIV/AIDS (GNP+) (then International Steering Committee of People Living with HIV/AIDS) founded

7 The World Health Organisation launches the Global Programme on AIDS

8 The first therapy for AIDS – zidovudine, or AZT -- is approved for use in the USA

9 In 1991-1993, HIV prevalence in young pregnant women in Uganda and in young men in Thailand begins to decrease, the first major downturns in the epidemic in developing countries

10 Highly Active Antiretroviral Treatment launched

11 Scientists develop the first treatment regimen to reduce mother-to-child transmission of HIV

12 UNAIDS is created

13 Brazil becomes the first developing country to provide antiretroviral therapy through its public health system

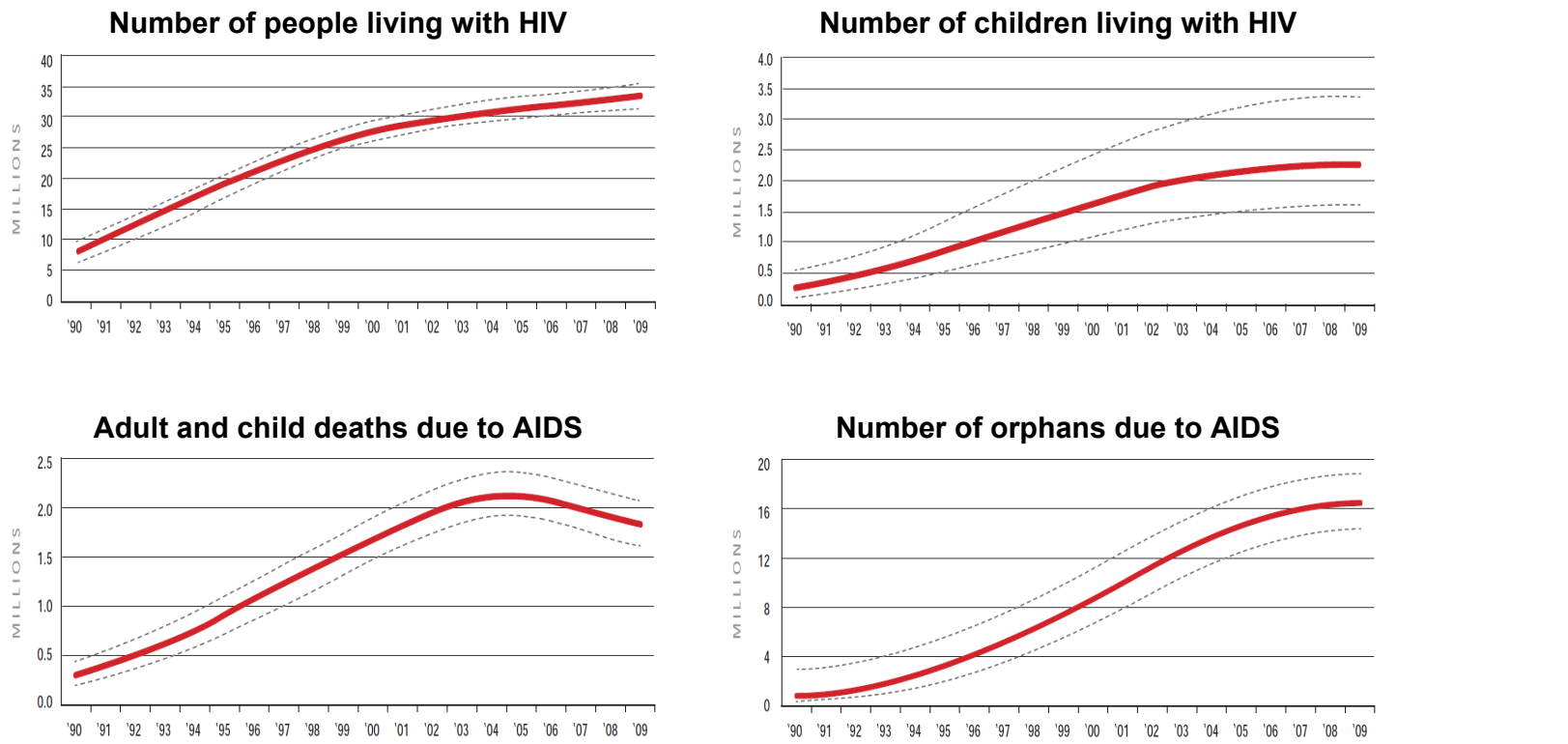
14 The UN General Assembly Special Session on HIV/AIDS. Global Fund to fight AIDS, Tuberculosis and Malaria launched

15 WHO and UNAIDS launch the "3 x 5" initiative with the goal of reaching 3 million people in developing world with ART by 2005

16 Global Coalition on Women and AIDS launched

Figure 2.5

Global HIV trends, 1990 to 2009



Dotted lines represent ranges, solid lines represent the best estimate.

Source: UNAIDS.

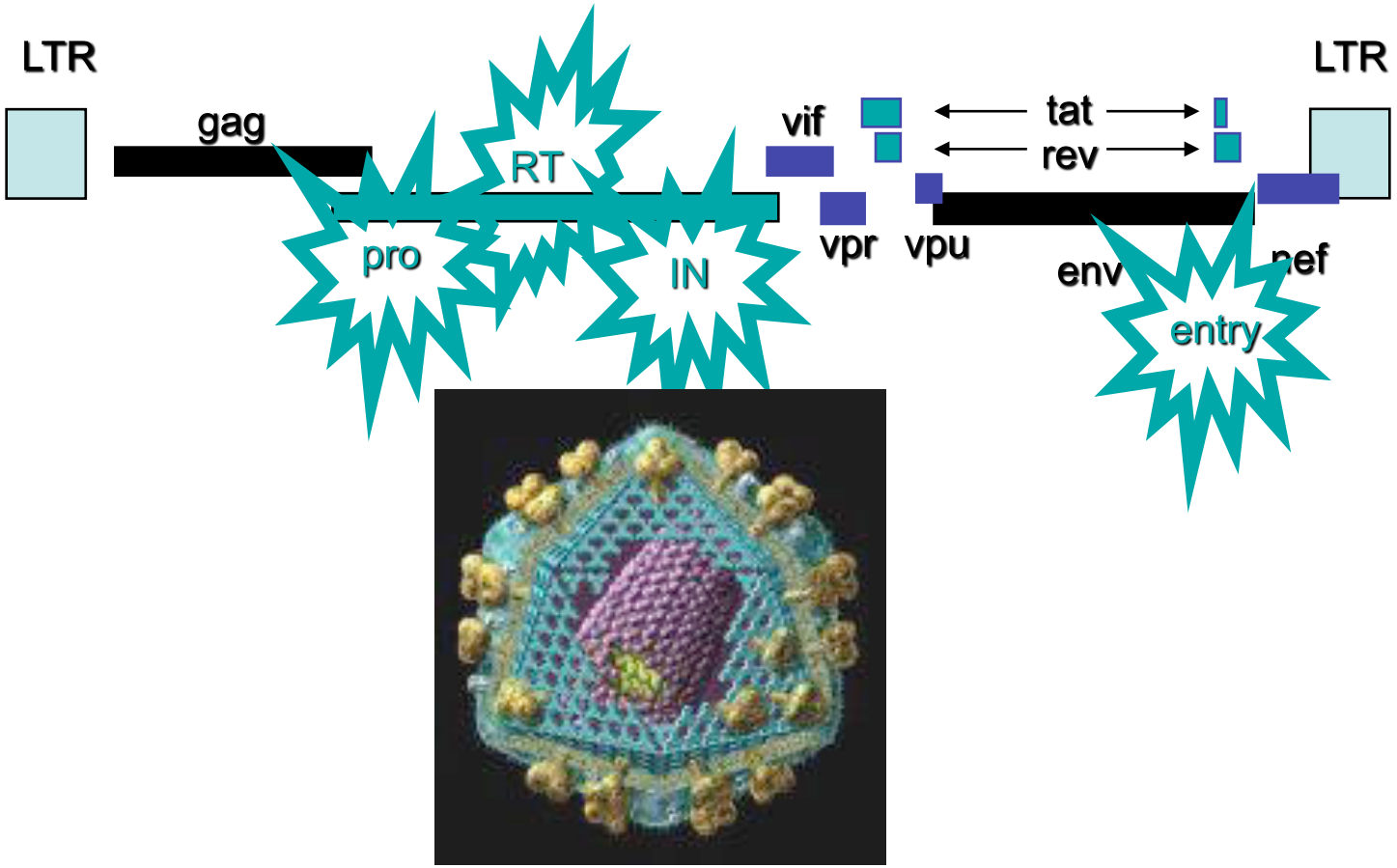
World Health Organization

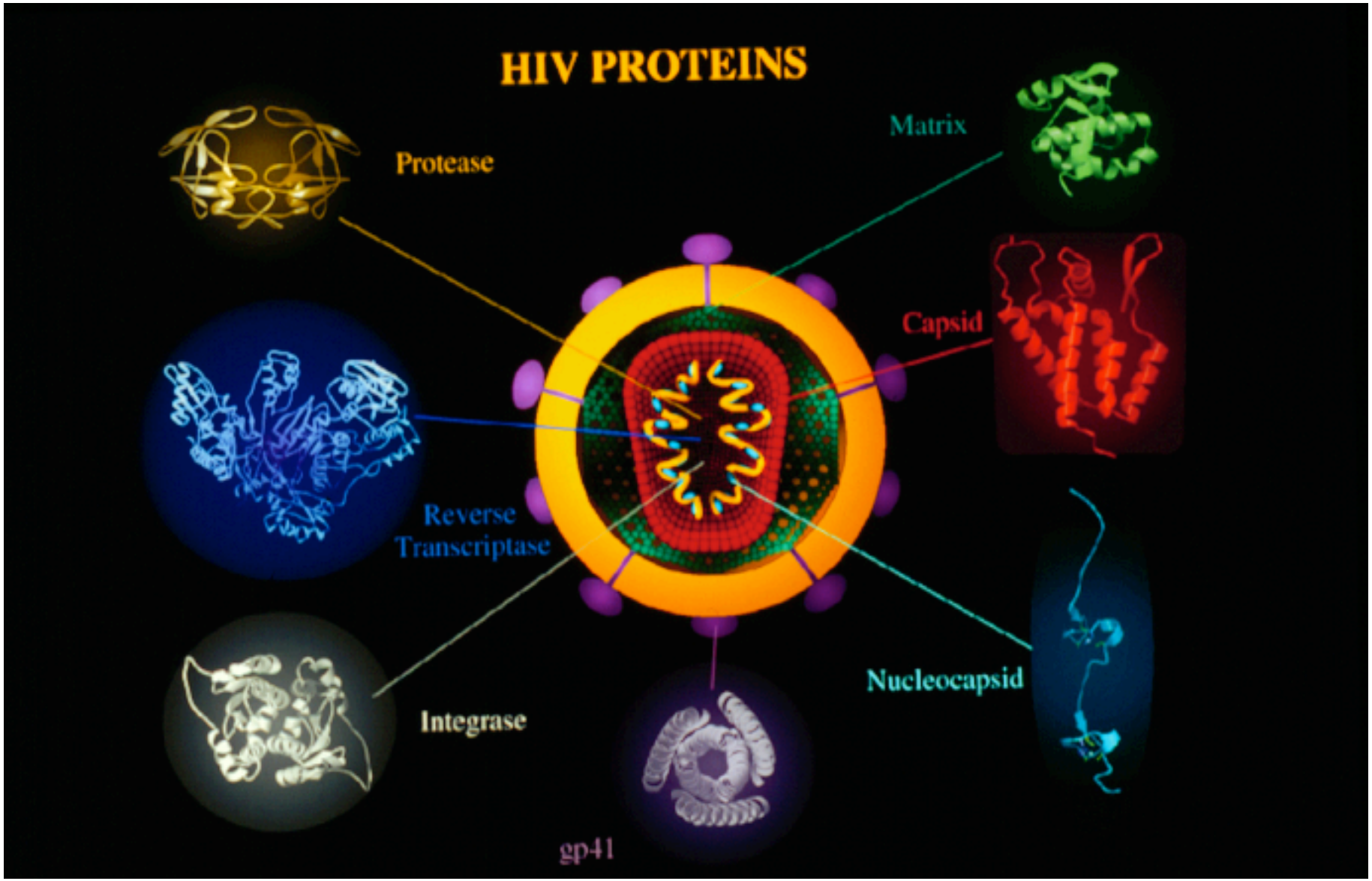
UNAIDS

30 Years Into The AIDS Pandemic:
Medical Victories Since The Beginning (~1981-84)

- Unknown Virus
- The most studied Virus
  - Virus discovery, Diagnostics
- Few drugs against viruses
- ~30 drugs against HIV
- No drug combinations
- Many drug combinations provide >15 years virus suppression
- Lack of infrastructure
- Millions of treated patients in poor countries
- For every 2 persons who begin ART, 5 persons become newly infected

The Most Studied Virus
HIV-1 Genome

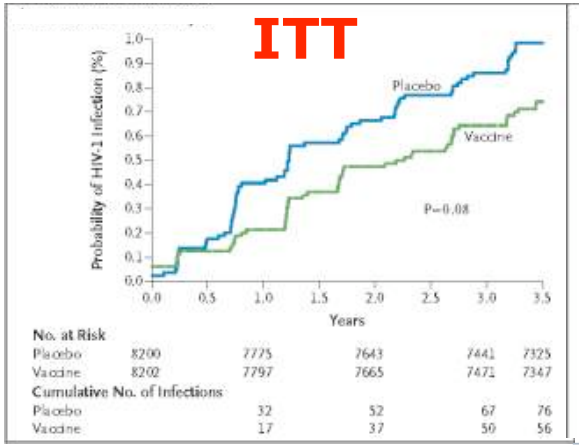


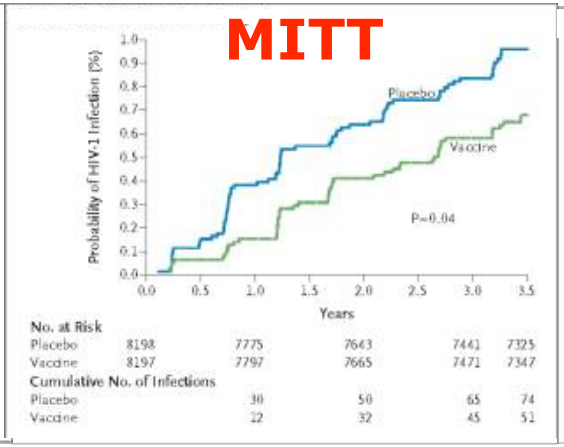

HIV PROTEINS
Protease
Matrix
Capsid
Reverse
Transcriptase
Integrase
Nucleocapsid
gp41

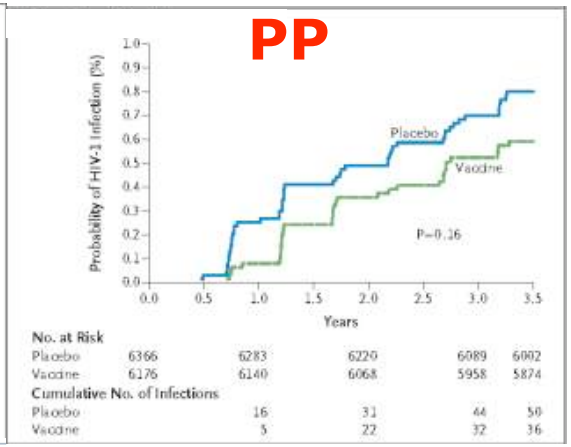
CAPRISA 2010
Antivirals As Prevention

- A vaginal microbicide (1% Tenofovir) could reduce a woman's risk of becoming infected during sexual intercourse: Hope for an effective prevention method that women may initiate on their own
- 2010, a multi-country study found that a daily tablet containing the antiretroviral drugs tenofovir and emtricitabine reduced the risk of infection among men who have sex with men by 44%

RV144 Prophylactic Vaccine Trial, Thailand (2009) 16,400 Randomized Subjects







Est. VE = 26%

(-4%, 48%)

p = 0.08

Est. VE = 31%

(1%, 51%)

p = 0.04

Est. VE = 26%

(-13%, 52%)

p = 0.16

UNAIDS, 2011

- Reshape the HIV response to reach:
  - zero new HIV infections
  - zero discrimination
  - zero AIDS-related deaths
- Saturate transmission hot spots with proven interventions such as female and male condom promotion, male circumcision, treatment as prevention, harm reduction for drug users
- Scale up research investments to accelerate the development of vaccines, female-controlled methods, microbicides and other prevention tools

Can We Think About AIDS Cures?

- "sterilizing" cure that completely eradicates the virus from the body
- "functional" cure that permanently suppresses the virus to a harmless level (EBV, CMV,…)
- Drugs not considered enough for sustainable progress, although they have converted the AIDS problem to a chronic disease management
- Need prophylaxis, especially vaccines

Realistic targets for AIDS vaccines

- Problem:
- HIV causes chronic active infection that is never cleared
- Post-infection immunity is not able to prevent re-infection
  - Virus mutates and recombines rapidly
  - Infected people can be re-infected with new HIV variants
- Many companies got out of the vaccine race (Merck, Wyeth)
  - Failure of Adenovirus vaccine had negative effects
- The recent low level of protection reported by the RV144 human trial in Thailand provides new hope that effective vaccines will be possible

Protection Gold Standards:

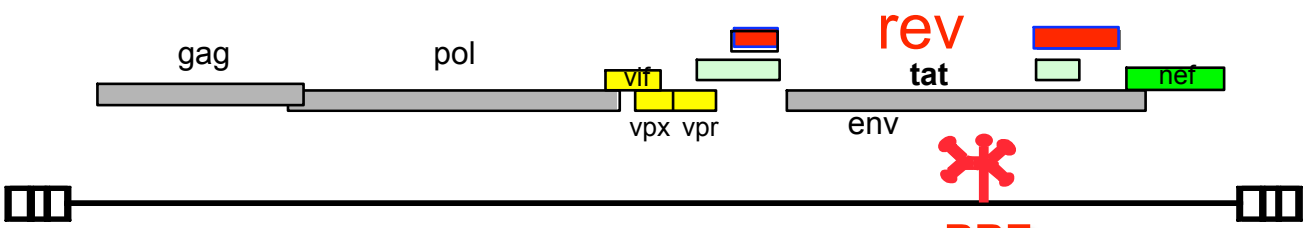
- Animals loaded with neutralizing Abs can resist infection
- Animals infected with attenuated strains of SIV resist disease progression upon pathogenic challenge and infection with WT

Live-attenuated SIV (LASIV) Strains provide the best known protection against pathogenic SIV
and
provide clues on benchmarks that need to be achieved by vaccination approach

Immune responses induced by LASIV serve as "gold-standard"

Use of non-pathogenic SIV to dissect components of the innate and adaptive immune system that are responsible to contain the virus

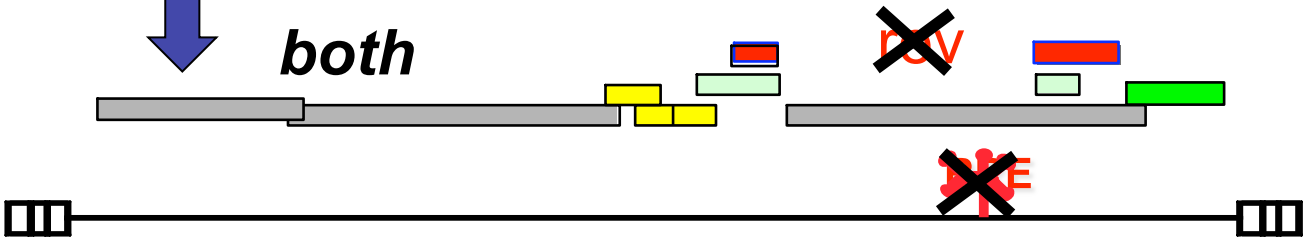
Live-attenuated SIVmac239



virus

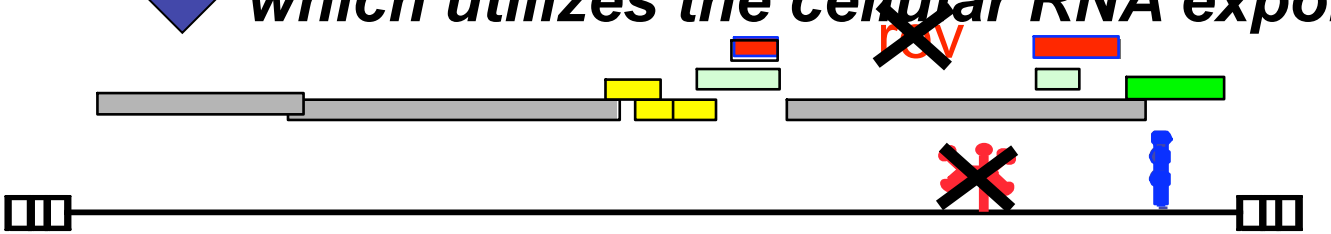
RRE Rev-Responsive Element

Mutation of rev or RRE or both



No virus

Replacement of Rev regulation by inserting CTE, the RNA export element of typeD retroviruses which utilizes the cellular RNA export machinery

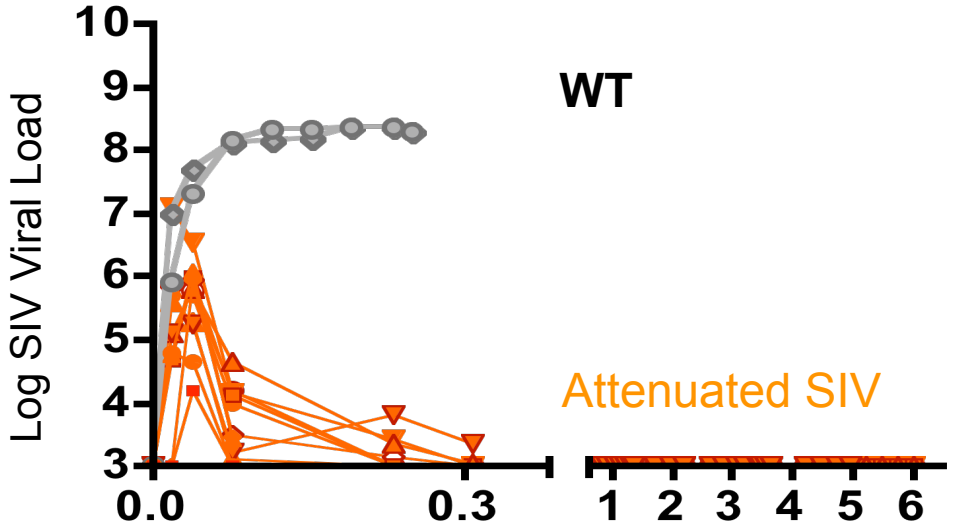


virus

CTE

constitutive RNA Transport element of SRV-1

Chronic Non-pathogenic Infection In Macaques With Rev-independent LASIV

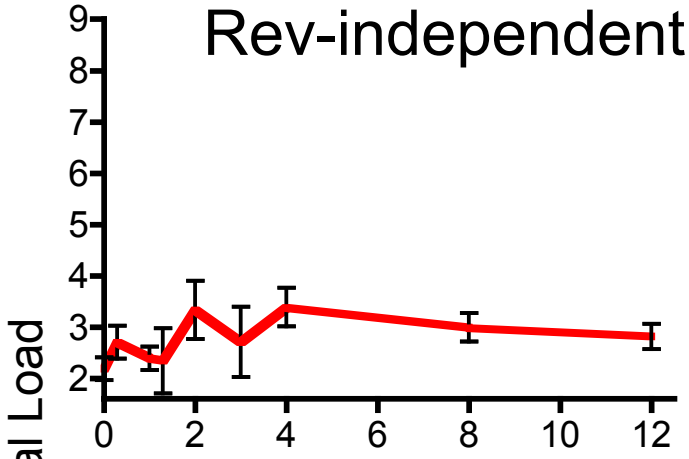


- ✔ Low or undetectable viral load
- ✔ Low levels of circulating SIV-specific T cells
- ✔ Persistent low levels of humoral immune responses

Von Gegerfelt 2002, 2006, 2010

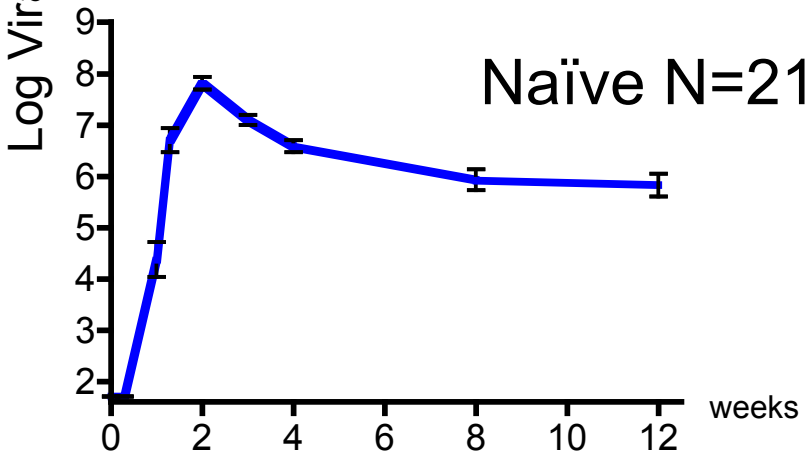
LASIV-infected Animals Control Mucosal Challenge by High Dose SIVmac251

LASIV



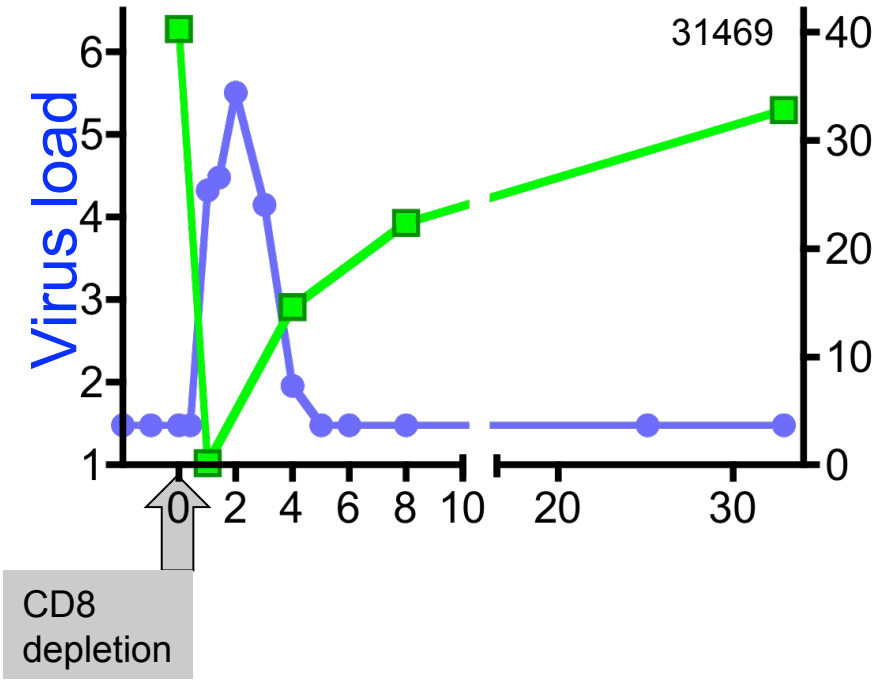
Long term (>7 years) control in 8 of 11 animals

CONTROL

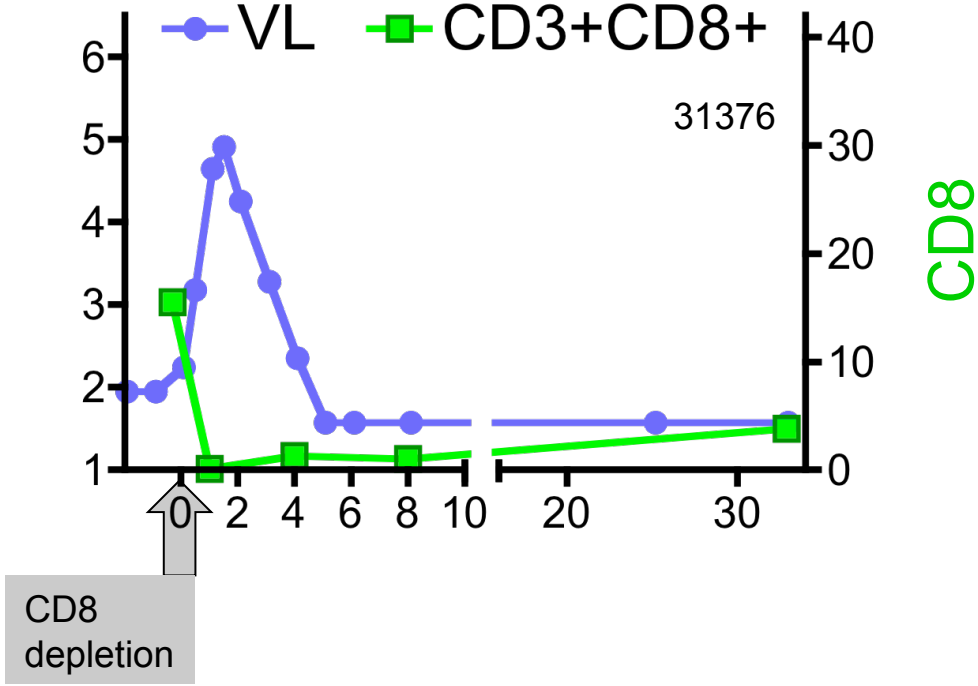


CD8+ cells contribute to virus suppression

Virus control coincides with CD8 recovery (1/5 animals)

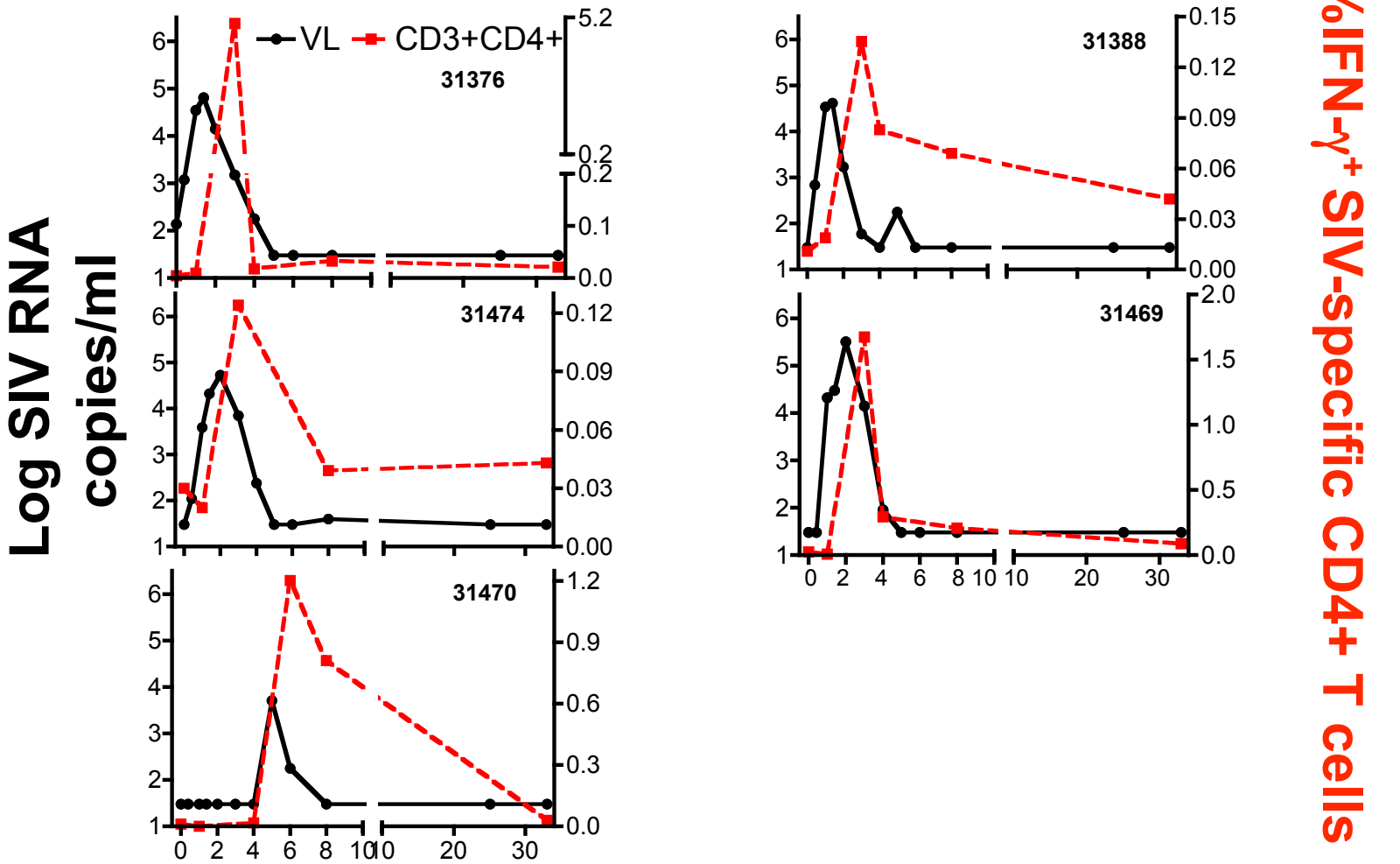


Virus control despite poor CD8 recovery (4/5 animals)



Von Gegerfelt et al JI, 2010

Control Of Viremia Correlates With The Emergence Of SIV-specific CD4+T Cells In The Absence Of Significant CD8 Responses



Von Gegerfelt et al, JI, 2010

Goals For Protective Vaccine

- Broad and sustained Neutralizing Ab
- Effective cellular Immunity

- What is the role of DNA vaccines?
  - Work in progress
  - Approved animal DNA vaccines, including the first cancer therapeutic vaccine, no approved human vaccines yet

Licensed DNA Vaccines

- Horse: West Nile-Innovator® DNA, Wyeth 2005
- Salmon: Apex-IHN® Novartis, 2005
- Canine Melanoma Cancer Vaccine, **Merial** 2007
  - First Therapeutic Cancer DNA Vaccine

DNA Vaccine Advantages/Disadvantages

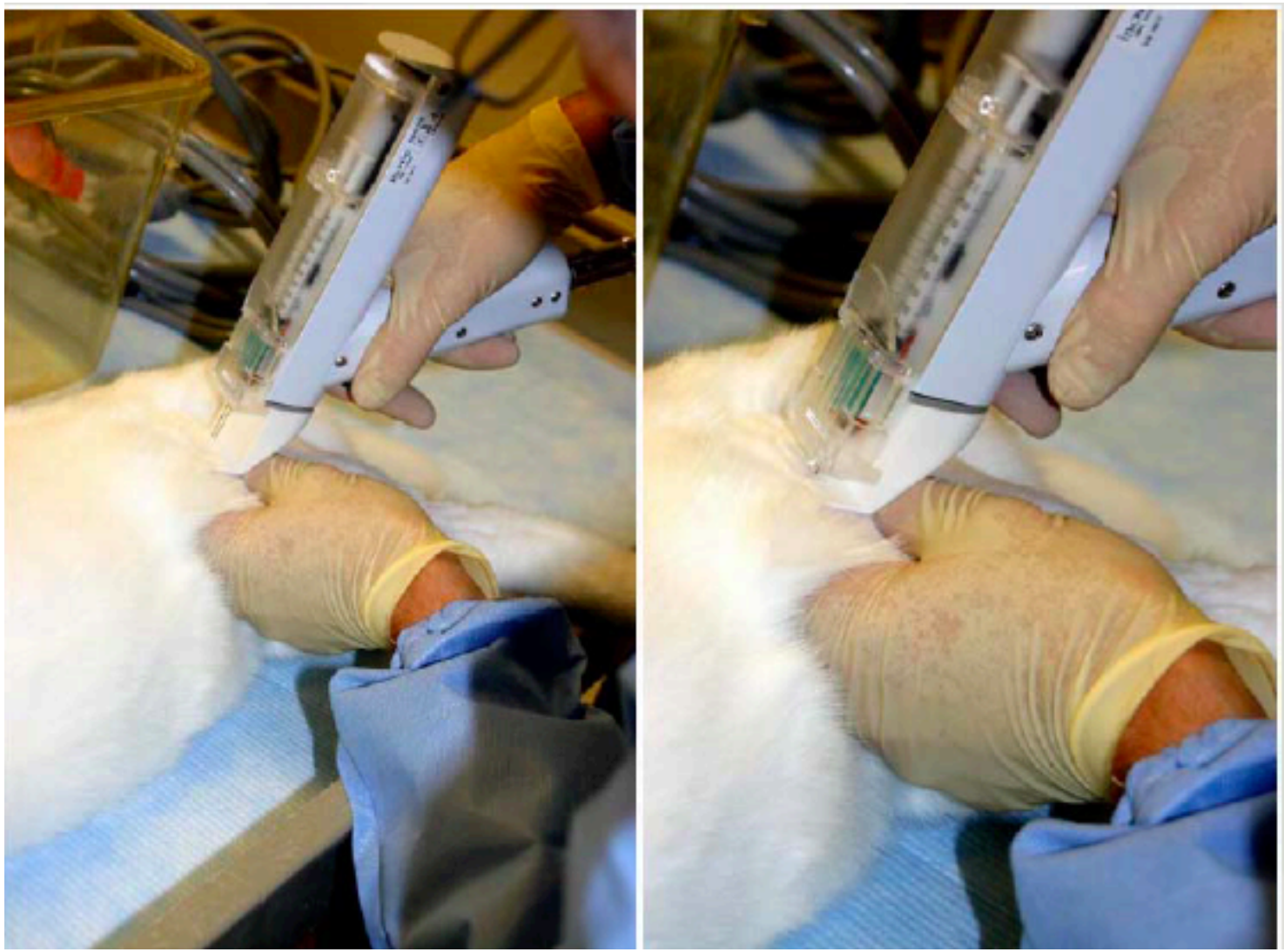
- Repeated Administration
  - Unlike viral vectors, DNA does not focus the immune response to the vector and can be effectively administered multiple times
- Rapid, Scalable, Safe
- Ideal platform for vaccine development due to rapidity and ease of DNA manipulations

- Immunogenicity in primates and humans is now vastly improved

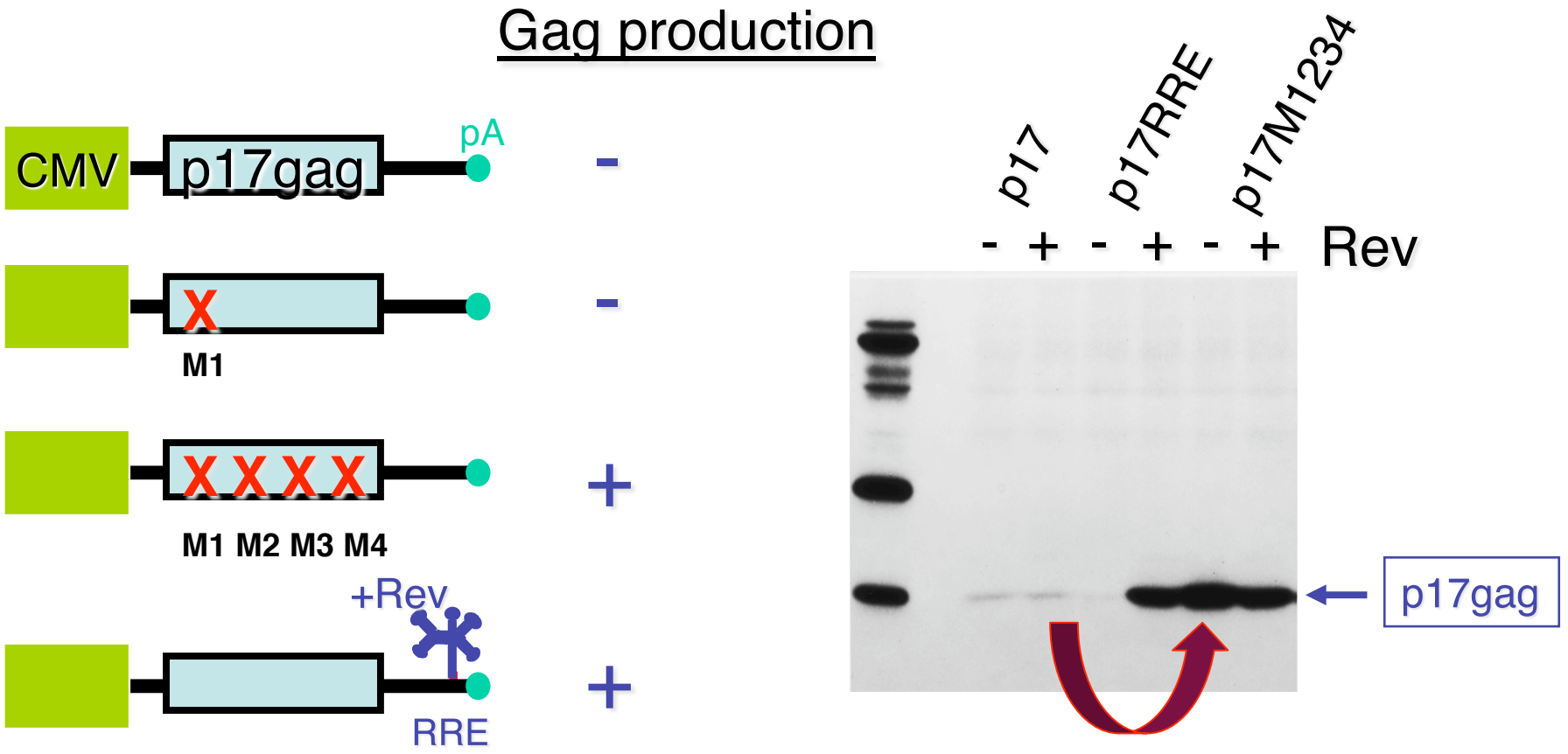
- Expression Optimization Is Critical for DNA vaccine immunogenicity
  - To increase expression, we developed general methodology, essential for progress in the field: RNA/codon Optimization to eliminate RNA instability

  - Increased Delivery by Electroporation and other methods

DNA Injection/Electroporation By Inovio's ELGEN Device



RNA (codon) Optimization Removes Inhibitory Sequences In Coding Regions of HIV-1 Gag



Posttrancriptional (RNA) Optimization: Stable mRNA=Better Protein Expression

…AAA AAA TAT AAA TTA AAA CAT ATA….. WT

…AAG AAG TAC AAG CTA AAG CAC ATC….. optimized

Lys Lys Tyr Lys Leu Lys His Ile

Changes in multiple codons result in stable mRNA, efficiently exported and translated in the ribosome

RNA/codon Optimization

- Codon optimization is not optimization of codons: all codons are translated well by the ribosome; for example, HIV uses “non-optimal” codons but is expressed highly in infected cells
- By changing mRNA codons, we optimize mRNA stability and export, eliminating a multitude of known negative-acting RNA signals, destabilizing sequences, repeats, splice sites, etc.
- Several NCI patents licensed to pharmaceutical companies
- This technology also allowed HIV DNA vaccine experiments in mice, a model system not accessible before, due to very low expression

Pavlakis/Felber: RNA/codon optimization USA Patents 1996-2002

6,414,132	Method of eliminating inhibitory/instability regions of mRNA
6,291,664	Method of eliminating inhibitory/instability regions of mRNA
6,174,666	Method of eliminating inhibitory/instability regions from mRNA
5,972,596	Nucleic acid constructs containing HIV genes with mutated inhibitory/instability regions and methods of using same
5,965,726	Method of eliminating inhibitory/ instability regions of mRNA

- **Licensed through NIH to Companies (Merck, Wyeth, Novartis,…)**
- **Priority dates 1992**
- **Cover codon/RNA optimization for improved expression**

DNA Vaccination Optimization

- Antigen
  - Different forms of antigen affect immune response
- Delivery
  - Electroporation increases both antigen expression and immunogenicity
- Adjuvant
  - Cytokine DNAs as molecular adjuvants IL-12, IL-15
- Combinations:
  - DNA+protein, DNA+viral vectors

Current Status:
DNA Vaccines Are Improving Rapidly

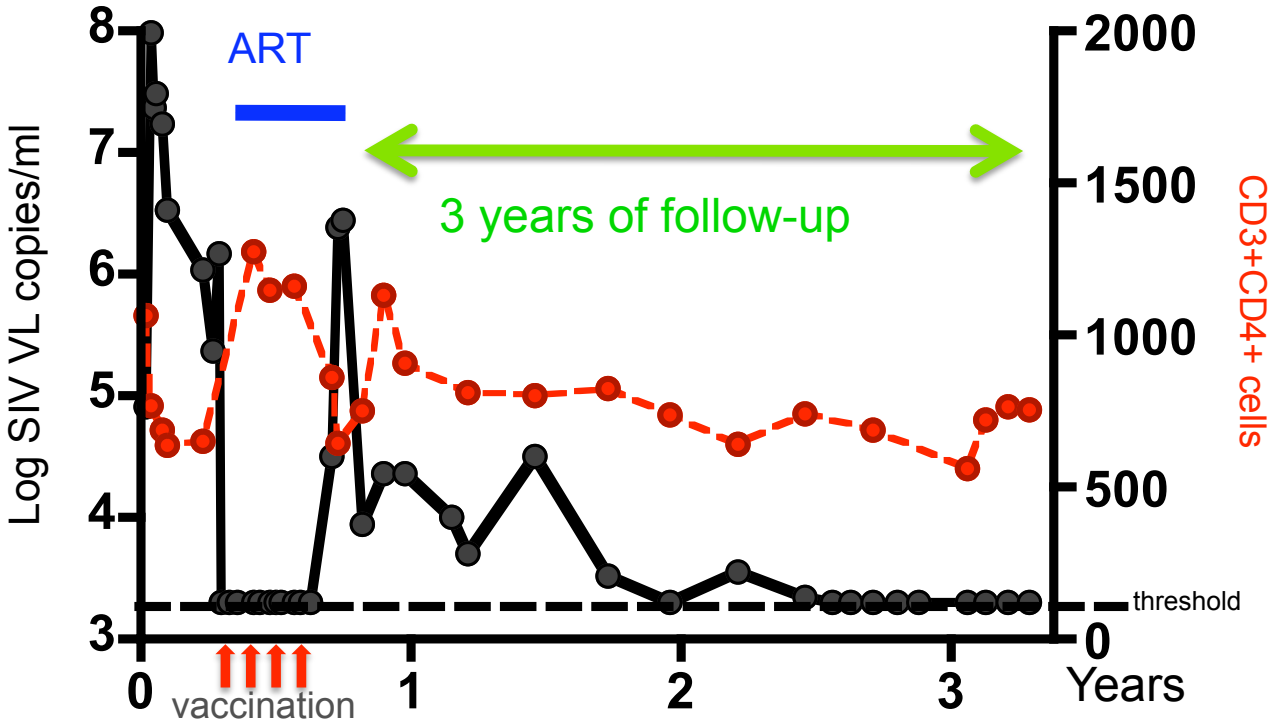
- DNA vaccination can achieve high levels of immune responses in primates
- Both Antibodies and Cell-Med. Immunity
- Dissemination to mucosal sites
- Protection from high viremia (prophylactic vaccination)
- Protection from high viremia and disease development after therapeutic vaccination

Control of viremia after antiretroviral treatment and therapeutic vaccination with novel forms of DNA vaccines in chronically SIVmac251-infected macaques

Agneta von Gegerfelt et al,

J Virol. 81, 1972 2007

Long-Term Benefit After DNA Therapeutic Vaccination



Long-term virus reduction (3-4 years follow-up), supports potency of vaccine-induced recall responses

von Gegerfelt, 2007, Valentin, 2009

DNA vaccination in rhesus macaques induces potent immune responses and decreases acute and chronic viremia after SIVmac251 challenge

Margherita Rosati[a], Cristina Bergamaschi[a], Antonio Valentin[a], Viraj Kulkarni[b], Rashmi Jalah[b], Candido Alicea[b], Vainav Patel[a], Agneta S. von Gegerfelt[a], David C. Montefiori[c], David J. Venzon[d], Amir S. Khan[e], Ruxandra Draghia-Akli[e], Koen K. A. Van Rompay[f], Barbara K. Felber[b,1], and George N. Pavlakis[a,1]

[a]Human Retrovirus Section, and [b]Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702-1201; [c]Department of Surgery, Laboratory for AIDS Vaccine Research and Development, Duke University Medical Center, Durham, NC 27710; [d]Biostatistics and Data Management Section, National Cancer Institute, Bethesda, MD 20892; [e]VGX Pharmaceuticals, LLC, The Woodlands, TX 77381; and [f]California National Primate Research Center, University of California, Davis, CA 95616

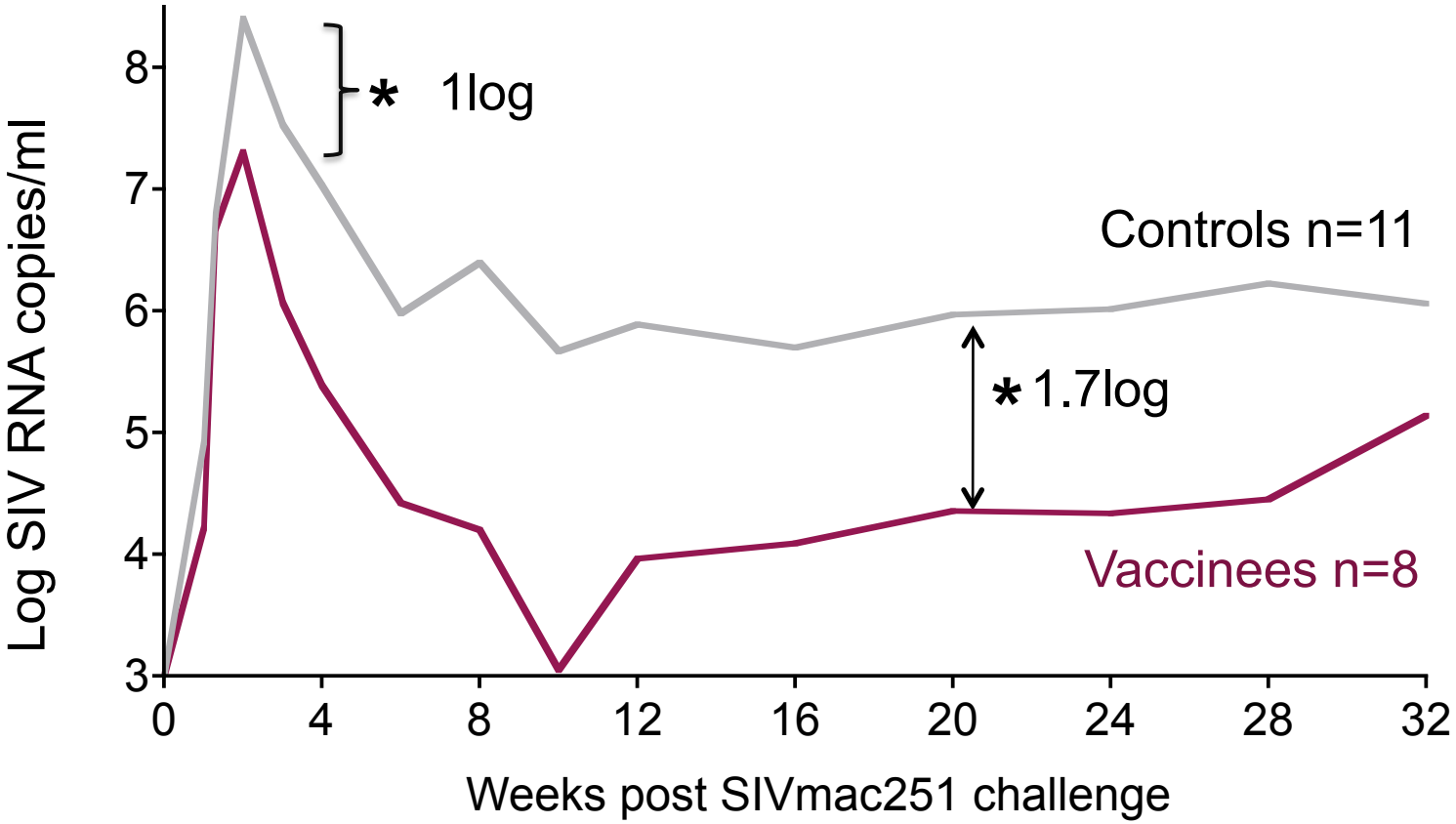
Edited by Robert C. Gallo, University of Maryland, Baltimore, MD, and approved July 23, 2009 (received for review March 9, 2009)

Optimized plasmid DNAs encoding the majority of SIVmac239 proteins and delivered by electroporation (EP) elicited strong immune responses in rhesus macaques. Vaccination decreased viremia in both the acute and chronic phases of infection after challenge with pathogenic SIVmac251. Two groups of macaques were vaccinated with DNA plasmids producing different antigen forms, "native" and "modified," inducing distinct immune responses. Both groups showed significantly lower viremia during the acute phase of infection, whereas the group immunized with the native antigens showed better protection during the chronic phase (1.7 log decrease in virus load, $P = 0.009$). Both groups

no decrease in viremia or modest and transient decrease during primary infection only, which, in some cases, could still benefit the animals (11). Promising strategies not involving DNA include nonreplicating recombinant adenovirus rAd26/rAd5 (12), replicating recombinant adenovirus plus protein boost (13), or recombinant rhCMV (14). Despite improvements of DNA vaccines, human trials have indicated that the magnitude of immune responses after DNA vaccination remains low (1, 15–18) compared to levels reported in macaques. The ability to increase the magnitude and quality of the immune responses and to achieve protection in a strict macaque SIV challenge model may provide

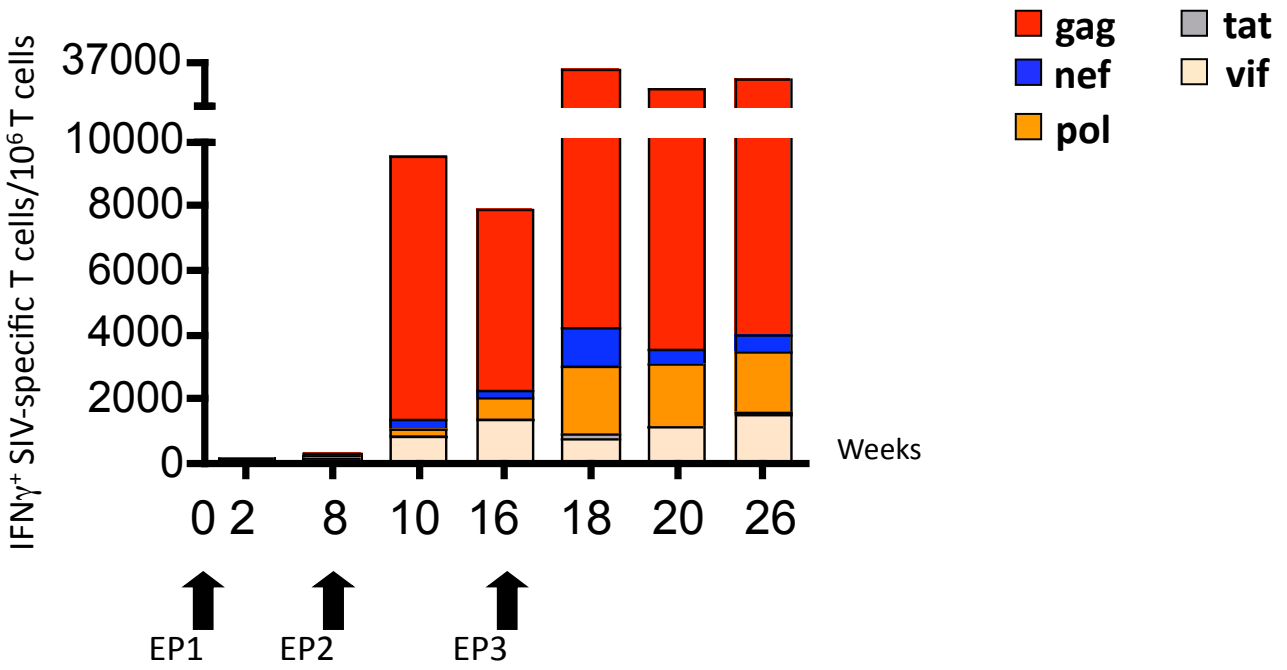
Rosati PNAS 09

DNA Prophylactic Vaccination Protects Macaques From High Viremia

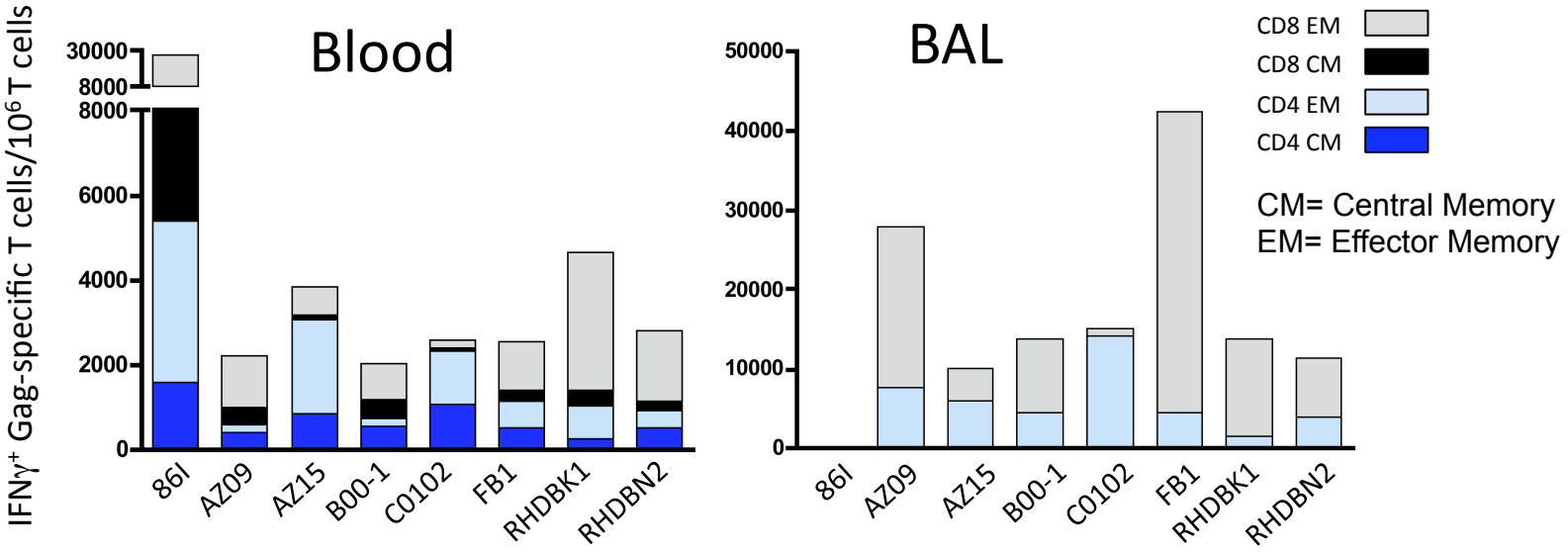


Rosati, JVir 2005, Rosati PNAS 2009

DNA Vaccination In Rhesus Macaques Induces Strong, Broad Systemic Cellular Responses



Vaccination With DNA Alone Induces Mucosal Dissemination Of Cellular Responses

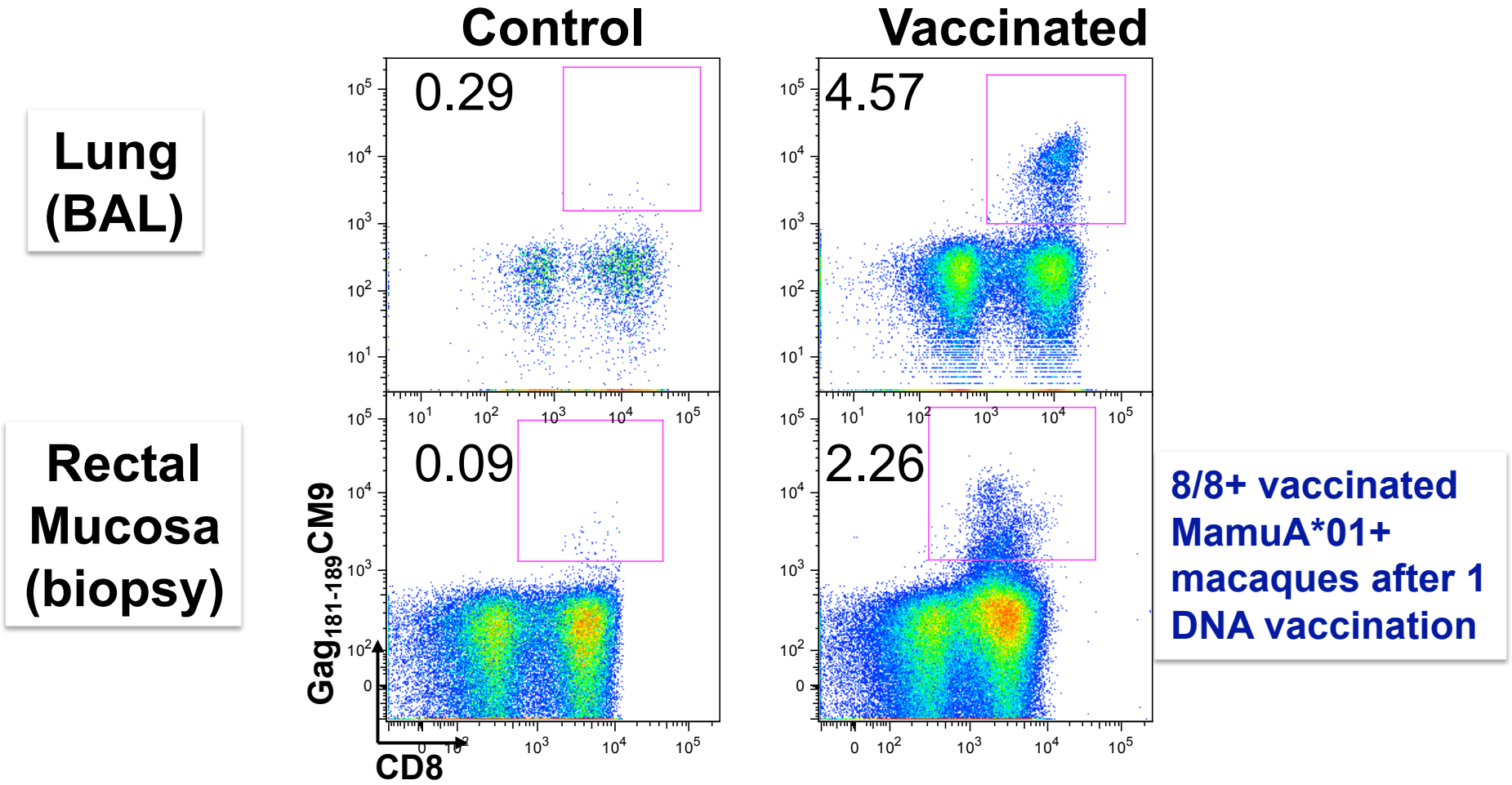


✔ Presence of both $CD4^+$ and $CD8^+$ Gag-specific T Cells with Central Memory (CD28+CD45RA-CD95+) and Effector Memory (CD95+CCR7-) phenotype in blood

✔ Gag-specific $CD8^+$ T Cells with Effector Memory phenotype in lung (Effector Site)

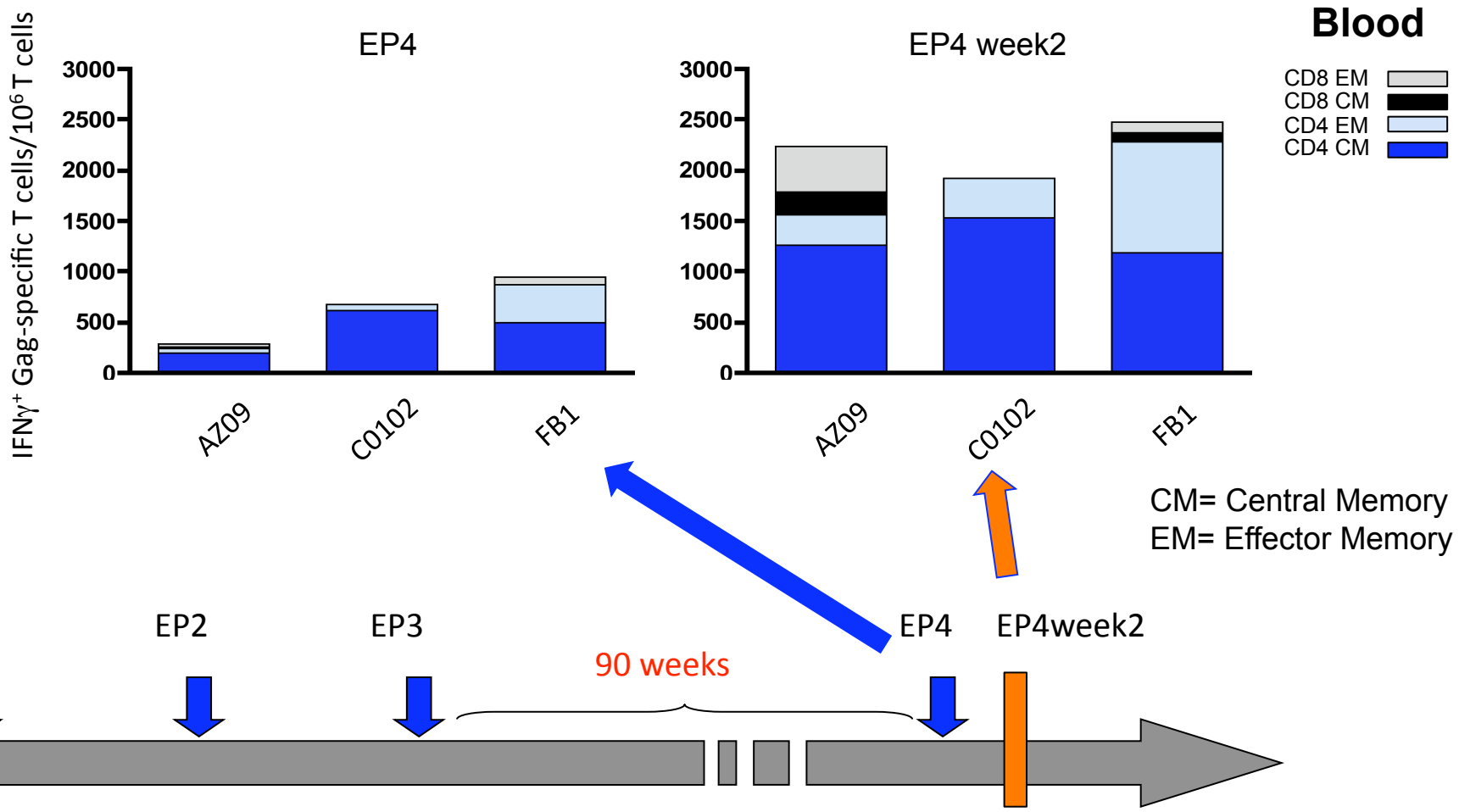
Patel, 2010 Vaccine

Strong Systemic Responses Induced By DNA Via IM Injection Result In Migration To Mucosal Sites

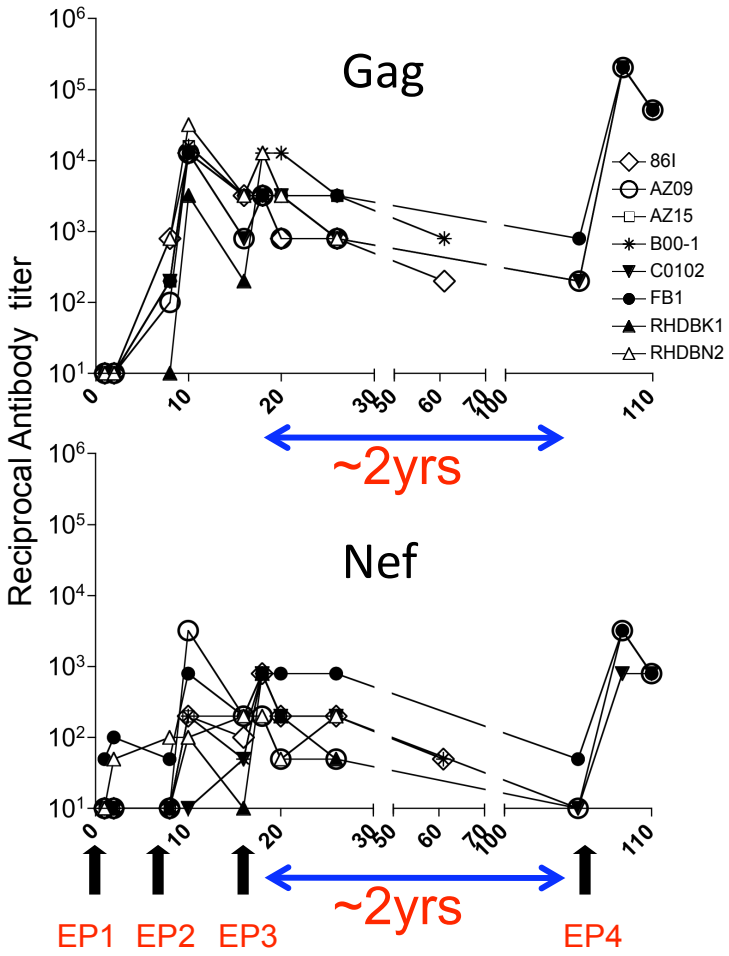


Gag-specific T Cells Measured By Tetramer Staining

Induced Responses Are Long-lasting (~2yrs) And Are Rapidly Boosted



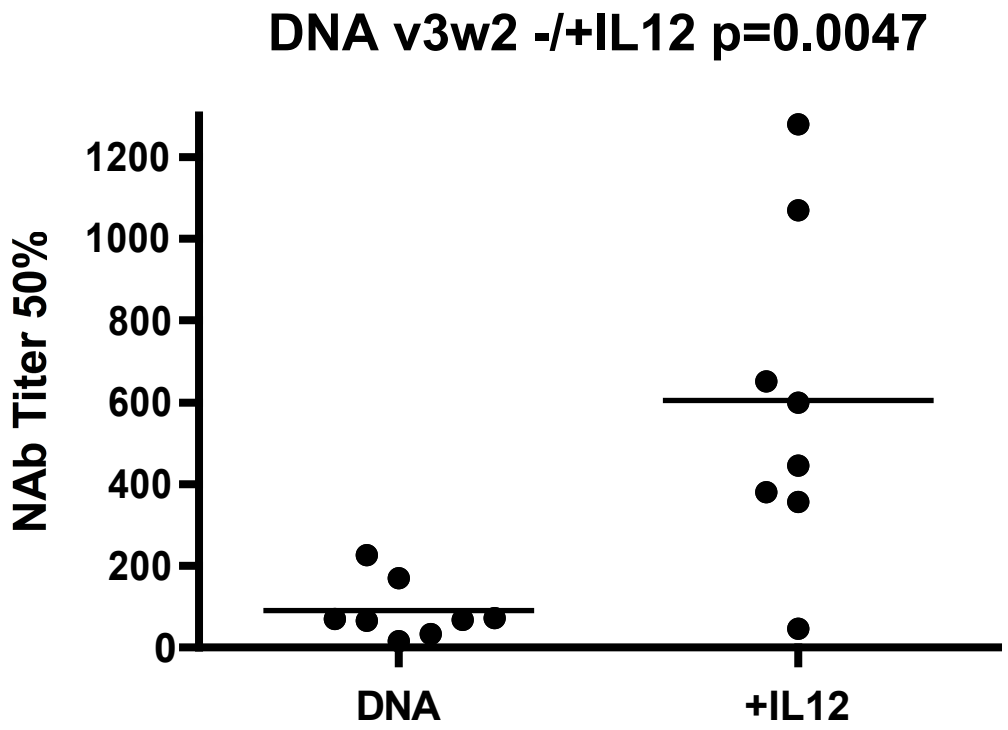
Presence Of Long-lasting SIV-specific Humoral Responses



Induction of Gag-specific IgA in Plasma and BAL

Patel, 2010 Vaccine

IL-12 DNA Enhances NAb



Vaccination with DNA as the ONLY Vaccine Modality Leads to:

- Protection from high viremia upon SIVmac251 high dose challenge

Rosati, et al., JVirol, 2007;
Rosati, et al., PNAS 2009

- Induction of long-lasting (~2 years) humoral and cellular immune responses, effectors

Patel, et al., Vaccine 2010

- Dissemination of antigen-specific T cells and Ab to mucosal sites

Patel, et al., Vaccine 2010

- Higher immune responses in the presence of IL-12 DNA as molecular adjuvant

Human Prophylactic Vaccination HVTN 080 (**NCT00991354)**
HIV gag pol env DNA + IL-12 DNA

- U Penn DNA Vaccine (PENNVAX-B™)
- Wyeth -> Profectus GENEVAX™ IL-12 DNA

- Two Arms:
- PENNVAX-B™
- PENNVAX-B™ plus GENEVAX™ IL-12 DNA

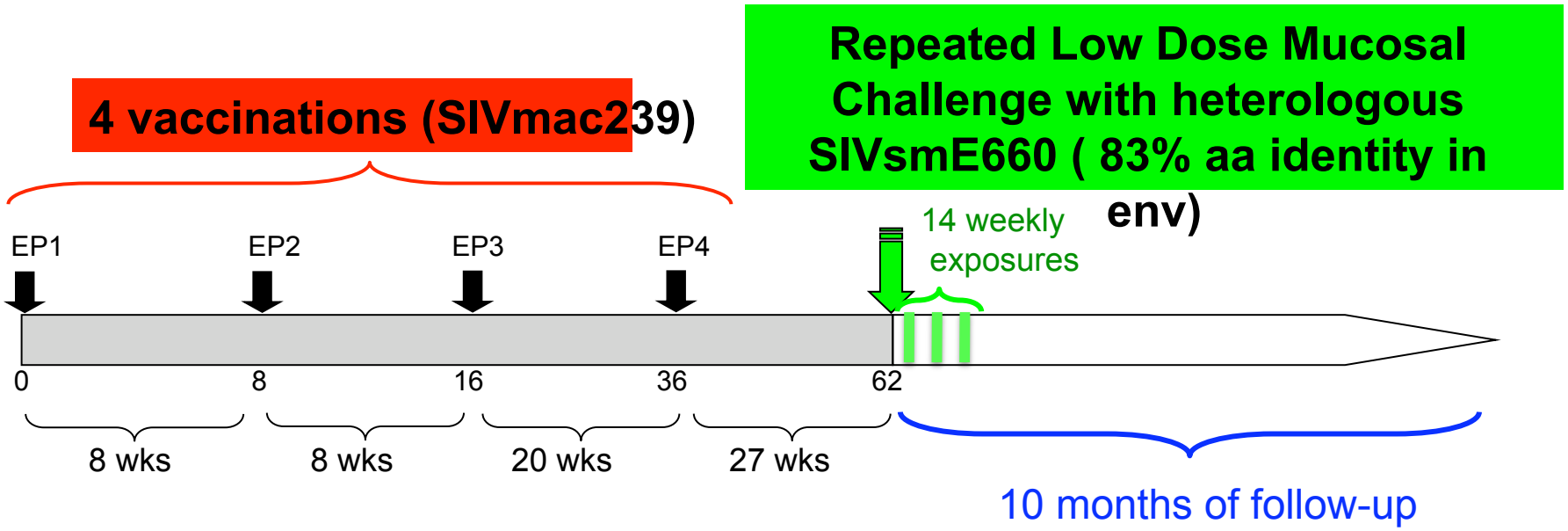
Results Of HVTN 080 (**NCT00991354)**

Vaccine:	**PENNVAX-B DNA**	**PENNVAX-B DNA + GENEVAX IL-12**
2 vaccinations:	30.0% (3 out of 10)	71.4% (20 out of 28) p=0.03
3 vaccinations:	66.7% (6 out of 9)	90.9 % (20 out of 22)

DNA + IL-12 gave better results than:
HVTN 049 DNA + protein (Novartis)
Geovax HVTN 065 (DNA + MVA)
SAAVI HVTN 073 (DNA + MVA)
HVTN 077 Adeno35/Adeno5

DNA And Protein Co-immunization Enhances The Magnitude And Longevity Of Immune Response

Study Design: Indian Rhesus Macaques Immunized with DNA or DNA-protein Followed by Repeated Low Dose SIVmacE660 Mucosal Challenge



- Test:
  - DNA
  - DNA & protein co-administration
  - DNA prime-Protein Boost

Vaccine: Optimized SIV Plasmid DNAs Alone Or with AT-2 SIV Particles Co-delivered Or As Prime Boost

Group (N=8)	Vaccine	Vaccination 1: Wk 0	Vaccination 2: Wk 8	Vaccination 3: Wk 16	Vaccination 4: Wk 36
1	**DNA Only**	DNA	DNA	DNA	DNA
2	**DNA + Protein**	DNA + AT-2 SIV	DNA + AT-2 SIV	DNA + AT-2 SIV	DNA + AT-2 SIV
3	**DNA prime- protein Boost**	DNA	DNA	AT-2 SIV	AT-2 SIV
4	**Sham-Vaccinated**	Sham DNA	Sham DNA	Sham DNA	Sham DNA

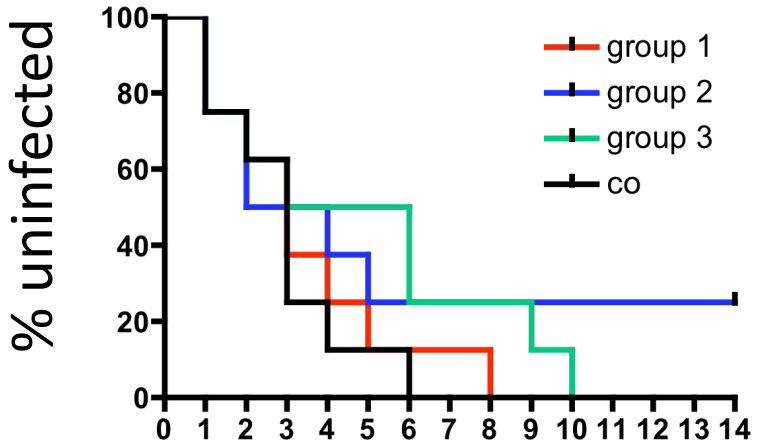
SIVmac239 DNA mixture:

- SIV immunogens (Env, Gag, Pol, Nef, Tat, Vif) coinjected with rhesus IL-12 DNA as molecular adjuvant
- intramuscular (IM) delivery by *in vivo* electroporation (Inovio)

Protein: AT-2 inactivated SIV239 particles

- protein delivery with needle/syringe at same place as DNA

Acquisition protection after 14 exposures

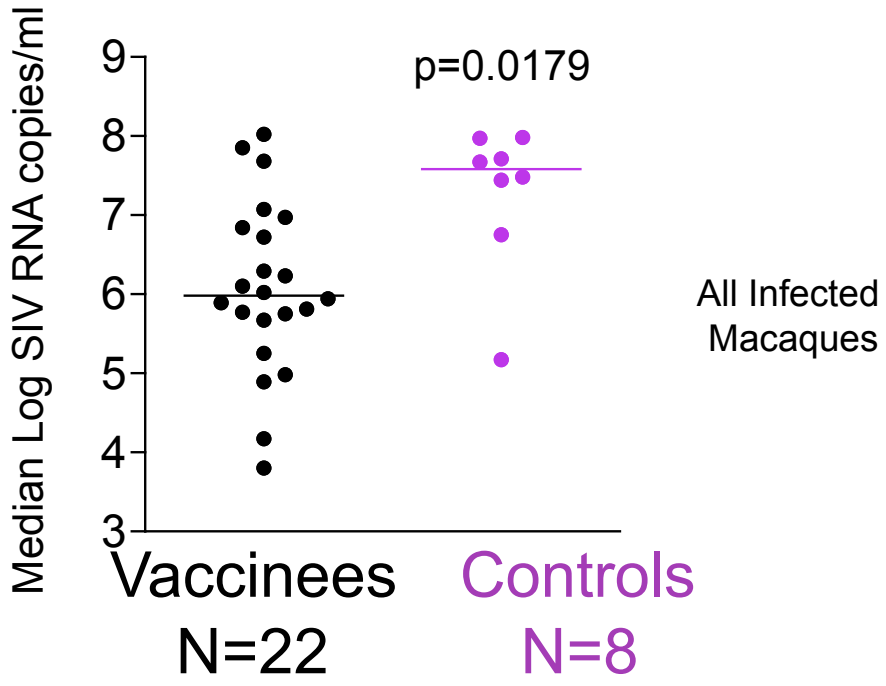


No of SIVsmE660 exposures

2/8 = 25% (DNA+AT-2 co-immunization) remain uninfected

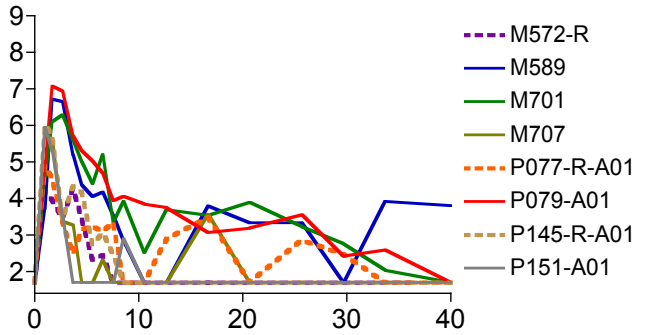
Delayed acquisition of all controls versus all vaccinees, p=0.05

Vaccinated Macaques Show Significant Lower Median Peak Viremia After Challenge with SIVmacE660

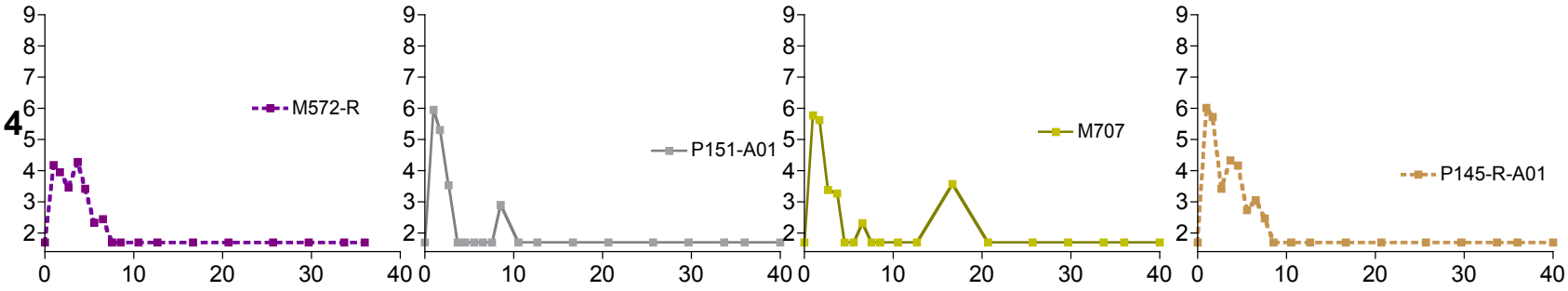


- Vaccination significantly decreases
  - peak viremia by 1.7 log
  - chronic viremia in 75% of vaccinees
    - Significant virological benefit after stratification of animals according to TRIM5a genotype

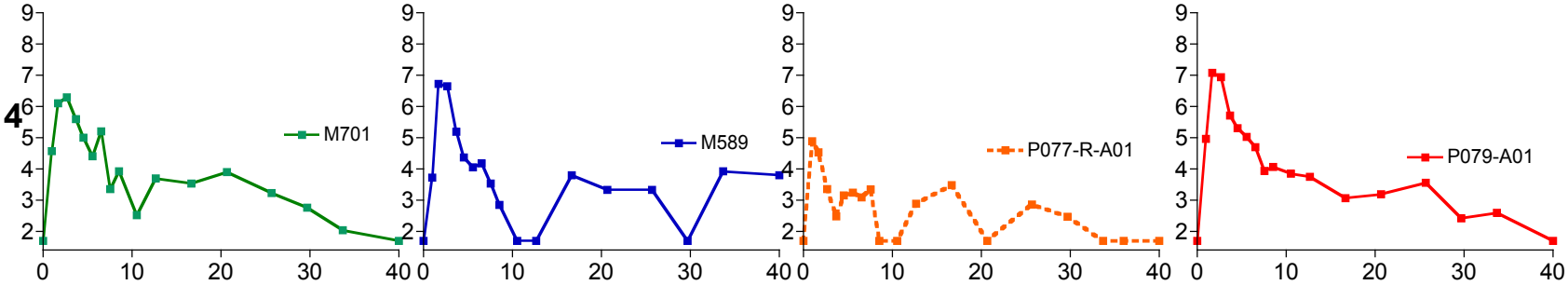
Viral Load after SIVsmE660 Challenge
Group 3: 2 X DNA+ 2x Protein boost



Elite controller: 4

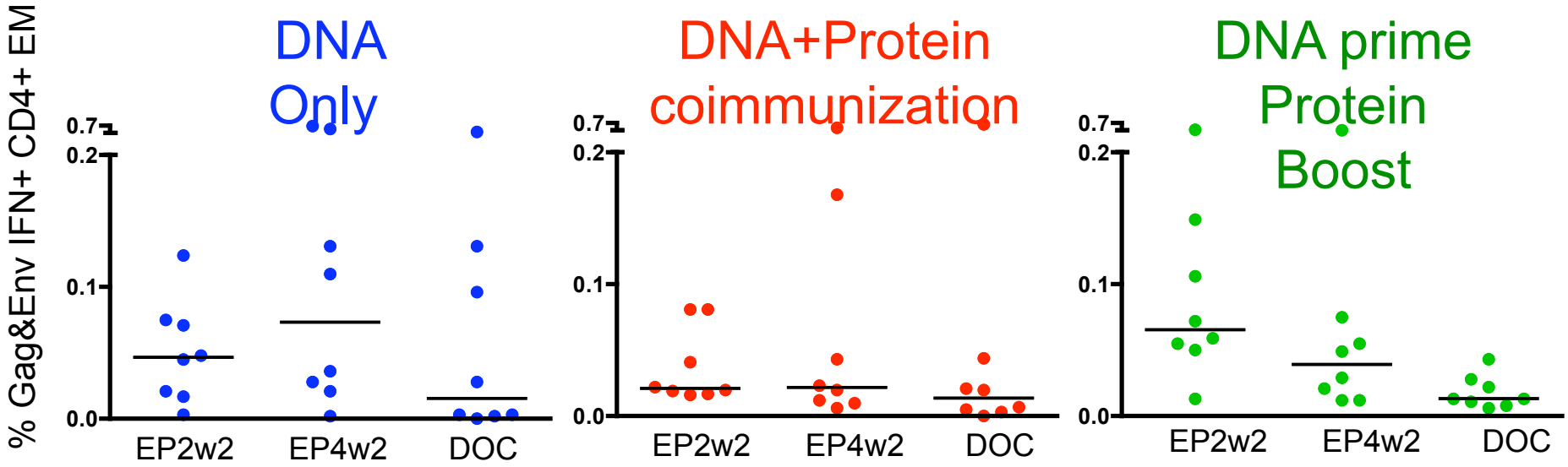


Viremic Controller: 4



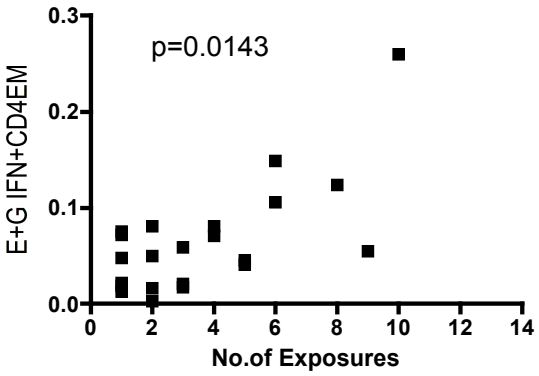
- **8 of the 8 vaccinees control the heterologous SIVsmE660 challenge and maintain CD4 cell counts**
- 4 elite controllers
- 4 viremic controllers

Persistence of Effector Memory responses

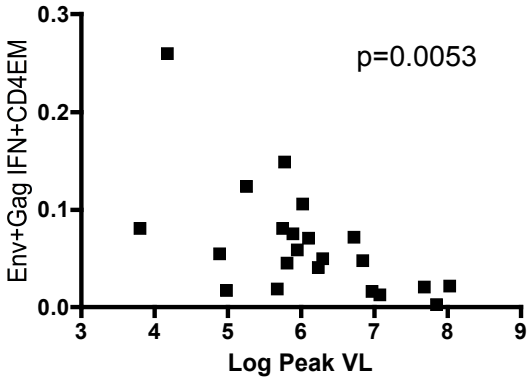


Correlation of SIV-specific CD4 Effector Memory Responses with Control of Viremia

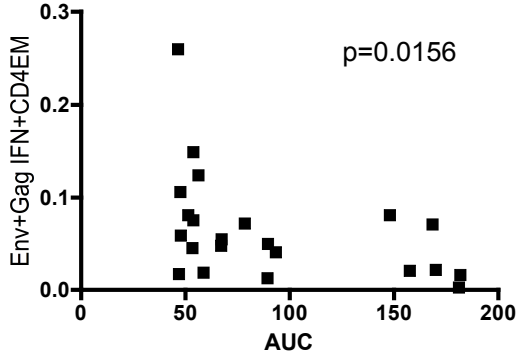
Acquisition



Peak viremia



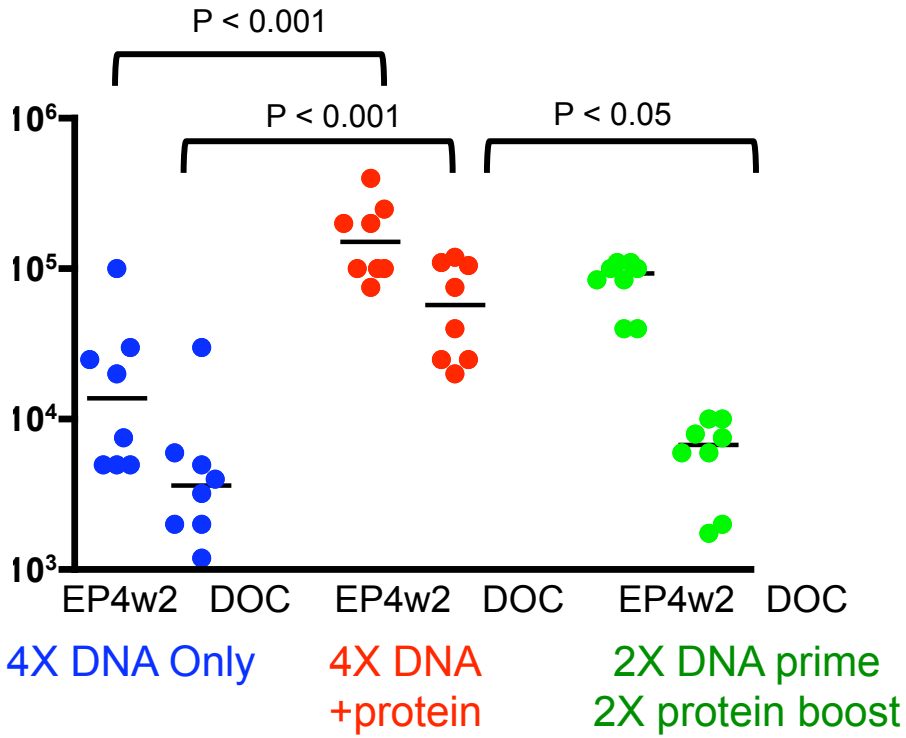
Chronic viremia



(responses from EP2w2)

EP4w2 correlation with acquisition and peak viremia
DOC correlation with acquisition

Binding Ab to SIVmac239 gp120

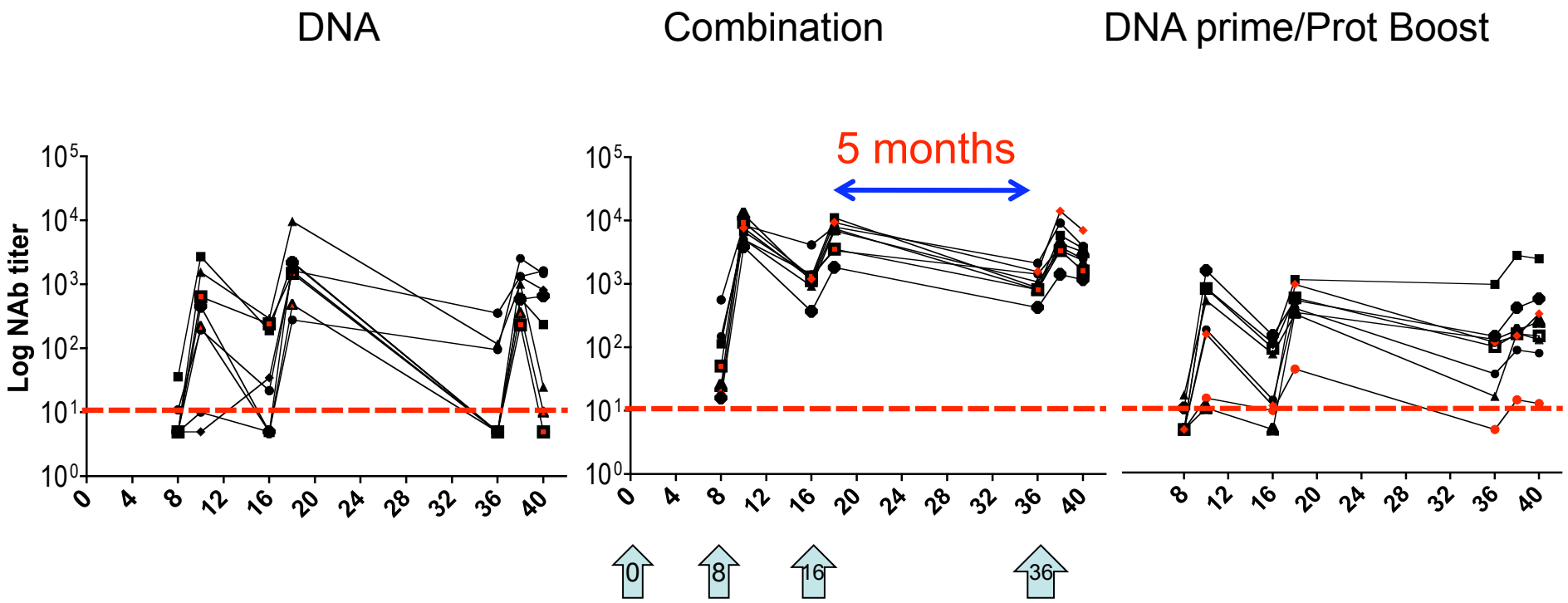


Macaques coimmunized with DNA+protein developed highest bAb to mac239 env (at peak and DOC)

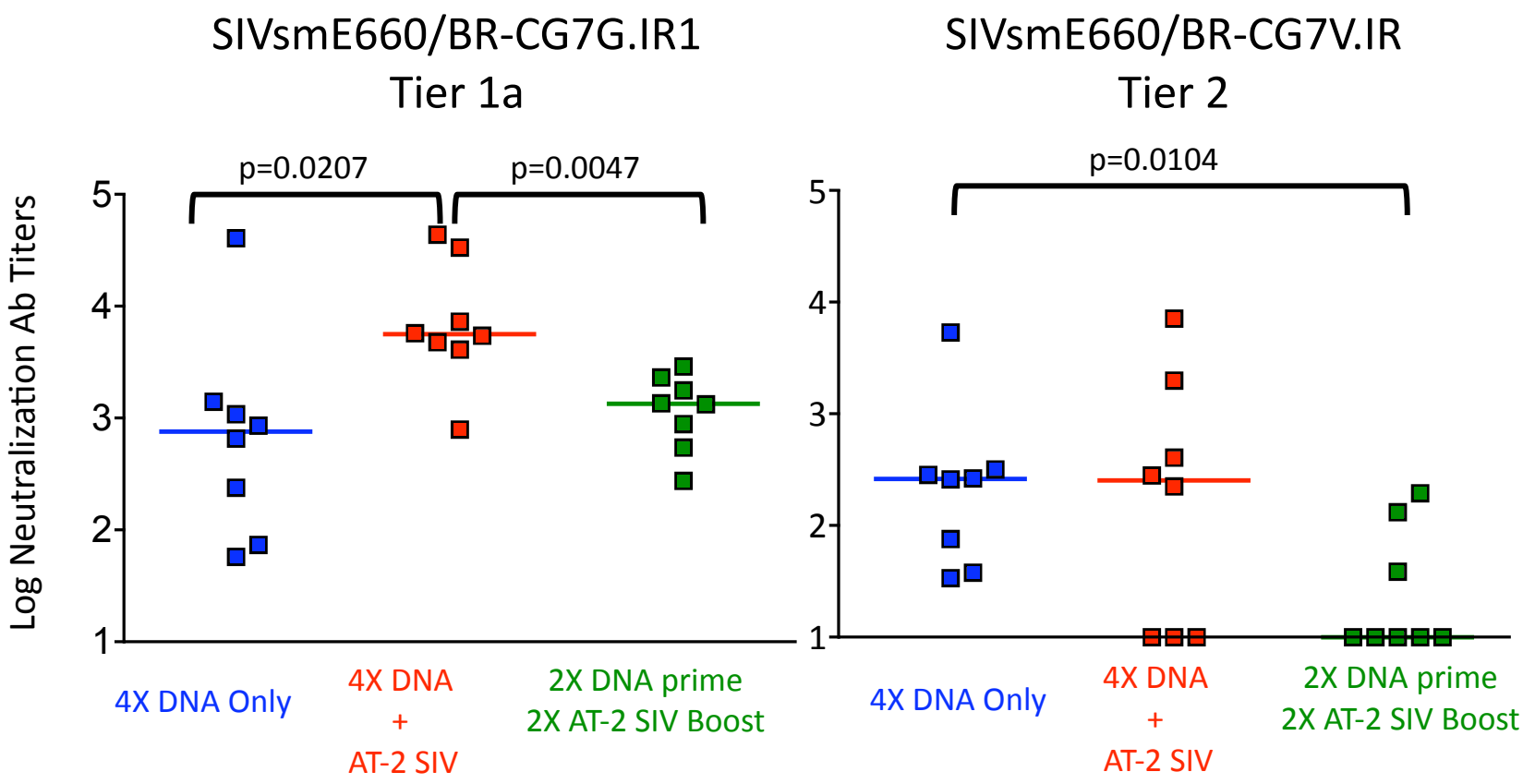
bAb decay between EP4w2 and DOC:

DNA only 3.8x; DNA+protein 2.6x; DNA prime-protein boost 13.7x

Combination Vaccine Increases The Levels And Longevity Of Neutralizing Ab



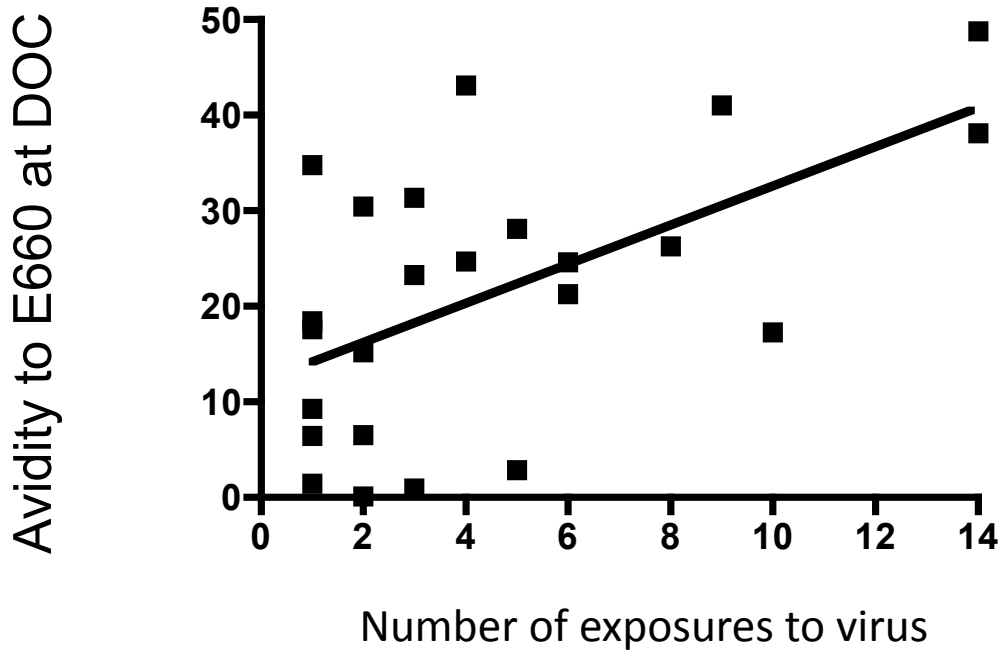
Immunization with either DNA or DNA+AT-2 SIVmac239 Induces Neutralizing Antibody Titers to Heterologous "Tier 1a" and "Tier 2" SIVsmE660



Mann Whitney two-tailed t test

Day of Challenge

Avidity of bAb to E660 env (gp120) correlates with challenge virus acquisition



p = 0.0039
R squared= 0.3211

Avidity of bAb to E660 at day of challenge correlates with # of virus exposures necessary to infect the vaccinated macaques

DNA Vaccines Continue To Improve

- Optimized **DNA-only vaccination** induces high level of immune responses
  - Central Memory and Effector CD4+ and CD8+ T cells, humoral responses
  - Dissemination to mucosal sites
  - Long lasting responses
  - Multifunctional SIV-specific T lymphocytes
  - Protection from high viremia after highly pathogenic challenge
  - Protection from infection in DNA + Protein co-immunization

Conclusions

- DNA vaccine delivered by electroporation in the presence of IL-12 is presently the most immunogenic method in humans (HVTN trials)
- Immature technology and low effective dose have hampered the applications in humans
- DNA provides a broad and durable immune response
- Interestingly, the Ab response also appears broad and durable

Way Forward

- Optimize antigens for a series of global AIDS vaccines based on DNA and protein immunization
  - Unique DNA – protein protocol not pursued by others
  - Propose to develop for human trials
- Big consortia necessary-different teams provide needed expertise
  - Although DNA vaccination is pursued by many, fewer groups have the expertise to optimize all critical steps
- DNA/RNA vaccines and immunotherapies are technologies important for cancer applications

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