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

• July 4, 1981
• 26 additional young men, all gay, from SF, with KS

• 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


Crude Mortality Rates for Syphilis And AIDS
Global estimates for adults and children | 2009

<table>
<thead>
<tr>
<th>Category</th>
<th>Estimate</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>People living with HIV</td>
<td>33.3 million</td>
<td>[31.4 million – 35.3 million]</td>
</tr>
<tr>
<td>New HIV infections in 2009</td>
<td>2.6 million</td>
<td>[2.3 million – 2.8 million]</td>
</tr>
<tr>
<td>Deaths due to AIDS in 2009</td>
<td>1.8 million</td>
<td>[1.6 million – 2.1 million]</td>
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</table>
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.

Highly Active Antiretroviral Treatment launched.

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

UNAIDS is created.

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

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

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

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.
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
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

**ITT**

Est. VE = 26%  
(-4%, 48%)  
p = 0.08

**MITT**

Est. VE = 31%  
(1%, 51%)  
p = 0.04

**PP**

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

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

Log SIV Viral Load
WT
Attenuated SIV

Von Gegerfelt 2002, 2006, 2010
LASIV-infected Animals Control Mucosal Challenge by High Dose SIVmac251

- Rev-independent N=11
  - Long term (>7 years) control in 8 of 11 animals

- Naïve N=21

Graph showing Log Viral Load over weeks for LASIV and control groups.
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

Gag production

CMV

p17gag

pA

- 

M1

X

- 

M1 M2 M3 M4

XXXX

+ 

+Rev

+ 

RRE

- + - + - + 

Rev

p17 p17RRE p17M1234

p17gag
Posttranscriptional (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,
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 Jalal 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 Dragha-Akli e, Koen K. A. Van Rompay f, Barbara K. Felber b, 1, and George N. Pavlakis a, 1

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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 developed strong cellular and humoral responses against the DNA. 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 initial information to humans. DNA vaccination offers a
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

IFNγ⁺ SIV-specific T cells/10⁶ T cells

Weeks

0 2 8 10 16 18 20 26

EP1 EP2 EP3

gag  nef  vif  pol
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

8/8+ vaccinated MamuA*01+ macaques after 1 DNA vaccination

Lung (BAL)

Rectal Mucosa (biopsy)

Gag-specific T Cells Measured By Tetramer Staining
Induced Responses Are Long-lasting (~2yrs) And Are Rapidly Boosted

**Blood**

- **CD8 EM**
- **CD8 CM**
- **CD4 EM**
- **CD4 CM**

**CM** = Central Memory  
**EM** = Effector Memory

**IFN**γ⁺ Gag-specific T cells/10⁶ T cells

**EP1**  
**EP2**  
**EP3**  
**EP4**  
**EP4 week2**

- **AZ09**  
- **C0102**  
- **FB1**

90 weeks
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

DNA v3w2 -/+IL12 p=0.0047

NAb Titer 50%

DNA

+IL12
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)

<table>
<thead>
<tr>
<th>Vaccine:</th>
<th>PENNVAX-B DNA</th>
<th>PENNVAX-B DNA + GENEVAX IL-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 vaccinations:</td>
<td>30.0% (3 out of 10)</td>
<td>71.4% (20 out of 28) p=0.03</td>
</tr>
<tr>
<td>3 vaccinations:</td>
<td>66.7% (6 out of 9)</td>
<td>90.9 % (20 out of 22)</td>
</tr>
</tbody>
</table>

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

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<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>16</td>
<td>36</td>
</tr>
<tr>
<td>8 wks</td>
<td>8 wks</td>
<td>20 wks</td>
<td>27 wks</td>
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</table>

Repeated Low Dose Mucosal Challenge with heterologous SIVsmE660 (83% aa identity in env)

14 weekly exposures

10 months of follow-up

4 vaccinations (SIVmac239)
<table>
<thead>
<tr>
<th>Group (N=8)</th>
<th>Vaccine</th>
<th>Vaccination 1: Wk 0</th>
<th>Vaccination 2: Wk 8</th>
<th>Vaccination 3: Wk 16</th>
<th>Vaccination 4: Wk 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA Only</td>
<td>DNA</td>
<td>DNA</td>
<td>DNA</td>
<td>DNA</td>
</tr>
<tr>
<td>2</td>
<td>DNA + Protein</td>
<td>DNA + AT-2 SIV</td>
<td>DNA + AT-2 SIV</td>
<td>DNA + AT-2 SIV</td>
<td>DNA + AT-2 SIV</td>
</tr>
<tr>
<td>3</td>
<td>DNA prime- protein Boost</td>
<td>DNA</td>
<td>DNA</td>
<td>AT-2 SIV</td>
<td>AT-2 SIV</td>
</tr>
<tr>
<td>4</td>
<td>Sham-Vaccinated</td>
<td>Sham DNA</td>
<td>Sham DNA</td>
<td>Sham DNA</td>
<td>Sham DNA</td>
</tr>
</tbody>
</table>

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

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

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

- **DNA Only**
- **DNA+Protein coimmunization**
- **DNA prime Protein Boost**
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
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

DNA                          Combination                    DNA prime/Prot Boost

![Graph showing the log NaB titer over time for DNA, Combination, and DNA prime/Prot Boost groups. The graph indicates that the combination vaccine increases the levels and longevity of neutralizing antibodies. The graph shows a significant increase in NaB titer levels after 5 months for the combination group compared to DNA and DNA prime/Prot Boost groups.](image)
Immunization with either DNA or DNA+AT-2 SIVmac239 Induces Neutralizing Antibody Titers to Heterologous “Tier 1a” and “Tier 2” SIVsmE660

<table>
<thead>
<tr>
<th>Challenge Day</th>
<th>Log Neutralization Ab Titers</th>
<th>4X DNA Only</th>
<th>4X DNA + AT-2 SIV</th>
<th>2X DNA prime 2X AT-2 SIV Boost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tier 1a</td>
<td>SIVsmE660/BR-CG7G.IR1</td>
<td>p=0.0207</td>
<td>p=0.0047</td>
<td></td>
</tr>
<tr>
<td>Tier 2</td>
<td>SIVsmE660/BR-CG7V.IR</td>
<td>p=0.0104</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mann Whitney two-tailed t test
Avidity of bAb to E660 env (gp120) correlates with challenge virus acquisition

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

\[ p = 0.0039 \]
\[ R \text{ squared} = 0.3211 \]
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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