

Novel Adenovirus Vector-Based Vaccines for HIV-1

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Pre-Existing Ad5 Vector-Specific Immunity

- **High prevalence of pre-existing immunity to Ad5 in human populations, particularly in the developing world**
- **Major limitation of current rAd5 vector-based vaccines**
- **Ad5 Seroprevalence:**
 - **U.S., Western Europe: 30-50% (low to moderate titers)**
 - **Sub-Saharan Africa, Southeast Asia: 80-95% (high titers)**
- **Blunts rAd5 vaccine immunogenicity in both preclinical and clinical studies and may limit their clinical utility**

Novel Adenovirus Vectors for HIV-1: Potential Applications

- **To replace Ad5 vectors in regions of the world where pre-existing anti-Ad5 immunity is limiting**
- **To be used together with Ad5 vectors or other vectors in heterologous prime-boost regimens**

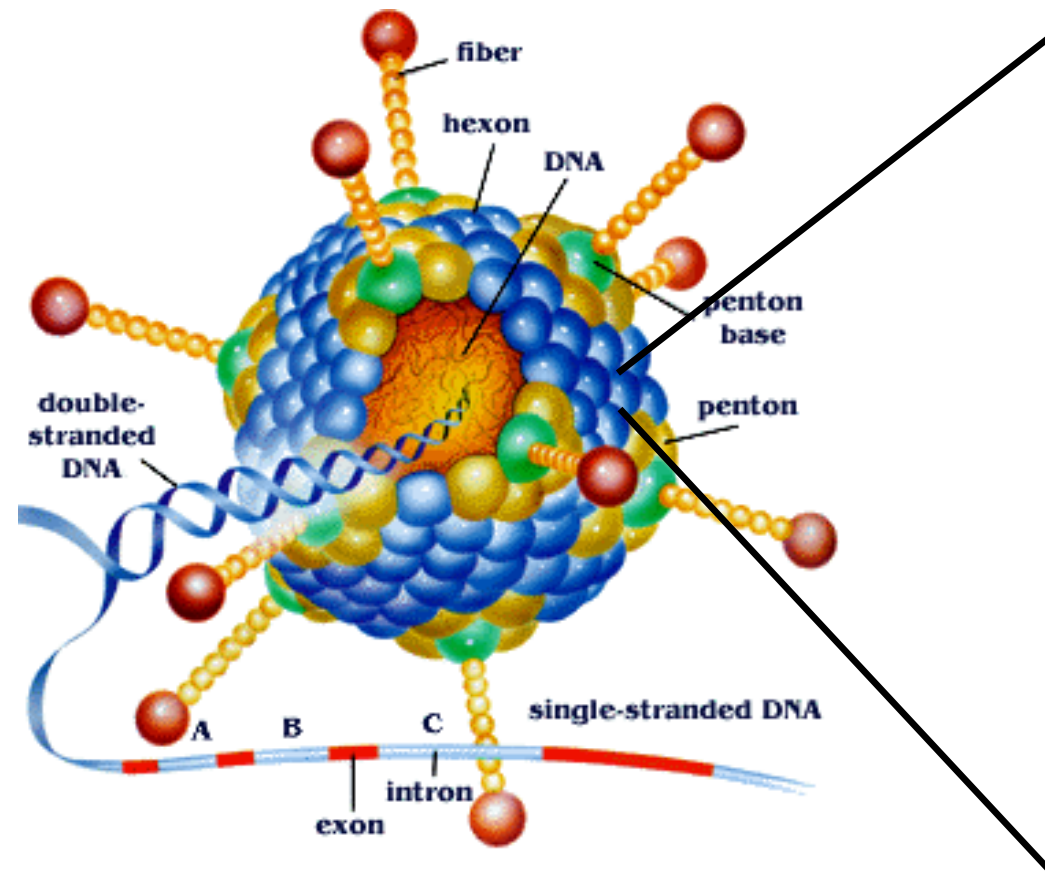
Development of Novel Adenovirus Vectors

- **Goal is to develop novel rAd vectors with:**
 - Immunogenicity of rAd5 vectors
 - Capacity to circumvent anti-Ad5 immunity
 - Large-scale manufacturability
- **Key strategies:**
 - Novel serotype rAd vectors
 - Novel chimeric rAd vectors

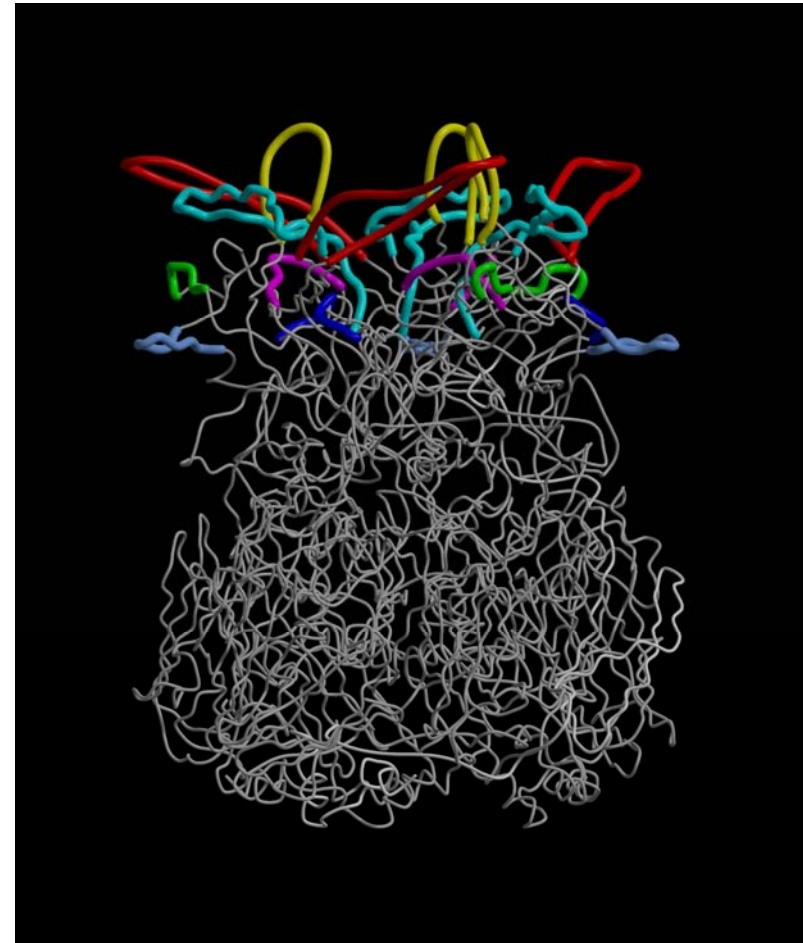
Comparative Evaluation of Six Novel Serotype rAd Vectors from Ad Subgroups B and D

- **Novel serotype rAd vectors evaluated:**
 - rAd11, rAd35, rAd50 (subgroup B)
 - rAd26, rAd48, rAd49 (subgroup D)
- **rAd26 selected as optimal rare serotype vector based on:**
 - Seroprevalence studies in U.S. and sub-Saharan Africa
 - Immunogenicity studies in mice and rhesus monkeys
 - Manufacturability studies

The 7 Hexon HVRs Form the Majority of the Solvent-Exposed Surface of the Ad5 Hexon Capsid Protein

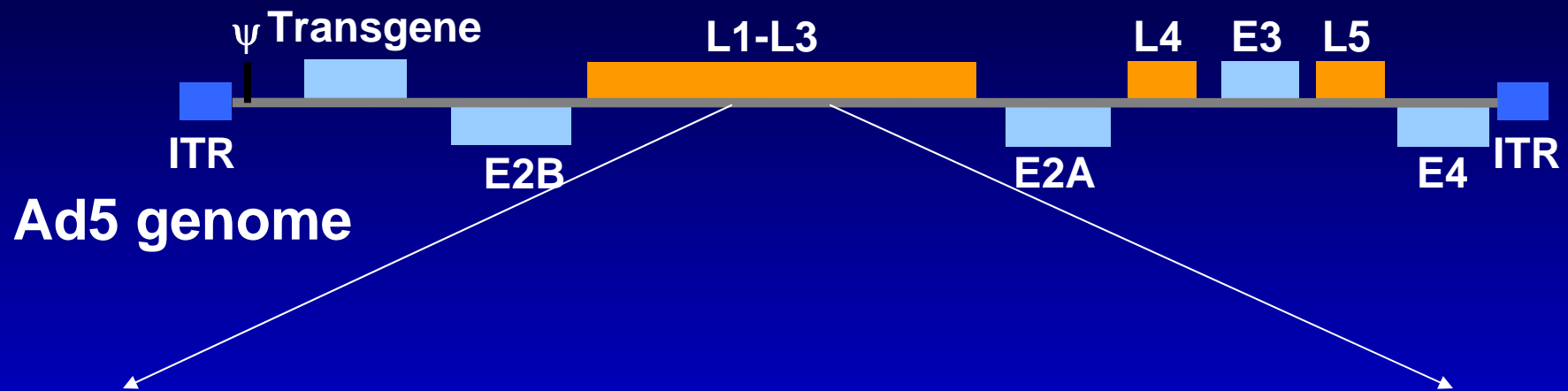


**Schematic Ad5 Particle
Hexons Shown in Blue**



**Hexon Protein Trimer
HVRs Shown in Color**

Hexon HVR-Chimeric rAd5 Vectors



Ad5 genome

Ad5 Hexon



Ad5HVR48(1) Hexon



Ad5HVR48(1-7) Hexon

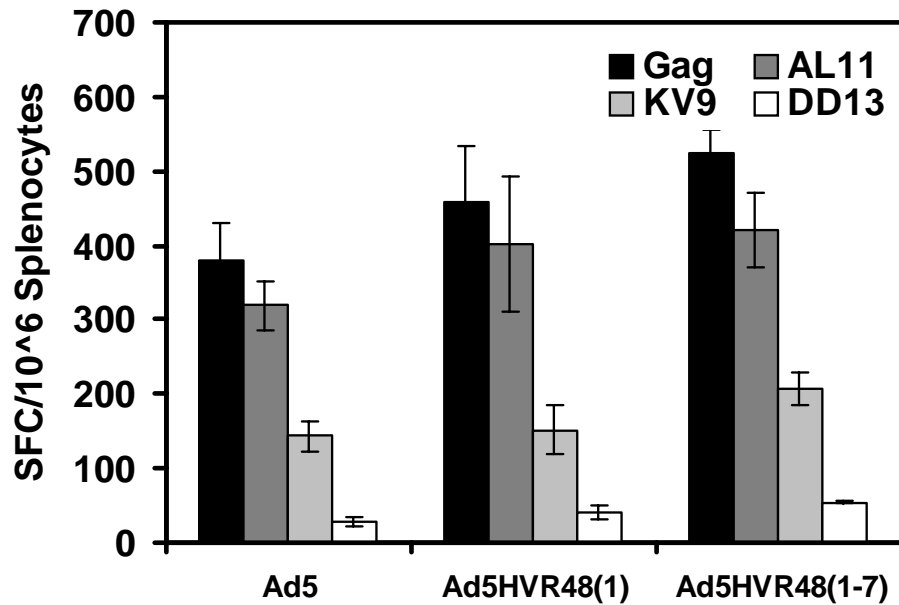


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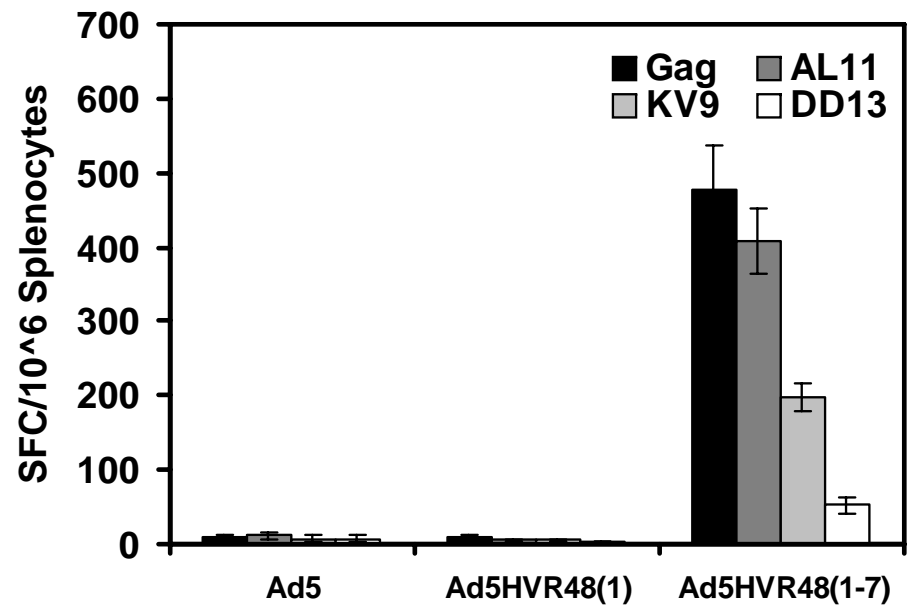
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Chimeric rAd5HVR48(1-7)-Gag Vector Effectively Circumvents Anti-Ad5 Immunity in C57BL/6 Mice

Naive



Anti-Ad5 Immunity

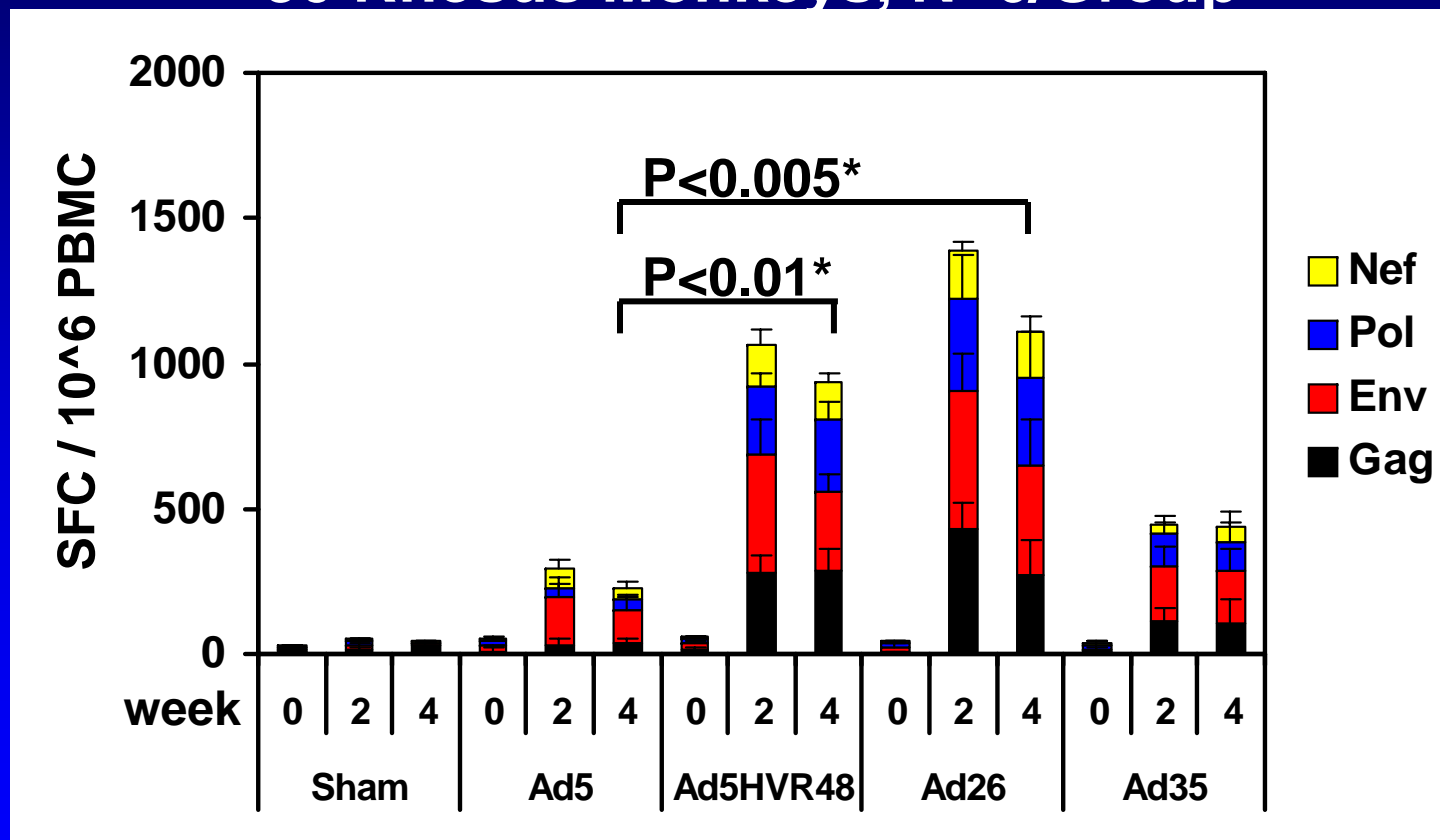


Comparative Assessment of Novel rAd Vectors in Rhesus Monkeys with Anti-Ad5 Immunity

- 30 rhesus monkeys pre-immunized with 2 injections of 10^{11} vp rAd5-Empty (median Ad5 NAb titers 16,384)
- Single injection of 10^{10} vp of the following rAd vectors expressing SIV Gag, Env, Pol, Nef (N=6/group):
 - 1) Sham
 - 2) rAd5
 - 3) rAd5HVR48
 - 4) rAd26
 - 5) rAd35

Novel rAd26 and rAd5HVR48 Vectors Elicit 5-Fold More Potent Cellular Immune Responses rAd5 Vectors in Rhesus Monkeys with Anti-Ad5 Immunity

30 Rhesus Monkeys, N=6/Group



* two-tailed Wilcoxon rank-sum test

Novel Serotype/Chimeric rAd Vectors for HIV-1: rAd26 and rAd5HVR48

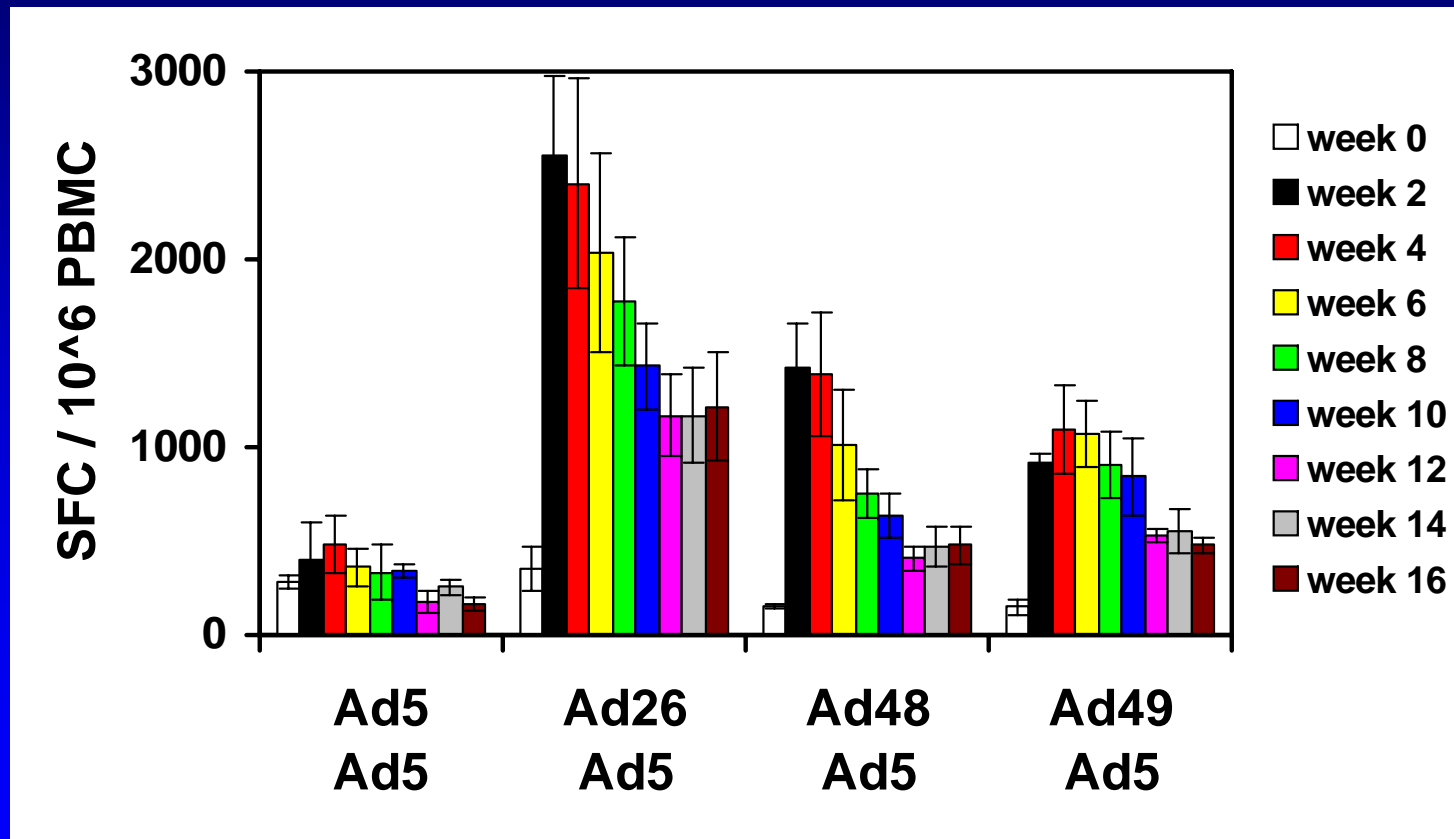
- **Significantly 5-fold more immunogenic than rAd5 vectors in rhesus monkeys with anti-Ad5 immunity**
- **More potent than five other rare serotype rAd vectors tested (Ad11, Ad35, Ad50, Ad48, Ad49)**
- **Immunogenic both as single modality vaccines and in the context of heterologous prime-boost regimens**
- **Elicit polyfunctional CD8+ and CD4+ T cell responses**
- **Generate both mucosal and systemic central memory T cell responses following intramuscular immunization**

Heterologous rAd Prime-Boost Regimens

- **DNA/rAd5 prime-boost regimens more immunogenic than rAd5 alone regimens but logistically complex**
- **Heterologous rAd/rAd prime-boost regimens using two serologically distinct rAd vectors offer a potentially more practical alternative**
- **Immunogenicity study in rhesus monkeys to compare rAd5/rAd5 with heterologous rAd/rAd prime-boost regimens**

8-Fold Greater Immunogenicity of rAd26/rAd5 vs rAd5/rAd5 Vaccine Regimens in Rhesus Monkeys

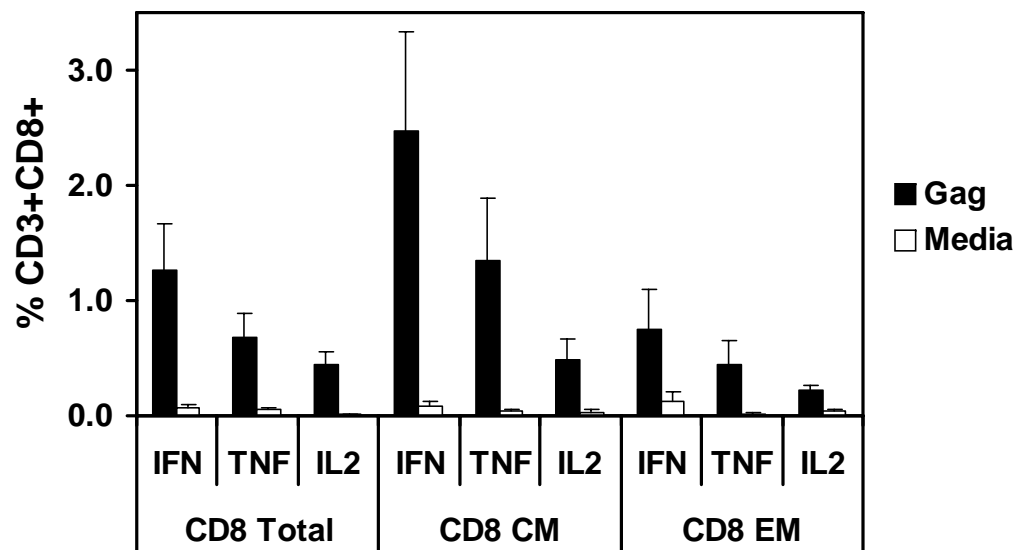
Optimal Regimen: rAd26 Prime, rAd5 Boost



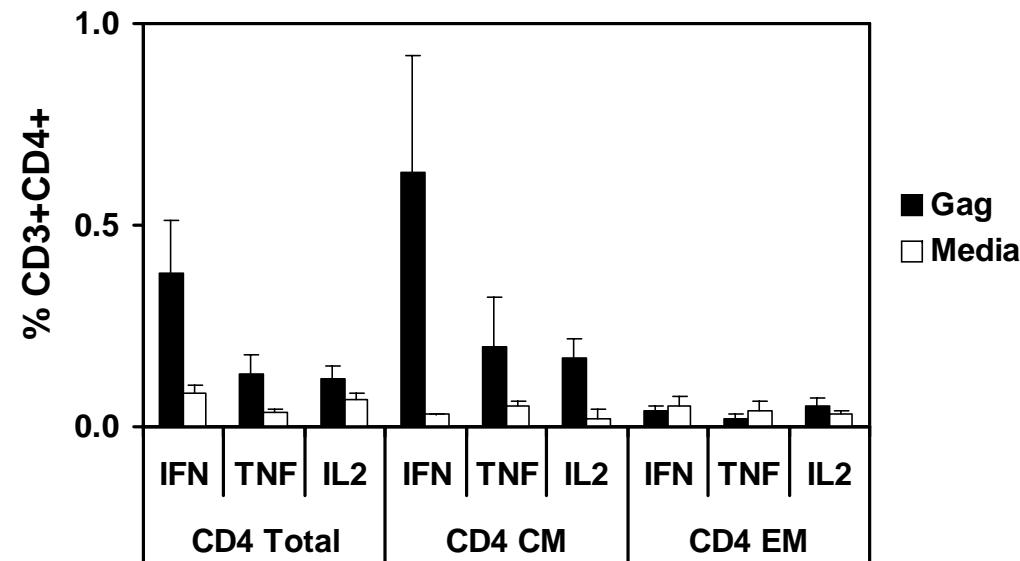
IFN- γ ELISPOT responses following boost immunization

Cytokine Secretion Profiles of Gag-Specific CD8 and CD4 T Lymphocyte Responses Elicited by Optimal rAd26/rAd5 Regimen in Rhesus Monkeys

CD8 Responses



CD4 Responses



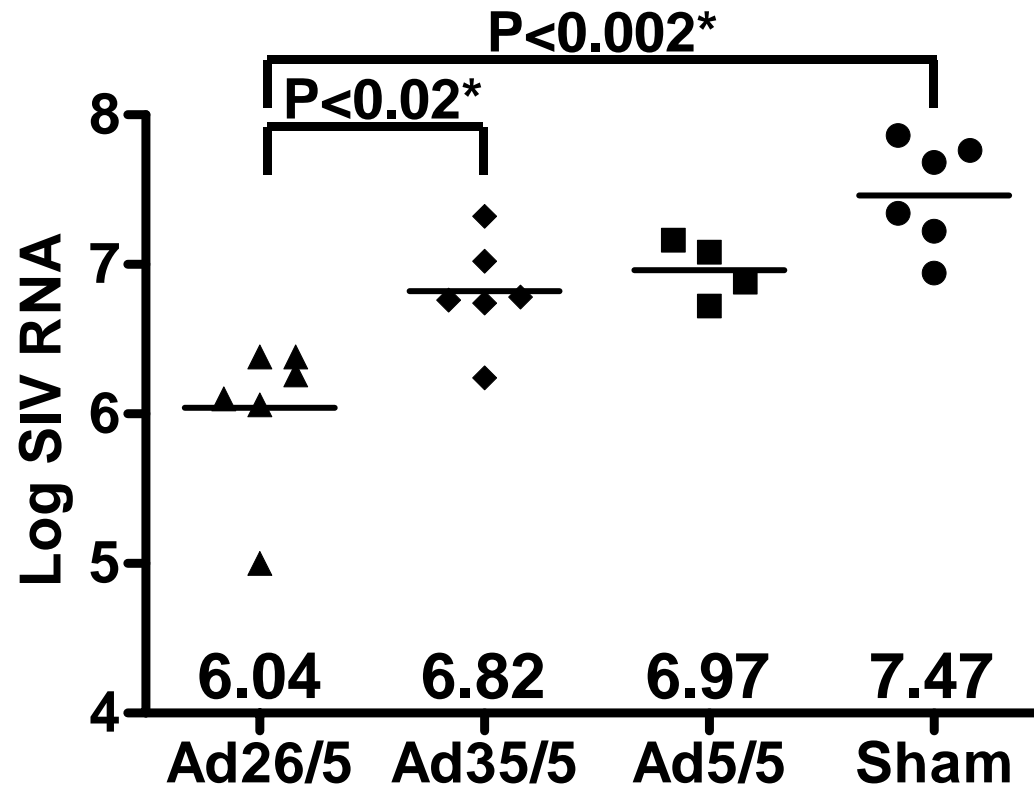
ICS responses at week 4 following boost immunization

Protective Efficacy of Heterologous rAd Prime-Boost Regimens Against SIVmac251 in Rhesus Monkeys

- Rhesus monkeys immunized with various rAd regimens expressing the single SIV Gag antigen:
 - rAd26-Gag prime, rAd5-Gag boost (N=6)
 - rAd35-Gag prime, rAd5-Gag boost (N=6)
 - rAd5-Gag prime, rAd5-Gag boost (N=4)
 - Sham (N=6)
- High-dose i.v. SIVmac251 challenge (provided by Norman Letvin) 6 months following the boost immunization
- Highly stringent challenge model:
 - Merck rAd5-Gag did not suppress SIV RNA post-challenge
 - 0.8-1.1 log decrease in peak SIV RNA observed with multiple vaccine antigens and DNA priming

Significant 1.4 Log Decrease in Peak SIV RNA Levels in rAd26/rAd5 Vaccinated Monkeys Post-Challenge

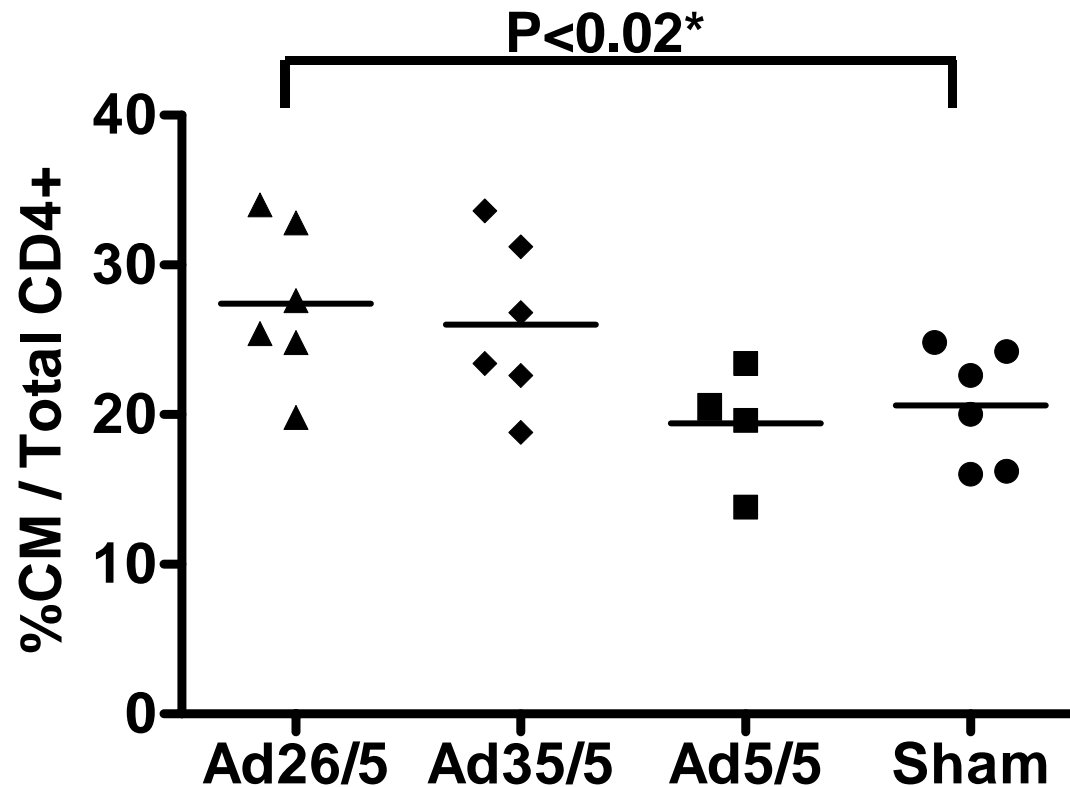
SIV RNA Levels; Day 14 Post-Challenge



* two-tailed Wilcoxon rank-sum test

Protection Correlated with Preservation of Central Memory CD4+ T Lymphocytes

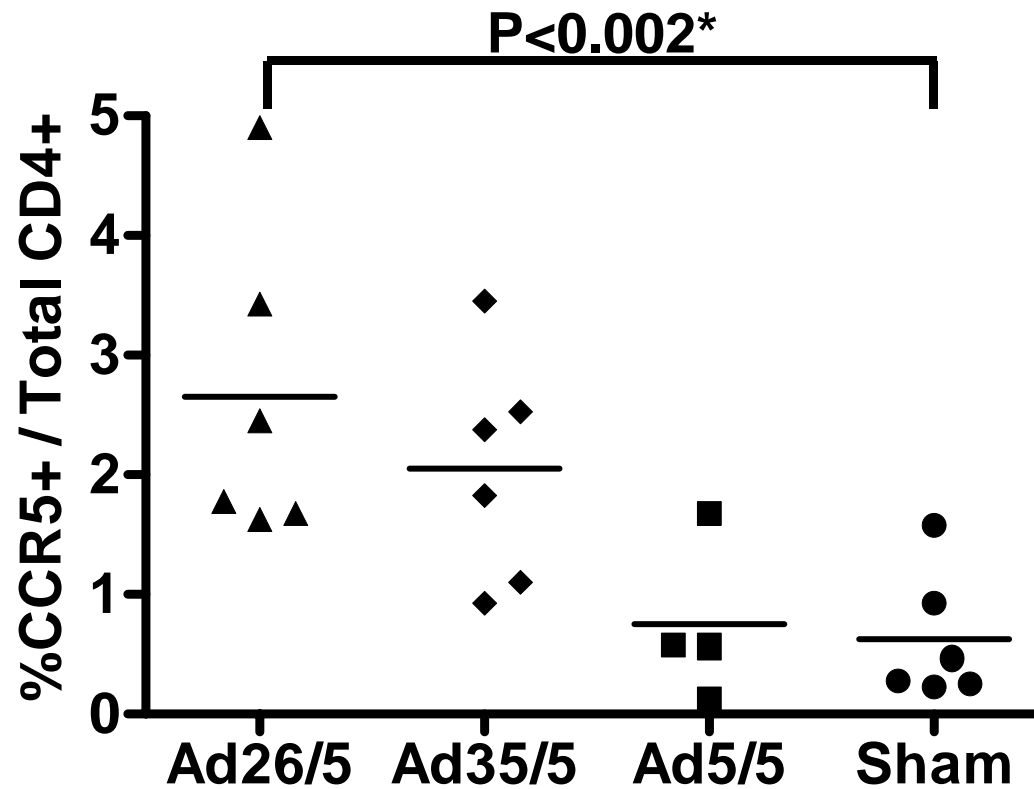
CD28+CD95+CD4+/CD4+ Cells; Day 14 Post-Challenge



* two-tailed Wilcoxon rank-sum test

Protection Correlated with Preservation of CCR5+CD4+ T Lymphocytes

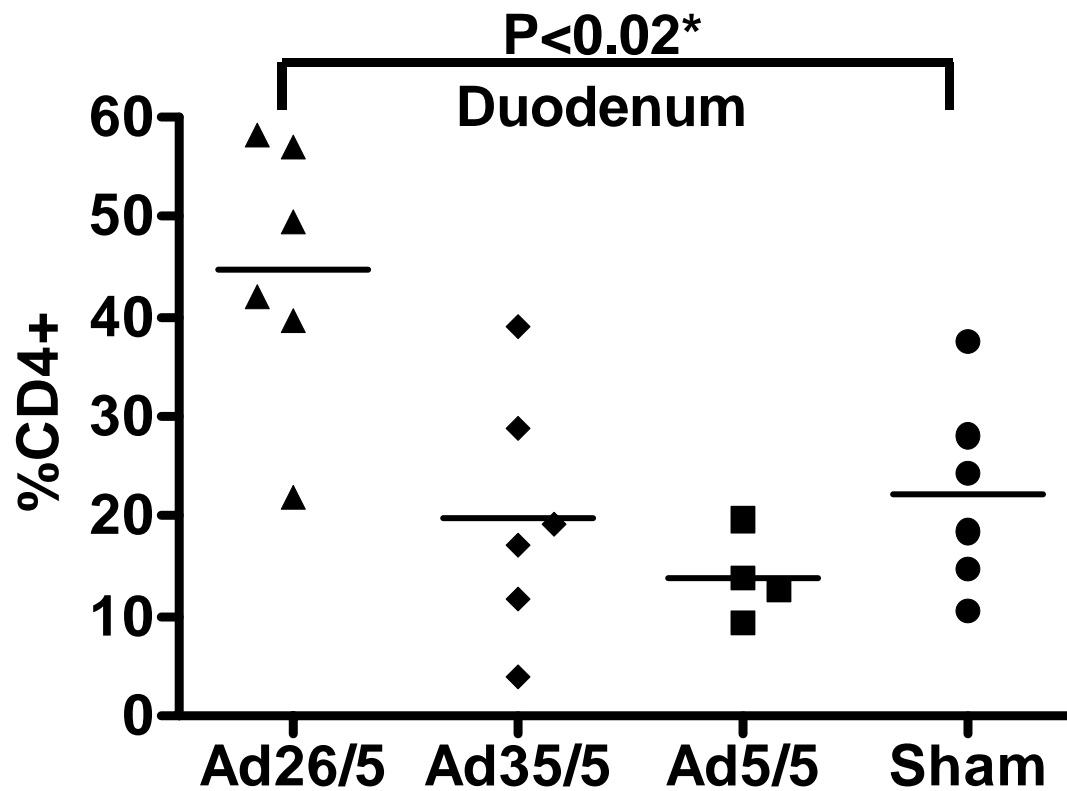
CCR5+CD4+/CD4+ Cells; Day 14 Post-Challenge



* two-tailed Wilcoxon rank-sum test

Protection Correlated with Preservation of Duodenal Memory CD4+ T Lymphocytes

CD4+/CD3+ Cells; Day 21 Post-Challenge



* two-tailed Wilcoxon rank-sum test

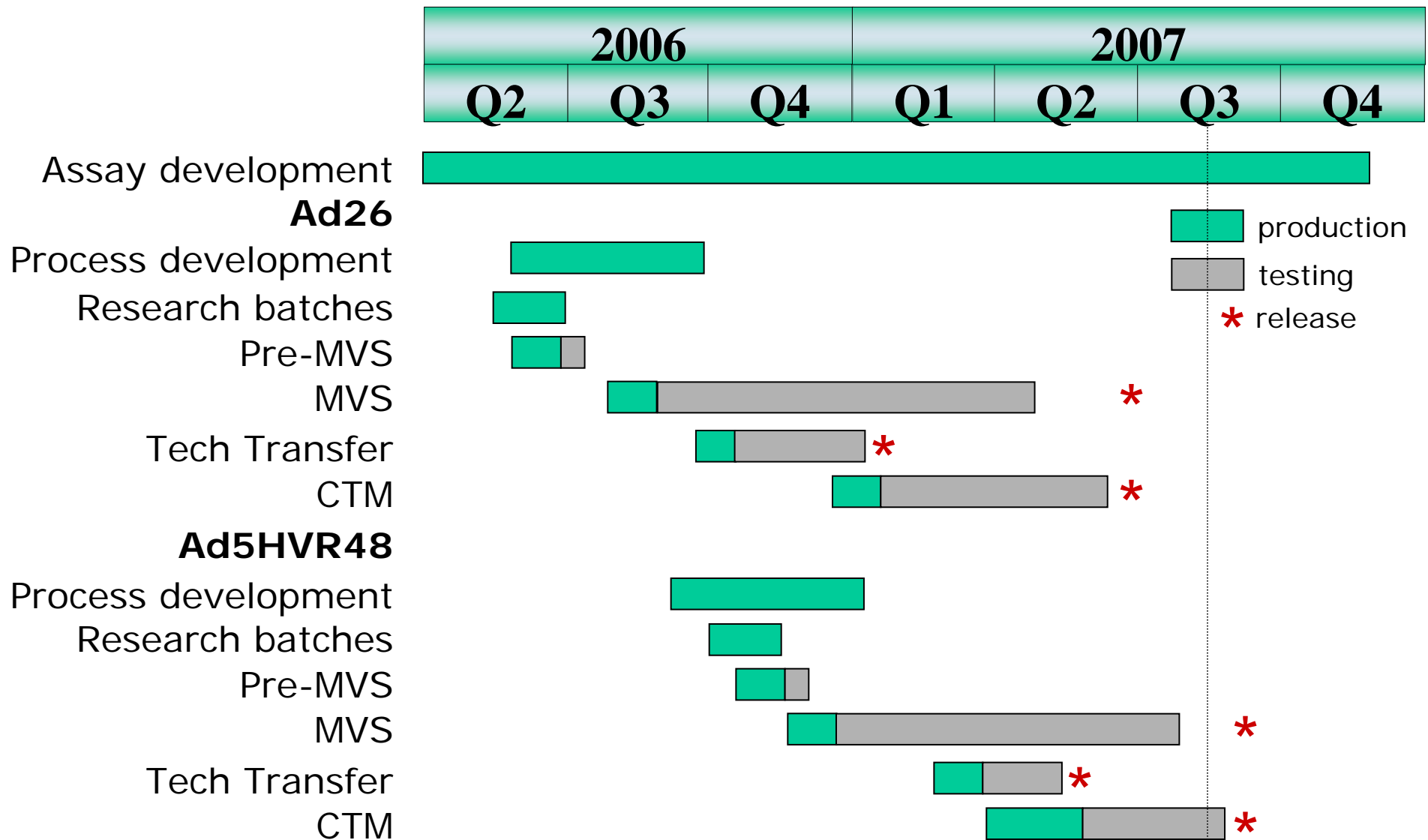
Protective Efficacy of Heterologous rAd Prime-Boost Regimens Against SIVmac251 in Rhesus Monkeys

- Results preliminary and restricted to acute infection; long-term follow-up data not yet available
- Protective efficacy correlates with immunogenicity:
 - rAd26/rAd5 > rAd35/rAd5 > rAd5/rAd5 > Sham
- Demonstrates potential of heterologous rAd prime-boost regimens, particularly rAd26/rAd5 regimen
- Protection correlated with preservation of CCR5+CD4+ T cells and duodenal memory CD4+ cells, as well as total central memory CD4+ cells

NIAID Integrated Preclinical/Clinical AIDS Vaccine Development (IPCAVD) Program

- **Vectors selected for advancement into clinical trials:**
 - **Ad26 - optimal rare serotype Ad vector**
 - **Ad5HVR48 - optimal chimeric Ad vector**
- **Antigen selected for phase I studies of prototype vectors:**
 - **Clade A HIV-1 Env gp140 (Gary Nabel, VRC, NIH)**
 - **Plans for a complete multivalent vaccine in progress**
- **HER.96 cells selected for GMP manufacturing (Crucell, IAVI)**

Manufacturing Clinical-Grade rAd26 and rAd5HVR48 Expressing Clade A HIV-1 Env gp140 (Crucell)



Current Timeline to Clinical Trials

- **Ad26.ENVA.01**
 - ✓ Pre-IND package reviewed by FDA (Q4 2006)
 - ✓ GMP clinical trial material manufactured (Q1 2007)
 - ✓ GLP toxicology studies completed (Q2 2007)
 - IND submission planned (Q3 2007)
 - Phase I study planned (Q4 2007)
- **Ad5HVR48.ENVA.01**
 - ✓ Pre-IND package reviewed by FDA (Q1 2007)
 - ✓ GMP clinical trial material manufactured (Q2 2007)
 - ✓ GLP toxicology studies in progress (Q2-Q3 2007)
 - IND submission planned (Q4 2007)
 - Phase I study planned (Q1 2008)

Phase I Study of Ad26.ENVA.01

Group	Number	Dose	Injection schedule in months (days)		
			0 (0)	1 (28)	6 (168)
1	10	10^9	rAd26	rAd26	rAd26
	2		FFB	FFB	FFB
2	10	10^{10}	rAd26	rAd26	rAd26
	2		FFB	FFB	FFB
3	10	10^{11}	rAd26	rAd26	rAd26
	2		FFB	FFB	FFB
4	10	10^*	rAd26		rAd26
	2		FFB		FFB
Total	48 (40/8)				

Conclusions

- **rAd5 vectors may be limited by high levels of anti-Ad5 immunity in the developing world**
- **Novel serotype/chimeric rAd vectors can be constructed with:**
 - **Immunogenicity of rAd5 vectors**
 - **Capacity to circumvent anti-Ad5 immunity**
 - **Large-scale manufacturability**
- **We propose that novel rAd vectors should be advanced into clinical trials as candidate HIV-1 vaccines:**
 - **To replace rAd5 vectors as single modality vaccines**
 - **To be used with rAd5 vectors in prime-boost regimens**

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