Vaccins anti-VIH: un défi!

Vaccins anti VIH : un développement complexe
….et extraordinairement difficile!

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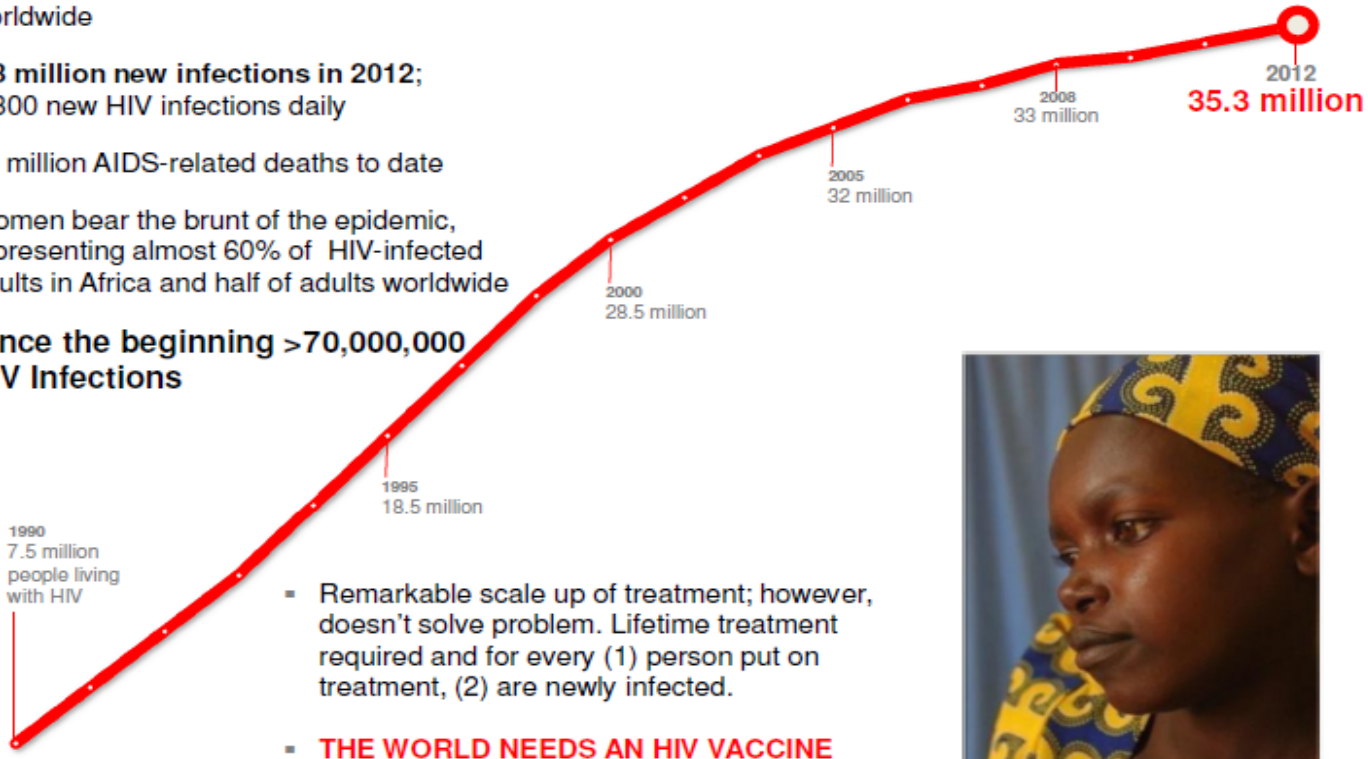
HIV continues to devastate….

- **35.3 million** people living with HIV worldwide
- **2.3 million new infections in 2012**; 6,300 new HIV infections daily
- 36 million AIDS-related deaths to date
- Women bear the brunt of the epidemic, representing almost 60% of HIV-infected adults in Africa and half of adults worldwide
- **Since the beginning >70,000,000 HIV Infections**



- Remarkable scale up of treatment; however, doesn't solve problem. Lifetime treatment required and for every (1) person put on treatment, (2) are newly infected.
- **THE WORLD NEEDS AN HIV VACCINE**

Source: Joint United Nations Programme on HIV/AIDS

avi

Quelques chiffres

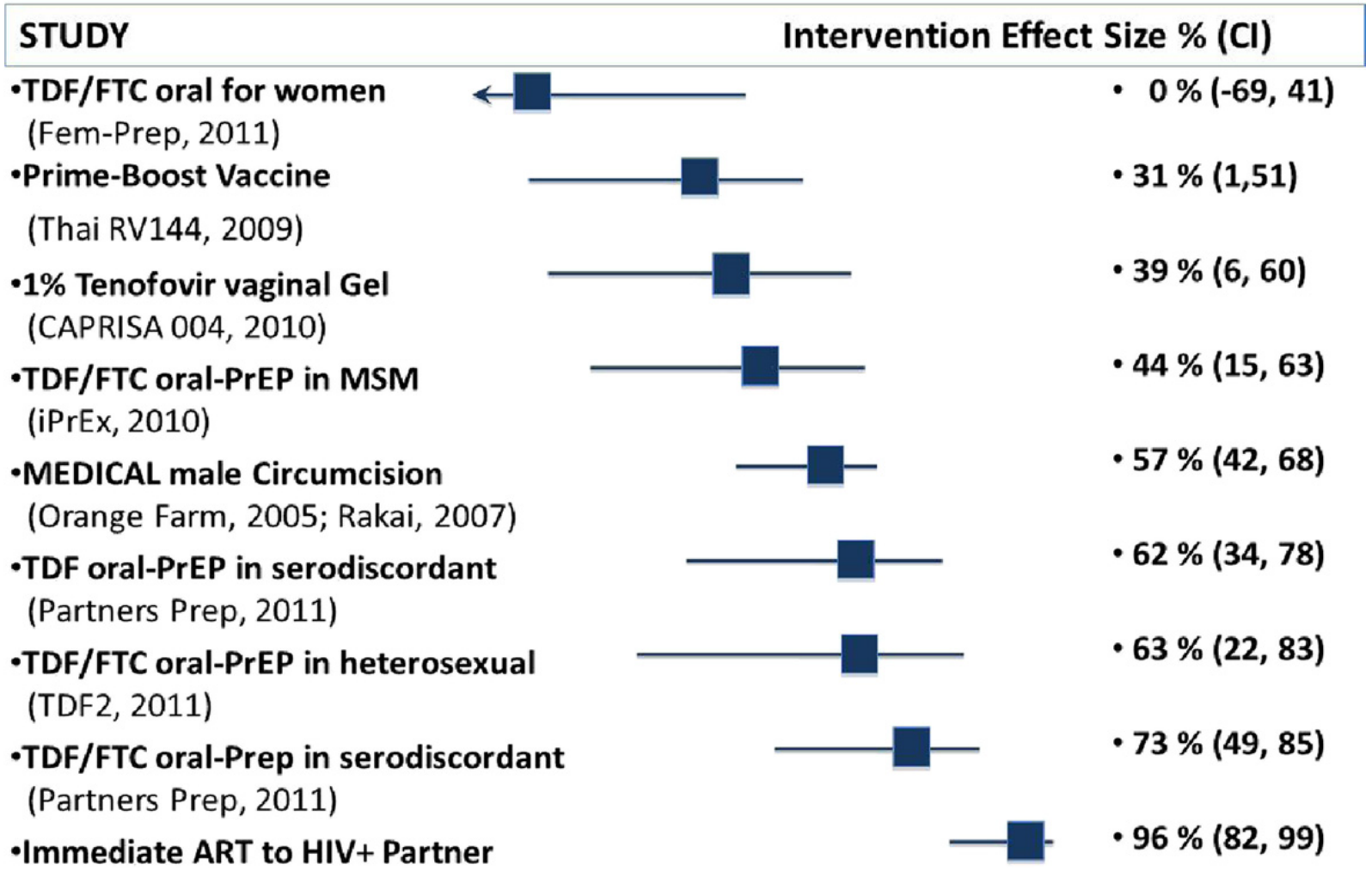
- On a dénombré, en **2016**, **une nouvelle infection HIV-1 dans le monde toutes les 17 secondes**, soit **5000 nouvelles infections par jour**

  → **1,8 millions de nouvelles infections dans l'année**

- **En 2016 aussi, on dénombrait 36,7 millions de personnes vivant avec le VIH dans le monde.**
  Mais seules 19,5 millions d'entre elles avaient accès à un traitement antirétroviral (ART)

- **La mortalité due au SIDA a été de 1 million de personnes en 2016**
  (Elle était de plus de 2 millions de personnes/an au début de la décennie)

WHAT WORKS in HIV Prevention



Why don’t we have a vaccine against HIV?

- No-one has ever recovered from HIV infection
- HIV is a rapidly moving target
- Diversity of mechanisms of transmission
- HIV integrates into human DNA
- It is difficult to neutralize HIV (complex surface envelope glycoprotein)
- Current vaccines are unable to stimulate broadly neutralizing antibodies

Le VIH échappe aux défenses immunitaires de l'hôte

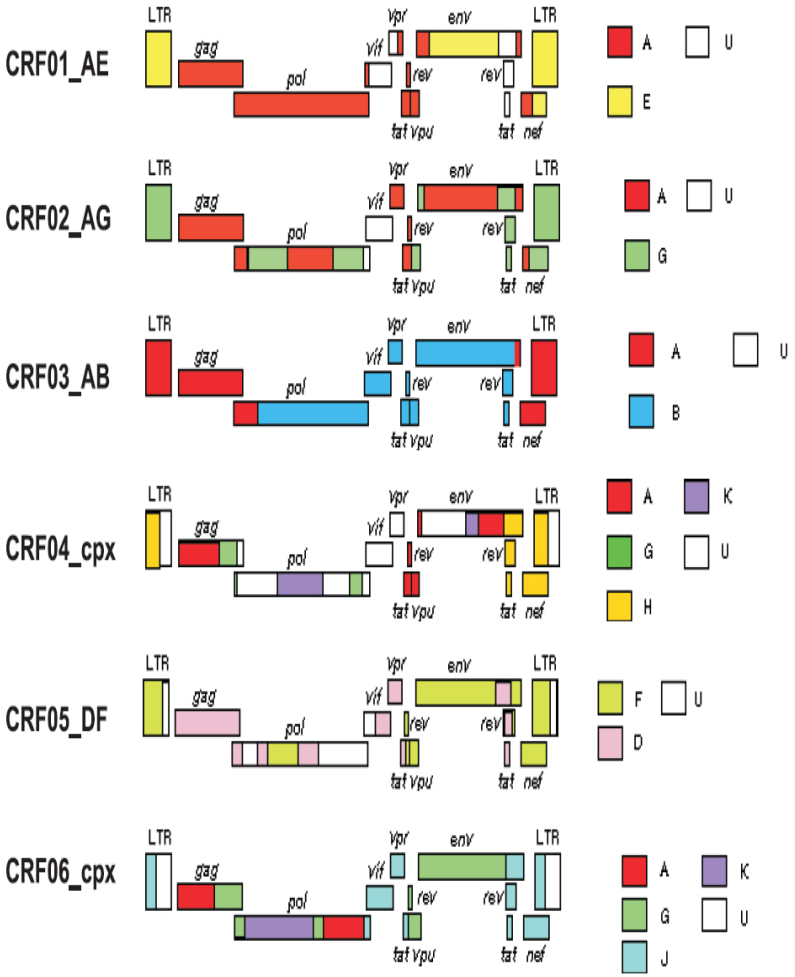
- Le virus a développé plusieurs stratégies d'échappement :
  - Il **persiste à l'état masqué** (« provirus latent») dans des « cellules réservoir » (**les lymphocytes T mémoire**)
  - Il **mute constamment**, échappant ainsi aux anticorps et aux CTL
  - Il génère **une hyperstimulation du système immunitaire**, qui conduit à l'épuisement de ce dernier, sans parler de la destruction des lymphocytes T4 dans lesquels il se réplique
  - **Il provoque l'effacement des marqueurs (HLA) de surface** de la cellule infectée, lui permettant ainsi d'échapper à la surveillance par les CTL

Diversité et variabilité du VIH-1

Quasiespèces
Chez un même patient

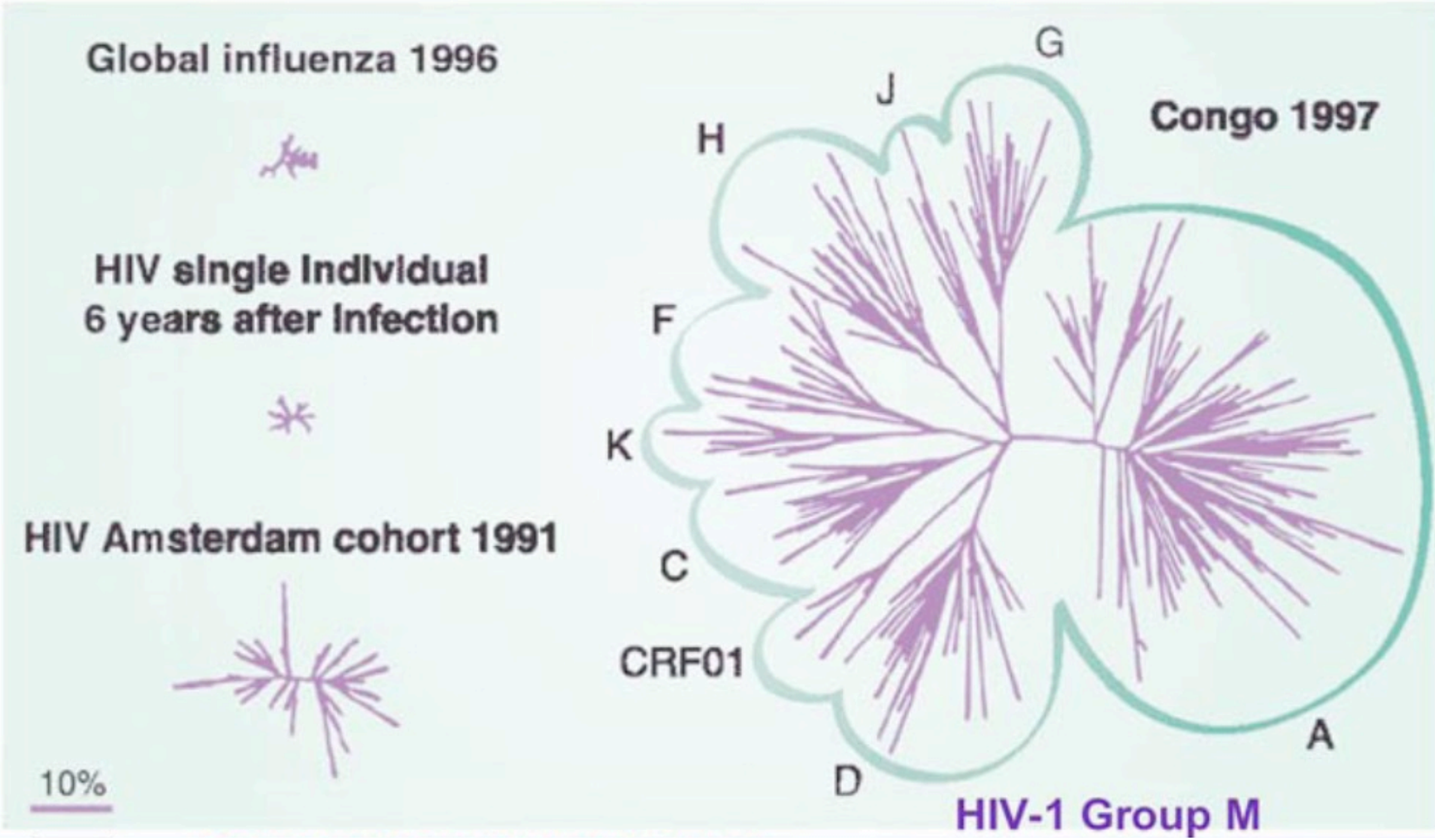
(Troyer et al, 2005)

Recombinaison



(Lal et al, 2005)

Diversité et variabilité du VIH-1 au sein d'une population

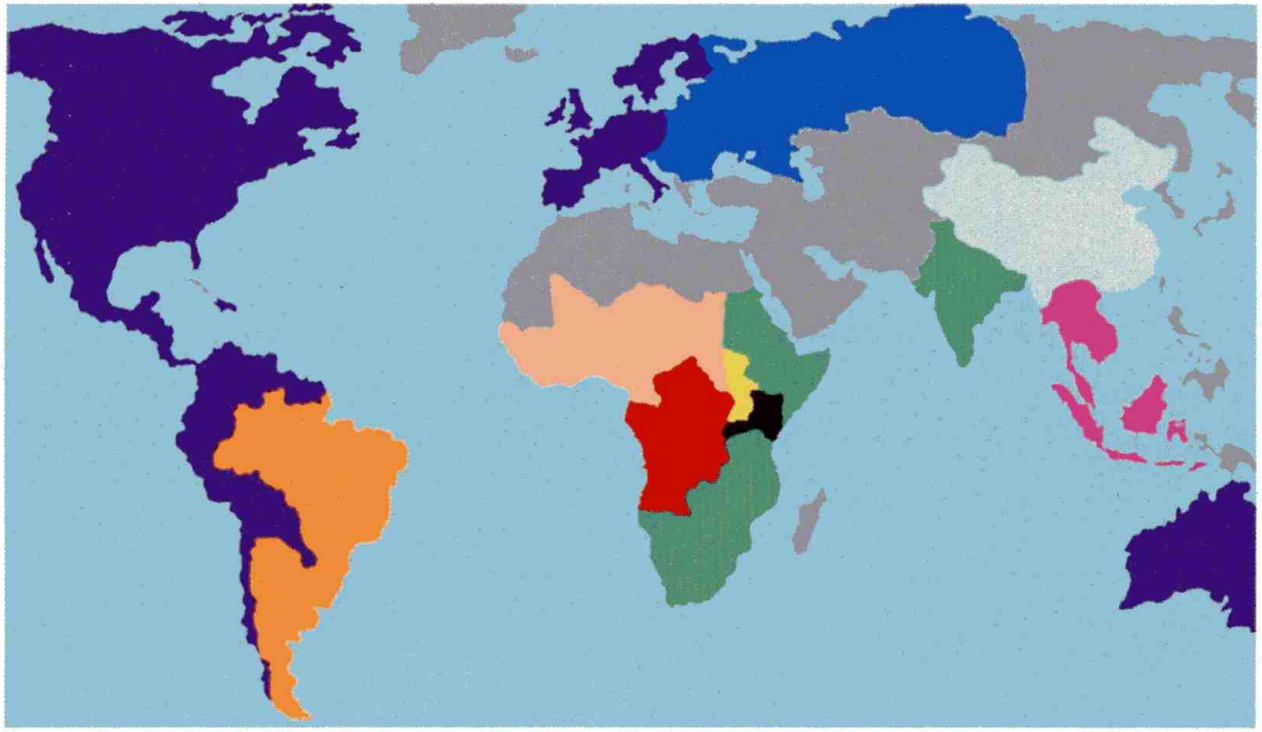


HIV-1 Variability

- HIV-1 is subdivided into **4 groups**: **M** (pandemic), **N** and **O,** the three of which are related to **SIV cpz, and** group **P**, recently identified, which is related to **SIV gor** **(Plantier, Nat Med 2009)**;

- **Group M , which is the most prevalent worldwide,** can be subdivided into **10 subtypes**, or **clades** (**A, B, C, D,…**), and a variety of « **recombinant forms** » or « **CRFs** » **(CRF01_AE in SE Asia; CRF02_AG in West Africa; CRF07 and 08_BC in China…**)

- Amino acid sequence of the Env glycoprotein shows **25-35% divergence between clades** in group M and **up to 20% divergence between isolates from the same clade**: **a formidable challenge to vaccine development!**



Global distribution of HIV-1 subtypes and recombinants. Ten different epidemic patterns have been observed, as indicated by the different colours. Data from: Global distribution of HIV-1 subtypes and recombinants. New York: IAVI, 2003.

Table 1. Antibody neutralization titers (subject TN-1, treatment naive) (Richman DD et al, PNAS April 03)

Virus, months	Plasma, months								
	0	3	6	9	12	15	18	21	25
0	**26**	219	675	1403	2670	2089	2190	2363	2411
3	29	**179**	1024	2151	3733	3152	2808	2953	3086
6	27	35	**78**	358	1769	1939	2247	3112	4345
9	36	67	82	**200**	795	1078	1371	2208	3375
12	19	48	36	64	**76**	166	556	937	1407
15	29	43	64	76	90	**119**	374	721	1234
18	42	65	61	152	117	134	**122**	289	526
21	41	66	82	84	85	113	78	**107**	296
25	42	62	56	62	85	77	55	61	**95**
Controls									
NL43	17	138	294	956	1172	953	1584	1868	2143
JRCSF	24	37	35	60	87	97	105	152	209
AMPHO	<10	32	14	13	14	13	<10	<10	31

Neutralizing HIV antibody titers of sequential plasma specimens against autologous virus. Serial plasmas were obtained from three untreated patients presenting with primary HIV infection. The titer of each plasma against its concurrent virus specimen is in bold type. Control viruses include an amphotropic murine leukemia virus (AMPHO), a neutralization-sensitive X4-tropic virus (NL4-3), and a relatively neutralization-resistant R5-tropic virus (JR-CSF).

Autre difficulté: les modèles animaux sont imparfaits

- **The chimp model**: HIV-1 ‘ takes’ in chimpanzees → the animals seroconvert and remain viremic b**ut do not develop any sign of immunodeficiency!**
- **The macaque model**: **HIV-1 does not infect macaques**. Hence the need to either **develop SIV vaccines** and use **the SIV / macaque model**; or to **develop HIV-SIV chimeric viruses** (« **SHIVs** ») which grow in rhesus macaque monkeys and carry the enveloppe spike of HIV-1 (and its antigenicity) in a SIV genetic backbone
- **The humanized mouse model**: knock-out mice devoid of a mouse immune system but grafted with human cells from the bone marrow, liver and thymus from human embryos (« **BLT mice** »)

« **Mice lie and monkeys exagerate** »→ **« ALL MODELS ARE WRONG BUT MOST ARE USEFUL! »**

Obvious consequence

- **The only way to test an HIV vaccine is in human volunteers :**

→ Phase III (or Phase IIb) clinical trials

But this implies:

- **To select a population at risk (drugs users, prostitutes, gays)**
- **To determine the infection incidence (>1%/an)**
- **To determine the number of volunteers required (16,000 in the case of the RV144 trial)**
- **To determine the duration of the study (4.5 years for RV144)**
- **To recruit the appropriate volunteers**
- **Consequences: To develop a HIV Vaccine is a major endeavior, a long-lasting, complex, difficult, and very expensive process.**

Les essais vaccinaux de Phase III depuis 1983

- 1/ **Vaxgen** (Etudes **Vax 003 et Vax 004**) **à base de gp120**:

  → 0 protection
- 2/ **Merck (**Etude **STEP**) **à base d'Ad5 recombinant**:

  → 0 protection
- 3/ **NIH (**Etude **HVTN 505**) **à base de DNA + Ad5 recombinant**:

  → 0 protection
- 4/ **Sanofi (**Etude **RV144) à base de canarypox recombinant (ALVAC) + gp120**: → **protection 31%**
- 5/ Plusieurs nouveaux essais ont été lancés en 2016-17; d'autres vont être lancés en 2018.

The quest for an AIDS vaccine started with Env vaccines (Induction of NAbs)

Early 1990s: recombinant gp120 or gp140 env subunit vaccines alone or in association with V3 peptides were shown to protect chimpanzees against homologous HIV-1 challenge using an « X4 » (TCLA) virus strain (Berman et al, Nature 1990; Fultz et al, Science 1992)

- They could not, however, protect the animals against heterologous challenge (HIV-1 DH2; CRF A-E vs clade B) because of the restricted specificity of the neutralizing antibodies elicited by the vaccines

  (Girard et al, J Virol 1995 and 1996; Mascola et al, J Infect Dis 1996).

First Phase III clinical trials

- **In spite of the limited protection observed in chimpanzees, VaxGen decided to test the concept of gp120 vaccines in two Phase III trials:**
  - **Vax003 in the Americas with a mixture of two gp120 clade B**
  - **Vax004 in Thailand with a mixture of gp120 clade B and CRF A-E**

    (both with alum as an adjuvant)

Both trials showed gp120 was unable to provide protection against HIV infection

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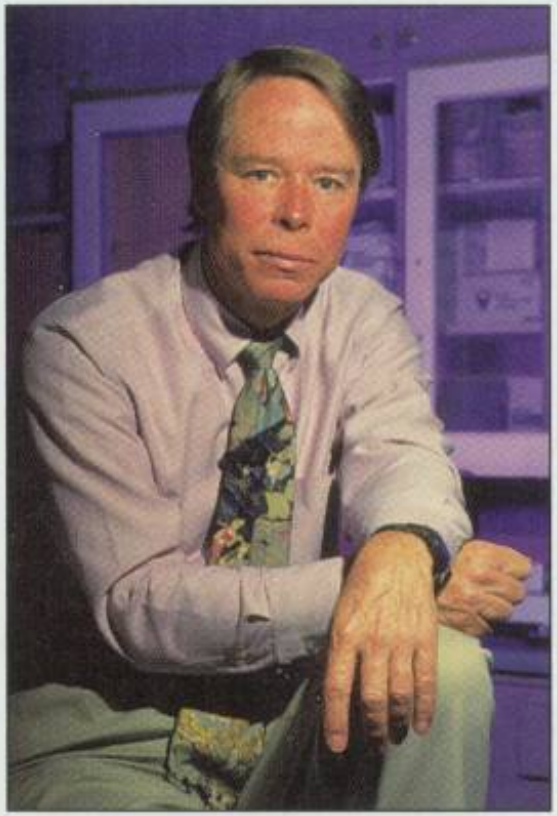

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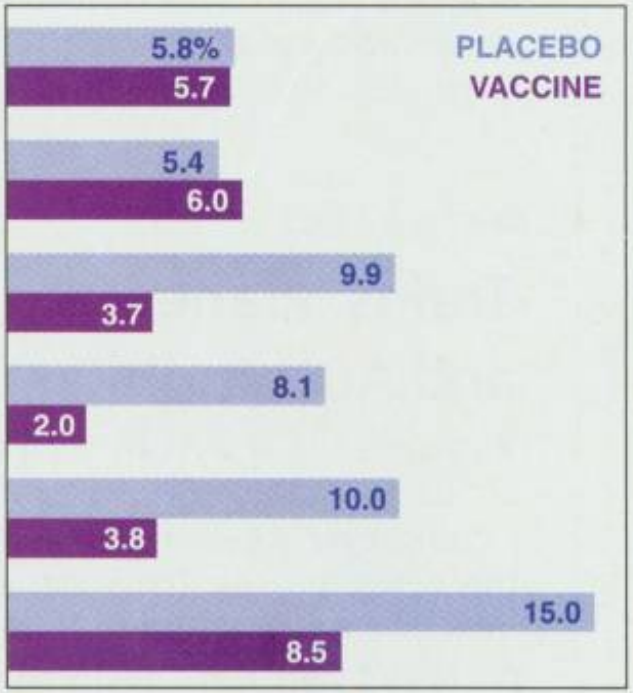
The trial, which took place in the United States, Puerto Rico, Canada, and the Netherlands, predominantly involved gay men at high risk of becoming infected. The researchers reported that 5.7% of the vaccinated group and 5.8% of those who received the placebo became infected with HIV, strongly indicating that the vaccine offered no benefit whatever. But VaxGen CEO Lance Gordon argued in general. "Subset analyses are notoriously difficult to interpret, and they're doubly difficult when the overall result is nil, which is the case here," says Self.

Seth Berkley, head of the International AIDS Vaccine Initiative, a nonprofit organization that bankrolls development of products, is more blunt. He notes that VaxGen's subanalysis hinged on "just 13 infections"

	Total	Infected
All subjects	1679	98
	3330	191
White and Hispanic	1508	81
	3003	179
Black, Asian, and Other (combined)	171	17
	327	12
Black	111	9
	203	4
Asian	20	2
	53	2
Other minorities	40	6
	71	6



Black and white? Despite overall negative results, VaxGen's Donald Francis sees hope in the subgroup analyses.

ORBIS; SOURCE: VAXGEN

II. Could T cell responses be an alternative?…

Initial demonstration of the protective role of CD8+ T cells in the SIV / macaque model : depletion of CD8+ T cells by anti-CD8 Mabs in SIV-infected animals → immediate, large increase in virus load and accelerated disease progression leading to premature death (Schmitz, Science 1999; Letvin, Immunity 2007)

Immunization of macaques with attenuated live SHIV 89.6 or with attenuated SIV Δnef elicits protection against intravaginal SIV challenge.

Protected animals showed polyfunctional, degranulating, SIV-specific CD4+ and CD8+ T cells in the vaginal mucosa (Genescà, Mucosal Immunol 2008 and J Intern Med 2009)

Depletion of CD8+ T cells (by injection of an anti-CD8 Mab) completely abrogated protection → protection from vaginal SIV challenge was indeed mediated by effector CD8+ T cell responses (Genescà, J Virol 2008)

T cell reponses in seropositive humans

- Human « elite controllers », whose viral load remains <75 copies/mL in the absence of antiviral treatment, show potent, multi- functional, viral infection- suppressing CD8+ CTL responses (Almeida, J Exp Med 2007; Migueles, Immunity 2008)

- Polyfunctional CD8+ T cells are also found in HIV controllers (<2000 copies/mL), including in mucosal tissues. Controllers frequently have a B27, B52 or B57 HLA haplotype (Betts, Blood 2006; Saez-Cirion, PNAS 2007; Emu J Virol 2008; Ferre, Blood 2009). Similarly, protective haplotypes have been described in monkeys (MHC Mamu B08, Mamu B17…).

- HIV-specific CD8+ CTL were initially found in the cervical tissue in HIV-1-exposed, persistently seronegative ('HEPS') commercial sex workers (Rowland-Jones, 2000).

The Merck Ad5-HIV gag, pol, nef Phase IIb « STEP » trial

An Ad5-HIV gag,pol, nef vaccine was administered three successive times to volunteers at risk.

The trial however had to be prematurely stopped because of **an increased number of infections** in the **uncircumcised volunteers with previous immunity to the Ad5 vector** :

29/532 infections reported in the Ad5-HIV vaccinated group vs 13/528 in **the placebo group** **(Schoenly , Weiner J Virol 2008)**

The reason for this facilitation phenomenon remains unclear (Sekaly, J Exp Med 2008; Watkins, Nat Med 2008; O'Brien, Nat Med 2009 ; Hutnick, Nat Med 2009)

- **Quite surprizingly, the Ad5 vaccine elicited no decrease in viral loads in the vaccinees who got infected, in spite of a measurable T cell response !**

Failure of the « STEP » trial

- The Merck Ad5 vaccine had been tested in **the macaque/ SHIV model**, **where it showed some efficacy at controlling viral loads when the challenge virus was SHIV 89.6P, an X4 SHIV.**

  It however showed no efficacy when tested in the more demanding macaque/ SIV model (Casimiro, J Virol 2005; Mattapallil , J Med Primatol 2006; Suh, Vaccine 2006; Wilson, J Virol 2007)

- Indeed, the Ad5 vaccine elicited **IFN-γ-secreting, circulating T cells**, but **protection in NHP models does not correlate with PBMC IFN-γ ELISPOT (Zhou, Vaccine 2007; Mansfield , J Virol 2008) , it needs high affinity, high-avidity, multi-cytokine T cell responses (Abel, J Virol 2003; Betts, Blood 2006; Belyakov, J Immunol 2007; Saez-Cirion , PNAS 2007; Almeida, J Exp Med 2007; Sui et al, Proc Nat Acad Sci USA 2010).**

- **Protective T cells stain positive for IL-2, TNF, MIP-1β, IFN-γ and granzyme; and they actively suppress viral replication through cell killing . The Merck recombinant Ad5 vaccine was not able to elicit this type of a T cell response.**

Other live vectored vaccines

- **Adenoviruses:**
  After the failure of the STEP trial, Ad5 will never be used again.
  Still some hope in Ad26, Ad35 and Chimp Ad 3, especially if they express mosaic antigens.

- **Pox viruses:**
  Vaccinia (MVA, NYVAC); Fowlpox; Canarypox (ALVAC).
  → CD4 cellular immune responses > CD8 cellular immune responses

- **Venezuelan Equine Encephalitis (VEEV);**
- **Adeno-associated virus (AAV) → weakly immunogenic**

- **Others: Measles (MV), Rubella virus, Stomatite vésiculaire (VSV) …**

DNA vaccines

- **Naked DNA (→ Cellular immune responses (CD4>CD8), after multiple immunizations)**
- **Adjuvanted DNA : CRL005, IL-15, IL-12 (→ No significant enhancement detected)**
- **Delivery by electroporation → Electroporation greatly enhances DNA immunogenicity**

- **As stand-alone candidates, DNA vaccines have generally been less immunogenic in humans compared with small animal or NHP.**
- **Their major interest lies in prime-boost immunization regimens:**

  DNA + live vectored vaccine (MVA, Ad26…) ; DNA + protein vaccine (gp120; SOSIP)

Prime-boost immunization regimens

- Canarypox prime + gp120 (RV 144 trial in Thailand) : ALVAC-HIV + gp120 B/E
- Ad35 prime + gag-pol-nef fusion protein (AS01B); Ad26 prime + gp140 (mosaic Ag) boost
- DNA + MVA; DNA + NYVAC (Both induce multifunctional CD4+ >CD8+ T cells)

  DNA + gp120 (elicits binding Ab, ADCC, neutralizing Ab against Tier 1 isolates)

- Ad26 prime + Ad35 boost
- MVA prime + Ad35 (or Ad26) boosts
- Ad26 prime + MVA boost

Vaccine clinical trials database www.iavi.org,

The RV144 Phase III trial

- **ALVAC-HIV *env, gag, pol* + gp120 clade E**: **prime-boost regimen tested on 16,400 volunteers in Thailand : 51 infections** in the vaccinated group versus **74** in the placebo group**,** i.e. **a statistically significant 31% reduction** in the number of infections**.**

  - **Protection actually was 61% at year 1 and appeared to progressively wane with time**

  - **There were no detectable CD8+ CTL responses** (no cellular immunity effect)**. Low titer NAbs** were **irregularly detected in some vaccinees.**

    The basis of protection was therefore neither CMI responses nor NAbs!

RV144 in Detail

First trial to show any efficacy of an HIV vaccine candidate

THE TRIAL

A Phase IIb test-of-concept trial, based on the expected number of HIV infection endpoints, conducted by Thailand Ministry of Public Health

Co-primary endpoints: Prevention of HIV infection and ability to reduce viral load

Duration: Six years
- September 2003–screening starts
- October 2003–first vaccination
- July 2009–data analysis begins

Sponsor: US Army, Surgeon General

Trial Cost/Funders: US$105 million; US National Institute of Allergy and Infectious Diseases (NIAID) (75%), US Army (25%)

THE VOLUNTEERS

16,402 Thai citizens (60% male, 40% female) enrolled, 16,395 received at least one dose of vaccine or placebo

Inclusion criteria:
- Male or female Thai citizen, 18-30 years of age
- Available for participation for 3.5 years
- Can understand study and give written informed consent
- Completed enrollment in screening protocol

Exclusion criteria:
- HIV infected, active tuberculosis, or chronic use of immune-modifying therapy
- History of anaphylaxis or other serious adverse reactions to vaccines

THE VACCINE CANDIDATES

Prime

ALVAC-HIV (vCP1521)

A live, recombinant, non-replicating canarypox viral vector vaccine encoding clade B *gag/pro* and clade E *env*

(Vaccine Developer: Sanofi Pasteur)

Boost

AIDSVAX gp120 B/E

A genetically engineered version of HIV gp120 (*env*) from clade B and E

(Vaccine Developer: Genentech; its spin-off, VaxGen, tested AIDSVAX previously; intellectual property rights now owned by Global Solutions for Infectious Diseases)

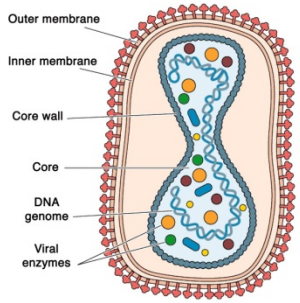
Dosing schedule

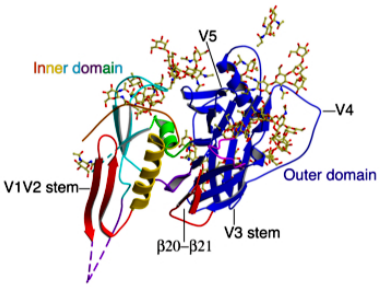
THE TEAM

Key collaborators:
- NIAID
- Sanofi Pasteur
- Global Solutions for Infectious Diseases
- US Military HIV Research Program, a branch of Walter Reed Army Institute of Research

Other collaborators:
- Mahidol University in Thailand
- Armed Forces Research Institute of Medical Science—US and Thai components

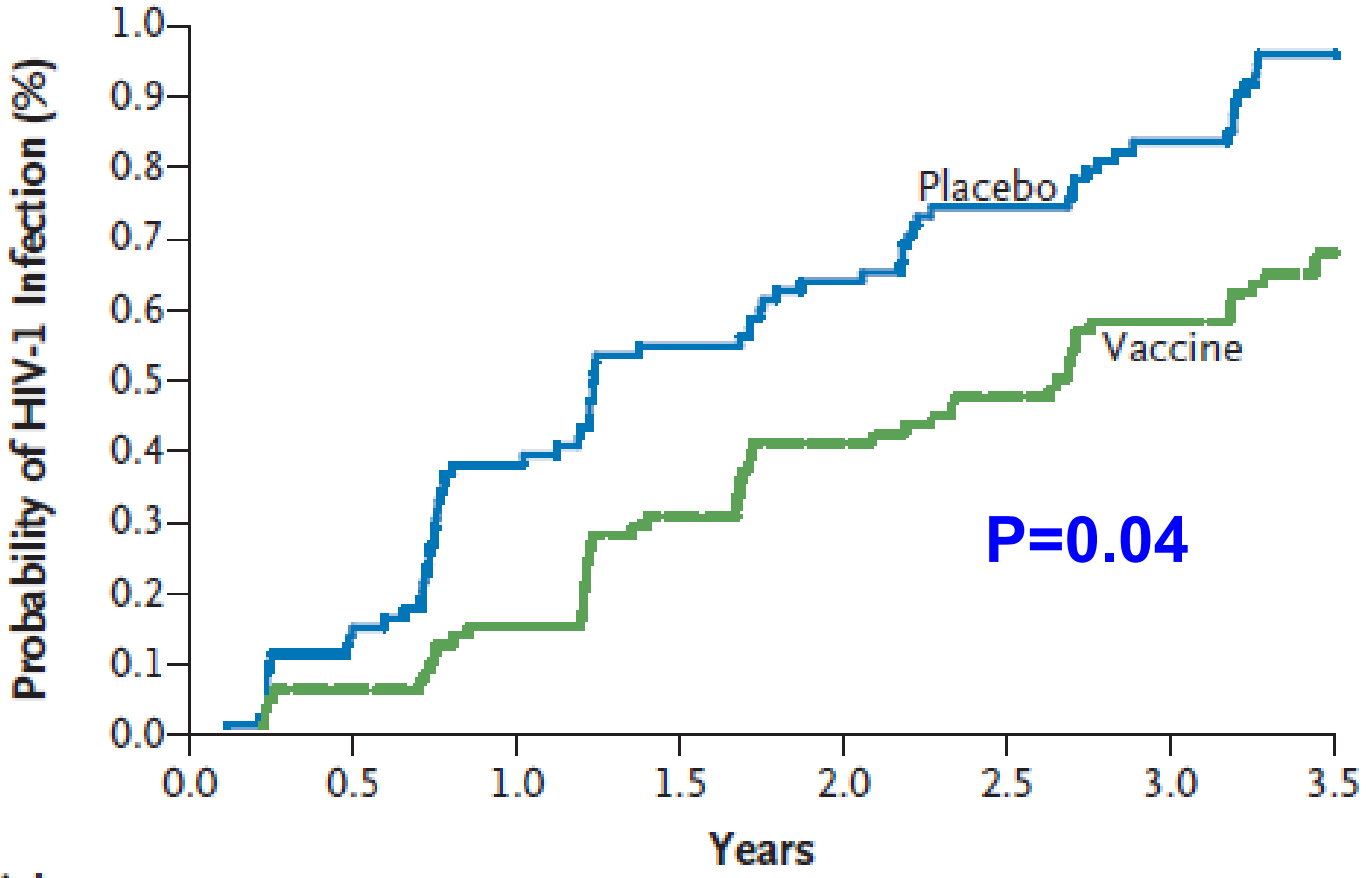
Principal investigator: Supachai Rerks-Ngarm, Thailand Ministry of Public Health





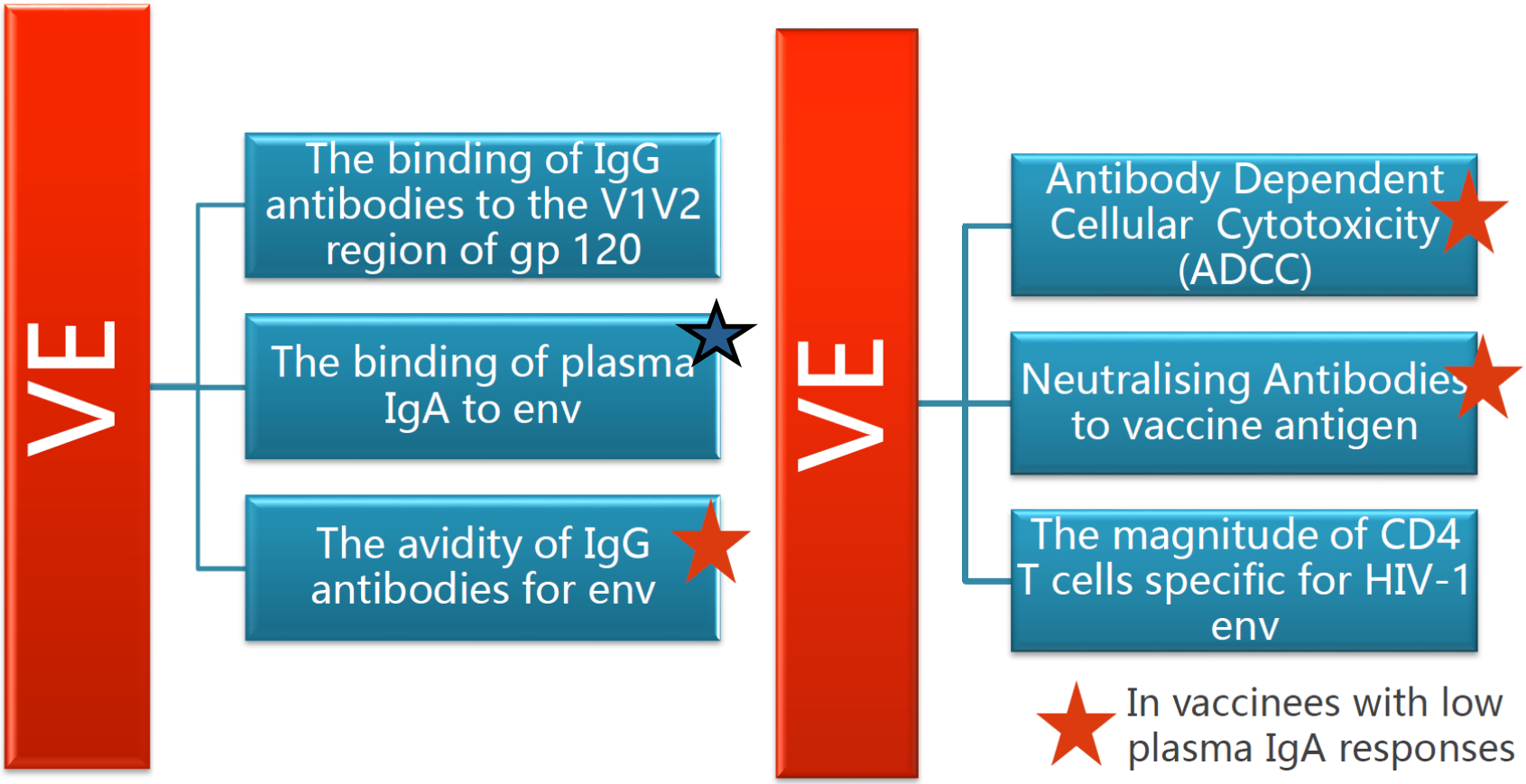
- **16,395 Volonteers**
- **18-30 Years**
- **12,542 Completed study**
- **$105 Million**

RV144 : 31.2% Reduction of Infection risk

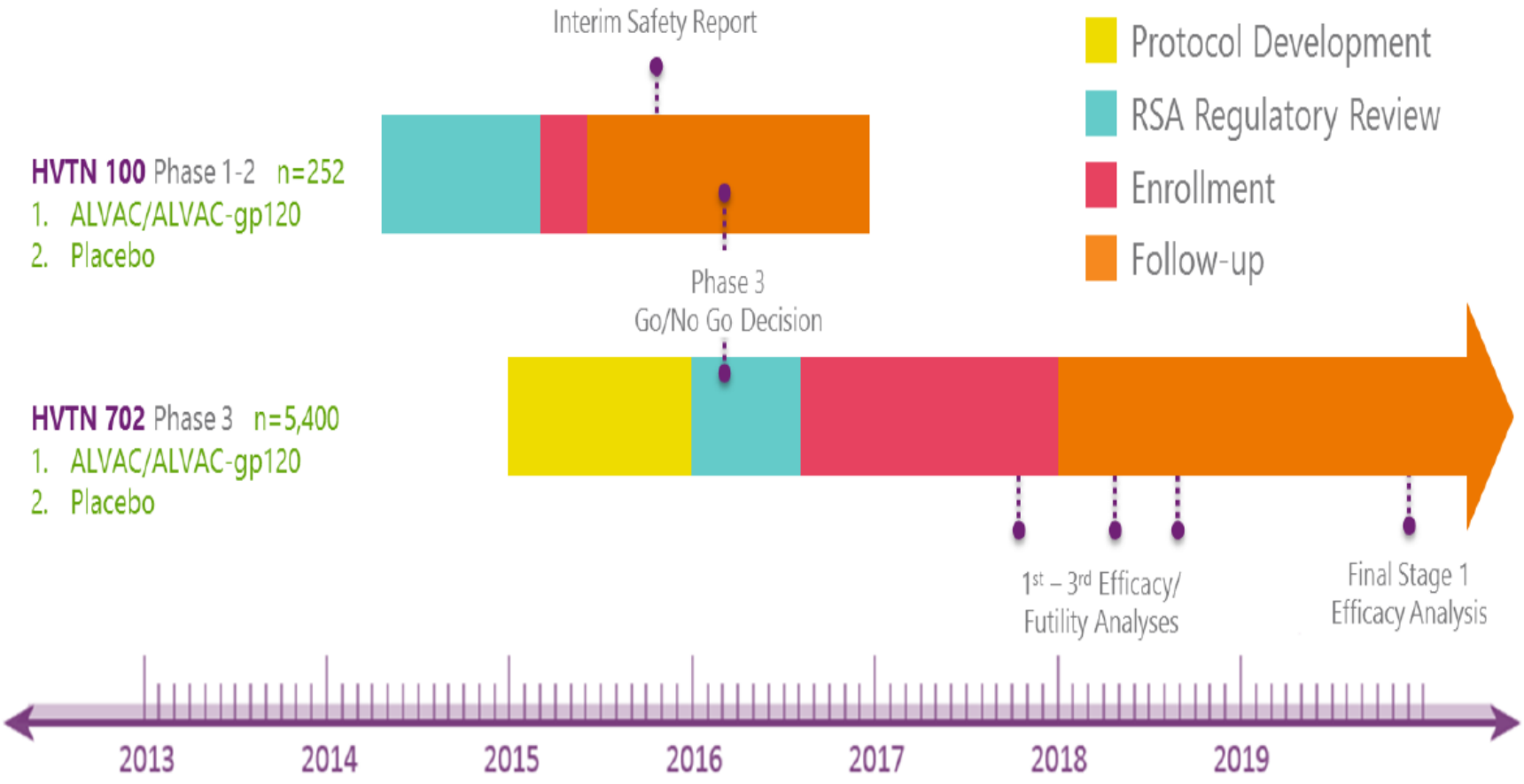


No. at Risk					
Placebo	8198	7775	7643	7441	7325
Vaccine	8197	7797	7665	7471	7347
Cumulative No. of Infections					
Placebo		30	50	65	74
Vaccine		12	32	45	51

Key correlates that emerged from RV144 that appeared to be related to Vaccine Efficacy (Haynes, 2012)



Timelines


Interim Safety Report
Protocol Development
RSA Regulatory Review
Enrollment
Follow-up
HVTN 100 Phase 1-2 n=252
1. ALVAC/ALVAC-gp120
2. Placebo
Phase 3
Go/No Go Decision
HVTN 702 Phase 3 n=5,400
1. ALVAC/ALVAC-gp120
2. Placebo
1st – 3rd Efficacy/
Futility Analyses
Final Stage 1
Efficacy Analysis
2013
2014
2015
2016
2017
2018
2019

New surrogate markers of protection

- **The RV144 trial showed correlation between protection and IgGs that target the C1 domain of gp120 and promote ADCC.**
  It also demonstrated a clear correlation between protection and V1-V2 targeted IgGs.

ADCC (Antibody-dependent cellular cytotoxicity) occurs when an Ab molecule bound by its Fab segment to a cognate viral Ag on the surface of an infected target cell interacts through its Fc portion with the Fc receptor of an effector cell (NK cell, monocyte), leading to death of the target cell

Antibody-dependent cell-mediated viral inhibition (ADCVI) is similar to ADCC but the read-out is the inhibition of virus production rather than the death of the target cell

(Hessel, Nature 2007; Forthal & Moog, Curr Opin HIV AIDS 2009; Perez, J Virol 2009; Moldt J Virol 2011 and 2012)

ADCC/ADCVI activities in passive immunization NHP models

- Le rôle de l'ADCC dans la protection contre une infection lentivirale a déjà été relatée dans le passé dans le modèle simien:
- **Protection of newborn monkeys** against **oral SIV infection by passive immunization with a nonneutralizing anti-SIV serum** strongly correlated with **ADCVI activity** of the serum **(Van Rompay , J Infect Dis 1998).**
- Passive immunization with **BNAb b12 induced protection in 8/9 monkeys against vaginal SHIV challenge. A variant of b12 that bound poorly to FcR retained full neutralizing activity** but protected only about **50% of the animals,** implying **ADCC/ ADCVI as an important mechanism in the protection provided by passive immunization with b12 (Hessell, Nature 2007; Hessell, Nat Med 2009).**
- Rhesus macaques immunized using a **Ad5 hr-SIV recombinant /SIV env prime-boost** regimen were protected against **intrarectal challenge with SIV mac251 in spite of total absence of NAb induction (Patterson, J Virol 2003 and 2004). A significant correlation** was found between protection **and ADCC activity in serum and mucosal secretions (Gomez-Roman J Immunol 2005; Hidajat, J Virol 2009; Xiao, J Virol 2010)**

- **On change d’orateur…**

Historical retrospective of HIV vaccine Phase III efficacy trials

1) NAb approach: VaxGen Phase III trials with gp120 clade B or CRF A-E→ Only type-specific neutralizing Ab (NAb) responses

→ no protection

2) CMI approach: Merck Phase IIb STEP trial with Ad5-HIV recombinants,

→ weak CTL response → No protection against infection nor against disease

NIH HVTN 505 trial (DNA prime-Ad5 boost): no protection either

3) Combined approach: Thai RV144 Phase III trial with ALVAC env,gag,pol prime and gp120 boosts→ 31% protection against infection (39% in women, 26% in men). No NAb, no CTL, but V1-V2 loops-targeted non-neutralizing antibodies and ADCC.

I. HIV antibodies

Three types of antibodies (Abs) are known that can play a role in protection:

1. **Neutralizing Antibodies (NAbs) , that neutralize a limited number of Tier-1 virus strains in the autologous clade**

2. **Recently discovered Broadly neutralizing antibodies (BNAbs) that neutralize the great majority of known virus strains in a cross-clade manner (Tier-2 as well as Tier-1 strains)**

3. **Non-neutralizing antibodies that act through recruitment of cytotoxic NK cells or monocytes via their Fc portion → ADCC, ADCVI..**

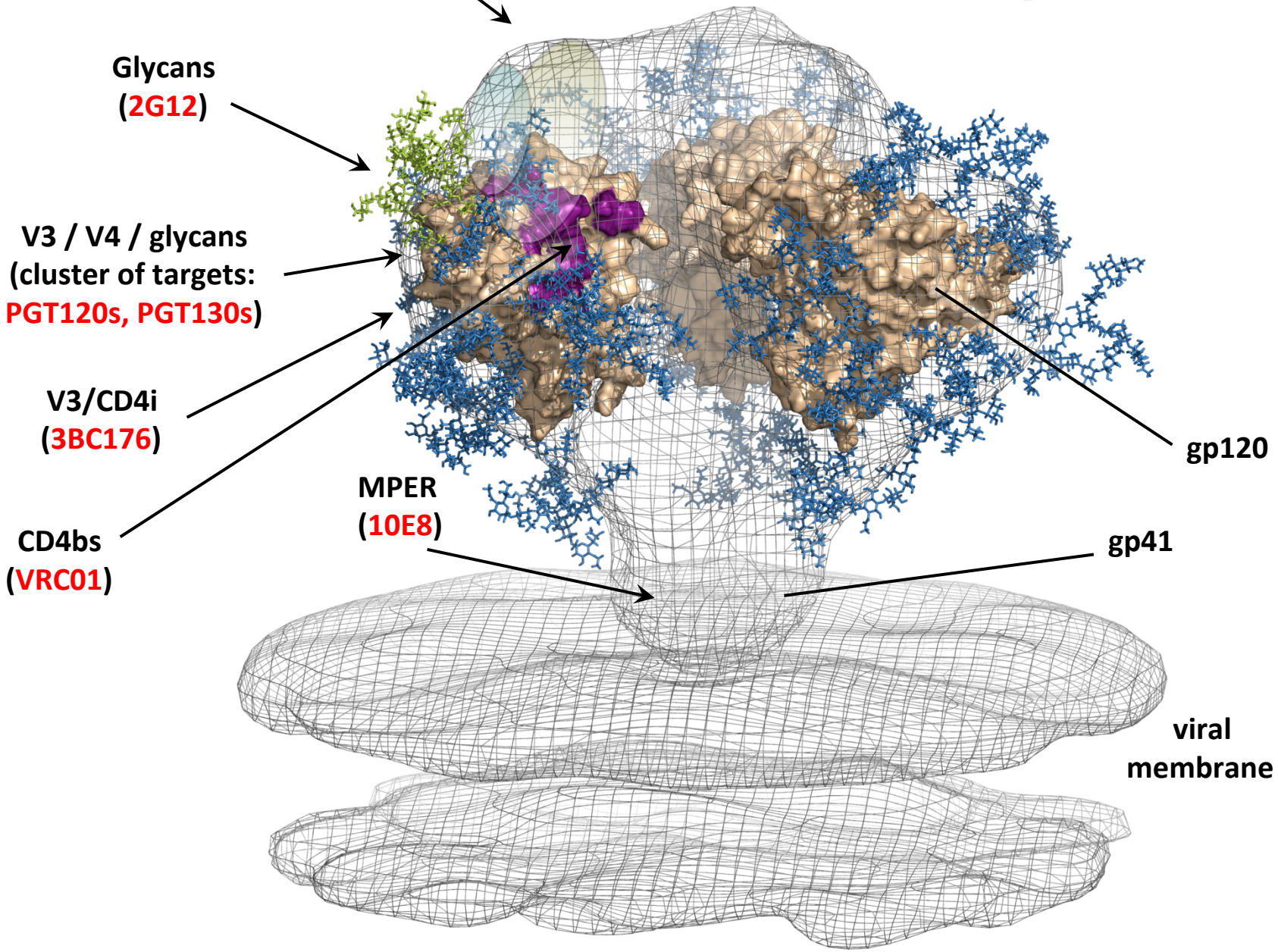
The issue of bNAbs

- **Experience shows that BNAbs develop over a period of a few years (2.5yrs av) in 15-20% of HIV-1 infected persons. They are the result of a long affinity maturation of B cells and extensive mutation of the B cell lineage that seem to be driven by long antigenic exposure**

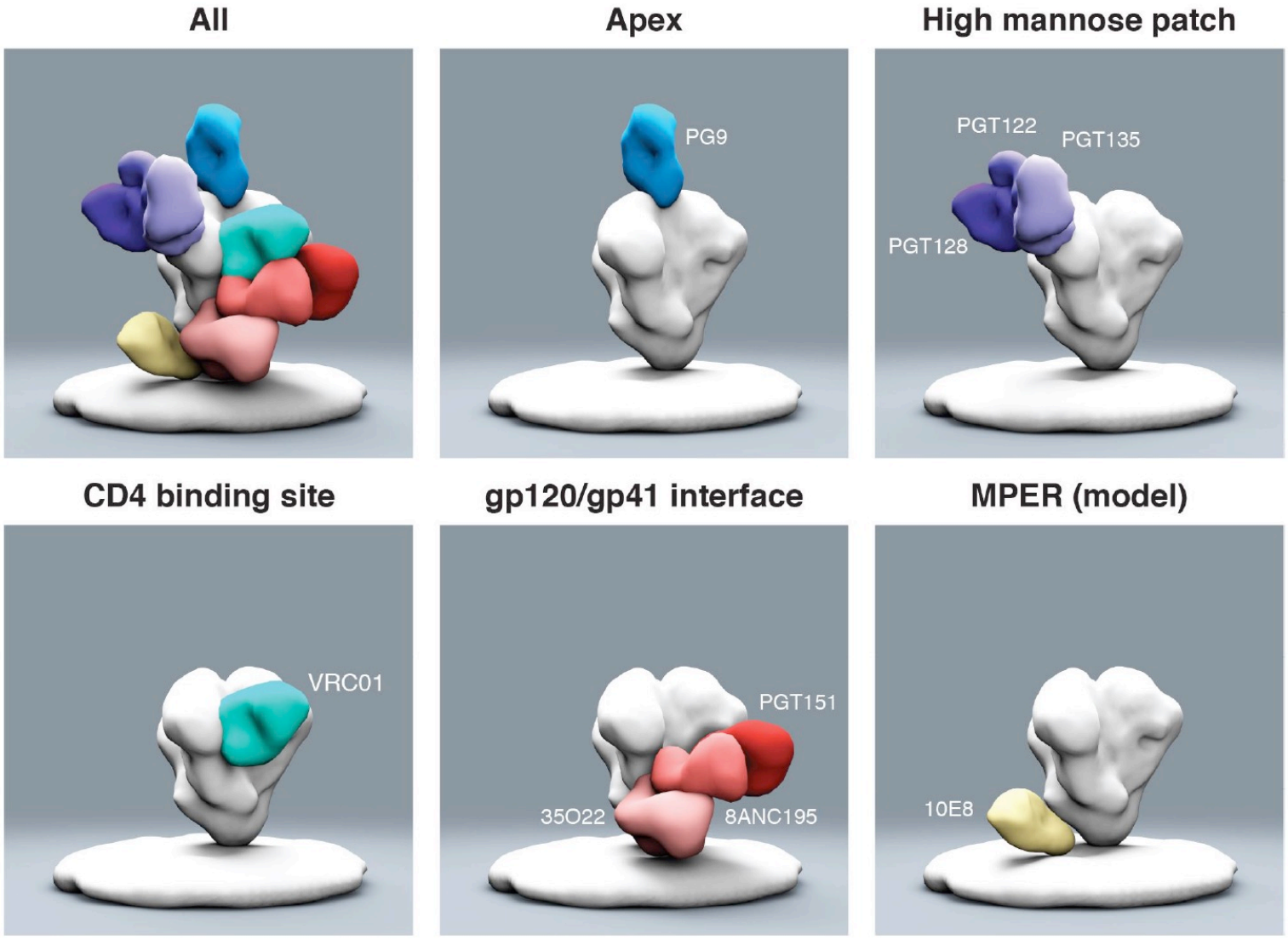
  (Stamatatos, Nat Med 2009; Sather J Virol 2009; Doria-Rose, J Virol 2009; Simek, J Virol 2009; Zhou, Science 2010; Wu, Science 2011; Huang, Nature 2012; Kwong, Immunity 2012; Doria-Rose, Nature 2014).

- **Many BNAbs have long protruding anionic heavy chain complementarity-determining region 3 loops (CDR H3) that allow the Ab to penetrate the HIV-1 glycan shield and engage protein epitopes on the V1V2 or V3V4 loops.**

HIV vaccine targets defined by bnMAbs,

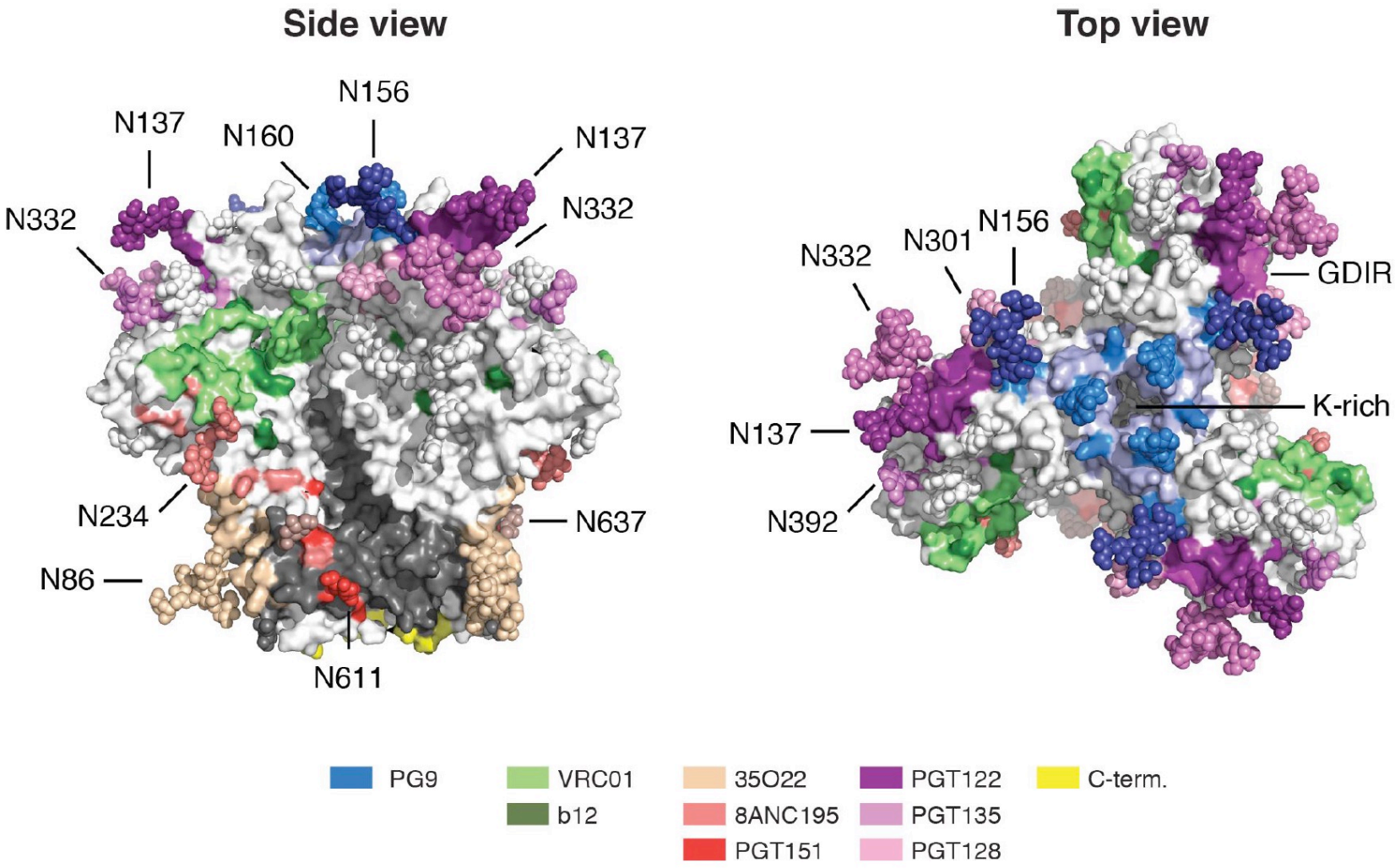


Prototype bnAbs: binding regions

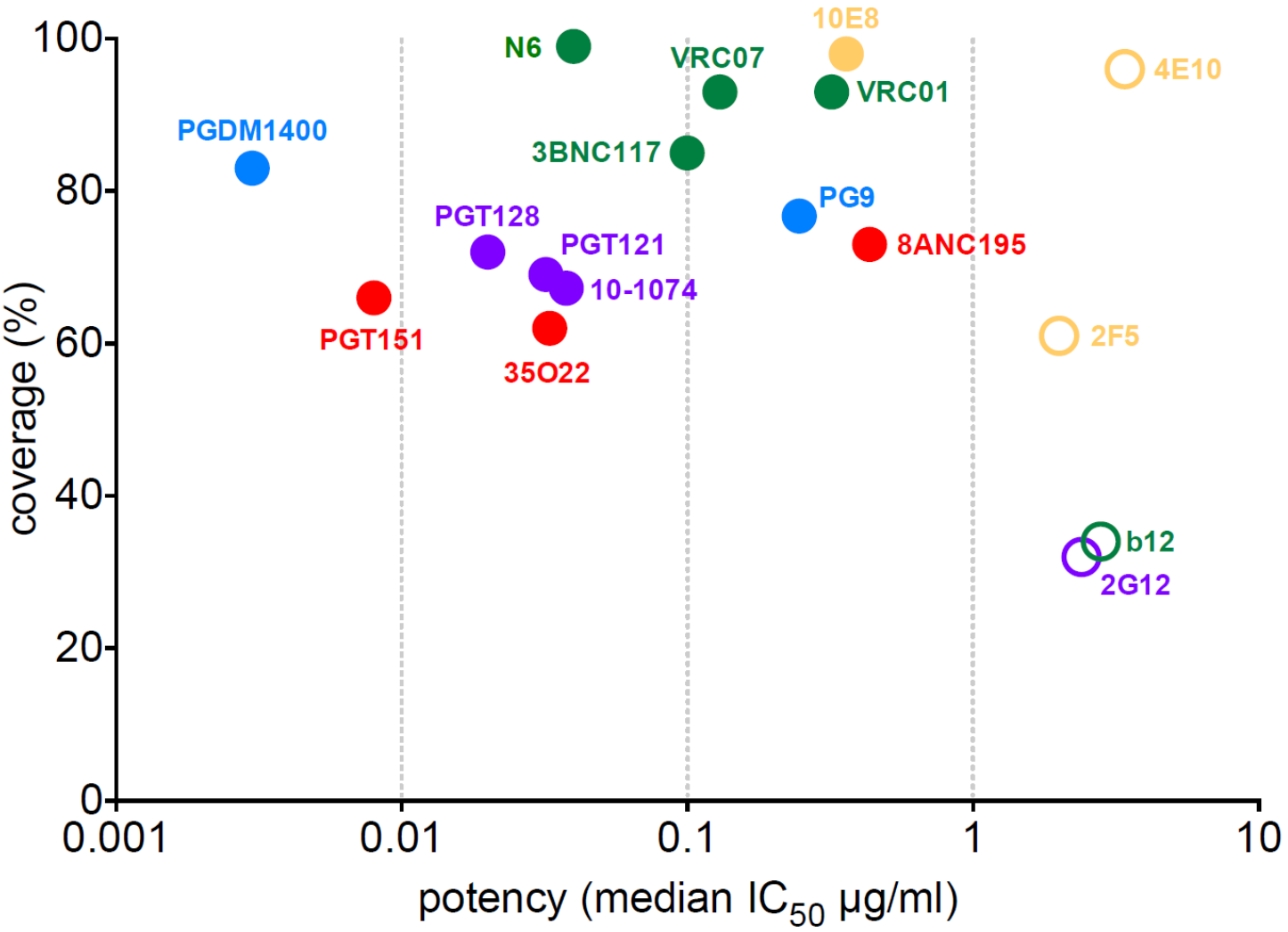


A. Ward & C. Corbaci

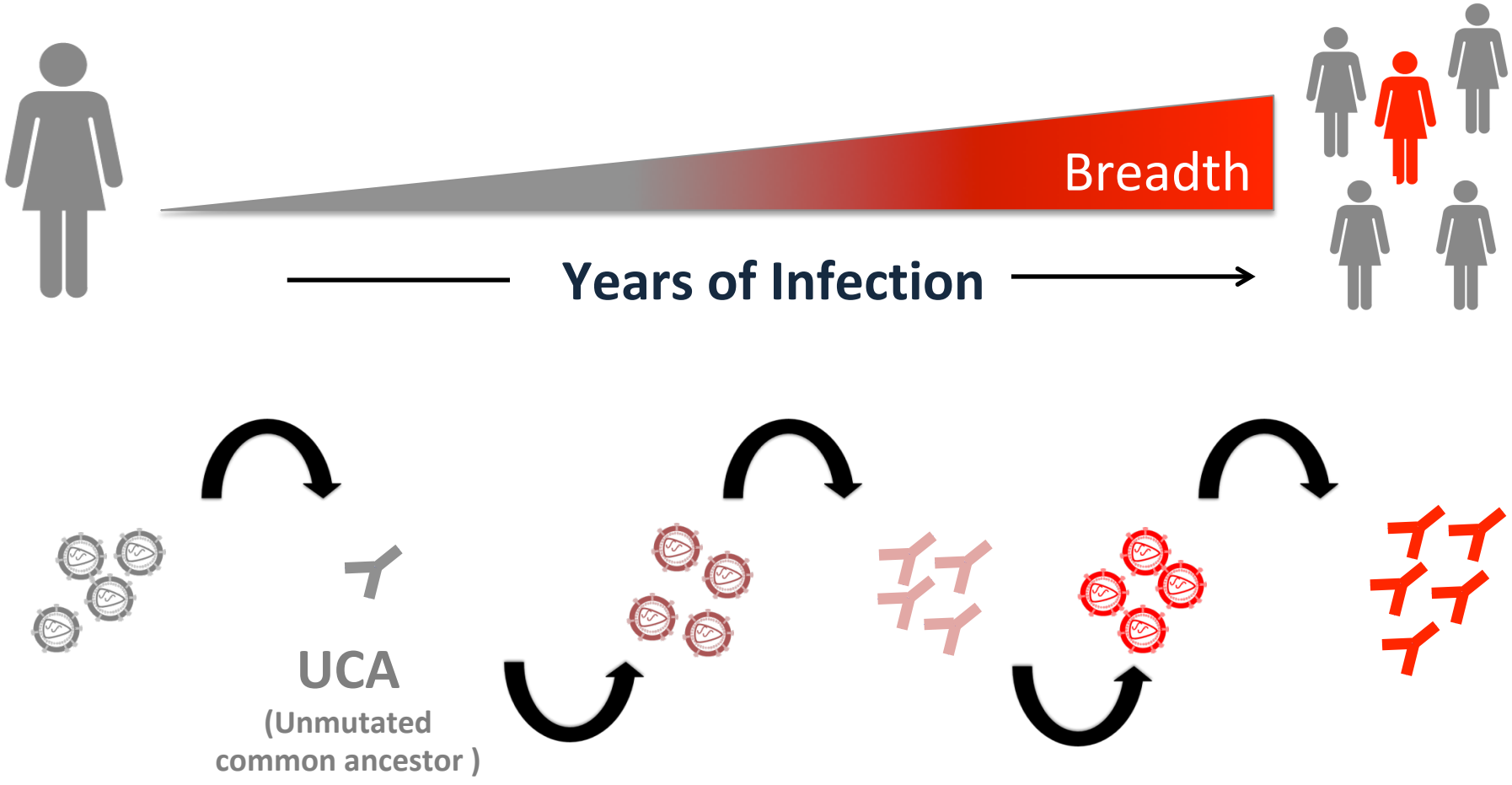
Prototype bnAbs: binding regions, including glycans



Prototype bnAbs: coverage vs IC_{50} potency-Scripps plot



Understanding how broadly neutralizing antibodies develop in HIV infection



Eliciting Broadly Neutralizing Abs

- **BNAbs show an unusually high level of somatic hypermutation (Kwong et al, Nat Rev Immunol 2013; Mascola & Haynes, Immunol Rev 2013; West et al, Cell 2014).**
- **The level of somatic mutation** in the B cell lineage directly **correlates with the neutralization activity of the Ab (Klein et al, Cell 2013; Sok et al, PLoS Pathog 2013; Bhiman et al, Nat Med 2015).**

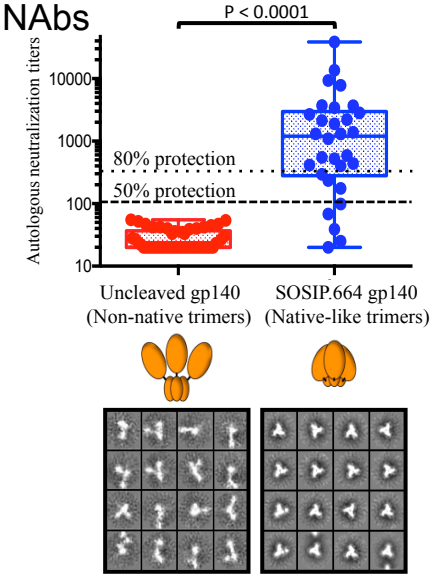
- In the infected host, HIV-1 escapes immune pressure by **continuously mutating, which results in a continuously evolving Ag presentation** → this generates in turn **a continuous evolution of the Ab response:** bNAbs develop with time **in response to the constant mutational modifications of the infecting virus.**

- **Designing a vaccine able to induce bNAb remains the major challenge in HIV vaccine research: It has been impossible so far to induce bNAbs by immunization with any of the Env antigens or Env scaffolds tested. (The same applies to bNAbs against influenza or RSV)**

B cell immunogen design: SOSIP trimers

SOSIP.664 trimers

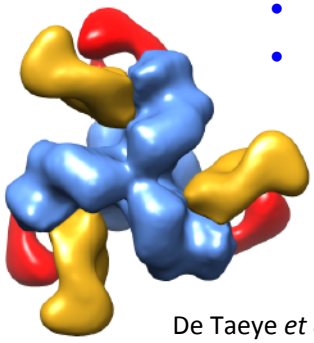
Induction of autologous Tier 2 NAbs



Sanders *et al*. 2013. *PLoS Path*. **9**:e1003618
Sanders *et al.* 2015. *Science* **349**:aac4223

SOSIP.v5 trimers

- Improved trimerization
- Increased stability
- Reduced V3 non-NAb epitope exposure
- Reduced CD4i non-NAb epitope exposure
- Improved bNAb exposure
- Reduced V3 immunogenicity and Tier 1A NAb induction



- Improves existing trimers
- Allows making new trimers

AMC008 SOSIP.v4.2
bNAb PGV04
bNAb 35O22

De Taeye *et al.* 2015. *Cell*

Sanders: AMC

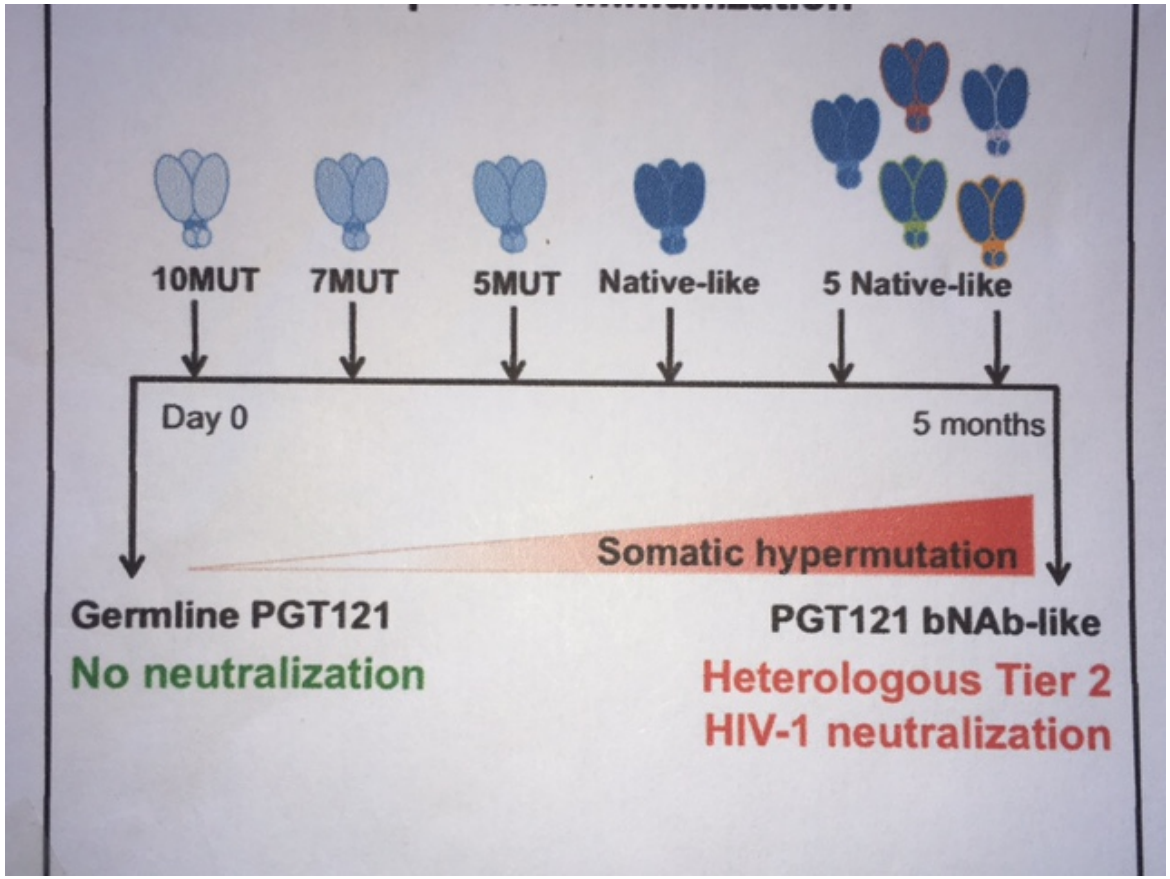
A possible answer

- **Hence the idea that sequential immunizations with a series of Env immunogens, with a gradually changing epitope structure ,** should elicit **the evolution of the B cell germline** through the **evolving presentation of the Ag (Haynes et al, Nat Biotechnol 2012; Jardine et al, Science 2013; Steichen et al, Immunity 2016)**

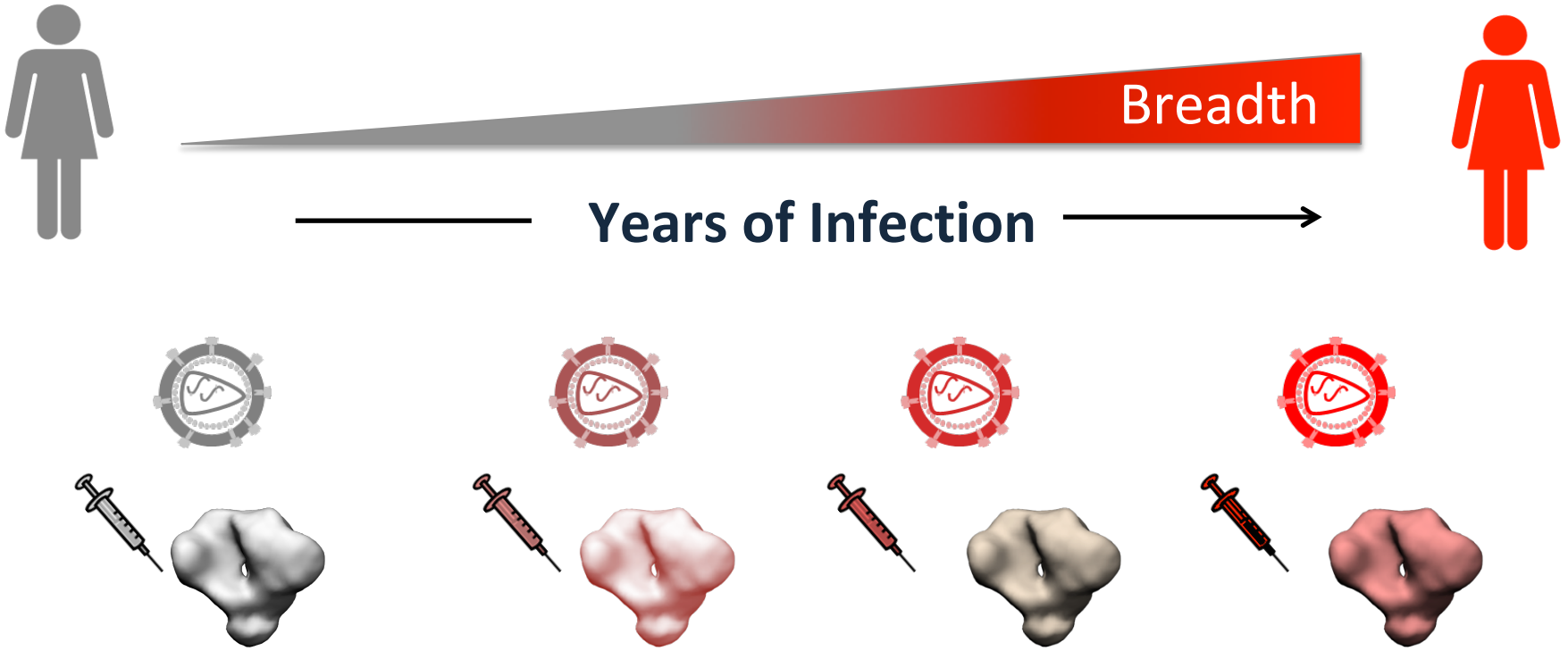
- Mice were immunized sequentially, using a family of Env trimers **(BG505-SOSIP)** corresponding to the **BNAb PGT121 B cell lineage with** a **progressively decreasing number of mutations** in the gp120 gene **(10MUT, 7MUT, 5 MUT, then wt) →** induction of NAb **that could neutralize several Tier-2 viruses (Escolano A, et al. Cell 2016)**

- **This suggests this might be the right way to go?…**

Eliciting PGT121 bNAb by sequential Immunization



Sequential immunization strategies



Malherbe et al, 2011; Haynes et al., 2012; Moore et al, 2012; Liao et al, 2013

Immunisation passive

- A défaut de pouvoir induire des Ac neutralisants à large spectre **(BNAb**) par la vaccination, on **peut les utiliser avec succès** en **immunisation passive.** On a testé notamment leur usage comme **microbicide vaginal.**
- **Problème**: comment les produire en quantité suffisante?

- **Bonne nouvelle**: contrairement à la grande majorité des vertébrés qui fabriquent des Ac avec des domaines HCDR3 de seulement 12-16 ac aminés, **les Ac des bovins ont naturellement un domaine HCDR3 très long** (26 ac aminés en général, jusqu'à 70 ac aminés)!

- **Indeed, BG505 SOSIP immunization resulted in rapid elicitation of broad and potent serum antibody responses in cows. Longitudinal serum analysis for one cow showed the development of neutralization breadth (20%, *n = 117 crossclade* isolates) in 42 days and 96% breadth (*n = 117) at 381 days.***

  A monoclonal antibody isolated from this cow harboured an ultralong HCDR3 of 60 amino acids and neutralized 72% of crossclade HIV isolates (*n = 117) with a potent median IC50 of 0.028 µg ml−1.*

(***D Sok et al, Nature 2017, 548, 108-11).***

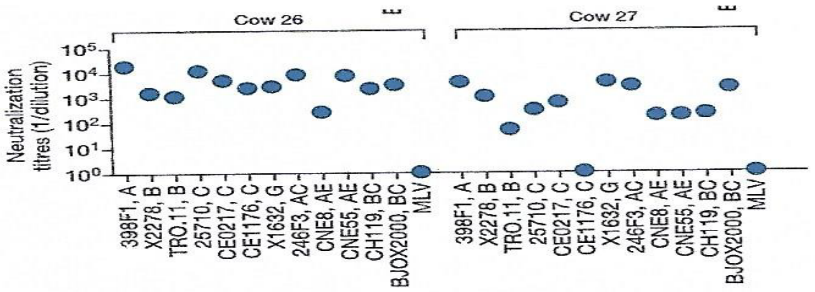


Figure 1 | Cows develop broadly neutralizing serum antibodies when immunized with the HIV envelope trimer mimic BG505 SOSIP.

Immunisation passive (2)

- **Immunisation passive du macaque avec un seul BNAb**: → protection contre l'infection par un SHIV R5… **mais rapide apparition de souches virales résistantes** (**mutants d'échappement**).

- D'où l'idée de combiner plusieurs déterminants de BNAbs **sur une seule molécule d'IgG** **(Xu L, et al, Science 2017, 358, 85-90)**
  - **→ Ac bi-spécifique (VRC01 sur un bras, PGT128 sur l'autre)**
  - **→ Ac tri-spécifique (VRC01 sur un bras, PGDM1400 +10E8 sur le 2ème) → epitopes reconnus: CD4bs, glycanes V1/V2, et MPER (gp41)**

→ Essai de protection du macaque contre une épreuve SHIV BaLP4 (voie I/R):

Anticorps	Protection
VRC01 seul	2 animaux sur 8
PGDM 1400 seul	3 animaux sur 8
Ac trispécifique	8 animaux sur 8

(*Xu et al, Science 2017; 358: 85-90*)

Une alternative: « Genetic immunization »

- A promising **alternative to active immunization** is the so-called **« vectored immunization »** approach (also called **'vector immunoprophylaxis' = 'VIP'),** which relies on the **IM injection of a recombinant AAV vector that can express the genes encoding the H and L chains of a bNAb**
- → Persistence de l'AAV recombinant dans l'organisme

  → **life-long expression of the H and L genes → broadly neutralizing MAb.**

  → **protection against HIV or SHIV challenges in humanized mice and/or monkey models (Johnson, Nat Med 2009; Balazs et al, Nature 2012).**

  → **Phase I/II clinical studies** using AAV-vectored **VRC01, PG9**, and/or **VRC07** Mabs have been started

Another role of nonneutralizing antibodies in protection

Multiple mechanisms for HIV to pass through mucosal barriers have been proposed that include **transcytosis of HIV-1 across simple columnar epithelial layers** (endocervix, rectum, GI tract). This can readily be demonstrated *in vitro* **(Tudor, Mucosal Immunol 2009; Tudor, Nat Immunol 2009)** : the **virions penetrate into the cell by endocytosis** and are **transported across the cell** wrapped in a **transcytosis vesicle** that releases them **on the baso-lateral side of the epithelium**

Mucosal IgAs specific for HIV-1 gp41 MPER have been shown to **block HIV-1 transcytosis across epithelial barriers** ***in vitro*** **(Alfsen, J Immunol 2001; Nguyen, J AIDS 2006; Shen, J Immunol 2010) = Transcytosis inhibition**

Such transcytosis-blocking IgAs can be found in the **cervicovaginal secretions of highly exposed, persistently seronegative (HEPS) women**

GP41-virosome immunization

(Bomsel et al, Immunity 2010)

- **Female macaque monkeys** were immunized with **rgp41 and an MPER peptide (P1) grafted onto virosomes**: either 4 times by the IM route or twice IM then twice intranasally (IN).

- **The animals were then challenged 13 successive times by the vaginal route with a low dose (30 TCID50) of SHIV SF162P3 (once- or twice-a-week)**

- **None of the IM/IN immunized females, and only 3/6 IM immunized females, became infected vs 6/6 placebos**

- **All the protected animals had developed transcytosis-blocking IgAs in their vaginal secretions.** None showed evidence of NAbs in their serum

So, what do we have in the pipe at this time?

A variety of prime-boost regimens using DNA as a prime and live vectored vaccines (Ad26, Ad35, Ad48; and/or MVA) as a boost. These vaccine approaches are at various stages of clinical trials**.**

So is a study of DNA vs MVA prime+ gp140 boosts

Mixed modality prime-boost regimens : **Ad26 followed by MVA then gp140** as compared to **Ad26 followed by gp140,** using **mosaic antigens→** expected to go into Phase III efficacy trial in **2018.**

A Phase III clinical trial (HVTN702) based on the RV144 model, which **is on-going in South Africa with a clade C canarypox (ALVAC) vaccine as a prime and a clade C gp140 as a boost,** using **MF59** as an adjuvant **.** The trial involves 5 clade C injections over 12 months (vs 4 clade AE injections over 6 months in the RV144 trial)

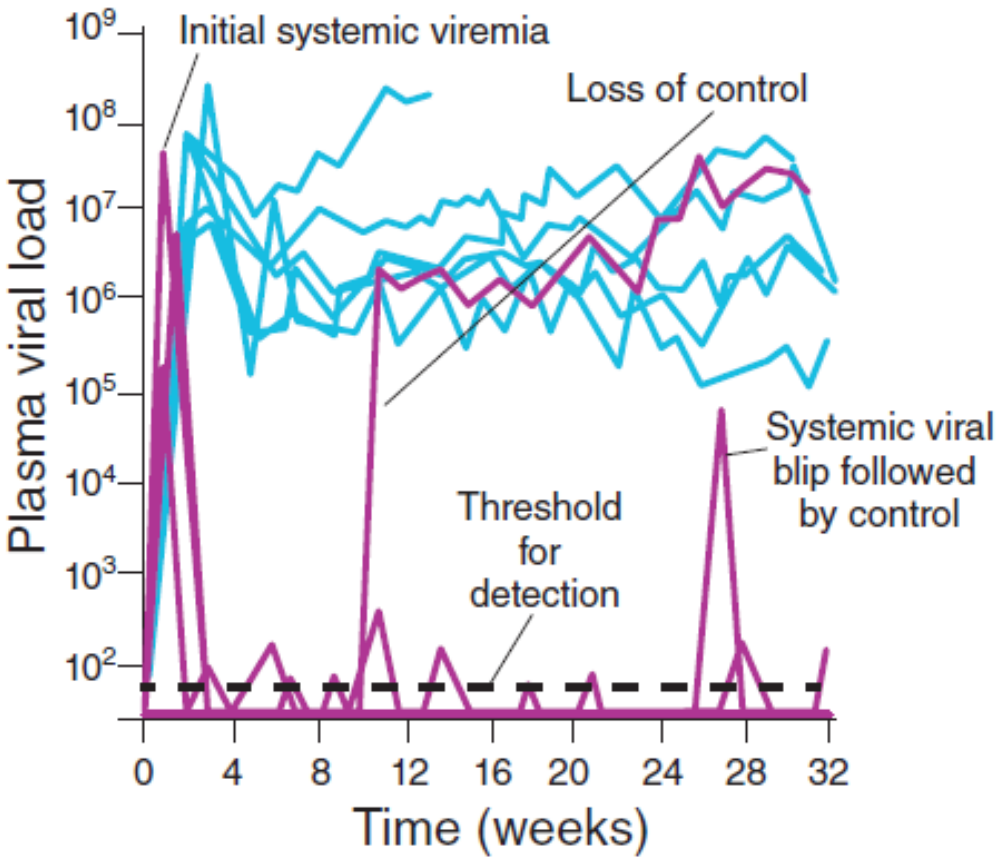
A new vector: CMV

Simian cytomegalovirus (Rh CMV) was **t**ested as a vector **(Louis Picker's group)**→ Simian CMV recombinant vaccine that expressed **SIV Gag, Rev, Tat, Nef, and Env → SIV challenge: Viral loads in 50% of the challenged animals remained mostly undetectable** (except temporary blips) for one year follow-up.

After one year, **no more virus blips were observed and no viral RNA nor DNA could be recovered from the protected animals = A cure!!**

Protection correlated with **mucosal T cell responses**, especially **CD4+ and CD8+ TEM cells.**

Surprizingly, the cytotoxic $CD8^+$ TEM cells elicited by the recombinant RhCMV /SIV vaccine were reactive to epitopes presented by **nonclassical major histocompatibility complex E** (**MHC-E**) molecules!..., not MHC-A nor –B!



Walker B. Nat Med 2011

Nouveaux vaccins potentiels

- **Plusieurs nouveaux vaccins recombinants** sont aussi en développement, notamment:

  1. **Un virus de la rougeole recombinant**, **MV-p55GagSIV,gp160EnvHIV**→ protection de 50% des macaques Cynomolgus contre une épreuve SHIV162P3. **(F Tangy, Institut Pasteur)**

  2. **Un lentivirus recombinant, LV-p24GagSIV**→ protection de macaques Rhesus contre une épreuve SIV voie rectale **(P. Charneau, Institut Pasteur)**

  3. **Un virus de la fièvre jaune** (souche 17D) recombinant **YF17D-CH505gp120 HIV (J-S Yu Duke University Med Ctr) (J-S Yu et al, J Virol Methods 2017, 249: 85-93)**

- D'autres approches sont aussi en cours de développement:
  - Des **IgA recombinantes type IgA-SOSIP**, ou, mieux encore, des complexes trivalents **IgA-SOSIP-p24** → induction d'Ac neutralisants **(S. Paul, GIMAP, St Etienne)**

Conclusion-1: The T-cell response

- **Control of viremia** in SIV/SHIV/HIV infection **correlates with** and is dependent on **CD8+ CTLs in macaques and chimpanzees (Belyakov Blood 2006 and J Immunol 2007),** especially with **high-avidity, polyfunctional, degranulating mucosal tissue-based CD8+ CTLs**

- **The polyfunctional , continuous CD8+ TEM cell response** elicited in macaque monkeys by **live recombinant CMV- SIV vaccine** controlled SIV infection and made 50% of the animals **virus-free** after one year infection**.**

- **Question: could one develop a similar vector suitable for human populations?** (A HCMV-HIV vaccine using a CMV Toledo/Towne chimeric vector was planned to enter Phase I clinical trials in 2017?)

Conclusion-2: Neutralizing antibodies

- Passively transferred, **broadly neutralizing antibodies** **efficiently protected NHPs against experimental SHIV challenge (Hessel , Nat Med 2009). Passive immunization trials** in human volunteers are on-going.

- **However, we still do not know how to induce bNAbs by active immunization! The search for a possible immunogen or a combination of immunogens is actively going on**. **The sequential use of SOSIP trimers with a evolutionnary sequence** seems to be able to elicit Abs that neutralize Tier-2 virus strains in small animals: a possible approach?

- As an alternative, could « **Vectored immunoprophylaxis** » using **recombinant AAV that express bNAbs genes** be a key to success? Future, upcoming clinical trials will tell.

Conclusion-3: Non-neutralizing Abs

- Non-neutralizing mucosal Ab (essentially **gp41-specific IgAs**) that **inhibit HIV/SIV transcytosis** **across an intact epithelial cell layer** correlated with **reduced chronic viremia** **after rectal SIV challenge or full protection against vaginal SHIV challenge in NHP models.**
- These Ab thus play an important role in mucosal protection.

- **Nonneutralizing Env-specific IgGs** can also play a major role in protection through **ADCC and ADCVI,** as seen in a variety of SIV and SHIV vaccine protection experiments in rhesus macaques.
- **IgGs targeting the V1-V2 domain of gp120 were the only correlate of protection in the RV144 trial in human volunteers.** Rabbits immunized with a V1V2-scaffold immunogen developed **V1V2- focussed Ab with marked ADCC activity** **(Zolla-Pasner et al, J Virol 2016)**

Final conclusion

- **An ideal HIV vaccine** should elicit:
  - **broadly neutralizing IgGs**,
  - **mucosal IgAs with transcytosis inhibiting capacities**,
  - **Ab-dependent ADCC /ADCVI activities**,
  - **V1-V2-targeting IgGs**
  - As well as **potent**, **multifunctional CD4+ and CD8+ T cell responses in mucosal tissues.**

- **At this time, we simply do not know how to achieve all of that, nor do we know whether it will ever be possible!…**

En guise de fin

« Teaching the immune system how to outwit a virus that itself survives by outwitting the immune system is a huge scientific hurdle. »
(J Cohen, Science 21 septembre 2012)

« What is success? It 's going from failure to failure with undiminished enthusiasm » (Winston Churchill)

« Oser toujours, douter parfois, ne renoncer jamais »
(Always dare, doubt at times, but never quit!)
(Maud Fontenoy, French solitary navigator)