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Long-term safety and vaccine-induced seropositivity in healthy volunteers from HIV vaccine trials: a French cohort study

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Introduction

Research on preventive HIV vaccines has assessed multiple vaccine candidates administered to several thousand healthy volunteers worldwide, mainly as part of phase I/II trials evaluating their safety and immunogenicity. A study conducted in 3,189 volunteers in 51 vaccine centers in the United States has focused on their long-term safety and collected all clinical adverse events (AEs) that occurred during a 7-year follow-up[1, 2]. No serious AEs (SAEs) related to the various vaccine preparations used were observed. Similarly, for vaccine trials completed between 2001 and 2007 in Africa, no safety signal after a median time of 5.2 years in 287 volunteers was identified[3]. However, these results cannot be extrapolated to studies assessing other vaccine candidates and other cohorts of volunteers.

Over the 1992-2009 period, 17 phase I/II clinical trials assessing the safety of several HIV-1 vaccine candidates and their ability to induce immune responses have been conducted by the French National Agency for Research on AIDS and Viral Hepatitis (ANRS) in about 500 healthy volunteers[4, 5]. The short-term safety has been studied throughout the trials[6]. One case of uveitis potentially related to a vaccine combination has been reported and one volunteer has experienced neurological symptoms six weeks after immunization in the VAC19/HVTN042 trial conducted by the NIH using the same vaccine candidates[7]. In this context, it seems important to investigate the occurrence of ophthalmological and neurological AEs in order to identify any possible link with vaccination. Regarding vaccines in general, the occurrence of autoimmune complications remains controversial[8].

Thus, we implemented a systematic prospective follow-up of all healthy volunteers who participated in preventive phase I/II HIV vaccine trials in France to assess the long-term clinical safety of the vaccine combinations used by the ANRS. The persistence of HIV vaccine-induced antibodies and the psycho-social consequences were also addressed. Our data could help to improve knowledge about the safety of HIV vaccines and the experience of volunteers in preventive clinical trials[9-11].

Methods

Setting

Between 1992 and 2007, several thousand subjects participated in the selection process of the ANRS Volunteer Network with a screening rate of 10-15%. Inclusion criteria were: 21-54 years old, seronegative and at anticipated low risk of HIV infection (supplementary table), and not experiencing any serious health problems. The selection was performed by a multi-disciplinary committee[12]. There was no financial compensation for participating in the trial apart from their travel expenses.

HIV vaccine candidates included recombinant envelope protein (rgp160), canarypox vectors (vCP) expressing Env, Gag, Pro, the CTL domain of Pol, lipopeptides including CTL epitopes of Gag, Pol and Nef and DNA prime/poxvirus NYVAC boost combination, both expressing a

common HIV-1 clade C immunogen of Env and Gag-Pol-Nef [4, 5]. All vaccine candidates adjuvanted or not were injected intramuscularly or intradermally, except in one mucosal rgp160 trial, between 1992 and 2008.

Created in 2007, the ANRS COV1-COHVAC cohort was a prospective, multicenter cohort of participants included in phase I/II preventive HIV-vaccine trials. All volunteers (n=496) who received at least one dose of a vaccine candidate were invited to participate in the cohort by mail. They signed an informed consent form only for the retrospective collection of data or for a retrospective and prospective follow-up. In order to minimize the non-response bias, participants known to be deceased or who did not respond to the invitation were included by derogation of the French Data Protection Authority (CNIL) for the retrospective collection of data. The vital status of participants lost to follow-up was updated at the end of the prospective study.

All-grade AEs of interest (suggestive of neurological, ophthalmological, or autoimmune disease) and other grade 3 or 4 AEs, occurring from the first injection of a candidate vaccine, were recorded. Clinical AEs were coded using the MedDRA and their severity was graded using the ANRS scale[13]. No clinical confirmation of a causal relationship between AEs and HIV vaccine candidates was needed, except for SAEs. Data were retrospectively extracted from the trial databases. For the period between the end of the trial and the inclusion in the COHVAC cohort, data were based on participant memory and, in some cases, on a medical monitoring. The prospective collection (up to 7 years) was based on yearly clinical examination and interview, and a self-administered diary was used by participants.

During the prospective follow-up, the presence of vaccine-induced anti-HIV antibodies was locally verified each year using HIV EIA tests[14] and positive results were confirmed by Western-blot. Participants had to complete each year a self-administered questionnaire on sexual behaviors, based on a methodology used in the ANRS-VESPA survey[15].

The “CPP Ile-de-France III” (French Ethics Committee, Paris, France) approved the conduct of this study. The study was registered on ClinicalTrials.gov under NCT00789789.

Statistical analysis

Summary statistics are presented as percentages, medians, interquartile ranges (IQR) and incidence rates. Ninety-five percent confidence intervals (95% CI) for incidence rates of AEs were calculated using exact Poisson limits[16].

The follow-up duration was calculated from the date of the first vaccination to the date of the last known follow-up visit or the date of diagnosis of the AE of interest or death, whichever came first. For volunteers included in the prospective cohort, the contribution was also calculated separately: from the first vaccine injection to the inclusion in the prospective cohort and from the inclusion in the prospective cohort. Age-and-sex-adjusted Standardized Mortality Ratio (SMR) and Standardized Incidence Ratio (SIR)[17] were used to

compare mortality rates and incidence rates of the cohort with the French population mortality rates over the 1992-2015 period [18], the French register for breast cancer[19] and Sentinelles data for herpes zoster, separately for men and women[17, 20].

The frequency of vaccine-induced seropositivity was determined with 95% binomial exact CIs. Seropositivity[21] was defined as at least one reactive HIV EIA regardless of Western-blot results.

The studied behavioral indicator was having sexual relationships without using condoms in the past 12 months with casual partners with unknown HIV status. Detailed results for all psycho-behavioral aspects have been previously reported[9, 10].

Results

Population

From December 2008 to January 2013, 488 out of 496 eligible participants (98%) were included in the cohort, and 8 participants refused to participate (Table 1). Among included participants, 55% were men. The median age (IQR) at the time of inclusion in the first vaccine trial was 44.7 years (39-50) and the median age (IQR) at the time of the last follow-up visit in the cohort was 58.0 years (51-63). Participants received a median of 4 injections (IQR=[3-5]). The consent for the prospective follow-up of 355 (72.7%) participants was obtained, varying from 20% to 89% depending on trial and 133 (27.3%) volunteers only consented to the retrospective collection of data (Figure 1).

Behavioral characteristics at the time of vaccine trial screening

Of the 488 participants included in the cohort, the median age at the time of vaccine trial start was 41.0 years for men and 45.5 years for women. About 80% of selected volunteers lived as a couple. The nature of sexual relations was, for men and women respectively, heterosexual (84%; 90%), homosexual (12%; 6%) or bisexual (4%; 4%). Multi-partnership (at least 2 partners) in the previous year was reported by 11% of homosexual men, 4% of heterosexual men and 2% of heterosexual women. The main motivations for participating in these trials were altruism in 85% of cases, being close to a person living with HIV in 47% of cases, and 'for the next generation' in 48% of cases. The other motivations were an interest in research or medicine (n=8), to help Africa (n=6), a connection with the HIV/AIDS community (n=4) and a desire to be helpful (n=5).

Socio-demographics at the time of inclusion in the cohort

At the time of inclusion in the prospective follow-up, 87% of volunteers had a level of education higher than or equal to the baccalaureate, 80% were employed, 72% lived with a partner, 43% had children living in the household, 74% owned their home, 76% were involved in an association, political party or labor union, 40% were registered as blood

donors and 18% as bone marrow donors, and 62% reported not being interested in religion. About half of the subjects reported knowing personally at least one person living with HIV.

Post-vaccination follow-up

For the 488 volunteers, the median follow-up duration (IQR) from the first vaccine candidate administration was 9.6 years (5.4-14.2) and the maximum was 24.3 years. This total follow-up period was 4,934 person-years (PY). The prospective follow-up was initially scheduled for 7 years (8 annual consultations). In 2016, due to the absence of safety signal, an amendment was made to terminate the study earlier. Regardless of this amendment, 85/355 (24%) subjects did not complete the follow-up: 3 died after their inclusion, 45 were lost to follow-up, 35 withdrawn their consent, 1 was unavailable and 1 moved abroad (Figure 1).

Safety

The overall incidence rates (95% CI) of all-grade and grade 3-4 AEs were 15.3 per 100 PY and 8.0 per 100 PY respectively (Table 2). These incidence rates were similar in the population with prospective and retrospective follow-up and in the population with retrospective follow-up only. No difference was found for grade 3-4 AEs (6.4 vs 7.5 per 100 PY) during the retrospective follow-up. The incidence rate of all-grade AEs during the retrospective follow-up was lower for subjects included in the prospective cohort compared to those who were not included (12.5 vs. 18.2 per 100 PY). The incidence rate (95% CI) of SAEs during the prospective follow-up was 8.1 (6.9-9.6) per 100 PY. None of the 148 SAEs was considered as possibly related to preventive HIV vaccine candidates by the investigator or the sponsor. Among them, one primary HIV infection occurred 7 years after inclusion in a HIV lipopeptide trial. When the vaccine trial groups defined in Table 1 were considered, the incidence rates of all-grade AEs were similar during the total follow-up and those of SAEs were similar during the prospective follow-up.

Eight subjects died, including 5 during the retrospective follow-up (breast cancer, ovarian carcinoma, amyotrophic lateral sclerosis, drowning, and suicide) and 3 during the prospective follow-up (bladder cancer, lung cancer, refractory anemia with excess blasts).

Regarding AEs of special interest, the incidence rate of neurological problems, including sciatica and migraine, was 4.6 per 100 PY (number of AEs = 229); 41 were of grade 3 and 3 of grade 4 (generalized tonic-clonic seizure, amyotrophic lateral sclerosis, venous meningeal hemorrhage). The incidence rate of ophthalmological AEs was 2.1 per 100 PY (N = 103), and the incidence rate of cataract, glaucoma and autoimmune diseases was 0.2 per 100 PY (N = 11; 4 autoimmune thyroiditis, 2 rheumatoid arthritis, 1 allergic cough, 1 allergy to an angiotensin-converting-enzyme inhibitor, 1 Basedow's disease, 1 immune thrombocytopenic purpura, 1 urticaria).

Among the 3 cases of grade 2 uveitis, one case was anterior uveitis occurring 1 year after the last injection of vCP and HIV lipopeptides (VAC03/09 trials) and another case occurred 3

years after vCP and rgp160 injections (VAC01 trial). The third case occurred 4 months after the last administration of vCP and HIV lipopeptides (VAC03/09bis trials) and as previously described[6]. Among nervous system disorders, a case of narcolepsy (in a 58-year old man with a family history of narcolepsy) occurred 4 years after DNA and NYVAC administrations in the VAC20 trial.

The mortality of women did not differ from that of the French population[18] (4 observed, 2,047 PY, 6.11 expected, SMR=0.65; 95% CI, 0.18-1.67; p=0.28) while that of men was significantly lower (4 observed, 2,757 PY, 15.48 expected, SMR=0.26; 95% CI, 0.07-0.66; p=0.0003).

The most common AEs (with at least 5 occurrences) are described in Table 3. Among them, 6 cases of invasive breast cancer were included based on the MedDRa preferred terms of breast cancer (N=4), breast cancer metastatic (N=1), invasive ductal breast carcinoma (N=1). The expected number of cases was 4.07 and the corresponding age-adjusted SIR was 1.47 (95% CI, 0.54–3.20; p=0.45) which did not differ from that of the French women general population. In situ breast cancer was not considered for comparison to the general population (N=1) due to a lack of homogeneity with the French register[19]. For herpes zoster, reference data were available from 2005 to 2015 for the first consultation during the acute phase, except for post-herpetic pain, and the subsequent visit for the same episode[20]. Over this period and with this definition, 13 cases were observed (herpes zoster (N=6), herpes zoster infection neurological (N=5), herpes zoster cutaneous disseminated (N=1), ophthalmic herpes zoster (N=1)). In women, 7 cases were observed, 8.32 cases were expected and the corresponding age-adjusted SIR was 0.84 (95% CI, 0.34, 1.73; p=0.55). In men, 6 cases were observed, 7.48 cases were expected and the corresponding age-adjusted SIR was 0.80 (95% CI, 0.29, 1.75; p=0.49). No increased incidence rates were observed compared to the French population.

No evidence of long-term vaccine-related AEs was observed following subject participation in a HIV preventive vaccine trial.

Vaccine-induced antibodies at the end of the follow-up

At the time of the inclusion in the prospective cohort (Y0), the centralized ARCHITECT® assay was used to re-test all subjects (except those who received DNA/NYVAC vaccines)[22]. Over the 2008-2012 period, two routine test kits were used for each subject following the recommendations of the French authorities. As many vaccine combinations were used (13 in 355 participants) and a good agreement was found with the centralized ARCHITECT® results (Kappa= 0.683, 95% CI: 0.527-0.838), we only considered the results obtained with the centralized ARCHITECT® test.

At the last follow-up visit, in line with the results found at Y0 using the centralized ARCHITECT® assay[22], only participants who received rgp160 had a positive HIV serology:

21/29 volunteers in the VAC01/05 and VAC02/06 trials (72.4%, 95% CI: 52.8%, 87.3%) after a median time (range) of 23.6 years (17.6-24.3) following vaccination (Figure 2a).

Among the 29 volunteers who received rgp160, the serological status at Y0 and at the last follow-up visit did not change significantly as shown in Figure 2b (Mc Nemar p-value=0.65). In the VAC02/06 trials (Figure 3), the median signal-to-cut-off ratios using the Architect® EIA were 1.70 (range: 0.19-14.04) and 1.33 (range: 2.13-15.24) at Y0 and at the last follow-up visit, respectively. In the VAC01/05 trials, the median S/CO ratios were 3.59 (range: 0.84-13.33) using the Architect® EIA and 14.05 (range: 1.5-31.8) using the LIAISON® XL MUREX assay at Y0 and at the last follow-up visit, respectively.

At the last follow-up visit, 4 volunteers of the VAC01/05 trials and 4 volunteers of the VAC02/06 trials had 1 or 2 positive bands among the p55, p18, p24, p68, and gp160 proteins. These bands, p55, p18, p24 (3 from HIV Gag protein), p68 (HIV Pol), gp160 (HIV Env) corresponded to components of the vaccine candidates received by these participants.

Behavior following participation in preventive HIV vaccine trials

Overall, 25% of men and 11% of women reported having had at least one casual partner in the past 12 months and 10% of men and 1% of women had condomless sexual intercourses with partners of unknown HIV status. One participant was infected with HIV by homosexual transmission after his third yearly follow-up visit. Thus, a small and stable percentage of volunteers indicated risky sexual practices several years after their screening based on a low risk of exposure to HIV.

Discussion

In the French ANRS COV1-COHVAC cohort, volunteers showed a sustained high commitment to research (73% consented to the prospective follow-up) and a high retention rate (76% completed their follow-up). The follow-up was carried out for 4,934 person-years (median: 9.6 years, up to 24 years) in 488 volunteers who participated in 17 trials assessing HIV envelope protein, canarypox vectors, lipopeptides, and other vaccines (see Table 1). This is the second largest study apart from the report of 3,189 volunteers during 12,340 person-years in the United States[1]. No long-term safety concerns were identified in the US report in which similar vaccines than those administered to the French cohort were tested.

Our analysis did not identify any safety signals. No death, SAE or AE suggesting an ophthalmological, neurological, or autoimmune disease was classified as probably or definitely related to the vaccines. As breast cancer is the most common cancer in women in France, its incidence could be assessed in the COHVAC cohort since the collection of cases was complete and the source population was exhaustive.No excess in the number of cases was observed as compared to the French register. Similar findings were found for the

incidence of herpes zoster infection. Neurological and ophthalmological AEs reported by more than 2% of our participants are commonly reported in the general population, including migraine, sciatica, carpal tunnel syndrome, cervical radiculopathy, cataract, and glaucoma. No autoimmune complications were identified. Anterior uveitis may be associated with inflammatory diseases that may result from an infection such as herpes virus infection or from autoimmune diseases[23, 24]. Narcolepsy, which has a potential autoimmune mechanism[25], has been described during the 2009 pandemic flu vaccination campaign in young people[26]. In our cohort, one 50-year old participant was diagnosed with narcolepsy several years after completion of the VAC20 trial and the diagnosis was made in a context of family history, supporting an absence of relationship with the HIV vaccine received.

Regarding the sustained presence of vaccine-induced antibodies, a recent study conducted in participants included in HVTN studies and in the HVTN 910 (VISP) cohort has shown that vaccine-induced serum IgG responses persisted in some subjects for more than 10 years after vaccination[27]. In a long-term extension study of participants who received mosaic-based, prime-boost vaccine regimens for HIV-1, the seropositivity persisted up to week 120 post-vaccination[28]. In the COHVAC cohort, the seropositivity persisted for up to 23 years after vaccination in participants who received rgp160 with or without recombinant vCP, whereas the antibody levels were similar to those of false positives[29]. This was especially observed when vCP125 and then recombinant gp160 were administered (in the VAC01 trial), as previously shown with the combination used in the RV144 trial, i.e. a recombinant canarypox vector vaccine (ALVAC-HIV [vCP1521]) plus two booster injections of a recombinant gp120 subunit vaccine (AIDSVAX B/E)[30]. As with other vaccines (hepatitis B vaccine[31], human papillomavirus vaccine[32]), recombinant proteins may induce the production of persistent antibodies. Post-vaccination seropositivity does not confer any HIV protection and this was explained to the participants, but social impacts in regards to HIV testing for bank loans and blood donation are possible.

Volunteers of this French cohort had a high social level and a strong involvement in social life and they could be not representative of other cohorts of volunteers who received financial compensation[33]. Their participation in vaccine trials did not increase their exposure to HIV infection and only one case of HIV infection occurred during the follow-up. Only one study has reported an increase in risky sexual behaviors[34], unlike several other studies conducted in different countries[35-37]. Regarding sexual behaviors, the questionnaires used in the cohort were different from those used at the time of the selection. It was not deemed possible to directly compare these two periods. However, the overall results did not indicate any significant change in behaviors compared to the pre-vaccination period.

This study has some limitations. Due to the absence of control subjects, whenever possible data from the general population (mortality and some specific morbidities) were used as a reference group, standardized for age. Potential vaccine-associated AEs are rare and the estimation of their expected incidence rates in the reference French population is needed,

based on the same definition. This limited the comparisons we could perform for breast cancer and herpes zoster, apart from mortality. A potential “healthy volunteer effect” can be suspected based on the comparison of the mortality rate with that of the general population[38]. Regarding vaccine-induced seropositivity, some participants were alternately negative and positive over time due to changes in commercial tests and low levels of antibodies. The functionality and specificity of vaccine-induced antibodies could not be characterized due to their low levels.

The main strength of this study was that it could include almost all the participants in preventive HIV vaccine trials in France, even for trials conducted in the nineties. As the incidence of AEs did not differ between participants who had a prospective follow-up and those who only had a retrospective follow-up, the approach combining both types of participants was validated. The difference in incidence rates of all AEs during the retrospective follow-up between the prospective and retrospective populations can be explained by that, for most participants who only had a retrospective follow-up, the follow-up was restricted to the trial period during which the monitoring and frequency of AE collection were very close. Of note, the incidence of grade 3-4 AEs was similar. Retention was particularly remarkable despite the absence of financial compensation due to a high commitment of the volunteers.

The ANRS COV1-COHVAC cohort provided unique data on the long-term follow-up of subjects who received preventive HIV vaccines. Data on safety showed that HIV vaccine candidates tested in France were well tolerated. The sustainability of vaccine-induced seropositivity with a possible impact on HIV testing results should be taken into account when informing future participants.

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Author contributions

CD, CDe, J-DL, BS, GP, LC, BB, IPM, AB, SG, BSp, LM, and OL contributed to the design of the study. CDe, J-DL, BS, GP, LC, BB, IPM, AB, CP, and OL contributed to data collection. CD, CDe, J-DL, CP, BSp, LM, and OL contributed to data analysis and interpretation of results. CD, CDe, J-DL, LM, and OL contributed to manuscript preparation. All authors reviewed and approved the final draft of the manuscript.

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Figure 1:

Study flow chart showing the selection, inclusion and follow-up steps of subjects participating in the COHVAC cohort. Eligible participants were healthy volunteers who received at least one dose of a HIV vaccine candidate except a placebo. Y0 to Y7: years 0 to 7

Figure 2:

(a) Positive HIV serology in the COHVAC cohort at the start of the follow-up (Y0) and at the last follow-up (Last FU) visit according to the group (see Table 1).

(b) Individual HIV serology (9 volunteers from the VAC01 trial, 20 volunteers from the VAC02 trial) during the follow-up of the cohort according to the time (years) from the beginning of the vaccine trial and Western-Blot bands at the last FU visit when the HIV ELISA was positive. Tests used at the last FU visit were LIAISON® XL MUREX HIV Ab/Ag HT except *VIDAS® HIV DUO Ultra for the VAC01 trial and ARCHITECT® HIV Ag/Ab combo except **cobas® HIV combi PT assay for the VAC02 trial. Y0-Y7 values are Signal-to-cut-off Ratios. Grey: at least 1 positive EIA test; white: 0 positive EIA test; np: Visit not performed.

Table 1. ANRS HIV preventive vaccine trials and inclusions in COHVAC cohort. Number (N) and percentage (%) of participants

Group	Trial 1	& Trial 2	Vaccine intervention Trial 1	Vaccine intervention Trial 2	Year of Start	Trial N	Cohort Refusal N (%)	Prospective cohort N (%)	Retrospective cohort only* N (%)
rgp160	VAC01	-	vCP125+ rgp160	-	1992	5		1 (20)	4 (80)
	VAC01	& VAC05		vCP125 or vCP65 or vCP205	1995	15		8 (53)	7 47)
	VAC02	-	rgp160+ peptide V3	-	1992	9		8 (89)	1 (11)
	VAC02	& VAC06		vCP65 or vCP125	1995	16		12 (75)	4 (25)
rgp160 muc	VAC14	-	rgp160muc ± DC-Chol	-	2003	34		29 (85)	5 (15)
vCP	VAC03	-	vCP205 + CLTB-36-alum	-	1994	9		6 (67)	3 (33)
	VAC03	& VAC08		vcP205 + CLTB-36-alum or CLTB-36-QS21 or CLTB-36-alum	1995	13		12 (92)	1 (8)
	VAC03	& VAC09		LIPO-6 ± QS-21	1997	8		8 (100)	
	LIP03	-	vCP1452	-	1999	3		1 (33)	2 (66)
	VAC07	-	vCP300	-	1995	5		1 (20)	4 (80)
	VAC07	& VAC09		LIPO-6 ± QS-21	1997	15		11 (73)	4 (27)
vCP or LIPO	VAC10	-	vCP1452 or LIPO-5 or LIPO-6T	-	1999	34		22 (65)	12 (35)
	VAC10	& VAC17		LIPO-6T	2002	21		12 (57)	9 (43)
LIPO	VAC04	-	LIPO-6 ± QS-21	-	1996	28		13 (46)	15 (54)
	VAC12	-	LIPO-4	-	2001	15		11 (73)	4 (27)
	VAC16	-	LIPO-4	-	2004	68	2 (3)	45 (66)	21 (32)
	VAC17	-	LIPO-6T	-	2002	10		6 (60)	4 (40)
	VAC18	-	LIPO-5	-	2004	114	6 (6)	90 (79)	18 (17)
DNA /NYVAC	VAC20	-	DNA/NYVAC	-	2007	74		59 (80)	15 (20)
	Total					496	8 (2)	355 (72)	133 (27)

rgp160 group: candidate recombinant envelope glycoproteins (rgp160) alone or with canarypox (vCP) vectors; rgp160muc group: mucosal recombinant glycoproteins (rgp160); vCP group: canarypox vectors (vCP) alone or in combination with other products than recombinant glycoproteins; LIPO group: lipopeptides (LIPO) alone; DNA/Nyvac group: DNA prime/poxvirus NYVAC boost. Trial 2 : - for none

rgp160: recombinant HIV-1 envelope protein (rgp160)

peptide V3: synthetic HIV-1 peptide (V3 loop)

vCP125, vCP205, vCP65, vCP300, vCP1452 : canarypox ALVAC vectors expressing Env, Gag, Pro and CTL domains of Pol and Nef

CLTB-36: synthetic peptide comprising a T-helper epitope (p24) and a B-cell epitope (V3)
LIPO-6, LIPO-4, LIPO-6T, LIPO-5 : HIV-1 lipopeptides whose sequences represent CTL-epitopes of HIV-1 Gag, Pol and Nef proteins
DNA/NYVAC: DNA prime/poxvirus NYVAC boost both expressing a common HIV-1 clade C immunogen consisting of Env and Gag-Pol-Nef polypeptide
alum, QS-21, DC-Chol : adjuvants
*No response to the invitation to participate (N=114) or deceased before inclusions in the prospective cohort (N=5)

Table 2. Adverse events (AEs) incidence rates (IR) for the entire COHVAC cohort and by vaccine trial group for all AEs, grade≥3 events in total follow-up (FU) and SAEs in prospective FU

		All volunteers (N=488; 4934 PY)		Volunteers with Prospective and Retrospective FU (N=355 ; 4466 PY)		Volunteers with Retrospective FU only (N=133; 468 PY)	
		No. AEs	IR per 100 PY (95% CI)	No. AEs	IR per 100 PY (95% CI)	No. AEs	IR per 100 PY (95% CI)
All AEs		756	15.3 (14.2-16.5)	671	15.0 (13.9-16.2)	85	18.2 (14.5-22.5)
Grade≥3 AEs		397	8.0 (7.3-8.9)	362	8.1 (7.3-9.0)	35	7.5 (5.2-10.4)
				Prospective FU (N=355; 1802 PY)		Retrospective FU (N=355; 2664 PY)	
All AEs				337	18.7 (16.8-20.8)	334	12.5 (11.2-14.0)
Grade≥3 AEs				192	10.7 (9.2-12.3)	170	6.4 (5.5-7.4)
SAEs				148	8.1 (6.9-9.6)		
	Vaccine Trial Group						
All AEs	rgp160	119	14.9 (12.4-17.9)				
	rgp160muc	57	17.2 (13.0-22.2)				
	vCP	162	13.7 (11.7-16.0)				
	LIPO	351	16.1 (14.5-17.9)				
	DNA/NYVAC	67	14.9 (11.5-18.9)				
Grade≥3 AEs	rgp160	79	9.9 (7.9-12.4)				
	rgp160muc	33	9.9 (6.8-14.0)				
	vCP	91	7.7 (6.2-9.5)				
	LIPO	163	7.5 (6.4-8.7)				
	DNA/NYVAC	31	6.9 (4.7-9.8)				
SAEs	rgp160			22	12.4 (7.8-18.8)		
	rgp160muc			18	11.2 (6.6-17.7)		
	vCP			24	8.2 (5.3-12.2)		
	LIPO			71	7.2 (5.6-9.1)		
	DNA/NYVAC			13	6.8 (3.6-11.7)		

FU, follow-up; PY, person-years; SAE: serious adverse events; IR (Incidence Rate) Events/100 PY; 95% CI: 95% confidence interval; Vaccine trial groups (see Table 1): rgp160 (N=46, 796 PY); rgp160muc (N=34, 332 PY); vCP (N=77, 1182 PY); LIPO (N=257, 2174 PY); DNA/NYVAC (N=74, 450 PY); in prospective FU: rgp160 (N=29, 177 PY); rgp160muc (N=29 , 161 PY); vCP (N=56, 292 PY) ;LIPO(N=182, 982 PY) ;DNA/NYVAC (N=59, 190 PY).

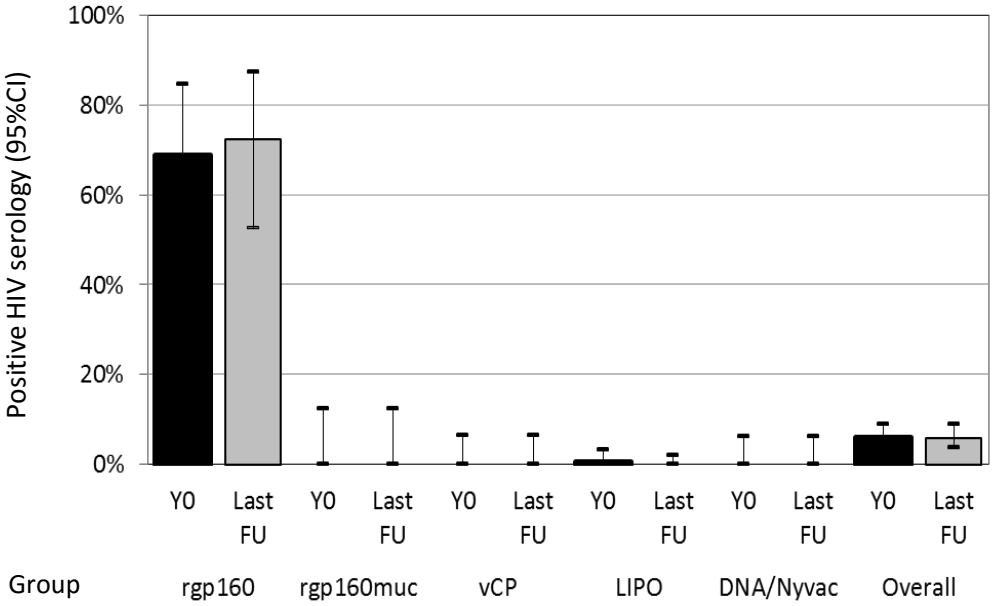
Table 3. Most frequent Adverse Events (AE). Preferred Terms with at least 5 occurrences by System Organ Class in alphabetical order

				No. AEs by Grade				
System Organ Class	Preferred Term	No. AEs	Volunteers with ≥ 1 AE (%)	NA	1	2	3	4
Cardiac disorders	Myocardial infarction	6	6 (1.2)				2	4
Eye disorders	Cataract	19	14 (2.9)		3	11	5	
	Glaucoma	11	11 (2.3)		2	9		
	Ocular hypertension	8	7 (1.4)		1	7		
	Chalazion	5	5 (1.0)		3	2		
	Retinal detachment	5	4 (0.8)			3	2	
Gastrointestinal disorders	Inguinal hernia	10	9 (1.8)			2	8	
	Large intestine polyp	7	7 (1.4)				7	
General disorders and administration site conditions	Death	8	8 (1.6)					8
Infections and infestations	Herpes zoster	7	7 (1.4)			6	1	
	Herpes zoster infection neurological	5	5 (1.0)			4	1	
	Influenza	5	5 (1.0)				5	
Musculoskeletal and connective tissue disorders	Intervertebral disc protrusion	17	15 (3.1)				17	
	Osteoarthritis	16	14 (2.9)			2	14	
	Back pain	11	11 (2.3)				11	
	Rotator cuff syndrome	7	6 (1.2)			3	4	
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Basal cell carcinoma	9	5 (1.0)			1	8	
	Uterine leiomyoma	5	5 (1.0)				4	1
Nervous system disorders	Migraine	50	30 (6.1)	4	13	26	7	
	Sciatica	43	38 (7.8)	1	6	30	6	
	Carpal tunnel syndrome	17	16 (3.3)		5	6	6	

System Organ Class	Preferred Term	No. AEs	Volunteers with ≥ 1 AE (%)	No. AEs by Grade				
				NA	1	2	3	4
	Cervical radiculopathy	12	11 (2.3)		1	11		
	Dizziness	10	9 (1.8)		8	2		
	Hypoaesthesia	8	7 (1.4)		7	1		
	Paraesthesia	8	8 (1.6)		7	1		
	Muscle contractions involuntary	7	4 (0.8)		5	1	1	
	Ischaemic stroke	5	3 (0.6)			3	2	
	Migraine with aura	5	5 (1.0)		1	4		
	Presyncope	5	4 (0.8)		2	2	1	
Vascular disorders	Hypertension	6	6 (1.2)			1	5	

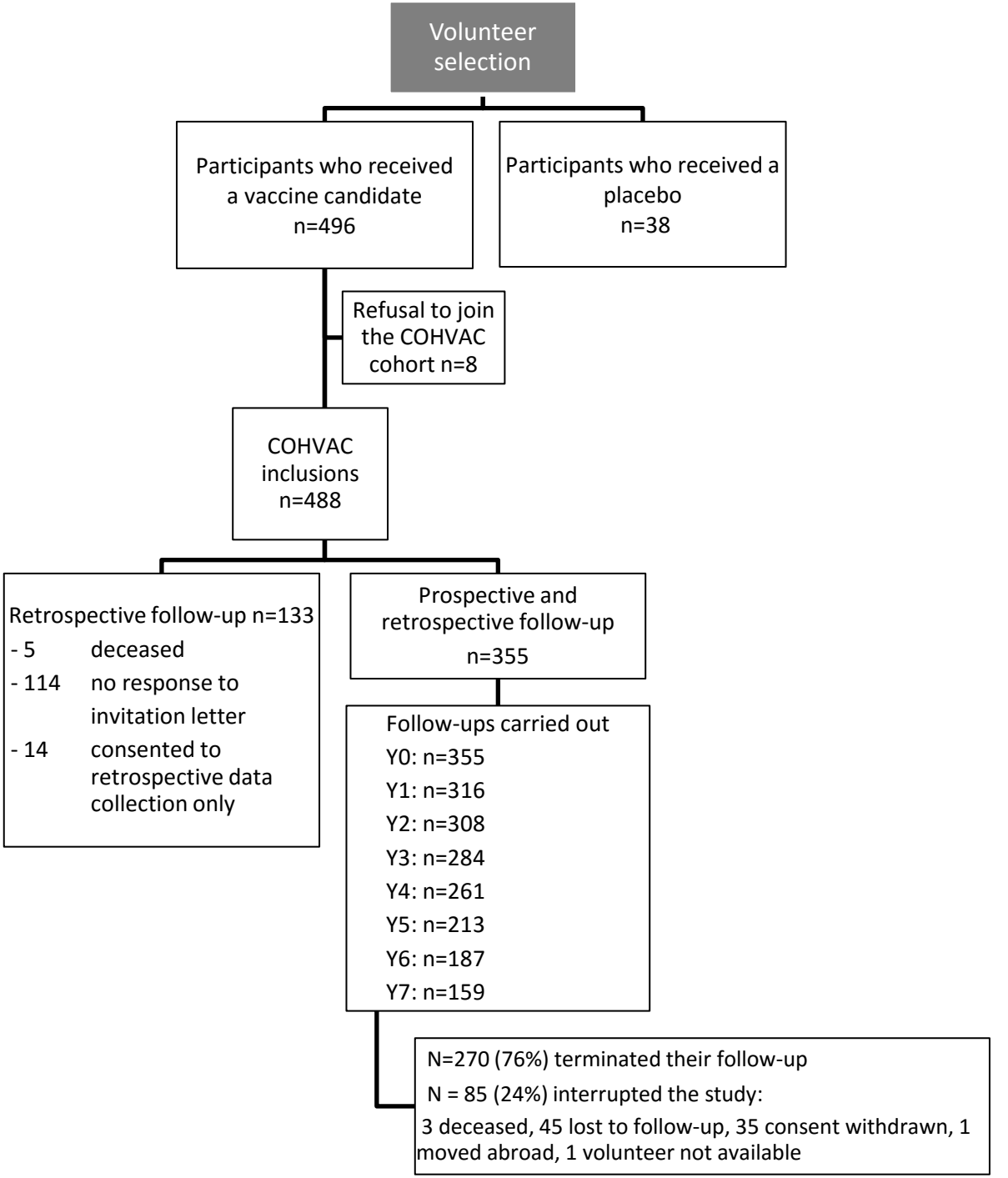
NA: grade not available.

(a)



(b)

VAC	VAC	Vaccine candidates	Architect SC/Ratio Y0	Local EIA test Y0	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Last FU and positive EIA: Years since trial	WB bands
01	-	vCP, rgp160	3.02						nd	25.6	nd	24.3	-
01	05	vCP, rgp160, vCP	5.63							9.2	nd	22.7	P55
01	05	vCP, rgp160, vCP	0.84								1.5	23.6	-
01	05	vCP, rgp160, vCP	7.6						16.5		nd	21.6	P68
01	05	vCP, rgp160, vCP	1.02		9.5*	nd	nd	nd	nd	nd	nd	17.6	-
01	05	vCP, rgp160, vCP	3.59								11.6	23.7	-
01	05	vCP, rgp160, vCP	13.33				nd		18.0	nd	nd	21.6	P24, P55
01	05	vCP, rgp160, vCP	2.06				nd	4.4	nd	nd	nd	20.5	P24, P55
01	05	vCP, rgp160, vCP	12.58								31.8	23.7	-
02	-	rgp160, peptV3	2.03				nd		nd		1.33	23.8	P18, P55
02	-	rgp160, peptV3	2.5								2.13	23.7	-
02	-	rgp160, peptV3	1.48								1.05	23.7	-
02	-	rgp160, peptV3	0.97								0.82		
02	-	rgp160, peptV3	14.04							15.24	nd	23.8	GP160
02	-	rgp160	3.44				1.4	nd	nd	nd	nd	19.9	P18, P55
02	-	rgp160, peptV3	0.32								0.24		
02	-	rgp160, peptV3	0.19						0.27	nd	nd		
02	06	rgp160, vCP	0.94								na		
02	06	rgp160, vCP	0.61								0.54		
02	06	rgp160,peptV3,vCP	13.51				nd				9.16	24	-
02	06	rgp160,peptV3,vCP	13.39								8.41	23.3	-
02	06	rgp160,peptV3,vCP	1.68								1.30	23.5	P18
02	06	rgp160,peptV3,vCP	0.84								1.00	23.6	-
02	06	rgp160,peptV3,vCP	3.14								2.53	23.6	-
02	06	rgp160,peptV3,vCP	0.5								3.25**	23.7	-
02	06	rgp160,peptV3,vCP	1.88				0.9	nd	nd	nd	nd		
02	06	rgp160,peptV3,vCP	1.96								1.92	23.6	-
02	06	rgp160,peptV3,vCP	1.72					0.92	nd	nd	nd		
02	06	rgp160,peptV3,vCP	0.66							0.33	nd		


Volunteer selection
Participants who received a vaccine candidate n=496
Participants who received a placebo n=38
Refusal to join the COHVAC cohort n=8
COHVAC inclusions n=488
Retrospective follow-up n=133
- 5 deceased
- 114 no response to invitation letter
- 14 consented to retrospective data collection only
Prospective and retrospective follow-up n=355
Follow-ups carried out
Y0: n=355
Y1: n=316
Y2: n=308
Y3: n=284
Y4: n=261
Y5: n=213
Y6: n=187
Y7: n=159
N=270 (76%) terminated their follow-up
N = 85 (24%) interrupted the study:
3 deceased, 45 lost to follow-up, 35 consent withdrawn, 1 moved abroad, 1 volunteer not available

Supplementary Table : Definition of "Low risk of HIV infection" in ANRS preventive HIV vaccine trials

ANRS Volunteer Network **ANRS VAC01 to VAC18 trials**	**ANRS VAC20 trial**
No history of injecting drug use in the past	No history of injecting drug use in the previous ten years No gonorrhoea or syphilis in the last six months
No high risk partner (e.g. injecting drug use, HIV positive partner) in the past	No high risk partner (e.g. injecting drug use, HIV positive partner) either currently or within the past six months
No more than one sexual partner in the last two years	
No history of unprotected sex	No unprotected anal intercourse in the last six months, outside a relationship with a regular partner known to be HIV negative No unprotected vaginal intercourse in the last six months outside a relationship with a regular known/presumed HIV negative partner