

INTRODUCTION

EuroVacc is a scientific programme to design, manufacture and assess pre-clinically 7 candidate HIV vaccines, and to conduct primate and human clinical trials.

The first to emerge from this pipeline into a randomised, blinded clinical trial is a pox-derived viral vector vaccine, NYVAC-HIV-C, a recombinant NYVAC (vP986) strain with an insert containing gag-pol-nef polyprotein and env both derived from the Chinese R5 HIV-1 clade C virus (97CN54) [1]. The multigenic recombinant NYVAC VP2010 (env, gag, pol, nef of HIV-1 clade C) has been shown to be stable for the HIV genes. Moreover, HIV clade C genes are also representative of strains circulating in China, India and South Part of Africa.

The best studied vaccine vectors in humans are the pox viruses. Vaccinia virus engineered with HIV-1 genes have been shown to induce virus-specific cellular and humoral immune responses in immunized macaques and protection against simian immunodeficiency virus (SIV) infection when immunization with such constructs have been followed with boosting by recombinant proteins [2].

However, due to the development of life-threatening disseminated vaccinia infections in immunosuppressed individuals [3], there is a reluctance to use vaccinia virus as a vector system in large human trials, where unsuspected HIV infection may occur. Particular attention has been, however, focused on pox viruses with limited in vivo replicative capacity and, therefore, limited pathogenicity, e.g. NYVAC, which has deletions of the genes associated with pathogenicity, and which do not complete an entire replication cycle in human cells, but initiate protein synthesis and thus elicit immune responses.

This proposal to study a modified pox viral vector is innovative in the following:

1. HIV subtype C accounts for the most rapidly emerging HIV epidemics in southern Africa, India and China. This novel vaccinia construct expressing HIV subtype C gag, pol, env and nef antigens is to be studied in humans for the first time.
2. The induction of cross-reactive CTL in relation to priming with C subtype NYVAC will be examined and compared to priming with B subtype NYVAC on frozen specimens when the equivalent NYVAC B trial has been completed.

PRIMARY OBJECTIVES OF EV01 CLINICAL TRIAL

To evaluate the **safety and immunogenicity** of two intramuscular injections of NYVAC-HIV-C in healthy female and male volunteers at low risk of HIV infection.

METHODS

We vaccinated 24 healthy low risk HIV-negative volunteers, recruited from two clinical sites: 12 in Switzerland (CHUV, Lausanne) and 12 in the United Kingdom (Imperial College, London).

Randomisation to NYVAC-HIV-C (n=20) or placebo (n=4) was stratified by clinical site. Scheduled immunisations – at weeks 0 and 4 – began in August 2003.

Local and systemic adverse events were assessed at 10 minutes, 1 hour, and 1 to 7 days after vaccination. The primary safety endpoints are grade 3 (severe) or 4 (extreme) adverse events (AE) reported within 28 days of an immunisation, presented as a proportion of participants exposed to NYVAC-HIV-C with 95% confidence intervals, and any events leading to discontinuation of the immunisation schedule.

The immunogenicity was evaluated by interferon- γ ELISpot assays on cryo-preserved blood mononuclear cells at weeks 0, 4, 6, 8, 24, and 48 with 8 pools of 49 to 61 peptides (15-mers overlapping by 11) encompassing the gag-pol-nef, and env regions. These peptides were obtained from the Chinese R5 HIV clade C virus (97CN54). All ELISpot assays had a background below 50 spots/10⁶ cells and a positive control above 500 spots/10⁶ cells but responses were regarded as positive only if they were at least 4-fold the background and higher than 55 spots/10⁶ cells.

STUDY DESIGN

Volunteers were randomised to receive two injections of either vaccine or placebo at week 0 and 4. The role of placebo was not to act as a parallel comparison in the analysis, but to minimise observer bias in clinical and laboratory assessments and to further reduce the possibility that volunteers may believe themselves to be "protected" from HIV. Participants, clinical investigators and cellular immunology were blind to the allocation throughout the study.

Weeks	-4	0	1	4	6	8	24	48
Immunisation		X		X				
ELISpot		X		X	X	X	X	X
Antibodies		X		X			X	X

Table 1 : Schedule of immunisation, timepoints for ELISpot and antibodies assays of EV01.

RESULTS

24 (11 female) of 36 volunteers screened have been enrolled and received both immunisations.

Characteristic	Centre		
	CHUV	St. Mary's	Total
Sex			
Female	6 (50%)	5 (42%)	11 (46%)
Median age (range) in years	35 (21-52)	33 (22-50)	33 (21-52)
Median weight (range) in kg			
Male	68 (57-70)	80 (67-94)	70 (57-94)
Female	65 (55-86)	62 (57-70)	64 (55-86)
TOTAL	66 (55-86)	73 (57-94)	68 (55-94)
Median height (range) in cm			
Male	175 (169-185)	180 (170-189)	178 (169-189)
Female	167 (153-171)	168 (159-177)	168 (153-177)
TOTAL	171 (153-185)	176 (159-189)	172 (153-189)
No. participants who have received:			
one vaccination	0	0	0
two vaccinations	12 (100%)	12 (100%)	24 (100%)

Table 2 : Trial population, and vaccinations received.

Safety

Solicited adverse effects (pain, itching, redness, swelling, temperature, chills/rigors, malaise, myalgia, headaches, nausea) were observed in 92% of the 24 participants after both vaccinations – 71% grade 1 and 21% grade II – and more frequently after the second vaccination. No grade III or IV adverse effect was reported.

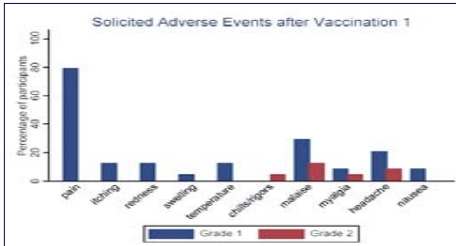


Figure 1 : Solicited adverse events after first vaccination

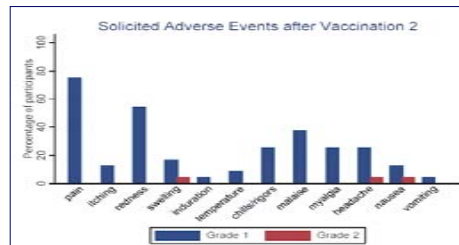


Figure 2 : Solicited adverse events after second vaccination.

Immunogenicity

The immunogenicity was evaluated by interferon- γ ELISpot assays on cryo-preserved blood mononuclear cells. A mix of CMV, EBV and Flu peptides (CEF) was used as positive control. The inter-timepoints variability of the peptide pool control was assessed : mean of the relative SD was 22.9%.

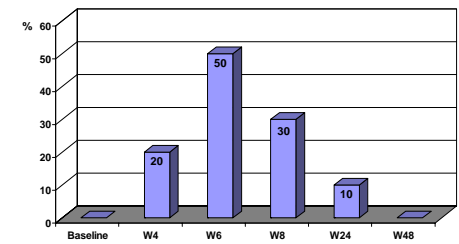


Figure 3 : Percentage of vaccinees (swiss volunteers) per timepoint showing positive specific responses induced following immunization

Vaccine-specific responses were generated in 5 of the 10 vaccinees from Switzerland (for technical reasons, results are not available for the 10 vaccinees from the United Kingdom until week 24; results for week 24 and 48 from UK volunteers are not shown; however, we can mention that vaccine-specific responses were obtained in 2 vaccine recipients (out of 10) at week 24, and 1 at week 48).

All 5 volunteers (50%) gave vaccine-specific positive responses at week 6, while only 2 were positive at week 4, 3 at week 8, and 1 at week 24. In addition, positive ELISpot responses were observed at week 24 in 2 of 10 vaccinees from the United Kingdom. No response was detected at week 48.

Of interest, 4 volunteers showed consistent positive responses to 1 (ENV1) of the 2 peptide pools covering env, while responses to the other env pool (ENV2) were also found in 2 volunteers. Furthermore, responses to both gag pools were also observed in 2 subjects, and responses to the nef pool in 2 volunteers.

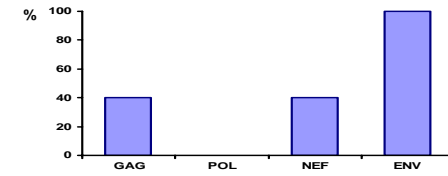


Figure 4 : Percentage of HIV proteins recognized by responders at any timepoint.

The fine mapping of the epitopes showed that both CD4 and CD8 T-cell responses were generated and some of these epitopes had already been described in HIV-infected patients.

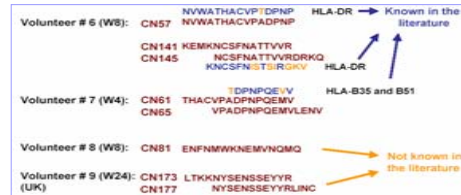


Figure 5 : Results from the mapping of ENV1c responses

Furthermore, CD4 and CD8 specific responses were also determined by flow cytometry (data not shown).

CONCLUSIONS

- NYVAC – HIV-C vaccine was **safe and well tolerated**
- Vaccine-specific immune responses were generated in **50%** (5 out of 10) of the vaccine recipients
- There is a peak of the responses at **week 6** which is the primary endpoint
- Env-specific responses were found in all subjects but additional responses against peptide of other proteins (eg. Gag and Nef) were detected in 40% of the responders
- Mapping of these peptides indicate that these responses are mediated by both **CD4** and **CD8** T cells

REFERENCES

- Su L et al. J Virol 2000;74:11367-76
- Excler J-L and Plotkin S. AIDS 1997;11:1517
- Redfield RR et al. NEJM 1987;316:673