Optimal priming of poxvirus vector (NYVAC)-based HIV vaccine regimens requires 3 DNA injections.

*Long term Results of the EV03/ANRS Vac20 Phase I/II Trial.*

Trial Objectives

A Phase I/II trial to compare the **immunogenicity** and **safety** of 3 DNA-C prime followed by 1 NYVAC-C boost to 2 DNA-C prime followed by 2 NYVAC-C boosts in healthy volunteers at low risk of HIV infection
Clinical Trial Design

- Randomized trial with a parallel group design
- No significant differences between groups in demographic characteristics at entry
- Open to the participants and investigators but blind to laboratory personnel
- Attendance to clinics at least 14 occasions over 72 weeks

Weeks:
0 4 8 20 24 28 48 72

Group 1 (n=74):
- DNA-C (4 mg) priming at week 0, 4 and 8 for group 1 at week 0 and 4 for group 2

Group 2 (n=73):
- NYVAC-C (10^{7.5} PFUs) boosting at week 24 for group 1 and at week 20 and 24 for group 2
### Endpoints

#### Immunogenicity
- The presence of CD8/CD4 T-cell responses to Env plus at least one of the Gag, Pol, Nef peptide pools at weeks 26 or 28 (*primary end point*)
- Long term immunogenicity at weeks 48 and 72

#### Safety
- Grade 3 or above Local & Systemic adverse event
- Grade 3 or above other clinical or laboratory adverse event confirmed at examination or on repeat testing respectively
- Any event attributable to vaccine leading to discontinuation of the immunisation regimen
Solicited and Non-Solicited Local and Systemic Adverse Events

ANRS VAC20 / EV03: Participants with non solicited/solicited events

Randomisation Group
- 3DNA + 1 NYVAC
- 2 DNA + 2 NYVAC
Peptides Pools for IFN-γ ELISpot

- Peptide pools:
  - Overlapping peptides (15-mers with 11 aa overlap, n=474) spanning the entire Gag/Pol/Nef polygene, and the Env clade C of HIV-1 97CN54.
    - Grouped in 8 pools: Gag1/Gag2/Gag/Pol
      Pol1/Pol2
      Nef
      Env1/Env2
  - Responders were defined as:
    > 4xfold the negative control and > 55 SFU/10^6 cells
Proportion of Responders at Primary Endpoints (Week 26/28)

<table>
<thead>
<tr>
<th>ITT Analysis</th>
<th>3 x DNA</th>
<th>2 x DNA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 70</td>
<td>n = 70</td>
<td>n = 140</td>
</tr>
<tr>
<td>Response</td>
<td>64 (91%)</td>
<td>56 (80%)</td>
<td>120 (86%)</td>
</tr>
</tbody>
</table>

**Chi² Test:** $p = 0.053$;  
**Risk difference:** 11.4% (95% CI 0.0 – 22.9%)

<table>
<thead>
<tr>
<th>PP Analysis</th>
<th>3 x DNA</th>
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<tr>
<td></td>
<td>n = 67</td>
<td>n = 68</td>
<td>n = 135</td>
</tr>
<tr>
<td>Response</td>
<td>63 (94%)</td>
<td>55 (81%)</td>
<td>118 (87%)</td>
</tr>
</tbody>
</table>

**Chi² Test:** $p = 0.021$;  
**Risk difference:** 13.1% (95% CI 2.2 – 24.1%)
# Proportion of Responders at Week 26/28 per Peptide Group

## ITT Analysis

<table>
<thead>
<tr>
<th>Peptide Group</th>
<th>3 x DNA n = 70</th>
<th>2 x DNA n = 70</th>
<th>Total n = 140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env</td>
<td>63/70 (90%)</td>
<td>54/69 (78%)</td>
<td>117/139 (84%)</td>
</tr>
<tr>
<td>Gag/Pol/Nef</td>
<td>27/70 (39%)</td>
<td>17/70 (24%)</td>
<td>44/140 (31%)</td>
</tr>
</tbody>
</table>

## PP Analysis

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</tr>
</thead>
<tbody>
<tr>
<td>Env</td>
<td>62/67 (93%)</td>
<td>53/67 (79%)</td>
<td>115/134 (86%)</td>
</tr>
<tr>
<td>Gag/Pol/Nef</td>
<td>26/67 (39%)</td>
<td>17/68 (25%)</td>
<td>43/135 (32%)</td>
</tr>
</tbody>
</table>
Primary ImmunogenicityEndpoints
*(Env + at least one Gag, Pol or Nef pools)*

<table>
<thead>
<tr>
<th>ITT Analysis</th>
<th>3 x DNA n = 70</th>
<th>2 x DNA n = 69</th>
<th>Total n = 139</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response</td>
<td>26 (37%)</td>
<td>15 (22%)</td>
<td>41 (30%)</td>
</tr>
</tbody>
</table>

Chi² Test: $p = 0.047$; Risk difference: 15.4% (95% CI 0.5 – 30.3%)
Risk ratio: 1.7 (95% CI 1.0 – 2.9)

<table>
<thead>
<tr>
<th>PP Analysis</th>
<th>3 x DNA n = 67</th>
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<th>Total n = 134</th>
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<tr>
<td>Response</td>
<td>25 (37%)</td>
<td>15 (22%)</td>
<td>40 (30%)</td>
</tr>
</tbody>
</table>

Chi² Test: $p = 0.059$; Risk difference: 14.9% (95% CI -0.3 – 30.2%)
Risk ratio: 1.7 (95% CI 1.0 – 2.9)
# Magnitude of IFN-\(\gamma\) ELISpot Responses at Week 26/28 Overall (SFUs/10\(^6\) cells)

<table>
<thead>
<tr>
<th>ITT Analysis</th>
<th>Week 26</th>
<th>Week 28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ITT</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>774 (622)</td>
<td>398 (318)</td>
</tr>
<tr>
<td>(IQR; range)</td>
<td>(340-1101; 75-3454)</td>
<td>(178-488; 63-1514)</td>
</tr>
<tr>
<td>Mann-Whitney test</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Note: Sum of SFU/Mio cells from all peptide pools with a positive response per participant
Proportion of Env Responders at W48 and W72

\[ P = 0.0106 \]

\[ P = 0.0131 \]
Proportion of Gag/Pol/Nef Responders at W48 and W72

<table>
<thead>
<tr>
<th></th>
<th>W48</th>
<th>W72</th>
</tr>
</thead>
<tbody>
<tr>
<td>3xDNA + 1x NYVAC</td>
<td>20%</td>
<td>15%</td>
</tr>
<tr>
<td>2xDNA + 2x NYVAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage of responders
Magnitude of Responses at W48 and W72 (mean of responding pools per subjects)
27 participants analyzed

- **14** participants within Gr#1 (3xDNA+1xNYVAC)

- **13** participants within Gr#2 (2xDNA+2xNYVAC); only **11** participants were considered in the present analyses
Functional Profile of HIV-Specific T-Cell Responses (Env/Gag/pol/Nef pools)

**CD4 T-cell responses**

- GR#1 (3xDNA)
- GR#2 (2xDNA)

**CD8 T-cell responses**

- GR#1 (3xDNA)
- GR#2 (2xDNA)

Frequency of CD4 T-cells

- IFN$\gamma$
- IL-2
- TNF$\alpha$

Frequency of CD8 T-cells

- IFN$\gamma$
- IL-2
- TNF$\alpha$
Distribution of HIV Regions Targeted by CD4 T-Cell Responses

Gr#1
N=42 responses

Gr#2
N=22 responses

GAG
POL
NEF
ENV

Number of responses

ENV
Gag, Pol or Nef
ENV
Gag, Pol or Nef

P* Fischer test

P* = NS
Distribution of HIV Regions Targeted by CD8 T-Cell Responses

Gr#1
N=22 responses

Gr#2
N=8 responses

Gag, Pol or Nef

ENV

Number of responses

P* Fischer test

P* = 0.01
Breadth of CD4 and CD8 T-Cell Responses
*(ICS assay, Env/Gag/pol/Nef pools)*

Responders were defined as:
> 0.03% CD4+Cyt+ or CD8+Cyt+T cells and 2x fold background
Total Magnitude of T-Cell Responses (Sum of Responding Pools)

<table>
<thead>
<tr>
<th>Group</th>
<th>T-Cell Type</th>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>Percentage of T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr1</td>
<td>CD4 T cells</td>
<td>3xDNA</td>
<td>14</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>CD8 T cells</td>
<td>2xDNA</td>
<td>11</td>
<td>1.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

P = 0.02
Conclusions

- Both the 3xDNA+1xNYVAC and 2xDNA+ 2xNYVAC vaccine regimens are safe and well tolerated

- The DNA/NYVAC prime/boost vaccine combination is highly immunogenic (94% responders in the PP analysis)

- Optimal priming of poxvirus-based vaccine regimens requires 3 DNA injections:
  - superior for both proportion of polyepitopic responders and magnitude of the response
  - elicits sustained responses
  - Extends the breadth of CD8+ T cell responses
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  - ANRS Centers (France)
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    - Cochin
    - Tenon
    - Toulouse
    - Marseille

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  - Yves Levy

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