Therapeutic vaccination (p24-VLP) of patients with advanced HIV-1 infection does not alter CD4 cell decline.

Running head  p24-VLP vaccination in advanced HIV.

Don Smith1, Irene Gow2, Robert Colebunders3, Ian Weller4, Stephen Tchamouroff5, Jonathon Weber6, Fiona Boag7, Gillian Hales1, Sally Adams2, Gary Patou2 and David A Cooper1, for the 006 Study Group.

Abstract word count: 233
Text word count: 1,907

Affiliations

1 National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia
2 British Biotechnology Ltd, Oxfordshire, UK
3 Institute of Tropical Medicine, Antwerp, Belgium
4 Middlesex Hospital, London W1N, UK
5 Royal Sussex Country Hospital, Brighton, UK
6 St Mary’s Hospital, London W2, UK
7 Chelsea & Westminster, London SW10, UK

Address for correspondence:

Dr Don Smith,
NCHECR, Faculty of Medicine, UNSW,
Level 2, 376 Victoria Street,
Sydney 2010, Australia.
Ph 61 2 9331 6320
Fax 61 2 9332 2485
e-mail don.smith@unsw.edu.au
Therapeutic vaccination (p24-VLP) of patients with advanced HIV-1 infection does not alter CD4 decline

Abstract
The primary objective of this study was to evaluate the impact of vaccination with p24-VLP on CD4 counts in patients with advanced HIV disease.

A double-blind, placebo controlled trial in three countries randomised 304 patients with CD4 counts below 300 cells/mm³ to receive alum placebo (Group A), 500µg p24-VLP (Group B) or 1000µg p24-VLP (Group C) at 0,1,2,3,4 and 5 months. Patients were followed for a year with regular CD4 counts, disease progression and adverse reactions recorded. Patients were allowed to receive background therapy of zidovudine plus or minus didanosine or zalcitabine.

The average change in CD4 counts at 52 weeks, compared to baseline for groups A, B and C, were –32.35, -40.03 and –51.64 cells/mm³ respectively. CD4 counts at six months and one year were not significantly different between the treatment groups. At week 24 a > 4 fold increase in p24 antibody titre was noted in 3/83 group A patients, 4/82 group B patients and 2/77 group C patients.

60 patients progressed to CDC category B or C, with no significant differences between the three arms (18 in arm A, 22 in arm B, 20 in arm C). No significant adverse reactions were seen that could be attributed to vaccination.

In conclusion, while therapeutic vaccination with p24-VLP appears to be safe and well tolerated, no improvement in immunological markers could be detected in this study of patients with advanced HIV infection.

Key words: HIV, vaccine, p24, VLP, immunisation, disease progression
Introduction

Therapeutic vaccination has been proposed as a mechanism for stimulating the immune response in patients chronically infected with the Human Immunodeficiency virus (HIV)\(^1\). Some studies have suggested that immunisation with HIV antigens can delay CD4 cell decline and lower the rate of viral DNA increase in asymptomatic HIV-1 infected people\(^2\). However, other studies have failed to show any significant improvement in surrogate markers with the use of therapeutic vaccination\(^3,4\).

There is a considerable body of evidence that suggests that antibody responses to viral p24 antigen correlate with immune preservation\(^5,6\), as waning p24 antibody responses are associated with clinical disease progression\(^7,8,9,10\). Therefore, stimulating p24 antibody production with the use of therapeutic immunisation may delay clinical progression. We have previously shown that immunization with p24 virus like particle (p24-VLP) is safe and well tolerated\(^11,12\). Immunisation with p24 VLP and zidovudine appears to augment HIV specific CTL activity in asymptomatic HIV infected individuals\(^13\). Such T-lymphocyte responses are thought to be critical in achieving immunological control of virus\(^14,15\).

Increased understanding around the best use of antiretroviral therapy has resulted in a significant improvement in surrogate marker response and clinical outcomes for chronically infected patients. However it has become clear that virological rebound occurs quickly in patients ceasing therapy\(^16\) and hence there is a need to determine whether immunological control of HIV is possible.

While most therapeutic vaccines have been evaluated in early asymptomatic HIV infection, those patients in greatest need of protective immunity are those with waning immunological responses to the virus and moderate to advanced immunodeficiency. We therefore evaluated p24 VLP on a background of antiretroviral therapy in patients with moderate HIV induced immunodeficiency.

Methods

This was a phase II, dose ranging placebo controlled trial of HIV p17-p24: Ty-VLP (p24-VLP) conducted at 13 sites in Sydney, Melbourne, London, Brighton and Antwerp. The study was approved by an ethics committee at each site and all patients gave informed consent.
p24-VLP was supplied by British Biotechnology Limited, (Oxfordshire, UK). This immunogen consists of codons 100-308 of the gag component of HIV-1IIIb. These HIV-1 proteins were fused with Ty encoded yeast protein p1 to self assemble into virus like particles.

Patients were randomly assigned in a 1:1:1 ratio to each of the following treatment arms:

Group I - aluminium hydroxide adjuvant (placebo),
Group II - p24-VLP 500 μg with aluminium hydroxide adjuvant,
Group III - p24-VLP 1000 μg with aluminium hydroxide adjuvant.

All subjects received intra-muscular injections, into the deltoid muscle, at monthly intervals for six months.

Subjects were allowed to receive background antiretroviral therapy, which at the time of study enrolment consisted of zidovudine (AZT) with or without didanosine (ddI) or zalcitabine (ddC).

The primary study endpoint was change in CD4+ lymphocyte count at one year. Secondary endpoints were safety, number of opportunistic infections, survival, change in p24 antibody titre post-immunisation compared to baseline and other surrogate markers. Study endpoints were assessed at monthly intervals over the six month treatment period with a further six months follow up. Assays for antibodies to p24, p17 and Ty were used to assess immunogenicity. Markers of disease activity: CD4+ lymphocyte counts, CD8+ lymphocyte count, beta-2 microglobulin and p24 antigen were also measured.

Patients were considered eligible for enrolment if they met the following criteria:

- documented HIV-1 antibody positive and over 18 years
- average CD4+ lymphocyte count < 350 cells per mm³ from 3 visits
- No concurrent opportunistic infection at screening
- Patients who were allergic to yeast or actively using injection drugs were excluded.
- Women were required to be using adequate contraception.
Clinical Evaluation

Patients had a complete system review and clinical examination during the screening period and immediately after each immunisation. In addition, the injection site was examined and pulse, temperature and blood temperature were monitored. Details of any adverse event were recorded and routine biochemistry and haematology profiles were performed three times prior to the first immunisation and at 12 weekly intervals there after. HIV disease was classified as category A, B, or C (AIDS) using the 1993 CDC AIDS case definition18.

Immunogenicity

Serum was stored at -70°C for batch testing by quantitative enzyme linked immune absorbent assay for antibodies to p24 and Ty at week 6, 24 and 48. Results were expressed as reciprocal endpoint with a >4-fold increase in endpoint titre considered to be a positive result.

CD4+ and CD8+ lymphocyte counts were assayed by a flow cytometry using monoclonal antibodies.

Statistical Analyses

Patients were randomly allocated one of the 3 study arms, using a minimisation process based on CD4 strata, CDC disease stage and antiretroviral therapy. The primary study endpoint was difference in CD4 T-lymphocyte count between treatment arms, assessed at baseline and 1 year. To avoid the phenomenon of regression to the mean, the average of 3 CD4 counts prior to vaccination and at weeks 48, 50 and 52 were used in the analysis. Change in CD4 lymphocyte count was analysed on an intention to treat basis.

Results

Between July 1993 and August 1994, 314 patients were randomised through a mixture of tertiary academic centres and high caseload primary care sites across three countries.
Patient disposition

Of the 314 patients entering the study, 10 did not receive any vaccination (5 failed to attend, 2 had disease progression, 1 withdrew consent, 1 had an average CD4 count >350 cells and one was deported from the country). 304 patients received study treatment: 102 received placebo, 101 received 500μg p24-VLP and 101 received 1000μg p24-VLP. Of these 304 patients, 197 completed the study, of the remainder: 31 did not attend all visits or moved away, 27 had disease progression, 18 died, 13 withdrew to enter another trial, 5 withdrew consent, 4 had adverse events, 4 withdrew to receive excluded medications, 1 withdrew due to perceived lack of efficacy, 1 stopped all medications as he was feeling so well, 1 was a protocol violation, 1 became emotionally unstable and 1 stopped due to increased alcohol abuse.

Patient demographics by treatment arm are shown in table 1 and baseline HIV disease stage, antiretroviral therapy and CD4 T-lymphocyte counts are shown in table 2. The 3 arms were well matched arms for baseline variables.

A decline in CD4 lymphocyte count over time was noted in all 3 study arms, with no statistically significant difference between arms throughout the study period (figure 1). Although CD4 declines appeared to diverge between the 500 and 1000μg arms at 48 weeks, this difference was not significant (p=0.063, t-test).

No differences were found between study arms for other immune activation markers: B2-microglobulin, CD8 lymphocyte counts and CD4/CD8 ratio. Levels of p24 antigen (free and acid-dissociated) also did not differ between arms (data not shown). At the time of this study HIV-RNA PCR testing was not available.
Antibody responses to vaccination

While there was a statistically significant dose related antibody response to the yeast Ty protein over time similar improvements in antibody responses were not seen with p17 and p24, table 3. The Ty antibody responses noted at week 24 were greatly diminished at week 48.

HIV disease progression

HIV-related illnesses were noted during the course of the study, in 84 patients in the placebo group, 85 patients in the 500μg group and 89 patients in the 1000 μg group. The number of patients progressing from CDC category A to B during the study period for the placebo, 500 μg and 1000 μg arms was 6, 8 and 6 respectively. Progression from CDC categories A or B to AIDS occurred in 12, 14 and 14 patients respectively.

Safety

Injection site reactions were noted in 15 patients in the placebo group, 20 patients in the 500μg group and 15 patients in the 1000 μg group. Common adverse events recorded during the study are shown in table 5. Serious adverse events, considered to be possibly or probably related to study treatment occurred in 9 patients in the placebo group, 6 patients in the 500μg group and 6 patients in the 1000 μg group. None of the 27 deaths during the study period were considered to be study drug related; again no differences between arms was noted (table 4).

Discussion

This randomised double blind study failed to show any significant benefit of immunization with p24-VLP on CD4 cell count decline in patients with moderately advanced HIV disease. The obvious explanations for this finding are that: i) p24-VLP is not highly immunogenic, ii) patients with moderate HIV disease have lost their capacity to mount a clinically relevant immune response to HIV. Vaccine induced antibody responses to p17 and p24 were poor to non-existent in most patients, however almost half of the patients receiving the highest dose of vaccine mounted an antibody response to the carrier protein Ty (although on a molecular basis, more Ty is expressed in the VLP construct than p24 or p17). This would indicate that patients with moderate immunodeficiency can still make a response to novel
antigens, although this response is short lived. Although p24-VLP has been shown to be immunogenic in man19,20; poor antibody responses were also noted in another study of p24-VLP21, involving 74 patients with CD4 counts above 350 cells/mm³, suggesting that the lack of p24/p17 antibody response may not due to the advanced clinical stage of the patients; but may be seen at any stage of chronic HIV infection.

The strategy of therapeutic immunization was posulated at a time when there was little awareness of the high levels of circulating virus in asymptomatic patients22 and patients would have greater exposure to HIV antigens though natural infection than is seen with immunisation. More recently, greater understanding of the immunological control of HIV suggest that HIV specific CTL responses, best able to control chronic HIV production, are lost early in the course of HIV infection23. In the face of on-going viral replication it is unlikely that HIV specific immune responses can be regenerated in patients with moderate immunodeficiency.

Other studies to date on therapeutic vaccination have yielded mixed results. Vaccination with inactivated gp120 depleted HIV-1 has been shown to increase the delayed type hypersensitivity reactions to HIV-1 antigens24. Patients who do develop HIV specific DTH responses to this antigen appear to have a delayed disease progression25, however, results of a placebo controlled trial of MNrgp120 HIV-1 vaccine failed to show any difference in CD4 decline in one large study4. A more recent study of gp160 suggested a significant CD4 benefit at six months compared with placebo patients26. However another placebo controlled study of a rgp-160 envelope vaccine failed to show any clinical benefit, despite induction of new lymphoproliferative responses27. The difference between these results seen with other vaccines compared to p24 VLP is unlikely to be related to poorer immune response to p24 VLP. Klein et al, have shown that this immunogen can enhance specific CTL precursor frequencies28, although CTL responses to p24 were not seen in seronegative subjects20.

The majority of studies showing either an increased antibody response or recovery of DTH responses to therapeutic vaccines have been performed in HIV-infected individuals with CD4 counts usually above 300, the majority of whom have been asymptomatic. This study has looked at a much more advanced patient group with CD4 counts below 350 cell/mm³, the majority of whom were symptomatic. These
individuals would be expected to be less likely to mount any HIV specific immune response compared to asymptomatic individuals with higher T-cells\textsuperscript{29}, many of whom may belong to the long term non-progressor group and may have reasonable antibody or CTL responses to HIV.

This study was performed in the era of inadequate antiretroviral therapy and so patients receiving vaccination were doing so in the face of considerable viral replication. It is possible that in patients who have received more potent antiretroviral therapy in whom HIV-RNA levels are very low, that there may be some regeneration of naive CD4 cells\textsuperscript{30} that could be refocused to better target HIV. In these well controlled patients HIV specific immune responses have been noted to wain over time, implying that their immune systems might not be able to contain HIV if therapy was ever withdrawn\textsuperscript{31}. The concept of immunotherapy is far from proven and further studies are required to determine whether a therapeutic vaccination approach is viable in patients with undetectable viral loads.

Acknowledgements

We wish to acknowledge the efforts of the 006 study investigators (listed below) and their patients. This study was funded by British Biotechnology Ltd. The National Centre in HIV Epidemiology and Clinical Research is funded by the Commonwealth Department of Health and Aged Care, and is affiliated with the Faculty of Medicine, the University of New South Wales.

006 study investigators:

Dr DE Smith, Ms G Hales, Ms J Mitchell (University of New South Wales, Sydney), Australia: Prof DA Cooper, Dr A Kelleher (St. Vincent’s Hospital, Sydney), Dr M McMurchie, Dr L Todhunter (229 Oxford Street, Sydney), Dr B Genn, Dr A Beveridge, Dr A Pethebridge (Grosvenor Street Clinic, Sydney), Dr C Duncombe, Dr M Bloch, Dr D Quan (Holdsworth House General Practice, Sydney), Prof A Mindel, Dr J Knox, Dr C O’Connor, (Sydney Hospital, Sydney), Dr J Anderson, Dr D Russell, Dr P Murray (Carlton Clinic, Melbourne), Dr P Meese, Dr I Chenoweth (Middle Park, Melbourne), Dr H Wraight, Dr A Buchanan, Dr N Roth, (William St/Prahran Market Clinic, Melbourne).

United Kingdom: Dr F Boag, Dr G Ronney, Dr D Hawkins, Prof B Gazzard (Chelsea and Westminster Hospital, London), Prof J Weber, Dr V Kitchen, Dr L Dorrell (St Mary’s Hospital, London), Prof I Weller, Dr I Williams, Dr J Richens, Dr P Noble (Middlesex Hospital, London), Dr S Tchamouropp (Royal Sussex Country Hospital, Brighton).

Belgium: Dr B Colebunders, Dr J Goeman (Institute of Tropical Medicine, Antwerp).

Dr G Patou, Dr I Gow, Ms K Hodgkins, Dr S Adams – British Biotech Pharmaceuticals Ltd, Oxford.
Legend to Figure:

CD4 cell counts – change from baseline – all subjects

Mean (+/- SEM)
Table 1. Baseline demographics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>500µg p24-VLP</th>
<th>1000µg p24-VLP</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>5</td>
<td>97</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>Mean</td>
<td>38</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>25-62</td>
<td>24-54</td>
<td>21-63</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>white</td>
<td>97</td>
<td>93</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>black</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>oriental/other</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Risk group</strong></td>
<td>homosexual</td>
<td>85</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>heterosexual</td>
<td>9</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2. Baseline CD4 counts, antiviral therapy and HIV disease category

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>500µg p24-VLP</th>
<th>1000µg p24-VLP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antivirals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>34</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Monotherapy</td>
<td>44</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>Dual Therapy</td>
<td>24</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Combination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HIV disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>27</td>
<td>27</td>
<td>26</td>
</tr>
<tr>
<td>B</td>
<td>44</td>
<td>45</td>
<td>44</td>
</tr>
<tr>
<td>C</td>
<td>31</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td><strong>CD4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>151.0</td>
<td>164.5</td>
<td>155.9</td>
</tr>
<tr>
<td>(sem)</td>
<td>(10.3)</td>
<td>(10.8)</td>
<td>(10.0)</td>
</tr>
<tr>
<td>N</td>
<td>102</td>
<td>101</td>
<td>101</td>
</tr>
</tbody>
</table>

- **baseline** = average of screening 1, screening 2, and pre-dose
- **values shown for all subjects**

* as defined by 1993 CDC case definition for AIDS
Table 3
Antibody response to Ty, p24, p17

(*Positive response is > 4 x increase in titratable antibody*)

<table>
<thead>
<tr>
<th>Ty responses</th>
<th>Week 12</th>
<th>Week 24</th>
<th>Week 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0/91 (0%)</td>
<td>0/83 (0%)</td>
<td>0/71 (0%)</td>
</tr>
<tr>
<td>500 µg</td>
<td>7/90 (7.8%)</td>
<td>25/82 (30.5%)*</td>
<td>5/67 (7.5%)~</td>
</tr>
<tr>
<td>1000 µg</td>
<td>10/92 (10.9%)</td>
<td>33/76 (43.4%)*</td>
<td>8/66 (12.1%)#</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p24 response</th>
<th>Week 12</th>
<th>Week 24</th>
<th>Week 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5/92 (5.4%)</td>
<td>3/83 (3.6%)</td>
<td>4/72 (5.6%)</td>
</tr>
<tr>
<td>500 µg</td>
<td>6/90 (6.7%)</td>
<td>4/82 (4.9%)</td>
<td>4/67 (6%)</td>
</tr>
<tr>
<td>1000 µg</td>
<td>3/94 (3.2%)</td>
<td>2/77 (2.6%)</td>
<td>2/66 (3%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>p17 response</th>
<th>Week 12</th>
<th>Week 24</th>
<th>Week 48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>ND</td>
<td>1/40 (2.5%)</td>
<td>ND</td>
</tr>
<tr>
<td>500 µg</td>
<td>ND</td>
<td>3/38 (7.9%)</td>
<td>ND</td>
</tr>
<tr>
<td>1000 µg</td>
<td>ND</td>
<td>1/38 (2.6%)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*p<0.001 for placebo vs 500mg and 1000mg arms
# p=0.002 for placebo vs 1000mg arms
~ p=0.025 for placebo vs 500mg arms
Fisher’s Exact Test
ND= not done
Table 4. Adverse events

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>500µg p24-VLP</th>
<th>1000µg p24-VLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deaths</td>
<td>6</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Serious adverse events</td>
<td>40</td>
<td>47</td>
<td>45</td>
</tr>
<tr>
<td>Any adverse events</td>
<td>99</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Withdrawals due to Aes</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Most common AEs:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site pain</td>
<td>70</td>
<td>71</td>
<td>80</td>
</tr>
<tr>
<td>Headache</td>
<td>26</td>
<td>27</td>
<td>16</td>
</tr>
<tr>
<td>Injection site inflammation</td>
<td>13</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Injection site reaction</td>
<td>15</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Fever</td>
<td>17</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Fatigue</td>
<td>13</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>8</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Rash</td>
<td>9</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>
Figure 1

CD4 cell counts - change from baseline
mean (+/- SEM)

Patient observations:
placebo
102 96 94 90 89 83 82 76 73 68 64
500mg
101 95 91 88 88 85 83 76 71 64 67
1000mg
101 99 98 94 90 89 78 77 68 61 65
References


