Immunological Characterization of Subjects from the Step Study (Merck V520 Protocol 023/HVTN 502)

A Phase II Test-of-Concept Trial of the MRKAd5 HIV-1 Gag/Pol/Nef Trivalent Vaccine

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Major Scientific Questions

• What are the reasons for lack of vaccine efficacy?
  – Was the vaccine immunogenic?
  – Were immune responses lower in subjects who became infected?
  – Were the quantity, quality or breadth elicited immune responses sub-optimal?

• Are there potential biological mechanisms which could explain the increased number of infections in the vaccine group?
ELISPOT Responses for subjects receiving Vaccine: Ad5 ≤ 200

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Non-cases</th>
<th>Cases</th>
<th>Non-cases</th>
<th>Cases</th>
<th>Non-cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>gag</td>
<td>74%</td>
<td>76%</td>
<td>63%</td>
<td>73%</td>
<td>74%</td>
<td>70%</td>
</tr>
<tr>
<td>nef</td>
<td>n: 19</td>
<td>n: 143</td>
<td>n: 19</td>
<td>n: 143</td>
<td>n: 19</td>
<td>n: 143</td>
</tr>
</tbody>
</table>

Week 8 ELISPOT (SFCs/10^6 PBMCs)

ELISPOT responder: ≥ 55 SFC/10^6 PBMC and ≥ 4-fold over negative control
ELISPOT Responses for subjects receiving Vaccine: Ad5 > 200

Week 8 ELISPOT (SFCs/10^6 PBMCs)

<table>
<thead>
<tr>
<th>Cases</th>
<th>Non-cases</th>
<th>Cases</th>
<th>Non-cases</th>
<th>Cases</th>
<th>Non-cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>gag</td>
<td>3</td>
<td>10</td>
<td>30</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>pol</td>
<td>181</td>
<td>168</td>
<td>296</td>
<td>241</td>
<td>149</td>
</tr>
<tr>
<td>nef</td>
<td>13</td>
<td>173</td>
<td>13</td>
<td>173</td>
<td>13</td>
</tr>
</tbody>
</table>

%Resp: GM:
n:

46% 181 13
54% 168 173
38% 296 13
47% 241 173
46% 149 13
51% 163 173

Responder
Non-responder
GM (all subj.)

ELISPOT responder: ≥ 55 SFC/10^6 PBMC and ≥ 4-fold over negative control
Ongoing studies to characterize the quality and breadth of elicited immune responses

• Multi-color flow cytometry to characterize HIV-specific T-cell functionality (in progress)
  – Cytokine production and lytic potential panel
    ▪ Vital dye, CD3, CD4, CD8, IFN-γ, IL-2, TNF-α, perforin
  – Memory panel
    ▪ CD3, CD4, CD8, CD57, CD27, CD28, CD45, CD103, CCR5, CCR7
  – Activation panel
    ▪ CD3, CD4, CD8, CCR5, CD38, HLA-DR, Ki67, Bcl2
• Epitope mapping (in progress)
• HLA typing (in progress)
• Herpes simplex serology (in progress)
STEP Trial: Vaccine-induced CD4+ T cells in cases and non-cases (Week 30)

CD4+ T Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (Ab titer)</th>
<th>Non-Cases (Ab titer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad5 Ab titer ≤ 18</td>
<td>4/8 (50%)</td>
<td>19/44 (43%)</td>
</tr>
<tr>
<td>Ad5 Ab titer &gt; 18</td>
<td>6/9 (67%)</td>
<td>22/49 (45%)</td>
</tr>
</tbody>
</table>

% T Cells Producing IFN-γ or IL-2

- Red dots: Positive response
- Blue dots: Negative response
STEP Trial: Vaccine-induced CD8+ T cells in cases and non-cases, Week 30
Major Scientific Questions

• What are the reasons for lack of vaccine efficacy?
  – Was the vaccine immunogenic?
  – Were immune responses lower in subjects who became infected?
  – Were the quantity, quality or breadth elicited immune responses sub-optimal?

• Are there potential biological mechanisms which could explain the increased number of infections in the vaccine group?
Activated Blood CD4+ T Cells (Ki67+/BcL2-) Expressing the HIV-1 Co-receptor CCR5, Week 30

- Ad5 Titer > 200
- Ad5 Titer ≤ 200

Placebo Vaccinee Non-Cases

% activated CD4+ T cells expressing CCR5

- N=166
- N=140

p=0.355

p<0.001

p<0.001

P<0.001

- Ad5 Titer > 200
- Ad5 Titer ≤ 200
Ad5-specific CD4+ and CD8+ T cell responses
(week 30, 10,000 MOI of empty VRC non-replicating Ad5 Vector)

Cases (n = 62)

Vaccinee n=37
Placebo n=25

% T Cells Expressing IFN-γ+ or IL-2+

CD4+

p = 0.027

% T Cells Expressing IFN-γ+ or IL-2+

CD8+

p = 0.004

Non-Cases (n = 87)

Vaccinee n=76
Placebo n=11

% T Cells Expressing IFN-γ+ or IL-2+

CD4+

p = 0.045

% T Cells Expressing IFN-γ+ or IL-2+

CD8+

p < 0.001

*: two out scale values at 0.73 and 2.3 %; **: one out scale value at 0.77; *** three out scale values at 0.57, 0.94 and 0.99
STEP Trial: PBMC Response to 10,000 MOI Empty VRC Non-Replicating Ad5 Vector, Vaccinee only

CD4⁺ Non-Cases

Non-Cases

CD4⁺

CD8⁺

≤18
n=37

>18
n=39

≤18
n=37

>18
n=39

≤18
n=13

>18
n=24

≤18
n=13

>18
n=24

% T Cells Expressing IFN-γ⁺ or IL-2⁺

p = 0.009

p = 0.588

p = 0.171

p = 0.651

p = 0.009

p = 0.651

p = 0.009

p = 0.651

% T Cells Expressing IFN-γ⁺ or IL-2⁺

% T Cells Expressing IFN-γ⁺ or IL-2⁺

% T Cells Expressing IFN-γ⁺ or IL-2⁺

% T Cells Expressing IFN-γ⁺ or IL-2⁺

* : one out scale value at 4 %; **: one out scale value at 2.3%; ***: two out scale value at 0.9 and 0.8%
Unanswered questions raised by the Step Study

• Can other “T cell vaccines” provide immune protection in the absence of neutralizing antibodies?
• Were vector-specific immune responses responsible for the increased number of infections observed in Ad5 seropositive vaccinees?
  – If so, will this be a problem for all adenovirus vectored vaccines? For any vectored HIV-1 vaccine?
• What can non-human primate teach us in predicting efficacy and safety in humans?
Other potential investigations

- Functional phenotype of epitope-specific T-cells
  - Anti-viral cytokines/chemokines
  - Central/effector memory phenotypes
  - Proliferative capacity (Ki67+, CFSE, tetramer staining)
  - TCR repertoire
  - Functional avidity

- Anti-HIV Activities
  - T-cell viral neutralization
  - Cytolytic potential (perforin, granzymes)

- Transcriptional and Proteomic Analysis
  - Microarray
  - Bead array

Prioritization will be essential because of limited specimen quantities.
Defining Potential Mechanisms to Further Address the Scientific Questions

Our goal is to provide an externally reviewed, expeditious process to address these issues

• Established a scientific committee of investigators from Merck, HVTN, NIAID and the scientific community to develop a scientific agenda to explain the vaccine’s lack of efficacy and apparent increased risk of acquisition
  – Committee established, Bruce Walker serving as chair, includes members of HVTN Lab Sciences Advisory Committee and 3 external experts
  – Laboratories of HVTN, Merck, CHAVI, USMRP and VRC will be enlisted to help in these endeavors
  – Contract funds for outside laboratories will also be sought

• Use HVTN web site for unsolicited proposals for ancillary studies involving trial specimens and related studies
Summary

- Immune responses elicited by the vaccine, as measured by $\gamma$-interferon ELISPOT, were as expected.
- Immune responses were similar in infected and uninfected subjects.
- No clear explanation for increased number of infections observed in vaccinees in the Ad5 seropositive volunteers.
  - Four weeks after the 3rd study injection, there were more activated PBMC in volunteers with high Ad5 antibody titers at baseline, but no difference between vaccinees and placebo recipients.
    - Would be of interest to look at mucosal sites, but no mucosal samples collected in Step.
  - Some evidence of more vector specific T-cells post vaccination, but more studies are needed.
- Process in place to prioritize further studies.
Special thanks to

• Other members of the Step Protocol Team

• Staff and community representatives at the trial sites

• Especially to all of the trial participants