T cell mediated immunity elicited in volunteers following immunization with the live oral *Salmonella Paratyphi* A attenuated vaccine strain CVD 1902

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S. Paratyphi A infection (paratyphoid A fever) has emerged as a health problem in enteric disease endemic areas.

Disease caused by S. Paratyphi A strains showing resistance to multiple clinically-relevant antibiotics are common.

Well-tolerated effective licensed vaccines are available to prevent S. Typhi disease (typhoid fever) but those do not provide effective cross-protection against paratyphoid A or B fevers.

Currently no vaccine is available to prevent S. Paratyphi A disease

Development of a vaccine against S. Paratyphi A is a public health priority.
Candidate vaccines against S. Paratyphi A developed at CVD-Maryland

- **Subunit vaccine**: S. Paratyphi A O polysaccharide linked to carrier proteins.

- **Live oral vaccine**: CVD 1902: A wild type S. Paratyphi A strain attenuated by
  - Introducing deletions in the *guaBA* chromosomal operon (which impairs the biosynthesis of guanine nucleotides).
  - An additional mutation in the *clpX* gene (encodes a chaperone ATPase) for safety and enhanced expression of flagellar antigen.

- **Pre-clinical study**: CVD 1902 immunized mice were protected against intraperitoneal wt-type S. Paratyphi A challenge.

- **Dose-escalating phase 1 clinical trial in healthy adults**: Single doses as high as $10^9$ and $10^{10}$ CFU were well tolerated and immunogenic.
Dose-escalating phase 1 clinical trial with CVD 1902 in healthy adults

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Setting</th>
<th>Vaccine Inoculum size</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CVD 1902 vaccine</td>
<td>Placebo</td>
</tr>
<tr>
<td>1</td>
<td>Inpatient</td>
<td>$10^6$ CFU</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Inpatient</td>
<td>$10^7$ CFU</td>
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<tr>
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<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Inpatient</td>
<td>$10^9$ CFU</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Inpatient</td>
<td>$10^{10}$ CFU</td>
<td>6</td>
</tr>
</tbody>
</table>
Dose-escalating phase 1 clinical trial with CVD 1902 in healthy adults

- Volunteers were immunized with a single dose of $10^9$ (n=6) or $10^{10}$ (n=6) CFU or placebo (n=4) of CVD 1902
- Blood samples were drawn before (day 0) and 28 days after vaccination
- Purified PBMC were cryo-preserved until used in CMI assays
Experimental Design

Generation of autologous B-LCL (EBV-B cells)

EBV-Infection

Wt-S. Paratyphi A Infection

γ-irradiation

Infected Target Cells (Autologous B cells)

Over night culture: Ex-vivo stimulation

Thawed PBMC (effectors)

Flow cytometry (Intracellular staining)
Experimental Design

Flow Cytometry assay

CD8+ T cell Memory Subsets

CD107a | IFN-γ | IL-2 | TNF-α

FCOM analysis

Single (S+) cells: Expressing / producing only one of the functions measured
Multifunctional (MF) cells: Concomitantly producing two or more functions

S. Paratyhi A specific CD8+T_{EM} cells

IFN-γ producing T cells
CVD 1902 elicited S. Paratyphi A specific CD8+ T_{EM} Responses

Post-vaccination increases in S. Paratyphi A specific CD8+ T_{EM} cells

Post-vaccination increase: Post-vaccination (day 28) minus Pre-vaccination (day 0) levels
CVD 1902 elicited S. Paratyphi A specific CD8+ T_{EM} Responses

**CD8+ vaccine responders:** Volunteers showing post-vaccination increases of \( \geq 0.1\% \) in PA target-specific CD8+ CD69+ T_{EM} cells producing and/or expressing at least 2 functions (IFN-\( \gamma \), TNF-\( \alpha \), IL-2 and/or CD107a)

* \( p=0.02 \) compared to Placebo, Chi-square test
Comparisons of vaccine elicited responses in CD8+ responders vs non-responders or placebo

Mann-Whitney test, Compared with NR (*, p<0.05; **, p<0.01) or P (#, <0.05, ## p<0.01)
Multifunctional Characteristics of the CVD 1902 CD8+ T_{EM} cell responses in CD8+ responders

* p<0.05 compared to respective S+ cells: Wilcoxon paired test
Gut homing potential of CVD 1902 elicited multifunctional CD8+ T$_{EM}$ cells in CD8 vaccine responders
Induction of multifunctional (MF) CD4+ T_{EM} cells following immunization with CVD 1902

**CD4+ Vaccine responders**

Cohort 4  Cohort 5  Combined

<table>
<thead>
<tr>
<th></th>
<th>% of CD4 responders</th>
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<tbody>
<tr>
<td>V</td>
<td>3/6</td>
</tr>
<tr>
<td>P</td>
<td>0/2</td>
</tr>
<tr>
<td>V</td>
<td>0/2</td>
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<tr>
<td>P</td>
<td>6/12</td>
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**Multifunctionality of CD4+ Response**

<table>
<thead>
<tr>
<th></th>
<th>% of CD4+ T_{EM} subset</th>
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<tbody>
<tr>
<td>VR</td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td></td>
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<tr>
<td>P</td>
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</tbody>
</table>

**CD4+ vaccine responders**: Volunteers showing post-vaccination increases of ≥0.1% in PA target-specific CD4+ CD69+ T_{EM} cells producing and/or expressing at least 2 functions (IFN-γ, TNF-α, IL-2 and/or CD107a)
Comparisons of vaccine elicited CD8+ and CD4+ responses

Magnitude of Functional cells

Correlation of IFN-γ+ Cells

% CD4 TEM

% CD8 TEM

r = 0.6
p = 0.02
Summary

❖ A single dose of either $10^9$ or $10^{10}$ CFU of CVD 1902 elicited S. Paratyphi A specific T effector memory ($T_{EM}$) responses mediated by both CD8+ and CD4+ T cells in almost two third of the vaccinees.

❖ CVD 1902 induced T-CMI predominately mediated by S. Paratyphi A specific-Multifunctional (MF) cells.

❖ A significant proportion of CD8+ MF $T_{EM}$ cells expressed the gut homing molecule integrin $\alpha 4\beta 7$.

❖ Cytokine production patterns by both CD8+ and CD4+ cells are suggestive of robust Th1 responses.

❖ Future challenge studies with wt S. Paratyphi A and field studies will establish the importance of these vaccine elicited T memory responses in protection.

❖ These results, together with the observed safety and humoral immunogenicity data elicited by CVD 1902, suggest that a single or multiple doses have the potential to protect against S. Paratyphi A infection.
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