Development of a Nontyphoidal *Salmonella* Controlled Human Infection Model

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Outline

• Rationale for an NTS CHIM

• Clinical Study Design

• GMP Manufacture and Characterisation

• Study Objectives and Progress
  • Dose-escalation and safety
  • Clinical and microbiological responses
  • Immunological responses

• Exploratory sub-studies
Nontyphoidal *Salmonella* (NTS)

- Spectrum of disease
  - iNTS vs. dNTS
- iNTS serovars dominant in Africa
- High case-fatality
  - 15-20%
- Vaccine development in progress

Feasey et al., Lancet 2012; 379: 2489–99
Stanaway et al., Lancet Infect Dis 2019; 19: 1312–24
Susceptible Host vs. Pathogen

Host

Primary immunodeficiency
- MSMD
- CGD

Adults
- HIV

Children
- Malaria
- Malnutrition
- Sickle cell disease
- Anaemia

Pathogen

- Host-adaptation
- ST313 Lineage II D23580
- Loss of multicellular behaviour and adaptation to extra-intestinal lifestyle

Canals et al., PLoS Biol 17(1): e3000059
Controlled Human Infection Models

- Unique opportunity to obtain early efficacy data of candidate vaccines or therapeutics
- Opportunity to dissect physiological and immunological responses to infection

Jin et al., Lancet 2017; 390: 2472–80
The CHANTS Consortium

- Overarching aim to develop a first-in-human NTS CHIM
- Support and accelerate NTS vaccine development
• Phase 1, randomised, double-blind, first-in human

• *Salmonella* Typhimurium (GMP)
  • ST19 (4/74) vs. ST313 (D23580)
  • NaHCO3 pre-treatment

• Healthy, *Salmonella* naïve, UK resident adult volunteers, aged 18-50 (n = 40-80)

• Primary Outcome > Systemic Salmonellosis
  • Sustained fever >38°C >12hrs and/or bacteraemia

• Dose-escalation model – target attack rate 60-75%
  • 1-5 x 10^3 to 10^6 CFU
### Development of non-Typhoidal *Salmonella* controlled human infection model

#### Genomes: 95% identical

<table>
<thead>
<tr>
<th></th>
<th>4/74</th>
<th>D23580</th>
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<tbody>
<tr>
<td>Genome degradation</td>
<td>some</td>
<td>Increased compared to 4/74</td>
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<tr>
<td>Invasiveness of epithelial cells</td>
<td>$\uparrow$</td>
<td>$\downarrow$</td>
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<td>Uptake in macrophages</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Survival &amp; replication in macrophages</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Apoptosis and inflammatory response in macrophages</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
</tr>
<tr>
<td>Resistance to killing by macrophages</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
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<td>Amount of complement required for antibody mediated bactericidal activity</td>
<td>$\downarrow$</td>
<td>$\uparrow$</td>
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**Healthy volunteers identified at screening**

Randomly assigned to challenge with one of two strains

- **Strain #1**
  - *Salmonella Typhimurium 4/74*
  - "UK strain"
  - Associated with gastroenteritis/diarrhoea.

- **Strain #2**
  - *Salmonella Typhimurium D23580*
  - Originally isolated in Malawi.
  - Associated with iNTS in vulnerable patients.

R. Canals, 2019
Development of non-Typhoidal *Salmonella* controlled human infection model

- Healthy volunteers identified at screening
- Randomly assigned to challenge with one of two strains

**Partnered with Walter-Reed Army Institute for research (WRAIR) to generate GMP quality stocks of both strains 4/74 & D23580**

1. Manufacture of GMP stocks
2. Characterization of GMP stocks for challenge
3. Preparation of Challenge Agent dose

**ST19**
- *Salmonella Typhimurium* 4/74
- "UK strain"
- Associated with gastroenteritis/diarrhoea.

**ST313**
- *Salmonella Typhimurium* D23580
- Originally isolated in Malawi.
- Associated with iNTS in vulnerable patients.

Eddie Suvarnapunya
1) MANUFACTURE OF GMP STOCKS (and quality control)

Walter-Reed Army institute for research (WRAIR)

Generation of Master Cell Bank from Research Cell Bank (RCB) ampoule

**PRE-FREEZE VIABILITY NUMBERS**

- 4/74: $1.24 \times 10^9$ CFU/ml
- D23580: $9.95 \times 10^8$ CFU/ml

**Verification of purity**
**Gram staining**
**Pre-freeze viability**

**Walter-Reed Army Institute of Research (WRAIR)**

**Generation of Master Cell Bank from Research Cell Bank (RCB) Ampoule**

- **TSA plates**
- **Select APS LB broth**
- **OD600 = 2 ± 0.2**
- **200 RPM 37°C**
- **Addition of glycerol (final concentration 16% v/v)**
- **300 vials of each strain**

**GMP stocks**

**Eddie Suvarnapunya**
### 2) CHARACTERISATION OF GMP STOCKS

<table>
<thead>
<tr>
<th>Specified Contaminant</th>
<th>Enrichment Broth</th>
<th>Selective Agar</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Fluid Lactose Medium</td>
<td>MacConkey Agar Medium</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Fluid Lactose Medium, Fluid Soybean-Casein Digest Medium</td>
<td>Cetrimide Agar Medium, Bacillus cereus Agar</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Fluid Soybean-Casein Digest Medium</td>
<td>Baird-Parker Agar Medium</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Fluid Soybean-Casein Digest Medium</td>
<td>Baird-Parker Agar Medium</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>N/A</td>
<td>Acidified Potato Dextrose Agar Medium</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>N/A</td>
<td>Sabouraud Dextrose Agar Medium + Kanamycin</td>
</tr>
</tbody>
</table>
2) CHARACTERISATION OF GMP STOCKS

- biochemical ID
- growth curves (A)
- RDAR phenotype (B)
- antimicrobial sensitivity testing (C)
- motility (D)
- whole genome sequencing

NO UNEXPECTED RESULTS
numbers of *Salmonella* bacteria remain relatively stable for at least an hour (at RT and on ice)
Adjusting D23580 stock concentration estimates:

1. Dilutions based on official BPR stock concentrations of 4/74 and D23580 GMP vials

\[
\begin{align*}
\text{Dilutions based on official BPR stock concentrations of 4/74 and D23580 GMP vials:} \\
\text{4/74: } 1.24 \times 10^9 \text{ CFU/ml} \\
\text{D23580: } 9.95 \times 10^8 \text{ CFU/ml}
\end{align*}
\]

- Some variability in concentrations of GMP master stocks (within strains)

2. Adjusting D23580 stock concentration estimates:

- D23580 GMP master stocks have higher than expected number of CFUs (between 20-50% higher)
OUR AIM WAS / IS TO ADMINISTER DOSE IN THE RANGE 1 – 5 \times 10^3 \text{ CFU / 30 ml}

(after dose escalation \times 10^4 etc.)

- Very low level of variability of the GMP stock concentrations

**TAKEAWAY**

- We are able to successfully administer doses within a very narrow range.
Dose-escalation

Dose-escalation process:

1. **Challenge 5 at the target dose**
   - Update the dose-attack rate model

2. **Recommend the dose closest to the target attack rate**
   - DSMC review and recommend a target dose
   - Were 20 participants challenged at this dose or has the max sample size reached?

3. **Stop**
   - Estimated attack rate at the target dose between 60-75%?
     - Yes: **DOSE CONFIRMED**
     - No: All candidate doses are too low/high. Additional dose levels are needed

4. **Unblinded review by DSMC**
   - Update dose-attack rate model

5. **Recommend dose closest to target attack rate**

6. **Repeat the process**
**Day 0 to 7: Inpatient Quarantine**
- Daily blood & stool cultures
- Treatment if severe gastroenteritis or other treatment criteria
- Screen for Salmonella
- Consent
- Medical history
- Examination
- Blood test
- Urine sample
- Pregnancy test
- Questionnaire
- Ultrasound scan
- Consent quiz
- Pre-Challenge
- Continued consent
- Issue study pack

**Day 8 to 14: Daily Visits**
- Daily blood & stool cultures
- Follow up visit
- Medical review
- Blood sample
- Stool sample
- Questionnaire

**Day 28**
- Medical review
- Blood sample
- Stool sample
- Questionnaire

**Day 90**
- Follow up visit
- Medical review
- Blood sample

**Day 180**
- Follow up visit
- Medical review
- Blood sample

**Day 365**
- Follow up visit
- Medical review
- Blood sample

**Salmonella diagnosis**
Criteria: Temperature ≥38°C for ≥12hrs or positive blood culture for Salmonella Typhimurium
May occur at any point from Day 0 to Day 14
Treatment started at time of diagnosis

**Treatment at Day 14 if not diagnosed**
Safety

1. Gastroenteritis
   - Very common risk: Developing Gastroenteritis
   - How is it minimised?
     - Treated with antibiotics to shorten duration.
     - Monitored for dehydration and given fluids to rehydrate.

2. Invasive Infection
   - Uncommon risk: Invasive Salmonella infection
   - How is it minimised?
     - High risk people are excluded from the study.
     - Daily blood samples to test for bacteria in the blood.
     - Immediate treatment to prevent severe symptoms.

3. Antibiotic side effects
   - Common risk: Antibiotic side effects
   - How is it minimised?
     - People with known allergies are excluded.
     - Treatment is as short as possible.
     - We monitor for and treat any side effects.
     - We can use alternative antibiotics if side effects occur.

4. Irritable bowel syndrome
   - Common risk: Post infectious irritable bowel syndrome (IBS)
   - How is it minimised?
     - Screening questionnaire for symptoms of IBS.
     - If symptoms don’t resolve, refer to a specialist.

5. Reactive arthritis
   - Uncommon risk: Reactive arthritis (joint pain/swelling)
   - How is it minimised?
     - A screening blood test (HLA-B27).
     - If symptoms occur, refer to a specialist.

6. Transmission & Shedding
   - Uncommon risk: Transmission or shedding
   - How is it minimised?
     - Quarantine until no longer infectious.
     - Participants given information and soap/disposable towels for good handwashing.
     - Testing at follow up visits.
     - Close contacts can be tested for Salmonella.
Clinical and Microbiological Responses

Clinical Response

• Gastroenteritis Rate
• Severe gastroenteritis
• Persistent fever
• Safety
• Clinical features – symptom diaries
• Haematological and biochemical parameters

Microbiological Response

• Colonisation
• Stool shedding
• Stool culture/PCR (Quantitative)
• Blood culture
Regulatory and Ethical Approvals

Late 2022

GMP Strain Manufacture and Characterisation

March 2023

Screening Open

Aug 2023

1st Challenge

Oct 2023
 screened
N = 33

Excluded (Ineligible)
n = 10*

Cohort 1
n = 2

Cohort 2
n = 2

Pending Challenge
n = 19

*Gallstones; Sickle cell trait; HLA-B*27 positive; Orthopaedic prostheses; Valvular murmur; SARS-CoV-2 unvaccinated
OUR AIM WAS / IS TO ADMINISTER DOSE IN THE RANGE 1 – 5 x 10³ CFU / 30 ml

(after dose escalation x 10⁴ etc.)

- Very low level of variability of the GMP stock concentrations

TAKEAWAY

- We are able to successfully administer doses within a very narrow range.
Challenge Summary

• Colonisation Achieved

• 0/4 primary outcome (Systemic Salmonellosis)

• 0/4 gastroenteritis

• No safety signals/SAEs (clinically or by blood parameters)
Next Steps – Immunological Responses

**Mucosal Immunity**
- Saliva
- Stool
- Secretory IgA/IgG

**Humoral Immunity**
- Serum LPS O-Ag IgG/IgA
- Serum bactericidal activity
- ALS Assay
- ASC and memory B-cell

**Cell-Mediated Immunity**
- Lymphocyte populations
- Intracellular cytokine staining
• **Progress thus far**
  - First NTS CHIM
  - Evidence of reliable colonisation > dose-escalation pending for disease endpoints
  - No safety signals

• **Future studies**
  - Vaccine efficacy
  - Novel therapeutics
  - Heterologous and homologous re-challenge
  - Transfer model to endemic setting
### Acknowledgements

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<thead>
<tr>
<th>CHANTS Study Team</th>
<th>Imperial College London</th>
<th>University of Liverpool</th>
<th>University of Oxford</th>
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<td>Amanda Bravery</td>
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<td>Emma Smith</td>
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<td>NWLP Microbiology Staff</td>
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Rossett-Rice model

• Rossett-Rice model is in vitro measure to mimic the passage through the stomach on the bench top.

• Allows for monitoring of the simulated stomach pH after antacid dosing and samples to be testing for viability of the challenge organism at various timepoints after dosing.

• Image on the left shows the image from GSK’s rota patent for the Baby Rossett-Rice model.

  • The volumes and secretion rates are adjusted for adults. We have substituted simulated gastric fluid for HCl.