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Innovations to accelerate vaccine development and manufacture

Workshop On Refining The Regulatory Context Of Controlled Human Infection Models

Developing a *C. difficile* CHIM

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C. difficile CHIM rationale

- *C. diff* leading cause of health-care associated diarrhoea
- Toxin mediated disease
- Risk factors: antibiotics, hospital stay, immunosuppression
- Treatment: antibiotics
  - High relapse rates &
    rise of antimicrobial resistance
- New treatments needed

→Develop a CHIM for *C. diff (toxigenic)*[1]

- New targets for (preventive) products
- Model to test efficacy of new (preventive) products

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[1] Hensen et al "How to develop a Controlled Human Infection Model for Clostridioides difficile", Clinical Microbiology and Infection, MAR 2025

Non-toxigenic *C. diff* challenge study (pre-study)

CloDiCo (*Clostridioides difficile* colonisation) study[1]

- Randomised double-blind controlled clinical trial, exposing healthy volunteers to **non-toxigenic** *C. difficile* (**NTCD**)
- SEP 2023- FEB 2025, outpatient, 69 healthy participants included, 3 consecutive phases
  - Phase 1 (n=24): **5D 10^4** OR **10^7** CFU **NTCD** spores OR 5D **placebo** capsule **(D0-D4)**
  - Phase 2 (n=21): **1D vancomycin pretreatment** 4dd250mg **(D-7),** followed by 5D 10^4 OR 10^7 NTCD spores OR 5D placebo (D0-D4)
  - Phase 3 (n=22): **5D vancomycin pretreatment** 4dd250mg **(D-11** followed by 5D 10^4 OR 10^7 NTCD spores OR 5D placebo (D0-D4)

No safety concerns

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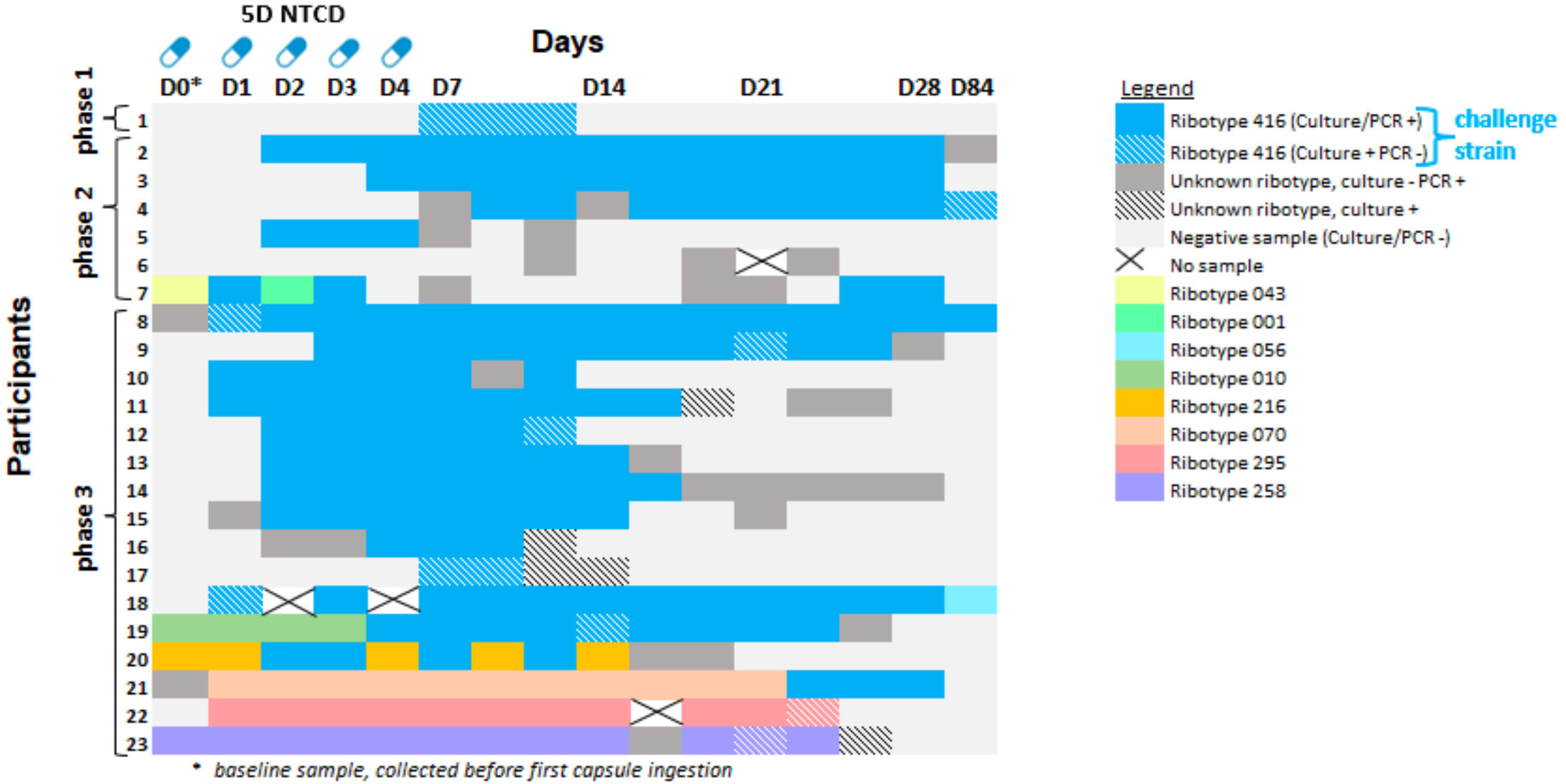
[1]ClinicalTrials.gov NCT05693077

CloDiCo results – colonisation mostly in vancomycin pretreated participants, no dose response relation

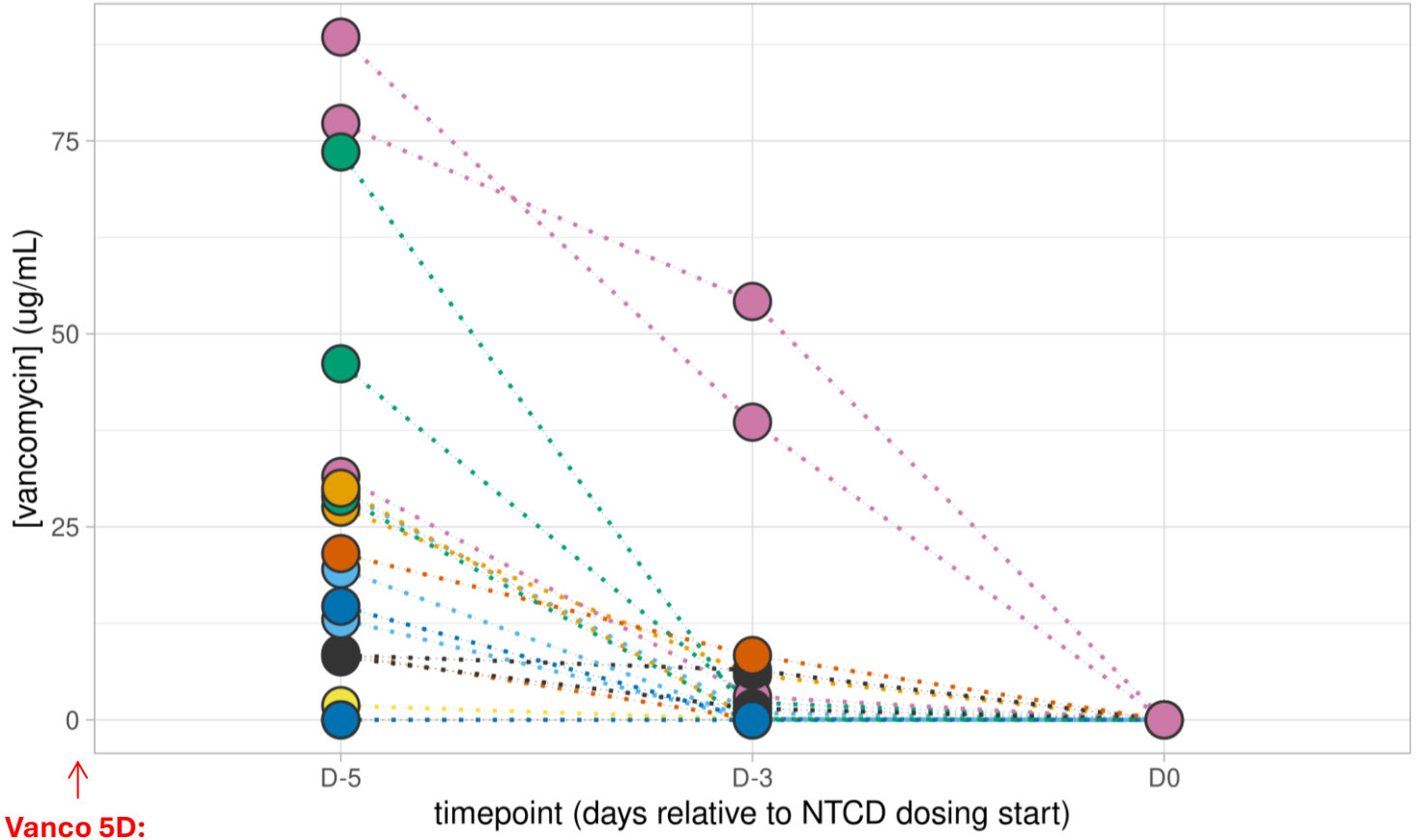
Intervention groups	Colonisation		
	Phase 1 No vanco (N=24)	**Phase 2** 1D vanco (N=21)	**Phase 3** 5D vanco (N=22)
5D 10^4 NTCD spores	**0%** (0/10)	**40%** (4/10)	**80%** (8/10)
5D 10^7 NTCD spores	**10%** (1/10)	**22%** (2/9)	**88%** (8/9)
Attack rate	**5%** (1/20)	**32%** (6/19)	**84%** (16/19)
5D placebo	0% (0/4)	0% (0/2)	33% (1/3)

CloDiCo results - heterogeneous colonisation patterns, including colonisation with field *C. diff* strains



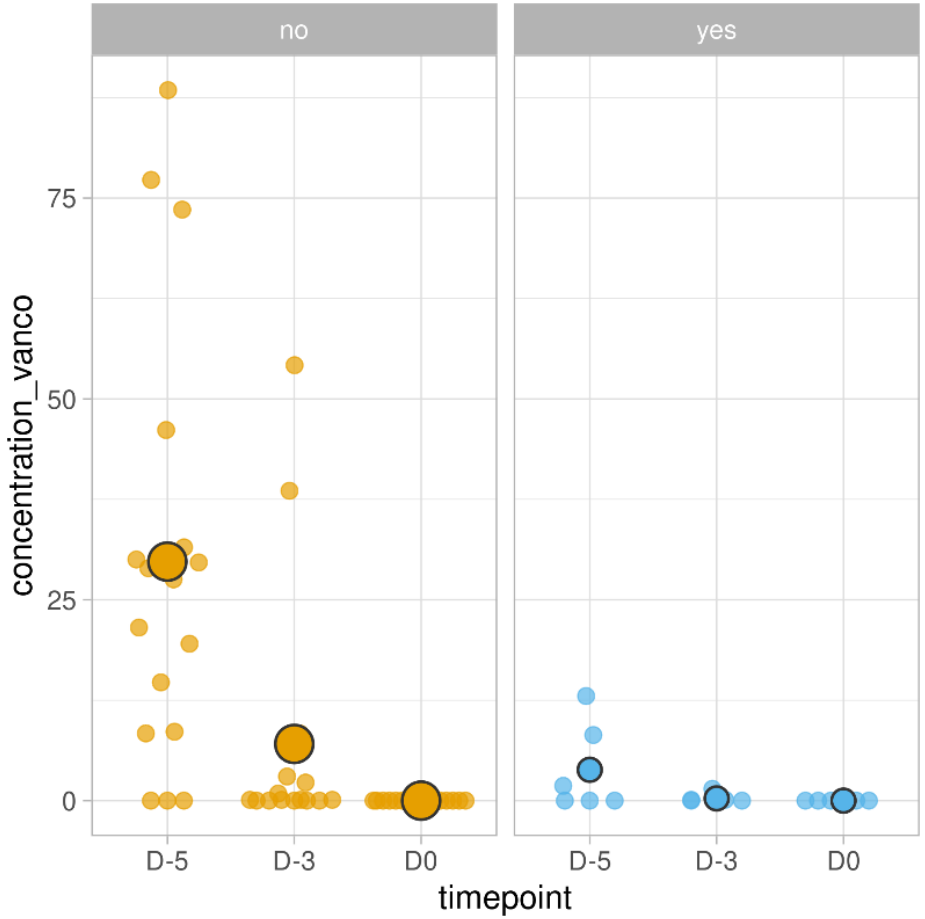
CloDiCo results phase 3 – vancomycin: 7D wash-out period, variance in clearance may affect *C. diff* colonisation


Fecal vancomycin clearance



Vanco 5D:
D-11-D-7

Cdiff positivity D0



[1] Hu et al (2022) Microbiol Spectr.
[2] Ringeling et al (2022) J Chromatogr B Analyt Technol Biomed Life Sci.

Lessons learned for design toxigenic *C. diff* CHIM

1. No dose response relation: continue with one Cdiff dose of 10^4 spores
2. To colonise majority of healthy participants: antibiotic pretreatment needed
3. Duration and dose needed: 5D vancomycin 4dd250mg
4. Total wash-out period of vancomycin is 7D, to prevent colonisation with non-challenge Cdiff strains; start Cdiff dosing directly after antibiotic pretreatment and continue for 12D (7D wash out and 5D ‘normal’ dosing)

Toxigenic *C. difficile* CHIM trial timelines

= screening visit with stool, blood and urine collection

= inclusion visit with safety assessment and urine pregnancy test (for women of child-bearing potential)

= TCD exposure + visit for AE, vital sign, and stool collection (on D0 sample collected before TCD exposure) and once in four days a safety blood draw

= TCD exposure at home

= follow-up visit every other day with AE, vital sign and stool collection and once in four days a safety blood draw

* = immunology samples collected on D0 (baseline sample before TCD ingestion),D2,D20, D35 and D84

1 = first day of CDI symptoms, hospital visit same day for physical check-up and stool, blood. serum collection

= vancomycin ingestion + visit with stool collection

= vancomycin ingestion at home, with stool collection on D-3 and D-1

= clindamycin ingestion + visit with stool collection

= clindamycin ingestion at home with stool collection on D-3 and D-1

= SMC review, planned after D35 visit

2 = unless still colonised with TCD, then follow-up until decolonisation

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Toxigenic *C. difficile* CHIM trial design

CloDiCHI : *Clostridioides difficile* Controlled Human Infection study [1]

First-in-human, open label, ad cal trial, investigating oral expos ifficile spores (TCD, 10^4 C ers

Pilot g atory group (N=15)

E on:

- Safety
- 2. Microbiological AND clinical endpoint in ideally ≥ 70%

> Decision made with the SMC

Approved by METC MAR-2025

*threshold for a microbiological (proven and probable) AND clinical (proven and probable) endpoint. After D35, safety, microbiological and clinical data will be discussed with the SMC to decide upon trial continuation according to above schedule.

Abbreviations used: 12D=12 days, 5D= 5 days, TCD= toxigenic C. difficile

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[1]ClinicalTrials.gov NCT06702345

C. difficile challenge material production

According to GMP principles at Experimental Bacteriology laboratory of LUMC [2]

- Starting material: L-TCD-01 strain from faecal material of patient with uncomplicated CDI
- Drug product: direct release capsules with 10^4 CFU L-TCD-01 spores

MCB
- From GLP material
- Full genome
- **QCs**
  - Identity
  - Potency
  - Purity
  - Safety
- Storage: production lab

Recover and amplify on GMP compatible media

WCB
- Generated with GMP grade chemicals
- **QCs**
  - Identity
  - Potency
  - Purity
  - Safety
- Storage: production lab

Aliquot for production run on GMP compatible media

Production run
- **IPC**: growth within expected parameters

Aliquot for production run on GMP compatible media

Drug substance
- **QCs**
  - Identity
  - Potency
  - Purity
  - Safety
- Storage: production lab

Potency adjustment in capsulization compatible buffer

Drug product
- **QCs**
  - Identity
  - Potency
  - Purity
  - Safety
  - Capsule controls
- Storage: Pharmacy

Samples tested at predefined intervals

Stability testing
- **QCs**
  - Identity
  - Potency
  - Purity
  - Capsule controls

[2] Hensen et al "Manufacture of Clostridioides difficile spores for experimental infection of human volunteers", JID, 2025, under revision

Conclusion and Outlook

- Insights gained from the NTCD colonisation model greatly informed the design of the TCD CHIM
- First pilot cohort TCD CHIM (12D TCD spores): no safety issues, no colonisation/infection → continue with second cohort (vanco 5D + 12D TCD)
- It is feasible to produce Cdiff challenge material for CHIM studies at small scale aligning to GMP principles
- Looking forward to the microbiota and immunological analyses within the consortium

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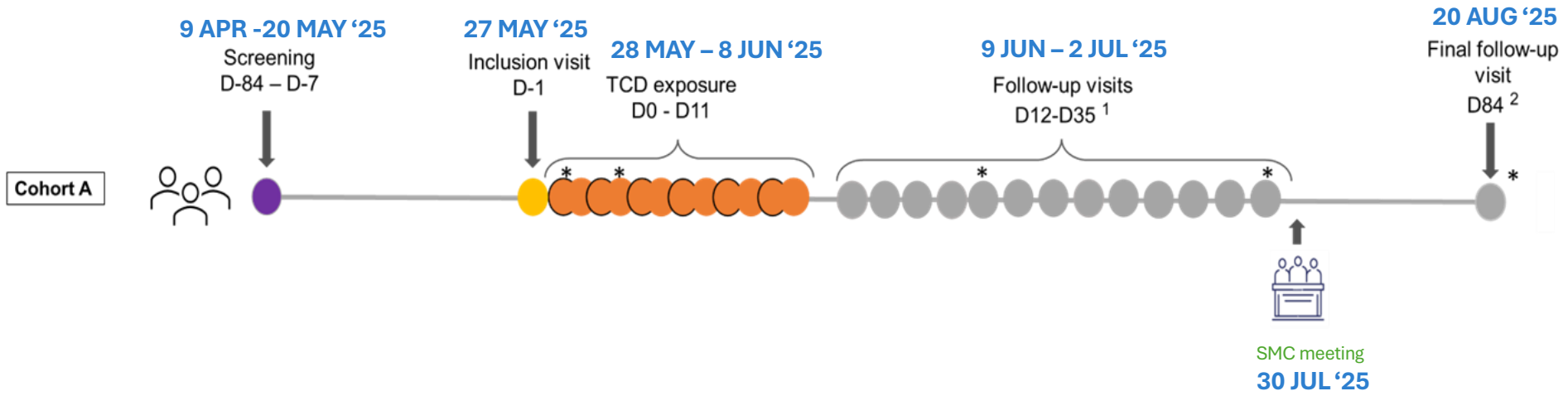
CloDiCo results – colonisation mostly in vancomycin pretreated participants, no dose response relation

Definition NTCD colonisation:

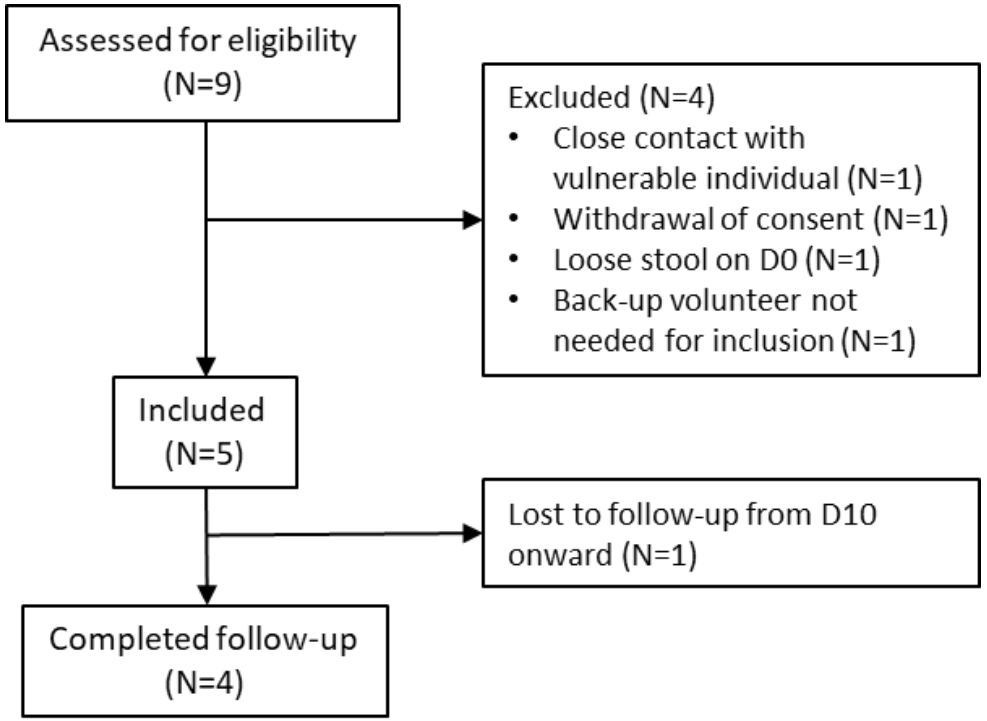
A positive *gluD* qPCR OR culture on at least two timepoints between three days and two weeks after last day of NTCD exposure

Intervention groups	Colonisation		
	Phase 1 No vanco (N=24)	Phase 2 1D vanco (N=21)	Phase 3 5D vanco (N=22)
5D 10^4 spores	**0%** (0/10)	**40%** (4/10)	**80%** (8/10)
5D 10^7 spores	**10%** (1/10)	**22%** (2/9)	**88%** (8/9)
Attack rate	**5%** (1/20)	**32%** (6/19)	**84%** (16/19)
5D placebo	0% (0/4)	0% (0/2)	33% (1/3)

CloDiCHI pilot group cohort A (N=5) - timelines



CloDiCHI pilot Cohort A – 1 lost to follow- up, median age 24 years, male/female ratio 1:4



	Cohort A (N=5)
Gender	
Male (%)	1 (20)
Female (%)	4 (80)
Age	
Mean (SD)	28 (10)
Median (Min, Max)	24 (22, 45)
BMI	
Mean (SD)	25.0 (3.3)
Median (Min, Max)	23.6 (21.8, 29.1)

Intention to treat population: N=5 (safety analysis)

Per protocol population: N=4 (microbiological and clinical endpoint analyses), because of less than 75% visit attendance of 1 participant (lost to follow-up (withdrew consent) due to non-study related issues)

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CloDiCHI pilot Cohort A – exposure to TCD capsules

Subject ID	Number of ingested capsules TCD	Days of missed ingestion (if applicable)
CDC-160-FR	12	NA
CDC-233-YV	12	NA
CDC-526-KK	12	NA
CDC-782-IS	10	D9 and D10
CDC-993-EG	12	NA

CloDiCHI pilot cohort A – no safety issues

Intention to treat population (N=5)

Exposure to 10^4 CFU TCD spores (one capsule daily for 12 consecutive days) was safe and well tolerated in all participants, with no study-related serious adverse events (SAEs).

A total of 28 AEs have been reported of which 14 were related. The severity of related AEs included 12 mild, 2 moderate and none severe.

CloDiCHI pilot Cohort A – related AEs

Description of AE	Related adverse events, number (proportion)				
	Mild	Moderate	Severe	Caused by TCD spores	Caused by study related procedures
Change in bowl habit (*not meeting criteria for diarrhoea or constipation*)	2 (40)	1 (20)	-	3	-
Abdominal pain	1 (20)	1 (20)	-	2	-
Flatulence	2 (40)	-	-	2	-
Abdominal distension	1 (20)	-	-	1	-
Fever	1 (20)	-	-	1	-
Hematoma after vena puncture	1 (20)	-	-	0	1
Anemia	1 (20)	-	-	1	-
Neutropenia	1 (20)	-	-	1	-
Leukopenia	1 (20)	-	-	1	-
Elevated CRP	1 (20)	-	-	1	-
Total	12	2	-	13	1

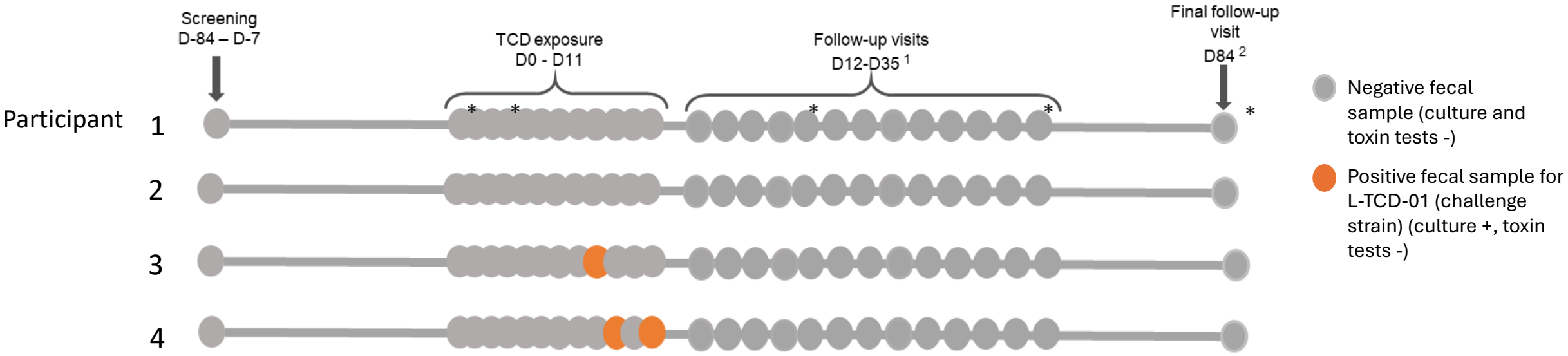
CloDiCHI pilot Cohort A – unrelated AEs

Description AE	Participants with unrelated adverse events, number (proportion)		
	Mild	Moderate	Severe
Hypernatremia	1 (20)	2 (40)	-
Headache	-	2 (40)	-
Common cold	1 (20)	-	-
Fatigue	-	1 (20)	-
Change in bowl habit	1 (20)	-	-
Dizziness	-	1 (20)	-
Abdominal pain	1 (20)	-	-
Sore throat	1 (20)	-	-
Anemia	1 (20)	-	-
Dysmenorrhea	1 (20)	1 (20)	-
Total	7	7	-

CloDiCHI pilot Cohort A – no microbiological or clinical endpoint reached

Per protocol population N=4

None of the participants reached the microbiological or clinical endpoint (no diarrhoea reported) (0/4)



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CloDiCHI - Main objectives

Primary

- To establish a controlled human infection model for *C. difficile* which is safe, well tolerated and results in microbiological AND clinical endpoint in ideally ≥ 70% of volunteers

Secondary

- Assess colonisation and/or infection with non-challenge *C. difficile* strains
- Measure the kinetics of colonisation and/or infection over time
- Investigate the mucosal and systemic immune response following *C. difficile* colonisation and/or infection

CloDiCHI - Exploratory objectives

Exploratory

- To determine factors in the host microbiota at baseline which determine risk of *C. difficile* colonisation and/or infection.
- Determine changes in the host microbiota following *C. difficile* colonisation and/or infection.
- Investigate the association between bile acid metabolism (and possible other metabolites) and *C. difficile* colonisation and/or infection.
- Investigate *C. difficile* in-vivo evolution and adaptation.
- Investigate the intestinal pharmacokinetics of orally administered vancomycin and clindamycin and its pharmacodynamic effects, including the association with *C. difficile* colonisation and/or infection.
- Investigate the effects of a *C. difficile* challenge on patient reported outcome measures.

CloDiCHI - Primary endpoints

Safety: number and grade of (related) adverse events until D35

Microbiological (colonisation):

1. Proven: positive TCD culture (with challenge strain ribotype 020), ≥2 timepoints from D14-D35
2. Probable: a positive Cdiff toxin test (EIA or *tcdB*-PCR), ≥2 timepoints from D14-D35

Clinical (infection) *(aligned with ESCMID guideline)*

1. Proven: symptoms of CDI (**at least diarrhoea**) and a positive TCD culture (with challenge strain ribotype 020) from D0-D35.
2. Probable: symptoms of CDI (**at least diarrhoea**) and a positive toxin test (EIA or *tcdB*-PCR) from D0-D35.

CloDiCHI - Secondary endpoints

Secondary

- Proven microbiological endpoint/colonisation with non-challenge strain: a positive culture of C diff with another molecular identity than the challenge strain, on at least two timepoints from D14 until D35, irrespective of CDI symptoms.
- Proven clinical endpoint/infection with non-challenge strain: clinical findings compatible with CDI (clinical findings should include at least diarrhoea: Bristol stool chart type 6-7 plus ≥ 3 stools in 24 hours) and a positive culture of Cdiff with another molecular identity than the challenge strain, from D0 until D35.
- Quantitative measurement of Cdiff by qPCR at samples taken every other day to determine peak value (lowest Ct value) and duration (in days) of PCR positivity until D35.
- Anti-Toxin A and anti-Toxin B neutralizing antibody titers by toxin neutralization assay (TNA) on serum samples at D0, D20, D35 and D84. **(GSK)**
- ELISA for antibody responses against various antigens of Cdiff in serum and fecal samples at D0, D20, D35, D84 and first day of CDI symptoms. **(Oxford University)**
- Cytokine measurement in serum and fecal samples at D0, D2, D20 and first day of CDI symptoms**. (Oxford University)**
- Calprotectin and potential other biomarkers for inflammation (like lactoferrin) in fecal samples on D0, D20 and D35. **(Oxford University)**

CloDiCHI - Exploratory endpoints

Exploratory

- Transcriptomics of PBMCs at D0, D2, D20 and D35, and first day of CDI symptoms. **(Oxford University)**
- Identification of microbiome markers (microbiota, metabolites etc) before exposure to Cdiff spores which are associated with subsequent colonisation and/or infection. **(Helmholtz center for Infectious Research)**
- Identification of changes in stool microbiota and components following Cdiff colonisation and/or infection. **(Helmholtz center for Infectious Research)**
- Identification of bile acid metabolism markers (and possible other metabolomic markers) associated with colonisation and/or infection. **(Helmholtz center for Infectious Research)**
- Identification of genetic changes in Cdiff after passage through the human host.
- Pharmacokinetic indices of fecal vancomycin and clindamycin (if escalation to cohort B/C) and the association of these indices with PD endpoints (including but not limited to colonisation or infection). Composite PK/PD variables will be calculated to identify a PK/PD target for successful colonisation and/or infection (for example time above MIC, AUC/MIC etc.) **(Helmholtz center for Infectious Research)**
- Effects of Cdiff challenge on self-reported quality of health questionnaires.

Exclusion criteria (1)

1. Any **physical or psychiatric illness** or conditions that could threaten or compromise the health of the subject during the study, influence their ability to participate in the trial or interfere with the interpretation of the study results, as determined by the trial physician;
2. Use of **systemic (IV or oral) antibiotics** within **three months prior to inclusion** (D-1 for cohort A, D-6 for cohort B/C); or use of other **microbiota influencing medication**, including probiotics, that could influence the trial (based on the Investigator's opinion), **within 1 month prior to inclusion** (D-1 for cohort A, D-6 for cohort B/C). Prior use of topical antibiotics is permitted if there is no clinically relevant systemic expected following assessment of the trial physician and are expected to be discontinued during the start of the first study activity.
3. Has had a **recent hospitalization** (e.g. **3 months prior to inclusion** (D-1 for cohort A, D-6 for cohort B/C)) and/or has someone in **immediate social circle who is frequently hospitalized** (≥3 times in a 12-month period) or frequently exposed to hospital settings (≥ one time a month, e.g. dialysis units);
4. **Regular use** (defined by more than once weekly) **of proton-pump inhibitors or H2-blocker**s during one month prior to inclusion (D-1 for cohort A, D-6 for cohort B/C);
5. **Chronic use of immunosuppressive drugs,** e.g. systemic corticosteroids or other immune modifying drugs (with exception of oral anti-histamines and topical/inhaled corticosteroids);
6. Positive HIV, Hepatitis B or C screening tests;
7. Known immunodeficiency disorders;
8. The use of strong P-glycoprotein-inhibitors (like ciclosporin, ketoconazole, erythromycin, clarithromycin, verapamil and amiodaron) during the trial;
9. Known allergy to vancomycin, clindamycin, fidaxomicin (and macrolides), or metronidazole;

Exclusion criteria (2)

10. Any known significant **allergy** against the excipients of ***C. difficile*** **inoculum** or inability to swallow capsules;
11. **Known gastro-intestinal disease** including but not limited to inflammatory bowel diseases (Crohn's disease, Colitis ulcerosa), a history of bowel resection or any other gastro-intestinal surgery which has significantly changed the anatomical structure or physiological function of the gastro-intestinal tract, bile acid secretion abnormalities, constipation defined by bowel movements less than every second day or chronic use of laxatives;
12. **Positive fecal culture or PCR with toxigenic or non-toxigenic *Clostridioides* spp. or SSYC** (Salmonella spp., Shigella spp., Yersinia spp. or Campylobacter spp.) at screening, or **recent** (<14 days) **history of diarrhoea** (i.e. as ≥3 loose stools (Bristol stool scale 6–7) in 24 hours);
13. Any condition that would put household members or close contacts at a greater risk for transmission e.g. **no access or use of flush toilet**;
14. **Individuals living, working or having close contact with people who belong to vulnerable populations** such as hospitalized patients, pregnant women, immune compromised individuals, children younger than 2 years, residents of nursing homes, elderly older than 70 years of age, or any person with a medical condition at risk of developing severe CDI.
15. Individuals **working in healthcare or food preparation**;
16. Individuals **having close contact with healthcare personal/individuals** working in food preparation;
17. For women of childbearing potential; a **positive serological pregnancy test** at screening or **lactating at screening/** during the trial;
18. **History of drug or alcohol abuse** interfering with normal social functioning in the period of one year prior to study onset, **positive urine toxicology test** for illicit drug use at screening;
19. **Receipt of another investigational agent within 90 days prior** or **60 days after *C. difficile* ingestion**;
20. Any condition or situation that could influence the independent consent of participant (e.g. being a direct colleague or family member of study personnel).