1 STUDY SYNOPSIS

Name of Sponsor/Company: 4D Pharma Research Ltd	**Individual Study Table Referring to Part of the Dossier** **Volume:** **Page:**	*For National Authority Use Only*
Name of Finished Product: Thetanix®		
Name of Active Ingredient: *Bacteroides thetaiotaomicron*		

TITLE OF STUDY: A Phase 1, Randomised, Double-Blind, Placebo-Controlled Study to Assess the Safety and Tolerability of *Bacteroides thetaiotaomicron* in Young People Aged 16 to 18 years with Stable Crohn's Disease

INVESTIGATORS: Dr Richard Hansen (Professor Richard Russell), Professor David Wilson, Dr Rafeeq Muhammed, Professor Ian Sanderson, Professor Stephen Allen, Dr Christos Tzivinikos

STUDY CENTRES: Five (5) centres in the United Kingdom.

PUBLICATIONS: None

STUDY PERIOD:	**DEVELOPMENT PHASE:** 1
First subject recruited 23 February 2016 Last subject completed 10 April 2018	

OBJECTIVES:

Primary (Part A and Part B): To assess the tolerability and safety profile of Thetanix when administered as a single dose and in multiple doses.

Exploratory (Part A and Part B): To assess the effect of administering Thetanix on the gastrointestinal microbiome.

Exploratory (Part B): To assess the effect of multiple doses of Thetanix on faecal calprotectin.

METHODS:

This was a randomised, double-blind, placebo-controlled, single and multiple dose study in subjects with Crohn's disease (CD), aged 16 to 18 years and consisted of 2 parts (Part A with single dose and Part B with multiple doses). Subjects suitable for this study were identified from current patient lists at appropriate gastroenterology clinics. Subjects and parents, as applicable, were approached during routine clinical visits to determine their interest in participation.

In Part A it was planned for 10 subjects (8 subjects post protocol Amendment 5) to receive a single oral dose (consisting of 3 capsules) of either placebo or Thetanix. After successful dosing of all subjects, a Safety Review Committee (SRC) reviewed the safety data up to Day 7. If it was deemed appropriate further subjects were to be recruited into Part B, the multi-dosing phase.

Part A Single Dose

Part A consisted of Screening (-28 days), when a check was made of the subject's ability and willingness to swallow 3× size 0 placebo capsules, and 3 research visits: for single dose treatment on Day 0 (Visit 1), and post-treatment visits on Day 1 (Visit 2) and Day 7 (Visit 3). In addition, 2 stool samples were requested: a baseline stool in the 72 hours prior to Day 0, and a post-treatment stool from 48 hours following dosing.

During Day 0 (Visit 1) subjects arrived in the morning approximately 2 hours after having consumed a light breakfast (e.g., cereal, fruit, or toast). Following eligibility and Baseline tests subjects received a single oral dose (3 capsules), of Thetanix or placebo. No food was allowed for 4 hours after dosing, though water was freely available. Subjects were monitored for 8 hours. A meal was provided 4 hours after dosing. If more than 1 subject was dosed on a single day the dosing interval between concurrent subjects was not less than 30 minutes.

Subjects were assessed (vital signs and adverse events [AEs]) at 2, 4, and 8 hours (plus or minus 30 minutes) post-dosing.

After 8 hours, and if deemed fit, subjects were discharged with access to electronic diaries in which to record body temperature twice daily for 7 days. Subjects were also provided with a stool collection kit.

Subjects who developed a fever (2 body temperatures ≥38.0°C in 12 hours or a single temperature ≥38.5°C at any time) were told to contact the site and attend an unscheduled assessment for blood cultures. If required, subjects were treated with metronidazole or co-amoxiclav.

Subjects returned 24 hours (±2 hours) and 7 days (±1 day) after Day 0 Visit 1 for a safety check.

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Part B Multiple Doses

After approval from the SRC, Part B recruitment commenced. Subjects who participated in Part A were also allowed to participate in Part B; following re-screening and re-consenting, they were allocated a new subject number. For subjects that did not participate in the single dose phase, a check was made of the subject's ability and willingness to swallow 3× size 0 placebo capsules. Subjects were provided with a stool collection kit and instructions. A total of 8 subjects were planned.

Part B consisted of Screening (-28 days), and 5 research visits: to commence treatment on Day 0 (Visit 1), 24 hours post-treatment on Day 1 (Visit 2), and visits 7 days post-Day 0 (Visit 3), 14 days post-Day 0 (Visit 4) and a final follow-up at Day 56 (Visit 5). In addition, 3 stool samples were requested: a baseline stool in the 72 hours prior to Day 0, a post-treatment stool from the 48 hours following Day 7, and a follow-up stool in the in the 72 hours before Day 56.

Part B treatment consisted of a twice daily dosing period of 7.5 days where the first dose was taken in clinic; the next 13 doses were taken at home and the 15th dose was taken in the clinic.

Subjects were asked to send samples in the normal post to a central laboratory.

Eligible subjects returned to the clinical unit on Day 0 (Visit 1; within 28 days of Screening visit) for a 4-hour stay arriving in the morning. Subjects were asked to have had a light breakfast (e.g., cereal, fruit or toast) about 2 hours prior to arriving at the clinic. Following a brief physical examination and confirmation of eligibility (including confirmation that they were not experiencing an exacerbation), the following were also recorded: changes in medication, assessment of vital signs and a urine pregnancy test for all female subjects who were post-menarche, height, weight and calculation of body mass index (BMI) Z scores, recalculation of Physician's global assessments and weighted paediatric Crohn's disease activity index (wPCDAI) scores.

Subjects received a dose of Thetanix or placebo (an hour before food) every 12 hours (15 doses in total) during the 7.5-day dosing period of Part B.

NUMBER OF SUBJECTS:
Planned: n=18 (8 in Part A [originally 10 in Part A but reduced as per protocol Amendment5] and 10 in Part B)

Screened: n=23 (10 in Part A and 13 in Part B)

Randomised: n=18 (8 in Part A and 10 in Part B)

Completed: n=18 (8 in Part A and 10 in Part B)

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION: Subjects with CD in clinical remission, aged 16 to 18 year were included.

Subjects were excluded if they had undergone surgery for resection of bowel in the last 12 months, or subjects, who had undergone resection of bowel more than 12 months ago, and had experienced an exacerbation in the last 12 months or developed fistulae. (Subjects who had resection of bowel more than 12 months ago, with no further requirement for surgery within the last 12 months, with stable medications as per other inclusion/exclusion criteria; and subjects who had surgery for perianal abscess more than 6 months prior to dosing remained eligible.). Had active fistulisation. Had a significant change in their immune-modulating maintenance medication in the 3 months prior to Screening and/or the start of dosing. Had taken systemic steroids in the last 3 months. Were unable to take any oral feeding or with feeding gastrostomies.

TEST PRODUCT:
Thetanix® is formulated as off white intrinsically enteric size 0 capsules containing lyophilised *B. thetaiotaomicron*. Each capsule contains $10^{7.73\pm1.43}$ colony forming units (CFUs) and microcrystalline cellulose. Three capsules contain $10^{8.21\pm1.43}$ CFUs and microcrystalline cellulose.

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COMPARATOR PRODUCT:
The placebo capsules contained microcrystalline cellulose and no *B. thetaiotaomicron* and were comparable in size, weight, and appearance to the test product.

DURATION/FOLLOW-UP:
Part A maximum total duration for each subject: 35 days, including a 7 day follow-up.

Part B maximum total duration for each subject: 84 days, including a 42 day follow-up

CRITERIA FOR EVALUATION:
There were no efficacy, pharmacokinetic or pharmacodynamic evaluations in this study.

Safety parameters included: physical examination, vital signs, electrocardiogram, wPCDAI, Physician's global assessment, symptom diary cards, AEs, clinical laboratory assessments, and stool sample assessment (including faecal bacterial count for Thetanix and faecal calprotectin).

STATISTICAL METHODS:
Safety Analysis Set (SAS): consisted of all randomised subjects that received at least one dose of study medication - used for primary safety analysis.

Full Analysis Set (FAS): consisted of all the subjects that received at least 1 dose of study medication and had at least one post Baseline assessment - used for secondary and exploratory safety analysis.

Exposure to study medication included the total number of doses, summarised by treatment group, for participants who withdrew, participants who completed and overall.

The primary analysis for Part A and Part B was the safety profile of AEs. All AEs were coded by the MedDRA. An overall summary of AEs was provided including the number of AEs, number of serious adverse events (SAEs), number of treatment-emergent adverse events (TEAEs) and number of treatment related AEs/SAEs. The incidence of TEAEs was summarised by system organ class (SOC), preferred term (PT) and treatment group. A separate summary of TEAEs was provided by SOC, PT, severity and treatment group. AEs that were related to the study treatment were also summarised separately.

For the safety analyses described below, categorical or qualitative variables were summarised by absolute counts (n) and percentages (%).

For vital signs, continuous variables were summarised by descriptive statistics. Vital signs summaries were grouped by treatment, visit and, if applicable, time point within visit. Additionally, changes from Baseline were presented in the summary tables and listings for Part A and Part B.

The wPCDAI raw values and changes from Baseline were summarised by descriptive statistics. wPCDAI summaries were grouped by treatment, visit. The individual scores were summarised on the categorical and continuous scale. Total scores were summarised for wPCDAI as a continuous variable.

Clinical laboratory parameters included collected and derived quantitative and qualitative parameters for haematology, chemistry and urinalysis. Quantitative parameters were summarised by raw values and change from Baseline. All clinical laboratory summaries were grouped by treatment and visit. Shift tables summarised n and % shift from Baseline to each post Baseline reference range category, for clinical chemistry and haematology parameters.

The Physician's global assessment (4-point scale) of the subject's CD, was summarised by n and % for each category, grouped by treatment and visit. Additionally, for Part B only, shift tables summarised the n and % shift in Physician's global assessment from Baseline to Day 7 (Visit 3) and Day 56 (Visit 5) by treatment group.

The continuous variables for inflammatory biomarkers, bacterial count and other microbiome constituents reported from these analyses were summarised by descriptive statistics. Summaries were grouped by treatment, visit and, if applicable, time point within visit. The parameters summarised were *B. thetaiotaomicron* count, faecal calprotectin (for Part B only) and any other microbial communities of interest.

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Electrocardiogram (ECG) parameters were summarised by descriptive statistics for absolute results and change from Baseline. The overall interpretation of ECG was summarised by treatment group for both parts of the study. Pregnancy test results and HIV, hepatitis B and hepatitis C results were listed.

RESULTS:

Disposition and Demographics: Overall, 23 subjects were screened, 18 subjects were treated during the study, 8 subjects in Part A (single dose) and 10 subjects in Part B (multiple dose). All subjects completed the study and no subjects discontinued. Three subjects were included in and completed both Part A and Part B. All subjects receiving at least 1 dose of study drug were included in the safety analysis and all subjects receiving study drug and who had at least 1 post Baseline assessment were included in the FAS; therefore, 8 subjects were included for Part A and 10 subjects for Part B for all analyses. Demographic and Baseline characteristics were similar for Parts A and B. The majority of enrolled subjects were male (Part A 75.0% and Part B 80.0%). All subjects were white and aged from 16 to 18 years.

Adverse Events: In Part A, a third of the subjects receiving a single Thetanix dose experienced at least 1 TEAE, of these 1 subject experienced 3 related TEAEs (eructation, flatulence, and gastrooesophageal reflux). No TEAEs were reported for subjects receiving placebo. In Part B, half of all subjects receiving a multiple Thetanix or placebo dose experienced at least 1 TEAE; 1 subject receiving Thetanix, experienced 3 related TEAEs (upper abdominal pain, dizziness and headache).

Other Safety Assessments: There were no clinically significant changes from Baseline in physical examinations, vital signs, electrocardiograms, Physician's global assessment, wPCDAI, or laboratory parameters.

Faecal calprotectin: Although overall mean changes in faecal calprotectin from Baseline to Visit 3 and Visit 5 were similar for Thetanix and placebo treated subjects, 1 of the 2 subjects on Thetanix treatment with a Baseline faecal calprotectin level >250 mg/kg showed a decrease from Baseline in faecal calprotectin levels over the study period. The decrease in faecal calprotectin over the whole study period indicates a potential anti-inflammatory effect.

Microbiome: No significant differences (p-values >0.05) in the microbiota profiles were found between Thetanix and placebo treatment groups. A temporal effect within each group across the study time points was not observed and no significant difference (p-values >0.05) in microbiota profiles was identified in either the Thetanix or placebo treated groups across the time points. No significant difference (p-values >0.05) in abundant taxa or functional pathways across the study time points were observed as a result of either treatment. A significant change in Shannon diversity and evenness was reported at Day 7 in the Thetanix treated group in Part B, however there was no increase in observed diversity (richness). This observation was mirrored in the Thetanix treated group in Part A where there was a non-significant increase (trend) in Shannon diversity and evenness at Day 1 compared to Day 0 which was of a similar effect size to that seen in the Thetanix treated group in Part B at Day 7.

B. thetaiotaomicron specific strain was not detected in the majority (88%) of the samples in both Part A and B using qPCR. The strain was detected in 5 of the 42 samples where 2 of those were in a placebo-treated subject in Part A and 3 samples were in Thetanix-treated subjects in Part B.

CONCLUSIONS:

This Phase 1 study has shown Thetanix to be well tolerated with a good safety profile. The results from a small number of subjects suggest a potential effect on the microbiome and inflammatory response. Further work is warranted to develop the safety profile and assess efficacy.

- Single and multiple doses of Thetanix were safe and well tolerated. There were no SAEs, deaths or TEAEs leading to study discontinuation.
- After multiple doses of Thetanix, a change in faecal calprotectin levels over time was observed in 1 subject, suggesting a potential anti-inflammatory effect.
- After multiple doses of Thetanix, an increase in evenness (more consistent relative abundance in

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taxa) in gut microbiome was observed. This evenness in taxa is considered a property of a healthy gut microbiome.

- Through qPCR the *B. thetaiotaomicron* specific strain was detected in 12% of all faecal samples tested (including a single placebo treated subject). However, *B. thetaiotaomicron* at the species level was detected in microbiome sequencing data and therefore further investigation is required.

DATE OF FINAL REPORT: 01 October 2018