A phase I randomized, double-blind, placebo-controlled study to assess the safety and tolerability of (Thetanix™) *Bacteroides thetaiotaomicron* in adolescents with stable Crohn's disease [Sa1905]

Authors: Hansen R[1], Sanderson I R[2], Muhammed R[3], Allen S[4], Tzivinikos C[5], Henderson P[6], Gervais L[1], Jeffery I B[7], Mullins D P[7], O'Herlihy E A[7], Weinberg J D[8], Kitson G[9], Russell R K[1], Wilson D C[10]

[1]Royal Hospital for Children, Paediatric Gastroenterology, Glasgow, United Kingdom; [2]Queen Mary University of London, Blizard Institute, London, United Kingdom ; [3]Birmingham Children's Hospital, Paediatric Gastroenterology, Birmingham, United Kingdom; [4]Liverpool School of Tropical Medicine, Paediatrics, Liverpool, United Kingdom; [5]Alder Hey Children's Hospital, Paediatric Gastroenterology, Liverpool, United Kingdom ; [6]Royal Hospital for Sick Children, Paediatric Gastroenterology, Edinburgh, United Kingdom; [7]4D pharma Cork Limited, University College Cork, Cork, Ireland; [8]4D pharma, Leeds, United Kingdom; [9]ProPharma Partners Limited, Horsham, United Kingdom; [10]University of Edinburgh, Child Life and Health, Edinburgh, United Kingdom

Introduction

Bacteroides thetaiotaomicron is a non-motile, purely anaerobic symbiotic bacterium with the ability to digest dietary fibers and host glycans, whilst producing short chain and organic acids, and a cell surface that both interacts with and evades the host immune system[1].

The organism has been shown to support and stimulate mucus production within the colon, enhancing an important innate immune mechanism against bacterial invasion[2].

B. thetaiotaomicron is also known to attenuate gut inflammation via enhancement of NF-κB subunit RelA nuclear export and subsequent antagonism of transcription factor NF-κB[3], a key component in TNF-α release following NOD2 expression[4].

B. thetaiotaomicron has therefore been identified by the trial sponsor as an ideal organism for consideration as a live biotherapeutic specific to Crohn's disease (CD), offering the compelling prospect of, essentially, a topically active anti-TNF-α probiotic.

Preclinical data has supported this concept, with *B. thetaiotaomicron* proving protective in two rodent models of colitis (dextran sodium sulphate (DSS) and IL-10 knockout), leading to amelioration of weight loss, histological damage, and immunological activation, including reduced expression of TNF-α in the DSS model[5].

We therefore set out to perform a first-in-humans safety study of lyophilized *B. thetaiotaomicron* (Thetanix™) in teenage patients with known CD in clinical remission.

Methods

This was a randomized, double-blind, placebo-controlled, single and multiple dose study in subjects with CD, aged 16 to 18 years, which consisted of 2 parts (Part A with single dose and Part B with two doses daily for 7.5 days). Patients who participated in Part A were also able to participate in Part B if inclusion/exclusion criteria were achieved after rescreening. Inclusion/exclusion criteria were designed to recruit adolescent patients with known Crohn's disease on stable therapy and in clinical remission.

Each capsule of Thetanix™ contains $10^{7.73\pm1.43}$ colony forming units (CFU) and microcrystalline cellulose. A single dose of Thetanix™ consisted of 3 capsules, comprising $10^{8.2\pm1.4}$ CFU of *B. thetaiotaomicron.*

The placebo capsules contained microcrystalline cellulose but no *B. thetaiotaomicron*, and were comparable in size, weight, and appearance. Randomization was performed a 4 active: 1 placebo basis for both parts of the study.

The occurrence of adverse events (AEs) was sought by non-direct questioning of the subject during study visits and by completion of a diary card in between times.

The trial protocol for follow-up is shown in **Table 1 (Part A)** and **Table 2 (Part B)**. Serial stools were analysed for calprotectin, microbiota profiling (16S rRNA amplicon sequencing) and quantification of *B. thetaiotaomicron* (qPCR).

Bloods were also taken serially. Subjects' temperature was recorded twice daily.

Study schedule

Table 1 (Part A)

Assessment	Screening Day -28 to Day -1	Visit 1 Day 0	Visit 2 Day 1	Visit 3 Day 7
Informed consent	X			
Inclusion/ Exclusion criteria	X	X		
Medical history	X			
Concomitant medication	X	X	X	X
Height/weight	X	X		
Vital signs (BP, HR, RR and temperature)	X	X[a]	X	X
Full physical examination	X			
HIV/Hepatitis B and C screen	X			
Electrocardiogram	X			
Hemoglobin	X		X	X
Clinical chemistry	X		X	X
Blood culture[e]		←	X	→
wPCDAI	X	X		X
Physician's global assessment	X	X		X
Brief physical examination		X		X
Stool sample		X[b]	X[c]	
Admission for 8 hours		X		
Randomization		X		
Dosing		X		
Urine pregnancy test		X		
Adverse events		X[a]		

Schedule of Events for Single Dose Thetanix™ Study (Part A)

BP=blood pressure, HR=heart rate, RR=respiratory rate, HIV=human immunodeficiency virus, wPCDAI=weighted pediatric Crohn's disease activity index.
[a] Predose, 2, 4, and 8 hours post□dose.
[b] Stool sample collected within 72 hours prior to Visit 1.
[c] Stool sample collected within 48 hours post-dosing.
[d] If clinically indicated due clinical suspicion of infection or fever >38.5°C on 1 occasion or >38.0°C ×2 in a 12-hour period.

Table 2 (Part B)

Assessment	Screening Day -28 to Day -1	Visit 1 Day 0	Visit 2 Day 1	Visit 3 Day 7	Visit 4 Day 14	Visit 5 Day 56
Informed consent	X					
Inclusion/Exclusion criteria	X	X				
Medical history	X					
Concomitant medication	X	X	X	X	X	X
Height/weight	X	X	X	X	X	X
Vital signs (BP, HR, RR and temperature)	X	X[a]	X	X[a]	X	X
Full physical examination	X					
HIV/Hepatitis B and C screen	X					
Electrocardiogram	X					
Hemoglobin			X	X		X
Clinical chemistry	X		X	X		X
Blood culture[e]			←	X	→	
wPCDAI	X	X	X	X	X	X
Physician's global assessment	X	X		X		X
Brief physical examination		X		X		X
Stool sample		X[b]		X[b]		X[c]
Admission for 4 hours		X		X		
Randomization		X				
Dosing[d]		X----------X				
Urine pregnancy test			X			X
Adverse events		X[a]	X	X[a]	X	X

Schedule of Events for Multiple Dose Thetanix™ Study (Part B)

BP=blood pressure, HR=heart rate, RR=respiratory rate, HIV=human immunodeficiency virus, wPCDAI=weighted pediatric Crohn's disease activity index.
[a] Predose, 2 and 4 hours post□dose.
[b] If clinically indicated due clinical suspicion of infection or fever >38.5°C on 1 occasion or >38.0°C ×2 in a 12-hour period.
[c] Stool sample collected within 72 hours prior to visit.
[d] Stool sample collected within 48 hours of the last dose of each 7.5-day twice daily administration.
[e] Dosing, 3 capsules twice daily for 7. 5 days (total of 15 doses).

Results

23 subjects were screened for this study: 10 in Part A and 13 in Part B. 8 completed Part A (6 active, 2 placebo) and 10 completed Part B (8 active, 2 placebo). Recruits to Part A were 75% male, median age 16.5 years (range 16-18) and in Part B were 80% male with a median age of 16.5 years (range 16-18). 3 participants completed both Part A and Part B.

All participants were on concomitant medication, the most common being immunosuppressant agents including anti-TNF in 75% of Part A (66.7% active arm, 100% placebo) and 90% of Part B (100% active arm, 50% placebo).
Reported treatment compliance in Part B was 99%, with one subject on active Thetanix™ reporting 14/15 doses and all others reporting full compliance.
No serious adverse events (SAEs) or deaths occurred within the study.

Two subjects reported AEs deemed related- one in Part A with eructation, flatulence and reflux; one in Part B with dizziness, abdominal pain and headache.
All physician's global assessment (PGA) scores were 'quiescent' in Part A and 'quiescent' or 'mild' in Part B. wPCDAI[6] scores ranged from 0 to 20 in Parts A and B, with all scores achieving remission (<12.5) throughout the study with the exception of one Day 0 score of 20, in a participant with a remission score at baseline, and one Day 56 (Visit 5) score of 18, both in the active group.

There was no significant change in mean (+/- standard deviation) calprotectin values across 5 visits in Part B: 145.8 +/-164.5 to 114.0 +/- 119.0, p=0.44 by t-test in active group and 64.6 +/-70.3 to 123.5 +/-163.0, p=0.69 in placebo group. However, a reduction in fecal calprotectin levels was observed for a few of the Thetanix™ treated subjects.

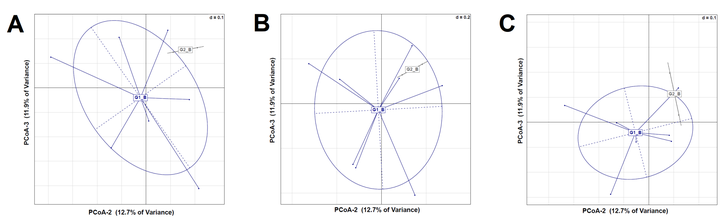

Figure 1: Microbiota Profiles of Treatment Groups in Multiple Dose Thetanix™ Study (Part B) on Days 0, 7 and 56 Based on Bray-Curtis Dissimilarities. A= Day 0 (n=10); B= Day 7 (n=10); C= Day 56 (n=9) G1= Active treatment; G2= Placebo PCoA= Principle components analysis. No significant differences were found in microbiota profiles between the groups at Day 0 (p=0.09), Day 7 (p=0.589) or Day 56 (p=0.299) in Part B.

Microbiome analysis demonstrated no significant differences between active and placebo groups by Bray-Curtis dissimilarity in Part B at Days 0, 7 and 56 (**Figure 1 A-C**).

Microbiome analyses

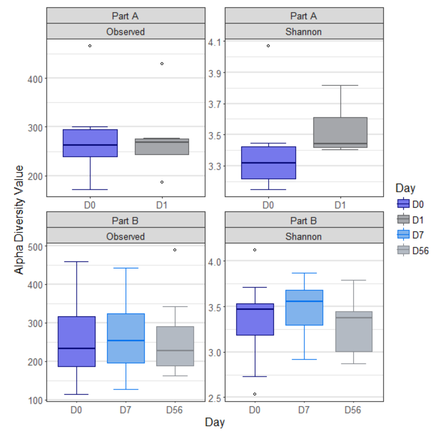

Figure 2: Effect of Thetanix™ on Microbiota Diversity in Part A and Part B Using Observed Species and Shannon Metrics

There was no significant difference in microbiota diversity for the active group in Part A (observed p value=0.236, Shannon p-value=0.140) from Day 0 to Day 1. Shannon diversity was however found to be significantly different (observed p value=0.436, Shannon p-value=0.023) across the study time points in Part B (Day 0, Day 7 and Day 56) with an increase in diversity from Day 0 to Day 7 which decreased at Day 56 (**Figure 2**).

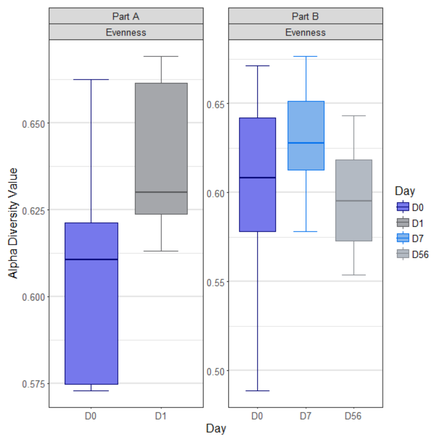

Figure 3: Effect of Thetanix™ Treatment on Microbiota Evenness by Shannon Diversity Index (Parts A and B).

A non-significant increase in microbiota evenness was observed across the timepoints in Part A (Day 0 to Day 1, p=0.07) and a significant difference was observed in Part B (p=0.03) across the study timepoints (Day 0, Day 7 and Day 56) with an increase in evenness from Day 0 to Day 7 which decreased again at Day 56 (**Figure 3**).

All stool samples were screened for the presence of *B. thetaiotaomicron* by qPCR , but this was only quantified in 5/42 (11.9%) samples, hence further analysis of this data was not possible.

Discussion

We have shown that *B. thetaiotaomicron* (as Thetanix™) is well tolerated by teenagers with CD in remission as a single dose and as twice daily doses over a 2-week period.

It does not result in significant adverse events or any significant toxicity over this time period and does not detrimentally alter clinical parameters or fecal calprotectin. Whilst exploratory, limited evidence points to a positive effect on microbial diversity and evenness.

Future studies should continue to explore the optimum dose of Thetanix™ as well as signals of its efficacy as an induction and maintenance agent in CD.

References

1. Porter NT, Luis AS, Martens EC. *Bacteroides thetaiotaomicron*. *Trends Microbiol.* 2018;26(11):966-967. doi:10.1016/j.tim.2018.08.005.
2. Wrzosek L, Miquel S, Noordine M-L, *et al. Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol.* 2013;11:61. doi:10.1186/1741-7007-11-61.
3. Kelly D, Campbell JI, King TP, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shutting of PPAR-γ and RelA. *Nat Immunol.* 2004;5(1):104-112. doi:10.1038/ni1018.
4. Rosenstiel P, Fantini M, Bräutigam K, et al. TNF-alpha and IFN-gamma regulate the expression of the NOD2 (CARD15) gene in human intestinal epithelial cells. *Gastroenterology.* 2003;124(4):1001-1009. doi:10.1053/gast.2003.50157.
5. Delday M, Mulder I, Logan ET, Grant G. *Bacteroides thetaiotaomicron* ameliorates colon inflammation in preclinical models of Crohn's disease. *Inflamm Bowel Dis.* 2019;25(1):85-96. doi:10.1093/ibd/izy281.
6. Turner D, Griffiths AM, Walters TD, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. *Inflamm Bowel Dis.* 2012;18(1):55-62. doi:10.1002/ibd.21649.