Impact of a Live Biotherapeutic on the Structure of the Gut Microbiota: Results from the Blautix® Phase Ib study in Irritable Bowel Syndrome

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4D pharma is a pharmaceutical company focussed on developing Live Biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D pharma has clinical stage programmes in cancer, asthma, IBS and IBD, and a strong pipeline of pre-clinical programmes in oncology, CNS disease, autoimmunity and inflammation. We have developed a multi-disciplinary functional screening platform, **MicroRx®**, to target specific biological functions and select bacterial candidates from our library of human commensal isolates.   We have also developed a complimentary microbiome analysis platform, **MicroDx**, which utilises metagenomic sequencing and our proprietary machine learning technology to provide deep insights into the functional microbiome in healthy and diseased states

Introduction

Evidence from microbiome research increasingly supports the potential clinical value of therapeutic manipulation of the microbiome, using live bacteria to treat disease, known as Live Biotherapeutic Products (LBPs). Irritable Bowel Syndrome (IBS) is a chronic, debilitating, functional gastrointestinal disorder with an estimated global prevalence of 10-20% [1]. The gut microbiota has increasingly been implicated in the development of IBS [2], with a number of studies reporting alterations in the diversity, stability and metabolic activity of the microbiota in IBS patients compared with healthy individuals [3].
Using 16S rRNA amplicon sequencing we profiled the microbiota of healthy and IBS subjects in i) an observational study and ii) a Phase Ib randomized control clinical trial with the LBP **Blautix**, a strain of *Blautia hydrogenotrophica* to investigate if a) the microbiota structure of healthy and IBS subjects differ and b) a LBP could affect the structure of the microbiota in IBS subjects.

Methods

Microbiota analysis was performed on faecal samples from our i) observational study (Healthy: n=64 and IBS: n=78) and Phase Ib Blautix clinical trial (Healthy-Blautix: n=16; Healthy-placebo: n=8; IBS-Blautix: n=16 and IBS-placebo; n=8) using 16S amplicon sequencing of the V3-V4 variable region and the MiSeq (2x250 bp) chemistry platform. Subjects in the Phase Ib study were randomized into the placebo or Blautix treatment group where they received twice daily doses of >10[10] CFUs of Blautix over a 16-day treatment period followed by 2-4 weeks washout. The microbiota was assessed over the study timepoints from baseline (D1), end of treatment (D16) to washout/end of study (EOS). Microbiome data analysis was performed using an in-house bioinformatics pipeline in our MicroDx® platform which included the USEARCH global alignment algorithm and UPARSE algorithm. Microbiome beta diversity was analysed using Principal Component Analysis (PCoA) with Spearman distance. Between Class Analysis (BCA) was performed to investigate the alteration of the microbiota. Investigation of the microbiome structure was based on co-occurrence networks, generated using the ReBoot method.

Results

Observational Study

The microbiota of IBS subjects was distinct from that of healthy subjects (Fig. 1A) but the microbiota profile of the clinical IBS subtypes did not differ (Fig. 1B) in the observational study.
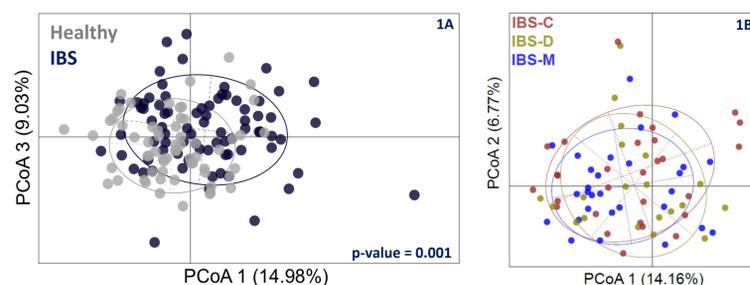

Fig. 1A-B: Difference in microbiota between healthy and IBS subjects and no difference among the IBS clinical subtypes in observational study

The structure of the microbiome was less interconnected in the IBS cohort when compared to the healthy cohort (Fig. 2A-B).
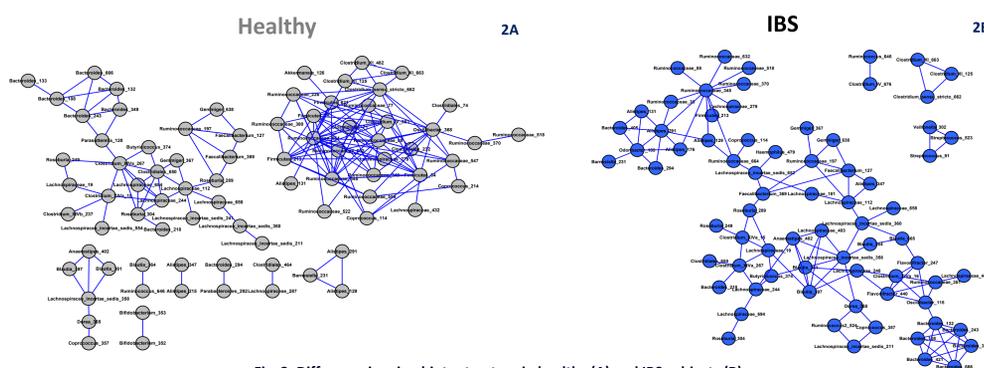

Fig. 2: Difference in microbiota structure in healthy (A) and IBS subjects (B)

Blautix Phase Ib RCT

The microbiota of IBS subjects differed significantly from healthy subjects (not shown) and moved towards that of healthy subjects after Blautix treatment (Fig. 3A) but not after receiving placebo (Fig. 3B).
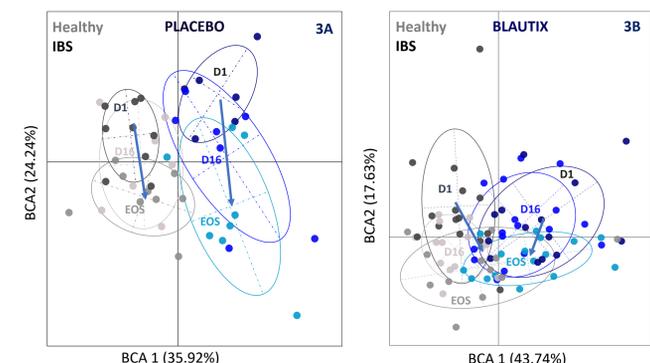

Fig. 3A-B: Effect of Blautix treatment or placebo on microbiota movement over study timepoints (D1(baseline), D16 (end of treatment) & end of study (EOS)) in IBS and healthy subjects

An increase in network connectivity and microbiota structure was observed in the Blautix-treated IBS subjects which was retained at washout (Fig. 4A-C). Blautix treatment had less of an impact on the healthy subjects with a slight increase in connectivity being observed which reverted back towards baseline at washout (Fig. 4D-F).
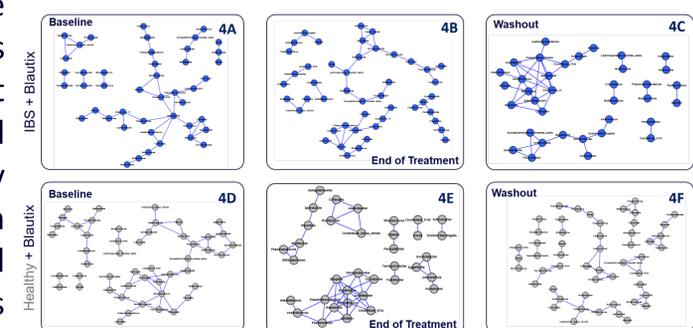

Fig. 4: Effect of Blautix treatment on microbiota structure over study timepoints (D1(baseline), D16 (end of treatment) and end of study (EOS)) in IBS (A-C) and healthy subjects (D-F).

Conclusions

- Global microbiota profiles differ between IBS and healthy subjects
- A less dense ecological network is observed in the IBS microbiome
- Blautix treatment altered the microbiome and improved network connectivity in IBS in a Phase Ib study
- Structural changes in the microbiota need to be considered when developing therapeutic candidates
- Any effect of therapeutics on the microbiota structure is likely to impact on the functional properties of the microbiome

References

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