Abstract 627: Oral Administration of MRx0518 in Treatment-Naïve Cancer Patients is Associated with Compositional Taxonomic and Metabolic Changes Indicative of Anti-Tumorigenic Efficacy

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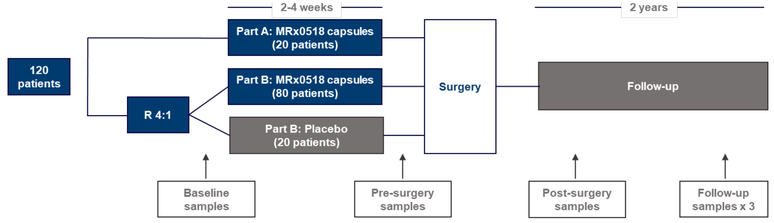
Background

- Live biotherapeutic products (**LBPs**) are recognised as a novel class of therapeutics, which have the potential to transform the way in which many diseases are treated.
- 4D pharma's LBPs are single strains of gut commensal bacteria which have been originally isolated from healthy human donors.
- **MRx0518** is a novel, gut microbiome-derived LBP, consisting of a single strain of ***Enterococcus gallinarum***, which has demonstrated strong anti-cancer and anti-tumorigenic effects in pre-clinical studies.
- Previous studies have demonstrated that **MRx0518 monotherapy** is associated with significant anti-tumorigenic genomic and immune responses and better clinical outcomes in treatment-naïve cancer patients.
- Although host-microbe interactions are typically initiated in the gut, the resulting changes in downstream pathways are diverse and produce systemic activity with effects in distal areas of the body.
- Efficacy is believed to be partly due to the interaction with human biological systems through a wide range of mechanisms, such as compositional **changes in the gut microbiome and metabolomic profile** with a particular interest in the anti-cancer efficacy of **microbial fermentation products and effector molecules**, including short-chain fatty acids (**SCFAs**).

Methods

The **MICROBIOME** trial (NCT03934827) is a Phase 1B single-centre study in patients with histologically confirmed cancer undergoing surgical resection:



- Patients receive 1 capsule of **MRx0518** (1 x 10^{10}-1 x 10^{11} CFU) *BID* from inclusion until surgery (maximum 28 days therapy).
- **Part A** has been completed, with 17 patients receiving 7-28 days of MRx0518 therapy across a range of cancers including breast (n = 8), prostate (n = 4), endometrial (n = 3), bladder (n = 1) and melanoma (n = 1).
- Exploratory outcomes included microbiome analysis of longitudinal fecal samples, with 16S rRNA amplicon sequencing, and investigation of metabolomic changes in SCFAs in plasma samples, by liquid chromatography-mass spectrometry.

Results: Taxonomic Changes

- MRx0518 was detected in all post-treatment fecal samples ($p < 0.0001$) (***Figure 1A***)
- Treatment with MRx0518 was associated with small, but significant alterations in beta-diversity.
- Significant zOTU level taxonomic changes in *Bacteroides* after treatment (30 days - Visit 6), and Lachnospiraceae, *Clostridium sensu*, Enterocloster, Ruminococcaece and *Anaerobutyricium* after surgery (60 days - Visit 8) were identified ($p < 0.05$) (***Figure 1B***).

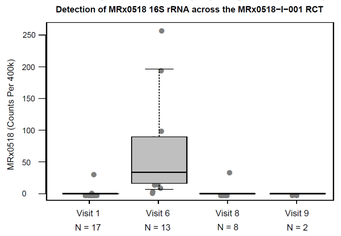


Figure 1A: Detection of MRx0518 16S rRNA amplicon in fecal samples by visit number

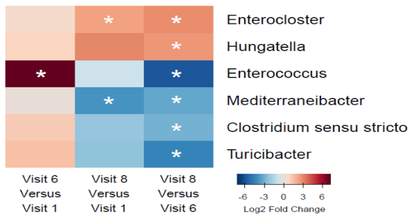


Figure 1B: Heatmap showing changes in the bacterial genera by visit number

Results: Metabolic Adaptations

- Analysis of plasma metabolomics on paired pre- and post-treatment samples at 30-days demonstrated a significant reduction ($p < 0.05$) in levels of acetic acid, with positive trends (non-significant) identified in 2-methyl butyric acid, butyric acid and propionic acid (***Figure 2A-E***).

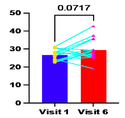


Figure 2A: 2-methyl butyric acid

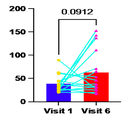


Figure 2B: Butyric acid

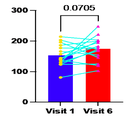


Figure 2C: Propionic acid

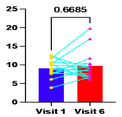


Figure 2D: Valeric acid

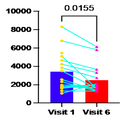


Figure 2E: Acetic acid

- Sub-analysis of subjects (n = 11) who received MRx0518 for > 15 days showed significant ($p < 0.05$) post-treatment increases in 2-methyl butyric acid, butyric, propionic and valeric acid, with a concordant significant reduction in acetic acid levels (***Figure 2F-J***).

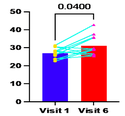


Figure 2F: 2-methyl butyric acid

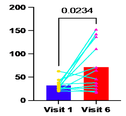


Figure 2G: Butyric acid

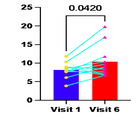


Figure 2H: Propionic acid

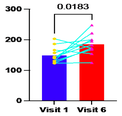


Figure 2I: Valeric acid

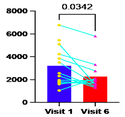


Figure 2J: Acetic acid

Treatment with MRx0518 as a monotherapy is associated with significant compositional taxonomic changes and alterations in short-chain fatty acids, indicative of anti-cancer efficacy

About MRx0518

- As both a monotherapy and in combination settings, MRx0518 has demonstrated robust efficacy as an immuno-stimulant and anti-tumor agent in multiple tumor models, such as breast cancer, renal cell carcinoma, and lung cancer.
- The mechanism of action of MRx0518 is identified as the bacterial flagellin as a potent stimulant of the innate and adaptive immune systems that interacts with the host TLR5 pathway known to be associated with the body's response to cancer.

Conclusions

- Effects are more pronounced in patients who receive MRx0518 treatment for a longer time period.
- Changes in SCFAs, such as butyric and propionic acid, may contribute to the anti-tumorigenic efficacy demonstrated by MRx0518.
- Further investigation is required to link post-treatment metabolomic changes to taxonomic changes in the gut microbiome

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