

ORAL ADMINISTRATION OF MRX0518 IN TREATMENT-NAÏVE CANCER PATIENTS IS ASSOCIATED WITH COMPOSITIONAL TAXONOMIC AND METABOLOMIC CHANGES INDICATIVE OF ANTI-TUMORIGENIC EFFICACY

¹Mark Lythgoe*, ¹Benjamin Mullish, ²Adam Frampton, ³Paola Dama, ¹Emily Pickford, ¹Laura Tookman, ¹Paula Cunnea, ¹Christina Fotopoulou, ⁴Ian Jeffery, ⁴Gayle Fyvie, ⁴Alexander Stevenson, ¹Jonathan Krell. ¹Imperial College London, London, UK; ²University of Surrey, Guildford, UK; ³University of Sussex, Brighton, UK; ⁴4D Pharma, Leeds, UK

Background Live biotherapeutic products (LBPs) are promising novel anti-cancer therapies. Their mechanism of action has not yet been fully elucidated but is thought to be partly due to compositional changes in the gut microbiome and metabolomic profile. There is particular interest in the anti-cancer efficacy of microbial fermentation products, including short chain fatty acids (SCFAs), including butyrate and propionate.

MRx0518 is a novel, gut microbiome-derived LBP, consisting of a single strain of *Enterococcus gallinarum*, which has demonstrated potent anti-tumorigenic efficacy preclinically.¹ We have previously shown MRx0518 therapy is associated with significant anti-tumorigenic genomic, cytokine and immune modulatory changes in treatment-naïve cancer patients.^{2,3} Here, we report compositional taxonomic and metabolomic changes following MRx0518 treatment.

Methods NCT03934827 is a Phase 1B single-centre study in patients with histologically confirmed cancer undergoing surgical resection. Patients receive 1 capsule of MRx0518 (1x10¹⁰-1x10¹¹CFU) BID from inclusion until surgery (maximum 28 days therapy). Exploratory outcomes included microbiome analysis of longitudinal faecal samples, with 16S rRNA gene sequencing, and investigation of metabolomic changes in SCFAs in plasma samples, by liquid chromatography-mass spectrometry.

Results 17 patients received 7-28 days of MRx0518 therapy. Significantly (P=0.0001) increased levels of MRx0518 16S rRNA levels were detected in all faecal samples at time of therapy cessation (Visit 6). 30-days after treatment stoppage (Visit 8), levels of MRx0518 16S rRNA were undetectable. MRx0518 therapy was associated with a small but significant increase in beta-diversity. Post-therapy samples showed significant microbiota changes, with a visual trend (non-statistical) back towards baseline profile at 6-month follow-up (Visit 9). Significant (P<0.05) taxa changes in *Bacteroides* at Visit 6, and Lachnospiraceae, Clostridium sensu, Enterocloster, *Ruminococcaceae*, and *Anaerobutyricum* at Visit 8, were identified.

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, butyrate and propionate. Sub-analysis of subjects (n=11) who received MRx0518 for >15 days showed significant (P<0.05) post-treatment increases in 2-methyl butyrate, butyrate, propionate and valerate, with a concordant significant reduction in acetic acid levels.

Conclusions MRx0518 therapy is associated with significant compositional taxonomic changes and alterations in SCFA, indicative of anti-cancer efficacy. This effect is more pronounced in patients who receive treatment for longer periods. Changes in SCFAs, such as butyrate and propionate, may contribute to the anti-cancer efficacy of MRx0518. Further investigation is required to link post-treatment metabolomic changes to taxonomic changes in the gut microbiome.

Trial Registration NCT03934827

REFERENCES

1. Lauté-Caly DL, Raftis EJ, Cowie P, *et al.* The flagellin of candidate live biotherapeutic *Enterococcus gallinarum* MRx0518 is a potent immunostimulant. *Scientific Reports*. 2019;**9**(1):1–14. doi:10.1038/s41598-018-36926-8
2. Lythgoe M, Stebbing J, Pickford E, *et al.* 805 Safety and emerging evidence of immune modulation of the live biotherapeutic MRx0518 in the neoadjuvant setting for patients awaiting surgical removal of solid tumours. *Journal for Immunotherapy of Cancer*. 2020;**8**(Suppl 3):A854–A854. doi:10.1136/jitc-2020-sitc2020.0805
3. Lythgoe M, Adriani M, Stebbing J, *et al.* 543P Neoadjuvant MRx0518 treatment is associated with significant gene and metagene signature changes in solid tumours. *Annals of Oncology*. 2021;**32**:S607. doi:10.1016/j.annonc.2021.08.1065

Ethics Approval This study has been approved by the UK Research Ethics Committee (East of England – Cambridge East Research Ethics Committee – C/35/2017). All patients gave informed consent for participation in this research.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

<http://dx.doi.org/10.1136/jitc-2022-SITC2022.0627>