Neoadjuvant MRx0518 treatment is associated with significant gene and metagene signature changes in solid tumours

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BACKGROUND

- Accumulated data has demonstrated a direct impact of specific bacteria strains on immune cell subsets activation and polarization, and ultimately on the efficacy of cancer immunotherapies such immune checkpoint inhibitors (ICIs) and adaptive cytotoxic T-cell therapy.
- MRx0518 is a novel, human gut microbiome-derived single strain, live biotherapeutic product in clinical development for the treatment of solid tumours.
- MRx0518 monotherapy demonstrated immunostimulatory activity and anti-tumorigenic effects (**Figure 1**) in a range of murine tumour models including EMT6 (breast cancer) and Renca (Renal cancer).
- MRx0518 monotherapy has been shown to increase tumour infiltration by cytotoxic cells, CD8+ T cells and other immune subsets associated with anti-tumour activity, as well as preliminary clinical activity in combination with ICI pembrolizumab in patients refractory to anti-PD-(L)1 ICI therapy.
- We investigated immune and metagene signature changes in solid tumours associated with MRx0518 neoadjuvant monotherapy.

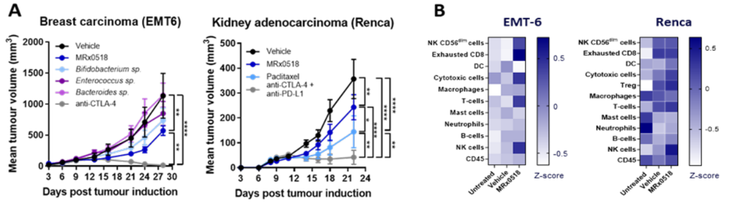
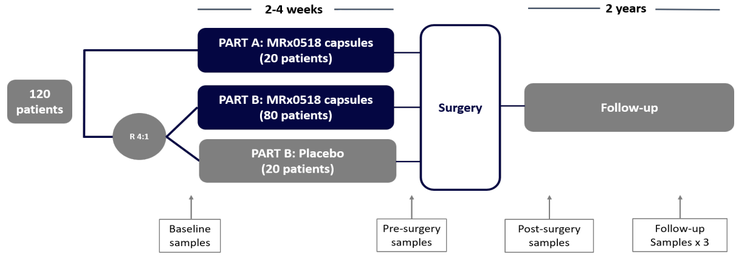


Figure 1. *Inhibition of tumour growth in murine models **(A)** and quantification of cell subsets utilizing tumour tissues and analysis via NanoString PanCancer IO360™ Gene Expression Profile **(B)**. (n=8 mice per group)*

RATIONALE & CLINICAL STUDY DESIGN

The **MICROBIOME** trial is designed to investigate the effects of oral MRx0518 for 2-4 weeks in patients with solid tumours awaiting surgical removal of the tumour



Part A: *1 capsule of MRx0518 (1x10[10]- 1x10[11]CFU) twice daily for 2-4 weeks prior to tumour resection*
Part B: *1 capsule of MRx0518 or placebo twice daily for 2-4 weeks prior to tumour resection*

METHODS

- In Part A of the study 17 patients have completed treatment across a broad range of cancer types including breast (n=8), prostate (n=4), endometrial (n=3), bladder (n=1) and melanoma (n=1)
  - No severe adverse events or grade 3/4 Common Terminology Criteria for Adverse Events reported
  - Independent Data Monitoring Safety Committee review has permitted transition to Part B
- 31 tumour samples were obtained for paired analysis (15 patients) including 16 pre-treatment (diagnostic biopsies) and 15 post-treatment (surgical specimens)
- Gene expression profiling (GEP) was performed using the NanoString IO 360 panel to evaluate both the gene and metagene changes
  - Analysis was correlated with cytokine and chemokine changes present in paired pre- and post-treatment plasma samples

GENOMIC MODULATION

- GEP analysis identified 96 differentially expressed genes (DEGs; $p<0.05$) between pre- and post-treatment tumour samples. The majority of DEG (n=92) were upregulated after treatment and a small number (n=4) were down-regulated (**Figure 2**)
- Pathway analysis was performed (using the nSolver software) and both undirected and directed global significance scores (GSS) calculated (**Table 1**). DEGs pathway analysis showed that treatment with MRx0518 was associated with anti-tumour immune activity including:
  - Antigen Presentation (**AXL** & **CXCL12**)
  - Innate Immune Processes (**CHUK**, **RELA**, **PPARG** & **HRAS**)
  - Interferon Response (**IFNGR1** & **IFNGR2**)

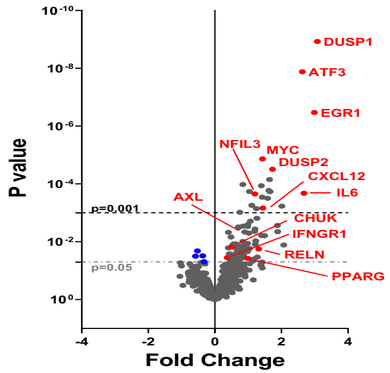
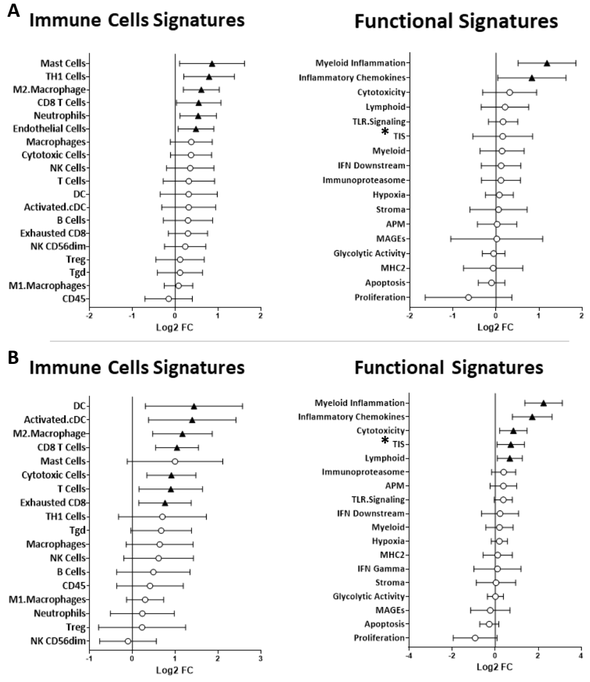


Table 1: Surgical vs Diagnostic Samples GSS (top 10 pathways)

Pathway	Undirected Scores	Directed Scores
Angiogenesis	2.003	1.688
Antigen Presentation	1.909	1.706
Apoptosis	1.376	-0.256
Autophagy	1.338	0.721
Cell Proliferation	1.889	1.016
Costimulatory Signaling	1.804	1.522
Cytokine and Chemokine Signalling	2.045	1.875
Cytotoxicity	1.315	0.877
DNA Damage Repair	1.374	0.386
Epigenetic Regulation	1.091	0.304

Figure 2: Comparison of the gene expression profiles in paired tumour samples collected pre- and post treatment with MRx0518. *Selected genes with significant ($P<0.05$) changes in expression are highlighted (red indicates increased and blue indicates reduced expression)*

IMMUNE & FUNCTIONAL SIGNATURES



* Tumour Infiltration Score (TIS); Ayers M, et al. J Clin Invest. 2017;127:2930–40.

- Analysis of paired tumour samples identified an increase in mast cells, Th1, CD8+ T-cells, endothelial cells and inflammatory chemokine metagene signatures following MRx0518 therapy (**Figure 3A**).
- Effects were particularly pronounced in the breast cancer cohort (n=7), with a significant increase in total and activated dendritic cells (DC), CD8+ T-cells, cytotoxic cells and mast cells in the tumour micro-environment.
- Functional metagene analysis also identified positive changes in prognostic indicators and metagene signatures predictive of immunotherapy response, including the cytotoxicity and lymphoid scores, and Tumour Inflammation Signature (TIS), demonstrated to retrospectively predict clinical benefit of anti-PD-(L)1 ICI therapy efficacy in various cancer types (**Figure 3B**).

Figure 3: Changes in expression of metagene signatures in paired tumour samples. *Forest plots show the Log2 mean fold change, 95% confidence intervals, of immune cell type and functional score signatures in pre- vs post-treatment in all paired tumour samples **(A)** or breast cancer cohort paired tumours **(B)**. Triangles (▲) indicate significant difference ($p<0.05$) as assessed by univariate analysis.*

CONCLUSION

- Analysis of paired tumour samples shows oral administration of the single strain live biotherapeutic MRx0518 monotherapy increases expression of genes associated with anti-tumour immune activity.
- Furthermore, metagene changes predictive of immunotherapy response and favourable disease prognosis, such as increased TIS, lymphoid and cytotoxicity scores were observed post-therapy
- These results show evidence of immune activation after MRx0518 therapy, further validation in treatment naïve patients is planned.

For additional information on clinical studies involving MRx0518 see poster **1024P**

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