# Trial in progress: A Phase I study of live biotherapeutic MRx0518 in the neoadjuvant setting for patients awaiting surgical removal of solid tumours

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## Background

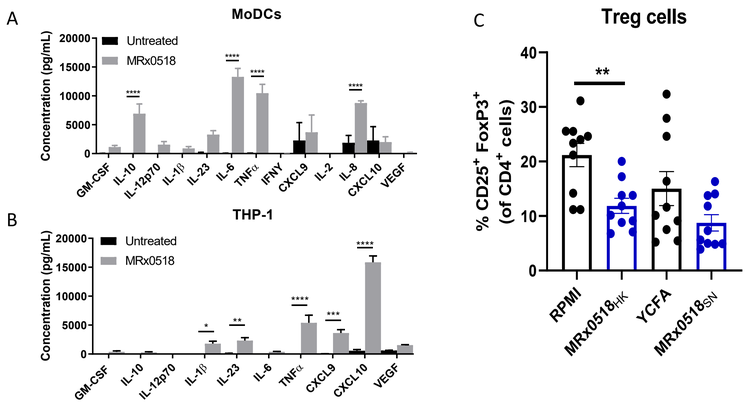
The gut microbiome has emerged as a novel therapeutic approach to immune-stimulation treatment of solid tumours.[1 2 3] MRx0518 is a gut microbiome-derived, oral Live Biotherapeutic, identified as inducing a strong immunostimulatory response, particularly in stimulating immune cell populations and signalling pathways relevant for anti-cancer therapy. For example, the bacterial flagellin moiety leads to NFκB-related cytokine responses.[4] Preclinical studies have demonstrated that MRx0518 reduced tumour growth in syngeneic models of kidney, lung and breast cancer. MRx0518 increased $CD8^+$ T cell infiltration into the tumour and decreased $T_{regs}$ along with up-regulation of tumour TLR5.

This study, one of the first oncology trials conducted with a Live Biotherapeutic, is a single-centre, double blind, neoadjuvant window study in patients with resectable solid tumours to evaluate the safety, tolerability and preliminary efficacy of MRx0518.

The study was approved by the East of England – Cambridge East Research Ethics Committee, reference 18/EE/0091.

## 1) MRx0518 has broad immunostimulatory effects on immune cell populations *in vitro*

Strain MRx0518 was identified for its potent immunostimulatory effect on host immune cells, inducing the production of cytokines/chemokines associated with both innate and adaptive immunity (A, B). MRx0518 treatment also decreased the proportion of Tregs in human PBMCs (C).

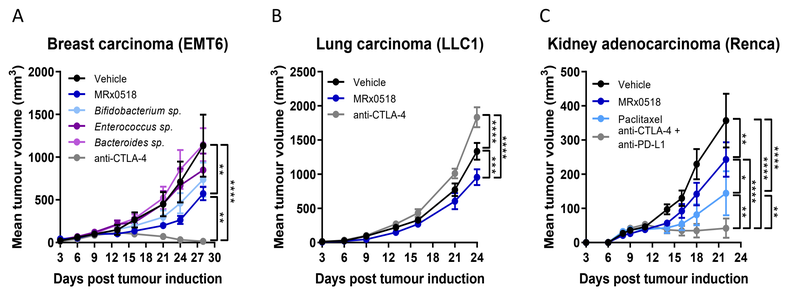



Human peripheral blood mononuclear cells (PBMCs) were used to isolate monocyte populations and differentiated into immature dendritic cells (MoDCs) in presence of rhIL-4 and rhGM-CSF for 7 days (A). Human THP-1 cells (monocytes) were differentiated into macrophages with PMA for 48 hours (B). PBMCs were stimulated by anti-CD3/CD28, TGFß and IL-2 for 4 days (C). Cells were plated at $2x10^5$ cells/well (A-B) or $4x10^5$ cells/well (C) and stimulated by MRx0518. Supernatants were collected and protein concentrations were quantified using MagPix (A-B). Regulatory T cells, $CD25^+FoxP3^+$, were detected by flow cytometry (C). RPMI or YCFA medium were included as negative controls.

## 2) Efficacy in preclinical syngeneic tumour models

### MRx0518 was tested in a number of different preclinical cancer models

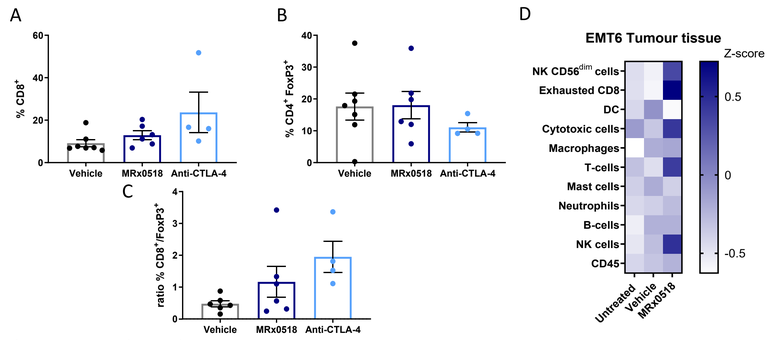
Breast carcinoma (EMT6), lung carcinoma (LLC1) and kidney adenocarcinoma (Renca).




From D-14, mice received vehicle (culture medium or PBS) or $2x10^8$ bacteria daily until termination. On D0, mice were engrafted with EMT6 (n=8-9) (A), LLC1 (n=8-9) (B) or Renca (n=12) (C) tumour cells subcutaneously. Anti-CTLA-4 (10 mg/kg, IP, TWx2), Paclitaxel (15 mg/kg, IP, Q4D) or a combination of anti-CTLA-4 and anti-PD-L1 (10 mg/kg each, IP, BID) were used as controls. Tumour length and width were measured 2-3 times a week.

### MRx0518 affects tumour immune cell populations

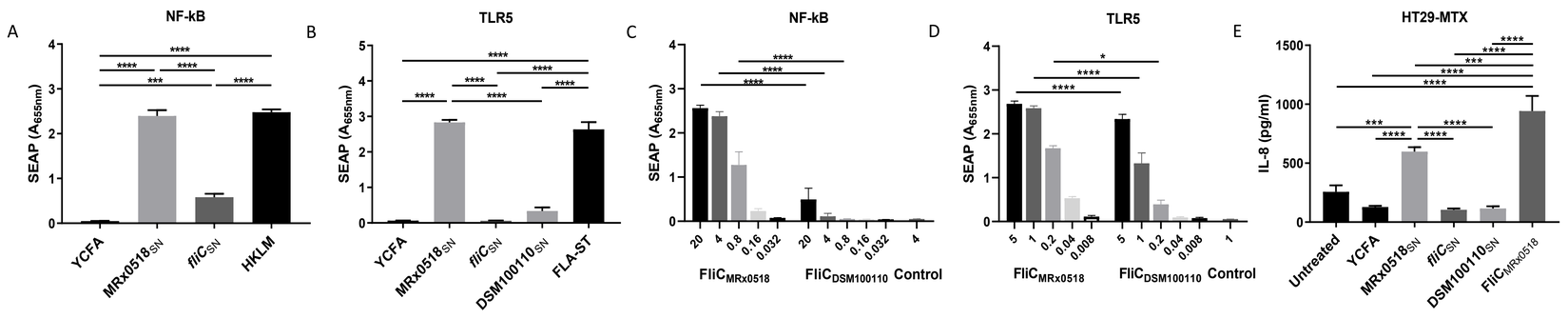
MRx0518 increased tumour infiltrating $CD8^+$ T cells, natural killer (NK) cells and ratio of $CD8^+$ : $CD4^+FoxP3^+$ Tregs.




$CD8^+$ (A), and $CD4^+FoxP3^+$ (B) cells were analysed by flow cytometry in the tumour tissue in the mouse model of breast cancer (EMT6). The ratio of the $CD8^+/CD4^+FoxP3^+$ cells (C) was calculated. (D) Tumour tissue analysis was conducted using NanoString technology. Heat map represents tumour cell populations abundance for untreated, vehicle-treated and MRx0518-treated animals in the EMT6 model. Note — not all cell populations reached statistical significance.

## 3) Mode of action mediated by bacterial surface molecules

Bacterial flagellin is a known TLR5 agonist, and reporter assays indicated the immunostimulatory effect of MRx0518 was at least partly mediated by TLR5 signaling. Genetic knock out of the flagellin protein (FliC) resulted in little to no NF-κB (A) or TLR5 signalling (B). The effect of purified recombinant MRx0518 flagellin was compared to that of a reference strain and was significantly more potent at lower concentrations (C, D). Genomic analysis identified broad *fliC* sequence divergence between the MRx0518 strain and reference strain (data not shown). MRx0518 culture supernatant and recombinant MRx0518 flagellin protein induced significantly upregulated expression of inflammatory cytokine IL-8 by intestinal epithelial cells (E).




NF-κB (A) and TLR5 (B) reporter assays with MRx0518 ($MRx0518_{SN}$), MRx0518 *fliC*::pORI19 ($fliC_{SN}$) and DSM100110 ($DSM100110_{SN}$) culture supernatants (MOI 100:1 equivalent) after 22h incubation. NF-κB (C) and TLR5 (D) reporter assays with a range of concentrations of MRx0518 and DSM100110 purified recombinant flagellins ($FliC_{MRx0518}$ and $FliC_{DSM100110}$). The control corresponds to the empty vector. (E) IL-8 concentrations detected by ELISA assay in HT29-MTX cell-free supernatant after 24h stimulation with MRx0518 ($MRx0518_{SN}$), MRx0518 *fliC*::pORI19 ($fliC_{SN}$) and DSM100110 ($DSM100110_{SN}$) culture supernatants (MOI 100:1 equivalent), and 1 µg/mL purified MRx0518 recombinant flagellin ($FliC_{MRx0518}$). YCFA was included as a negative control.

## Clinical Study Design

### Part A

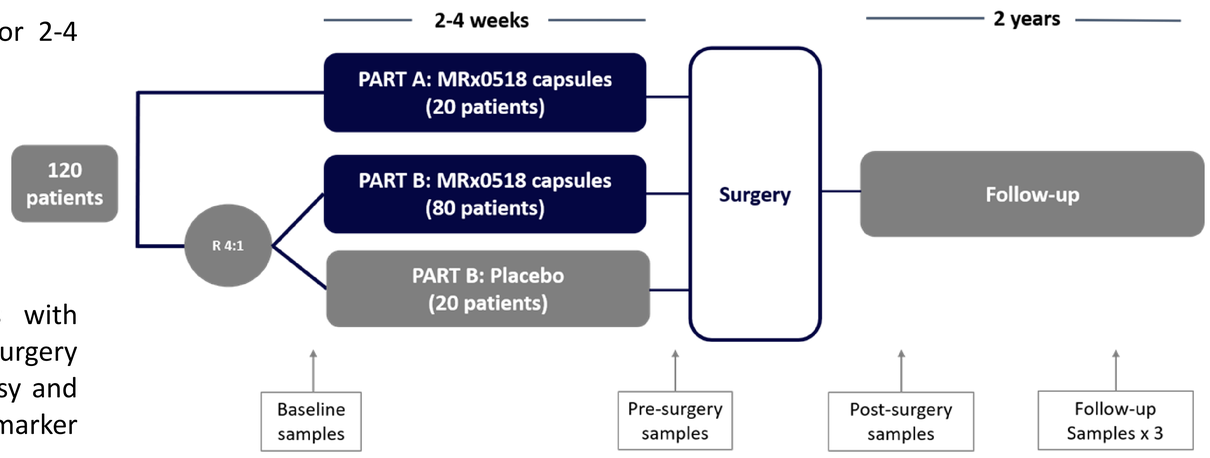
20 patients - 1 capsule of MRx0518 twice-daily for 2-4 weeks prior to tumour resection

### Part B

80 patients - 1 capsule of MRx0518 twice-daily
20 patients - 1 capsule of placebo twice-daily
For 2-4 weeks prior to tumour resection

### Eligibility

Eligibility is limited to treatment-naïve patients with confirmed solid tumours amenable to primary surgery resection with a comparison of the diagnostic biopsy and surgical excision specimen to assess the biomarker response




## Clinical Study Objectives

### Primary

- Safety and tolerability of MRx0518

### Secondary

- Responses in respect of intra-tumoural biomarkers compared to placebo
- Clinical outcomes including overall survival

### Exploratory

- Surrogate biomarkers of treatment effect
- Microbiome analysis
- Impact on tumoural T cell populations including CD8+ Ts, NKs, Tregs.
- Impact on systemic T cell populations

References 1 Nelson KE, An Update on the Status of Current Research on the Mammalian Microbiome., ILAR K., 2015, 56: 163-168; 2 Dzutsev A, Goldszmid RS, Viaud S et al., The role of the microbiota in inflammation, carcinogenesis, and cancer therapy., Eur J Immunol., 2015, 45: 17-31; 3 Sivan A, Corrales L, Hubert N et al., Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy., Science, 2015, 350: 1084-1089; 4 Lauté-Caly DL, Raftis EJ, Cowie P et al., The flagellin of candidate live biotherapeutic Enterococcus gallinarum MRx0518 is a potent immunostimulant., Sci Rep., 2019, 9: 801-814

## Clinical Study Status

- The study commenced in April 2019
- Subjects are being enrolled into Part A in a staggered fashion
- All subjects are currently tolerating the treatment well
- A safety review will be performed after completion of Part A before moving into Part B

The trial is being sponsored by Imperial College London
4D pharma plc is a collaborator on the trial
For further information please see:
www.clinicaltrials.gov — **NCT03934827**
Or email clinicaltrials@4dpharmaplc.com