A Phase I/II study of live biotherapeutic MRx0518 in combination with pembrolizumab in patients who have progressed on prior anti-PD-1 therapy

Authors: Shubham Pant[1], Imke Mulder[2], Amishi Y Shah[1], Pavlos Msaouel[1], Mehmet Altan[1], John Weinberg[2], Alex Stevenson[2], Funda-Meric Bernstam[1], Timothy A Yap[1], Vivek Subbiah[1], Gayle Fyvie[2], Michael Chisamore[3], Jordi Rodon[1]

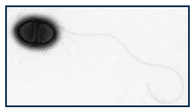
[1]MD Anderson Cancer Centre, Houston, TX; [2]4D pharma plc, Leeds, UK; [3]Merck & Co., Inc., Kenilworth, NJ

Background

The Microbiome and Immunotherapy

The gut microbiome has emerged as a new therapeutic target to augment the efficacy of immune checkpoint blockade, and has been shown to influence therapeutic responses in both preclinical models and patient cohorts.[1, 2, 3]

MRx0518

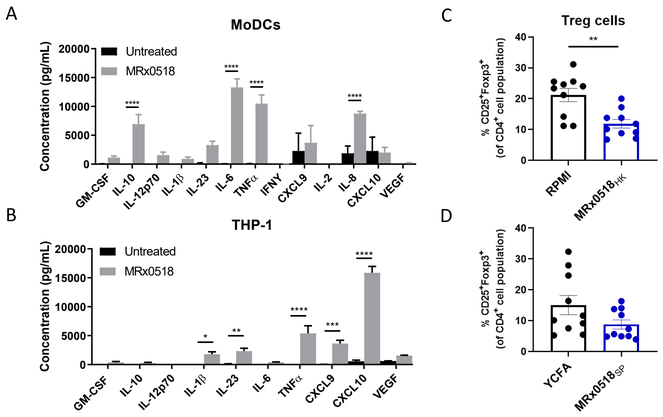


MRx0518 is a gut microbiome-derived, oral live biotherapeutic, identified as inducing a broad immunostimulatory response.

Preclinical studies showed that MRx0518 reduced tumour growth in models of kidney, lung and breast cancer. MRx0518 increased CD4 and CD8 T cell and NK cell infiltration into the tumour and decreased Tregs. Upregulation of tumour TLR5 in the tumour microenvironment was observed and linked to the bacterial flagellin moiety, which was shown to strongly induce NF-κB signalling, cytokine responses and IFNγ+ CD4 and CD8 T cells.

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 (C, D).

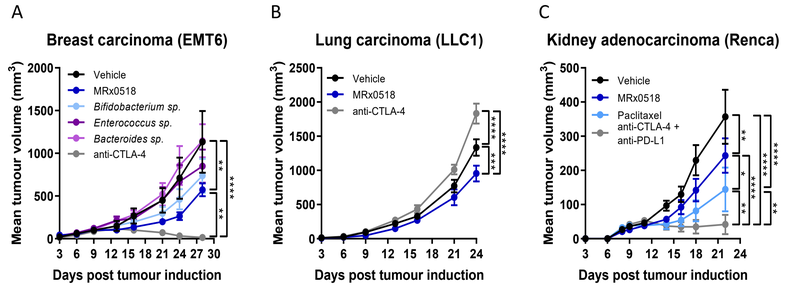


Human peripheral blood mononuclear cells (PBMCs) were used to isolate monocyte populations and differentiated into immature dendritic cells (MoDCs) in presence of rhIL-4 and rhGM-CSF for 7 days (A). Human THP-1 cells (monocytes) were differentiated into macrophages with PMA for 48 hours (B). PBMCs were 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, D) and stimulated by MRx0518 (A, B), heat killed MRx0518 (C) or MRx0518 culture supernatant (D). Supernatants were collected and protein concentrations were quantified using MagPix (A, B). Regulatory T cells, $CD25^+FoxP3^+$, were detected by flow cytometry (C, D). 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

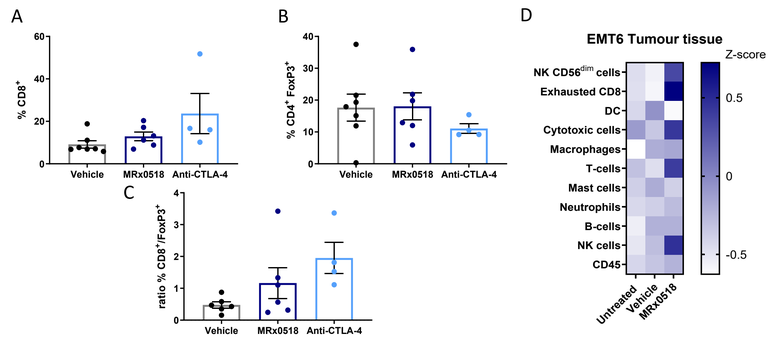
Breast carcinoma (EMT6), lung carcinoma (LLC1) and kidney adenocarcinoma (Renca).



From D-14, mice received vehicle (culture medium or PBS) or $2x10^8$ bacteria daily until termination. On D0, mice were engrafted with EMT6 (A), LLC1 (B) or Renca (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

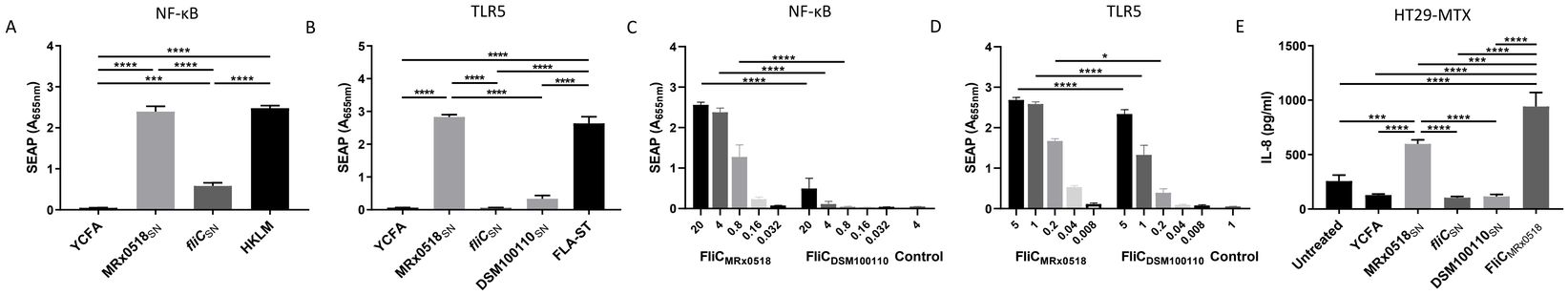
MRx0518 increased tumour infiltrating $CD8^+$ T cells, natural killer (NK) cells and ratio of $CD8^+$: $CD4^+FoxP3^+$ Tregs.



$CD8^+$ (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 signalling. 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 more potent at lower concentrations (C, D). Genomic analysis identified *fliC* sequence divergence between the MRx0518 strain and reference strain (data not shown). MRx0518 culture supernatant and recombinant MRx0518 flagellin protein induced 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 rationale

Checkpoint inhibitor immunotherapies are highly effective in some patients but efficacy is limited to a minority. Furthermore, around 40% of patients who respond to checkpoint blockade therapy become refractory over time.[4, 5]

There is a significant need for therapeutics that increase response to immunotherapy and re-engage the immune system in patients who have responded but then lost efficacy.

MRx0518 has potent immunostimulatory activity, and demonstrated anti-tumour activity as a monotherapy in multiple preclinical models. Oral administration of the strain increases tumour infiltration of immune cells known to be critical in response to checkpoint blockade immunotherapy.

Clinical study status

The study was activated in January 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 4D pharma plc.

Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA provided pembrolizumab for the study.

For further information:
clinicaltrials@4dpharmaplc.com
www.clinicaltrials.gov — NCT03637803

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Clinical study objectives

Primary

Part A:

- To assess the safety and tolerability of MRx0518 in combination with pembrolizumab

Part B:

- To assess the safety and clinical benefit of MRx0518 in combination with pembrolizumab

Secondary

Part B:

To evaluate the antitumour effect of study treatment per RECIST 1.1

- Objective Response Rate
- Duration of Response
- Disease Control Rate
- Progression-Free Survival

Exploratory

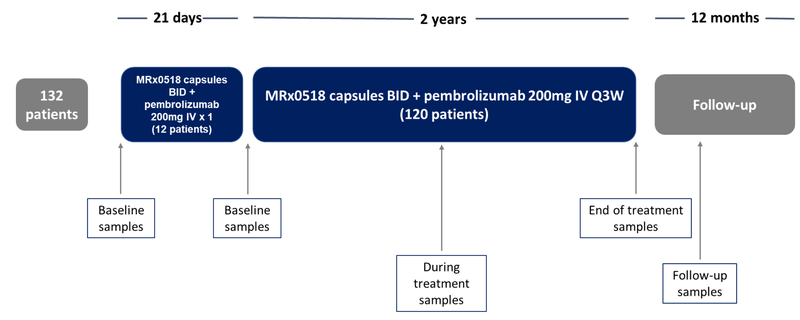
- Blood biomarkers of immune status and treatment effect
- Tumour biomarkers of immune status and treatment effect
- Faecal and urine microbiota and metabolomics analysis
- To evaluate the anti-tumour effect of study treatment per iRECIST
- Overall survival, determined from the start of combination therapy until death due to any cause

Clinical study design

The study, one of the first oncology trials conducted with live biotherapeutics, is a single centre, open label, safety and preliminary efficacy study.

The study will enrol patients who have progressed on prior anti-PD-1 therapy and who have a diagnosis of:

- Non-small cell lung cancer
- Renal cell carcinoma
- Bladder cancer
- Melanoma



Part A: 12 patients with any of the above tumour types will receive pembrolizumab 200mg every 3 weeks plus 1 capsule (bid) of MRx0518 with a DLT period of 1 cycle (21 days).

Part B: Up to 30 patients per cohort will receive pembrolizumab 200mg every 3 weeks plus 1 capsule (bid) of MRx0518 for up to 35 cycles or until disease progression per RECIST 1.1.

References: 1. Routy et al., *Science* 2018, 359(6371), 91-97; 2. Gopalakrishnan et al., *Science* 2018, 359(6371), 97-103; 3. Matson et al., *Science* 2018, 359(6371), 104-108; 4. O'Donnell et al., *Cancer Treatment Reviews* 2017, 52, 71-81; 5. Schachter et al., *Lancet* 2017, 390(10105), 21-27