Live biotherapeutic *Enterococcus gallinarum* MRx0518 is a potent immunostimulant and inhibits tumour growth in syngeneic mouse models

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4D Pharma PLC is a pharmaceutical company focussed on developing live biotherapeutic products (LBPs) from the human gut microbiome. LBPs represent a new class of drugs that contain live organisms for the prevention, treatment or cure of disease. 4D Pharma currently has four clinical stage programmes (in oncology, IBS, asthma and IBD) and a strong pipeline of pre-clinical programmes in oncology, autoimmunity, inflammation and CNS disease.

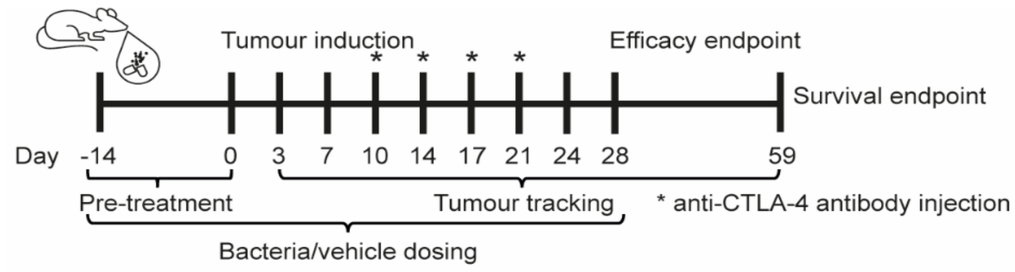
Introduction

The importance of the intestinal microbiota in cancer is increasingly recognized as it can affect the efficacy of immune checkpoint inhibitors. We have identified specific single-strain LBPs as having uses in the growing field of oncobiotics.

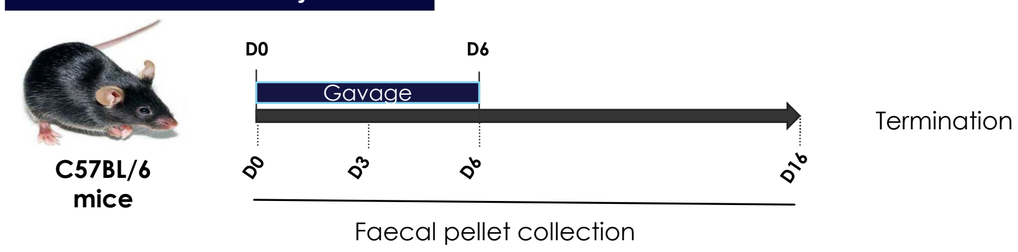
Enterococcus gallinarum MRx0518 was isolated from the intestinal tract of a healthy human donor and is a promising next generation live biotherapeutic for the treatment of cancer. MRx0518 displays strong anti-tumorigenic effects in a range of murine solid tumour models, including the EMT6 breast carcinoma and RENCA kidney carcinoma syngeneic tumour models. Here we studied the mechanism of action by which MRx0518 exerts its anti-tumorigenic effects *in vivo,* through investigating the immune populations and cytokine signalling responses induced by the strain in different host components.

Study Design

Syngeneic mouse models



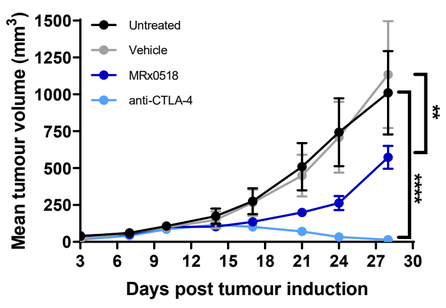
SPF *in vivo* study



Efficacy in Syngeneic Tumour Models

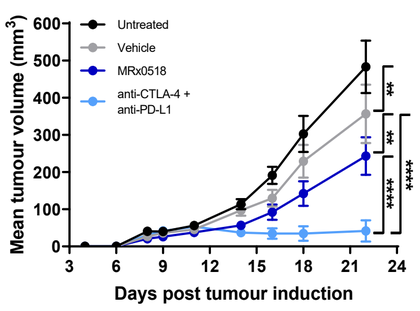
MRx0518 shows efficacy in syngeneic tumour models of breast and kidney cancer

Breast carcinoma (EMT6)



Group	Tumour volume (Mean±sem)	T/C (%) at D28	
		vs Untreated	vs Vehicle
Untreated	1010.88 ± 282.98	-	-
Vehicle	1134.43 ± 362.03	106.37	-
MRx0518	572.93 ± 77.61	55.29	51.97
Anti-CTLA-4	14.33 ± 8.57	0.00	0.00

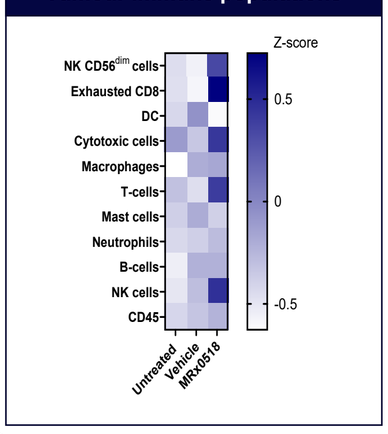
Kidney carcinoma (RENCA)



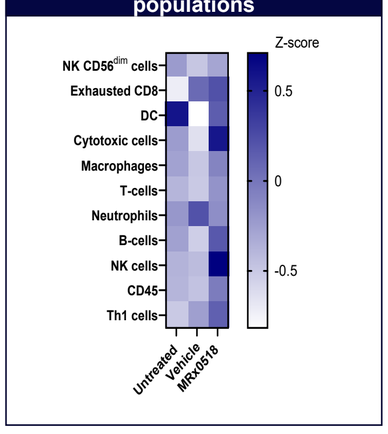
Group	Tumour volume (Mean±sem)	T/C (%) at D22	
		vs Untreated	vs Vehicle
Untreated	483.36 ± 70.56	-	-
Vehicle	356.93 ± 78.86	49.77	-
MRx0518	243.29 ± 50.53	40.06	80.49
Anti-CTLA-4 + Anti-PD-L1	41.79 ± 28.52	0.00	0.00

EMT6 dosed mice show increased NK cells, T cells and cytotoxic cells, with few changes in ileal immune populations.

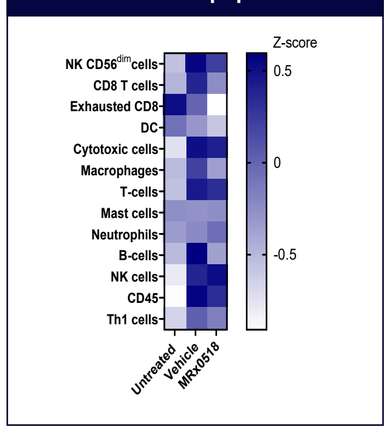
Tumour immune populations



Ascending colon immune populations



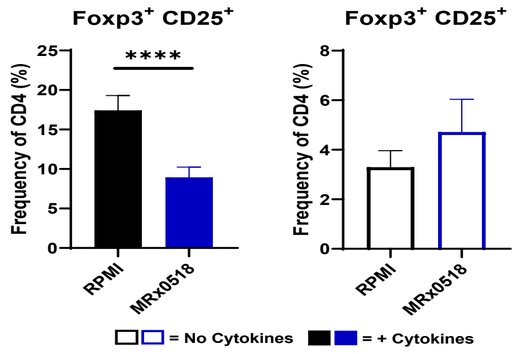
Ileum immune populations



Immunostimulatory effects *in vitro* and *in vivo*

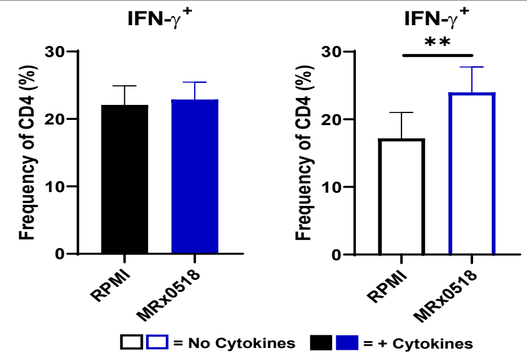
MRx0518 treatment reduces the differentiation of Tregs in the presence of polarising cytokines.

Treg differentiation assay



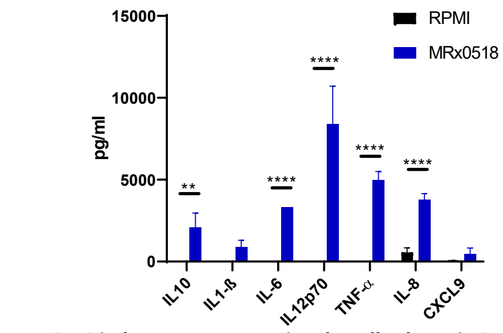
MRx0518 treatment increases the CD4⁺ IFNγ⁺ population in absence of polarising cytokines.

Th1 differentiation assay

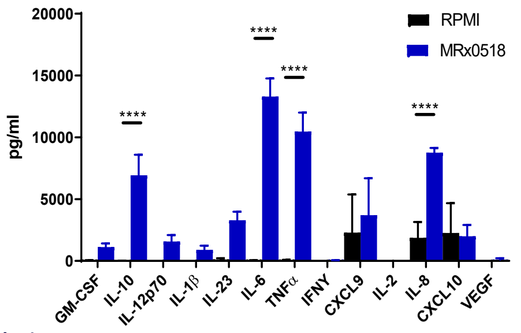


MRx0518 increases the production of cytokines and chemokines in primary cells and a human monocytic cell line

PBMCs

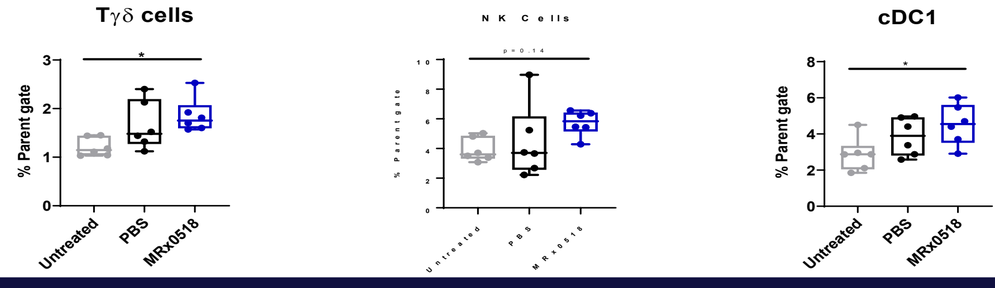


MoDCs



MRx0518 induces non-conventional T cell subsets in SPF unchallenged mice

Systemic immune cells specifically upregulated by MRx0518



Key Findings

- MRx0518 monotherapy significantly reduces tumour burden in EMT6 and RENCA syngeneic models.
- SPF mice dosed with MRx0518 show increased frequency of systemic immune cell populations associated with anti-tumour immunity (Tγδ, NK and cDC1 cells).
- MRx0518 increases the production of a cytokine/chemokine signature in THP-1, MoDC and PBMCs that includes IL-8, IL-12p70, IL-6, IL-10, TNF-α, IL-1β, IL-23, CXCL9 and CXCL10.
- MRx0518 treatment reduces differentiation of PBMCs into $CD4^+$ $CD25^+$ $Foxp3^+$ Tregs and increases T-cell production of IFN-γ.

Future Direction

- Further *in vitro* and *in vivo* studies are underway to further elucidate the mechanism of action of MRx0518.
- Data infer that flagellin may play a role in the therapeutic properties of *E. gallinarum* MRx0518 and derivatives are currently being investigated in murine cancer models.
- MRx0518 is currently in a Phase I/II clinical trial combination study with anti-PD-1 (KEYTRUDA®) as well as a Phase I neoadjuvant monotherapy study. Both of these studies are conducted in solid tumour cancer patients.

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