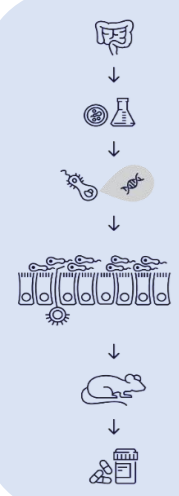


Live biotherapeutics and immuno-oncology: from discovery to efficacy studies

Anna Ettorre¹, Margaret Delday¹, Meenakshi Pradhan², Ian Jeffery², Alessio Panzica¹, Amy Holt¹, Philip Cowie¹, Sam Yuille¹, Emma Raftis¹, Nicole Reichardt¹, Helene Savignac¹ and Imke Mulder¹

¹4D Pharma Research Ltd, Life Sciences Innovation Building, Cornhill Road, AB25 2SZ, Aberdeen, UK;

²4D Pharma Cork Ltd, Cavanagh Pharmacy Building, University College Co. Cork, Western Road, Cork, Ireland



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 two clinical stage programmes (in IBS and IBD) and a strong pipeline of pre-clinical programmes in autoimmunity, inflammation, oncology and CNS disease.

Introduction

The gut microbiota is now known to be a key player in immune system development, disease susceptibility and therapeutic response. Recent studies have pointed out how gut bacteria can improve the efficacy of checkpoint inhibitors in preclinical oncology models (Sivan *et al.*, 2015; Vétizou *et al.*, 2015) and treatment response in patients (Routy *et al.*, 2018; Gopalakrishnan *et al.*, 2018).

MRx0518

MRx0518 is the lead LBP candidate for the immuno-oncology programme of 4D Pharma. It is a commensal bacterial strain isolated from the healthy human gut microbiota. Its antitumorigenic effects are linked to its distinct and species-specific *in vitro* immune-stimulatory phenotype.

Results

Anti-tumour activity of MRx0518 was tested *in vivo* in a number of different syngeneic mouse tumour models using the following cell lines injected subcutaneously: EMT6 - Breast carcinoma in BALB/c mice; LLC1 - Lewis lung carcinoma in C57BL/6 mice; RENCA - Renal adenocarcinoma in BALB/c mice (Fig. 1). MRx0518 treatment reduced tumour volume with T/Cs of 44% in EMT6 (day 24) and 51% in RENCA (day 18) compared to the vehicle-treated group.

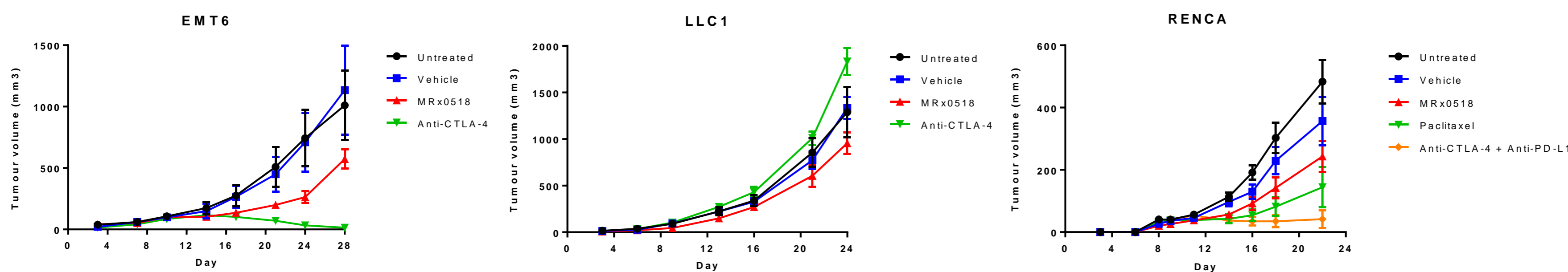


Fig. 1: Measurement of tumour volume (mm³) in syngeneic models of breast, lung and kidney cancer. Data are presented as Mean ± SEM.

In the EMT6 model, MRx0518 promoted an increase in the percentage of necrotic area and reduced the number of dividing cells (Fig. 2, left and right panel). Gut immuno-staining showed that more CD8 α ⁺ cells localized in the crypt region of the ileum after MRx0518 and anti-CTLA4 treatment than vehicle (Fig. 3). Analysis of immune cell populations in tumour microenvironment shows a decrease in the relative percentage of CD4 cells and increase of CD8 cells in MRx0518 and anti-CTLA-4 treated mice compared to vehicle. Moreover the CD8/Tregs ratio was increased in MRx0518 and anti-CTLA-4 treated mice (Fig. 4).

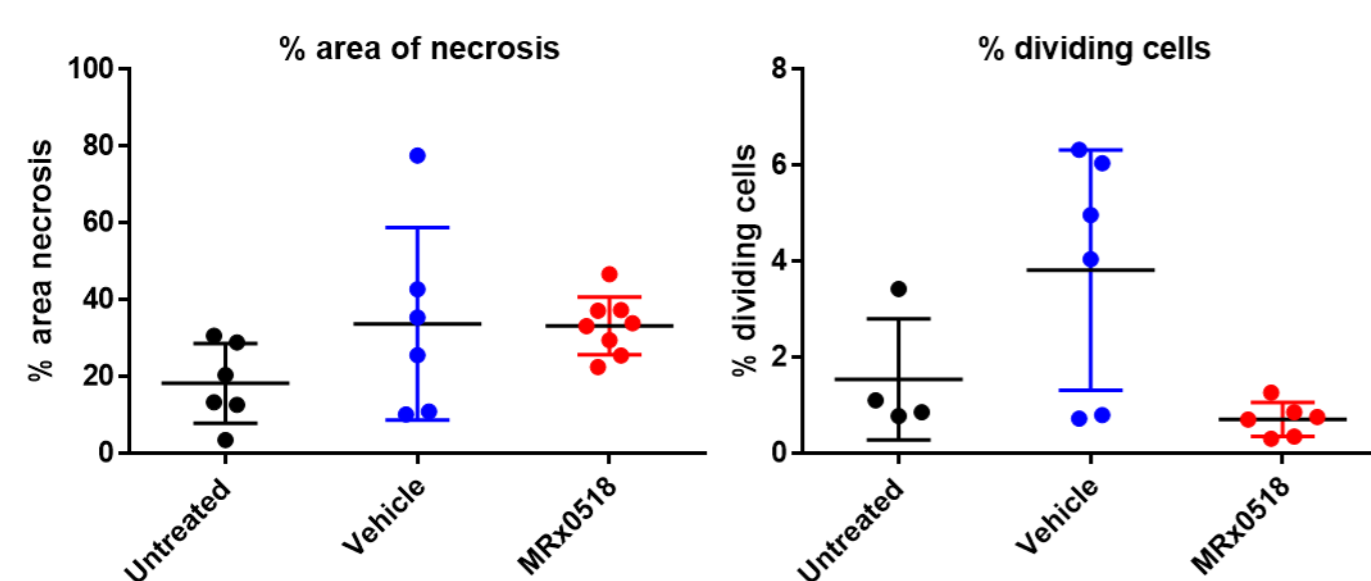


Fig. 2: Percentage of necrotic area (left) and dividing cells (right) in EMT6 tumours. Data are presented as Mean ± SD. Anti-CTLA-4 group had no tumour at the termination of the study.

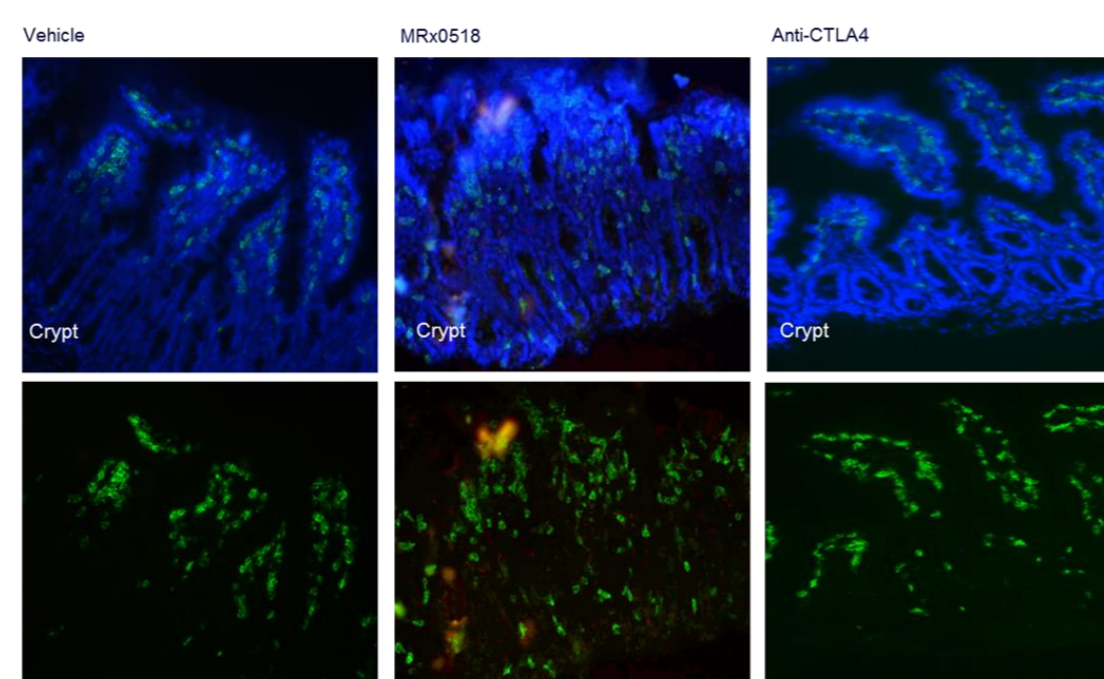


Fig. 3: Ileum cryo-sections stained with antibodies against CD8 α (green) and DAPI (blue). Original magnification x200.

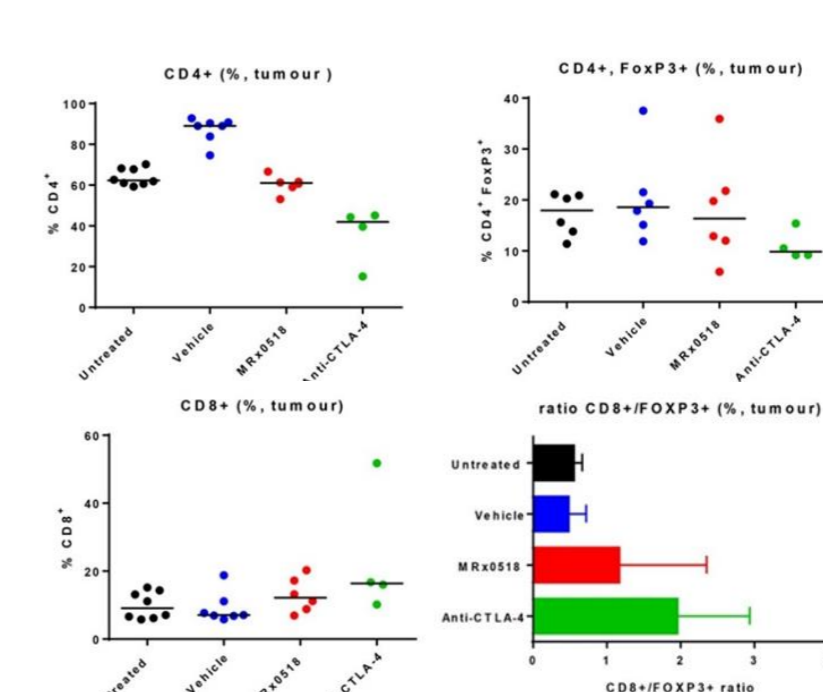


Fig. 4: Flow cytometry analysis of immune cells in tumour microenvironment. Data are presented as Mean.

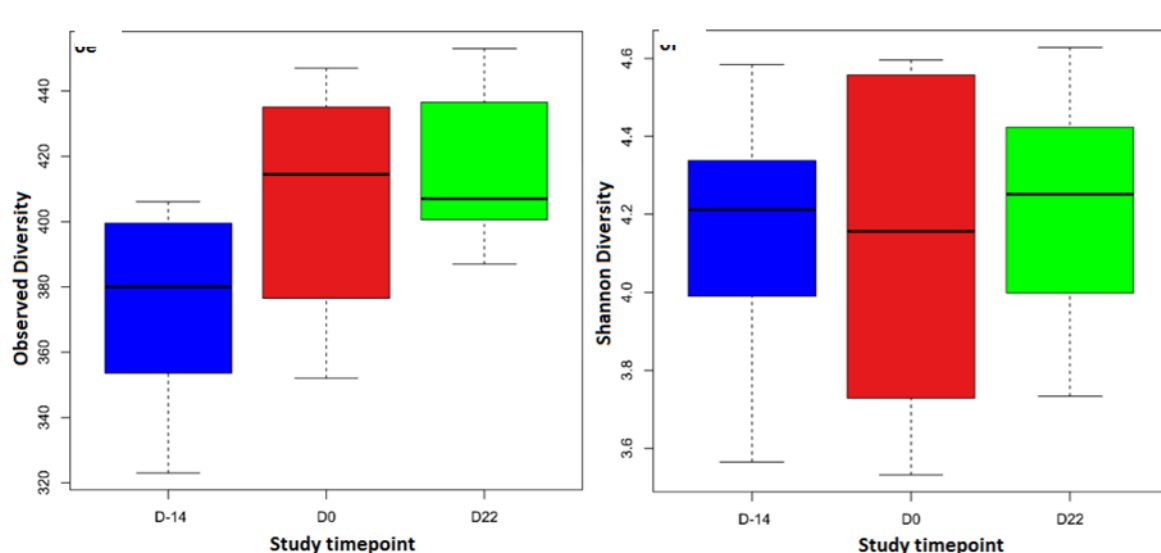


Fig. 5: Microbiota diversity for observed species detected at different time-points with MRx0518 treatments. Data are presented as Mean ± SE.

TIME-POINT	MRx0518	Anti-CTLA-4
D-14	<i>Eubacterium dolichum</i>	<i>Roseburia faecis</i> <i>Clostridium saccharolyticum</i> <i>Clostridium XIVa Spp.</i>
D0	<i>Barnesiella intestinihominis</i> <i>Oscillospira guilliermondii</i> <i>Odoribacter splanchnicus</i>	0
D22	<i>Lachnospiraceae Spp.</i> <i>Clostridium XIVa Spp.</i> <i>Firmicutes Spp.</i> <i>Eubacterium Spp.</i> <i>Bacteroides acidifaciens</i> <i>Barnesiella intestinihominis</i>	<i>Lachnospiraceae Spp.</i> <i>Clostridium XIVa Spp.</i> <i>Clostridium saccharolyticum</i>

Table 1: Differentially abundant bacterial taxa in MRx0518 and anti-CTLA-4 treated animals. Red: more abundant taxa; Black: less abundant taxa.

Microbiota profiling

To investigate the impact of MRx0518 and anti-CTLA-4 treatments on gut microbiota diversity and composition, 16S taxonomic microbiota profiling of faecal samples was conducted using a bioinformatics workflow. A significant difference in microbiota diversity for Observed Species ($p=0.03617$) was detected across timepoints in MRx0518-treated animals but this was not significant for Shannon index ($p=0.9075$) (Fig. 5). Significant differentially abundant taxa were observed in the treated groups at all three timepoints compared to the vehicle group (Table 1).

Key Findings

MRx0518 has therapeutic value in the treatment of cancer, evidenced by:

- Reduction of tumour size in *in vivo* models
- Increased necrotic area/reduced dividing cells in tumours
- Increased CD8:Treg ratio in the tumour microenvironment
- CD8⁺ immune cell localisation in the ileum crypts
- Increased microbiota diversity

Future Studies



- Further *in vivo* studies are underway to determine the molecular and cellular mechanism through which MRx0518 exerts its antitumorigenic effects.
- MRx0518 is currently in development for clinical studies.