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

Anna Ettorre1, Margaret Delday2, Meenakshi Pradhan3, Ian Jeffery2, Alessio Panzica1, Amy Holt1, Philip Cowie1, Sam Yuille1, Emma Raftis1, Nicole Reichardt2, Helene Savignac3 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; Vétiouz 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. The antitumorigenic effects of MRx0518 are linked to its distinct and species-specific in vitro immuno-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.

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 panels). Gut immuno-staining showed that more CD8a+ 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).

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).

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

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.