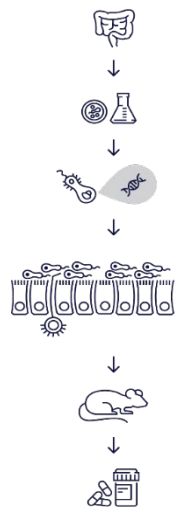


Understanding the immunological phenotyping of *Bifidobacterium breve* MRx-4DP0004

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4D Pharma PLC is a pharmaceutical company focused 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.

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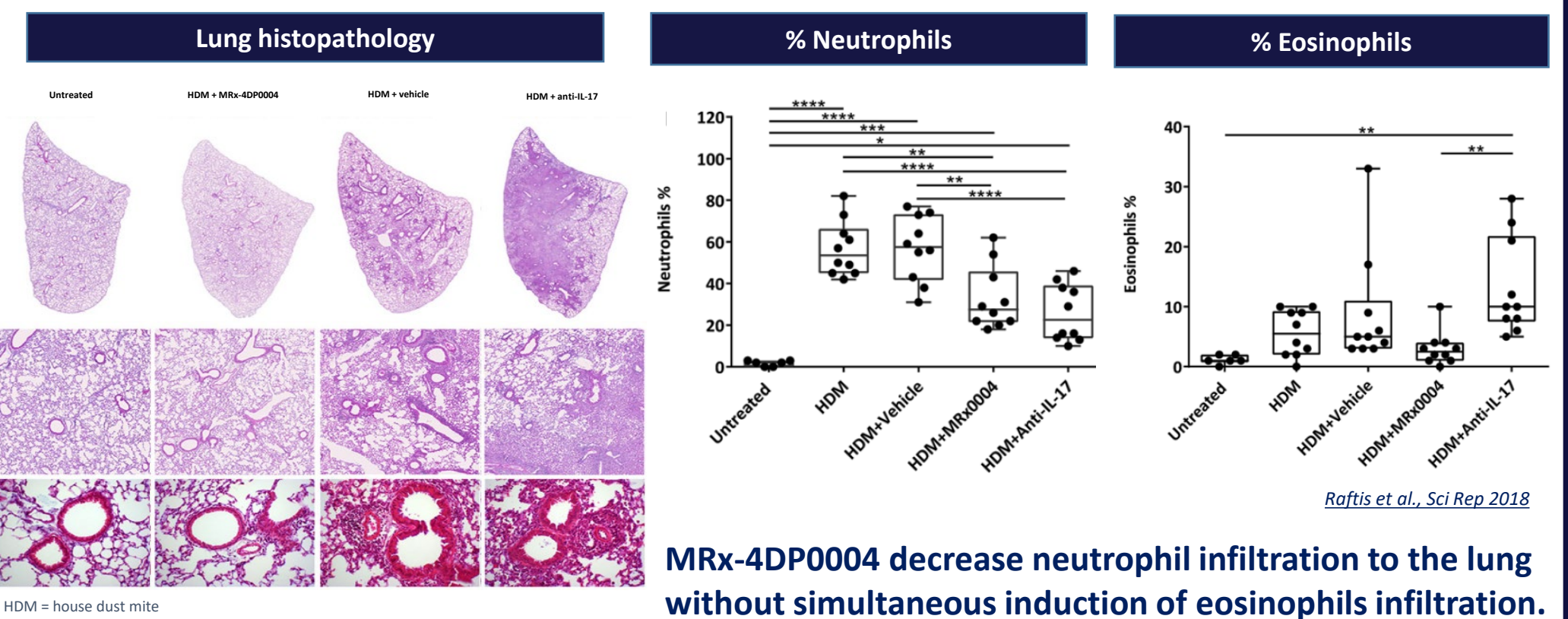
Introduction

Bifidobacterium species have been reported to modulate T-helper immune responses in a species-specific manner. The promising next-generation candidate live biotherapeutic bacterial strain *Bifidobacterium breve* MRx-4DP0004 isolated from the intestinal tract of a healthy human donor, has shown efficacy in a preclinical model of severe asthma. This complex inflammatory disease is associated with imbalanced Th1/Th2 polarisation. The activity of the strain in asthma has been correlated with reduced neutrophil and eosinophil infiltration in the lung bronchoalveolar fluid and increased abundance of FoxP3⁺ T regulatory cells (Tregs) in lung tissues.

Objective: To investigate the immunomodulatory activity of MRx-4DP0004 *in vitro* using human peripheral blood mononuclear cells (PBMCs) and *in vivo* using SPF mice

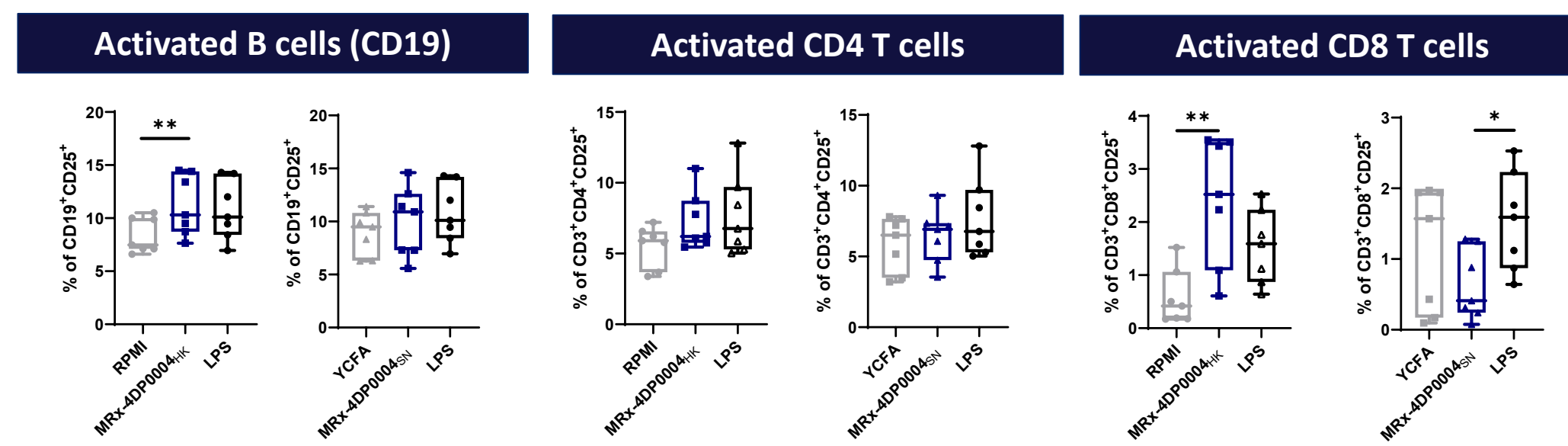
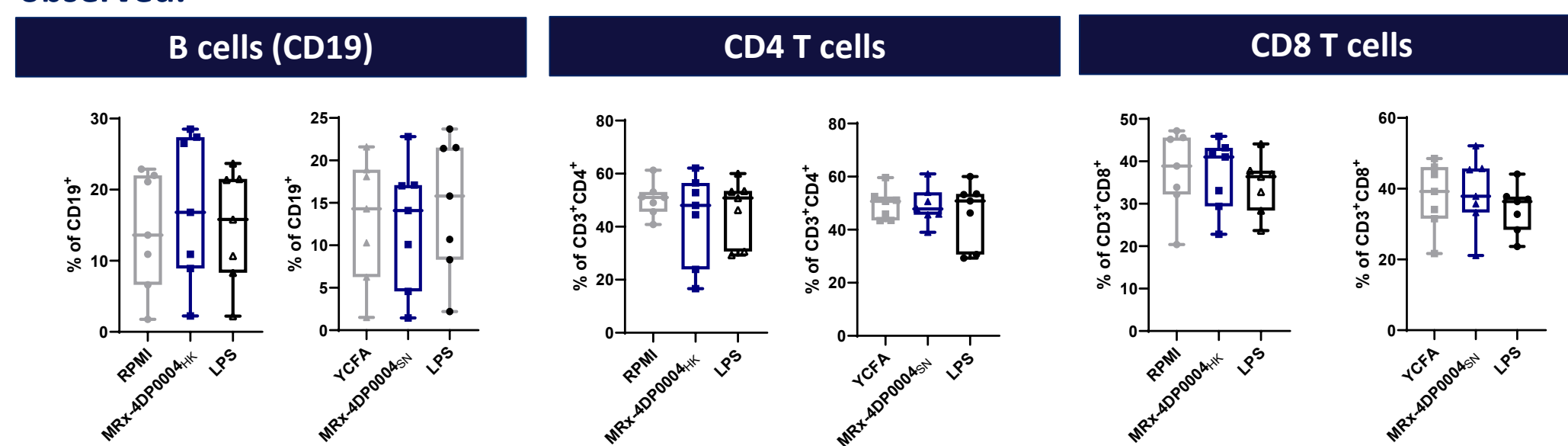


Neutrophilic asthma model

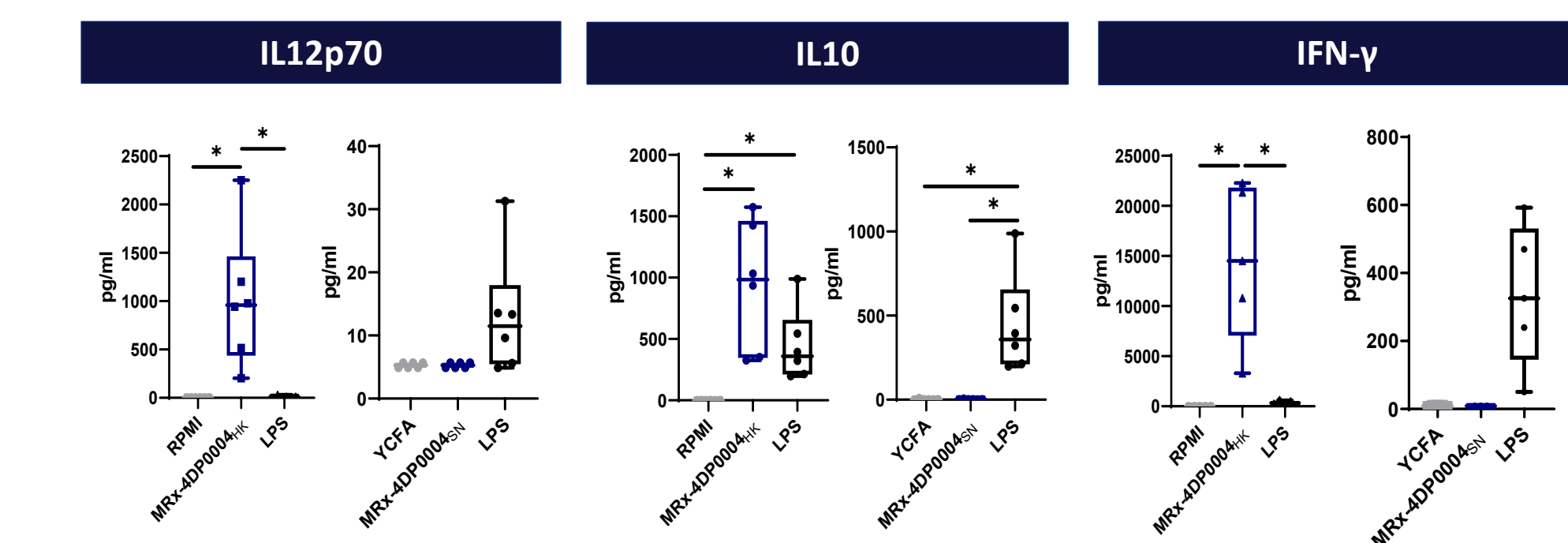


Results

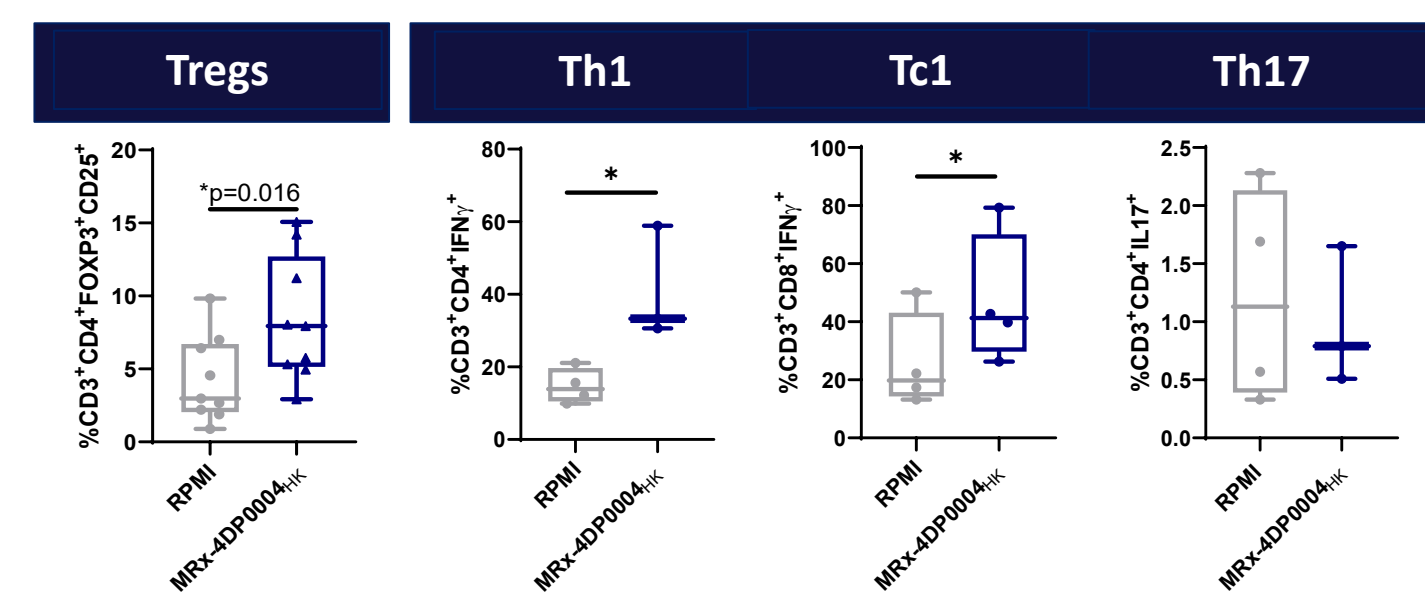
1. *In vitro* MRx-4DP0004_{HK} induced PBMC activation of CD19⁺ B and CD8⁺ T cells with no effect on the frequency of total cells. No difference in the frequency of CD4⁺ T cells was observed.



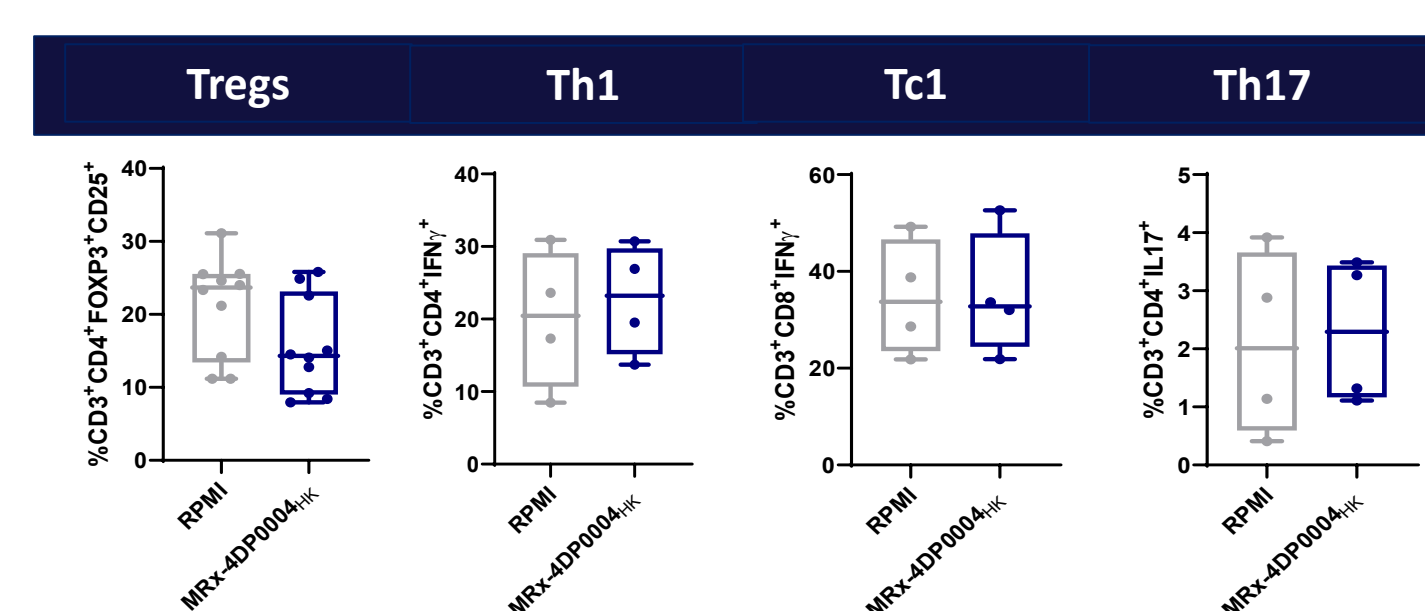
2. MRx-4DP0004_{HK} treated PBMCs increased both pro- and anti-inflammatory cytokine secretion suggesting that MRx-4DP0004_{HK} leads to a balanced immune response.



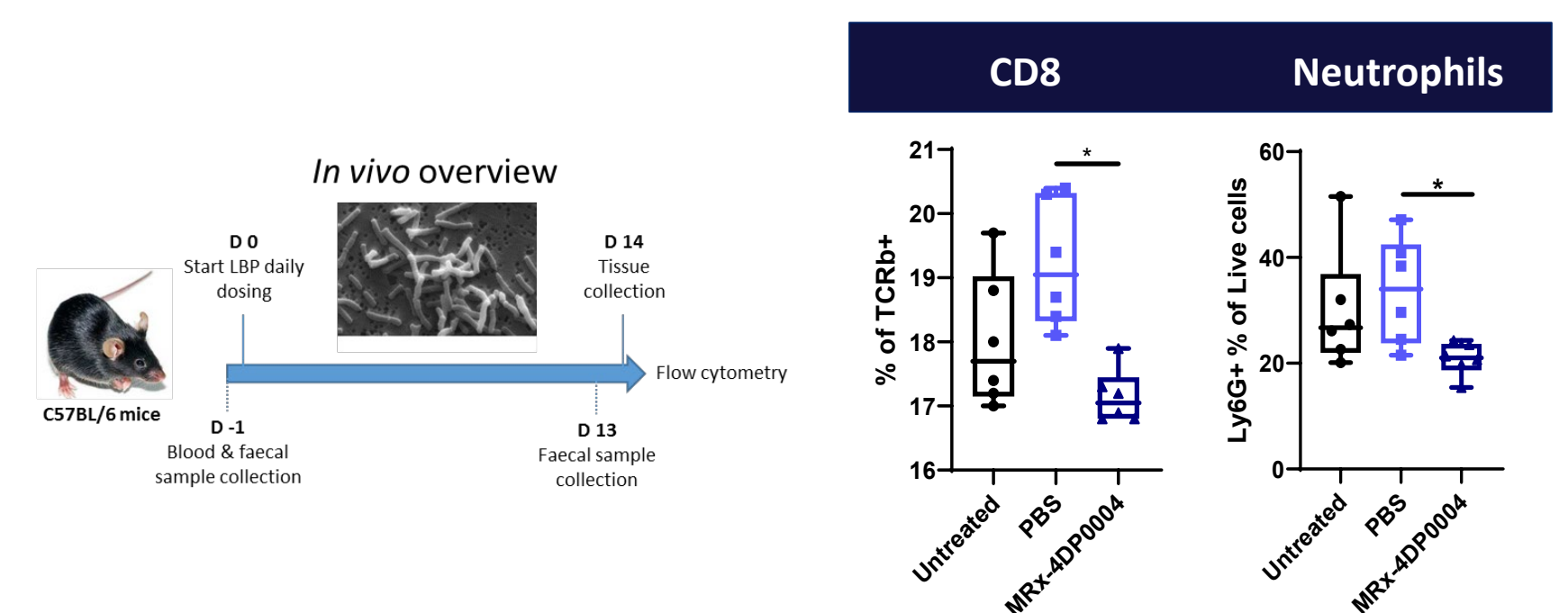
3. MRx-4DP0004 in presence of a stimulant (anti-CD3 or anti-CD3/CD28) induced increase of Tregs, Th1 and Tc1 *in vitro* in PBMC co-cultures.



4. No differences were observed in the PBMC subsets in the presence of polarising cytokines suggesting that the addition of cytokines bypasses the innate immune response.



5. SPF mice treated with MRx-4DP0004 downregulated splenic CD8 and neutrophils suggesting that even in absence of disease MRx-4DP0004 can affect neutrophil population.



Key Findings

- Our *in vitro* data suggest that MRx-4DP0004 activity in asthma may be associated with the ability to reshape T cell polarization.
- MRx-4DP0004 induces activation of B and CTL cells and production of both pro-inflammatory and tolerogenic cytokines without affecting the frequency of total B and T cell subsets.
- MRx-4DP0004 induces FOXP3⁺ Treg, Th1 (CD4+IFN γ +) and Tc1 (CD8+IFN γ +) cells after *in vitro* stimulation of PBMCs with anti-CD3 or anti-CD3/CD28, respectively.
- In vivo* MRx-4DP0004 induced a specific reduction of CD8⁺ T cells and neutrophils in the spleen of SPF mice. These data suggest that whereas MRx-4DP0004 dosing may reduce the frequency of neutrophils in peripheral tissues even in absence of disease its effect on CD8 and Treg frequency may be tissue and/or disease specific.

Future Direction

- Further studies to identify the specific molecule(s) involved in MRx-4DP0004 activity are under investigation using mutant strain knockouts for single genes with predicted roles in host interaction
- MRx-4DP0004 is currently being tested in a first-in-man Phase I clinical trial in asthma patients