



**4D pharma plc** is a pharmaceutical company focused on developing Live Biotherapeutic products (LBPs) derived from the human gut microbiome. LBPs are a regulated, emerging and disruptive new class of medicines, which have the potential to transform the way in which we treat many diseases. 4D pharma currently has clinical stage programs in cancer, asthma, irritable bowel syndrome (IBS) and Crohn's disease, and a strong pipeline of pre-clinical programs including immuno-oncology, CNS and autoimmune diseases.

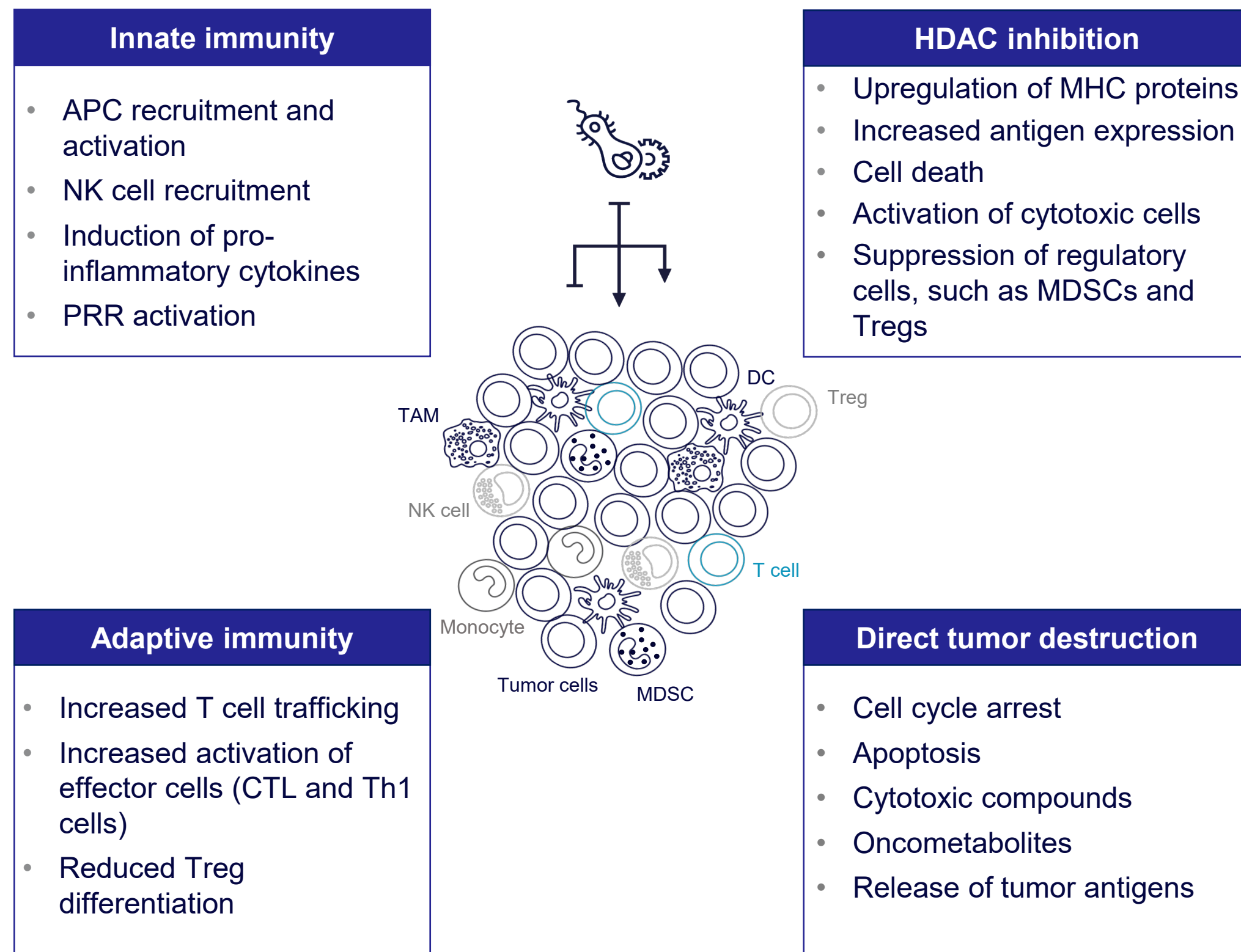
## Introduction

The human gut microbiota plays an essential role in modulating both intestinal and systemic immunity. Its importance in regulating anti-tumorigenic responses is now being explored, supported by evidence that functional components of the gut microbiome influence response to cancer therapy, including but not limited to immunotherapies.

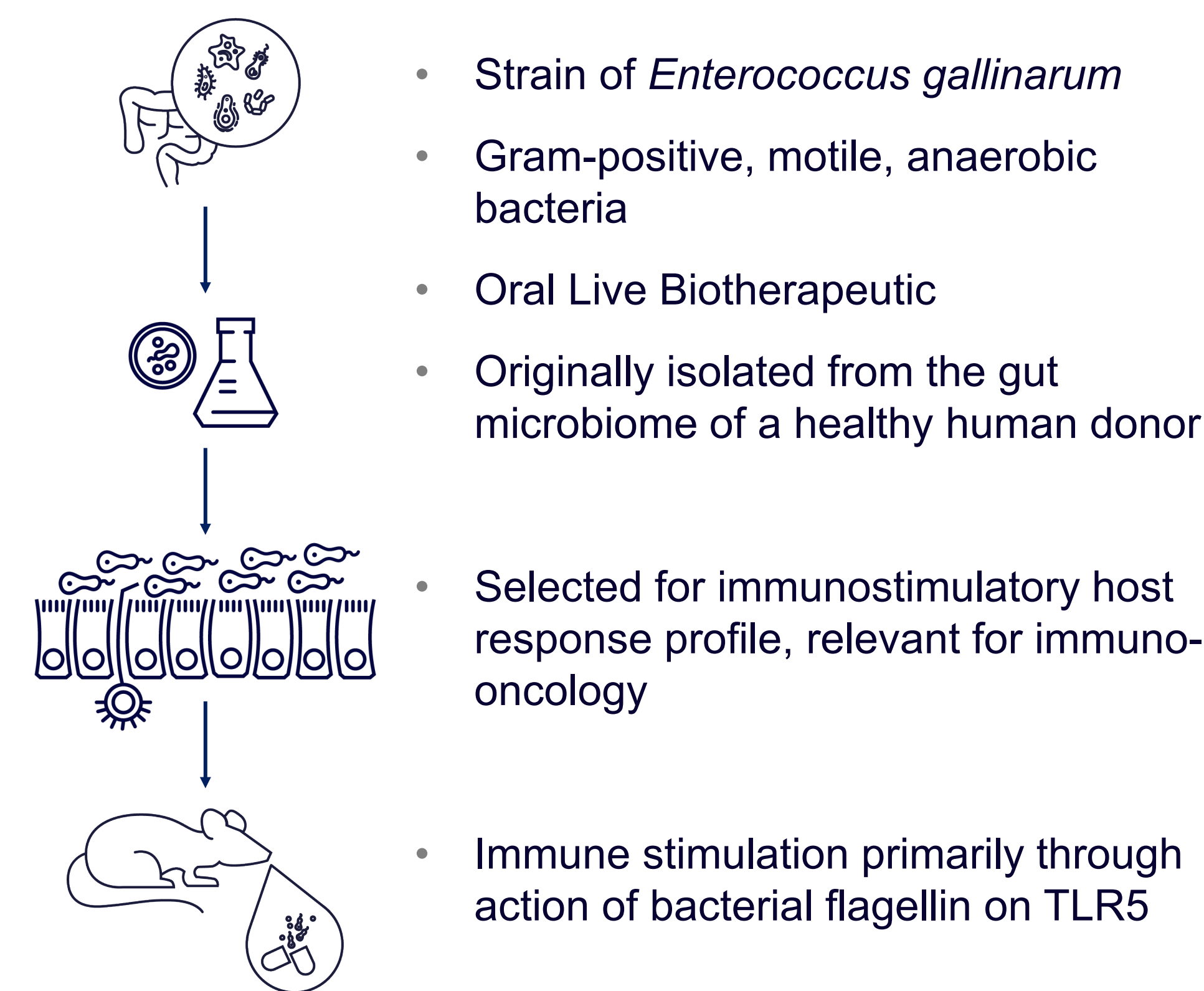
We previously demonstrated that the single strain Live Biotherapeutic, *Enterococcus gallinarum* MRx0518, had strong immunostimulatory properties *in vitro*, and was a TLR5 and NF-κB activator.

We further investigated whether this bacterial strain displayed anti-tumor efficacy in a murine mammary carcinoma model.

## LBPs and the tumor microenvironment



## MRx0518

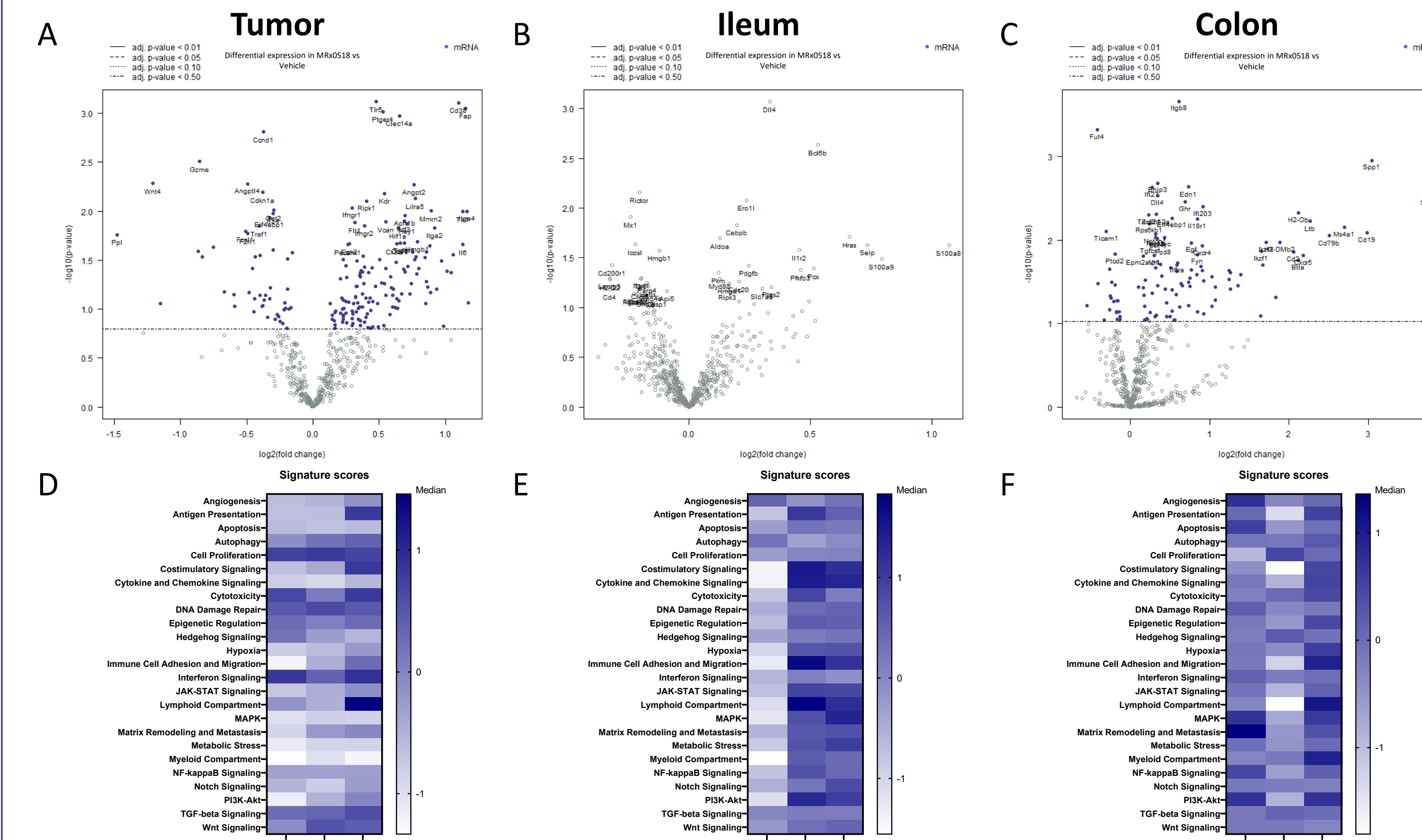


**Oncodesign** is a biopharmaceutical company dedicated to precision medicine. With its unique experience acquired by working with more than 800 clients along with its comprehensive technological platforms combining state-of-the-art medicinal chemistry, pharmacology, bioanalysis, pharmaco-imaging and artificial intelligence, **Oncodesign** orients, drives & executes the development of drug candidates from hit findings up to early clinical phases in oncology and autoimmune/inflammatory diseases.

## Results: Transcriptional analysis

### MRx0518 significantly modulates gene expression in the tumor and the colon

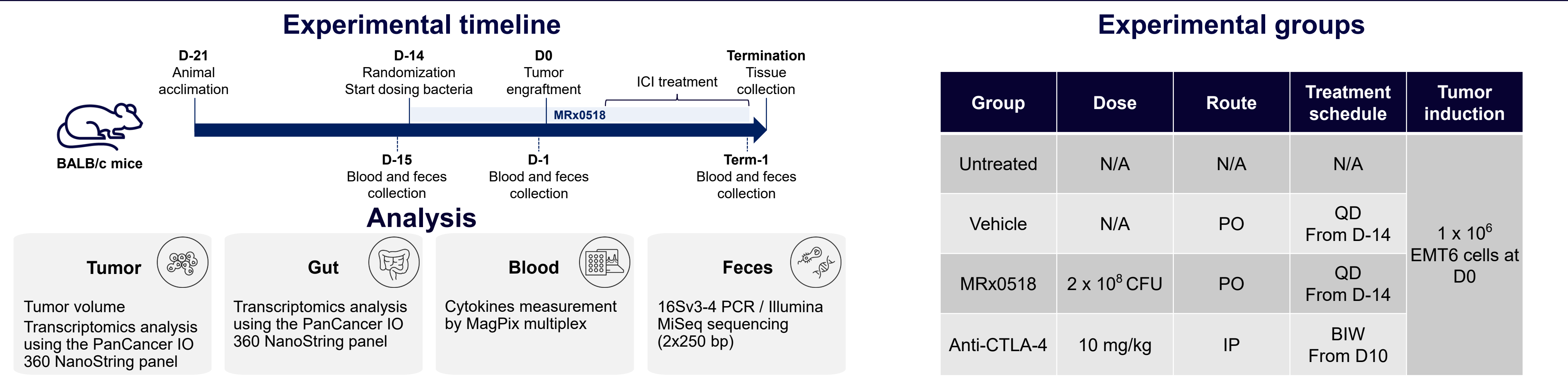
MRx0518 therapeutic activity was associated with upregulation of genes involved in immune cell adhesion and migration, such as *Cd38* and *Clec14a*, and increased *Tlr5* (innate immune receptor) expression in the tumor. At the intestinal level, lymphoid compartment and cell adhesion pathways were upregulated in the colon, in particular *Spp1*, *Cd19*, *Ms4a1* (CD20) and *Sell* (CD62L) genes.



Transcripts analysis of the tumoral (A), ileal (B) and colonic (C) tissue in the mouse model of breast cancer (EMT6) was conducted using the PanCancer IO 360 NanoString gene expression panel.

KEGG database was used to annotate the transcripts isolated from tumoral (D), ileal (E), and colonic (F) tissues and to conduct pathway identification.

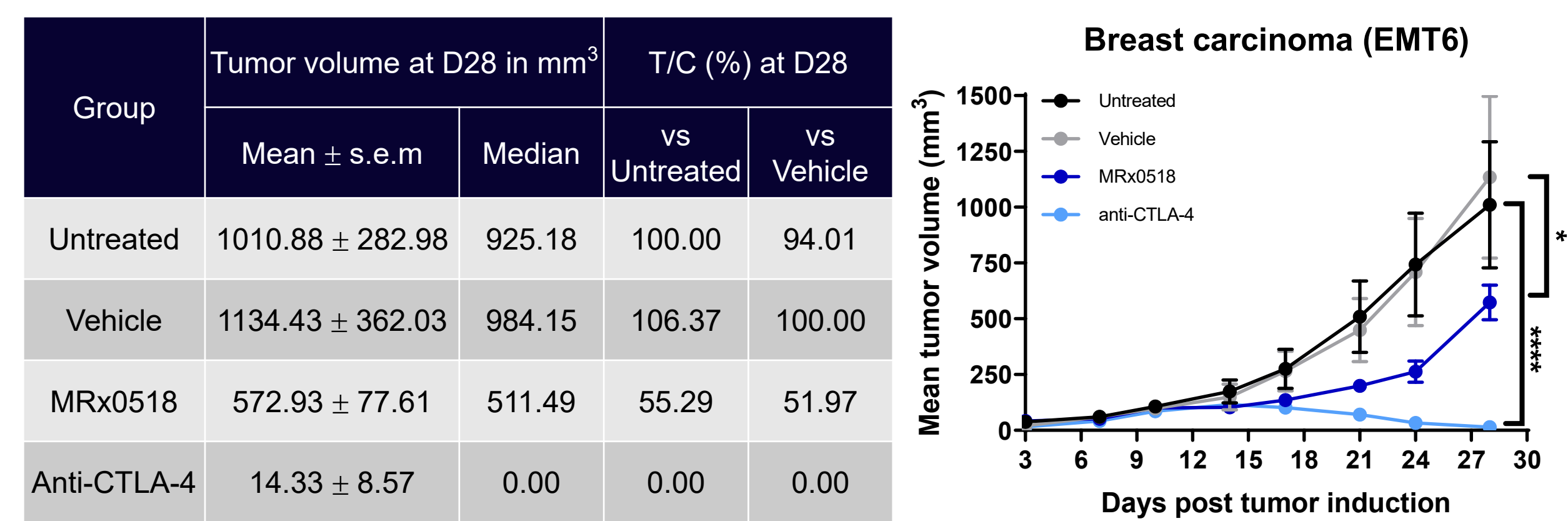
## Experimental design: EMT6 model



## Results: Tumor growth

### MRx0518 monotherapy induces a reduction in EMT6 murine tumor burden

- From D-14, mice received vehicle or 2x10<sup>8</sup> CFU MRx0518 daily until termination.
- On D0, mice were engrafted with EMT6 tumor cells subcutaneously.
- Anti-CTLA-4 (10 mg/kg, IP, BIW) was used as positive control.
- Tumor length and width as well as body weight were measured 2-3 times a week. No side effect of the treatment was observed.



## Conclusions

- MRx0518 *in vivo* Efficacy**
  - Reduces tumor growth in EMT6 model (also LLC and RENCA models, data not shown)
  - Increases tumor and intestine immune infiltration: NKs, T cells and cytotoxic cells
  - Increases TLR5 expression within the tumor tissue
- MRx0518 Mechanism of Action**
  - Modulation of both the gut immune system and the tumor microenvironment
  - Flagellin TLR5 agonism (Lauté-Caly *et al. Sci Rep* 2019)

## MRx0518 Clinical development

- Phase I/II, open-label, combination with pembrolizumab in anti-PD-(L)1 secondary resistant NSCLC, RCC, melanoma, bladder
  - Early signals of activity and clinical response, no serious drug-related adverse events
  - Part A enrollment complete
- Phase I, neoadjuvant monotherapy study in treatment-naïve patients awaiting surgical resection of solid tumors; enrolling
- Phase I, pancreatic cancer in combination with radiotherapy; enrolling