LB-283

# Leveraging gut microbiota to impact tumor immunotherapy

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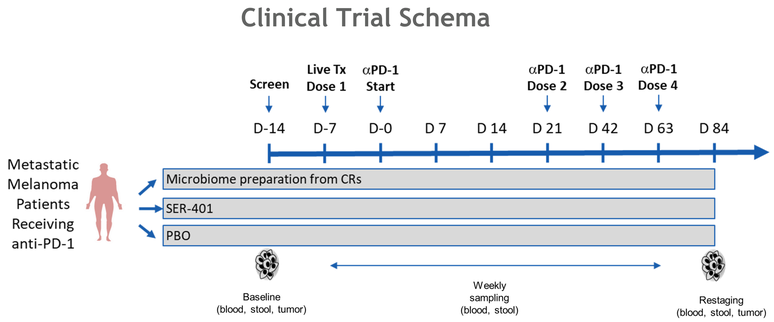
## Abstract

**Background:** The human gut microbiome is a diverse, dynamic and complex ecosystem that modulates numerous host processes including metabolism, inflammation and cellular and humoral immune responses. Recent publications have suggested that the gut microbiota of cancer patients is predictive of response to immune checkpoint inhibitors (ICI). To better understand how the microbiome may impact response to ICI, we have developed and validated robust tumor models using both conventional mice treated with antibiotics as well as germ free mice.

**Results:** We show that germ-free mice lacking a microbiome, as well as antibiotics-treated mice fail to mount an effective anti-tumor immune response following treatment with anti-PD-1. The response to anti-PD-1 can be restored in germ free mice by introduction of a microbiome using fecal material prepared from healthy donor stool, and is driven by increased entry of tumor-infiltrating lymphocytes (TILs) into the tumor; specifically CD8+ T cells. Importantly, for the first time, we show that the bacterial spore fraction from healthy donor stool can restore response to anti-PD-1 and increase CD8+ TILs in both conventional mice treated with antibiotics as well as germ free mice.

Based on these encouraging animal model data we plan to initiate a randomized, placebo-controlled clinical study at MD Anderson Cancer Center in 2018, sponsored by the Parker Institute for Cancer Immunotherapy, in patients with advanced metastatic melanoma. The clinical trial will evaluate the impact of an anti-PD-1 checkpoint inhibitor with adjunctive microbiome therapy on patient outcomes. Seres is developing SER-401, a preclinical stage oral microbiome therapy to improve the efficacy and safety of immunotherapy. Our drug discovery strategy iterates computational analyses with machine learning approaches, as well as empirical *in vitro*, *in vivo* and *ex-vivo* screening of strains and consortia to inform selection and drive microbiome drug design. Data from such a comprehensive approach is invaluable for designing compositions of bacteria that form “functional ecological networks” that can impact response to ICI therapy. We believe these data will provide insight into how microbiome drugs can be discovered and developed in the setting of immunotherapy to augment the efficacy of ICIs by altering the cancer-immune set point.

### Clinical Trial Schema




- Primary readouts: (i) safety/ tolerability (ii) tumor response and T cell infiltration vs. baseline
- Exploratory readouts: microbiome and metabolome correlates of clinical measures

## Conventional animal model

### Anti-PD-1 promotes anti-tumor immune response

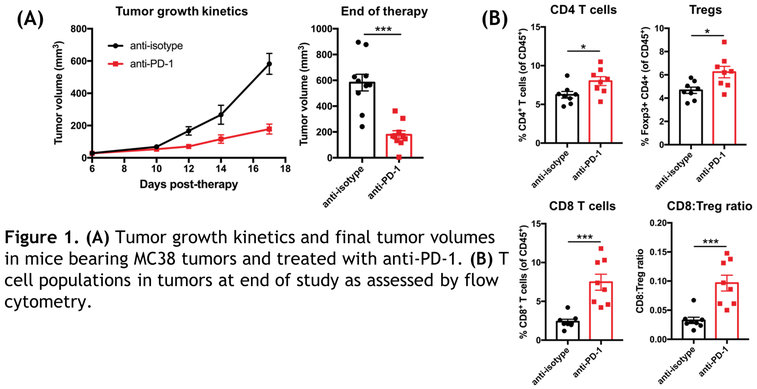



**Figure 1.** **(A)** Tumor growth kinetics and final tumor volumes in mice bearing MC38 tumors and treated with anti-PD-1. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

### Treatment with antibiotics negates anti-tumor efficacy of anti-PD-1

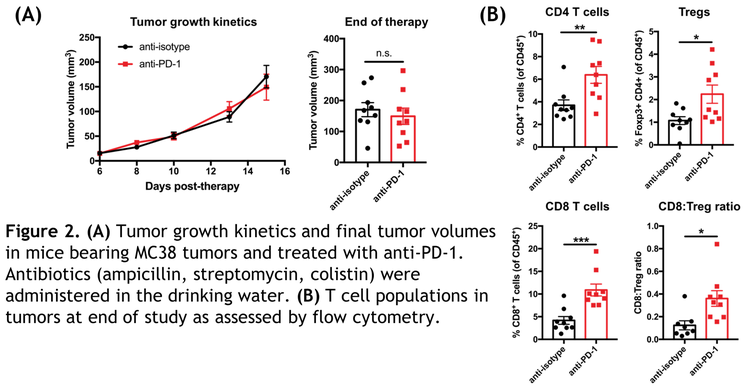



**Figure 2.** **(A)** Tumor growth kinetics and final tumor volumes in mice bearing MC38 tumors and treated with anti-PD-1. Antibiotics (ampicillin, streptomycin, colistin) were administered in the drinking water. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

### Spore fraction of healthy donor stool restores anti-tumor efficacy of anti-PD-1 after treatment with antibiotics

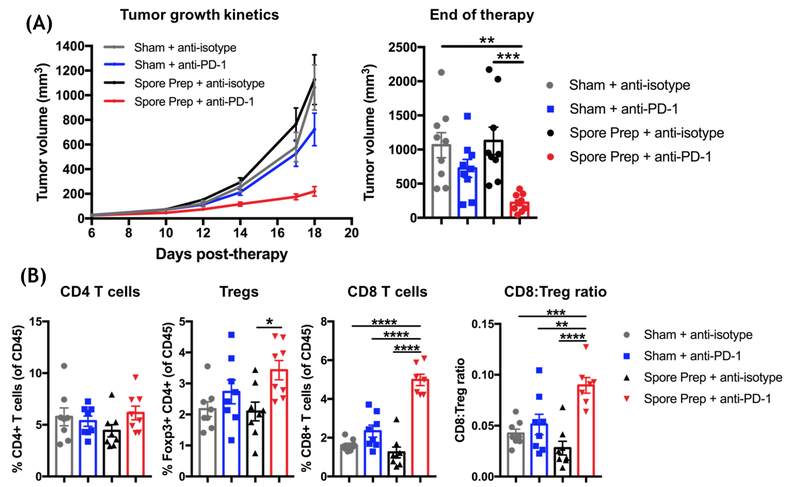



**Figure 3.** **(A)** Tumor growth kinetics and final tumor volumes in mice bearing MC38 tumors. Antibiotics were delivered in the drinking water prior to administration of spore fraction prepared from healthy donor stool. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

## Germ-free animal model

### Germ-free mice cannot promote anti-tumor immune response after anti-PD-1 therapy

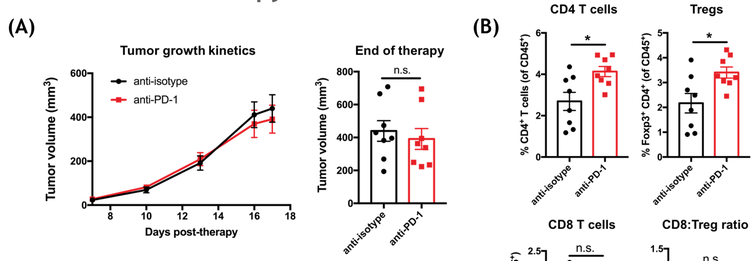



**Figure 4.** **(A)** Tumor growth kinetics and final tumor volumes in germ-free mice bearing MC38 tumors and treated with anti-PD-1. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

### Healthy donor FMT promotes anti-tumor immune response to anti-PD-1




**Figure 5.** **(A)** Tumor growth kinetics and final tumor volumes in ex-germ-free mice bearing MC38 tumors and treated with anti-PD-1. Mice were colonized with FMT prepared from healthy donor stool a few weeks prior to tumor cell injection. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

### Spore fraction of healthy donor stool promotes anti-tumor immune response with anti-PD-1 treatment

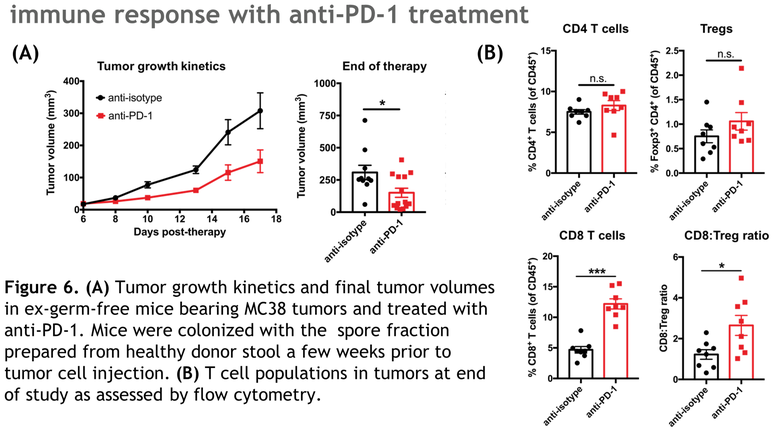



**Figure 6.** **(A)** Tumor growth kinetics and final tumor volumes in ex-germ-free mice bearing MC38 tumors and treated with anti-PD-1. Mice were colonized with the spore fraction prepared from healthy donor stool a few weeks prior to tumor cell injection. **(B)** T cell populations in tumors at end of study as assessed by flow cytometry.

n=8-10 mice per group for all studies
Statistical analysis was performed using two-tailed Student’s T test or one-way ANOVA with p<0.05 considered significant (*p<0.05; **p<0.01, ***p<0.001, ****p<0.0001).