Discovery and functional validation of a rationally selected, orally administered, live biotherapeutic consortium of commensal bacteria for the treatment of solid tumors

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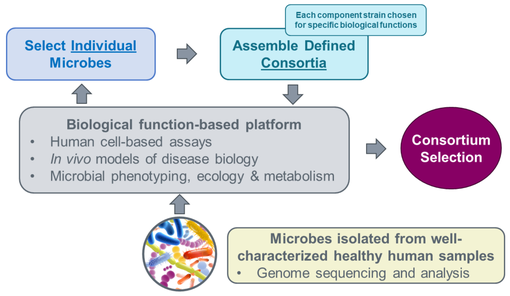
Introduction

Recent evidence supports the role of gut commensal microbes in mediating patient response to immune checkpoint inhibitor therapies (ICT) and chemotherapy. Fecal transplants from ICT responder or non-responder patients into mice modulate syngeneic tumor growth and response to checkpoint antibody treatment, recapitulating the response patterns seen in the human donors. These and other data support the conclusion that certain gut commensal bacteria can promote a systemic immune response to tumors distant from the gut mucosa. We hypothesize that a well-defined consortium of bacteria, rationally selected based on disease-relevant immunological mechanisms, could provide meaningful clinical benefit upon oral administration and delivery to the intestine of cancer patients. To this end, we established a biological function-based platform to identify bacterial strains with immune stimulatory activities *in vitro* and significant anti-tumor efficacy alone or in combination with checkpoint antibody in syngeneic tumor models.

Methods

Rationally Selecting Consortia of Live Microbes with Defined Pharmacological & Biological Functions



Human cell-based assays
- Validated *in vitro* assays established with human PBMCs, MoDCs & macrophages
- Human cells co-cultivated with freshly grown live microbes
- Culture supernatant cytokines/chemokines evaluated by MSD multiplex system & ELISA. Immune cell activation assessed by multi-color flow cytometry

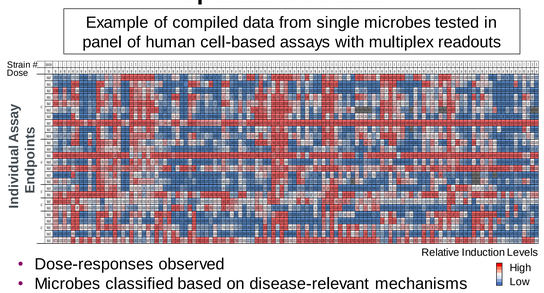
B16F10 and CT26 syngeneic tumor models
- n = 10 mice per test group, n = 15 for vehicle control group
- Tumors established prior to indicated treatments: oral gavage of microbes or vehicle; anti-PD-1 or anti-PD-L1 antibodies administered IP
- Efficacy demonstrated in context of native mouse microbiota (without antibiotic pre-treatment)
- Tumors at endpoint: IHC, flow cytometry, MSD cytokine profiling and Nanostring RNA-based profiling with pathway analysis

Summary

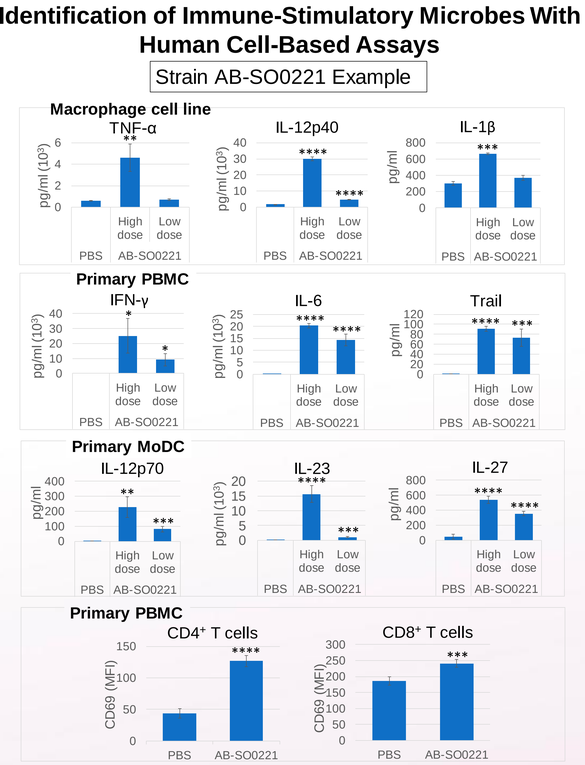
- Bacterial strains were purified from well-curated healthy human stool samples, identified by 16S and whole genome sequencing and phenotypically characterized.
- Individual strains were evaluated by co-incubating live bacteria in panel of validated human *in vitro* assays with cancer-relevant immune-stimulatory readouts.
  - Strains reproducibly inducing immune-stimulatory cytokines from PBMCs or MoDCs, activating T cells or M1-shifting macrophages were selected for *in vivo* evaluation.
- Oral administration of bacterial strains or defined consortia in B16F10 and CT26 syngeneic mouse tumor models demonstrated reproducible anti-tumor activities.
- Rationally-selected, defined consortia significantly enhanced checkpoint antibody efficacy, and their combination induced hallmarks of immune activation in tumors *e.g.*, increased IFN-γ and CD8⁺ T cell infiltration.
- Using well-defined, robust GMP processes, a candidate therapeutic consortium will be prepared as viable lyophilized formulation retaining biological function, encapsulated for oral administration and evaluated in human subjects.

Results

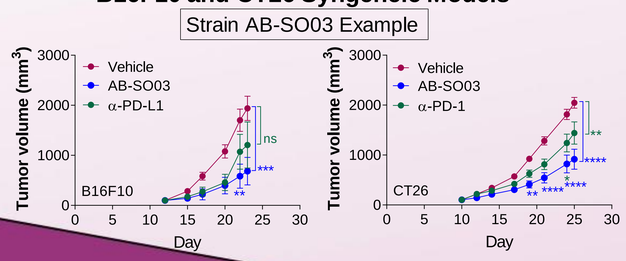
Reproducible Cell-Based Assay Results Demonstrate Unique Functional Differences Between Individual Species & Strains



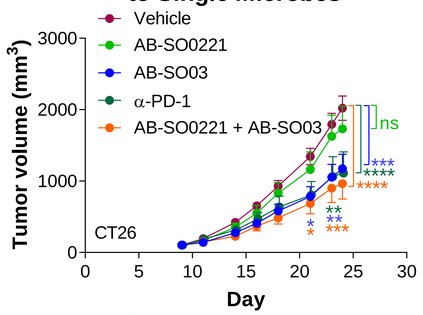
Identification of Immune-Stimulatory Microbes With Human Cell-Based Assays



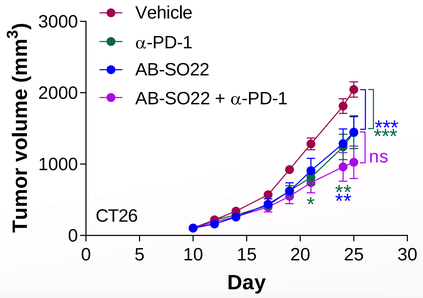
Individual Microbes Show Anti-Tumor Activity in B16F10 and CT26 Syngeneic Models



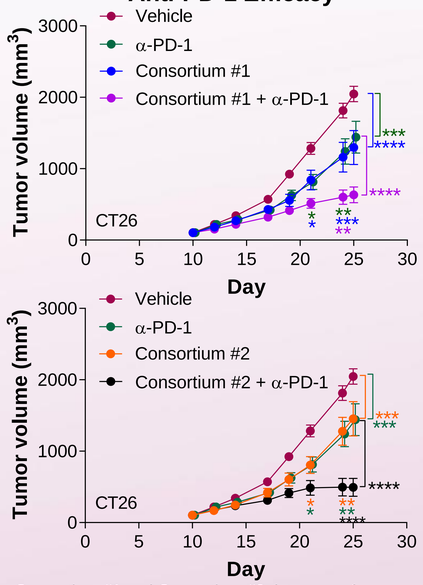
Combinations of Microbes Can Mediate Increased Anti-Tumor Activity Compared to Single Microbes



Individual Efficacious Microbes Can Enhance Anti-PD-1 Efficacy in CT26 Tumor Model

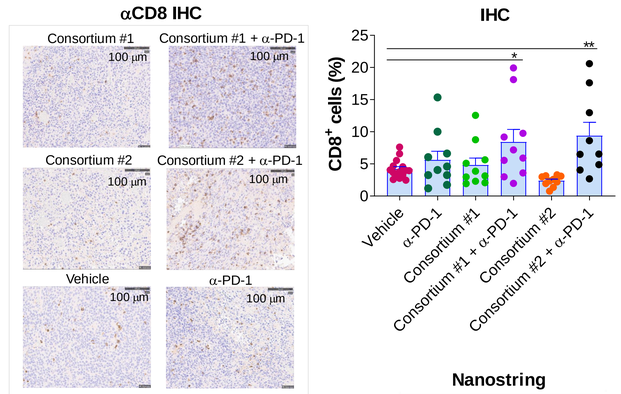


Rationally-Selected Defined Microbial Consortia Significantly Enhance Anti-PD-1 Efficacy

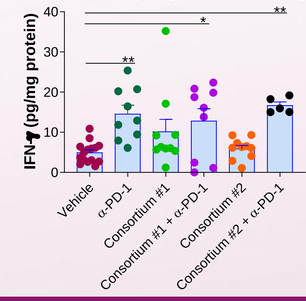


Consortium #1 and Consortium #2 data came from same experiment but are plotted separately for ease of visualization.

Consortium #1 or Consortium #2, in Combination with Anti-PD-1, Significantly Increase Tumor-Infiltrating CD8⁺ T Cells



Anti-PD-1 and Anti-PD-1 in Combination with Consortium #1 or Consortium #2 Significantly Increase Tumor IFN-γ by MSD Analysis



Statistics

- * P<0.05, **P<0.01. ***P<0.001, ****P<0.0001; ns, not significant, mean ± SEM; unpaired 2-tailed Student's T test for comparison to PBS in *in vitro* assays; tumor endpoints: one-way ANOVA with Dunnett's post hoc test for ≥3 sample comparison or two-way ANOVA with Dunnett's post hoc test for ≥3 sample comparison across multiple time points, relative to Vehicle or α-PD-1 where indicated
- Results are reproducible across multiple independent experiments.

Disclosures

- LPN, JH, JM, KK, ARA, ZR and JP are employees, and PA and KD are former employees, of Assembly Biosciences.