Saccharomyces boulardii as a Treatment for Colonic-inflammatory Conditions such as Chemotherapeutic-induced Mucositis and Colorectal Cancer

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Abstract

Chemotherapeutic-induced mucositis (CIM) is a prevalent and debilitating side effect of cancer treatment, characterized by inflammation and ulceration of the gastrointestinal (GI) tract. Current treatments focus on controlling symptoms; however, recent research has focused on using probiotics such as *Saccharomyces boulardii (S. boulardii)* as a potential treatment for CIM given its ability to restore gut dysbiosis, facilitate cell growth, and strengthen the mucin barrier. CIM shares pathophysiological features such as gut dysbiosis and intestinal inflammation with inflammatory bowel disease (IBD) as well. Patients with IBD are at increased risk for developing colorectal cancer (CRC), highlighting the importance of understanding the relationship between inflammation and tumorigenesis in the GI tract. Probiotics such as S. boulardii have shown promise in alleviating the symptoms of IBD and reducing CRC risk; however, S. boulardii does not stably colonize and is not retained well in the GI tract thereby necessitating daily and high dosing. Our team has developed an engineered *S. boulardii* designed to attach to the fibronectin moieties that are highly expressed on ulcerated tissue in the GI tract and secrete nanobodies against pro-inflammatory TNFα. To validate the CIM model, female FVB mice were intraperitoneally injected with irinotecan or saline three times a week for two weeks. The measured outcomes included body mass, Bristol Stool scores, fecal occult blood, and fecal lipocalin-2 concentrations. To validate the AOM/Il10-/- model for colitis-associated cancer, germ-free 129SvEv Il10-/- mice were colonized with fecal microbiota transplant to begin inducing inflammation and then intraperitoneally injected once weekly for six weeks with AOM to induce tumorigenesis. As a probiotic treatment, mice received oral gavages of engineered *S. boulardii* once or twice weekly, unmodified *S. boulardii* twice weekly, or PBS once weekly. The measured outcomes included body mass, macroscopic tumor counts and sizes, histological neoplasia scores to quantify the severity of neoplasia and tumor invasion, and histologic inflammation scores. For CIM model validation, mice injected with irinotecan did not exhibit significant weight loss, have higher Bristol Stool scores, test positive for fecal occult blood, or have significantly higher concentrations of fecal lipocalin-2 than irinotecan-untreated mice. our inability to validate the model prevented us from evaluating the efficacy of *S. boulardii* in treating CIM. We were able to successfully run the AOM/Il10-/- model for colitis-associated cancer, where both inflammation and invasive tumors (adenocarcinoma) were successfully induced. In this model, *S. boulardii* decreased tumor multiplicity and tumor load in a dose-dependent manner regardless of engineered properties. In addition, there was no significant difference between unmodified and engineered *S. boulardii* in terms of colonization efficiency. These findings suggest that *S. boulardii* exhibits anti-cancer activity and should also be evaluated for ameliorating mucositis associated with other anti-cancer regimens like chemotherapy following model validation. Our findings also suggest that engineered *S. boulardii* may also hold promise as a treatment for inflammation-associated CRC, but we must identify a targeting ligand to improve colonization.

Introduction

- 10 million deaths are attributed to cancer (1). Nearly 40% of Americans will be diagnosed with some form of cancer resulting in economic costs of upwards of $21 billion (2).
- Chemotherapy is a common cancer treatment that involves systemic infusions of antibodies targeting proinflammatory cytokines (3). The resulting immunosuppression increases the risk of opportunistic infections, among other side effects such CIM (4).
- CIM causes inflammation, apoptosis, ulceration, mucosal damage, and severe diarrhea (5). Irinotecan hydrochloride is a chemotherapeutic agent used to treat advanced CRC and its active form has been associated with CIM (6).
- Current treatments for CIM are supportive and focus on controlling existing symptoms (7).
- Probiotic supplementation has also been studied as an adjunctive therapy for IBD but has not been successful in inducing remission (8).
- The inflammation characteristic of IBD increases IBD patients' risk of developing CRC (9).
- Engineered probiotic organisms are an emerging treatment modality for CIM and CRC in part because of the local rather than systemic treatment that targets ulcerated tissue and tumors, respectively, without causing systemic side effects (10, 11).
- We hypothesize that an engineered *Saccharomyces boulardii* will mitigate symptoms of CIM in a mouse model of mucositis and decrease inflammation and tumor burden in a mouse model of CRC.

Study Design – Aim 1

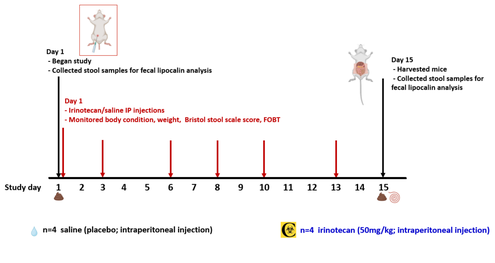

Figure 3: The FVB model for mucositis. For cohort 2, the model was extended by one week and mice received three extra doses of IP injections. For cohort 3, mice also received 150μL *E. coli* LF82 treatment via oral gavage on days that IP injections were administered. Created with BioRender.com.

Results – Aim 1

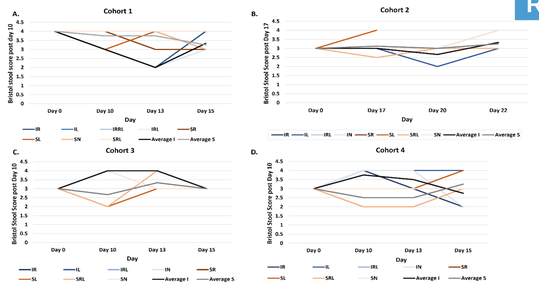

Figure 8: Bristol stool scores over time for the irinotecan-induced mucositis model. No significant change was seen in stool conditions for all cohorts of mice over time indicating that irinotecan did not effectively induce diarrhea.

Study Design – Aim 2

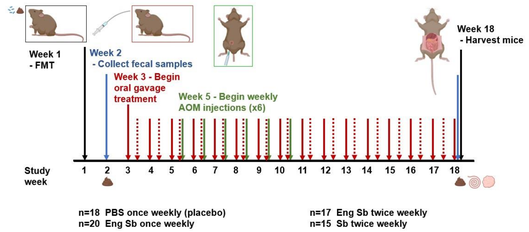

Figure 4: The azoxymethane (AOM)/*Il10*$^{-/-}$ model for colitis-associated cancer. FMT=fecal microbiota transplant, PBS=phosphate buffered saline, Sb=unmodified *S. boulardii*, Eng Sb=engineered *S. boulardii*. Created with BioRender.com.

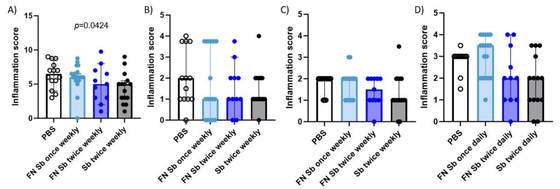

Figure 12: Histologic inflammation scores by colonic region for (A) total colon, (B), proximal colon, (C) mid colon, and (D) distal colon. Median total inflammation scores were lower in both treatment groups that received *S. boulardii* twice weekly, regardless of engineered properties (p=0.0424 for overall effect). Median regional inflammation scores were highest in the distal colon, but there were no significant differences between any treatment group and the placebo group within the colonic region.

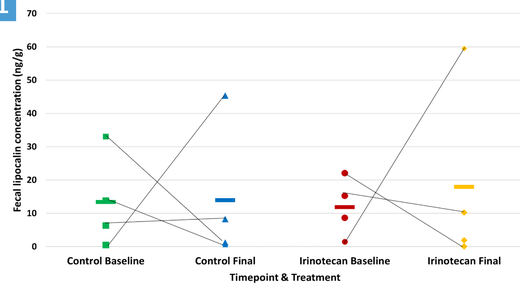

Figure 9: Cohort 1, Fecal lipocalin-2 concentration change over time. No significant difference in fecal lipocalin-2 concentrations was observed between experimental and control groups over time indicating that irinotecan was ineffective in inducing inflammation.

Results – Aim 2

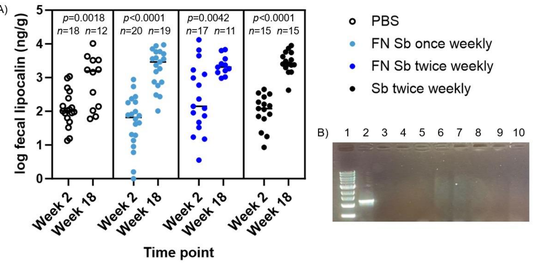

Figure 11: (A) Fecal lipocalin concentrations over time. All treatment groups had significant increases in fecal lipocalin concentrations over time indicating the progression of inflammation. **(B) Representative agarose gel demonstrating the absence of Helicobacter in samples.** *Helicobacter hepaticus*, an enterohepatic murine pathogen increases AOM-induced colon tumor incidence.

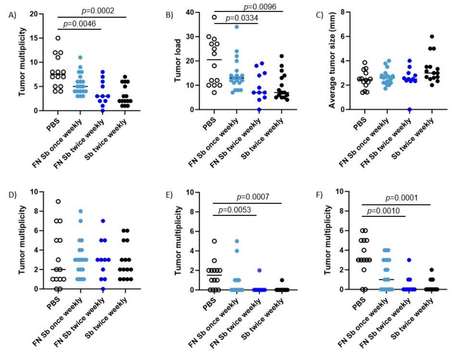

Figure 14: Tumor multiplicity, size, and load by treatment group. (A) total colon tumor multiplicity, (B) total colon tumor load, (C), total colon average tumor size, (D) proximal colon tumor multiplicity, (E), mid colon tumor multiplicity, (F) distal colon tumor multiplicity. *S. boulardii* decreased overall colon tumor multiplicity and tumor load in a dose-dependent manner. Most tumors among all treatment groups were in the proximal region (181). When analyzed by region, *S. boulardii* decreased overall tumor multiplicity in the mid and distal colon.

Methods

- For aim 1, four cohorts of 8 mice were used for model validation. Cohort 1: Enterobacteriaceae-free mice over a 15-day model, Cohort 2: Enterobacteriaceae-free mice over a 22-day model, Cohort 3: Enterobacteriaceae-free mice colonized with supplemental *Escherichia coli* LF82 via oral gavage over the 15-day model, and Cohort 4: Enterobacteriaceae-harboring mice over the 15-day model.
- Four mice were assigned to the experimental cohort which received intraperitoneal injections of 50mg/kg irinotecan.[14] Four mice were assigned to the control group which received 50mg/kg of 0.9% saline. Mice received injections three times a week.
- Qualitative body scoring and stool scoring were recorded using the Body Condition Scoring of Mice Scale and the Bristol Stool Score Scale, respectively.
- Stool samples from each mouse were collected following each dose of treatment to test for fecal occult blood using Beckman Counter Hemoccult Sensa slides.
- An additional set of stool samples was collected for fecal lipocalin-2, a non-invasive marker of intestinal inflammation, via Lcn2 ELISAs (R&D Systems, manufacturer #DY-1857-05).
- For aim 2, germ-free 129/SvEv *IL10*$^{-/-}$ mice were aged to 8-10 weeks and subsequently colonized with a specific pathogen free fecal microbiota transplant.
- Yeast cultures were grown overnight in YPD broth with penicillin (100U/mL) and streptomycin (100ug/mL), centrifuged, and pellets were resuspended in 0.5 volumes of PBS.
- Oral gavage was performed with 150uL PBS (placebo) versus 150uL yeast suspension with dosing ranging from 6×10^{9} 6 to1.3×10^{11} colony forming units.
- Azoxymethane was delivered via intraperitoneal injection at a dose of 10 mg/kg.
- Upon harvest, colons were flushed, rolled into Swiss rolls, and fixed in 10% formalin for ~48 hours before sectioning. A small piece of both proximal and distal colon was removed before fixation and snap frozen for later RNA extraction.
- Fecal lipocalin concentrations were determined by ELISA (R&D Systems #DY1857-05). Tissue RNA was extracted using the Qiagen RNeasy mini kit (#74106), cDNA synthesized using the Maxima first stand cDNA synthesis kit (ThermoScientific, #K1672), and qPCR performed using SYBR Green (Genese #17-505B).

Discussion

- We were unable to establish mucositis in the FVB model across all outcomes contrary to established literature. Therefore, we were unable to test our hypothesis that *S. boulardii* would mitigate symptoms of CIM.
- Future steps may include increasing irinotecan dosage and/or frequency or changing the animal model.
- There was successful validation of the (AOM)/*Il10*$^{-/-}$ model.; however, this is an immune-driven inflammation model that is more representative of IBD pathophysiology.
- *S. boulardii* may decrease inflammation mediated CRC as shown with both reduction in inflammation and CRC.
- There was a discrepancy between the location of the inflammation and the location of the tumors. This may be attributed to the overall gut microbial community composition.
- *S. boulardii* decreased tumor burden and inflammation regardless of engineered properties due to similar colonization efficiencies.
- Exploring other cell surface markers, secreting a different nanobody, or secreting antagonists of immunosuppressive proteins warrants investigation.
  The engineered yeast with its immunosuppressive properties may not increase tumor invasion and appears to be safe against an industry standard.

Conclusions

- Irinotecan did not induce significant mucositis symptoms in the FVB mucositis model. Extending the experimental duration and introducing Enterobacteriaceae did not alter this outcome.
- Model validation may require adjustments to irinotecan dosage or frequency and highlighting the importance of considering model-specific factors in experimental design.
- *S. boulardii* reduced inflammation and tumor burden but did so independent of any engineered properties suggesting a need to improve the engineered probiotic's ability to target the affected tissue.
- The discrepancy between the location of inflammation and tumor development suggests a complex underlying mechanism highlighting the need for future investigations into other cell surface markers or antagonists of immunosuppressive proteins.
- Engineered *S. boulardii* does not worsen cancer outcomes. Even if used to reduce inflammation in IBD patients, we expect it will not increase their risk of developing cancer.

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