I. SIGNIFICANCE

Just as penicillin was a life-saving drug to combat infections at the turn of the century, food fortification was critical in preventing life-threatening disease and fetal deformities. Despite these discoveries, we are now plagued with both antibiotic-resistant 'superbugs' [1,2], and our low-fiber Western diet is further increasing mortality and disease risk [3,4]. **We hypothesize that precision dietary prebiotic fibers may prevent or reduce deadly infections and improve cancer outcomes.** Dietary fiber is critical to support the health of the bacteria in the gut, the microbiome, especially microbes which possess the ability to evict pathogens [5]. Deaths from pathogen infections accounts for approximately 700,000 death each year globally, with 23,000 U.S. deaths attributed to antibiotic resistant bacteria [1]. Among the major culprits are *C. difficile,* which is related to approximately 15,000 deaths per year in the U.S., and enterotoxic *B. fragilis* (ETBF), which is a major cause of hospital-related infections. The standard treatment for these infections is a combination of antibiotics, however, as we run out of antibiotics to combat these 'superbugs', we must look for alternatives and preventative strategies, which has ignited a new effort to develop pre- and probiotics to address this critical situation. Currently, we have very little understanding of *which types* of dietary fibers support those microbes that convey protection from infection nor the mechanisms by which specific dietary fibers are protective [6-8]. To address this issue, we have designed a multi-phase strategy to develop precision medicine prebiotics by **utilizing a new tool the Mini-Bioreactor Array system (MBRA)[9].** This new system will allow us to test dietary fibers in a controlled gut microbiome community setting. The purpose of this research is to identify the prebiotics and microbial factors conveying resistance to infection while accounting for inter-individual microbiome variability. Indeed, a recent study showed a 5-fold improved response to cancer immunotherapy with a high fiber diet, which supports our focus on cancer treatment [10].The results from our initial study (Phase I) will allow us to pursue a multi-phase research strategy (**Fig. 1**) designed to address an outstanding need in our field to identify critical microbes and metabolites that are altered as a result of exposure to dietary fibers and pathogens. *Ultimately, this evidence will expand our understanding of the fiber-microbiome relationship, and allow us to develop preventative interventions using prebiotics*. (**Initiative=Health/Data Science)**.

INNOVATION: **1)** Application of the *MBRA system* to study diet-microbiome interaction, as well as, drug-microbe, microbe-microbe, or nanomaterial-microbe interaction in a controlled environment **(Initiative=Health)**. **2)** Innovative use of microbial populations to study dietary fibers through *metabolic modeling* **(Initiative=Data Science/Health)** and their effects over time on resilience, stability, and resistance to pathogens. **3)** Development of a precision medicine prebiotic; a) **Phase I,** will set the stage for several more powerful studies **(Initiative=Health)**: b) **Phase II -** *in vitro* studies to investigate the differential response to dietary fibers between stool communities from healthy individuals and those with colon cancer, and c) **Phase III -** *in vivo* studies to determine the response to fiber-treated microbiota in healthy and cancer models.

IMPACT: The NIH released its draft Strategic Plan for Nutrition Research; this research will position Baylor to take advantage of these funding opportunities. Specifically, our research addresses 8 priorities (**see Appendix).** *Results from this pilot data will be used to develop a large (R01) multi-phase strategy (**Fig. 1**) that includes multiple study inputs and outputs poised to address questions regarding host-microbe and fiber-microbe interactions.* Thus, this study, in combination with future studies, has the potential to lead to development of precision medicine prebiotics for infection resistance and improvement of cancer treatment.

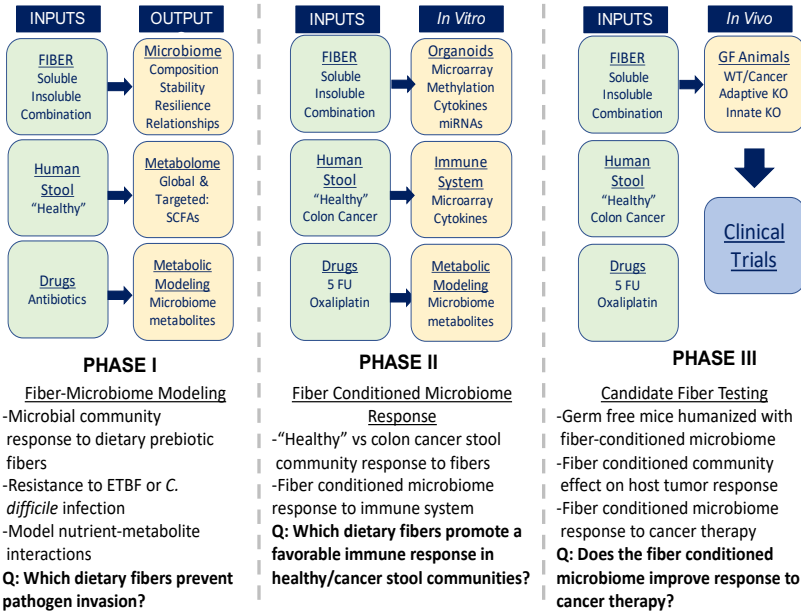

Fig. 1. Three Phase Strategy for Developing Precision Medicine Prebiotics. Phase I is described in this proposal.

II. RESEARCH QUESTIONS ADDRESSED

This proposal seeks to illuminate the dietary fiber-microbiome relationship using a **powerful new tool - fecal mini-bioreactor arrays (MBRAs)**[9]. First used to study *Clostridium difficile* infection, the MBRA allows for continuous cultivation of complex fecal microbial communities to study infection [9]. Using the MBRA system, developed by **Co-PI Dr. Robert Britton**, we seek to develop a prebiotic that is capable of preventing or reducing infection, focusing initially on the effects of two types of fermentable fiber, inulin and resistant starch in Phase I of this research strategy (**Fig. 2**).

Studies indicate that fermentable dietary fibers (prebiotics) are paramount in maintaining a healthy microbiome that is resistant to disease, including infection and cancer [8,11]. Little is known; however, about *which types* of dietary fiber are responsible for these protective effects nor their mechanisms [12]. With the global prebiotics market size anticipated to reach $7.91 billion by 2025, we are facing a critical need to move beyond our current 'one size fits all' application of dietary fiber to that of a tool for precision medicine [13]. The bacteria in the gut, collectively termed the microbiome, is significantly affected by our diet [14]. Our understanding of how this dietary fiber-microbiome relationship impacts response to infection however remains rudimentary, in particular with respect to the effect on the microbiome. Dietary fiber is one of the main sources of carbohydrates the microbiome uses for fuel in the production of short chain fatty acids (SCFAs), which supports gut barrier function [8,15]. Without fiber, the gut bacteria feed off the mucus lining the gut, leaving the host susceptible to infection and disease, including cancer [11,13,15] .

Our long-term goal is to develop prebiotics for precision medicine using the MBRA system in our multi-phase strategy (**Fig. 1**). The goal of this proposal (**Fig. 2**) is to operationalize the MBRA system by testing two types of dietary fibers in healthy stool communities, and their ability to resist pathogen colonization**.** We are choosing to focus on two of the most common dietary fibers, insulin and resistant starch, because they have consistently demonstrated the ability to favorably modify the gut microbiome to produce SCFAs [7,16]. Additionally, by establishing this new tool, **we will be able to train graduate and undergraduate students in using the MBRA** system (**see Appendix)**, which will give them critical skills to enhance learning and the ability to be competitive for fellowships or jobs in the STEM fields. To meet these goals, we have developed the following specific aims:

A.1. Specific Aim 1: Quantify changes in model stool community composition and fatty acid metabolism after exposure to inulin or resistant starch. The gut microbiome is reliant upon dietary fiber for nutrients, which in turn supplies energy for the intestinal epithelial cells. Data show that lack of dietary fiber results in dysbiosis, changes in metabolites (e.g. SCFAs), and increased disease risk [15,17]. However, human dietary fiber intervention studies with inulin or resistant starch each show different effects on the microbiome and SCFA production [18-21]. *Thus, we hypothesize that the gut microbiome composition and SCFA production will be differentially altered in response to inulin or resistant starch, which will be dependent upon the donor microbial stool community supplied.*

A.2. Specific Aim 2: Measure the ability of fiber-conditioned stool communities to resist pathogen colonization. The ability of microbial communities to resist pathogen colonization involves mechanisms that are, in part, reliant upon fermentation of prebiotic fibers to produce SCFAs. Specifically, commensal *Bacteroides spp.* confer colonization resistance again *Salmonella* infection through the production of the SCFA propionate [22]. Further, the combinations of prebiotic fibers with the probiotic *Lactobacillus reuteri* 1063 prevents colonization of the pathogenic strain of *E. coli* by limiting mucus adhesion [23]. *Thus, we hypothesize that inulin and resistant starch will confer differential colonization resistance to the pathogens, enterotoxic B. fragilis (ETBF), and C. difficile.*

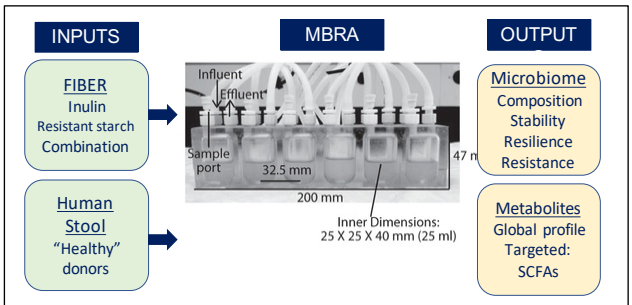

Fig. 2. Study Design Schema for Phase I.

Our research plan is supported by a highly-experienced team of scientists at Baylor University, Baylor College of Medicine, and Mayo Clinic, with expertise in microbiology, metabolic modeling, and metabolite analysis [24-28]. Completion of this research will provide a tool (MBRA) to **enhance the current space available** and allow recruitment of students and faculty of high research caliber. This research will also provide the foundation for an externally funded research program dedicated to elucidating the dietary fiber-microbiome relationship. *These findings will impact our field by not only answering outstanding questions that remain with regards to microbiome resilience to infection, but also lead to novel prebiotic interventions to prevent or reduce infection.* ***Our research will benefit Baylor by providing cutting-edge tools, collaboration, student training, and a sustainable research focus that can be used to make Baylor a leader in prebiotics and infectious disease research.***