Development of a Novel Synthetic Glycan to Prevent Bacterial Infections and Ameliorate Respiratory Viral Infections

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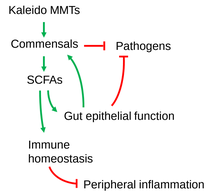
INTRODUCTION

- The prevention and treatment of bacterial infections is a significant challenge to human health, and broad use of antibiotics is driving an increase in multidrug-resistant (MDR) pathogens.
- Antibiotic-resistant bacteria such as carbapenem-resistant *Enterobacteriaceae* (CRE) and vancomycin-resistant *Enterococcus* (VRE) are an increasing concern in hospitalized patients worldwide. Colonization of the gut by these antibiotic-resistant pathogens often precedes systemic infection.
- In addition to selecting for resistance, another disadvantage of antibiotics is that they often kill beneficial commensal bacteria in addition to their pathogenic targets. Indiscriminate killing disrupts the homeostasis between commensal bacteria and the host gut epithelium, allowing colonization of the gut by pathogenic bacteria, and increases susceptibility to infections.
- This research was done to develop a non-antibiotic modality to reduce pathogen colonization by selectively growing, rather than killing, commensal bacteria in the gut. This modality represents an antibiotic-sparing approach to infectious disease.
- Commensal bacteria grown on carbohydrates produce short-chain fatty acids (SCFAs) that support the maintenance of gut homeostasis and promote resistance to pathogen colonization.
- SCFAs have direct and indirect effects on the gut and lung mucosal immune system. They have also been linked to respiratory viral infection reduction and shown to influence macrophage function to mitigate pro-inflammatory neutrophil-mediated tissue damage.

MMT PROPOSED MECHANISM OF ACTION (MoA)

FIGURE 1. PROPOSED MoA



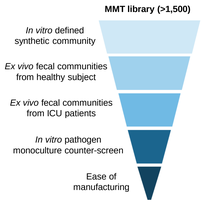
- Microbiome Metabolic Therapies (MMTs™) selectively support the growth and metabolism of commensal bacteria (Fig 1).
- Commensal growth consumes nutrients and creates a competitive ecosystem that leads to the exclusion of pathogenic bacteria. Commensals can also produce antimicrobial factors and transform host-derived bile acids to further limit the growth of pathogens.
- The metabolic products of MMT utilization, particularly SCFAs, stimulate the gut epithelium to exert colonization resistance on pathogens through the production of antimicrobial factors.
- SCFAs also promote immune homeostasis by stimulating the differentiation and function of regulatory T cells.
- These beneficial immune responses can dampen inflammation both locally and peripherally.

METHODS

- A library of over 1,500 synthetic proprietary glycans, termed MMTs, was synthesized using a wide array of chemical and enzymatic approaches (Fig 2).
- An *ex vivo* platform using fecal bacterial communities from human subjects and patients with a variety of diseases was devised to screen MMTs for their abilities to deplete pathogenic bacteria and enrich commensal bacteria. Changes in the taxonomic composition of fecal communities, including pathogen depletion and commensal enrichment, were measured using 16S rRNA gene sequencing according to the 16S Earth Microbiome Project protocols.
- This platform was also employed to screen for the ability of MMTs to modulate multiple aspects of bacterial metabolism, e.g. ammonia production, SCFA production, and bile acid conversion.
- In addition to the *ex vivo* platform, *in vitro* bacterial monoculture growth assays were employed to examine the responses of individual bacterial strains to MMTs. This *in vitro* approach was combined with an dbCAN2 CAZyme genome analysis to understand how carbohydrate utilization genes vary across taxa of interest.

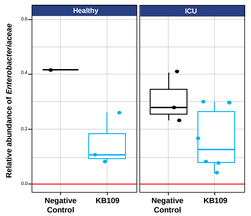
RESULTS

Figure 2. KB109 identification



- KB109 was identified using a high-throughput screening cascade involving both *in vitro* and *ex vivo* methodologies.

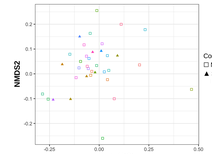
Figure 3. KB109 reduced pathogenic bacteria in fecal communities in an *ex vivo* system



- Fecal communities from different healthy subjects and ICU patients were spiked with CRE and then incubated for 45 hours without (negative control) and with KB109.

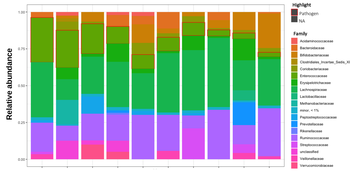
RESULTS (CONT'D)

Figure 4. Taxonomic ordination of fecal communities from healthy subjects in Kaleido collection



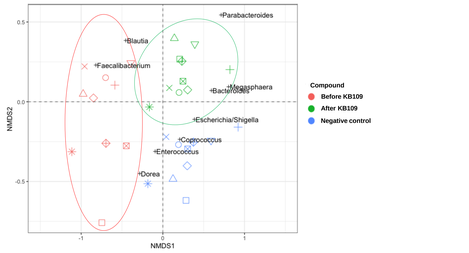
Bray-Curtis NMDS of genera OTU table. Distance of selected samples >0.3 (replicate sample max distance).

Figure 5. Taxonomic composition of fecal communities selected for testing



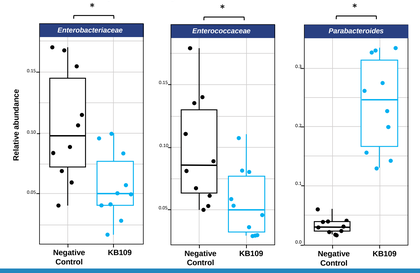
- Fecal communities were selected from an in-house library of healthy stool samples based on their continuity (i.e. previously used, or future planned usage in assays) for further *ex vivo* testing with KB109.
- The selected communities represent taxonomic diversity across a set of fecal communities from healthy subjects from the Kaleido collection (Fig 4).
- The communities can be distinguished by their relative composition of bacterial families (Fig 5). VRE (*E. faecium* ATCC 700221) was spiked into samples prior to use and are highlighted (*Enterococcaceae*) in red.

Figure 6. Incubation with KB109 resulted in a distinct shift in the composition of a diverse set of fecal communities



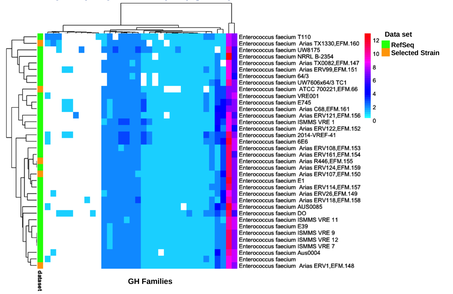
- VRE spiked fecal communities from different healthy subjects (Fig 5) were incubated for 45 hours without (negative control) and with KB109. Different communities (marker, Fig 6) showed clear compositional shift, including a depletion of pathogens and enrichment of commensals (Fig 7).

Figure 7. KB109 consistently depleted pathogens *(Enterobacteriaceae and Enterococcaceae)* and enriched commensal *Parabacteroides* across these compositionally diverse fecal communities



*P<0.01 MWU paired.

FIGURE 8. Glycosyl hydrolase (GH) gene profiles across *E. faecium* strains



- To ensure that KB109 did not promote the growth of pathogenic bacteria, we sought to confirm that pathogenic bacteria lacked the ability to utilize KB109 as a carbon source.
- Using functional genomics, we selected a subset of *Enterococcus faecium* strains that represented the diversity of glycosyl hydrolase gene profiles (Fig 8). These strains were tested in an *in vitro* monoculture assay (Fig 9).

FIGURE 9. KB109 did not support the growth of a diverse set of antibiotic-resistant *E. faecium* strains in an *in vitro* monoculture assay

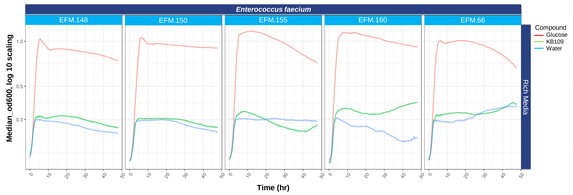
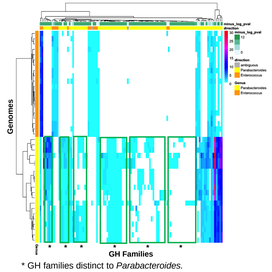


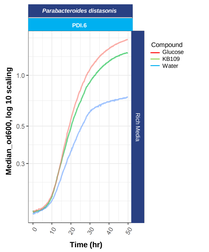
Figure 10. Genomics heatmap of commensals and pathogens



* GH families distinct to *Parabacteroides*.

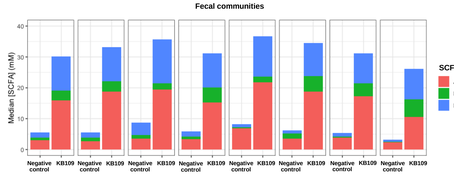
- *E. faecium* has a narrow set of GHs compared with *Parabacteroides*.
- *Parabacteroides* has many GHs that *E. faecium* lacks.

Figure 11. KB109 supports growth of *Parabacteroides distasonis*



- KB109 may exploit the limited ability of pathogens to utilize complex oligosaccharide MMTs that are readily utilizable by commensal bacteria.

FIGURE 12. SCFA production in fecal communities



- KB109 consistently increased the production of the SCFAs acetate, propionate, and butyrate in each of the fecal communities tested.
- SCFAs support the maintenance of gut homeostasis and promote resistance to pathogen colonization, as well as stimulate immune homeostasis and dampen inflammation.

CONCLUSIONS

- KB109 depletes antibiotic-resistant pathogenic bacteria by enriching commensal bacteria of genus *Parabacteroides* in an *ex vivo* system designed to model the human gut microbiome.
- In addition to shifting the gut microbiome taxonomic composition, KB109 also changes the gut microbiome metabolic output. This change results in increased production of SCFAs.
- SCFAs have been shown to promote healthy gut epithelial function, support immune homeostasis and dampen inflammation locally in the gut, as well as peripherally in the lungs.
- This novel synthetic glycan has an appealing activity profile and offers an opportunity to promote gut homeostasis and colonization resistance, and supports immune health.
- Future research will focus on identifying and understanding the enzymatic machinery responsible for MMT utilization by commensals.
- KB109 is also a promising candidate for stimulating immune homeostasis. KB109 is under evaluation in two COVID-19 clinical studies, K031 (NCT04414124) and K032 (NCT04486482).

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