Identification of Novel Glycans That Target Gut Microbiota-Associated Ammonia Production

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INTRODUCTION

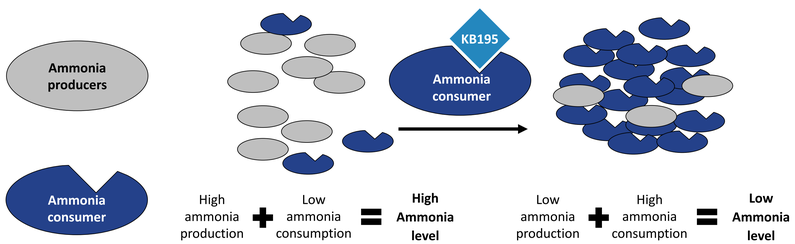
The gut microbiome plays a significant role in the production and consumption of ammonia, which is central to the pathogenesis of hepatic encephalopathy (HE). Existing therapies, such as lactulose, reduce blood ammonia levels but are poorly tolerated.

We sought to develop Microbiome Metabolic Therapies (MMT™) that are synthetic, novel oligosaccharide compositions (glycans) to reduce net ammonia production by the gut microbiome with good tolerability. MMTs are related to a class of compounds that is "Generally Recognized as Safe," or GRAS, or are determined to be GRAS, based on their history of safe human exposure when utilized for particular uses as food substances, and are commonly accepted as safe by regulators for use in food. This classification allows for the collection of early human data; for HE this human data is collected in a nitrogen metabolism model system.

An *ex vivo* screening platform was established to collect data on the effect of glycans as MMTs. Via this platform, fecal samples from healthy human microbiomes were incubated with more than 300 glycans. Levels of microbiota-derived metabolites, including ammonia, were measured.

One of the top performing glycans from the screen was KB195. One proposed mechanism of action for KB195 is to change the composition of bacterial species in the gut to modulate levels of metabolites including ammonia (Figure 1).

FIGURE 1. ONE PROPOSED MECHANISM OF ACTION FOR KB195



OBJECTIVES

The objectives of the study were to assess the ability of KB195 to

- Reduce ammonia levels in fecal microbiome samples from patients with HE in an *ex vivo* assay.
- Reduce ammonia levels in a clinical food study in healthy human subjects. Safety and tolerability were also evaluated.

METHODS

***Ex vivo* assay**

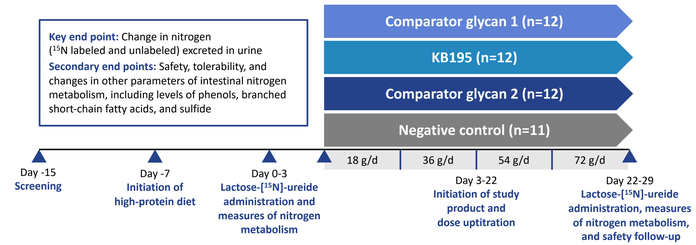
- KB195 and 9 other glycans were tested in an *ex vivo* assay with 19 fecal microbiome samples from patients with hepatic impairment.

Clinical food study

- A controlled, randomized, double-blind clinical food study of KB195 was conducted in healthy human subjects.
- Subjects were between 18 and 50 years of age, with a BMI of 20 to 40 and no history of GI conditions (inflammatory bowel disease, irritable bowel syndrome, GI malignancy) or GI-related medications or supplements (including probiotics, fiber supplements, and bismuth).
- As perturbations in blood ammonia after protein challenge are difficult to measure in healthy subjects, a model system using a lactose-[^{15}N]-ureide tracer was used. After screening and initiation of a high-protein diet, subjects were given a standard oral dose of lactose-[^{15}N]-ureide for bacterial nitrogen metabolism. In this model, ^{15}N excretion in the urine was a surrogate marker of nitrogen metabolism in the gut; a reduction in urinary ^{15}N is consistent with a reduction in net ammonia production by the gut microbiome.
- Subjects were administered KB195 (n=12) or 1 of 3 comparator compounds: comparator glycan 1 (n=12), comparator glycan 2 (n=12), or negative control (n=11). Subjects were again given lactose-[^{15}N]-ureide at the end of the 21-day study period (Figure 2).

METHODS (CONT)

FIGURE 2. STUDY DESIGN

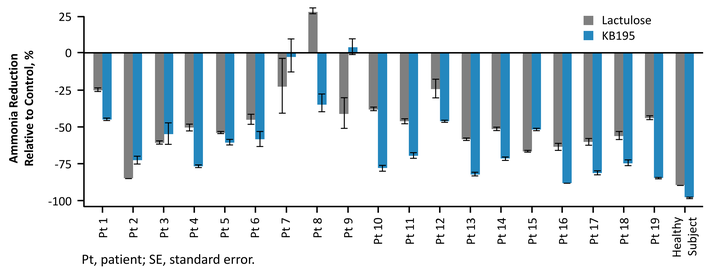


Safety was reported as treatment-emergent adverse events (TEAEs). In addition to standard adverse event reporting, we used sensitive measures to assess GI tolerability including the GI tolerability questionnaire (GITQ) and the Bristol Stool Scale (BSS). The GITQ assesses the severity and frequency of GI symptoms, including nausea, vomiting, abdominal cramping, gas/flatulence, and reflux, on 4-point scales (range is 0 to 64). Two additional questions were added to this questionnaire to assess frequency and urgency of bowel movements. Subjects were expected to complete the questionnaire daily from 1 to 3 days after screening until the end of the study. The BSS assesses stool consistency (range is 0 to 7; scores of 6 or 7 were scored as diarrhea); subjects scored all bowel movements according to the scale for the duration of the study.

RESULTS

In the *ex vivo* assay with 19 fecal microbiome samples from patients with hepatic impairment, KB195 reduced ammonia levels in 95% (18/19) of samples, and in 74% (14/19) of samples, KB195 resulted in greater reductions in ammonia levels than lactulose (Figure 3).

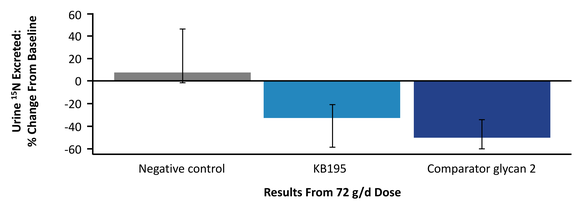
FIGURE 3. AMMONIA LEVELS: KB195 VS LACTULOSE (MEAN ± SE)



Pt, patient; SE, standard error.

Forty-seven subjects were randomized in the clinical food study. The study population was 49% male and 77% white, with a mean age of 35 years and a mean body mass index of 27. All 47 subjects completed the study. The study also included an additional comparator glycan arm (comparator glycan 1). Operational challenges resulted in interruptions in the glycan 1 dosing schedule. As such, the incomplete glycan 1 arm data are not reported. After ingestion of KB195, subjects showed a decrease in ^{15}N excretion vs the negative control (P=.0126; Figure 4), independent of the microbiome structure at baseline.

FIGURE 4. DECREASE IN ^{15}N EXCRETION (KB195 VS NEGATIVE CONTROL)



RESULTS (CONT)

Overall there were no safety signals (Table 1). Most TEAEs were mild in severity; no subject discontinued due to a TEAE. Of the 3 subjects who reported moderate TEAEs, none were deemed related to study product. All adverse events resolved or were improving over the 7-day follow-up period after the end of study product consumption. There were no clinically relevant abnormal laboratory values.

TABLE 1. TEAEs IN THE CLINICAL FOOD STUDY

	KB195 (n=12)	Comparator glycan 2 (n=12)	Negative control (n=11)	Overall (N=35)
Total number of TEAEs, No.	17	35	10	62
Subjects reporting ≥1 TEAE, No. (%)	4 (33.3)	6 (50.0)	4 (36.4)	14 (40.0)
Maximum TEAE intensity, No. (%)				
Mild	3 (25.0)	6 (50.0)	7 (63.6)	16 (45.7)
Moderate	2 (16.7)	1 (8.3)	0	3 (8.6)
Severe	0	0	0	0

Tolerability of KB195 was comparable to that of negative control at all doses according to both GITQ (Figure 5) and BSS (Figure 6). Treatment with KB195 resulted in fewer subjects reporting diarrhea compared with comparator glycan 2.

FIGURE 5. GI TOLERABILITY (GITQ)

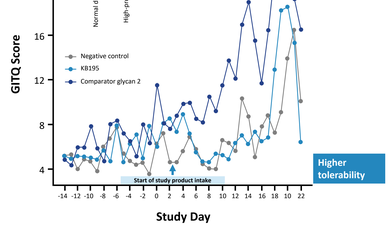
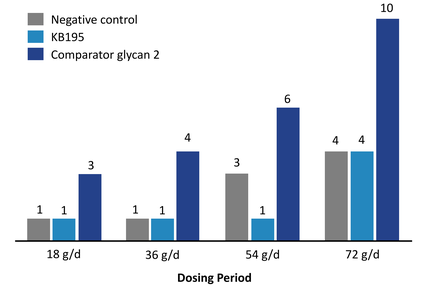


FIGURE 6. NUMBER OF SUBJECTS REPORTING DIARRHEA (BSS)



CONCLUSIONS

An *ex vivo* screening in human microbiome samples was effective in identifying an MMT that showed a significant effect on ammonia reduction. These results were confirmed in a clinical food study of healthy subjects, where net ammonia production was decreased relative to a control glycan, consistent with the effect of lactulose. KB195 was well tolerated, with mostly mild adverse events. Subject-reported tolerability was similar to that with negative control.

These studies with KB195 have informed our understanding of the activity and tolerability of MMTs in the reduction of ammonia levels. Future clinical studies aim to show improved management of patients with hyperammonemia, including those with urea cycle disorders and hepatic encephalopathy. A phase 2 clinical trial in patients with urea cycle disorders will assess the ability of KB195 to achieve the primary endpoint of ammonia reduction. A clinical food study to assess the effect of another MMT (KB174) on reducing ammonia levels in patients with well-compensated cirrhosis is also underway (NCT03855956).

ACKNOWLEDGEMENTS AND REFERENCES

Acknowledgements: Ruth Thieroff-Ekerdt and Anastasia Murphy contributed to the design and conduct of the study. ETHOS Health Communications provided editorial support.

Reference: Data on file. Kaleido Biosciences Inc.

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