composition in non-alcoholic fatty liver disease (NAFLD) patients.

Methods This is a randomized, double-blind, controlled clinical trial. Twenty-nine patients were randomly assigned into probiotics and placebo groups. Multi-strain probiotics containing six different *Lactobacillus* and *Bifidobacterium* species at the concentration of 30 billion CFU were administered during the 6-month study. Anthropometric measurements like body mass index (BMI) and waist circumference (WC) were recorded. The duodenal biopsies from pre-and post-intervention were analyzed for microbiota compositions using amplicon sequencing based on the V3 region of 16s rRNA.

Results The average mean age of recruited patients was 52 ± 13 years. Both groups displayed the same BMI and WC at the baseline and after the intervention. The 16s rRNA analysis revealed three main prokaryotic phyla, namely Actinobacteria, Proteobacteria, and Firmicutes, with genera *Streptococcus*, *Methylobacterium*, *Cutibacterium*, and *Prevotella* being particularly common after intervention for both groups. Beta-diversity analysis of the probiotics group disclosed a significant change of gut microbiota composition upon intervention procedures ($p < 0.05$). Notably, the probiotics group also presented a decrease of Firmicutes/Bacteroidetes (F/B) ratio upon 6-month of intervention. Conversely, the placebo group illustrated a stable diversity upon intervention procedures as Alpha and Beta-diversity analyses showed no significant difference.

Conclusions We anticipated a potential pattern of dysbiosis among NAFLD patients through the high prevalence of *Streptococcus* in core microbiome analysis for both groups. Interestingly, we managed to obtain a decrease in Firmicutes/Bacteroidetes ratio in the probiotics group which suggested a balance of microbial compositions through the presence of *Lactobacillus* and *Bifidobacterium* species. Hence, probiotics could be adopted as a potent preventive strategy in NAFLD patients

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METABOLIC INHIBITOR OF PFKFB3 AND ITS IMPLICATION IN IMMUNE EVASION BY UPREGULATING PD-L1 EXPRESSION VIA THE PHOSPHOPFKFB3-HIF1A-PD-L1 AXIS

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Background PFKFB3 is a crucial metabolic enzyme which is highly upregulated in cancer cells, prompting much research on potential selective inhibitors of PFKFB3 which all have shown promising tumor inhibitive effects. However, clinical trials such as NCT02044861 on PFKFB3 inhibitors seem resultless. With recent implications of cancer metabolism and tumor immune evasion, we hypothesized that PFKFB3 inhibition and subsequent metabolic reprogramming might affect tumor immune evasion.

Methods We examined the function of PFK-15 on tumor cells in vitro and in vivo in immune-deficient nude-mice models and immune-competent mouse models such as C57/BALB/c-CDX models and huPBM-CDX and PBX models. CO-IP, fluorescent immunohistochemistry, CHIP and dual-luciferase assays were used to investigate the underlying mechanism.

Results Inhibition of PFKFB3 using PFK-15 suppressed cell growth in human esophageal cell lines in vitro and in vivo in immune-deficient xenografts. However, inhibition of PFKFB3 caused a marked upregulation in PD-L1 which inactivated cocultured T-cells in vitro and compromised anti-tumor immunity in immune-competent mouse, an effect which could be reversed when PFK-15 was combined with PD-1 mab. Mechanistically, we identified ERK pathway upregulation after treatment with PFK-15, which increased PFKFB3 phosphorylation levels, causing its transformation and increased its binding with HIF-1 α , then Phospho-PFKFB3 co-translocated into the nucleus together with HIF-1 α by binding with importin α 5, whereby nuclear HIF-1 α attaches itself to HRE regions on PD-L1 promoter, upregulating PD-L1 expression. Clinically, we observed higher Phospho-PFKFB3 in tumor tissues from non-responders to PD-1 mab treated ESCC patients.

Conclusions This study shows that inhibition of PFKFB3 increases immune evasion via the phospho-PFKFB3-HIF-1 α -PD-L1 axis. The translational significance of this study lies in the fact that in-vivo experiments in immune-competent mice using the closest to human immune tumor microenvironment model huPBM-CDX/PDX combining PFKFB3 inhibitors and PD-1 mab resulted in a marked decrease in tumor development. These findings may be of relevance for the design of anti-cancer treatment trials with PFKFB3 inhibitors.

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THE EFFECT AND IMMUNE CELL ANALYSIS OF CLOSTRIDIUM BUTYRICUM ON DEXTRAN SULPHATE SODIUM INDUCED COLITIS IN MICE PRETREATED WITH ANTIBIOTIC COCKTAIL

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Background Inflammatory bowel disease is a chronic inflammatory disease characterized by recurrent abdominal pain and diarrhea, and gut microbiota is one of its causes. Probiotics, as one of the intervention methods of microbiota, play an important role in disease treatment. This study aims to observe the effect and immune mechanism of *Clostridium butyricum* MIYAIRI 588 (CBM) and its supernatant on colitis mice which pretreated with antibiotic cocktail (ABx).

Methods Twenty four Balb/c mice aged 6-8 weeks were randomly divided into four groups, and CBM and its supernatant was used to treat colitis mice which pretreated with ABx. The body weight, disease activity index (DAI) and colon length of mice were observed respectively. The relative expression of inflammatory cytokines in colon tissue was detected by qPCR. And the number of neutrophils, macrophages, Th1 and Th17 cells in lamina propria of mucosa was detected by flow cytometry to observe the changes of pro-inflammatory immune cells in mouse mucosa.

Results The DAI of mice were decreased, the shortened colon length was improved after CBM and its supernatant intervention (IDDF2021-ABS-0196 Figure1A). *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; DAI scores of mice after *C. Butyricum* gavaging with

Abstract IDDF2021-ABS-0196 Figure 1

antibiotic cocktail pretreatment at the end of experiment)(IDDF2021-ABS-0196 Figure1B. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The macroscopic images of colon)(IDDF2021-ABS-0196 Figure 1C. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The statistical analysis of colon length). The results of qPCR showed that the relative expression of pro-inflammatory cytokines TNF- α , IL-6, IL-1 β , IL-17a were down-regulated after CBM and its supernatant treatment. And the relative expressions of anti-inflammatory cytokines TGF- β , IL-10, IL-4, IL-5, IL-13 were up-regulated (IDDF2021-ABS-0196 Figure1F. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The amount of Th1 cells based on flow cytometry, and its statistical analysis). Flow cytometry showed that the amount of neutrophils, macrophage, Th1, Th17 cells were down-regulated which were the pro-inflammatory immune cells in intestinal mucosa lamina propria after CBM and its supernatant treatment (IDDF2021-ABS-0196 Figure1D. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The amount of neutrophil based on flow cytometry, and its statistical analysis) (IDDF2021-ABS-0196 Figure1E. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The amount of macrophage based on flow cytometry, and its statistical analysis) (IDDF2021-ABS-0196 Figure1F. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The amount of Th1 cells based on flow cytometry, and its statistical analysis) (IDDF2021-ABS-0196 Figure1G. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The amount of Th17 cells based on flow cytometry, and its statistical analysis) (IDDF2021-ABS-0196 Figure1H. *Clostridium butyricum* and its supernatant ameliorated colitis and regulated immune cells; The relative expression of inflammatory cytokines)

Conclusions CBM can alleviate the symptoms and colon shortening of mice with colitis after pre-treatment with ABx, improve the expression of inflammatory factors, and might play an anti-inflammatory role by down-regulating the infiltration of inflammatory immune cells in intestinal mucosa lamina propria.

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DELAYED INTERVENTION OF AGAROPECTIN-DERIVED OLIGOSACCHARIDES ALLEVIATE LIPID ACCUMULATION BY MODULATING INTESTINAL FLORA HOMEOSTASIS

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Background Polysaccharides from marine red algae possess a variety of biological activities. And, our previous study

illustrated that the agarpectin-derived oligosaccharides from *Gloiopeltis furcata* (SAOs) activated AMPK signaling pathway in vitro. However, the effects of SAOs on alleviating lipid accumulation in vivo and its underlying mechanism are not clear.

Methods C57BL/6J mice were randomly divided into five groups of ten mice each. Control group: mice were fed a normal chow diet. And, the other four groups were continuously fed an HFD for 24 weeks to establish the obesity model. After 24 weeks, the Metf, SAOs-L, and SAOs-H groups received either metformin (225 mg/kg/d dissolved in saline) or SAOs (100 or 300 mg/kg/d dissolved in saline), while the Control and Model groups were given aliquots of saline. Saline and the drugs were given by oral gavage for the final six weeks of the experimental period.

Results We found that SAOs decreased the body weight (about 3 grams) and the adiposity index (from 7 to 5) after a 6-week treatment. In addition, SAOs alleviated lipid accumulation in the liver, perirenal fat, and epididymal fat tissues. Investigation of the underlying mechanism showed that the cecal microbiota dysbiosis in HFD-fed mice were ameliorated after SAOs treatment, including significantly increasing the relative abundance of *Alistipes*, while reducing the relative abundance of *Helicobacter*. In addition, Spearman's correlation analysis indicated that changes in the cecal microbiota could regulate lipid accumulation, oxidative stress, inflammation.

Conclusions The present study confirmed that SAOs could effectively alleviate HFD-induced fat accumulation, partly through regulating cecal microbiota by fostering the preferential growth of probiotics and suppressing the relative abundance of harmful bacteria. In summary, our study illustrated that SAOs could be further developed as a potential pharmaceutical agent for obesity.

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CANAGLIFLOZIN ALLEVIATES DIABETIC CARDIOVASCULAR DISEASE VIA LIPID LOWERING, MITOCHONDRIAL HOMEOSTASIS, AND GUT MICROBIOTA REGULATION

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Background T2DM is associated with CVD. SGLT2i represent the most effective class of oral medications for the treatment of T2DM, and have been shown to improve generalized vascular dysfunction.

Methods T2DM was induced by HFD to male C57BL/6J mice for 24 weeks. After that, HFD-fed mice were treated with Cana (50 mg/kg/d) by oral gavage for 6 weeks. Myocardial mitochondrial morphology and cardiovascular abnormalities were assessed by TEM, immunofluorescence staining and Masson's staining; lipids profile and oxidative stress state were assessed by corresponding biochemical assay kits and immunohistochemical staining; the myocardial injury was characterized by H&E staining, TUNEL, and ELISA assays; colonic gut microbiota was assessed by 16S rDNA sequencing.