Supplemental material

Supplemental methods

Ginseng polysaccharide extraction

Extraction was performed according to a previously described method [1]. Dried *Panax ginseng* C. A. Mey. roots (300 g) were pulverized and extracted with hot water (2.4 L) under reflux for 2 h. The extraction was performed twice. The extract was concentrated in a vacuum and centrifuged at 3000 rpm for 30 min to remove insoluble substances. The supernatant was subjected to EtOH (80%) precipitation, and the precipitate was dissolved in water and then dialyzed against distilled water (cut off, 3.5 kDa). The non-dialysate was lyophilized to yield polysaccharides (yield 25%). The high-performance liquid chromatography (HPLC) chromatogram [Agilent 1200 HPLC-RID, Thermo Acclaim SEC 300 Å (5 μm, 7.8 × 300 mm), mobile phase 0.1 M $NaNO_3$] of the polysaccharides showed a major peak with a molecular weight of 627 kDa.

Isolation of intraepithelial lymphocytes IELs

Briefly, Peyer's patches (PPs) were removed; then, the small intestine was washed with PBS containing 1% FBS to remove the contents and cut into pieces of approximately 5 mm per piece. The intestines were then shaken with prewarmed predigestion solution containing HBSS (w/o), 10 mM HEPES, 5 mM EDTA, 1 mM DTT, and FBS for 30 min at 220 rpm and 37 °C twice. Supernatants were filtered with a 70 μm filter. The IELs were further purified by 20/40/70% Percoll density gradient, and the cells layered between the 40 and 70% fractions were collected as IELs.

RNA sequencing

Purified RNA libraries were constructed using a NEBNext® Ultra RNA Library Prep Kit and run on an Illumina machine (Illumina PE150). Raw data were processed and qualified. The reads were mapped, and the gene expression level was analyzed using HTSeq v0.6.1 and DESeq2 software to determine transcript abundance and the differentially expressed genes between samples. GO enrichment analysis was implemented with the cluster Profiler R package, in which gene length bias was corrected. GO terms with corrected p values less than 0.05 were considered significantly enriched with differentially expressed genes.

Antibiotic treatment

Mice were orally gavaged with antibiotics for 2 weeks before tumor inoculation and continued until the end of the experiment. The antibiotic cocktail was comprised of ampicillin (100 mg/kg, Maclin), vancomycin (50 mg/kg, Maclin), neomycin (100 mg/kg, Maclin), and metronidazole (100 mg/kg, Maclin). The antibiotic activity was confirmed routinely. Tumor growth evaluation was following the conventional mouse model.

Meta-fermentation of microbiota by an *in vitro* batch fermentation system

To investigate the effect of GPs on the microbiota, we performed a meta-fermentation analysis of the microbiota by an *in vitro* batch fermentation system. Using an *in vitro* batch fermentation system, fresh fecal samples from a healthy individual were collected and inoculated into YFCA glucose medium supplemented with or without GPs (final concentration, 50 mg/ml) for 24 h under aerobic and anaerobic conditions. Metabolomics analysis was performed for every sample, which was prepared according to the manufacturer's instructions. Gas chromatography time-of-flight mass spectrometry (GC-TOF/MS) analysis was performed using a LECO Pegasus 4D system (Leco Corporation, St Joseph, MI). The inlet temperature was 250 °C. The carrier gas helium was kept at a constant flow rate of 1.0 ml/min. The GC temperature program was set to 1 min isothermal heating at 70 °C, followed by a 5 °C/min temperature ramp to 280 °C, and was held for 10 min. The transfer line and ion-source temperatures were 250 °C and 220 °C, respectively. Electron impact ionization (70 eV) was set at a detector voltage of 1,575 V. Ten scans per second were recorded over the full mass range of 50-800 m/z. Chromatogram acquisition, library research, and peak area calculation were performed using ChromaTOF software (Version 4.5, LecoCorp.). Significantly different molecules were selected by FDR-adjusted p values.

Patient characteristics and clinical study details

Sixteen patients diagnosed with stage IV NSCLC and with PD-L1 expression or high TMB were included in this study according to the AJCC 8th edition criteria. The patients received pembrolizumab therapy at 2 mg/kg body weight, repeated every 3 weeks until progression was observed or the toxicity became intolerable. The response

rate (RR) was defined as the proportion of patients with a complete response (CR) or partial response (PR) to treatment, as assessed by the physician. The treatment response and disease progression were assessed by CT or positron emission tomography/computed tomography. We used RECIST 1.1 (investigator assessment) for efficacy evaluation. The progression-free survival (PFS) was calculated from the start date of pembrolizumab treatment. This study was approved by the Institutional Review Boards of Kiang Wu Hospital (approval number: 2018-007). The local independent ethics committee reviewed and approved the study protocol and documents used for informed consent before the study initiation. All study procedures were conducted in accordance with the Declaration of Helsinki and compliance with local regulatory requirements. All patients provided written informed consent before any study-related procedures were performed. This study is registered with ClinicalTrials.gov (identifier NCT04076228). The patient characteristics are detailed in Supplementary Table 2.

Patient consent for publication Patient consent was obtained, and this study was approved by the Institutional Review Board of the Kiang Wu Hospital, Macau (approval number: 2018-007).

Supplementary Table 1. GP has an influence on Trp/Kyn pathway *in vitro*.

Metabolite name	Cont O+	GP O+	Cont O-	GP O-
L-Tryptophan	0.118295	0.9801	0.24698	3.99539
L-Kynurenine	0.084101	0.0001	0.06126	0.00405
L-Kynurenine /L-Tryptophan	0.710943	0.000102	0.248036	0.001014

Cont O+: Control group in aerobic; GP O+: GP treatment in aerobic.

Cont O-: Control group in anaerobic; GP O-: GP treatment in anaerobic.

Supplementary Table 2. Characteristics of patients after treatment with pembrolizumab

	All patients (N=16)	**Responder (N=10)**	**Non-responder (N=6)**
Age in years, median (range)	65 (35-89)	66(35-89)	66(63-71)
Gender			
Female	2 (12.5)	1(10.0)	1(16.7)
Male	14 (87.5)	9(90.0)	5(83.3)
Smoking history			
Ever	4 (25.0)	1(10.0)	3(42.8)
Never	12(75.0)	9(90.0)	4(57.2)
Stage IV	16 (100)	10(100.0)	6(100.0)
WHO Performance Status			
0	9(56.3)	7(70.0)	2(33.3)
1	5(31.2)	2(20.0)	3(50.0)
2	2(12.5)	1(10.0)	1(16.7)
PD-L1 Status			
≥50%	4(25.0)	3(30.0)	1(16.7)
1-49%	8(50.0)	6(60.0)	2(33.3)
<1%	4(25.0)	1(10.0)	3(50.0)
Efficacy			
CR	1(6.3)	1(10.0)	0
PR	9(56.2)	9(90.0)	0
SD	2(12.5)	0	2(33.4)
PD	4(25.0)	0	4(66.6)

Values are presented as number (%) unless otherwise stated. Percentages may not sum to exactly

100 due to rounding.

Figure legends

Supplementary Figure 1. Combination therapy delays tumor progression in LLC-bearing mice and enhances the anti-tumor effect in B16-F10 tumor-bearing mice. (A) Tumor progression of the LLC-bearing mice. (B) Tumor growth curves were shown in each group. (C) Tumor weight of each group. Data are analyzed by Prism 8.0.2. Error bars represent Mean ± SEM. Tumor growth curves were assessed by two-way ANOVA. Tumor weight was assessed by one-way ANOVA. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure 2. Flow cytometry analysis of IFN-γ, TNF-α, and granzyme B among $CD8^+$ T cells and $Foxp3^+$ $CD4^+$ T cells.

Supplementary Figure 3. LEfSe analysis for differential abundant taxa detected between Vehicle and GP group. Threshold parameters were set as p=0.05 for the Mann-Whitney test and multi-class analysis=all against all. LDA score >2.0.

Supplementary Figure 4. Tryptophan metabolism contributes most among these metabolites in mice plasma examined by LC/MS between αPD-1 mAb alone group and the combination group. (A) A heatmap of 52 metabolites was generated by hierarchical cluster analysis with Pearson correlation and Ward clustering algorithm. Each rectangle represents a metabolite colored by its normalized intensity scale. Blue represents low, and red represents a high abundance of the metabolite relative to the mean. Data were representative of 9-11 independent biological replicates. (B) Orthogonal partial least squares discriminant analysis (OPLS-DA) scores plot of plasma from LLC tumor-bearing mice showing segregation of metabolites between αPD-1 mAb alone group and the combination group. Each dot represents an individual sample. (C) Loadings of the two main components in OPLS-DA. (D) Bubble diagram representing the significantly differed pathways.

Supplementary Figure 5. Relatively enriched bacteria in the R group are associated

with enhanced clinical efficacy and increased survival time. (A) Representative CT scan images of the patient's response to PD-1 inhibitor treatment. A CT scan image at baseline is shown on the left, and after treatment is shown on the right. (B) Representative histology and IHC profile of PD-L1 expression in tumor tissues. (C) Kaplan-Meier estimates of overall survival (OS) with PD-1 treatment for a follow-up of more than 30 months. (D-G) At the species level, *Bacteroides vulgatus*, *Parabacteroides distasonis*, *Bacterium LF-3*, and *Sutterella wadsworthensis HGA0223* were differentially abundant bacteria in the R group.

Supplementary Figure 6. LEfSe analysis for differential abundant taxa after FMT from NR. LLC tumor cells were inoculated and intervention of combination therapy, total fecal DNA was isolated and 16S rRNA genes were sequenced by 16S PacBio SMRT sequencing platform. (A) LEfSe analysis between αPD-1 mAb and combination group. (B) LEfSe analysis between Vehicle and GP group. (C) LEfSe analysis between Vehicle and combination group. Threshold parameters were set as p=0.05 for the Mann-Whitney test and multi-class analysis=all against all. LDA score >2.0.

Supplementary Figure 7. Alpha diversity observed in different time courses. (A) Richness (Chao1). (B) Diversity (Shannon index) for Day 0, Day 9, D15, and D24 after tumor inoculation in FMT experiment. Vehicle, αPD-1 mAb, GP and GP plus αPD-1 mAb. 1, 2, 3, 4 represent Day 0, Day 9, D15, and D24, respectively. Data were analyzed by Kruskal-Wallis test. ns: no significant difference.

Supplementary Table 1. GP has an influence on Trp/Kyn pathway *in vitro*.

Supplementary Table 2. Patient demographics and baseline characteristics.

Supplementary reference

1. Dong CX, Liu L, Wang CY, et al. Structural characterization of polysaccharides from Saposhnikovia divaricata and their antagonistic effects against the immunosuppression by the culture

supernatants of melanoma cells on RAW264.7 macrophages. *Int J Biol Macromol* 2018;113:748-56. doi: 10.1016/j.ijbiomac.2018.03.022 [published Online First: 2018/03/09]