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Lipid metabolism associated with efficacy of metronomic capecitabine and camrelizumab in gastrointestinal cancer: an exploratory clinical trial

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Abstract

Background Metronomic chemotherapy shows potential to enhance efficacy of PD-1 antibodies but has not been assessed in gastrointestinal (GI) cancer. Immunotherapy efficacy can be affected by body composition and lipid metabolism of patients. We aimed to evaluate the feasibility of metronomic capecitabine plus camrelizumab as a salvage treatment of late-stage GI cancer and to explore the roles of body composition and lipid metabolism in this regimen.

Methods This is a single-center, exploratory trial. Eligible GI cancer patients who had disease progression after standard chemotherapy were treated with metronomic capecitabine (500 mg twice daily) plus camrelizumab (200 mg on day 1 intravenously every 2 weeks). The primary endpoint was safety. Body composition indices analyzed by SliceOmatic software and lipidomics analyses using liquid chromatography-mass spectrometry were performed as exploratory investigation. Differentially expressed genes (DEGs) of C2C12 myocytes treated with metronomic dose 5-fluorouracil were detected by RNA sequencing.

Results A total of 26 patients were enrolled. Treatment emergent adverse events (TEAEs) grade ≥ 3 occurred in five patients (19.2%). Objective response rate was 19.2% (5/26), including two patients with complete response. High skeletal muscle radiation attenuation (SMRA) was associated with disease control and better survival. Differential plasma lipids were identified in disease-controlled patients compared with those who showed disease progression. High levels of a 6-lipid signature composed of SM40:1;3, TG54:4-FA20:2, LPC(16:0), TG52:0-FA20:0, TG56:3-FA20:2, and PE(P-18:1/18:2) were associated with better survival. SMRA and this plasma lipid panel were both increased in disease-controlled patients after treatment. DEGs including *prkg1*, *adora1*, and *Il15* in metronomic 5-FU treated C2C12 myocytes could be enriched into lipid metabolism pathways.

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Conclusions Metronomic capecitabine plus camrelizumab is well tolerated and shows promising efficacy in GI cancer. SMRA and specific plasma lipids are associated with efficacy of this regimen and indicate the modulation effect of metronomic capecitabine on lipid metabolism.

Trial registrations NCT04508686 (Aug 11, 2020), NCT04510818 (Aug 12, 2020), NCT04932187 (Sep 17, 2021).

Keywords Gastrointestinal cancer, Metronomic chemotherapy, Camrelizumab, Lipid metabolism

Background

Gastrointestinal (GI) cancers including esophageal, gastric, pancreatic, and colorectal cancer are common malignant diseases worldwide [1]. According to GLOBOCAN 2020 report, over 30% of cancer related deaths are caused by GI cancer [2, 3]. GI cancer patients with locally advanced unresectable or metastatic diseases can be treated with chemotherapy, molecular targeted therapy, and/or immunotherapy, but the overall treatment efficacy still needs to be improved [4].

In the last decade, targeting programmed cell death protein 1 (PD-1) by monoclonal antibodies has become an important part of the systemic treatment of cancer patients and is now approved for multiple types of GI cancer [5]. However, patients with refractory disease without biomarkers such as PD-L1 positive or high microsatellite instability (MSI-H) barely benefit from PD-1 antibodies [6]. To optimize PD-1 antibody application in GI cancer, strategies including drug combinations and novel target explorations are now under investigation. The immune phenotype of tumor microenvironment (TME) is one of the most important factors affecting the response to PD-1 antibodies [7]. In this regard, combination PD-1 antibodies with cytotoxic drugs, targeted agents, or antibodies targeting other immune checkpoints which can modulate the TME through variant mechanisms is a promising strategy to improve anti-tumor efficacy [8]. Currently, angiogenesis inhibitor is a common partner of PD-1 antibody as salvage therapy in GI cancer that shows promising efficacy in early-stage clinical trials, but it has not been validated by a randomized controlled trial [9, 10]. Moreover, toxicities of this combination regimen are also concerning, especially for patients who are elderly or have poor performance status [11, 12]. As such, optimizing combination strategies of PD-1 antibodies in late-stage GI cancer is still necessary.

Metronomic chemotherapy represents a new paradigm in pharmacological anti-cancer treatment. It refers to the administration of oral cytotoxic drugs with low dose, high frequency, and minimal drug-free breaks, and shows high tolerance as well as promising antitumor efficacy in cancer patients [13]. In addition to direct cytotoxic effect on tumor cells, metronomic chemotherapy can also modulate TME [14]. Metronomic capecitabine in combination with proton pump inhibitor rabeprazole

has been investigated in GI cancer by taking advantage of the TME modification feature of both drugs [15]. In our previous studies, we reported that metronomic capecitabine repressed tumor growth by inhibiting angiogenesis and regulating cancer-associated fibroblast phenotypes in xenografts of GI cancer cells [16, 17]. Immunomodulatory effects of metronomic chemotherapy such as activating immune effector cells and reducing immunosuppressive cells have also been identified [18]. Therefore, metronomic chemotherapy can be a promising partner of PD-1 antibodies.

The tumor immune microenvironment is also markedly influenced by the metabolic state of patients [19, 20]. Late-stage GI cancer patients have high prevalence of cachexia as a result of tumor-related symptoms like vomiting, anorexia, and dyspepsia [21]. Altered lipid metabolism is an important feature of cachexia which is characterized by body composition alterations such as myosteatosis, an ectopic fat accumulation in the skeletal muscle, which is closely associated with reduced survival [22]. Systemic deregulation of lipid metabolism can lead to reprogramming of immune cell activation in the TME and is associated with clinical outcomes of cancer immunotherapy [23]. Currently, the association between lipid metabolism and metronomic capecitabine is unclear.

In this study, we aimed to assess the safety and preliminary efficacy of metronomic capecitabine plus camrelizumab in refractory GI cancer patients based on a prospective, single-center, exploratory clinical trial. The role of body composition and lipid metabolism in this combination therapy was investigated.

Methods

Study design and patients

This was a prospective, single-center, exploratory trial conducted at the Department of Oncology, Ruijin Hospital. The study protocol had been published [24]. Eligible patients were assigned to cohorts according to primary tumor location: cohort 1 (HER2 negative gastric or gastroesophageal junction [G/GEJ] adenocarcinoma), cohort 2 (esophageal squamous cell carcinoma [ESCC] and head and neck squamous cell carcinoma [HNSCC]), and cohort 3 (hepatobiliary-pancreatic [HBP] and non-stomach, non-esophageal gastrointestinal carcinoma). Cohort

specific eligibility criteria included patients with HER2 negative advanced G/GEJ adenocarcinoma who had disease progression after ≥ 2 previous standard regimens (cohort 1); patients with advanced ESCC or HNSCC who had disease progression after first-line standard regimen (cohort 2); patients with HBP or non-stomach, non-esophageal gastrointestinal carcinoma who had disease progression after standard regimens (cohort 3).

Common eligibility criteria included age over 18 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; life expectancy ≥ 3 months; adequate organ functions. Hepatitis B virus (HBV) infected patients could be enrolled, while patients should be inactive/asymptomatic carriers or chronic/active infection with HBV DNA < 500 IU/mL (or 2500 copies/mL). Major exclusion criteria included patients with pregnancy or children bearing potential; brain and meningeal metastasis; second primary malignant diseases; uncontrolled auto-immune diseases; receiving long-term glucocorticoid treatment (> 10 mg/day prednisone); uncontrollable complications. All patients provided written informed consent. The protocols were approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China (2020, No.130; 2020, No.146; 2021, No.151) and were registered on Clinicaltrial.gov (protocol ID: NCT04508686, NCT04510818, NCT04932187). This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guideline for Good Clinical Practice.

Procedure

Capecitabine 500 mg was administered orally twice daily. Camrelizumab 200 mg was administered intravenously once every 2 weeks. Camrelizumab plus capecitabine were given until disease progression, intolerable toxicities, withdrawal of informed consent, or when patients could not benefit from study treatment as decided by the investigators. Treatment emergent adverse events (TEAEs) were assessed at every cycle before treatment. Severity of adverse events were graded according to the Common Terminology Criteria for Adverse Events (CTCAE criteria, v5.0). Severe adverse events (SAEs) were reported according to Good Clinical Practice. Drugs to relieve toxicities and as therapeutics for comorbidities were allowed. Drugs with potential anti-tumor effects were not allowed to be used. Treatment with camrelizumab was suspended when grade ≥ 3 immune-related adverse events (irAEs) occurred, and intravenous glucocorticoid was administered following clinical guideline. Camrelizumab could be re-started when irAEs recovered below grade 2. The treatment of camrelizumab was stopped when grade 4 irAEs occurred. If grade ≥ 3

capecitabine related adverse events occurred, administration of capecitabine was discontinued.

At baseline, comprehensive information of patients was recorded, including demography and medical history, physical examination, vital signs, and performance status. Routine hematological tests, cardiac biochemical markers, thyroid function, and serum cortisol were assessed at baseline and every 2 weeks after treatment. HBV markers and DNA copy number were detected at baseline. Electrocardiogram and echocardiography were performed before treatment. Imaging evaluation (contrast enhanced CT preferred) of chest, abdomen and pelvis was performed within 1 week before enrollment. Therapeutic efficacy was assessed every 8 weeks. Objective response was assessed by investigators according to Response Evaluation Criteria in Solid Tumors (RECIST, v1.1). Patient survival was followed up every 3 months.

Endpoints

The primary endpoint was safety defined as grade ≥ 3 treatment emergent adverse events (TEAEs). The secondary endpoints were progression-free survival (PFS), overall survival (OS), objective response rate (ORR), and disease control rate (DCR). PFS was defined as time from enrollment to first documented progressive disease (PD) or death, whichever occurred first. ORR was defined as the proportion of patients with complete response (CR) or partial response (PR). DCR was defined as the proportion of patients with CR, PR, or stable disease (SD). Exploratory endpoints were to analyze the associations of body composition parameters and lipidomics with treatment outcomes. Patients who achieved CR, PR, and SD were identified as good responders (GoRs), and PD patients were identified as poor responders (PoRs) for exploratory analysis.

Body composition analysis

Enhanced CT scans at the 3rd lumbar vertebral level (L3) before the first and fifth treatment cycle were collected retrospectively. Single slices of CT scan at the L3 level were analyzed by SliceOmatic software (v5.0, TomoVision). Skeletal muscle and adipose tissue segmentation was performed using predefined Hounsfield unit (HU) ranges for skeletal muscle (SM, -29 to 150 HU), visceral adipose tissue (VAT, -150 to -50 HU), and subcutaneous adipose tissue (SAT, -190 to -30 HU) [25]. Mean radiation attenuation value of the skeletal muscle (SMRA), visceral adipose tissue (VATRA), and subcutaneous adipose tissue (SATRA) were calculated. Index of skeletal muscle (SMI), visceral adipose tissue (VATI), and subcutaneous adipose tissue (SATI) were calculated using the area of each tissue compartment on the CT scan divided by square meter of patient's height.

Sample preparation and data collection of lipidomics

Five milliliters of peripheral blood were collected prospectively at baseline, radiological evaluation, and disease progression of patients. Blood samples from 16 GI cancer patients treated with PD-1 antibody-based regimens were collected as test cohort (Additional file 1: Table S1). Among them, 7 patients experienced ≥ 1 lines of therapy and 10 patients received PD-1 antibody plus chemotherapy. Plasma was prepared and stored at -80°C within 2 h.

The lipid extraction procedure utilized a modified MTBE method [26]. Briefly, 10 μL of plasma was mixed with 60 μL of methanol and 200 μL of MTBE, and the mixture was agitated at room temperature for 30 min. Subsequently, 50 μL of deionized water was introduced and thoroughly mixed for 1 min, then the sample was subjected to centrifugation (at 3000 g) for 10 min at 20°C . A 75 μL aliquot of the upper organic phase containing lipids was carefully transferred into a new 1.8 mL glass vial. To this lipid extract, 15 μL of a mixture of multiple internal standards representing 13 common lipid classes, sourced from an internal standards kit designed for the lipidizer™ platform, were added. The extract was gently dried under a nitrogen stream, then re-suspended in a 100 μL mixture of Dichloromethane-Methanol (1:1, v/v) containing 10 mM ammonium acetate. Subsequently, 25 μL of re-suspended extract was transferred into a 0.2 mL glass insert for lipidomic analysis. Plasma samples from all participants were combined and divided into multiple smaller aliquots as quality control (QC) samples. These QC samples underwent extraction and LC-MS analysis at the start and end of each daily sequence, as well as between every 10 sample runs. This rigorous QC approach was implemented to calibrate the analytical platform and measure intra-study analytical variability.

Lipid separation involved the utilization of the Shimadzu Nexera X2 LC-30AD ultra-performance liquid chromatography system, with the application of Waters CQUITY UPLC BEH HILIC Column (130 Å, 1.7 μm , 2.1 mm $\times$ 100 mm). This separation was achieved through an elution flow rate of 300 $\mu\text{L}/\text{min}$ over a 15-min linear gradient. Mobile phase A was composed of a mixture of acetonitrile and water in a 50:50 (v/v) proportion, to which 10 mM ammonium acetate was added. The pH of this solution was adjusted to 8.0 using ammonium hydroxide. Mobile phase B consisted solely of acetonitrile. Subsequent mass spectrometry analyses were conducted on a SCIEX 5500 QTRAP hybrid triple quadrupole/linear ion trap mass spectrometer. Electrospray ionization was employed in both positive and negative ion modes. Instrument settings included a curtain gas of 35 psi, a medium-level collision gas. The ion source gas1 was maintained at a pressure of 40 psi, while ion source gas2 was set to 50 psi. In the positive mode, the ion spray

voltage was set at 5.5 kV, whereas in the negative mode, it was -4.5 kV. The source gas temperature was maintained at 500°C . Data was acquired using the multiple reaction monitoring (MRM) mode. Following data acquisition, Analyst 1.6.3 software (Sciex, Foster City, CA) was employed for the manual examination of chromatograms and compound detection. The integration of peak areas was facilitated using Sciex's MultiQuant™ 3.0 Software (Sciex, Foster City, CA). To ensure precise quantitative comparisons, the signal intensity of each MRM value was normalized with reference to an internal standard from the Lipidizer™ internal standard kit, supplied by Sciex, Foster City, CA.

RNA sequencing of C2C12 cells

Mouse myoblast C2C12 cells were ordered from National Collection of Authenticate Cell Cultures, Shanghai, China (SCSP-505) and were cultured in DMEM media (1 g/L glucose, 10% fetal bovine serum) at 37°C with 5% CO_2 . C2C12 cells were differentiated using DMEM media with 5 g/L glucose and 1% heat-inactivated fetal bovine serum. Then, differentiated cells were treated with metronomic dose of 5-FU (5-FU/m, 5 $\mu\text{g}/\text{L}$) for 24 h (C2C12/5FU), and phosphate-buffered saline (PBS) treatment was used as control (C2C12/NC). Total RNA of cells was extracted using Trizol method to build cDNA library. The resulting cDNA library was sequenced using Illumina Novaseq6000 by Gene Denovo Biotechnology Co. (Guangzhou, China). Differential expression analysis between the treatment groups was performed by DESeq2 software. Differentially expressed genes (DEGs) were identified at false discovery rate (FDR) below 0.05 and absolute fold change (F_c) ≥ 2 . Pathway enrichment analysis was performed based on the KEGG database. Pathways with $FDR \leq 0.05$ were defined as significantly enriched pathways in DEGs. Total cell RNA of C2C12 cells treated with metronomic (5-FU/m, 5 $\mu\text{g}/\text{L}$), conventional (5-FU/c, 20 $\mu\text{g}/\text{L}$) dose of 5-FU or PBS was extracted. Real-time PCR was performed to detect the expression of IL-15. Supernatants of C2C12 cells treated with metronomic dose of 5-FU (5-FU/m, 5 $\mu\text{g}/\text{L}$) or PBS were collected. IL-15 levels were detected by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

According to previous reports, grade ≥ 3 TEAEs rate of camrelizumab plus apatinib was about 70–75%. The grade ≥ 3 TEAEs rate of camrelizumab plus metronomic capecitabine was assumed to be 40%. Based on the two-sided significant level of 5%, a sample size of 60 patients ensured at least 80% power. This trial was closed early due to changes of standard of care. PD-1/PD-L1 antibodies were approved to treat gastric cancer,

esophageal cancer, and biliary tract cancer as first-line treatment during the conduct of this study which reduced the number of eligible patients who did not expose to immunotherapy in our center, while these indications were not approved at the time of the study design. Following the principle of beneficence, the investigators decided to terminate this study. Finally, 26 patients were enrolled. Rate of TEAEs was described as frequency and percentage. The independent-samples *T* test was performed to analyze quantitative data. Paired-samples *T* test was performed to analyze quantitative data before and after treatment. Cut-off values of parameters were determined by receiver operating characteristics (ROC) curves. Crosstabs χ^2 analysis was performed to analyze qualitative data. Data analyses were performed using SPSS 26.0 software (Chicago, IL, USA).

Statistical analysis of lipidomic data was carried out in R (version 4.1.2), unless otherwise specified. Non-paired samples were subjected to the Wilcoxon rank-sum test, while paired samples were analyzed using the Wilcoxon signed-rank test. Principal component analysis (PCA) was performed using the R package “ggbiplot,” and heatmaps were generated using the “pheatmap” package in R. Other figures were generated in R using the “ggplot2” package. The network diagram illustrating differential lipid composition was constructed in Cytoscape (version 3.8.2) [27]. An elastic net regression model was built on the training set using the “glmnet” R package to predict treatment response in the test cohort. The survival curve was generated using the Kaplan–Meier method, and the difference between the curves was calculated using the log-rank test. R packages “survival” and “survminer” were employed for this analysis. $P < 0.05$ was considered statistically significant.

Results

Demographics and clinicopathological characteristics of patients

From December 2020 to February 2022, 26 patients were enrolled and received at least one cycle of treatment (Fig. 1A). In cohort 1, 8 gastric cancer patients were enrolled. In cohort 2, 9 esophageal cancer patients were enrolled. Patients with bile duct ($n=5$), pancreatic ($n=3$), and duodenal cancer ($n=1$) were enrolled in cohort 3. Clinical information of patients is listed in Table 1. Fifteen patients had ECOG performance status 1, and 11 patients were ECOG 2. Sixteen patients (61.5%) received at least two prior lines of therapy. All patients were enrolled in safety analysis, and 23 patients were eligible for response analysis. Disease progression was the major reason for treatment discontinuation (Fig. 1B).

Safety

Grade ≥ 3 TEAEs were reported by 5 patients (19.2%), including infection (11.5%), fatigue (7.7%), and AST/ALT increased (3.8%) (Table 2). Infection events included biliary duct infection and pneumonia occurred in 2 bile duct cancer patients and 1 duodenal cancer patient. Most patients experienced at least one type of TEAE (25/26, 96.2%). Reactive cutaneous capillary endothelial proliferation (RCCEP) was the most common adverse event (AE) (65.4%). Other TEAEs that occurred in $\geq 15\%$ of patients included fatigue (30.7%), anemia (23.1%), hypokalemia (23.1%), hypoalbuminemia (19.2%), neutropenia (19.2%), AST/ALT increased (15.4%), blood bilirubin increased (15.4%), and hyponatremia (15.4%). Three patients suffered SAEs, including bowel perforation ($n=1$), bowel obstruction ($n=1$), and bleeding ($n=1$), which were not related to treatment. No dose reductions of either capecitabine or camrelizumab due to TEAEs were recorded.

Efficacy

As of March 2023, treatment response could be assessed in 23 patients (Fig. 1C and D). For three patients, radiographical examinations were not performed after treatment due to early discontinuation (2 SAEs and 1 withdrawal of consent). ORR was 19.2% (5/26) and DCR was 50% (13/26) (Additional file 1: Table S2). One pancreatic cancer patient and one esophageal cancer patient achieved CR, and three esophageal cancer patients achieved PR (Fig. 1E). No objective response was observed in cohort 1 (gastric cancer). Median follow-up time was 6.3 months (Fig. 1F). Median PFS of all patients was 2.4 months (95%CI, 0.90 to 3.90 months), and median OS was 6.8 months (95%CI, 4.14 to 9.46 months). In cohort 2 (esophageal cancer), median PFS were 7.4 months ($P=0.005$, 95%CI, 5.10 to 9.70 months) and median OS did not reach ($P=0.044$), which were significantly better than other two cohorts. Median PFS and OS of cohort 1 and cohort 3 were similar (Fig. 1G).

High skeletal muscle radiation attenuation was associated with better clinical outcomes

Patients were stratified as good or poor responders according to treatment response. Good responders showed both higher baseline and posttreatment skeletal muscle radiation attenuation (SMRA) than poor responders (Fig. 2A), while SMI, VATRA, VATI, SATRA, and SATI were not different between good and poor responders (Additional file 1: Table S3). The representative images of body composition segmentation from patients with different response are illustrated in Fig. 2B. The cut-off value of baseline SMRA (37.75HU)

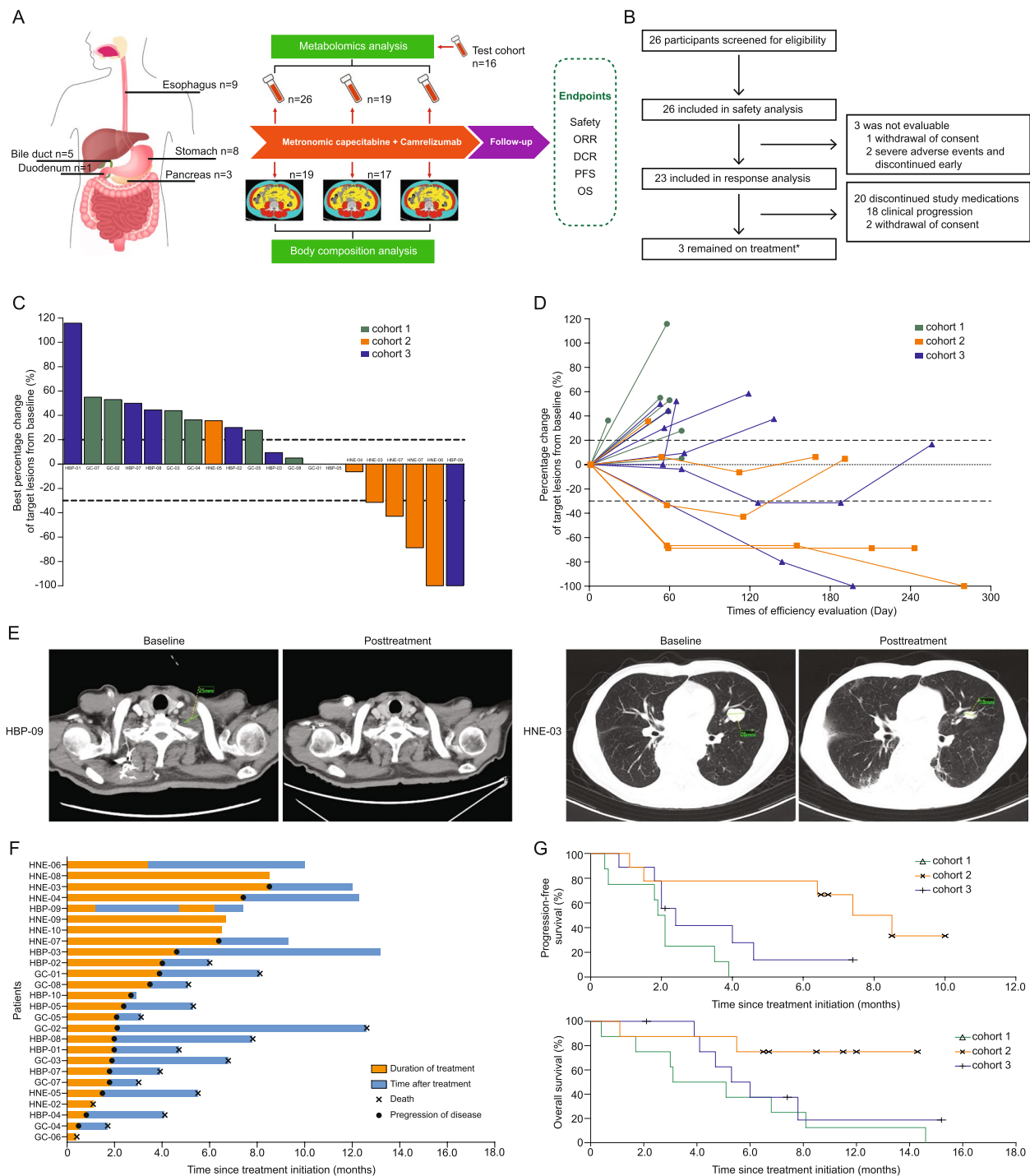


Table 1 Clinicopathological characteristics of patients

Clinicopathological characteristics	Cohort 1 <i>n</i> = 8	Cohort 2 <i>n</i> = 9	Cohort 3 <i>n</i> = 9	Total <i>n</i> = 26 (%)
Age	65 (49–74)	60 (44–77)	65 (54–74)	64 (44–77)
Gender				
Male	5	7	8	20 (76.9)
Female	3	2	1	6 (23.1)
ECOG				
1	2	4	9	15 (57.7)
2	6	5	0	11 (42.3)
Primary site				
Esophageal	–	–	9	9 (34.6)
Gastric	8	–	–	8 (30.8)
Bile duct	–	5	–	5 (19.2)
Pancreatic	–	3	–	3 (11.5)
Duodenal	–	1	–	1 (3.8)
Number of metastases				
1	5	6	6	17 (65.4)
≥ 2	3	3	3	9 (34.6)
Primary lesion resection				
Yes	6	7	6	19 (73.1)
No	2	2	3	7 (26.9)
Previous lines of treatment				
1	0	2	8	10 (38.5)
≥ 2	8	7	1	16 (61.5)
Molecular characteristics				
PD-L1 negative (CPS)	4	6	4	14 (53.8)
PD-L1 positive (CPS)	2	1	5	8 (30.8)
PD-L1 unknown	2	2	–	4 (15.4)
pMMR/MSS	8	9	9	26 (100)

ECOG Eastern Cooperative Oncology Group, CPS Combined positive score, pMMR Mismatch repair proficient, MSS Microsatellite stable

associated with good response was determined by ROC curve (AUC = 0.847, $P = 0.016$). Patients with high baseline SMRA (≥ 37.75 HU) showed a higher rate of good response than those with low baseline SMRA (72.7%, vs. 16.7%, $P = 0.05$), and achieved better PFS and OS (Fig. 2C). For good responders, SMRA was increased after treatment (43.09 ± 6.36 HU vs. 45.91 ± 4.53 HU, $P = 0.016$), but it was not changed in poor responders (36.11 ± 5.57 HU vs. 36.83 ± 7.26 HU, $P = 0.643$, Fig. 2D).

A plasma lipid signature associated with better survival of patients

Sixty-seven differential plasma lipids were detected between good responders and poor responders at baseline. Compared with poor responders, 66 upregulated lipids and 1 downregulated lipid were identified in good responders (Fig. 3A, Additional file 1: File S1). Categories of differential lipids included glycerolipids ($n = 46$), glycerophospholipids ($n = 9$), sphingolipids ($n = 9$), carnitine ($n = 2$) and sterol lipids ($n = 1$). Of the 67 differential

lipids, 46 lipids were associated with patient survival including glycerolipids ($n = 34$), sphingolipids ($n = 6$), glycerophospholipids ($n = 4$), carnitine ($n = 1$) and sterol lipids ($n = 1$) (Fig. 3B, Additional file 1: File S2). A specific 6-lipid signature comprised of SM(40:1;3), TG54:4-FA20:2, LPC(16:0), TG52:0-FA20:0, TG56:3-FA20:2 and PE(P-18:1/18:2) was significantly associated with patients' response (training cohort, AUC = 100%, Fig. 3C) and was confirmed in the test cohort (AUC = 97.9%, Fig. 3C). Median values of these 6 differential lipids were used to stratify patients with high and low levels. Patients with high levels of these lipids showed better PFS and OS (Fig. 3D).

Plasma lipids were upregulated after treatment

Changes of plasma lipids in good responders and poor responders were compared after treatment. In good responders, 20 lipids were upregulated, including glycerophospholipids ($n = 11$), glycerolipids ($n = 7$), fatty acyls ($n = 1$), and sphingolipids ($n = 1$), and no downregulated

Table 2 Treatment emergent adverse events (TEAEs) of patients

TEAEs (n=26)	All	G1	G2	G3/4
Any	25 (96.2%)	25 (96.2%)	9 (34.6%)	5 (19.2%)
RCCEP	17 (65.4%)	15 (57.7%)	2 (7.7%)	
Fatigue	8 (30.7%)	4 (15.4%)	2 (7.7%)	2 (7.7%)
Anemia	6 (23.1%)	4 (15.4%)	2 (7.7%)	
Hypokalemia	6 (23.1%)	5 (19.2%)	1 (3.8%)	
Hypoalbuminemia	5 (19.2%)	2 (7.7%)	3 (11.5%)	
Neutropenia	5 (19.2%)	3 (11.5%)	2 (7.7%)	
AST/ALT increased	4 (15.4%)	3 (11.5%)		1 (3.8%)
Blood bilirubin increased	4 (15.4%)	4 (15.4%)		
Hyponatremia	4 (15.4%)	3 (11.5%)	1 (3.8%)	
Infection	3 (11.5%)			3 (11.5%)
Hypophosphatemia	3 (11.5%)	2 (7.7%)	1 (3.8%)	
Anorexia	2 (7.7%)	1 (3.8%)	1 (3.8%)	
Hypocalcemia	2 (7.7%)	2 (7.7%)		
Hypothyroidism	2 (7.7%)	1 (3.8%)	1 (3.8%)	
Nausea	1 (3.8%)		1 (3.8%)	
Creatinine increased	1 (3.8%)	1 (3.8%)		

ALT Alanine transaminase, AST Aspartate transaminase, G Grade, RCCEP Reactive cutaneous capillary endothelial proliferation

lipid was identified (Fig. 4A, Additional file 1: File S3). In poor responders, 86 lipids were upregulated, and 3 lipids were downregulated, including glycerolipids ($n=48$), glycerophospholipids ($n=19$), sphingolipids ($n=18$), glycerolipids ($n=2$), and carnitine ($n=2$) (Fig. 4B, Additional file 1: File S4). Levels of differential lipids were mainly increased after treatment (Fig. 4C). Changes of LPC(16:0), PE(P-18:1/18:2), SM(40:1;3), TG52:0-FA20:0, TG54:4-FA20:2, and TG56:3-FA20:2 in good responders and poor responders were showed in Fig. 4D.

Differentially expressed genes in metronomic 5-FU treated C2C12 cells are enriched in lipid metabolism related pathways

Treatment of C2C12 cells with metronomic doses of 5-FU caused upregulation of 142 genes and downregulation of 44 genes in comparison with untreated control cells (Fig. 5A, Additional file 1: File S5). Metabolism pathways were the main enriched signaling pathways of differentially expressed genes (DEGs) in C2C12 cells (Fig. 5B). Pathways involved in metabolism of amino acids and regulation of lipolysis in adipocytes were enriched (Fig. 5C, Additional file 1: File S6). Examples of differentially expressed lipid metabolism related genes were *prkg1* and *adora1* (Fig. 5D). Interleukin 15 (*Il15*) was identified as an upregulated gene after treatment. IL-15 mRNA was significantly increased in C2C12 cells by metronomic dose (5-FU/m, 8.91 ± 0.20 , $P < 0.001$) rather than conventional dose of 5-FU (5-FU/c, 7.80 ± 0.07). Its protein level

was also increased by metronomic dose of 5-FU in ELISA test (10.89 ± 0.71 pg/mL vs. 8.58 ± 0.40 pg/mL, $P = 0.001$, Fig. 5E).

Discussion

In this study, metronomic capecitabine plus camrelizumab is well tolerated and effective in late-stage GI cancer patients with poor performance status. The SMRA and specific plasma lipids are associated with treatment outcomes, and can be regulated by metronomic capecitabine. Hence, modulation of lipid metabolism can be a novel mechanism of metronomic capecitabine in promoting the efficacy of PD-1 antibodies in GI cancer.

Toxicity is a pivotal factor to determine treatment strategies for late-stage GI cancer patients, especially for those who had poor performance status. Although promising responses to angiogenesis inhibitors plus PD-1 antibodies have been reported, grade ≥ 3 adverse events (AEs) rates of these regimens were about 40% to 70%, leading to a high frequency of treatment discontinuation [28–30]. In this study, we selected metronomic capecitabine as an alternative partner of PD-1 antibodies based on its safety profiles and modulation effect on the tumor microenvironment [14]. As expected, grade ≥ 3 TEAEs rate is less than 20% which is better than previously shown for angiogenesis inhibitors plus PD-1 antibodies. Infection was the most common grade ≥ 3 TEAEs which could be attributed to tumor location and/or poor performance status of patients. The most common TEAE is RCCEP that is closely related to camrelizumab [31]. Most patients who experienced RCCEP did not require special treatment and its frequency is comparable with previous report [32]. Furthermore, metronomic capecitabine did not increase the risk of other irAEs. Although sample size is limited, treatment lines and performance status of patients in our study are worse than previous studies, underscoring its high tolerance.

For late-stage GI cancer patients, the efficacy of PD-1 antibody monotherapy is variant according to tumor type, with ORR merely over 10% in patients without biomarkers [33–35]. Meanwhile, the ORR of metronomic capecitabine is about 10–20% and DCR is 40–50% [36, 37]. In our study, although the ORR and DCR are not superior to the historical data of either PD-1 antibody or metronomic capecitabine, CR can be achieved in some cases. Pancreatic cancer is commonly insensitive to PD-1 antibody [38]. In cohort 3, 1 pancreatic cancer patient who had distant lymphatic metastasis and got 2 prior lines of therapy achieved CR, with duration of disease control over 10 months. Of the 9 ESCC patients, 4 cases achieved objective response (1CR/3PR, 44.4%), and 3 cases achieved SD. The ESCORT trial, a randomized phase 3 study comparing camrelizumab

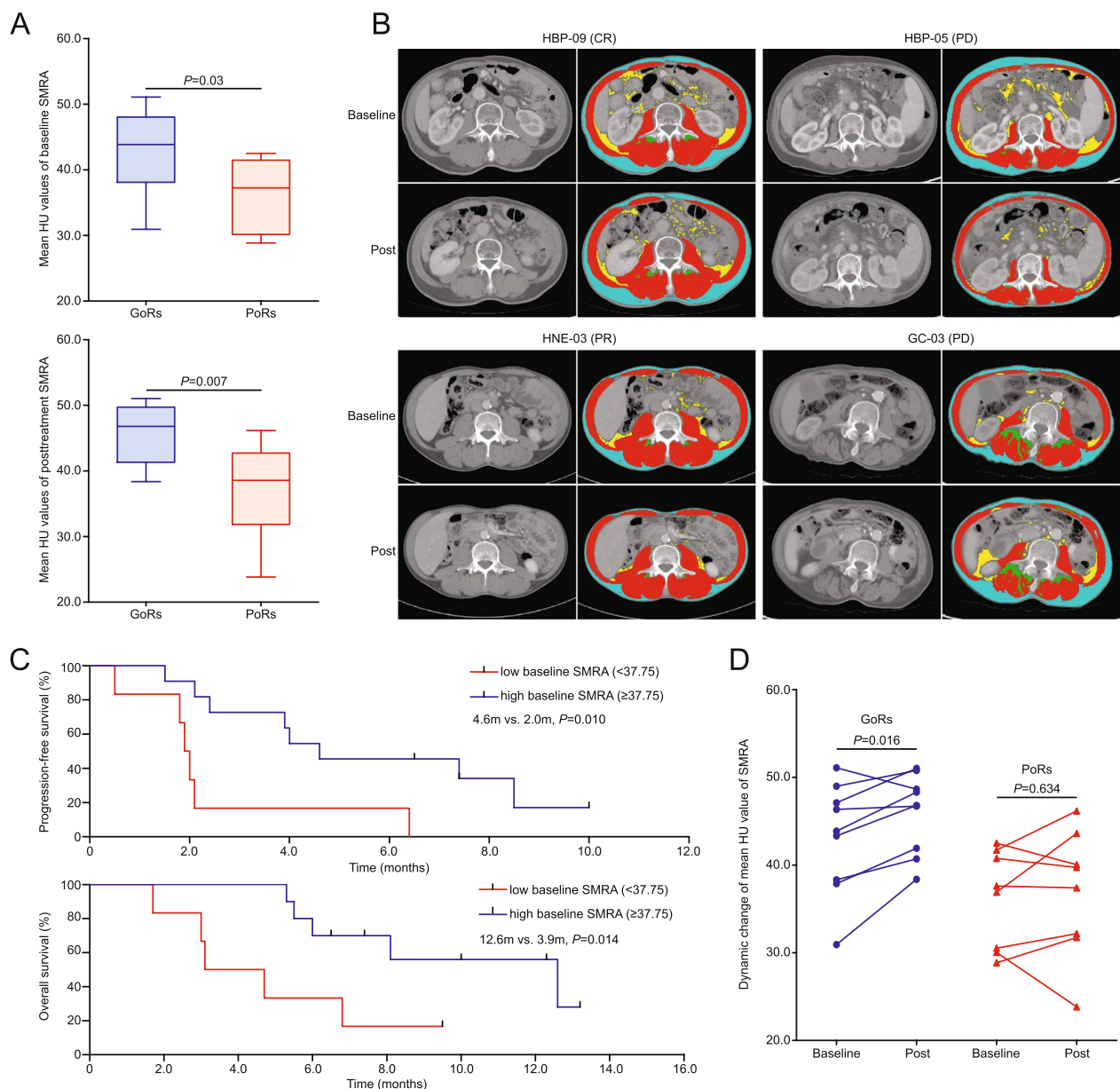


Fig. 2 Association between SMRA and patients' treatment outcomes. **A** Baseline and posttreatment HU values of SMRA in good responder (GoRs) were both higher than those in poor responders (PoRs). **B** Representative images of body composition analysis in patients with different tumor response. Red, skeletal muscle; Cyan, subcutaneous adipose tissue; Yellow, visceral adipose tissue; Green, intermuscular adiposis tissue. **C** PFS and OS of patients with high and low baseline SMRA. **D** Changes of SMRA before and after treatment in GoRs and PoRs

monotherapy versus standard chemotherapy as second-line treatment in ESCC patients, showed 20.2% of ORR [39]. These results indicate the promoting effect of metronomic capecitabine on camrelizumab. PD-1 antibody plus standard chemotherapy has been approved as first line treatment of ESCC [40]. Based on our results, metronomic capecitabine plus PD-1 antibody can be a maintenance strategy for ESCC patients to reduce toxicities and improve patients' quality of life.

Modulation of tumor immune microenvironment is one of the mechanisms of metronomic chemotherapy to promote efficacy of immunotherapy [41]. Increased infiltration of cytotoxic immune cells was observed in glioblastoma treated with metronomic capecitabine [42]. Standard doses of temozolomide were associated with increased T cell exhaustion compared to its metronomic doses [43]. However, the underlying mechanisms by which metronomic capecitabine regulates immune

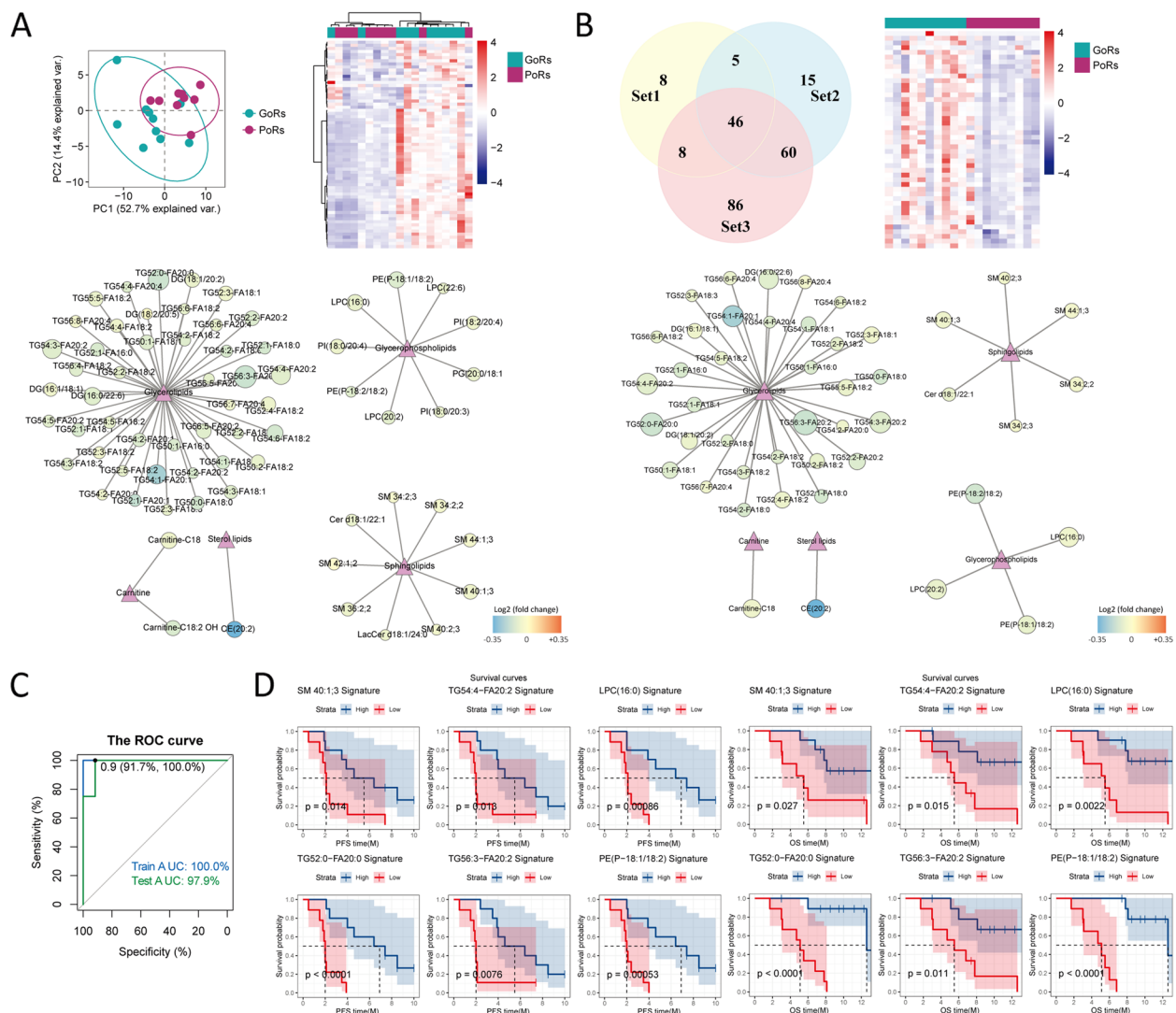
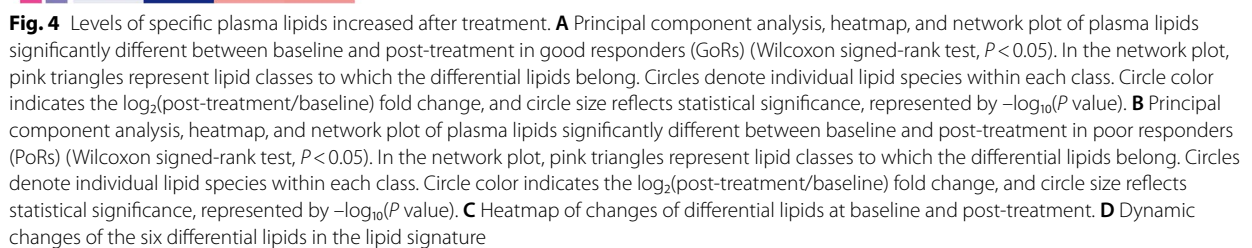


Fig. 3 Differential plasma lipids associated with patients' outcomes. **A** Principal component analysis, heatmap, and network plot of plasma lipids significantly different between good responders (GoRs) and poor responders (PoRs) (Wilcoxon rank-sum test, $P < 0.05$). In the network plot, pink triangles represent lipid classes to which the differential lipids belong. Circles denote individual lipid species within each class. Circle color indicates the log₂(PoRs/GoRs) fold change, and circle size reflects statistical significance, represented by $-\log_{10}(P \text{ value})$. **B** Venn diagram, heatmap, and network plot of differential plasma lipids associated with patient survival between good responders (GoRs) and poor responders (PoRs). Set 1 represents lipids that showed significant differences between GoRs and PoRs. Set 2 includes lipids associated with progression-free survival (PFS), and Set 3 comprises lipids associated with overall survival (OS). For Set 2 and Set 3, patients were stratified into high and low groups based on the median level of each lipid. Survival differences between groups were assessed using the log-rank test, with $P < 0.05$ considered statistically significant. In the network plot, triangles represent lipid classes, while circles represent individual lipid species. Circle color indicates the log₂(PoRs/GoRs) ratio, and size corresponds to $-\log_{10}(P \text{ value})$. **C** ROC analysis of 6-lipid signature associated with patients' response in training and test cohort. **D** PFS and OS of patients with high versus low 6 differential lipids, patients were divided into high and low groups based on the median level of each lipid

homeostasis in the TME have not been extensively investigated. Aberrant lipid metabolism, a common phenomenon in late-stage GI cancer patients with cachexia, can regulate the homeostasis of immunosuppressive cells and immune effector cells in the TME [44]. Excessive lipid accumulation in the TME can impair activation of immune effector cells including CD8⁺ T cells and NK

cells, while it seemed to be favorable for immunosuppressive cells like myeloid-derived suppressor cells, tumor associated macrophages, and regulatory T cells [21]. In this study, we applied body composition analysis and plasma lipidomics to explore the relationship between lipid metabolism and treatment efficacy. SMRA, an established indicator of ectopic fat accumulation in



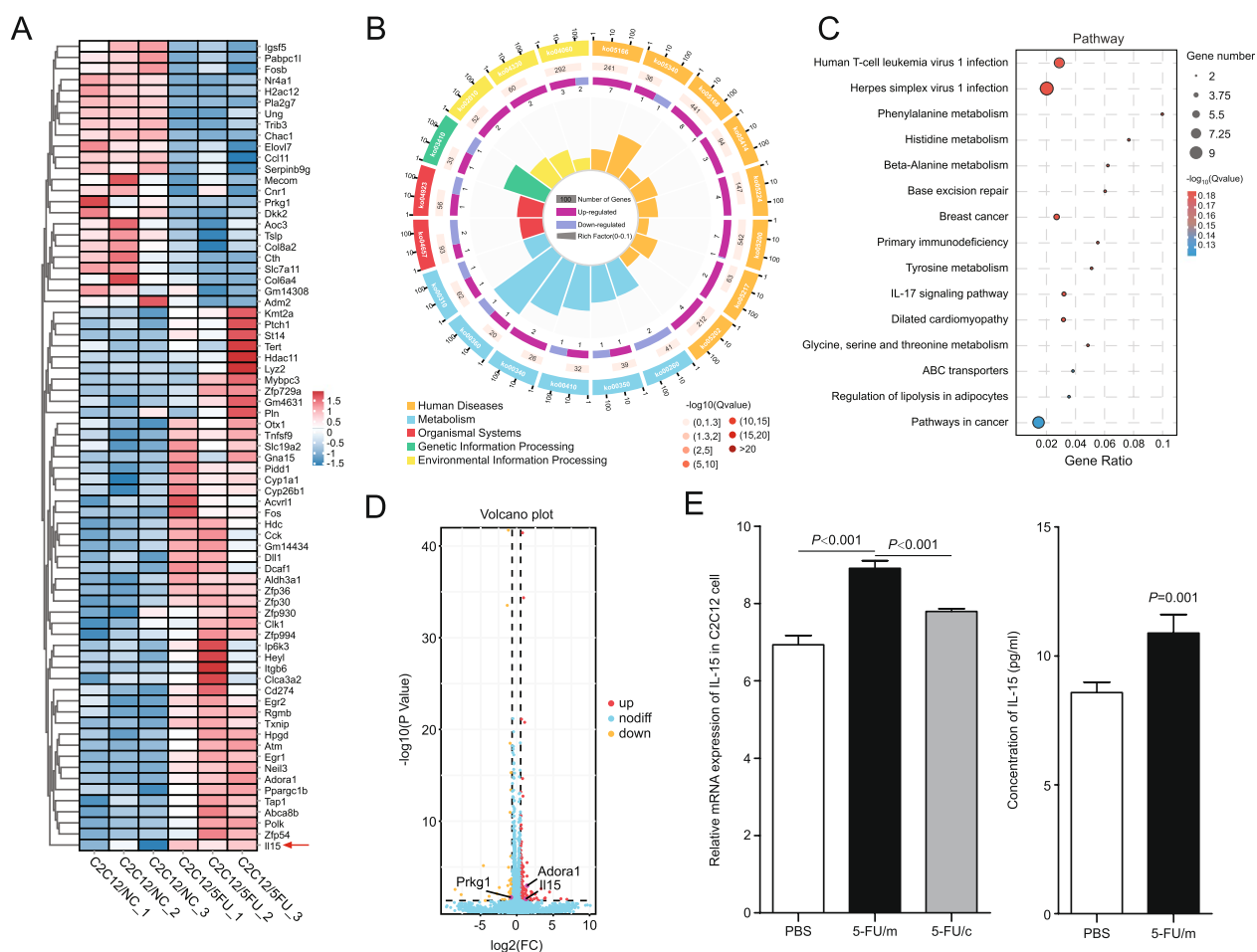


Fig. 5 RNA sequencing of C2C12 cells after treatment with metronomic 5-FU. **A** Heatmap of differentially expressed genes between C2C12/5FU and untreated control cells. *Il15* gene is marked. **B** Circular plot of top 15 enriched pathways. **C** Dot plot of top 15 enriched pathways. **D** Volcano plot of differentially expressed genes. *Il15*, *prkg1* and *adora1* are marked. **E** IL-15 mRNA was detected by real-time PCR in C2C12 cells treated with metronomic dose of 5-FU (5-FU/m) and conventional dose of 5-FU (5-FU/c). IL-15 protein level was detected by ELISA test using supernatant of C2C12 cells treated with metronomic dose of 5-FU (5-FU/m). PBS treatment was used as control group for both experiments

skeletal muscle and aberrant lipid metabolism of cancer patients [45], was associated with disease control and better survival of our patients. The association between SMRA and efficacy of immunotherapy has been reported in melanoma and lung cancer patients [46, 47], while this phenomenon is identified in GI cancer patients treated with metronomic capecitabine plus camrelizumab for the first time. Lipidomics analysis revealed that specific plasma lipids were correlated with both treatment efficacy and patient survival. Therefore, modulation of lipid metabolism can be a potential mechanism of metronomic capecitabine in regulating tumor immune microenvironment.

The skeletal muscle is the major organ that regulates lipids consumption [48]. To further validate the modulation effect of metronomic capecitabine in lipid metabolism, gene expression of C2C12 cells after metronomic

5-FU treatment was analyzed. The results show that regulation of lipolysis in adipocytes pathway-related genes, *prkg1* and *adora1*, are identified as DEGs after treatment. Protein kinase cGMP-dependent 1 (PRKG1) is key mediator of the nitric oxide/cGMP signaling pathway that can regulate activation of intracellular hormone-sensitive lipase [49]. Adenosine A1 receptor (ADORA1) belongs to the G-protein coupled receptor 1 family that also participates in regulating lipid deposition of skeletal muscle [50]. This finding shows that this pathway may participate in metronomic capecitabine regulated lipid metabolism of skeletal muscles.

Meanwhile, expression of *Il15* encoding interleukin-15 (IL-15) was upregulated. Skeletal muscle has been recognized as an endocrine organ that can regulate multiple physiological functions including anti-tumor immunity [51]. Physical exercise that enhances skeletal muscle mass

and function can not only improve patients' qualitative of life but also enhance the anti-cancer treatment efficacy by inducing expression of myokines [52]. IL-15 is a myokine and immune modulating protein which can promote survival and the cytotoxic phenotype of CD8⁺ T cells [53]. Exercise-induced IL-15 promotes the infiltration of IL-15Ra⁺ CD8⁺ T cells in the TME and improves anti-tumor immunity [54]. Furthermore, the expression of IL-15 is negatively correlated with trunk fat mass [55]. Based on these data, we suggest that muscle-derived IL-15 can be one of the downstream effectors of metronomic capecitabine to improve efficacy of PD-1 antibodies by modulating tumor immune microenvironment.

Association between plasma lipidomics and efficacy of PD-1 antibody-based treatment in GI cancer has not been reported. A 6-lipid signature was associated with treatment efficacy and was confirmed in a test cohort. Yu et al. identified a 9-lipid signature in lung cancer patients treated with chemotherapy plus immune checkpoint inhibitors that was associated with occurrence of irAEs, but its association with treatment efficacy was not assessed [56]. Sphingomyelin (SM), lysophosphatidylcholine (LPC), phosphatidylethanolamine (PE), and triacylglycerol (TAG) are the major lipid types detected in our signature. SM is a key component of cellular membrane and contain microdomains involved in intracellular signaling transduction [57]. The SM/ceramide balance regulates cancer cell proliferation, survival, and apoptosis [58]. In vivo studies showed that administration of SM impaired progression of colon cancer and potentiated the effect of 5-FU [59]. For immune cells, a SM-rich microdomain was necessary for the activation of T cells [60]. The associations of specific types of LPC and PE with tumor progression are also reported. Plasma levels of sn-1 LPC(18:3) and sn-2 LPC(20:3) were inversely associated with risk of gastric cancer [61]. Increased PE levels in plasma membrane could facilitate growth of breast cancer cells [62]. These evidences support the result of our study that a high plasma level of these lipids is associated with better response and survival of patients. For TAG, its potential role in immune modulation of GI cancer is unclear. The synthesis of SM is linked to glycerolipid metabolism which may explain the consistent changes of plasma TAGs and SM in our study [44]. Although lipolysis related pathway is enriched in C2C12 cells, the sources of these lipids and the mechanisms that increase their levels after treatment are unclear. Detailed biological effects of those differential plasma lipids on treatment efficacy of cancer immunotherapy should be further investigated.

There are several limitations of this study. This is a single center exploratory trial with limited sample size. Given the approval of PD-1 antibodies in GI cancer as

first-line therapy, the number of late-stage patients who were naïve to PD-1 antibodies decreased which limited patient enrollment. Nevertheless, this is the first prospective trial so far to assess the feasibility of metronomic capecitabine plus camrelizumab in late-stage GI cancer patients. Our data can provide useful information to help optimizing combination strategies of PD-1 antibodies, especially regarding safety profiles. In the exploratory analysis, peripheral blood samples and images of CT scan images were not available from all patients which might impair the significance of results. To avoid selection bias, a test cohort was enrolled to verify the results of the lipidomics analysis. Further studies with larger sample sizes should be carried out. Moreover, molecular mechanisms of metronomic capecitabine alone or combined with PD-1 antibodies with respect to lipid metabolism should be further investigated.

Conclusions

Metronomic capecitabine plus camrelizumab is well tolerated and shows promising efficacy to treat refractory late-stage GI cancer patients with poor performance status. SMRA and specific plasma lipids were associated with efficacy of this regimen. Modulation of lipid metabolism is a potential mechanism of metronomic capecitabine in regulating tumor immune microenvironment and promoting the efficacy of PD-1 antibodies.

Abbreviations

AEs	Adverse events
CR	Complete response
CTCAE	Common Terminology Criteria for Adverse Events
DCR	Disease control rate
DEGs	Differentially expressed genes
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assay
ESCC	Esophageal squamous cell carcinoma
FDR	False discovery rate
GI	Gastrointestinal
G/GEJ	Gastric or gastroesophageal junction
GoRs	Good responders
HBV	Hepatobiliary-pancreatic
HBV	Hepatitis B virus
HNSCC	Head and neck squamous cell carcinoma
HU	Hounsfield unit
irAEs	Immune-related adverse events
L3	The 3rd lumbar vertebral level
IL-15	Interleukin 15
MDSCs	Myeloid-derived suppressor cells
MRM	Multiple reaction monitoring
MSI-H	High microsatellite instability
ORR	Objective response rate
OS	Overall survival
PBS	Phosphate-buffered saline
PD-1	Programmed cell death protein 1
PFS	Progression-free survival
PoRs	Poor responders
PR	Partial response
QC	Quality control
RCCEP	Reactive cutaneous capillary endothelial proliferation
RECIST	Response Evaluation Criteria in Solid Tumors
SAEs	Severe adverse events

SATI	Subcutaneous adipose tissue index
SATRA	Subcutaneous adipose tissue radiation attenuation
SD	Stable disease
SM	Sphingomyelin
SMI	Skeletal muscle index
SMRA	Skeletal muscle radiation attenuation
SNVs	Single-nucleotide variants
TAG	Triacylglycerol
TEAEs	Treatment emergent adverse events
TME	Tumor microenvironment
VATI	Visceral adipose tissue index
VATRA	Visceral adipose tissue radiation attenuation

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12916-025-04377-4>.

Additional file 1: Table S1–S3 and File S1–S6. Table S1 Clinical characteristics of GI cancer patients in test cohort. Table S2 Treatment response of patients. Table S3 Body composition indices at baseline and posttreatment between GoRs and PoRs. File S1 Differential plasma lipids between GoRs and PoRs. File S2 Differential plasma lipids between GoRs and PoRs associated with patients' survival. File S3 Plasma lipids changed after treatment in GoRs. File S4 Plasma lipids changed after treatment in PoRs. File S5 List of differentially expressed genes between C2C12/5FU and untreated control cells. File S6 List of enriched pathways based on differentially expressed genes.

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Authors' contributions

CZ and JZ conceived the study. CZ, CS, and MS wrote the protocol of clinical trial. CZ and CS were responsible for patient recruitment and treatment with help from WX, LG, JJ1, JW. Data collection was performed by CZ and CS. Sample collection was performed by QC and JJ2. Images of CT scan were collected by XZ. Body composition was performed by CZ with help from YS, SR and SD. YY, QW and RZ performed lipidomic analysis. CZ and YY wrote the draft of the manuscript. JZ, SR and RZ reviewed and edited the manuscript. JZ, SD, RZ and ZZ supervised the study. All authors read and approved the final manuscript.

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Data availability

Data supporting the findings of this study are available within the paper and its Additional files. RNA-sequencing data is deposited in the NCBI's Gene Expression Omnibus database under accession number GSE268030 and is accessible using a reviewer token (wpsbqyiffejdcr) at the following URL: <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE268030>.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization guideline for Good Clinical Practice. The protocols were approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, People's Republic of China (2020, No.130; 2020, No.146; 2021, No.151) and were registered on Clinicaltrial.gov (protocol ID: NCT04508686, NCT04510818,

NCT04932187). Informed consent to participate was obtained from all participants before enrollment.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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