Association of Peripheral Blood Biomarkers with Response to anti-PD-1 Immunotherapy for Patients with dMMR Metastatic Colorectal Cancer: A Multicenter Cohort Study

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Research

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Abstract

Background: Mismatch repair deficient (dMMR) is an established biomarker for response to anti-PD-1 immunotherapy in metastatic colorectal carcinoma (mCRC). Although patients with dMMR mCRC could achieve a high incidence of disease control and favorable progression-free survival (PFS), reported response rates to PD-1 inhibitors are variable with 28%–52%, indicating that additional predictive biomarkers are warranted.

Methods: This multicenter cohort study enrolled patients with dMMR mCRC receiving anti-PD-1 immunotherapy at Sun Yat-sen University, the Sixth Affiliated Hospital and Sun Yat-sen University Cancer Center between December, 2016 to December, 2019. A total of 20 peripheral blood biomarkers, including T cells (frequency of CD4+ T cell, frequency of CD8+ T cell, ratio of CD4+/CD8+), carcinoembryonic antigen (CEA), inflammatory markers and lipid metabolism markers. The association between response or survival and peripheral blood parameters was analyzed.

Results: Among tested parameters, ratio of CD4+/CD8+, frequency of CD4+ T cell was significantly associated with PFS ($P=0.023$, $P=0.012$) and OS ($P=0.027$, $P=0.019$) in univariate analysis. Lower level of CD4+/CD8+ ratio or frequency of CD4+ T cell showed significant association with better overall response rates (ORR; $P=0.03$, $P=0.01$). Ratio of CD4+/CD8+, and frequency of CD4+ T cell maintained significance in multivariate Cox model for PFS ($HR=9.23$, $P=0.004$; $HR=4.83$, $P=0.02$) and OS ($HR=15.22$, $P=0.009$; $HR=16.21$, $P=0.025$).

Conclusions: Ratio of CD4+/CD8+ and frequency of CD4+ T cell might be crucial independent biomarkers within dMMR mCRC to better identify patients for response to PD-1 inhibitors. If validated in prospective clinical trials, ratio of CD4+/CD8+ and frequency of CD4+ T cell might provide aid in guiding the treatment of PD-1 inhibitors in dMMR mCRC.

Background

Colorectal cancer (CRC) is the fourth most common cause of cancer-related death globally and there is an increasing incidence of CRC, often metastatic, among younger patients [1, 2]. DNA mismatch repair-deficient (dMMR)/microsatellite instability-high (MSI-H) is a well-established biomarker for the response to programmed cell death (PD)-1 inhibitor in metastatic CRC (mCRC), which led the US Food and Drug Administration (FDA) to approve these drugs for this subset of patients[3]. Moreover, promising efficacy has been reported in locally advanced colon cancer with dMMR tumors[4]. However, overall response rates (ORR) in MSI-H mCRC patients are variable with 28–52%[3, 5, 6], which likely reflected tumor heterogeneity. Analysis of tumor mutational burden (TMB) in tumor sampling provided value in further identifying MSI-H mCRC patients who responds to PD-1 inhibitors[7], but invasively obtaining tissue might cause treatment delays. Hence, identification of new biomarkers from easily accessible peripheral blood is critical for selecting patients who respond most to PD-1 inhibitors.

Tumor infiltrating lymphocytes (frequency of CD8 + T cells) mainly contributes to the antitumor immune response and is a reliable prognostic indicator for CRC[8, 9], but it is not an optimal predictor for anti-PD-1 immunotherapy. Furthermore, several peripheral blood indexes- including T cells (CD4⁺, CD8⁺ T-lymphocytes), systemic inflammation (neutrophil-to lymphocyte ratio (NLR), absolute neutrophil count (ANC), C-reactive protein (CRP), and lactate dehydrogenase (LDH)) have been associated with response or survival outcomes in patients with melanoma and non-small cell lung cancer (NSCLC) receiving immune checkpoint inhibitors (ICIs)[10–15]. In addition, lipid metabolism has been demonstrated to have a crucial role in the promotion of migration[16], invasion[17] and be related with tumor immune milieu[18]. However, it remains unclear whether peripheral blood profiling could detect responses to anti-PD-1 immunotherapy in mCRC with MSI-H tumors. Thus, this multicenter study analyzed 41 mCRC patients with dMMR tumors to investigate the potential association between peripheral biomarkers with response to anti-PD-1 immunotherapy.

Methods

Patients

A total of 41 patients with mCRC with dMMR tumors treated with anti-PD-1 inhibitor (nivolumab, pembrolizumab, triprizumab, toripalimab and camrelizumab) were identified at Sun Yat-sen University, the Sixth Affiliated Hospital and Sun Yat-sen University Cancer Center between December, 2016 to December, 2019 (Figure 1). The end of follow-up was June 30, 2020. The study was approved by the institutional review board of Sun Yat-sen University, the Sixth Affiliated Hospital. Written informed consent from patients was waived due to the retrospective nature of our study.

Pretreatment clinicopathologic features and treatment history were collected from the individual database at these two institutions, which included age, sex, stage, tumor location, histologic subtype, carcinoembryonic antigen (CEA), mutational status (KRAS, BRAF), T cells (CD4⁺ T cell [CD3⁺ CD4⁺ T cell], CD8⁺ T cell [CD3⁺CD8⁺ T cell], ratio of CD4⁺/CD8⁺), inflammatory biomarkers (neutrophils, lymphocytes, monocytes, platelets, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR), lactate dehydrogenase (LDH), C-reactive protein (CRP), albumin (ALB)) and lipid metabolism markers (cholesterol (CHO), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB)). Pretreatment values were defined as the values obtained before the initiation of anti-PD-1 immunotherapy.

Flow cytometry

We obtained peripheral blood samples before anti-PD-1 immunotherapy. The Abs for staining are Ab anti-CD4 (APC-labeled CD4, clone SK3), anti-CD8 (PE-labeled CD8, clone SK1), anti-CD3 (FITC-labeled CD3, clone SK7), anti-CD45 (PerCP-labeled CD45, clone 2D1 [HLE-1]). All of the above Abs (BD Biosciences)

included isotype-matched negative controls. According to the procedure of BD Multitest™ CD3/CD8/CD45/CD4 kit (No. 340499, BD, USA), 100 µL of well-mixed, anticoagulated whole blood is vortex gently mixed with 20 µL of abs, and is incubated for 15 minutes in the dark at room temperature. 450 µL of 1X BD FACS lysing solution is added and incubated for 15 minutes in the dark at room temperature. The stained cells were analyzed on a BD FACS Canto II flow cytometry system with FACS Diva software (BD Biosciences) (Figure S1).

Statistical analysis

Tumor response was evaluated according to the Response Evaluation Criteria in Solid Tumors, version 1.1. Progression-free survival (PFS) was defined as the duration from the date of immunotherapy initiation to clinical or radiographic progression or death. Overall survival (OS) was defined as the duration from the date of immunotherapy initiation to death. Fisher's exact test and Mann-Whitney U test were performed to compare distribution between groups based on response for categorical variables and continuous variables, respectively. Univariate Cox regression model was performed to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) of survival based on clinicopathologic parameters and peripheral blood indexes. The receiver operating characteristics (ROC) curve analysis was used to determine the cut-off point for the continuous variables including peripheral blood parameters. Kaplan-Meier method was used to perform survival analysis, with P values compared by the log-rank test. Only parameters with statistical significance in univariate analysis were included in multivariable analysis. HRs and 95% CIs of survival was estimated multivariate Cox regression models. A two-tailed P value < 0.05 was considered statistical significance. All statistical analyses were performed in R software (version 3.5.1; <http://www.Rproject.org>).

Results

Patients characteristics

A total of 41 mCRC patients with dMMR tumors were identified to be treated with PD-1 inhibitors. Clinical outcomes are depicted in supplementary Table S1. Overall, 4 patients achieved a complete response (CR), 19 patients achieved a partial response (PR), 10 patients achieved stable disease (SD), and 8 patients achieved a progressive disease (PD), which led to an overall response rate (ORR) of 56% (23/41). Patients clinicopathological characteristics are detailed in Table 1. Median age for the entire cohort was 41 years old (range 20-77) and 54% of patients were male. KRAS mutations were observed in 73% (16/22) of patients and BRAF mutations in 9% (2/22). A total of 30 patients (73%) had colon tumor. The median values of frequency of CD4+ T cells, frequency of CD8+ T cell, and ratio of CD4+/CD8+ for the entire cohort were 37 (23-61), 27 (12-53), and 1.3 (0.5-4.6), respectively.

Association between biomarkers and objective response

Characteristics and overall response (OR) were compared between responders (CR/PR) and non-responders (SD/PD). frequency of CD4+ T cell and CEA as continuous variables were significantly associated with ORR (all P values <0.05, Table 1), while other investigated parameters were similar in spite of the significant association between lower level for the ratio of CD4/CD8 T cells with ORR (P=0.03). For mutation data, KRAS or BRAF mutations did not show any significant difference (Table 1).

Table 1

Patient’s characteristics

Characteristics	No (%) of patients (n=41)	CR/PR (n=23)	SD/PD (n=18)	P value ^a
Age, y, median (range)	41 (20-77)	35 (20-68)	47 (21-77)	0.16
Gender				0.13
Male	22 (54)	10 (43)	12 (67)	
Female	19 (46)	13 (57)	6 (33)	
Grade				0.40
High	5 (12)	4 (17)	1 (6)	
Moderate	14 (34)	8 (35)	6 (33)	
Low	15 (37)	6 (26)	9 (50)	
NA	7 (17)	5 (22)	2 (11)	
Tumor location				0.73
Colon	30 (73)	16 (70)	14 (78)	
Rectum	11 (27)	7 (30)	4 (22)	
Known KRAS status ^b				1.0
Mutant	16 (73)	8 (73)	8 (73)	
Wild-type	6 (27)	3 (27)	3 (27)	
Known BRAF status ^c				1.0
Mutant	2 (9)	1 (9)	1 (9)	
Wild-type	20 (91)	10 (91)	10 (91)	
Frequency of CD4+ T cells, %, median (range)	37 (23-61)	32 (23-51)	41 (25-61)	0.013
Frequency of CD4+ T cells, %				0.01
>39.5	16 (39)	5 (22)	11 (61)	
≤ 39.5	25 (61)	18 (78)	7 (39)	
Frequency of CD8+ T cells, %, median (range)	27 (12-53)	28 (15-53)	24 (12-46)	0.24
Ratio of CD4/CD8, %, median (range)	1.3 (0.5-4.6)	1.1 (0.5-2.3)	1.9 (0.6-4.6)	0.12
Ratio of CD4/CD8, %				0.03

>1.64	15 (37)	5 (22)	10 (56)	
≤ 1.64	26 (63)	18 (78)	8 (44)	
CEA, ng/mL, median (range)	9.3 (1.1-754.6)	5.0 (1.4-754.6)	44.0 (1.1-596.1)	0.03
CRP, mg/L, median (range)	14.4 (0.2-201.7)	12.5 (0.2-201.7)	16.3 (0.5-181.8)	0.47
LDH, U/L, median (range)	197.2 (130.9-931.2)	171.9 (135.5-931.2)	228.0 (130.9-567.3)	0.19
Neutrophils, 10E9/L, median (range)	4.1 (0.6-20.7)	3.3 (0.6-10.9)	4.7 (1.2-20.7)	0.17
Lymphocytes, 10E9/L, median (range)	1.3 (0.3-2.8)	1.3 (0.3-2.2)	1.3 (0.5-2.8)	0.88
NLR, median (range)	3.3 (0.6-26.0)	2.9 (0.6-26.0)	3.6 (1.0-17.6)	0.34
Monocytes, 10E9/L, median (range)	0.6 (0.2-1.8)	0.5 (0.2-1.8)	0.6 (0.3-1.0)	0.72
Platelets, 10E9/L, median (range)	272.0 (111.6-479.7)	265.0 (111.6-444.0)	276.9 (126.0-479.7)	0.82
PLR, median (range)	180.5 (61.5-900.0)	180.5 (61.5-900.0)	182.7 (66.8-622.4)	0.94
LMR, median (range)	2.1 (0.5-9.3)	2.1 (0.5-9.3)	2.2 (1.1-7.7)	0.81
ALb, g/L, median (range)	40.8 (23.0-49.5)	40.9 (26.4-49.5)	40.5 (23.0-48.1)	0.62
CHO, mmol/L, median (range)	4.4 (3.4-6.8)	4.8 (3.5-6.8)	4.0 (3.4-5.2)	0.45
TG, mmol/L, median (range)	1.2 (0.5-3.9)	1.1 (0.7-3.9)	1.4 (0.5-3.4)	0.79
HDL, mmol/L, median (range)	1.2 (0.5-5.1)	1.3 (0.6-5.1)	1.1 (0.5-1.7)	0.11
LDL, mmol/L, median (range)	2.8 (0.8-7.7)	2.8 (1.2-3.8)	2.4 (2.0-7.7)	0.63
ApoA1, g/L, median (range)	1.2 (0.3-1.7)	1.2 (0.7-1.7)	1.1 (0.3-1.5)	0.29
ApoB, g/L, median (range)	0.8 (0.4-1.5)	0.9 (0.4-1.5)	0.8 (0.4-1.2)	0.45

^a P values were estimated by Fisher's exact test and Mann-Whitney U test for categorical variables and continuous variables, respectively.

^b A total of 22 patients were tested with KRAS.

^c A total of 22 patients were tested with BRAF.

CR= complete response, PR= partial response, SD= stable disease, PD=progressive disease, CEA=carcinoembryonic antigen, CRP=C-reactive protein, LDH=lactate dehydrogenase, ALB= albumin, NLR=neutrophil-to-lymphocyte ratio, PLR=platelet-to-lymphocyte ratio, LMR=lymphocyte-to-monocyte

Association between biomarkers and survival

Among all tested parameters for their correlation with PFS using a Cox regression model, gender, age, tumor location, tumor grade, stage, KRAS and BRAF status did not affect PFS nor OS (Table 2, S2). Frequency of CD4+ T cell, ratio of CD4+/CD8+, high dense lipoprotein (HDL), and ApoA1 were associated with PFS in a univariate Cox regression model (Table 2). Frequency of CD4+ T cell, ratio of CD4+/CD8+, NLR, high dense lipoprotein (HDL), and ApoA1 were associated with OS in a univariate Cox regression model (Table S2). Using ROC curves, the cut-off values of the above variables for PFS were identified (Table 2, Figure S2). The potentially survival-related factors (HDL, ApoA1, NLR) were not significantly associated with frequency of CD4+ T cell or ratio of CD4+/CD8+ (Table S3). Frequency of CD4+ T cell and ratio of CD4+/CD8+ remained significance in multivariate analysis for both PFS and OS (Table 3, Table S4). The optimal predictive cut-points of CD4+/CD8+ratio and frequency of CD4+ T cell were 1.64 and 39.5. For group with low level of CD4+/CD8+ ratio, 18 of 26 (69%) cases had an OR (CR+PR), while only 5 of 15 (33%) had an OR (P=0.03) for group with higher value of CD4+/CD8+ ratio (Table 1, Figure 2). Log rank analysis revealed that lower level of CD4+/CD8+ ratio was associated with a better PFS (P=0.002) and OS (P=0.007) (Figure 2). In a multivariate analysis, ratio of CD4+/CD8+ remained the significance in predicting PFS (P=0.004, HR=9.23, 95% CI=2.04-41.7) and OS (P=0.009, HR=15.22, 95% CI=2.00-115.8) (Table 3, S3). For group with lower level of frequency of CD4+ T cell, 18 of 25 (72%) cases had an OR (CR+PR), while only 5 of 16 (31%) had an OR (P=0.01) for group with higher value of frequency of CD4+ T cell (Table 1, Figure 3). Log rank analysis revealed that lower level of frequency of CD4+ T cell was associated with a better PFS (P=0.017) and OS (P=0.0495) (Figure 3). In a multivariate analysis, frequency of CD4+ T cell remained the significance in predicting PFS (P=0.02, HR=4.83, 95% CI=1.28-18.27) and OS (P=0.025, HR=16.21, 95% CI=1.43-184.2) (Table 3, S4). Furthermore, NLR was significantly associated with OS in univariate and multivariate analysis.

Table 2

Progression-free survival and associations with clinicopathologic features using Cox regression

Clinicopathologic parameters	HR	95%CI	P value
Age (y)			
Continuous	1.03	0.99-1.07	0.11
Gender			
Female versus male	1.74	0.51-6.0	0.38
Location			
Rectum versus colon	0.53	0.16-1.83	0.32
Grade			
High versus moderate/ low	0.038	0.0-16.09	0.29
KRAS mutation			
Yes versus no	0.30	0.06-1.50	0.14
BRAF mutation			
Yes versus no	0.04	0.00-2165.88	0.56
Frequency of CD4+ T cell ^a (%)			
Continuous	1.09	1.02-1.16	0.012
>39.5 versus ≤ 39.5	4.05	1.17-13.97	0.027
Frequency of CD8+ T cell ^a (%)			
Continuous	0.94	0.87-1.01	0.09
Ratio of CD4+/CD8+ ^a (%)			
Continuous	1.81	1.08-3.01	0.023
>1.64 versus ≤ 1.64	5.99	1.58-22.70	0.008
CEA (ng/mL)			
Continuous	1.002	1.00-1.004	0.09
CRP (mg/L)			
Continuous	1.007	0.997-1.02	0.15
LDH (U/L)			
Continuous	1.002	0.999-1.004	0.26
Neutrophils ^a (10E9/L)			
Continuous	1.27	1.09-1.49	0.002

>4.35 versus ≤ 4.35	1.14	0.35-3.76	0.82
Lymphocytes (10E9/L)			
Continuous	0.77	0.25-2.35	0.65
NLR			
Continuous	1.09	0.99-1.19	0.07
Monocytes (10E9/L)			
Continuous	1.39	0.22-8.72	0.73
Platelets (10E9/L)			
Continuous	0.997	0.99-1.003	0.32
LMR			
Continuous	0.83	0.57-1.22	0.34
PLR			
Continuous	0.999	0.996-1.003	0.68
Alb (g/L)			
Continuous	0.92	0.84-1.01	0.09
CHO (mmol/L)			
Continuous	0.21	0.04-1.21	0.08
TG (mmol/L)			
Continuous	1.57	0.74-3.31	0.24
HDL ^a (mmol/L)			
Continuous	0.11	0.02-0.82	0.03
>0.875 versus ≤ 0.875	0.15	0.04-0.54	0.004
LDL (mmol/L)			
Continuous	1.84	1.11-3.05	0.07
ApoA1 ^a (g/L)			
Continuous	0.03	0.002-0.40	0.008
>0.865 versus ≤ 0.865	0.14	0.04-0.50	0.003
ApoB (g/L)			
Continuous	0.39	0.02-7.31	0.53

^a Optimal cut-off points were estimated by receiver operating characteristics (ROC) curve analysis.

HR= hazard ratio, CI= confidential interval, CEA=carcinoembryonic antigen, CRP=C-reactive protein, LDH=lactate dehydrogenase, ALB= albumin, NLR=neutrophil-to-lymphocyte ratio, PLR=platelet-to-lymphocyte ratio, LMR=lymphocyte-to-monocyte ratio, CHO=cholesterol, TG= triglyceride, HDL=high-density lipoprotein, LDL=low-density lipoprotein, ApoA1=apolipoprotein A1, and ApoB= apolipoprotein B

Table 3

Multivariate survival analysis after variable selection for progression-free survival

Clinicopathologic parameters [#]	HR	95%CI	P value	HR	95%CI	P value
HDL ^a (mmol/L)						
>0.875 versus ≤ 0.875	0.36	0.04-3.31	0.37	0.13	0.01-1.44	0.10
ApoA1 ^a (g/L)						
>0.865 versus ≤ 0.865	0.28	0.03-2.67	0.27	0.56	0.06-5.26	0.61
Frequency of CD4+ T cell ^a (%)						
>39.5 versus ≤ 39.5	4.83	1.28-18.27	0.02			
Ratio of CD4+/CD8+ ^a (%)						
>1.64 versus ≤ 1.64				9.23	2.04-41.69	0.004
[#] Since frequency of CD4+ T cell was strongly correlated with ratio of CD4+/CD8+ with rho value of 0.73 (p<0.001), these two parameters were separately included in the Cox model. ^a Optimal cut-off points were estimated by receiver operating characteristics (ROC) curve analysis. HR= hazard ratio, CI= confidential interval, HDL=high-density lipoprotein, ApoA1=apolipoprotein A1						

Discussion

Anti-PD-1 immunotherapy is approved by FDA for refractory dMMR CRC. In the present multicenter cohort study, response data for 41 patients with dMMR mCRC treated with anti-PD-1 inhibitors were analyzed and potential blood parameters were identified as predictive biomarkers for response. Although tumor mutational burden has potential predictive value for anti-PD-1 therapy in MSI-H mCRC patients, the identification of additional peripheral blood biomarkers is crucial because the access and assay of

biomarkers from blood is more easily than those from tumor tissue. Considering the limited experience with anti-PD-1 therapy in patients with dMMR mCRC, the evidence of potential blood biomarkers for these patients was scarce. To the best of our knowledge, the present study with 41 patients with dMMR mCRC is the first to show that baseline level of frequency of CD4 + T cell and the ratio of CD4+/CD8 + are independent potential biomarkers for ORR and survival. Moreover, the present study indicated the potential prognostic value for NLR with regard to OS.

Previously, pretreatment counts of peripheral blood cells or LDH have been investigated as potential biomarkers for clinical outcomes in patients with melanoma[10, 11, 19] and non-small cell lung cancer (NSCLC)[13, 14, 20] treated with ICIs. Even though the present study showed that NLR could potentially predict OS, it failed to indicate the predictive value of NLR for ORR and PFS in anti-PD-1 therapy, which indicated dMMR mCRC might be distinct with melanoma or NSCLC. As far as we known, this study with 41 patients with dMMR mCRC is the first to show that pretreatment frequency of CD4 + T cell and the ratio of CD4+/CD8 + are independent potential biomarkers for both ORR and survival. Our analysis thus showed that a low ratio of CD4 T cell (≤ 39.5) was significantly associated with a better ORR and PFS/OS in dMMR patients with mCRC. The potential mechanism may be that CD4 + lymphocytes are anergised rather than being stimulated and this might therefore correlate with a poor prognosis[21]. Moreover, the domain type of frequency of CD4 + T cell in the peripheral blood may be regulatory T cells, which have been recently reported to inhibit the anti-tumor activity of cytotoxic frequency of CD4 + T cell and then negatively affect the response and survival of patients undergoing anti-PD-1 immunotherapy[22]. Moreover, the ratio of CD4+/CD8 + is a predictor for ORR and survival. This may be explained not only by the potential pro-tumor activity of regulatory frequency of CD4 + T cell but also by the anti-tumor of frequency of CD8 + T cell. More frequency of CD8 + T cell in blood represents systematic anti-tumor immune features and they could migrate to the tumor site, lymph nodes and distal sites to enhance anti-cancer ability[17, 23, 24], which was consistent with the findings from a recent study[12] to investigate the peripheral cell to predict response to anti-PD-1 immunotherapy in melanoma. They found the reduction of frequency of CD8 + T cell in the peripheral blood responders as compared to that in the blood of non-responders, which also indicated the crucial role in response to anti-PD-1 therapy. Moreover, NICHE trial indicated that increase of frequency of CD8 + T cell counts in CRC might reflect an underlying immune activation[4].

Although our data revealed that HDL and ApoA1 were significantly associated with PFS and OS in univariate analysis, the significance of HDL and ApoA1 did not maintain in multivariate analysis. ApoA1, as a prominent protein component in HDL, not just has antiapoptotic, anti-inflammatory, and antioxidant functions[25], but also alters tumor associated macrophages (TAMs) from a protumor M2 to an antitumor M1 phenotype[26] and modulates regulatory T cells[27]. A very recent study[18] also inferred that high ApoA1 correlated with higher TIL, which might be the reason for its potential positive impact on PFS.

Limitations of the present study include relatively small sample size of patients and its retrospective nature. Another limitation lies in the absence of external validation of the associations detected in the

present study, which need further large-scale study to validate our findings. Since the present study has not performed the associations for other variables especially TMB that has been indicated predictive value in dMMR cancers, future integrative analysis of circulating immune-based biomarkers with genomic and epigenetic biomarkers for clinical response or survival and prospective trials of MSI-H cancers are warranted to validate their predictive potential. In addition, the specific subtypes of peripheral leukocyte excluding CD4 + and CD8 + immune cells have not been analyzed, although these immune cells had different roles and prognoses in response to anti-PD-1 therapy. Thus, these findings require high content data-generating technologies to provide potential mechanism for the circulating immune system and its correlation with the tumor-immune microenvironment.

This is the first multicenter study to reveal that frequency of CD4 + T cell and ratio of CD4+/CD8 + are biomarkers to predict response to anti-PD-1 therapy and survival within an dMMR population. The finding indicates that patients with very low frequency of CD4 + T cell or low ratio of CD4+/CD8 + might respond well, and this subset of patients might be further selected to receive first-line treatment with anti-PD-1 immunotherapy, which was consistent with the recent concept that anti-PD-1 immunotherapy is moved to first-line treatment for mCRC[28]. These findings might provide a potential explanation for the variability in response to anti-PD-1 inhibitors in numerous prospective clinical trials in dMMR mCRC and support the potential predictive role of frequency of CD4 + T cell and ratio of CD4+/CD8 + in anti-PD-1 immunotherapy.

Conclusions

Ratio of CD4+/CD8 + and frequency of CD4 + T cell might be crucial independent biomarkers within dMMR mCRC to better identify patients for response to PD-1 inhibitors. If validated in prospective clinical trials, ratio of CD4+/CD8 + and frequency of CD4 + T cell might provide aid in guiding the treatment of PD-1 inhibitors in dMMR mCRC.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of Sun Yat-sen University, the Sixth Affiliated Hospital. Written informed consent from patients was waived due to the retrospective nature of our study.

Consent for publication

Not applicable.

Availability of data and materials

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declare no potential conflicts of interest.

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Authors' contributions

Ping Lan, Yi-Kan Cheng, Shu-Biao Ye, Zhen-Sen Lin, Dong-Wen Chen planned the conception and design. Ping Lan, Yi-Kan Cheng, Shu-Biao Ye provided financial support. Ping Lan provided the study materials or patients. Yi-Kan Cheng, Shu-Biao Ye, Ping Chen, Shao-Xia Chen, Pei-Si Li collected and assembled data. Yi-Kan Cheng, Shu-Biao Ye, Ping Lan, Ping Chen, Pei-Si Li analysed and interpreted data. All authors drafted the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Flowchart depicting patient selection. PD-1, programmed cell death 1; MMR, mismatch repair; dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

Figure 2

The ratio of CD4+/CD8+ is predictive of response and survival outcome. Optimal cut-off point was calculated by receiver operating characteristics (ROC) curve analysis to dichotomize patients into high and low groups. (A) ratio of CD4+/CD8+ distribution is visualized by a histogram between treatment response groups (CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease). (B) Kaplan-Meier survival curves for progression-free survival and (C) Kaplan-Meier survival curves for overall survival.

Figure 3

Frequency of CD4+ T cell is predictive of response and survival outcome. Optimal cut-off point was calculated by receiver operating characteristics (ROC) curve analysis to dichotomize patients into high and low groups. (A) Frequency of CD4+ T cell distribution is visualized by a histogram between treatment response groups (CR, complete response; PR, partial response; SD, stable disease; PD, progressive

disease). (B) Kaplan-Meier survival curves for progression-free survival and (C) Kaplan-Meier survival curves for overall survival.

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