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B2M and *JAK1/2*–mutated MSI-H Colorectal Carcinomas Can Benefit From Anti-PD-1 Therapy

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Summary: β2-microglobulin (*B2M*) and Janus kinases 1 and 2 (*JAK1/2*) mutations have been suggested as genetic mechanisms of immune evasion for anti–programmed cell death protein 1 (PD-1) therapy. Whether *B2M* and *JAK*1/2 lose-of-function mutation can cause primary resistance to anti-PD-1 therapy in colorectal carcinoma (CRC) patients remains controversial. Here, we sought to compare the efficacy of anti-PD-1 therapy in DNA mismatch repair deficient/microsatellite instability–high CRC patients with or without *B2M* or *JAK1/2* mutations. Thirty-Five CRC patients who received anti-PD-1 therapy were enrolled in this study. All tumor samples underwent next-generation sequencing. The clinical and molecular data from 110 CRC patients sequenced with the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay and accessed through cBioportal were also analyzed in this study. Of the 35 CRC patients from our center, 10 (28.6%) had a *B2M* loss-of-function mutation, and 8 (22.9%) had a *JAK1/2* loss-of-function mutation. Compared with *B2M* wild-type CRCs, *B2M*-mutated CRCs did not show a higher frequency of resistance to anti-PD-1 therapy ($P = 0.71$). There was even better response to anti-PD-1 therapy in patients with *JAK1/2* mutation than in those without ($P = 0.015$). Of the 110 CRC patients in the MSK-IMPACT datasets, 13 (11.8%) had a *B2M* mutation, and 15 (13.6%) had a *JAK1/2* mutation. After analyzing the response to anti-PD-1 therapy in these 110 patients, we found similar results ($P = 0.438$ and 0.071, respectively). Moreover, patients with *B2M* or *JAK1/2* mutation had a lower tumor mutational burden score compared with those without. *B2M* and *JAK1/2* loss-of-function mutations occur frequently in microsatellite instability–high CRC. Our study demonstrated that patients with CRC harboring *B2M* or *JAK1/2* mutations should not be excluded from anti-PD-1 therapy.

Key Words: *B2M*, *JAK1/2*, colorectal carcinoma, anti-PD-1 therapy

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Colorectal carcinoma (CRC) is a genetically heterogenous disease, and ~10%–15% of all CRCs are identified as DNA mismatch repair deficient (dMMR)/microsatellite instability–high (MSI-H).[1,2] This feature can cause high mutational loads, high levels of tumor neoantigens, dense infiltration of $CD8^+$ T cells, and upregulation of programmed

TABLE 1. Baseline Characteristics

Factors	N = 35 [n (%)]
Age (y)	
≤40	20 (57.1)
>40	15 (42.9)
Sex	
Male	28 (73.6)
Female	7 (26.4)
Lynch syndrome	
Lynch	25 (71.4)
Sporadic	10 (28.6)
Tumor location	
Rectum	9 (25.7)
Left-sided	12 (34.3)
Right-sided	14 (40.0)
RAS status	
Wild-type	15 (42.9)
Mutant-type	20 (57.1)
PIK3CA status	
Wild-type	17 (48.6)
Mutant-type	18 (51.4)
MLH1	
Present	18 (51.4)
Absent	17 (48.6)
MSH2	
Present	19 (54.3)
Absent	16 (45.7)
MSH6	
Present	18 (51.4)
Absent	17 (48.6)
PMS2	
Present	17 (48.6)
Absent	18 (51.4)
Clinical settings	
Neoadjuvant	30 (85.7)
Metastatic	5 (14.3)
B2M mutation	
Wild-type	25 (71.4)
Mutant-type	10 (28.6)
JAK1/2 mutation	
Wild-type	27 (77.1)
Mutant-type	8 (22.9)
PD-1 response	
Response	23 (65.7)
No response	12 (34.3)

B2M indicates β2-microglobulin; *JAK1/2*, Janus kinases 1 and 2; PD-1, programmed cell death protein 1.

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TABLE 2. Comparison of Baseline Information Between 2 *B2M* Status

	n (%)		
	***B2M* Wild**	***B2M* Mutated**	***P***
Age (y)			0.46
≤40	13 (52.0)	7 (70.0)	
>40	12 (48.0)	3 (30.0)	
Sex			0.16
Male	22 (88.0)	6 (60.0)	
Female	3 (12.0)	4 (40.0)	
Lynch syndrome			0.69
Lynch	17 (68.0)	8 (80.0)	
Sporadic	8 (32.0)	2 (20.0)	
TMB > 20			0.54
No	3 (12.0)	0 (0.0)	
Yes	22 (88.0)	10 (100.0)	
Tumor location			0.47
Left-sided	16 (64.0)	5 (50.0)	
Right-sided	9 (36.0)	5 (50.0)	
RAS mutation			0.46
Wild-type	12 (48.0)	3 (30.0)	
Mutant-type	13 (52.0)	7 (70.0)	
PIK3CA mutation			0.71
Wild-type	13 (52.0)	4 (40.0)	
Mutant-type	12 (48.0)	6 (60.0)	
Clinical response			0.53
CR_PR	16 (64.0)	7 (70.0)	
SD_PD	9 (36.0)	3 (30.0)	

B2M indicates β2-microglobulin; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; TMB, tumor mutational burden.

TABLE 3. Comparison of Baseline Information Between 2 *JAK1/2* Status

	n (%)		
	***JAK1/2* Wild**	***JAK1/2* Mutated**	***P***
Age (y)			0.42
≤40	14 (51.9)	6 (75.0)	
>40	13 (48.1)	2 (25.0)	
Sex			1.00
Male	21 (77.8)	7 (87.5)	
Female	6 (22.2)	1 (12.5)	
Adenocarcinoma			0.70
Yes	10 (37.0)	4 (50.0)	
No	17 (63.0)	4 (50.0)	
Lynch syndrome			1.00
Lynch	19 (70.4)	6 (75.0)	
Sporadic	8 (29.6)	2 (25.0)	
TMB > 20			**0.009**
No	0 (0.0)	3 (37.5)	
Yes	27 (100.0)	5 (62.5)	
Tumor location			1.000
Left-sided	16 (59.3)	5 (62.5)	
Right-sided	11 (40.7)	3 (37.5)	
RAS mutation			0.42
Wild-type	13 (48.1)	2 (25.0)	
Mutant-type	14 (51.9)	6 (75.0)	
PIK3CA mutation			0.44
Wild-type	12 (44.4)	5 (62.5)	
Mutant-type	15 (55.6)	3 (37.5)	
Clinical response			**0.032**
CR_PR	15 (55.6)	8 (100.0)	
SD_PD	12 (44.4)	0 (0.0)	

Bold values are statistically significant at $P < 0.05$.

CR indicates complete response; *JAK1/2*, Janus kinases 1 and 2; PD, progressive disease; PR, partial response; SD, stable disease; TMB, tumor mutational burden.

death-ligand 1 (PD-L1),[3,4] making dMMR/MSI-H CRC patient suitable for immunotherapy such as anti–programmed cell death protein 1 (PD-1) therapy.[5] Recently, PD-1 inhibitors have been approved by Food and Drug Administration (FDA) for dMMR/MSI-H metastatic CRC patients.[6] However, nearly 40%–70% of these patients would not respond, with 10%–28% experiencing early progression.[7–9] There are several mechanisms of resistance to anti-PD-1 therapy in microsatellite instability (MSI) cancer, among which mutations in β2-microglobulin (*B2M*) and Janus kinases (*JAK1* and *JAK2*) have been widely reported.[4,10]

The *B2M* gene encodes the protein β2-microglobulin, an extracellular component of major histocompatibility complex (MHC) class I molecules that is present on every nucleated cell in the human body. MHC class I molecules are essential for proper and stable antigen presentation.[11] Therefore, the absence of MHC class I caused by *B2M* loss-of-function mutation theoretically may lead to resistance to PD-1 blockade, which has been supported by some preclinical models.[12,13]

Similarly, *JAK1/2* loss-of-function mutations can lead to resistance to anti-PD-1 therapy.[10,14] Inactivation of *JAK1* and *JAK2* impairs the downstream signaling of interferon (IFN) γ receptors and thus lessens the ability of IFN-γ to exert antitumor effects. In some preclinical models, cell lines with *JAK1/2* knockout became insensitive to IFN and resulted in nonresponse to anti-PD-1 treat.[13] *JAK* loss-of-function mutations are found in many types of cancers that show primary resistance to anti-PD-1 therapy, including dMMR/MSI-H CRCs.[14]

These results are meaningful because they suggest that *B2M* and *JAK* loss-of-function mutations could be used as a biomarker to identify patients who might not benefit from an anti-PD-1/PD-L1 therapy. We acknowledge that the biology of these loss-of-function mutations is not simple and clear, as several well-documented cases of patients with *B2M* mutations at baseline were reported to respond to anti-PD-1 therapy, and pharmacological *JAK1/2* inhibition can restore the response of immune checkpoint blockade-resistant tumors.[15–17] The aim of the current study was to compare the efficacy of anti-PD-1 therapy in CRC patients with or without *B2M* or *JAK1/2* mutations.

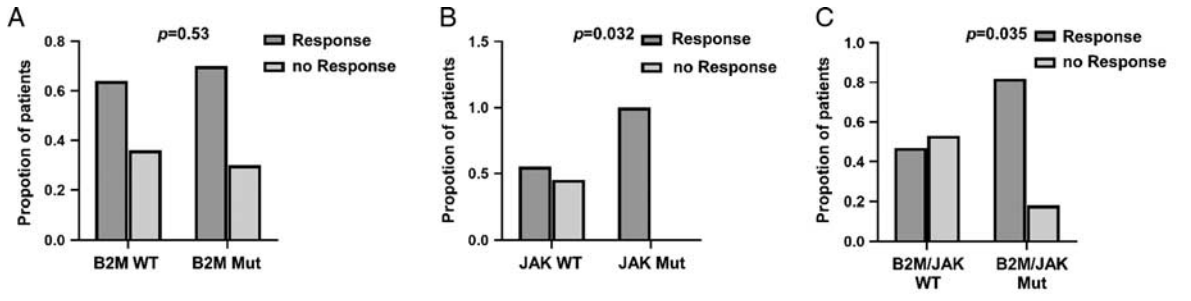
METHODS

Baseline Data Collection

We reviewed 78 patients who received anti-PD-1 therapy for CRC in Sun Yat-sen University Cancer Center. Of them, 40 underwent next-generation sequencing (NGS). Two identified as microsatellite stable and 3 receiving anti-PD-1 as adjuvant therapy after surgery were excluded. Finally, 35 patients who received anti-PD-1 therapy and underwent NGS were included in our study. Demographic and clinicopathologic data were collected from hospital records, including sex, age, and primary tumor site. *RAS*, *PIK3CA*, *MLH1*, *MSH6*, *PMS2*, *B2M*, and *JAK1/2* status were tested through NGS.

Treatment and Response Assessment

All patients enrolled in this study received at least 2 courses of anti-PD-1 therapy with or without chemotherapy. Anti-PD-1 therapy used included pembrolizumab,



Clinical response	B2M WT	B2M Mut	Total
Response	16	7	23
no Response	19	3	12
All	25	10	35

Clinical response	JAK WT	JAK Mut	Total
Response	15	8	23
no Response	12	0	12
All	27	8	35

Clinical response	B2M/JAK WT	B2M/JAK Mut	Total
Response	8	15	23
no Response	9	3	12
All	17	18	35

FIGURE 1. Association between β2-microglobulin (*B2M*]) and Janus kinases 1 and 2 (*JAK1/2*) mutation status and clinical response in our cohort. A, There was no significant difference between *B2M* mutation status and clinical response ($P = 0.53$). B, A better clinical response was observed in patients with *JAK1/2* mutation compared with wild-type ($P = 0.032$). C, Taken *B2M* and *JAK1/2* mutation status together, the better clinical response to anti–programmed cell death protein 1 therapy was also observed in patients with *B2M* or *JAK1/2* mutation type ($P = 0.035$).

nivolizumab, sintilimab, toripalimab, or camrelizumab. The recommended anti-PD-1 therapy dose was 200 mg for pembrolizumab, sintilimab, or camrelizumab, and 3 mg/kg for nivolizumab or toripalimab. Tumor responses to anti-PD-1 therapy were evaluated after at least 2 courses. Response Evaluation Criteria in Solid Tumors (RECIST) scores were assessed via radiologic data and classified as follows: complete response (CR), disappearance of all target lesions; partial response (PR), $\geq 30\%$ decrease; progressive disease, $\geq 20\%$ increase over smallest sum observed; and stable disease, meeting none of the other criteria. Responders were defined as patients achieving PR or CR.

cBioportal Datasets

The frequency of *B2M* and *JAK1/2* mutations was assessed using the cBioportal for a Memorial Sloan Kettering Cancer Center (MSKCC) database, which included the details for >1000 patients with various cancer types sequenced with the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay. Of them, 110 were CRC patients who received anti-PD-1 therapy. Further, we assessed the overall survival (OS) and tumor mutational burden (TMB) with the *B2M* and *JAK1/2* gene status as queried with cBioportal.

Statistical Analyses

The χ^2 test or Fisher exact test were employed to analyze the associations between the *B2M* or *JAK1/2* mutation status and clinicopathologic variables. A 2-sided *P*-value <0.05 was considered statistically significant. Statistical analysis was performed in SPSS (version 25.0). The key raw data have been uploaded to the Research Data Deposit public platform (www.researchdata.org.cn) with approval number RDDA2022997199.

RESULTS

Baseline Information

Table 1 shows the baseline information of the selected patients. All of the 35 patients were MSI-H/dMMR and received anti-PD-1 therapy. The median age was 39.0 years, and 7 (26.4%) were female. About two third of the patients (25/35) were Lynch syndrome carriers. Tumor sequencing was performed in all of the 35 patients, 10 (28.6%) of whom were *B2M* mutant-type, 8 (22.9%) were *JAK* mutant-type, and none of them had both *B2M* and *JAK1/2* mutation. Twenty-three of the 35 patients responded to anti-PD-1 therapy.

Comparison of Baseline Information Between 2 *B2M*/*JAK* Status

Table 2 shows comparisons between patients with different *B2M* status. There were no significant differences in age, sex, and other basic information between *B2M* wild and mutational types. TMB score have been reported as a predictive biomarker for anti-PD-1 therapy. Here, we did not find a statistic difference in TMB score between the 2 groups ($P = 0.54$).

Similarly, basic clinical information was compared in *JAK1/2* wild and mutational types (Table 3). There were also no significant differences in age, sex, histology, and other basic information between the 2 *JAK1/2* status. We observed a low TMB score in patients with *JAK1/2* mutation ($P = 0.009$). Other gene status like RAS and PIK3CA were not associated with *B2M* and *JAK1/2* mutation status.

Association Between *B2M* and *JAK1/2* Mutation and PD-1 Clinical Response

As for *B2M* status, the response to anti-PD-1 therapy had no significant difference between wild and mutational types ($P = 0.53$, Fig. 1A). As for *JAK1/2* status, we even observed a better clinical response in patients with *JAK1/2* mutation ($P = 0.032$, Fig. 1B), regardless of their low TMB score. Combined *B2M* and *JAK1/2* status together, the better clinical response to anti-PD-1 therapy was also observed in patients with *B2M* or *JAK1/2* mutation type ($P = 0.035$, Fig. 1C). These results are significant because it indicates that the resistance mechanism of anti-PD-1 therapy for CRC may be different from other solid tumors.

Verification With cBioPortal Datasets

To further verify the association between *B2M* and *JAK1/2* status with clinical response to anti-PD-1 therapy, we used the cohort from a MSKCC research on cBioPortal. This cohort included 110 CRC patients who received anti-PD-1 therapy. Of the 110 patients, 13 (11.8%) had a *B2M* mutation, 15 (13.6%) had a *JAK1/2* mutation, and 6 (5.5%) had both mutation (Fig. 2A). Patients with *B2M* mutation type showed no difference on OS compared with wild-type (69.2% vs.

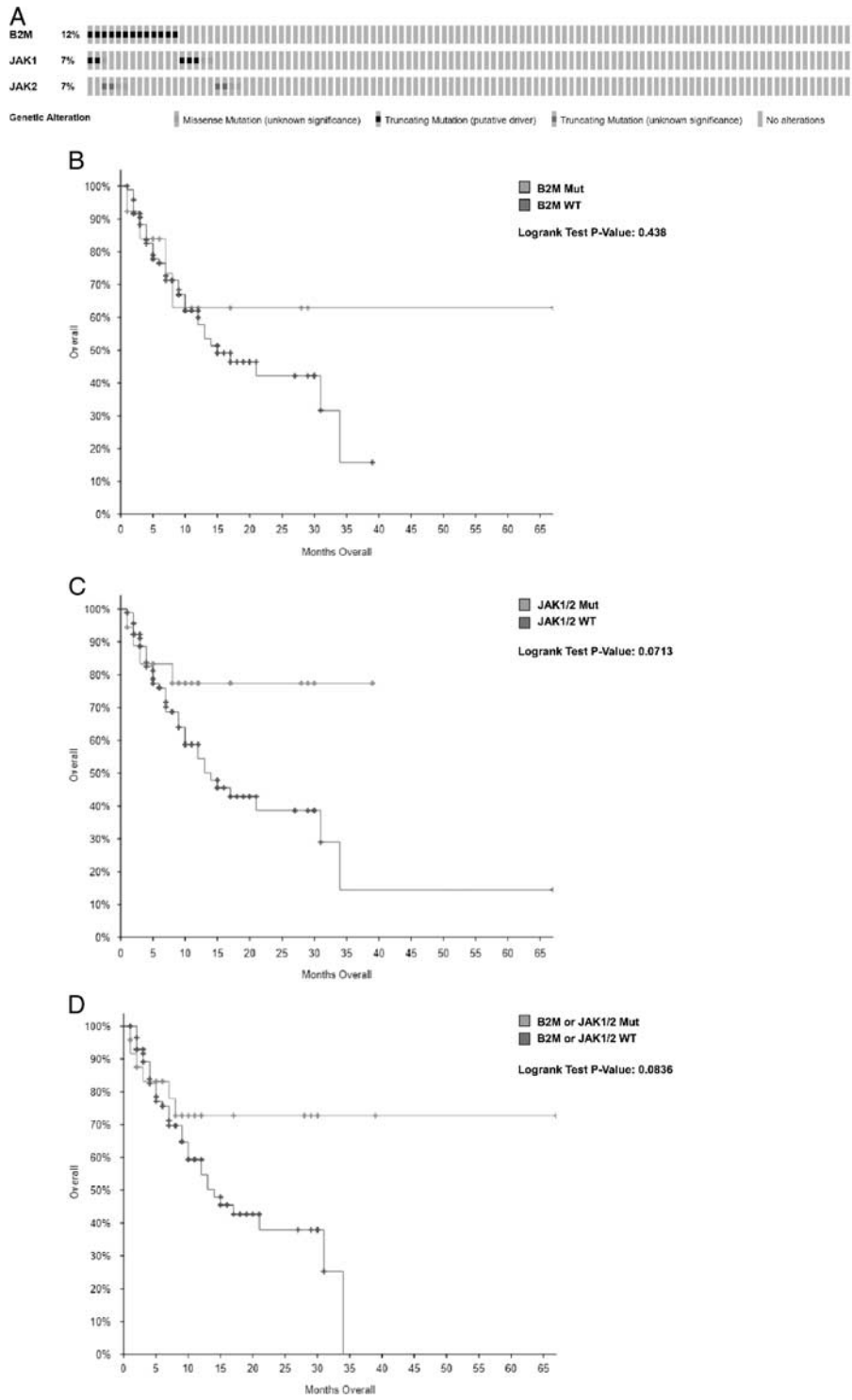


FIGURE 2. Association between β2-microglobulin (*B2M*) and Janus kinases 1 and 2 (*JAK1/2*) mutation status and overall survival. A, *B2M* and *JAK1/2* mutation rate was observed in a 110 colorectal carcinoma patients cohort from cBioportal datasets. B, Patients with *B2M* mutation type showed no difference on survival time compared with wild-type. C, Patients with *JAK1/2* mutation type seem to have a longer survival time compared with wild-type. D, A tendency shows that patients with B2M or *JAK1/2* mutation could benefit from anti–programmed cell death protein 1 therapy.

57.7%, respectively, $P = 0.438$, Fig. 2B). While patients with *JAK1/2* mutation seem to have a better OS compared with wild-type (73.3% vs. 56.8%, respectively, $P = 0.0713$, Fig. 2C). Taken *B2M* and *JAK1/2* status together, patients with *B2M* or *JAK1/2* mutation also seem to have a better OS compared with wild-type (72.8% vs. 55.7%, respectively, $P = 0.0836$,

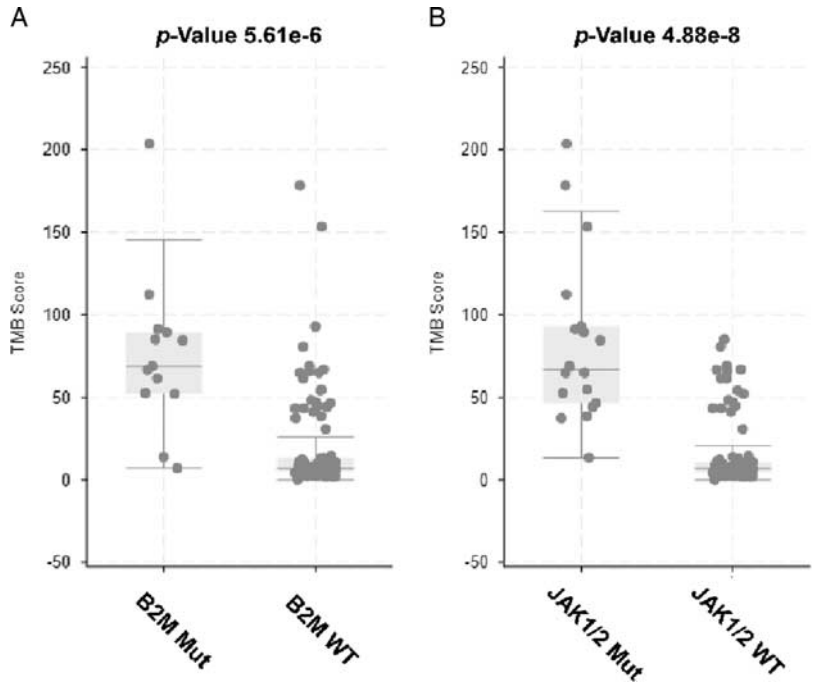


FIGURE 3. Tumor mutational burden score and β2-microglobulin (*B2M*) and Janus kinases 1 and 2 (*JAK1/2*) mutation status. A and B, Patients in this cohort with either *B2M* or *JAK1/2* mutation type had a higher tumor mutational burden score.

Fig. 2D). It is interesting to note that, these patients had a higher TMB than patients without *B2M* or *JAK1/2* mutations (Fig. 3). It seems that TMB rather than *B2M* or *JAK1/2* status is predictive of the efficacy of anti-PD-1 therapy.

DISCUSSION

Although anti-PD-1 therapy has proven to be an ideal therapy for dMMR/MSI-H CRC, <50% of the patients could reach CR or PR. Primary resistance to anti-PD-1 therapy is one of the most important reasons for treatment failure, but the mechanism remains unclear. Our study reveals that dMMR/MSI-H CRCs with *B2M* and *JAK1/2* mutations are responsive to PD-1 inhibitors. This finding is contrary to that in lung cancer and malignant melanoma, indicating that the mechanism of resistance to anti-PD-1 therapy in CRC may be different from that in other solid tumors. The results were echoed by those of MSKCC-IMPACT. In a MSKCC research, patients with *B2M* or *JAK1/2* mutation could benefit from anti-PD-1 therapy.

Defects in antigen presentation and disturbance of IFN-γ signal pathway are thought to be possible mechanisms of resistance to anti-PD-1 therapy.[18,19] *B2M* loss-of-function mutation cause the absence of MHC class I and *JAK1/2* loss-of-function mutation lead to impairment of the IFN-γ receptors. In some preclinical models, it has been shown that *B2M* and *JAK1/2* mutation can cause resistance to anti-PD-1 therapy. Moreover, Zaretsky et al[10] found resistance-associated loss-of-function *JAK1/2* mutation in 2 of the4 patients with melanoma and a *B2M* truncating mutation in a third patient. In 2 patients with non–small cell lung cancer not responsive to anti-PD-1 therapy, Rizvi et al[20] found one of them had a homozygous deleterious mutation. However, the sample sizes of these studies are small, so it remains debatable whether *B2M* and *JAK1/2* mutations are a contraindication for anti-PD-1 therapy.

Unlike with melanoma and lung cancer, *B2M* and *JAK1/2* loss-of-function mutations do not necessarily confer resistance to anti-PD-1 therapy in MSI-H CRC. Snahnicanova et al[12] found no significant difference in TILs and peritumoral lymphoid reaction between patients with *B2M* mutation and patients without. It was also shown that the infiltration of $CD3^+$ and $CD8^+$ T cells was not significantly correlated with the presence of *B2M* mutations.[21,22] Furthermore, Middha et al[23] reported that most patients with *B2M*-mutant MSI-H CRC could still benefit from anti-PD-1 therapy. In our study, we did not observe any correlation between *B2M* or *JAK1/2* mutations and a poor response. Consistent with our hypothesis, MSI status rather then *B2M* or *JAK1/2* status contribute to clinical response to anti-PD-1 therapy.

Despite the fact that *B2M* loss-of-function mutation can lead to the absence of MHC I class molecules and that *JAK1/2* loss-of-function mutation can cause disturbance of IFN-γ signal pathway, the absence of *B2M* mutation still cannot be used as a negative predictor of immune checkpoint therapy. One possible reason is that not all the *B2M* mutations cause T-cell ignorance. Middha et al[23] reported that *B2M* protein loss was not correlated with loss of MHC class I expression. Janikovits et al[21] established *B2M* mutation as a consequence of high PD-1-positive T-cell counts in MSI cancers. In addition, studies have shown that the activation of innate and adaptive immunity mediated by natural killer (NK) T cells, $CD4^+$ T cells, and $CD8^+$ T cells could overcome the influence of *B2M*/*JAK* knockout *in vivo*.[13] Germano et al[24] reported that the efficacy of immunotherapy against dMMR *B2M* null tumors did not require $CD8^+$ T cells but relied on the presence of $CD4^+$ T cells. In fact, CD1 family is required for NK recognition, and it is reported that CD1a, CD1b, and CD1c were absent from the surfaces of *B2M*-deficient cells.[25] Therefore, it seems that $CD4^+$ T cells rather than NK and $CD8^+$ T cells influence the immunotherapy response in *B2M*/*JAK* mutation CRC. However, the potential molecular mechanism needs to be further clarified.

KRAS and *PIK3CA* mutations are frequently found in CRC, which are present in 20%–35% and 14%–25% of CRC

patients, respectively.[26–30] *KRAS* and *PIK3CA* mutations have been associated with a worse clinical outcome and with a negative prediction of response to targeted therapy by anti-EGFR monoclonal antibodies.[31] However, whether the mutations of *KRAS* and *PIK3CA* influence the response to anti-PD-1 therapy have not been confirmed. Song et al[32] found that the mutation of *KRAS* gene in non–small cell lung cancer tissues was an independent predictor of the long-term benefit of immunotherapy.[13] Mishima et al[33] found that patients with *PIK3CA* mutations had a higher overall response rate in advanced gastric cancer. However, in subgroup analysis of the KEYNOTE 177 study, the RAS status did not influence the response rate of anti-PD-1 therapy compared with chemotherapy.[34] In our study, the *RAS* and *PIK3CA* status were not a prognostic factor of anti-PD-1 therapy. Therefore, the influence of *RAS* and *PIK3CA* status on the response of anti-PD-1 therapy need to be further verified.

There are some limitations in our study. First, receiving chemotherapy or not is associated with prognosis, but due to the scarcity of cases (n = 35), it was not analyzed in this study. Second, the radiologic assessment of tumor response to anti-PD-1 therapy is based mainly on the subjective judgment of the radiologist, and subjective volume estimation provides poor results. Last, some factors were dispensed with in the study due to a lack of samples, including the state of POLE mutation, the carcinoembryonic antigen level, and the dose of PD-1 inhibitors. Therefore, larger, prospective studies are needed to clarify if the response rate and duration of response vary by *B2M* and *JAK1/2* mutation status.

CONFLICTS OF INTEREST/FINANCIAL DISCLOSURES

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All authors have declared that there are no financial conflicts of interest with regard to this work.

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