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Gastrointestinal Cancer

Regorafenib and Nivolumab or Pembrolizumab Combination and Circulating Tumor DNA Response Assessment in Refractory Microsatellite Stable Colorectal Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Regorafenib • Nivolumab • Microsatellite stability • Colorectal cancer

Abstract

Background. Metastatic colorectal cancers (MCRCs) with microsatellite stability (MSS) are resistant to immunotherapy with programmed cell death protein 1 (PD-1) and programmed death-ligand 1 inhibitors. However, the addition of regorafenib to nivolumab was recently associated with a high response rate and a protracted progression-free survival in a small cohort of MSS Japanese patients with metastatic colorectal cancer.

Materials and Methods. We evaluated the outcome of patients with MSS metastatic colorectal cancer who were treated on a compassionate basis with PD-1 inhibitors in combination with regorafenib in a single U.S. center.

Results. A total of 18 patients were treated with a combination of regorafenib and PD-1 inhibitors. No treatment-related grade 3 or above toxicities were noted. Thirteen patients (69%) had progressive disease, and five patients (31%) experienced stable disease as best response. Four out of five stable diseases occurred in patients without liver metastases, whereas only 1 of 14 patients with history of liver metastases had a short disease stabilization. A rise in circulating tumor DNA (ctDNA) at the 4-week time pointuniversally predicted tumor progression at 2 months, whereas a decline was associated with radiographic disease stabilization.

Conclusions. Regorafenib and nivolumab combination was associated with modest clinical activity in patients with MSS chemotherapy-resistant metastatic colorectal cancer. Selection for patients without history of liver metastases may identify a cohort of patients with MSS colorectal cancer with a higher likelihood of benefit from this combination. ctDNA may represent a powerful tool for predicting early therapeutic efficacy of immunotherapy in the MSS colorectal cancer population. ***The Oncologist*** 2020;25:e1188–e1194

Implications for Practice: This study showed that the combination of regorafenib and nivolumab was associated with a modest clinical activity in patients with advanced microsatellite stability (MSS) metastatic colorectal cancer. This combination should be avoided in clinical practice, especially in patients with MSS colorectal cancer with liver metastases. Further investigation of regorafenib plus PD-1 inhibitors should be considered in MSS colorectal cancer without liver metastases.

Introduction

Colorectal cancers with microsatellite instability (MSI-H) are associated with high mutation load, increased tumor infiltrating lymphocytes, and high expression of checkpoints such as programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 and lymphocyte-activation gene 3 [1]. Microsatellite stable (MSS) colorectal cancers are considerably less mutated and are characterized by a less inflamed tumor immune microenvironment [1]. Although the targeting of PD-1 has been associated with robust clinical responses in colorectal cancer with MSI-H, limited antitumor activity was observed in MSS colorectal cancers even after selecting for programmed death-ligand 1 (PD-L1) expression [2, 3].

Regorafenib is a small molecule tyrosine kinase inhibitor with inhibitory activity against multiple targets involved

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Table 1. Patients characteristics

Age, yr	Sex	Race	Primary tumor location	Prior liver metastasis resection	Sites of metastases when on treatment	Prior PD-1/ PD-L1 inhibitor	Duration and response on prior PD-1/PD-L1 inhibitor, mo	ECOG PS	Regimen	TMB (mutations per Mb)	No. of Cycles	Response
55	M	White	Left	Yes	Lung	Yes	PD (2)	0	Rego + nivo	3	2	PD
75	M	White	Right	Yes	Lung	No		0	Rego + nivo	3	2	PD
53	M	White	Left	No	Lung, peritoneum	Yes	SD (16)	1	Rego + nivo	2	8	SD
67	M	Asian	Left	No	Lung	Yes	SD (8)	1	Rego + pem	4	9	SD
52	M	White	Right	No	Liver, lymph node	No		1	Rego + nivo	7	2	PD
73	M	Asian	Right	Yes	Lung	No		1	Rego + nivo	11	2	PD
65	M	White	Left	Yes	Liver, peritoneal, spleen, RPLN	No		1	Rego + nivo	4	2	PD
65	M	White	Right	No	Liver, lung, lymph node	Yes	PD (1)	1	Rego + nivo	NA	2	PD
60	M	NA	Left	No	Lung, RPLN	No		1	Rego + nivo	5	7	SD
57	M	NA	Left	Yes	Lung	No		0	Rego + nivo	7	4	SD
75	M	Asian	Left	Yes	Liver, lung, bone	No		1	Rego + nivo	6	2	PD
43	M	Asian	Left	Yes	Liver, lung, peritoneum, lymph node	Yes	PD (1)	1	Rego + nivo	NA	4	PD
48	M	White	Left	Yes	Liver, lung	No		1	Rego + nivo	6	3	PD
67	F	White	Left	Yes	Lung, RPLN, hilar adenopathy, brain	No		0	Rego + nivo	NA	2	PD
54	M	Asian	Left	Yes	Liver, lung, RPLN	No		1	Rego + nivo	2	2	PD
60	M	White	Right	Yes	Lung	Yes	SD (5)	1	Rego + nivo	2	1	PD
47	M	White	Left	No	Pelvic	No		0	Rego + nivo	4	4	SD
79	F	White	Right	No	Liver, lymph nodes	No		1	Rego + nivo	6	1	PD

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; F, female; M, male; NA, not available; Nivo, nivolumab; PD, progressive disease; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; Pem, pembrolizumab; Rego, regorafenib; RPLN, retroperitoneal lymph node; SD, stable disease; TMB, tumor mutation burden.

in tumor angiogenesis and oncogenesis. Preclinical studies have demonstrated the enhanced concomitant antitumor activity of regorafenib and anti–PD-1 in in vivo colorectal cancer models [4]. Mechanistically, this effect may be mediated by (a) a reduction of tumor-associated macrophages (TAMs), (b) reprogramming of TAMs toward an M1 phenotype by the inhibition of colony-stimulating factor 1 receptor by regorafenib [4], (c) suppression of interferon gamma–induced PD-L1 and indoleamine 2, 3-dioxygenase 1 expression [5], and (d) inhibition of vascular endothelial growth factor receptor and its signaling pathway, which may normalize tumor blood vessels and thereby improve cytotoxic T cell infiltration [6]. A recent study of regorafenib and nivolumab reported a 33% response rates and a median progression-free survival of more than 6 months in a cohort of 24 Japanese patients with MSS chemoresistant metastatic colorectal cancers [7]. These findings were met with hope and excitement within the medical oncology community in the U.S., with many centers considering the combination of regorafenib and PD-1 inhibitors on compassionate basis for refractory MSS colorectal cancers.

Here, we report on our experience with the compassionate administration of regorafenib plus PD-1 inhibitors in patients with advanced, refractory, MSS colorectal cancers.

Subjects, Materials, and Methods

We performed a retrospective review of all patients with MSS colorectal cancer treated at a City of Hope Comprehensive Cancer Center who received a PD-1 inhibitor in combination with regorafenib on a compassionate basis. Eligibility for inclusion included the receipt of the combination of regorafenib plus nivolumab or the combination of regorafenib plus pembrolizumab in the setting of MSS colorectal cancer and following disease progression on standard of care therapy, which included fluorouracil, oxaliplatin, and irinotecan. Because the intent of the combination was synergistic, patients with prior exposure to regorafenib monotherapy or to an anti–PD-1 monotherapy prior to receipt of the combination were also included. Responses were assessed by the study investigator using RECIST 1.1 guidelines. Progression-free survival was measured from start of treatment to time of progression. The study was conducted under an institutional review board–approved protocol, IRB14361.

Treatment

Patients received regorafenib at 80 mg p.o. once a day for 21 days every 28-day cycle. Patient receiving regorafenib plus nivolumab received nivolumab at 240 mg intravenously every 2 weeks starting day 1 of regorafenib. Patients receiving regorafenib plus pembrolizumab received pembrolizumab at 200 mg intravenously every 3 weeks.

Circulating Tumor DNA Assay

When feasible, patients underwent Guardant360 circulating tumor DNA (ctDNA) assay (Guardant Health, Inc, Redwood City, CA) before initiation of treatment and after cycle 1 of treatment. Guardant360 sequencing technique is based on a next-generation sequencing technology (Guardant Digital Sequencing) with a single-molecule analytical sensitivity and a 99.9999% specificity [8].

Table 2. Patient characteristics summary

Characteristics	Total (n = 18), n %
Median age, (range), yr	60 (43–79)
ECOG PS	
0	5 (27.8)
1	13 (72.2)
Sex	
Male	16 (88.9)
Female	2 (11.1)
Primary tumor location	
Left	12 (66.7)
Right	6 (33.3)
Liver metastases	
Yes	14 (77.8)
No	4 (22.2)
Prior anti-PD-1/PD-L1	6 (38)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

Results

Patient Characteristics

A total of 18 patients with MSS colorectal cancer (16 men, 88.9%) were treated with a combination of regorafenib and PD-1 inhibitors and were evaluable for response. Baseline characteristics are listed in Table 1. All patients had progressed on all prior standard therapies. A total of 17 patients received regorafenib plus nivolumab, and 1 patient received regorafenib plus pembrolizumab. Twelve patients (66.7%) had left-sided primary tumor, and six patients (33.3%) had right-sided primary tumor. Liver metastases were recorded in 14 (77.8%) patients (Table 2). For the four (22.2%) patients without a history of liver metastases, three patients were with lung metastases, and one patient was with pelvic metastases. Six patients had prior anti–PD-1/PD-L1 on clinical trials but no concurrent regorafenib exposure. Two patient had prior regorafenib exposure (2 months, with progressive disease [PD]) but without concurrent anti–PD-1/PD-L1 exposure. Tumor mutation burden (TMB) was available in 15 patients, with low TMB observed among all patients.

Safety

Treatment administration was well tolerated, with no significant toxicities being recorded while on treatment. Hematological toxicities were limited to grade 1 toxicities. Skin toxicities were limited to grade 1 except for one patient who developed a grade 2 immune related dermatitis that responded well to topical steroids (Table 3). No patient required dose reductions or interruptions. No grade 3 or above toxicities were noted on treatment.

Table 3. On-study adverse events (n = 18)

	All cycles				
Adverse event	**Grade 1 No. (%)**	**Grade 2 No. (%)**	**Grade 3 No. (%)**	**Grade 4 No. (%)**	**Grade 5 No. (%)**
Anemia	1 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
WBC decrease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Neutrophil decrease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Platelet decrease	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AST increase	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALT increase	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALP increase	1 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Skin rash	1 (5.5)	1 (5.5)	0 (0.0)	0 (0.0)	0 (0.0)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; WBC, white blood cell.

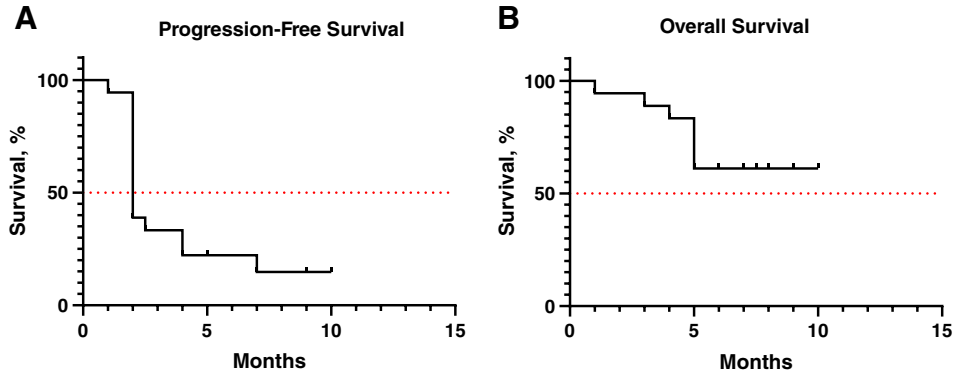

Figure 1. Progression-free survival and overall survival. **(A):** Progression-free survival. **(B):** Overall survival.

Efficacy

All patients were evaluable for treatment response assessment. Per investigator review, which concurred with the official radiology reviews, no objective responses were noted on treatment. Thirteen patients (72.2%) had progressive disease, and five patients (27.8%) experienced stable disease. (Table 1). Six patients included on this study had prior PD-1/PD-L1 therapy. Two of the three patients who had stable disease as a best response on prior immunotherapy experienced stable disease, whereas all three patients who had progressive disease as best response on prior immunotherapy had disease progression. Neither of the two patients who had received prior regorafenib exhibited a response. Median progression-free survival was 2 months (Fig. 1A). The duration of stable disease (SD) was 8+ months in one patient treated with pembrolizumab plus regorafenib (with prior pembrolizumab exposure), 7 months in one patient with prior atezolizumab exposure, 8+ months in one patient, 4 months in one patient, and 4+ months in one patient (Table 4). Notably, four out of five patients with SD had no history of liver metastases, and all 13 patients who had PD had history of liver metastases (Tables 1, 4). Three of the patients with SD were encountered in checkpoint-inhibitor naive patients, whereas two patients with SD (8+ months and 8 months) were seen among the six patients with prior PD-1 or PD-L1 exposures (Table 1). Overall survival analysis remains immature, as the median follow-up is only 7 months (Fig. 1B).

Table 4. Metastatic pattern and duration of benefit in patients with SD

Patient	Metastatic site when on treatment	Prior liver metastases	Duration of SD, mo
1	Lung, peritoneum	no	7
2	Lung	no	8+
3	Lung, RPLN	no	8+
4	Lung	yes	4
5	Pelvic	no	4+

Abbreviations: RPLN, retroperitoneal lymph node; SD, stable disease.

ctDNA and Carcinoembryonic Antigen Monitoring

ctDNA assays were evaluated at baseline and at 4 weeks of treatment in 13 patients (Fig. 2A–D). All 10 patients with a rise in ctDNA or emergence of new clones at 4 weeks had PD at the 2-month imaging point (Fig. 2E). Three patients with declining ctDNA at 4 weeks experienced SD for 8+ months, 7 month, and 4+ months each (Fig. 2F). To investigate the correlation of ctDNA and carcinoembryonic antigen (CEA) in monitoring tumor response, we analyzed 10 PD and 3 SD patients with paired CEA and ctDNA. The dynamics of CEA were shown at baseline, 4 weeks, and 8 weeks after treatment. For 10 patients with PD, 4 patients had a CEA decline at 4 weeks, and 6 patients had a CEA increase at 4 weeks (Fig. 3A). For three patients with SD, two patients had a CEA surge at 4 weeks, followed by a

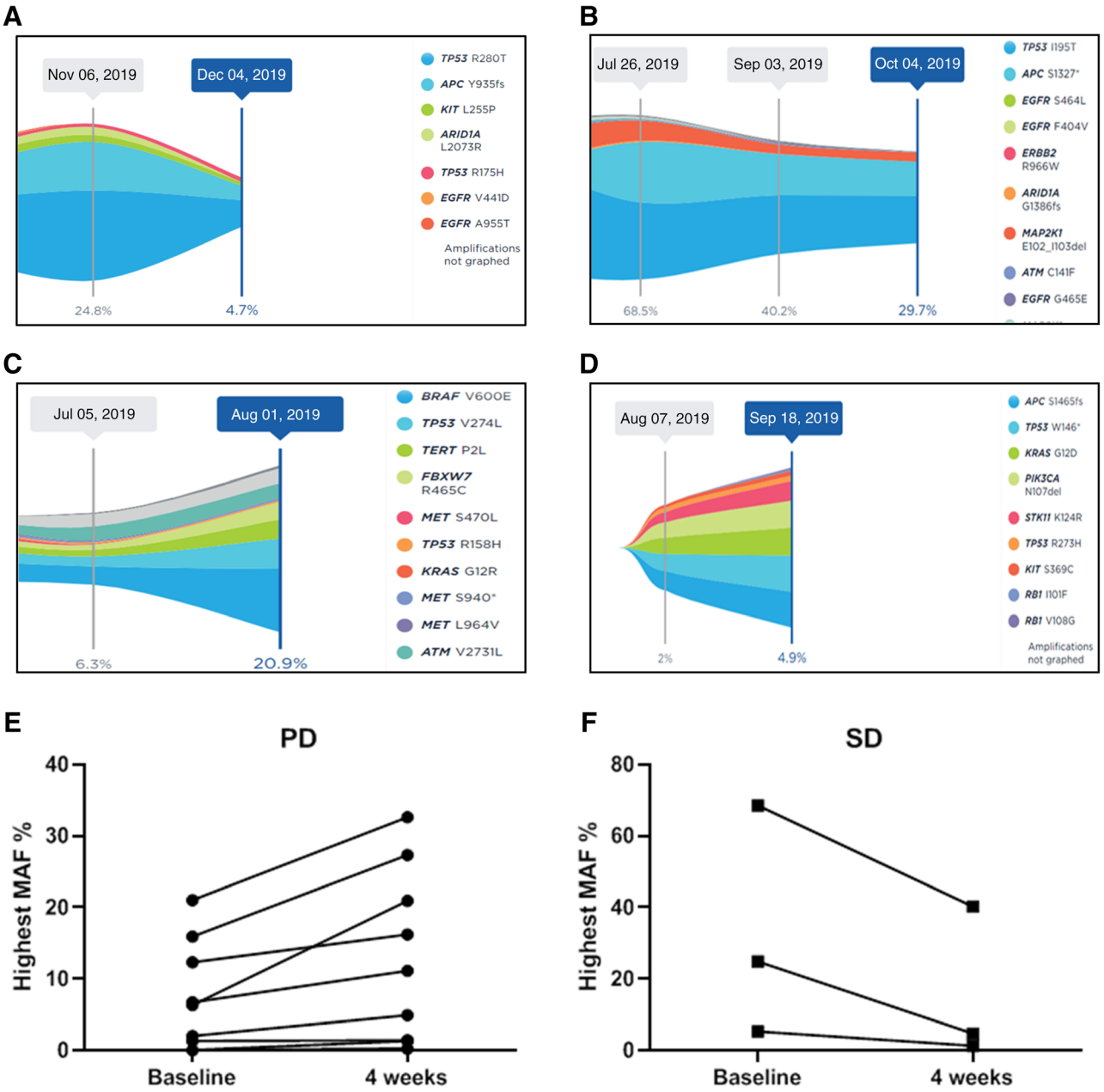

Figure 2. Guradant360 ctDNA assay of patients treated with regorafenib and PD-1 inhibitor. **(A, B):** ctDNA of SD patients. **(C, D):** ctDNA of PD patients. **(E):** The highest MAF change at baseline and 4 weeks after treatment in patients with PD. **(F):** The highest MAF change at baseline and 4 weeks after treatment in patients with SD.
Abbreviations: MAF, mutation allele frequency; PD, progressive disease; SD, stable disease.

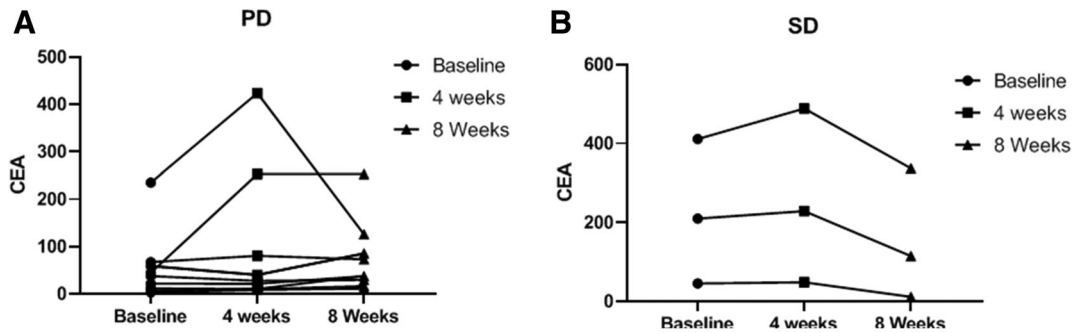

Figure 3. CEA response of patients treated with regorafenib and PD-1 inhibitor. **(A):** CEA changes in patients with progressive disease. **(B):** CEA changes in patients with stable disease.
Abbreviations: CEA, carcinoembryonic antigen; PD, progressive disease; SD, stable disease.

decline (Fig. 3B). ctDNA at 4 weeks was more accurate in predicting a radiographic benefit than CEA.

Discussion

Tumors with microsatellite instability have been associated with robust responses to PD-1 inhibitors [9–12]. Microsatellite stable tumors, known for low tumor mutational burden and lack of significant immune cell infiltration, remain resistant to immunotherapy [1, 2]. Numerous investigations are underway to explore the potential of combination immunotherapies to convert MSS colorectal cancer to an immune-responsive malignancy. Preclinical studies have demonstrated synergy between regorafenib and PD-1 inhibitors in colorectal cancer models [4]. In addition, a recent Japanese trial, the REGONIVO study, reported a robust response rate in Japanese patients with MSS metastatic colorectal cancer [7]. In our study, we did not observe any responses in our patients treated with this regimen. Stable disease was recorded in five (31%) patients, four of which occurred in patients without any existing or history of liver metastases. Progressive disease was observed in 13 (69%) patients, all of whom with existing or history of liver metastases. Interestingly, all clinical benefits occurred in patients with left-sided tumors. However, all six patients with right-sided tumors had history of liver metastases prior to or at the time of treatment with regorafenib and nivolumab. Given this limitation, we cannot assess an independent impact of sidedness on treatment outcome. Clinical benefit was observed in patients with *RAS* mutant (3) and *RAS* wild-type (2).

The pattern of metastatic disease may impact the responsiveness to PD-1/PD-L1 inhibitors. Prior clinical studies have shown that patients with melanoma and non-small cell lung cancer (NSCLC) with liver metastasis have a diminished likelihood of response and a shortened survival when treated with PD-1 inhibitors in comparison to patients with melanoma and NSCLC without liver metastases [13]. Tumor biopsies from these studies revealed lower CD8+ T cell infiltration in the primary tumor of the liver metastasis group when compared with the non-liver metastasis group. In addition, patients with liver metastases had lower CD8+ T cells in extrahepatic distant metastases, which suggest that patients with liver metastases suffer from a diminished antitumor immune response and may be less likely to benefit from checkpoint inhibition. It is also possible that liver metastases have a systemic immunosuppressive effect, which diminishes the immune response both intra and extrahepatically in patients with solid tumors. The fact that liver allografts are accepted without the need for histocompatibility suggests that liver can induce peripheral immune tolerance in immune-competent recipients [14]. In addition, liver transplantation makes liver recipients more tolerant to other organ transplantation from the same donor, suggesting that liver allografts can induce systemic immune suppression [15, 16]. Mechanistically, this phenomenon could be explained by the deletion of activated CD8+ T cells [17, 18], poor CD8+ and CD4+ T cell activation [19, 20], and the activation of regulatory T cells by the liver [21]. Therefore, we hypothesize that liver metastases take advantage of the liver immune tolerance that suppresses systemic antitumor immune response and renders PD-1 inhibitors less effective.

Multiple mechanisms have been proposed to explain the immunomodulatory effect of regorafenib, including reduction of tumor infiltrating macrophages, enrichment in M1 macrophage phenotype, enhanced T cell activation, decreased regulatory T cell infiltration, and decreased inhibitory checkpoints expression such as indoleamine 2,3-dioxygenase [4, 5, 7]. Although no response was recorded in our cohort, the disease control in five patients, four without liver metastases, suggests its clinical activity when combined with nivolumab in a subset of MSS metastatic colorectal cancer. In addition, the protracted SD of 7 months or more in two patients with prior progression on pembrolizumab and atezolizumab provides preliminary evidence suggesting potential synergy between regorafenib and PD-1 inhibitors. It also suggests that select patients who progressed on prior immunotherapy should not be necessarily excluded from consideration for the combination of regorafenib and PD-1 blockade in a clinical trial setting.

In addition to investigating the efficacy of regorafenib and PD-1 inhibitors, we evaluated the merits of ctDNA in predicting for radiographic response to this treatment combination. ctDNA has been suggested as a potential biomarker for monitoring and tailoring treatment in solid tumors. In patients with metastatic colorectal cancer receiving standard first-line or second-line chemotherapy, early reductions in ctDNA were correlated with radiological responses and disease outcome [22, 23]. In the setting of immunotherapy, a reduction in ctDNA levels was associated with clinical response in patients with non-small cell lung cancer, melanoma, and MSI-H colorectal cancer treated with checkpoint blockade. Patients who experienced initial response followed by disease progression had an initial decline at the time of response followed by an increase in ctDNA levels at the time of progression [24–26]. Here, we show that early ctDNA changes can predict for disease control in a small cohort of patients with MSS colorectal cancer treated with immunotherapy. Our paired CEA and ctDNA data analysis also suggest that ctDNA may be more accurate than CEA in monitoring early tumor response to immunotherapy. Given the expense associated with immunotherapy, and especially with the low potential of benefit in MSS colorectal cancers, the identification of an early biomarker of response is important to cut costs and decrease potential toxicities. Our findings will require further validation in a larger cohort of patients.

Our experience with regorafenib and nivolumab did not replicate the high response rate observed in the REGONIVO Japanese trial. We identify liver metastases as a predictive biomarker for lack of efficacy to this combination. It is possible that the discrepancy between our data and the Japanese study is related to differences in patient characteristics such as liver metastases. The REGONIVO trial has been presented at the American Society of Clinical Oncology but has not yet been published. The details of the demographics of that population have not been fully reported. Among 50 patients, equally divided among between colorectal and stomach cancer, 54% had liver metastases and 41% were PD-L1 positive. These data were not detailed by site of disease (colorectal vs. gastric). It does appear that a lower proportion of liver

metastases were noted on the REGONIVO trial. In addition, the percentage of PD-L1 positivity is higher than would be expected [3]. We have not evaluated PD-L1 expression in our cohort. Finally, significant differences exist in terms of race between REGONIVO trial and ours, as all REGONIVO patients were Japanese. However, an impact of race on immune response has not been confirmed previously. We suggest caution from implementing the combination of regorafenib and nivolumab in clinical practice until additional supportive data from prospective trials emerge.

Author Contributions

Conception/design: Chongkai Wang, Marwan Fakih
Provision of study material or patients: Marwan Fakih
Collection and/or assembly of data: Chongkai Wang, Dawnyel Chevalier, Janelle Saluja, Jaideep Sandju, Cecilia Lau, Marwan Fakih
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Manuscript writing: Chongkai Wang, Cecilia Lau, Marwan Fakih
Final approval of manuscript: Chongkai Wang, Dawnyel Chevalier, Janelle Saluja, Jaideep Sandhu, Cecilia Lau, Marwan Fakih

Disclosures

Marwan Fakih: Amgen, Array, Bayer (C/A), Amgen, AstraZeneca, Novartis (RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

References

1. Llosa NJ, Cruise M, Tam A et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov 2015;5:43–51.

2. Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509–2520.

3. O'Neil BH, Wallmark JM, Lorente D et al. Safety and antitumor activity of the anti-pd-1 antibody pembrolizumab in patients with advanced colorectal carcinoma. PLoS One 2017; 12:e0189848.

4. Hoff S, Grünewald S, Röse L et al. Immunomodulation by regorafenib alone and in combination with anti PD1 antibody on murine models of colorectal cancer. Ann Oncol 2017;28(suppl 5): v403–v427.

5. Wu RY, Kong PF, Xia LP et al. Regorafenib promotes antitumor immunity via inhibiting pd-l1 and ido1 expression in melanoma. Clin Cancer Res 2019;25:4530–4541.

6. Zopf D, Fichtner I, Bhargava A et al. Pharmacologic activity and pharmacokinetics of metabolites of regorafenib in preclinical models. Cancer Med 2016;5:3176–3185.

7. Fukuoka S, Hara H, Takahashi N et al. Regorafenib plus nivolumab in patients with advanced gastric (GC) or colorectal cancer (CRC): An open-label, dose-finding, and dose-expansion phase 1b trial (REGONIVO, EPOC1603). J Clin Oncol 2019;37:2522a.

8. Lanman RB, Mortimer SA, Zill OA et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS One 2015;10:e0140712.

9. Cristescu R, Mogg R, Ayers M et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based immunotherapy. Science 2018; 362:eaar3593.

10. Overman MJ, McDermott R, Leach JL et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. Lancet Oncol 2017;18:1182–1191.

11. Overman MJ, Lonardi S, Wong KYM et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair–deficient/microsatellite instability–high metastatic colorectal cancer. J Clin Oncol 2018;36:773–779.

12. Marabelle A, Le DT, Ascierto PA et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: Results from the phase II KEYNOTE-158 study. J Clin Oncol 2019;38:1–10.

13. Tumeh PC, Hellmann MD, Hamid O et al. Liver metastasis and treatment outcome with anti-pd-1 monoclonal antibody in patients with melanoma and NSCLC. Cancer Immunol Res 2017;5:417–424.

14. Calne RY. Mechanisms in the acceptance of organ grafts. Br Med Bull 1976;32:107–112.

15. Calne RY, Sells RA, Pena JR et al. Induction of immunological tolerance by porcine liver allografts. Nature 1969;223:472–476.

16. Qian S, Demetris AJ, Murase N et al. Murine liver allograft transplantation: Tolerance and donor cell chimerism. Hepatology 1994;19:916–924.

17. John B and Crispe IN. Passive and active mechanisms trap activated CD8+ T cells in the liver. J Immunol 2004;172:5222-5229.

18. Qian S, Lu L Fu F, et al. Apoptosis within spontaneously accepted mouse liver allografts: Evidence for deletion of cytotoxic t cells and implications for tolerance induction. J Immunol 1997;158:4654–4661.

19. Limmer A, Ohl J, Kurts C et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med 2000;6:1348–1354.

20. Wang JC, Livingstone AM. Cutting edge: CD4+ T cell help can be essential for primary CD8+ T cell responses in vivo. J Immunol 2003; 171:6339–6343.

21. Bamboat ZM, Stableford JA, Plitas G et al. Human liver dendritic cells promote T cell hyporesponsiveness. J Immunol 2009;182:1901–1911.

22. Tie J, Kinde I, Wang Y et al. Circulating tumor DNA as an early marker of therapeutic response in patients with metastatic colorectal cancer. Ann Oncol 2015;26:1715–1722.

23. Garlan F, Laurent-Puig P, Sefrioui D et al. Early evaluation of circulating tumor DNA as marker of therapeutic efficacy in metastatic colorectal cancer patients (PLACOL study). Clin Cancer Res 2017;23:5416–5425.

24. Anagnostou V, Forde PM, White JR et al. Dynamics of tumor and immune responses during immune checkpoint blockade in non–small cell lung cancer. Cancer Res 2019;79:1214–1225.

25. Cabel L, Riva F, Servois V et al. Circulating tumor DNA changes for early monitoring of anti-pd1 immunotherapy: A proof-of-concept study. Ann Oncol 2017;28:1996–2001.

26. Goldberg SB, Narayan A, Kole AJ et al. Early assessment of lung cancer immunotherapy response via circulating tumor DNA. Clin Cancer Res 2018;24:1872–1880.

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