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Original Article

Antibiotic Exposure Does Not Impact Immune Checkpoint Blockade Response in MSI-H/dMMR Metastatic Colorectal Cancer: A Single-Center Experience

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Abstract

Background: Immune checkpoint blockade (ICB) has improved outcomes for patients with microsatellite instability high (MSI-H)/deficient mismatch repair (dMMR) tumors. However, not all MSI-H/dMMR patients will exhibit the same ICB efficacy. Previous studies suggest that concomitant antibiotic use while receiving ICB may result in poorer outcomes. We aimed to evaluate this association in patients with MSI-H/dMMR metastatic colorectal cancer (mCRC).

Materials and Methods: A single-site, retrospective review of 57 patients with MSI-H/dMMR mCRC that received ICB was completed. Data collected included patient demographics, ICB information, and antibiotic use. Antibiotic exposure was considered from 90 days prior to ICB through 6 weeks after initiation. Primary endpoint was overall response rate (ORR).

Results: The majority of patients received pembrolizumab (27 [47%]) or nivolumab (17 [30%]) monotherapy as their ICB agent. Of the 57 patients, 19 (33.3%) had antibiotic exposure from 90 days prior to ICB initiation through 6 weeks after initiation with most (13 [68%]) having antibiotic use in the 30 days preceding ICB initiation. Similar ORRs were seen in both groups (*P*-value > .99). No difference was observed in OS (*P*-value .29) or PFS (*P*-value .36) between groups.

Conclusion: Our data show no association of lower response rates or survival in those MSI-H/dMMR patients with mCRC who receive antibiotics around the initiation of ICB. This information needs to be confirmed in a larger prospective cohort.

Key words: colon cancer; immunotherapy; microbiome; antibiotics.

Implications for Practice

Immune checkpoint blockade has changed standard of care in dMMR/MSI-H colorectal cancer, with response rates of 50% and many patients with a durable response, likely providing a cure for some. However, there is a need to understand why some patients do not respond. Prior studies have shown concomitant antibiotic use with immune checkpoint blockade is associated with lower response rates and poorer survival in certain tumor types. This is hypothesized to be due to the antibiotic effect on the gut microbiome. To our knowledge, this association has not previously been investigated in the MSI-H/dMMR colorectal cancer population.

Introduction

Colorectal cancer (CRC) is the third most common cause of cancer worldwide and, despite early screening and new treatment strategies, remains the second most common malignant cause of death.[1] Patients with metastatic disease or unresectable locally advanced disease tend to have poor outcomes with a 5-year survival of only 12%.[2] Patients with microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) tumors comprise approximately 4% of all metastatic CRC (mCRC). These patients have shown less benefit with conventional chemotherapy and historically have had a shorter survival than those with microsatellite-stable (MSS)/proficient MMR (pMMR) mCRC.[3,4] However, MSI-H/dMMR tumors are more responsive to immune checkpoint

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blockade (ICB) due to their higher mutational burden and tumor neoantigen load with dense immune cell infiltration. ICB has transformed the treatment of MSI-H/dMMR tumors, including MSI-H/dMMR mCRC. Response rates of approximately 50% are reported with many patients having a durable response which potentially allows for a proportion of patients to achieve cure.[5,6] With a reported 50% response rate, this does raise the question of what factors play a role in those patients who are refractory or develop resistance to ICB.

Recent data suggest that factors beyond tumor genomics influence therapeutic responses, including host factors such as the gastrointestinal (gut) microbiome.[7,8] Previous studies have shown a clear impact of the gut microbiome on the development of chronic inflammatory disease processes and pathogenesis of cancer, especially CRC.[7-9] CRC has most often been found to be associated with lower microbial diversity and higher abundance of certain bacteria, the most well documented being Fusobacterium and Bacteriodes.[10,11] Interestingly, early-onset CRC has been shown to be less associated with lower microbial diversity and to have a different microbial profile than later onset CRC.[12] The gut microbiome has also been shown to have an effect on response to various anti-tumor therapies such as cyclophosphamide and hematopoietic stem cell transplant[13,14] with additional studies showing a strong association between the gut microbiome and response to immune checkpoint blockade in various cancer types.[15-17] Furthermore, it has been well established through both pre-clinical and clinical studies that antibiotic use can lead to alteration and dysbiosis of the gut microbiome.[18,19] Multiple recent studies have shown that antibiotic use either prior to or shortly after the initiation of immune checkpoint blockade therapy is associated with lower response rates and survival.[20-23] However, most of these studies have been in melanoma, non–small cell lung cancer, and renal cell carcinoma patients. To our knowledge, this association has not previously been investigated in the MSI-H/dMMR colorectal cancer population.

Therefore, the purpose of this study was to evaluate the impact of antibiotic exposure on immunotherapy response rates and survival in our cohort of patients with MSI-H/dMMR colorectal cancer. We hypothesized that antibiotic use prior to or concomitantly with the initiation of ICB therapy would in fact be associated with lower response rates and overall survival.

Materials and Methods

Patients with unresectable, locally advanced, or metastatic colorectal adenocarcinoma treated with immune checkpoint blockade at the University of Texas MD Anderson Cancer Center (MDACC) from 2013 through 2021 were identified. Patients were included regardless of previous systemic chemotherapy or other anti-cancer therapy and all ICB agents were included. Patients were excluded if they did not have follow-up after first dose of ICB, if there was no data available prior to first dose of ICB, or if they only received one dose of antibiotics (such as pre-operatively). Patients were evaluated every 2 to 3 months with imaging while on therapy. Response was based on RECIST 1.1 criteria.

Based on preclinical and clinical evidence that antibiotic use can alter the microbiome for a prolonged amount of time,[24,25] a timeframe of antibiotic use was investigated from 90 days prior to initiation of ICB through 6 weeks following initiation. Given the variability of microbiome recovery after antibiotic use, we also analyzed the data using a 60-day and 30-day window of antibiotic use prior to ICB. Antibiotic use was determined through data gathered from the electronic medical record (EMR) either in structured data, such as prescriptions or the medication administration record, or provider note free text.

The primary outcome was overall response rate (ORR) and secondary outcomes included OS, progression-free survival (PFS), and response at first scan. ORR and response at first scan include both partial and complete response. Deaths were ascertained through EMR data and internet obituaries.

Prespecified covariates including age at the time of ICB, sex, race, Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor site, sites of metastatic disease, tumor grade, and BRAF status were gathered from EMR review. Covariates of ICB therapy such as agent, line of therapy, and duration as well as covariates of antibiotic use such as agent, single versus multiple course, and time of exposure were also collected from EMR manual review.

Comparisons for categorical and continuous variables were conducted using Fisher's exact test and Wilcoxon rank sum test (or Kruskal-Wallis test), respectively. OS was estimated as the time interval between the initiation date of ICB and last follow-up date. OS was censored on the last follow-up date. PFS was defined as the time interval between the initiation date of ICB and recurrence (or progression) date or last follow-up date, whichever occurred first. PFS was censored on the last follow-up date for patients who were alive without recurrence or progression. Cox proportional hazards regression and Log-rank test were applied to assess the association between patient characteristics and time-to-event outcomes. Statistical significance was achieved at P-value = .05.

Results

This retrospective cohort of 57 patients with MSI-H/dMMR mCRC included 33 men and 24 women with a median [range] age of 58 [26, 91] years. Most patients had right-sided tumors (33 [68%]), poorly differentiated tumors (31 [54%]), a single metastatic site (39 [68%]), and were BRAF wild type (44 [77%]). The majority of patients received pembrolizumab (27, [47%]) or nivolumab (17, [30%]) monotherapy as their ICB agent, received ICB as first (18, [32%]) or second (22, [39%]) line of therapy, and discontinued therapy due to patient choice/treatment break at 2 years (26 [46%]) or radiographical progression (15 [23%]) (Table 1).

Of the 57 patients, 19 (33.3%) had any antibiotic use from 90 days prior to ICB initiation through 6 weeks after initiation with most of these patients (13 [68%]) with antibiotic use in the 30 days preceding ICB initiation. Out of those patients with antibiotic use, most patients had exposure to a single course of antibiotics (15 [79%]) (Table 1).

Median follow-up was 41.8 months and 46 (80.7%) patients were alive at last follow-up. We found similar ORR and response at first scan in patients without antibiotic exposure and those with antibiotic exposure at any time point from 90 days prior through 6 weeks after ICB initiation (Fig. 1A and 1B, P-value >.99). The estimated 1 year OS was 89% (95% CI: 0.81, 0.98) with an estimated 1 year PFS of 81% (95% CI: 0.71, 0.92). We saw no difference in OS (Fig. 2

Table 1. Patient/tumor characteristics and characteristics of antibiotic use.

Category	*n* = 57	%
Patient characteristics		
Sex		
Male	33	57.89
Female	24	42.11
Ethnicity		
White	40	70.18
Hispanic	10	17.54
Black	4	7.02
Arabic	2	3.51
Asian	1	1.75
Primary tumor		
Colon	51	89.47
Rectal	6	10.53
Colon sidedness (*n* = 51)		
Right	33	68.42
Left	10	17.54
Transverse	8	14.04
Unknown	6	10.53
Metastatic sites*		
Single	39	68.42
Multiple	15	26.32
Advanced/unresectable	3	5.26
Differentiation		
Poor	31	54.39
Moderate	23	40.25
Unknown	3	5.26
MSI-H		
Yes	57	100
BRAF mutation		
No	44	77.19
Yes	13	22.81
Immunotherapy agent		
Pembrolizumab	27	47.37
Nivolumab monotherapy	17	29.82
Nivolumab + Ipilimumab	9	15.79
Other**	4	7.02
Line of therapy		
1st	18	31.58
2nd	22	38.60
third	11	19.30
fourth or more	2	3.51
Unknown	1	1.75
ECOG at treatment start		
0	21	36.84
1	27	47.37
Unknown	9	15.79
Reason for treatment discontinuation		
Patient choice	26	45.61
Radiographical progression	15	26.32
Toxicity	10	17.54
Clinical progression	1	1.75
Unknown	5	8.77
Antibiotic characteristics		
pABx within 90 days		
Yes	15	26.32
pABx within 60 days		
Yes	14	24.56
pABx within 30 days		
Yes	13	22.81
cAbx through 6 weeks		
Yes	9	15.79
Abx use (pAbx + cAbx)		
Yes	19	33.33
# of Abx course		
Single	15	26.32
Multiple	4	7.02
None	52	91.23
Beta lactams		
Yes	5	8.77
Fluoroquinolones		
Yes	8	14.04
Metronidazole		
Yes	2	3.51
Vancomycin		
Yes	1	1.75
Doxycycline		
Yes	5	8.77

Abbreviations: pABx, antibiotics prior to treatment; cAbx, antibiotics concomitant with treatment; Abx Use (pABx + cABx), any antibiotic use from 90 days prior to treatment through 6 weeks after starting treatment.
*Metastatic sites = number of organs/organ systems involved
**Other includes durvalumab, atezolizumab.

P-value .29) or PFS (Fig. 3, *P*-value .36) between those with antibiotic exposure at any time point and those without exposure. Multivariate Cox proportional hazards analysis was done controlling for age, sex, ECOG, primary tumor site, tumor grade, BRAF status, and ICB agent and no clinical factor was significantly associated with ORR, OS, or PFS (Supplementary Table S1).

Discussion

To our knowledge, this is the first comprehensive study evaluating the effect of antibiotic use on ICB response in MSI-H/dMMR mCRC. In contrast to previous studies of NSCLC, melanoma, and other tumor types,[20-23] we found no relationship to antibiotic exposure and subsequent response to ICB in this population of MSI-H/dMMR patients with mCRC. ORR, OS, and PFS showed no significant difference in patients with antibiotic use within either 90 days prior, 60 days prior, or 30 days prior to ICB initiation or within 6 weeks after ICB initiation when compared to those without exposure to antibiotics. Possible explanations for the contradicting results from prior studies include a smaller data set, variable antibiotic exposure timeframe in previous studies, and the high response rates to ICB in MSI-H/dMMR tumors. Previous studies have used

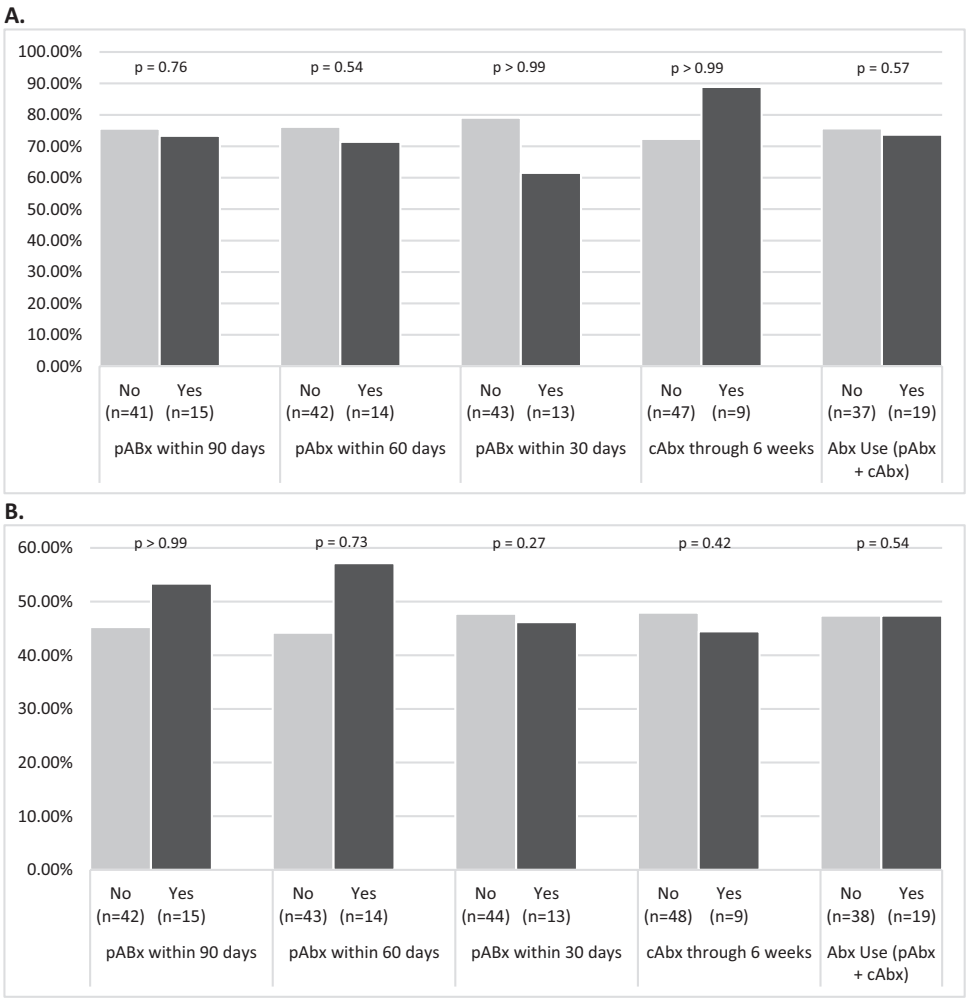

Figure 1: Response by antibiotic use. **(A)** Overall response by antibiotic use. **(b)** Response at first scan by antibiotic use.

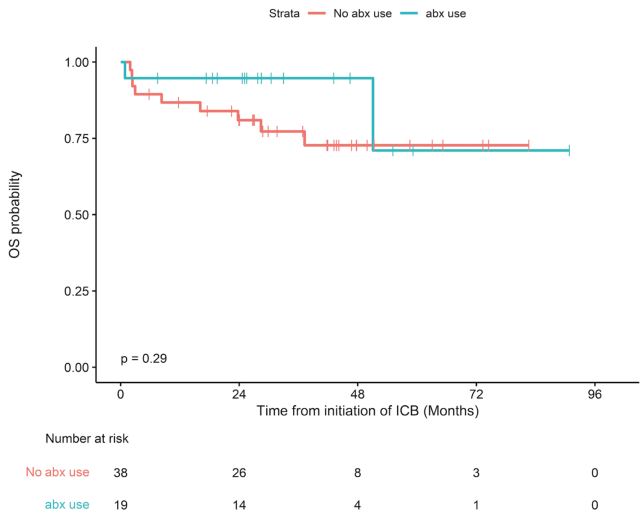

Figure 2: Kaplan-Meier curve for overall survival by antibiotic use.

antibiotic exposure windows from as early as 90 days prior to ICB initiation through up to 1 year after initiation or in some cases, entire duration of ICB therapy. In addition, the patients in our dataset have high response rates near 75%. Possibly most interestingly, our results also raise the question of whether the effect of antibiotic use may be different for tumors that are MSI-H/dMMR in comparison to traditionally immunologically "hot" tumors. For these MSI-H/dMMR tumors with such high response rates to ICB, while microbiome may still play a role in response, it is possible that the tumor neoantigen load may trump this effect. Further investigation needs to occur in this area.

We acknowledge our study has notable limitations. It is a retrospective study of a limited subset of CRC patients, a much smaller cohort than those evaluated in previous studies of melanoma, RCC, and other tumor types (range 30-1960, median 225 patients).[20-23] Additionally, our study relied on the retrospective retrieval of antibiotic use data via EMR documentation. We had to exclude any patient lacking data prior to being started on ICB, and were only able to include antibiotic data if documented in the MDACC EMR.

In addition to the limitations summarized above, there are general limitations in regards to studies on the effect of antibiotic exposure on response to ICB and the clinical impact of these studies. First, all of these studies are retrospective in nature. Although it is not feasible or ethical to do randomized studies in this area, a prospective cohort study with concurrent analysis of microbiome changes over time may provide

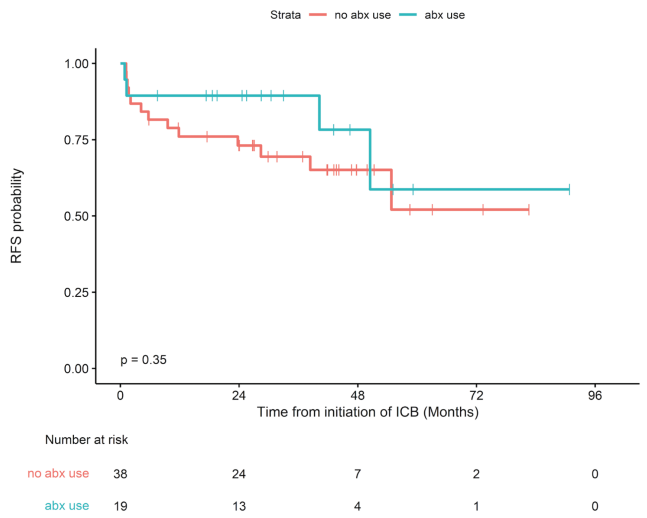

Figure 3: Kaplan-Meier Curve for progression-free survival by antibiotic use.

even more useful information. Secondly, the ideal timeframe or window of time to consider antibiotic use is still unclear. We suspect the recovery of microbiome dysbiosis is variable making it difficult to choose a clear cut off and to interpret the data generated. Finally, there is concern for cofounding variables as antibiotic use is often associated with infectious disease processes that may predispose the patient to a lower immune response and therefore lower ICB response regardless of antibiotic use.

As the gut microbiome seems to play a role in response to ICB therapy, alteration of the microbiome may be a potential treatment strategy. Although there are many methods of microbiome alteration including diet modification and provision of beneficial microorganisms through probiotics, fecal microbiota transplantation (FMT) is the most well-established method. A recently published trial in melanoma patients previously non-responsive to ICB showed that the addition of FMT from melanoma patients with response to ICB followed by ICB re-challenge resulted in response in 3 out of 10 melanoma ICB non-responders.[26] This study suggests that alteration of the microbiome may improve response to ICB. Based on these results, there is an ongoing clinical trial of FMT in addition to rechallenge of ICB in MSI-H/dMMR tumors previously non-responsive to ICB at MDACC (NCT04729322).[27] This study also provides an opportunity for further microbiome analysis in MSI-H/dMMR ICB non-responders which may provide further insight into how the microbiome plays a role in treatment response.

In conclusion, our data do not support an association of lower response rates in those MSI-H/dMMR patients with mCRC who received antibiotics in specific time intervals within initiation of ICB therapy. However, while these data are hypothesis generating, we understand this initial report in MSI-H mCRC needs to be confirmed in a larger cohort with prospective data collection.

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Conflict of Interest

Scott Kopetz: Genentech, EMD Serono, Merck, Holy Stone, Novartis, Lilly, Boehringer Ingelheim, Boston Biomedical, AstraZeneca/Medimmune, Bayer Health, Pierre Fabre, RedX Pharma, Ipsen, Daiichi Sankyo, Natera, HalioDx, Lutris, Jacobio, Pfizer, Repare Therapeutics, Inivata, GlaxoSmithKline, Jazz Pharmaceuticals, Xillis, Abbvie, Amal Therapeutics, Gilead Sciences, Mirati Therapeutics, Flame Biosciences. Servier, Carina Biotechnology, Bicara Therapeutics, Endeavor BioMedicines. Numab Pharma, Johnson & Johnson/Janssen, Genomic Health, Frontier Medicines, Replimune, Taiho Pharmaceutical (C/A), Sanofi, Biocartis, Guardant Health, Array Biopharma, Genetech/Roche, EMD Serono, MedImmune, Novartis, Amgen, Lilly, Daiichi Sankyo (RF), MolecularMatch, Lutris, Iylon, Frontier Medicines (OI); **Michael J. Overman:** Merck (C/A), Merck, Bristol Myers Squibb, AstraZeneca (RF); **Benny Johnson:** Gristone Bio, Incyte, Taiho Oncology, Insmed Oncology (C/A), Bristol Myers Squibb, Syntrix, Gateway for Cancer Research (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.

Author Contributions

Conception/design: V.S.H., J.R., B.J. Provision of study material or patients: A.D., K.M.R., V.K.M., R.A.W., K.R., R.H., C.P., J.W., S.K., M.J.O., B.J. Collection and/or assembly of data: V.S.H., J.R. Data analysis and interpretation: V.S.H., J.R., B.J. Manuscript writing, review, and editing: V.S.H., J.R., A.D., K.M.R., V.K.M., R.A.W., K.R., R.H., C.P., J.W., S.K., M.J.O., B.J. Statistical analysis: H.H., W.Q., L.X. Final approval of manuscript: All authors.

Data Availability

The data underlying this article are available in the article and in its online supplementary material.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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