ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2022 November 24; 13(11): 866-942

Published by Baishideng Publishing Group Inc

WJCO

World Journal of Clinical Oncology

Contents

Monthly Volume 13 Number 11 November 24, 2022

World Journal of Clinical Oncology

Monthly Volume 13 Number 11 November 24, 2022

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RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Clinical Oncology

ISSN
ISSN 2218-4333 (online)

LAUNCH DATE
November 10, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
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https://www.wjgnet.com/2218-4333/editorialboard.htm

PUBLICATION DATE
November 24, 2022

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World J Clin Oncol 2022 November 24; 13(11): 929-942

DOI: 10.5306/wjco.v13.i11.929

ISSN 2218-4333 (online)

SYSTEMATIC REVIEWS

Gut microbiota diversity and composition in predicting immunotherapy response and immunotherapy-related colitis in melanoma patients: A systematic review

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Specialty type: Oncology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: MI SC, China; Wen XL, China

Received: October 19, 2022
Peer-review started: October 19, 2022
First decision: October 28, 2022
Revised: October 30, 2022
Accepted: November 6, 2022
Article in press: November 6, 2022
Published online: November 24, 2022

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Abstract

BACKGROUND

Gut microbiome (GM) composition and diversity have recently been studied as a biomarker of response to immune checkpoint blockade therapy (ICB) and of ICB-related colitis.

AIM

To conduct a systematic review on the role of GM composition and diversity in predicting response and colitis in patients with melanoma treated with ICB.

METHODS

The review protocol was registered in PROSPERO: CRD42021228018. From a total of 300 studies, nine studies met inclusion criteria. Two studies were phase I clinical trials, while the remainder were prospective observational studies. All but one study has moderate risk of bias. In addition, we conducted a relevant search by Reference Citation Analysis (*RCA*) (https://www.referencecitationanalysis.com).

RESULTS

Fecal samples enriched in Firmicutes phylum were associated with good response to ICB, whereas the Bacteroidales family was associated with poor response to ICB. Samples with greater GM diversity were associated with more favorable response to ICB [hazard ratio (HR) = 3.57, 95% confidence interval = 1.02-12.52, $P < 0.05$]. Fecal samples with a higher abundance in Firmicutes were more susceptible to ICB-related colitis ($P < 0.01$) whereas samples enriched in *Bacteroidetes* were more resistant to ICB-related colitis ($P < 0.05$). Overall, there was limited concordance in the organisms in the GM identified to be associated with response to ICB, and studies evaluating GM diversity showed conflicting results.

CONCLUSION

This highlights the need for further prospective studies to confirm whether the GM could be used as a biomarker and potential intervention to modulate ICB response in melanoma patients.

Key Words: Melanoma; Gut microbiome; Microbiota; Immunotherapy; Biomarker; Immune checkpoint blockade therapy

Core Tip: Since the introduction of immune checkpoint inhibitors as part of standard of care for melanoma patients, there has been a growing interest in identifying biomarkers of response and immune related adverse events. Amongst these biomarkers, the composition of the gut microbiome has been one of the most intriguing discoveries. Our aim was to ascertain the current published evidence on the gut microbiome diversity and composition as a biomarker of response to immunotherapy. We demonstrated high variability in the results and limited concordance on the organisms identified. We highlight the conflicting aspects of these reports as well as their few commonalities.

Citation: Oey O, Liu YY, Sunjaya AF, Simadibrata DM, Khattak MA, Gray E. Gut microbiota diversity and composition in predicting immunotherapy response and immunotherapy-related colitis in melanoma patients: A systematic review. *World J Clin Oncol* 2022; 13(11): 929-942
URL: https://www.wjgnet.com/2218-4333/full/v13/i11/929.htm
DOI: https://dx.doi.org/10.5306/wjco.v13.i11.929

INTRODUCTION

Melanoma is the most lethal form of skin cancer accounting for 73% of skin-cancer related mortality and over 50000 deaths worldwide annually[1,2]. Survival for metastatic melanoma has significantly improved since the introduction of immunotherapy and targeted therapy with a 5-year survival rate of up to 50%[3-5]. Currently, the standard first-line therapy for metastatic melanoma include BRAF-targeted therapies and immune checkpoint blockade (ICB) consisting either anti-programmed death (PD)-1 monotherapy or combination of anti-PD-1 as well as anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) therapy[6]. Despite the considerable benefit of ICB, 40%-60% of melanoma patients do not experience objective responses to the therapy[7-9]. Thus, tremendous efforts are now focused on identifying novel biomarkers which could accurately predict the subset of patients who would benefit from ICB[10-14]. These biomarkers include tumor mutational burden, cytokines, circulating tumor DNA, human leukocyte antigen, gut microbiota (GM) diversity and composition, among many others [15].

The GM is a community of 100 trillion microorganisms of more than 1000 species mainly bacteria but also, archaea, viruses and fungi which colonize the human intestines[16]. The relationship which exists between GM and the host is a mutualistic relationship where one benefits the other[16]. In return for the nutrients derived from the host, the GM performs numerous critical functions such as fermentation of dietary fiber into short-chain fatty acids; synthesis of vitamins; protection against pathologic gut microbes; and induction and regulation of the immune system[17,18]. The gut microbial balance is pivotal in the optimal functioning of all of these roles and thus any discrepancy in this delicate

equilibrium could produce a state of dysbiosis which has been associated with many pathologies including cancer[19]. In the context of cancer, preclinical studies have demonstrated that some GM subpopulations have pro-tumorigenic effects, whereas others have tumor-suppressive effects[20-22]. Additionally, the GM has also been shown to modulate response to chemotherapy and immunotherapy [23-25]. This could be linked to the role of GM in metabolizing anti-cancer compounds and regulating the host's immune response[16]. Thus, GM has been studied intensely as a potential biomarker of response to ICB[12,26-31]. This is particularly relevant for melanoma, where ICB has become standard of care given its demonstrated pronounced effectiveness.

Studies investigating GM composition and/or diversity in patients with melanoma have identified distinct GM composition in responders to ICB compared to non-responders, offering hope of a novel biomarker for predicting response to ICB[12,26-32]. Additionally, studies exploring whether certain GM composition and diversity could be predictive of ICB-related colitis - one of the major factors of ICB treatment cessation and thus failure to derive full benefit of ICB - have also been conducted[27,33]. This systematic review will be the first to compile the existing data regarding the role of GM composition and diversity in predicting response to ICB and ICB-related colitis specifically in patients with melanoma. Notably previous reviews have combined multiple cancers.

MATERIALS AND METHODS

Literature search strategies

This review was conducted following the preferred reporting Items for systematic reviews and meta-analyses guidelines[34]. The review protocol was submitted to the international prospective register of systemic reviews (PROSPERO Registration number: CRD42021228018).

In this comprehensive literature search, original studies exploring the variation in GM community in fecal samples of melanoma patients who responded and did not respond to immunotherapy, experienced colitis and did not experience colitis were identified. Medline and Embase were searched for eligible papers published prior to December 2021 using the following search terms: (fecal OR gut) AND (microbiota OR microbiome) AND (melanoma) AND (immunotherapy OR checkpoint OR nivolumab OR ipilimumab OR pembrolizumab). OpenGrey and the Grey Literature Report were also searched for eligible unpublished papers and grey literature. The following keywords and its synonyms will be used for our search strategy: "fecal microbiota", "melanoma", "immunotherapy".

Duplicate and irrelevant publication types such as symposium agendas were removed from the initial search results. Titles and abstracts of relevant publications were screened independently by Oliver O and Simadibrata DM based on inclusion and exclusion criteria stated below. Subsequently, reference lists within each relevant publication were examined for further pertinent studies. The full texts of these publications were then reviewed.

Inclusion criteria

Inclusion criteria for the systematic review included randomized controlled trials (RCTs), original cohort, case-control studies published in a peer-reviewed journal exploring GM diversity and composition in fecal samples from melanoma patients treated with ICB which can be anti-PD-1 and/or anti-PD-L1 and/or anti-CTLA-4. Studies included should assess treatment outcome and/or ICB-related colitis incidence following treatment with ICB. Treatment outcomes should be determined by RECIST criteria and/or progression free survival (PFS) and/or overall survival (OS) and ICB-related colitis confirmed by colonoscopy.

Only studies which utilized fecal samples obtained from human subjects receiving ICB were included. Studies which assessed treatment response to immunotherapy in animal models were excluded. Two reviewers (Oliver O, Liu YY) independently screened and read the full text of the included articles for eligibility.

Data extraction

Two investigators (Oliver O, Liu YY) independently reviewed the eligible studies and extracted data from each study. Extracted variables included title, first author, year of publication, number of participants, type of immunotherapy received, GM analysis method, and study outcomes (GM composition and diversity in responders/non-responders and ICB-related colitis/non-ICB-related colitis patients). Extracted GM composition data included a list of the GM at the level of phyla, class, order, family, genus and species, whereas extracted GM diversity extracted included alpha diversity or the Shannon index. Any discrepancies found by the investigators on data extraction were resolved by consensus. In addition, we conducted a relevant search by Reference Citation Analysis (*RCA*) (https://www.referencecitationanalysis.com).

Quality assessment

Non-randomized studies, including cohort studies, case-control studies and single-arm clinical trial that

were included in this systematic review were independently evaluated by Oliver O and Simadibrata DM for any risk of bias using the Risk of Bias in Non-randomized Studies of interventions (ROBINS-I) assessment tool, a tool which assesses seven items: confounding, selection, intervention classification, deviation from intervention, missing data, measurement of outcome and selection of reported result. Each item was assessed according to the ROBINS-I guideline, where each bias domain can be classified as either low, moderate, serious or critical risk of bias, or no information mentioned.

RESULTS

Study selection and risk of bias assessment

The initial search from Medline, Embase, OpenGrey and Grey Literature retrieved 300 studies. After deduplication, the studies were screened by reviewing their abstracts and 10 articles selected for full assessment (Figure 1). One study by Vétizou *et al*[35] was excluded because while the fecal samples were obtained from patients treated with anti-CTLA-4, treatment response to ICB was assessed in an *in-vivo* mice model of melanoma following fecal transplantation rather than humans.

From the nine included studies, two studies were phase I clinical trials, while the remainder were prospective observational studies[26,28]. Unfortunately, no RCTs were available to date. According to the ROBINS-I assessment tool, all but one study was shown to have moderate risk of bias (Table 1). The study by Matson *et al*[29] had a serious risk of bias as there was a lack of clarity regarding the definition of intervention used.

GM composition and diversity in predicting immunotherapy response

Eight studies assessed the role of GM composition and/or diversity and response to ICB in melanoma patients (Table 2). Seven studies compared the GM between responders and non-responders to ICB, and two studies analyzed the GM in patients undergoing fecal microbiota transplant (FMT).

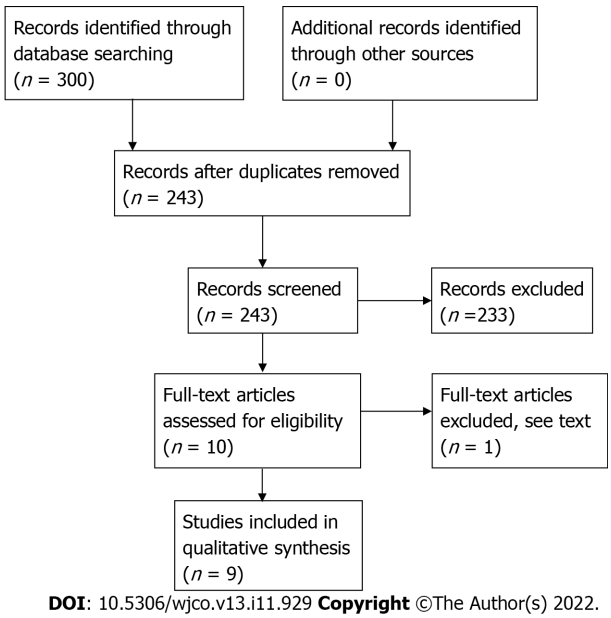
The study by Chaput *et al*[27] assessing fecal GM composition of 26 metastatic melanoma patients prior to and post commencing anti-CTLA-4 therapy revealed that GM composition varied according to response. Patients showing long term response to therapy (nine out of 26 patients) were found with fecal samples with significantly higher *Faecalibacterium* percentages ($P = 0.0092$) while patients with poor clinical benefit had higher proportions of *Bacteroides* ($P = 0.034$). When patients were grouped based on their microbiota composition, those with high prevalence of *Faecalibacterium* and other *Firmicutes* had a longer PFS ($P = 0.0039$) and to a lesser extent longer OS ($P = 0.051$) relative to patients whose fecal samples were abundant with *Bacteroides*. Additionally, these patient groups were noted to derive long-term clinical benefit compared to the latter (67% *vs* 0%; $P = 0.0017$)[28].

In an analysis of stool samples from 42 metastatic melanoma patients prior to treatment with anti-PD-1 ($n = 38$) and anti-CTLA-4 ($n = 4$) therapy, Matson *et al*[29] showed a significant difference in GM composition between responders (16 patients) and non-responders (26 patients) ($P < 0.01$). In responders, eight microbial species namely, *Enterococcus faecium*, *Collinsella aerofaciens*, *Bifidobacterium adolescentis*, *Klebsiella pneumoniae*, *Veillonella parvula*, *Parabacteroides merdae*, *Lactobacillus* sp. and *Bifidobacterium longum* were found to be more abundant in responders than in non-responders[29]. In non-responders, two microbial species, specifically, *Ruminococcus obeum* and *Roseburia intestinalis* were more abundant[29]. To further assess the applicability of GM composition as a biomarker of response to ICB, they explored the correlation between the ratio of total numbers of potentially "beneficial" and "nonbeneficial" operational taxonomic units (OTUs), and change in tumor size, as assessed by the RECIST[29]. Patients with an OTU ratio of greater than 1.5 demonstrated clinical response to ICB[29].

In another study by Gopalakrishnan *et al*[12], fecal samples of 43 metastatic melanoma patients prior to treatment with anti-PD-1 therapy were analyzed. In responders (30 patients), analysis of fecal samples revealed abundance of GM from *Ruminococcaceae* family of the *Clostridiales* order, whereas in non-responders (13 patients), abundance of GM from the *Bacteroidales* order was noted[12]. Further analyses demonstrated that *Faecalibacterium* genus was notably enriched in fecal samples from responders and *Bacteroides thetaiotaomicron*, *Escherichia coli*, and *Anaerotruncus colihominis* were enriched in non-responders[12]. In addition, to investigate durability of response, patients were stratified based on their fecal composition of *Faecalibacterium* genus and *Bacteroidales* order and correlated to their PFS [12]. Results demonstrated that patients with *Faecalibacterium*-enriched fecal samples have longer PFS than those with low abundance ($P = 0.03$) and patients with *Bacteroidales*-enriched fecal samples have shorter PFS than those with low abundance ($P = 0.05$). Beyond specific microbial taxa, GM diversity, as assessed by Simpson's reciprocal index, was higher in responders compared to non-responders ($P < 0.01$)[12]. Moreover, high GM diversity was significantly associated with anti-PD-1 therapy response, when compared to patient groups of intermediate diversity [hazard ratio (HR) = 3.60, 95% confidence interval (CI): 1.02-12.74, $P < 0.05$) and low diversity (HR = 3.57, 95%CI: 1.02-12.52, $P < 0.05$). Other important predictors of therapy response include abundance of *Faecalibacterium* (HR = 2.92, 95%CI: 1.08-7.89) and *Bacteroidales* (HR = 0.39, 95%CI: 0.15-1.03) in the fecal microbiome[12].

Table 1 Risk of bias assessment with Risk of Bias In Non-randomised Studies - of Interventions

Ref.	Confounding	Selection	Intervention classification	Deviation from intervention	Missing data	Measurement of outcome	Selection of reported result	Overall
Dubin *et al*[33], 2016	Moderate	Low	Low	Low	Low	Low	Low	Moderate
Chaput *et al*[27], 2017	Moderate	Low	Low	Moderate	Low	Low	Moderate	Moderate
Gopalakrishnan *et al*[12], 2018	Moderate	Low	No information	Low	Low	Low	Low	Moderate
Matson *et al*[29], 2018	Moderate	No information	Serious	No information	No information	Low	Moderate	Serious
Peters *et al*[30], 2019	Moderate	Low	No information	No information	Low	Low	Low	Moderate
Baruch *et al*[26], 2020	Moderate	Low	Low	Low	Moderate	Low	Low	Moderate
Wind *et al*[31], 2020	Moderate	No information	No information	No information	No information	Low	Low	Moderate
Davar *et al*[28], 2021	Moderate	Moderate	Low	Low	No information	Low	Low	Moderate
Andrews *et al*[36], 2021	Moderate	Low	Low	Low	Low	Low	Low	Moderate



DOI: 10.5306/wjco.v13.i11.929

Figure 1 Prisma flow diagram of study selection.

Peters *et al*[30] examined the correlation between GM taxa and PFS in pre-treatment fecal samples of 27 metastatic melanoma patients receiving anti-PD-L1 and/or anti-CTLA-4. GM which was associated with shorter PFS included genera *Bacteroides* and *Bilophila*, and species *Bacteroides ovatus*, *Blautia producta*, and *Ruminococcus gnavus*, whereas those which correlated with longer PFS included genera *Faecalibacterium* and *Parabacteroides* and species *Faecalibacterium prausnitzii*[12]. With regards to GM richness the authors compared the β-diversity or between-sample microbiome diversity relative to survival. Multivariate analysis adjusting for age, sex, BMI, stage, number of sites of metastases, and antibiotic use in the last 6 mo revealed that higher GM richness was correlated with longer PFS (number of 16S sub - OTUs: HR [95%CI] = 0.97 [0.95, 1.00], P = 0.02; number of shotgun subspecies: HR [95%CI] = 0.89 [0.79, 0.99], P = 0.03)[30]. Furthermore, analysis of the 16S but not shotgun dataset showed that higher diversity of GM, as assessed by the Shannon index, was associated with longer PFS (P = 0.02)[12].

Table 2 Characteristics of included studies exploring link between gut microbiome composition and diversity and response to immune-checkpoint blockade therapy in metastatic melanoma patients treated with immune-checkpoint blockade therapy

Ref.	Year	Therapy	Method	Sample size/ time point	Dominant microbes	Microbial diversity
Chaput *et al*[27], 2017	2017	Anti-CTLA-4	16S rRNA gene sequencing of fecal samples	26 before tx	Responders: *Faecalibacterium* and *Firmicutes*	N/A
Matson *et al*[29], 2018	2018	Anti-PD-1 or anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples; qPCR on selected bacteria	42 before tx	Responders: *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* Non-responders: *Ruminococcus obeum* and *Roseburia intestinalis*	N/A
Gopalakrishnan *et al*[12], 2018	2018	Anti-PD-1	16S rRNA gene and shotgun metagenome sequencing of fecal samples	43 before tx	Responders: *Clostridiales*, in particular *Faecalibacterium* Non-responders: *Bacteroidales*, in particular *Bacteroides thetaiotaomicron*; as well as *Escherichia coli*, and *Anaerotruncus colihominis*	Higher alpha diversity in patients with longer PFS
Peters *et al*[30], 2019	2019	Anti-PD-1 or anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	27 before tx	Responders: *Faecalibacterium, Parabacteroides*, and *Faecalibacterium prausnitzii* Non-responders: *Bacteroides* and *Biophilia*	Higher microbial community richness and diversity was associated with longer PFS
Wind *et al*[31], 2020	2020	Anti-PD-1 or anti-CTLA-4	Shotgun metagenome sequencing of fecal samples	25 before tx	Responders: *Ruminococcus gnavus, Streptococcus parasanguinis*, and *Bacteroides massiliensis*. Non-responders: *Bifidobacterium longum* and *Peptostreptococcaceae*	No significant difference in alpha-diversity between responder and non responders
Baruch *et al*[26], 2020	2020	Anti-PD-1 refractory	16S rRNA gene and shotgun metagenome sequencing of fecal samples	10 anti-PD-1 refractory patients	FMT donors (responders): *Lachnospiraceae, Veillonellaceae*, and *Ruminococcaceae* Post FMT Responders: *Enterococcaceae, Enterococcus*, and *Streptococcus australis* Non-responders: *Veillonella atypica*	No significant difference in GM composition prior to FMT, but significant difference post-FMT between responders and non-responders Lower microbial richness in the donor of responding recipients
Davar *et al*[28]. 2021	2021	Anti-PD-1 refractory	Shotgun metagenomic sequencing of fecal samples	15 anti-PD-1 refractory patients, before FMT	Responders: Firmicutes (*Lachnospiraceae* and *Ruminococcaceae* families) and *Actinobacteria* (*Bifidobacteriaceae* and *Coriobacteriaceae* families)	Higher GM diversity of donors who were complete responders compared to donors who were partial responders No significant difference in GM diversity between donors and recipients prior to FMT
Andrews *et al* [36], 2021	2021	Combined ICB - Anti-PD-1 and anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	38	Responders: *Bacteroides stercoris*, *Parabacteroides distasonis*, *Fournierella massiliensis*. Non-responders: *Klebsiella aerogenes* and *Lactobacillus rogosae*	No significant difference in GM diversity between responders and non-responders

Anti-CTLA-4: Anti-cytotoxic T lymphocyte-associated antigen-4; N/A: Not applicable; Anti-PD-1: Anti-programmed death-1; qPCR: Quantitative real-time polymerase chain reaction; FMT: Fecal microbiota transplant; GM: Gut microbiome; ICB: Immune checkpoint blockade therapy.

Similarly, Wind *et al*[31] analyzed fecal samples from 25 metastatic melanoma patients - 12 responders, 13 non-responders - prior to start of treatment with anti-PD-1 or anti-CTLA-4. Analysis revealed that the fecal samples of responders were mainly enriched in *Ruminococcus gnavus*, *Escherichia coli*, *Eubacterium biforme*, *Phascolarctobacterium succinatutens* and *Streptococcus salivarius*, whereas samples from non-responders were abundant in *Bifidobacterium longum*, *Prevotella copri*, *Coprococus* sp, *Eggerthella unclassified* and *Eubacterium ramulus*[31]. When correlated with survival, fecal samples of participants enriched in *Bacteroides massiliensis* and *Streptococcus parasanguinis* were associated with longer PFS (HR: 3.79, 95%CI: 1.06-13.52 P = 0.04) and OS (HR: 5.05, 95%CI: 1.33-19.21, P = 0.017) respectively, whereas those who were carriers of *Peptostreptococcaceae* were associated with shorter PFS (HR: 0.18, 95%CI: 0.05-0.62, P = 0.007) and OS (HR: 0.12, 95%CI: 0.01-0.96, P = 0.046)[31]. In terms of GM diversity, as assessed by Shannon index, no significant difference between responders and non-responders was noted[31].

The study by Andrews *et al*[36] analyzed gut microbiome samples from a subset of 77 metastatic melanoma patients - 27 responders, 11 non-responders - who underwent combined ICB. There was no significant association in *Firmicutes* phyla and *Clostridiales* order and response to ICB (P = 0.39 and P = 0.38, respectively) and no significant difference in alpha diversity between responders and non-responders to ICB[36]. Fecal samples from responders were mainly enriched with *Bacteroides stercoris*,

Parabacteroides distasonis and *Fournierella massiliensis* (P = 0.03, P = 0.04 and P = 0.008, respectively) while fecal samples from non-responders were abundant in *Klebsiella aerogenes and Lactobacillus rogosae* (P = 0.04 and P = 0.02, respectively)[36].

In a first clinical trial of its kind (phase 1), Baruch *et al*[26] demonstrated that FMT from anti-PD-1 treated metastatic melanoma patients who were complete responders (2 donors), triggered response to anti-PD-1 therapy in metastatic melanoma patients who were refractory to at least one line of anti-PD-1 therapy. Out of 10 patients included in the trial, 3 patients demonstrated objective responses with 1 achieving complete response and 2 patients achieving partial response[26]. Notably, the PFS milestone of 6 mo was reached in all responders[26]. Upon analysis of pre-treatment fecal samples of donors, donor of the responding recipients had a lower microbial richness than the other donor of the non-responding patients[26]. There was no significant difference on the GM composition prior to FMT of recipients who responded compared to those who did not respond[26]. Metagenome sequencing found that recipients post FMT have higher proportions of *Veillonellaceae* family and a lower relative abundance of *Bifidobacterium bifidum*. Donors were found with high amounts of *Lachnospiraceae, Veillonellaceae*, and *Ruminococcaceae*. Comparison of a small subset of non-responders with responders, found statistically significant higher abundance of *Enterococcaceae, Enterococcus*, and *Streptococcus australis*, and a lower relative abundance of *Veillonella atypica*. However clear deductions on specific GM taxa cannot be made, as there were non-responders and pre-treatment fecal samples with similar dynamics. it is crucial to note that this trial was primarily designed to assess safety of FMT and not statistically powered to assess efficacy[26].

In a separate trial, Davar *et al*[28] showed that fecal microbial transplant (FMT) from metastatic melanoma patients (7 donors) who had complete (4 donors) or partial response (3 donors) to anti-PD-1 therapy helped overcome resistance in anti-PD-1 treatment-refractory metastatic melanoma patients (15 patients). Following FMT and anti-PD-1 therapy, 6 out of 15 patients achieved clinical benefit, with 3 patients achieving objective responses and 3 patients experiencing stable disease lasting more than 12 mo[28]. Analysis of stools after FMT revealed that samples from responders were abundant in the phyla, *Firmicutes* (*Lachnospiraceae* and *Ruminococcaceae*) and *Actinobacteria* (*Bifidobacteriaceae* and *Coriobacteriaceae*) and had decreased proportions in phylum *Bacteroidetes*[28]. In terms of GM diversity assessed with inverse Simpson index, GM diversity of donors who were complete responders were more diverse than donors who were partial responders. There was no significant difference in GM diversity between donors and recipients prior to FMT[28].

Gut microbiota composition and diversity in predicting ICB-related colitis

To date only three studies have reported on the correlation between pre-treatment GM composition and/or diversity and ICB-related colitis (Table 3).

Firstly, a prospective study by Dubin *et al*[33] explored the link between GM composition, and subsequent colitis development in 34 metastatic melanoma patients treated with ipilimumab, showed that the *Bacteroidetes* phylum was more abundant (P = < 0.05) in fecal samples of the 24 patients who did not develop ipilimumab-induced colitis compared to those who did. Further analysis revealed that within the *Bacteroidetes* phylum, the population of *Bacteroidaceae, Rikenellaceae* and *Barnesiellaceae* was significantly more abundant in the former than the latter (P < 0.01, P < 0.05 and P < 0.05 respectively)[33]. However, there was no significant difference in microbial richness and diversity, as assessed by Shannon and inverse Simpson indices, between those who developed ipilimumab-induced colitis relative to those who did not[33].

In a similar study by Chaput *et al*[27], analysis of fecal samples of metastatic melanoma patients receiving ipilimumab demonstrated high proportions of *Firmicutes* in patients who developed ipilimumab-induced colitis (P = 0.009). In contrast, fecal samples of those that did not develop colitis were mainly enriched with *Bacteroidetes* (P = 0.011)[27]. Accordingly, patients with the former GM composition also tend to have a shorter colitis-free cumulative incidence compared with patients with the latter composition[27]. Several OTUs known to be predictive to colitis such as *F. prausnitzii* L2-6, *butyrate producing bacterium* L2-21 and *G. formicilis* ATCC 27749 were associated with longer OS[27].

Finally, Andrews *et al*[36], analyzed gut microbiome samples in metastatic melanoma patients undergoing combined ICB and their link to ileitis and colitis events. No significant difference in alpha diversity was observed between those that did and did not develop colitis[36]. Fecal samples of patients developing colitis were enriched in *Bacteroides intestinalis* and *Intestinibacter bartlettii* (P = 0.009 and P = 0.009, respectively) while those that did not were abundant in *Anaerotignum lactatifermentans* and *Dorea formicigenerans* (P = 0.016 and P = 0.06, respectively)[36]. For both *B. intestinalis* and *D. formicigenerans*, associations with their risk of colitis were still maintained after adjustment using a logistic regression model [OR = 4.54 (95%CI = 1.06-24.7) and OR = 0.35 (95%CI = 0.082-1.35), respectively][36].

DISCUSSION

Our review of current reports assessing the GM composition relative to response to ICB, indicated high variability in the results and limited concordance on the organisms identified (Figure 2). Amongst the

Table 3 Characteristics studies exploring link between gut microbiome composition and diversity and immune-checkpoint blockade therapy-related colitis in metastatic melanoma patients treated with immune-checkpoint blockade therapy

Ref.	Year	Therapy	Method	Sample size/ timepoint	Dominant microbes	Microbial diversity
Dubin *et al* [33], 2015	2015	Anti-CTLA-4 immuno-therapy	16S rRNA gene and shotgun metagenome sequencing of fecal samples	34	Colitis-resistant: *Bacteroidetes* (*Bacteroidaceae, Rikenel-laceae* and *Barnesiellaceae*)	No significant difference in microbial richness and diversity
Chaput *et al* [27], 2017	2017	Anti-CTLA-4 immuno-therapy	16S rRNA gene sequencing of fecal samples	26	Colitis-resistant: *Bacteroidetes*; Colitis-prone: *Firmicutes*	Decreased bacterial diversity was associated with colitis
Andrews *et al*[36], 2021		Combined ICB - Anti-PD-1 and anti-CTLA-4	16S rRNA gene and shotgun metagenome sequencing of fecal samples	38	Colitis resistant: *Firmicutes* ; Colitis prone: *Bacteri-odetes*	No significant difference in alpha diversity

Anti-CTLA-4: Anti-cytotoxic T lymphocyte-associated antigen-4; N/A: Not applicable; Anti-PD-1: Anti-programmed death-1; qPCR: Quantitative real-time polymerase chain reaction; ICB: Immune checkpoint blockade therapy.

few commonalities, we found that fecal samples enriched in organisms from the *Firmicutes* phylum (*Lachnospiraceae* and *Ruminococcaceae* family) especially the *Faecalibacterium* genus were associated with ICB responders in 4 of 9 studies[12,27,28,31], while *Bacteroidetes* phylum was found in higher proportions in non-responders in 2 of the studies[12,30]. However, other than these two findings, there was no clear correlation between specific GM composition and response to ICB.

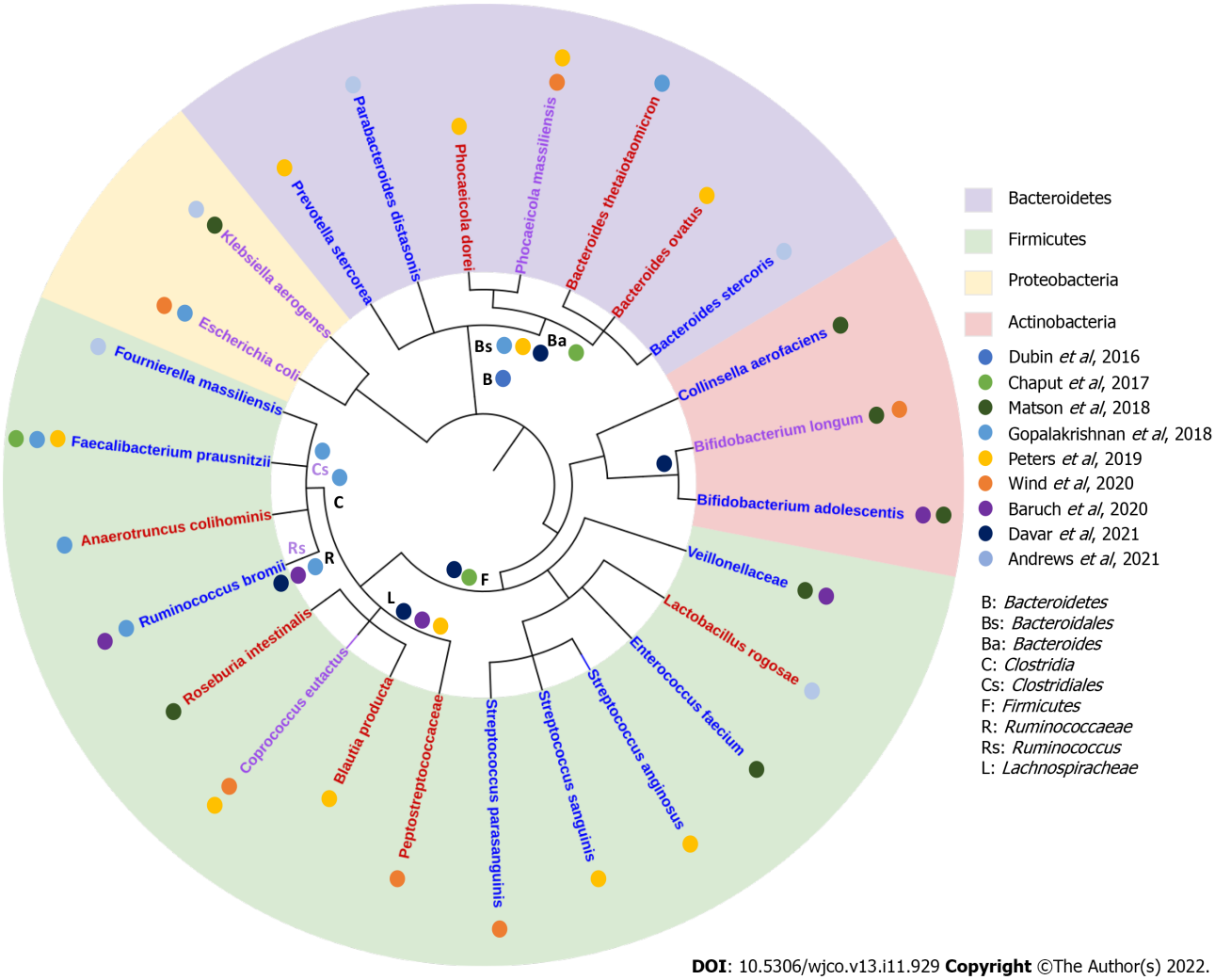
In fact, our analysis mainly identified inconsistencies in the GM composition reported to be associated with response to ICB. For instance, *Bifidobacterium longum* was found to be abundant in responders in the study by Wind *et al*[31], but found to be enriched in non-responders in the study by Matson *et al*[29]. Some species from the *Firmicutes* family were found in both responders and non-responders such as *Roseburia intestinalis*[29]. Similarly, species from the *Bacteroidales* order were found in both responders and non-responders, such as *Bacteroides massiliensis*[31]. The overlap in GM composition in responders and non-responders may suggest that the functional capacity of the GM may be more important than individual GM family/order/species in determining response to ICB[30].

In contrast to individual species or taxas, GM diversity have been heralded to a marker of good health[37]. Here four studies - Gopalakrishnan *et al*[12], Peters *et al*[30], Wind *et al*[31] and, Andrews *et al* [36] - assessed its potential to predict ICB responsiveness. The two first studies demonstrated that higher GM diversity in the responder group compared to non-responder arm[12,30]. However, the other two studies, Wind *et al*[31] and Andrews *et al*[36], found no differences in GM diversity between both groups. Nevertheless, in other cancer types such as renal cell carcinoma and non-small cell lung cancer, greater GM diversity has also been associated with improved responses to anti-PD-1 therapy[37-39].

Study results showing associations with GM diversity were consistent with previous studies which showed that greater GM diversity is prevalent in healthy state across multiple diseases, plausibly suggesting that a greater GM diversity produces the optimal immune environment needed for normal physiological functioning[40-42]. One major reason is the promotion of a favorable immune phenotype, as evidenced by the positive correlation between Shannon diversity index and several CD8+ T cell and NK cell signatures, required to produce a robust anti-tumoral response[38].

Previous studies have demonstrated that GM from *Firmicutes* family and *Bacteroidales* order play a significant role in mediating the response to immunotherapy in melanoma patients[12,27,29]. For instance, abundance of *Firmicutes* was associated with increased frequencies of CD4+, CD8+ T cells, CD 45+ myeloid and lymphoid tumor-infiltrating cells and preserved cytokine response to anti-PD-1 therapy[12]. Additionally, abundance of *Firmicutes* was linked with decreased frequency of intestinal and systemic regulatory T cells (Tregs) and $B7^+$ T cells, cells responsible for limiting immune response robustness[27]. This resulted in increased antigen presentation and effector T cell function in both the periphery and tumor microenvironment[12,27,29]. However, other GM such as *Bacteroidales* were unfavorable in terms of anti-tumoral response in that its abundance was associated with higher frequencies of Tregs and myeloid-derived suppressor cells and a blunted cytokine response[12]. These findings combined demonstrated that certain GM play a crucial role in mediating systemic and antitumor immune responses which have clear implications on efficacy on ICB therapy in metastatic melanoma patients.

Notably, GM has also been shown to potentially serve as not just a predictor of ICB therapy response, but also for boosting response to ICB therapy. FMT on anti-PD-1 treatment-refractory metastatic melanoma patients produced a complete response to anti-PD-1 therapy in one-third (9 out of 25 patients) of the otherwise therapy refractory patients[26,28].



DOI: 10.5306/wjco.v13.i11.929

Figure 2 Phylogenetic tree showing family and species of gut microbiome abundant in responders and non-responders to immune-checkpoint blockade therapy in all included studies. Gut microbiome species highlighted in red: Abundant in non-responders to immune-checkpoint blockade therapy; blue: Abundant in responders to immune-checkpoint blockade therapy; purple: Abundant in both responders and non-responders.

Another aspect of the GM analyzed here, was its association with ICB-related colitis. The three studies included in this review demonstrated that GM which was abundant in ICB-related colitis-prone patients was enriched in responders to ICB (*Firmicutes*) while GM which was abundant in ICB-related colitis-resistant patients was enriched in non-responders to ICB (*Bacteroidetes*). This is consistent with the understanding that a more effective anti-tumoral response will produce greater off-target effects. The *Bacteroidetes* phyla has been linked with low-grade systemic inflammation, which could explain the observation that *Bacteroidetes* phyla was abundant in ICB-related colitis-resistant patients[27]. In line with this observation is the finding that level of *Bacteroidetes* is lower in inflammatory bowel disease - an autoimmune condition which produces chronic inflammation of the digestive tract - patients relative to healthy patients[43]. Conversely, *Firmicutes* phyla, especially *F. prausnitzii* has been associated with induction of Tregs which express high levels of CTLA-4, fueling speculation that it may cause sequestration of Tregs within the intestine[44]. Since Tregs express high levels of CTLA-4, their actions are inhibited, thereby limiting self-tolerance and promoting the development of colitis. These findings reiterate that GM has an immunomodulatory role, giving them the potential to be utilized as biomarkers of ICB-related colitis, in addition to response to ICB.

Our systematic review has several strengths. Firstly, unlike previous reviews which combined studies in various cancer types, this review focused solely on the effect of GM composition and diversity only in patients with melanoma. Secondly, we conducted a comprehensive search for RCTs and observational studies, performed a risk-of-bas assessment and studied clinically important outcomes - clinical response and ICB-related colitis - an adverse event reported in up to 25% of patients treated with ICB [45]. Thirdly, we only included studies which assessed response to immunotherapy in humans, not animals. However, several limitations exist in our systematic review. Studies which we included used distinct approaches when segregating patients into the responder and non-responder groups, using different response criteria to evaluate treatment response in patients. Additionally, there were

differences in methods of stool collection and analysis of GM composition and diversity. For example, Chaput *et al*[46] collected multiple stool samples every 3 wk of ICB, while other studies such as Dubin *et al*[33] and Matson *et al*[29] collected stool samples only prior to initiation of ICB. Furthermore, only 4 studies considered confounding factors such as variation in diet and antibiotic use[27,29-31]. Therefore, inter-study comparison of the GM composition and diversity in responders *vs* non-responders and those who experienced colitis *vs* non-colitis should be addressed with caution. Furthermore, included studies only enrolled a small number of patients, which could explain inconsistent results between studies.

CONCLUSION

In conclusion, GM composition and diversity holds some potential as a biomarker of response and toxicity to ICB in melanoma. Larger prospective studies with standardized experimental protocol ought to be conducted to elucidate whether distinct GM signatures are required for robust response to different ICB regimens. Additionally, more studies correlating metagenomic and metatranscriptomic data of GM to outcomes of melanoma patients on immunotherapy ought to be performed as the functional capacity may be more important rather than individual GM family/order/species. In addition, we eagerly await the outcome of multiple large-scale RCTs involving FMT in the context of ICB-refractory melanoma such as NCT04577729 and NCT04988841 (PICASSO) (ClinicalTrails.gov).We foresee that together with other promising biomarkers, GM composition and diversity will be integrated into a multiparameter model to accurately predict which subset of melanoma patients are likely to respond to ICB[10,11,47].

ARTICLE HIGHLIGHTS

Research background

Survival for metastatic melanoma has significantly improved since the introduction of immune checkpoint blockade (ICB) therapy. However, despite their considerable efficacy, 40%-60% of melanoma patients do not experience objective responses to the therapy. Additionally, some patients experience ICB-related colitis as a consequence of ICB therapy, preventing them from deriving the full benefit of ICB therapy. Recent studies have demonstrated that the gut microbiome (GM) may affect tumor immunity by regulating the host immune system and tumor micro-environment, thus suggesting that GM may affect response to ICB therapy and susceptibility of ICB-related colitis.

Research motivation

The GM has shown great potential as a biomarker of response to ICB therapy in melanoma patients. Previous studies investigating GM composition and/or diversity in patients with melanoma have identified distinct GM composition and diversity in responders to ICB compared to non-responders, as well as those more susceptible to ICB-related colitis than those who are not.

Research objectives

To be the first to compile the existing data regarding the role of GM composition and diversity in predicting response to ICB and ICB-related colitis specifically in patients with melanoma.

Research methods

Comprehensive literature search was done in various platforms using the following search terms: (fecal OR gut) AND (microbiota OR microbiome) AND (melanoma) AND (immunotherapy OR checkpoint OR nivolumab OR ipilimumab OR pembrolizumab). From a total of 300 studies, nine studies met inclusion criteria. Two studies were phase I clinical trials, while the remainder were prospective observational studies. All but one study has moderate risk of bias. Data from these studies including but not limited to, number of participants, type of immunotherapy received, GM analysis method, and GM composition and diversity were collected and interpreted.

Research results

Fecal samples enriched in *Firmicutes* phylum were associated with good response to ICB therapy, however they were associated with increased susceptibility to ICB-related colitis. Fecal samples enriched in *Bacteroidales* family were associated with poor response to ICB. Samples with greater GM diversity were associated with more favorable response to ICB. Fecal samples enriched in *Bacteroidetes* were associated with decreased incidence of ICB-related colitis. Overall, there was limited concordance in the organisms in the GM identified to be associated with response to ICB, and studies evaluating GM diversity showed conflicting results.

Research conclusions

GM composition and diversity holds some potential as a biomarker of response and toxicity to ICB in melanoma. Further prospective studies, including several RCTs that are underway, are needed to confirm whether the GM could be used as a biomarker and potential intervention to modulate ICB response in melanoma patients.

Research perspectives

With other promising biomarkers, GM composition and diversity holds potential to be integrated into a multiparameter model to accurately predict which subset of melanoma patients are likely to respond to ICB.

FOOTNOTES

Author contributions: Oey O, Simadibrata DM, Gray E and Khattak MA contributed to the study conception and design; Oey O and Liu Y performed data extraction; Oey O and Simadibrata DM performed risk of bias assessment; Oey O, Liu Y, Sunjaya AF, Simadibrata DM, Khattak MA and Gray E performed data analysis; Oey O written the first draft of the manuscript; all authors commented on previous versions of the manuscript, read and approved the final manuscript.

Conflict-of-interest statement: Khattak MA reports receiving travel support from Merck Sharp and Dohme (MSD), Bristol-Myers Squibb and Merck Serono. Gray E reports receiving travel sponsorship from MSD. Oey O, Liu Y, Sunjaya AF, and Simadibrata DM report no competing interests.

PRISMA 2009 Checklist statement: All authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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S-Editor: Wang LL
L-Editor: A
P-Editor: Wang LL

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