April 08, 2021

World Journal of Gastrointestinal Oncology
Re: “Immune checkpoint inhibitor-mediated colitis and diarrhea in gastrointestinal malignancies and inflammatory bowel disease”

We appreciate the insightful comments from reviewers and welcome the opportunity to revise our manuscript. Please see individual comments and responses below.

Reviewer 1:
Suggestion: - Data on the incidence of colitis on patients who underwent endoscopy would be useful to report in the incidence section of the article.

We appreciate this suggestion from the reviewer, as information on the rates of endoscopy in studies of the incidence of IMC is important to appropriately critique the literature available on this disorder. Sadly, endoscopy rates are frequently not reported, particularly within clinical trials focused on efficacy of ICI and safety across a broad swath of irAE. Nonetheless, we have collected what information is available in the studies cited in our review, and discussed in the manuscript as below:

Unfortunately, most clinical studies focused on safety and use of ICI have not included information on endoscopy in those patients who develop diarrhea or colitis. As noted in multiple meta-analyses of IMC which incorporate these studies, lack of information on endoscopy is a significant limitation on accurate estimates of the rate of IMC[27,36]. As the common terminology criteria for adverse events (CTCAE) for diarrhea and colitis does not require endoscopic assessment (Table 1), it is probable that many of these patients do not undergo endoscopy to confirm diagnosis of IMC[22].

In cohort studies focused on IMC, patients were more likely to be included only if they had endoscopic and/or histologic confirmation of colonic inflammation. Across thirteen cohort studies of IMC, encompassing 863 patients, 626 (72.5%) underwent endoscopy to confirm the diagnosis, ranging from 40-100% of patients within each study[23,37–48]. However, because patients with symptoms of IMC but without endoscopy were frequently excluded from these studies, the ability to extract information on IMC incidence from this literature is limited.

Minor point: Table 4 – consider rounding the percentages to whole numbers or one decimal place and keep consistent in the table.

Thank you for noticing these errors, we have corrected this.

Reviewer 2:
Suggestion: The mechanism of immune checkpoint inhibitor mediated colitis needs further introduction.

Expanded information on the mechanisms of IMC is an excellent suggestion. Although at the present there is limited information on the mechanisms of IMC, we have modified and added the following paragraphs in our introduction to discuss what little is currently known:

The specific mechanisms of irAE remain unclear, but are thought to be related to removal of key peripheral tolerance pathways normally maintained by immune checkpoints, resulting in inflammation in several non-tumor tissues[13]. Some studies have speculated that anti-PD-1 treatment may modulate humoral immunity to enhance pre-existing anti-thyroid

antibodies, in the case of ICI-induced hypothyroidism[14]. In a report from one patient with myocarditis, similar T cell clones were found in myocardium and tumor[15]. In addition, the rate of vitiligo in patients treated with ICI for melanoma is higher compared to other tumor types[16]. These findings suggest that there may be cross-reactivity between tumor antigens and self-antigens that arises when immune checkpoints are removed.

Beyond cross-reactivity of antigens, there is some evidence that in the colon immune checkpoint blockade may shift the balance of regulatory T cells, responsible for peripheral immune tolerance, and Th17 cells, which produce interleukin (IL)-17 that can drive inflammation. For example, one study has found increased serum IL-17 in patients with colitis due to ICI[17]. In mouse models, administration of recombinant PD-L1 protein decreases the number of IL-17-producing cells in the colon and ameliorates colitis[18]. Meanwhile, mutations in the *Ctla4* gene in both humans and mice are associated with colitis[19]. Overall, it is currently speculated that loss of CTLA-4 and/or PD-1 may lead to decrease activation of regulatory T cells and increased activation of effector T cells, leading to unchecked inflammation in the colon and elsewhere[20].

Cytotoxic CD8+ T cells may also play a role in the development of IMC. A recent analysis of single-cell RNA sequences from patients with IMC revealed that tissue resident memory CD8+ T were more likely to have expanded into inflammatory populations within colon tissue compared to patients without IMC[21]. These findings suggest that activation or alteration of CD8+ T cell populations may also be a potential mechanism of colitis due to checkpoint inhibitors.

The format of the article needs to refer to the requirements of the magazine.

As below, we have updated the formatting of the references to fit the requirements of the journal.

Reviewer 3:
However, the managements were not clearly explored.

As mentioned in our review, an in-depth discussion of IMC management is beyond the goals of this manuscript. However, we recognize that what information had been provided was quite minimal, and therefore we have expanded the discussion of IMC management to include the following:

Several guidelines from both Oncology and Gastroenterology societies have recently emerged to direct therapy of IMC[57–61]. For grade 1 diarrhea and/or colitis, supportive therapy is recommended, including anti-diarrheal medications, dietary modifications, and hydration. For higher grade IMC, many guidelines recommend temporary cessation of ICI and treatment with steroids, which appears to be effective in 87.5% of patients[37]. Permanent discontinuation of anti-CTLA-4 is recommended with grade 3 IMC, and discontinuation of all ICI is recommended for grade 4 colitis[58,62].

If a patient does not respond to steroids in 2-3 days, current recommendations are to advance to an anti-inflammatory biologic, typically the anti-tumor necrosis factor antibody infliximab[56–58]. The anti-integrin antibody vedolizumab is emerging as an alternative to infliximab, particularly in those with concomitant hepatitis who should not receive infliximab[57,63,64]. There are also case reports of the use of the small molecule Janus kinase inhibitor tofacitinib[65] and fecal microbiota transplantation[66] in IMC refractory to the above therapeutic options. Finally, case reports have suggested that budesonide may be an option for treatment of microscopic colitis due to ICI[67].

Editors:

PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references.

We have updated the references to include this information.

Sincerely,

Alexa R. Weingarden, MD/PhD, on behalf of co-authors
Samuel J.S. Rubin, PhD
John Gubatan, MD