

Dear Editor,

I would like initially to thank the reviewers for the thorough review our manuscript was subjected to. The changes in the manuscript were highlighted in bold characters.

A Full copy of this paper with the corrections in bold appears in the supplemental material.

In response to reviewer #1 we added information regarding the possible mechanisms whereby FMT restores sensitivity to Anti-PD1 agents in the discussion section as follows:

The mechanism whereby FMT can restore sensitivity to anti-PD1 monoclonal antibodies is not entirely understood. Davar et al. demonstrated that FMT induced rapid and durable changes in the gut microbiota. The responders showed an increased abundance of specific taxa associated with response to anti-PD-1, increased activation of CD8+ T cells with higher cytolytic functions, and decreased frequency of interleukin-8-expressing myeloid cells, which may have immunosuppressive activity (5). Proteomic and metabolomic analyses revealed distinct signatures in the responders, and network analyses confirmed that the gut microbiome regulated these changes (5). In addition, Baruch et al. (4) reported that gut sample analysis demonstrated post-treatment up-regulation of gene sets related to APCs via MHC class 1 and IL-1 mediated signaling. Furthermore, tumor sample analysis showed post-treatment up-regulation of multiple immune-related gene sets (Interferon- γ , T cell activation, MHC Class II protein complex, dendritic cell differentiation, and T helper 1 type immune response)(4).

Regarding the English review, we rechecked the English once more for spelling and grammar errors with the help of AI language software to make all the corrections necessary to improve the quality of the text.

In response to reviewer 2:

By what mechanics can FMT restore sensitivity to Pembrolizumab?

See above answer to reviewer #1.

The text of the manuscript must be carefully and thoroughly revised by a native speaker.

We reviewed the manuscript with AI powered programs (Microsoft Word and Grammarly and Chat-GPT) to improve the text and make it more close to what a native English speaker would write.

Please avoid referring to the patient as him/he.

We changed all occurrences of him/he in the case report.

What clinically happened to the patient when the treatment was Ipilimumab plus Pembrolizumab?

We added to the case report that the patient was progressing on Ipilimumab and Pembrolizumab, as follows:

In December 2020, we started Ipilimumab plus Pembrolizumab with a new progression

What was the reason for taking FMT into consideration?

We added in the introduction that because of encouraging data regarding FMT action to restore immunotherapy sensitivity, we tried this procedure in our patient.

FMT can restore sensitivity to anti-PDL1 immunotherapy in about 40% of patients previously refractory to this medication (5,6). **Based on these encouraging preliminary data (5,6), we report a case of a 57-year-old patient refractory to immunotherapy who benefited from FMT added to Anti-PD1 immunotherapy with Pembrolizumab, which he was previously refractory to.**

Were the pathological characteristics of the two patients' disease similar? Please include the data related to this! Also, the characteristics of the two patients alongside their prior lines of therapy must be included!

We added this information to the case report, as follows:

Fecal material was obtained from a female metastatic melanoma patient donor who achieved a longstanding complete response to Ipilimumab and Nivolumab and was off therapy in remission for more than two years.

Was complete remission declared at last? Please declare the status of the patient as of 23 June, 2023!

We added this information as requested in the case report:

until this writing (06/23/2023), and the patient is presently in remission without evidence of melanoma recurrence.

All this information was added to the discussion section

Most sentences in the discussion section do not have any references to back them up. Please revise and add!

As suggested, we added references throughout the discussion section to contextualize the statements made.

Please expand the discussion on previous studies that have used FMT to reverse resistance to immunotherapy! Only two studies have been discussed which must be more!

Please refer to the above answer to reviewer #1. Please note that there are very few articles on FMT and reversal of immunotherapy resistance in refractory melanoma patients. We cited the 2 most important articles (Davar *et al* and Baruch *et al*) and a Systematic review that includes these two papers and all other reports by Ferreira *et al*.

This report has limitations. Besides the fact that we only reported one case of restoring sensitivity to anti-PD1 treatment through FMT, we did not pursue immunological studies or fecal microbiome analysis here.

Please discuss the shortcomings of the study and how they could influence the whole trajectory!

We added to the end of the last paragraph the following,

Despite these limitations, the favorable clinical and long-lasting effect we saw in our patient without significant toxicity suggests that this procedure should be considered in similar patients with immunotherapy refractory melanomas.

Please define all abbreviations of the figure captions.

We changed the legend to Figure 1 as follows,

Figure 1: Time sequence of the fecal microbiota transplantation (FMT) and the following PET images with their dates. As can be seen, the disease decreased in size after FMT showing that Pembrolizumab regained activity after the FMT procedure. Surgery (SO) done in September 2021 rendered the patient disease-free. (Figure Copyright of the author)

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