Gut Dysbiosis and Fecal Calprotectin Predict Response to Immune Checkpoint Inhibitors in Patients With Hepatocellular Carcinoma

Francesca Romana Ponziani [ID] ,[1,2] Angela De Luca,[1] Anna Picca,[3,4] Emanuele Marzetti,[3] Valentina Petito,[1,2] Federica Del Chierico,[5] Sofia Reddel,[5] Francesco Paroni Sterbini,[6] Maurizio Sanguinetti,[2,6] Lorenza Putignani,[7] Antonio Gasbarrini,[1,2*] and Maurizio Pompili[1,2*]

The gut microbiota is a well-known prognostic factor and a modulator of treatment sensitivity in patients with cancers treated with immune checkpoint inhibitors. However, data on hepatocellular carcinoma (HCC) are lacking. This study aimed to evaluate the prognostic role of the gut microbiota and changes produced by immunotherapy on the intestinal environment in patients with cirrhosis and HCC. Eleven patients treated with Tremelimumab and/or Durvalumab were included in the analysis. All study participants underwent gut microbiota profiling, quantification of fecal calprotectin, serum levels of zonulin-1, lipopolysaccharide binding protein (LBP), and programmed death-ligand 1 (PD-L1) at baseline and at each treatment cycle until the third cycle, then every three cycles until treatment discontinuation or last visit. The 6 patients who achieved disease control (DC) showed lower pretreatment fecal calprotectin (median, 12.5; interquartile range [IQR], 5-29 vs. median, 116; IQR, 59-129 μg/g; $P = 0.047$) and PD-L1 serum levels (median, 0.08; IQR, 0.07-0.09 vs. median, 1.04; IQR, 0.17-1.95 ng/mL; $P = 0.02$) than nonresponders. The relative abundance of *Akkermansia* (log2 fold change [FC], 2.72; adjusted P [Padj] = 0.012) was increased, whereas that of *Enterobacteriaceae* (log2 FC, –2.34; Padj = 0.04) was reduced in the DC group. During treatment, fecal calprotectin showed a temporal evolution opposite to the *Akkermansia* to *Enterobacteriaceae* ratio and gut microbiota alpha diversity, but similar to zonulin-1 and LBP. *Bifidobacterium* had a stable behavior in patients with a long follow-up, while *Akkermansia* was more variable. *Akkermansia* and *Bifidobacterium* showed similar temporal patterns and causative relationships with *Prevotella, Veillonella, Ruminococcus, Roseburia, Lachnospira, Faecalibacterium,* and *Clostridium*. Conclusion: A favorable composition of the gut microbiota and low intestinal inflammation are associated with achieving DC. The intestinal environment changes dynamically during therapy. (*Hepatology Communications* 2022;6:1492-1501).

I mmune checkpoint inhibitors (ICIs) are molecules able to enhance the immune system response; they represent the new frontier for treatment of various types of cancer, including hepatocellular carcinoma (HCC).[(1)] Recent findings suggest that nonalcoholic fatty liver disease (NAFLD) could be associated with

immune dysregulation and unfavorable response to ICIs treatment in patients with HCC, probably owing to nonalcoholic steatohepatitis-related aberrant T-cell activation and exhaustion.[2]

The gut microbiota has been linked to the response to ICIs in patients with solid tumors.[3] Chronic inflammation is a well-known risk factor for the development of HCC, and has been associated with the gut microbiota profile, intestinal inflammation, and immune system imbalance.[3,4] However, studies analyzing the gut microbiota of patients with cirrhosis and HCC treated with ICIs are lacking.

We report a case series of patients with cirrhosis and HCC treated with ICIs, in order to evaluate the prognostic role of the gut microbiota and changes produced by immunotherapy on the intestinal environment.

Patients and Methods

Patients affected by liver cirrhosis and unresectable HCC who were consecutively enrolled in clinical trials D4190C00022 (NCT02519348) and D419CC00002 (NCT03298451, HIMALAYA)[5] at the Fondazione Policlinico Agostino Gemelli, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) in Rome between May 2018 and December 2019 were included in this prospective observational study. Patients received Tremelimumab, an anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) monoclonal antibody, and/or Durvalumab, an anti-programmed death-ligand 1 (PD-L1) monoclonal antibody, every 4 weeks (one treatment cycle) at different doses and combination schedules, as described in the protocols of the previously mentioned clinical trials. As the interaction between the gut microbiota and ICIs was the target of the study, patients in the Sorafenib arm of trial D419CC00002 were excluded.

Stool and peripheral venous blood samples were collected at baseline and at each treatment cycle until the third cycle, then every three cycles until treatment discontinuation or the last visit. Gut microbiota analysis and quantification of fecal calprotectin, lipopolysaccharide binding protein (LBP), and zonulin-1 serum levels were performed at the above time points, as reported.[4,6] Commercial enzyme-linked immunosorbent assays (ELISA) for the detection of serum levels of PD-L1 (#DB7H10; R&D Systems Inc., Minneapolis, MN) and LBP (human LBP; Hycult Biotech, the Netherlands) were also performed. Clinical variables, adverse events, and reasons for treatment discontinuation were recorded.

Radiological tumor response was assessed every two cycles of treatment using the modified Response Evaluation Criteria in Solid Tumors (mRECIST). Disease control (DC) was defined as evidence of stable disease, partial response, or complete response based on the best radiological response recorded over the entire treatment.

In order to avoid bias in the analysis of the gut microbiota, patients with active alcohol intake and those treated with antibiotics, prebiotics, probiotics,

ARTICLE INFORMATION:

From the [1]Internal Medicine and Gastroenterology, Hepatology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [2]Università Cattolica del Sacro Cuore, Rome, Italy; [3]Department of Geriatrics, Neuroscience, and Orthopedics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [4]Aging Research Center, Department of Neurobiology, Care Sciences, and Society, Karolinska Institutet and Stockholm University, Stockholm, Sweden; [5]Area of Genetics and Rare Diseases, Unit of Human Microbiome, Bambino Gesù Children's Hospital IRCCS, Rome, Italy; [6]Microbiology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; [7]Department of Laboratories, Unit of Parasitology and Area of Genetics and Rare Diseases, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Francesca R. Ponziani, M.D., Ph.D.
Internal Medicine and Gastroenterology,
Hepatology Unit Fondazione Policlinico Universitario
Agostino Gemelli IRCCS

Via della Pineta Sacchetti, 217
00168 Rome, Italy
E-mail: francesca.ponziani@gmail.com
Tel.: +39 0630156018

nonabsorbable disaccharides, laxatives, or proton pump inhibitors in the last 3 months before enrollment were excluded. Bacterial DNA extraction, amplification of the 16s ribosomal RNA V3-V4 region, and sequencing were performed according to the described protocol.[7]

STATISTICAL ANALYSIS

Data were reported as median and interquartile range (IQR) for continuous variables or frequency and percentage for categorical ones. For the analysis of gut microbiota, the Chao1 index of alpha diversity was calculated on raw reads. Bray-Curtis distance was computed after applying the variance-stabilizing transformation for data normalization, and nonmetric multidimensional scaling (NMDS) plots were used to visualize the results. Baseline inflammatory features and the gut microbiota profile of patients who achieved DC and those who did not were compared using the Wilcoxon test, whereas differential abundance analysis of the gut microbiota at the family and genus levels was performed using DESeq2, as described.[7] To reduce error and uncertainty, $\log_2$ fold change (FC) of taxa that were highly variable between samples or had low read counts were subjected to shrinkage.

To explore the dynamics of the gut microbiota during ICIs treatment, variation in relative taxa abundance was analyzed to identify the core microbiota (taxa observed at all time points for at least 6 months/cycles), the persistent microbiota (taxa observed in 20% or more of time points for at least 6 months/cycles, with at least 75% consecutive observations), and the transient microbiota (taxa observed in 50% or more of time points for at least 6 months/cycles, but with at most 74% of consecutive observations).[8] The presence of taxa with nonstationary behavior (i.e., those with mean abundance, standard deviation, variance, covariance, and autocorrelation that change over time) was also assessed as an indirect marker of intermicrobial competition or other factors that may influence the dynamics of the gut microbiota community.

A pairwise modified dynamic time warping (DTW) algorithm was then used to identify sets of taxa with similar patterns over time.[8] The modified DTW distance ranged from 0 to 1, with values close to 0 showing similar temporal trends; results were plotted as hierarchical trees and heatmaps. The Granger algorithm was adopted to identify causal relationships between taxa that may be involved in gut microbiota temporal changes. Analyses were performed using the R statistics program (version 3.6.2) or the web application "TIME."[8]

Results

During the study period, 25 patients were evaluated for inclusion in the two clinical trials (19 in D4190C00022, 6 in D419CC00002). Nine of these patients did not meet the inclusion criteria, thus 16 were finally randomized; 2 patients in the Sorafenib arm of the D419CC00002 trial were excluded and 3 were unable to provide the baseline fecal sample. Therefore, 11 patients, 9 men and 2 women, were enrolled in this study (Table 1). Median age was 68 years (IQR, 66-80 years); the most common etiology of cirrhosis was viral (five cases), followed by NAFLD (three cases) and alcohol abuse (three cases). All participants showed good liver function (Child-Pugh A) and performance status (grade 0 according to the Eastern Cooperative Oncology Group [ECOG] classification). Endoscopic signs of portal hypertension were observed in approximately half of patients.

Seven patients presented a multinodular HCC, with a median overall tumor size of 12 cm (IQR, 10.5-15.5 cm); extrahepatic spread was recognized in 4 patients and macrovascular invasion in 1, whereas 1 patient presented both. Four patients received the combination of Tremelimumab plus Durvalumab; the others received either Durvalumab (4 patients) or Tremelimumab (3 patients) monotherapy.

At the time of the analysis, the median treatment duration was 5.97 months (IQR, 4.33-10.37 months), and treatment was still ongoing for 2 patients who showed stable disease at cycle 18 and cycle 3. Six patients achieved DC; these subjects showed lower fecal calprotectin concentrations (median, 12.5; IQR, 5-29 vs. median, 116; IQR, 59-129 μg/g; $P = 0.047$) and PD-L1 serum levels (median, 0.08; IQR, 0.07-0.09 vs. median, 1.04; IQR, 0.17-1.95 ng/mL; $P = 0.02$) at baseline compared with nonresponders, whereas zonulin-1 (median, 7.22; IQR, 0.63-9.05 vs. median, 5.13; IQR, 4.79-9.53 ng/mL; $P = 0.75$) and LBP (2.17; IQR, 1.77-2.52 vs. median, 2.22; IQR, 2.12-2.39 mg/L; $P = 0.99$) were similar between the two groups. No significant pretreatment difference was observed between responders and nonresponders

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF THE 11 PATIENTS INCLUDED IN THE STUDY

ID	Age (Years)	Sex	BMI	Etiology	Esophageal/ Gastric Varices	Size (cm, Overall)	Size (cm, Largest Nodule)	Number of Nodules	MVI	Extrahepatic Disease/ Type	ECOG	Child-Pugh	MELD	Treatment Duration (Months)	ICI Type	DC	Reason for Discontinuation
PT1	59	Male	19.7	HCV	Yes	7.5	5.5	Multinodular	No	Bone, adrenal	0	A	10	6.93	Tremelimumab plus Durvalumab	No	Progression
PT2	68	Male	32.1	HBV	0	12	6.4	3	No	No	0	A	7	4.33	Durvalumab	Yes	Side effects
PT3	81	Male	22.3	NASH	Yes	22.5	12	Multinodular	No	No	0	A	10	4.33	Tremelimumab	No	Progression/sepsis
PT4	68	Male	27.1	HBV	0	10	3.5	4	No	No	0	A	9	18.73	Tremelimumab	Yes	Progression
PT5	67	Male	23	Alcohol*	0	25	9.5	Multinodular	No	Adrenal	1	A	11	8.73	Tremelimumab plus Durvalumab	No	Progression/ decompensation
PT6	65	Male	28.3	NASH	0	9.7	2.7	Multinodular	No	No	0	A	7	18.97	Durvalumab	Yes	Ongoing
PT7	79	Male	31.22	NASH, alcohol*	Yes	13.1	9	3	No	Lymph Nodes	0	A	10	12.00	Tremelimumab	Yes	Decompensation
PT8	81	Female	20.4	HCV	Yes	16	16	Multinodular	No	No	0	A	9	2.13	Tremelimumab	Yes	Decompensation
PT9	65	Female	20.7	HCV	0	10.9	7.6	3	No	Bone Lymph Nodes	0	A	7	5.73	Tremelimumab plus Durvalumab	No	Progression/ decompensation
PT10	76	Male	22.6	Alcohol*	Yes	15	8	Multinodular	Yes	Peritoneal	0	A	7	3.67	Durvalumab	No	Progression
PT11	85	Male	23.4	Alcohol*	0	12	12	1	Yes	No	0	A	8	5.97	Durvalumab	Yes	Ongoing

Abbreviations: BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus; ICI, immune checkpoint inhibitor; MELD, Model for End-Stage Liver Disease; MVI, macrovascular invasion; NASH, nonalcoholic steatohepatitis.
*Alcohol consumption had been discontinued for at least 3 years before inclusion in the study.
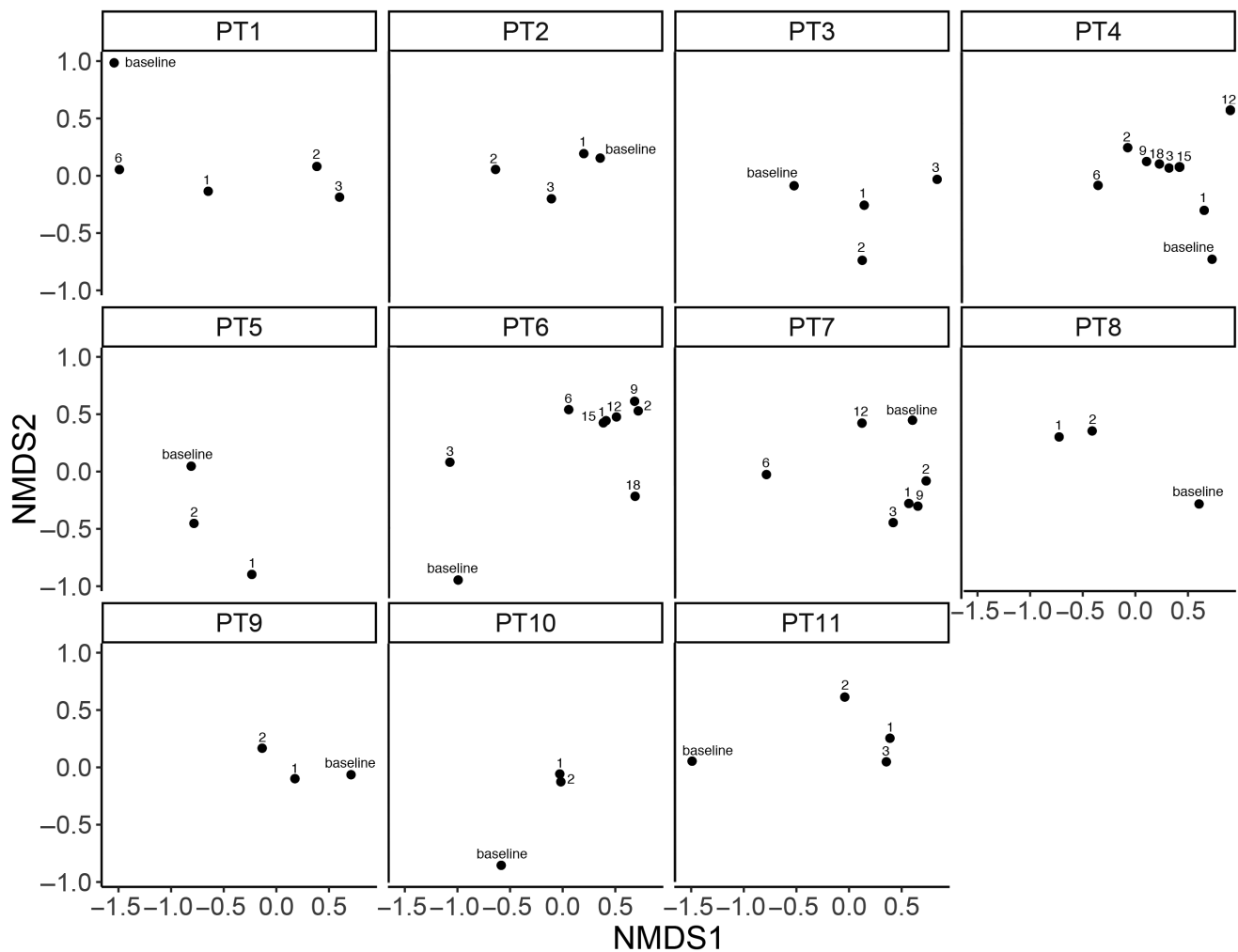
1495

FIG. 1. Nonmetric multidimensional scaling (NMDS) ordination plots on Bray-Curtis distance computed after data normalization using the variance-stabilizing transformation. Each plot shows treatment time points (indicated by labels) for an individual patient.

regarding the Chao1 index of alpha diversity ($P = 0.43$). Notably, in the DC group, pretreatment relative abundance of *Akkermansia* (log2FC, 2.72; adjusted P [Padj] = 0.012) was increased whereas *Staphylococcus* (log2FC, –2.90; Padj = 0.042), *Neisseria* (log2FC, –3.08; Padj < 0.0001), and *Enterobacteriaceae* (log2FC, –2.34; Padj = 0.04) were reduced.

Overall, the gut microbiota composition showed clear fluctuations during the treatment period (Figs. 1 and 2). Calprotectin followed a similar trend to zonulin-1 and LBP, but showed an opposite trend with respect to the *Akkermansia* to *Enterobacteriaceae* (AE) ratio and to the gut microbiota alpha diversity (Fig. 3). In responders or nonresponders who discontinued treatment due to decompensation, side effects, or progression, we observed a drop in the AE ratio at the last follow-up, whereas a progressive increase was observed in patient (PT)6 and PT11, who were the two responders still on treatment at the end of the study. Interestingly, serum levels of PD-L1 increased over time in patients who received the anti-PD-L1 monoclonal antibody Durvalumab.

To reliably assess the stability of the gut microbiota during treatment with ICIs and the microbial connections possibly linked by causal relationships, only PT1, PT4, PT6, and PT7, who were followed for more than 6 months were considered. As expected in a population of patients with cirrhosis, *Enterobacteriaceae* was a core
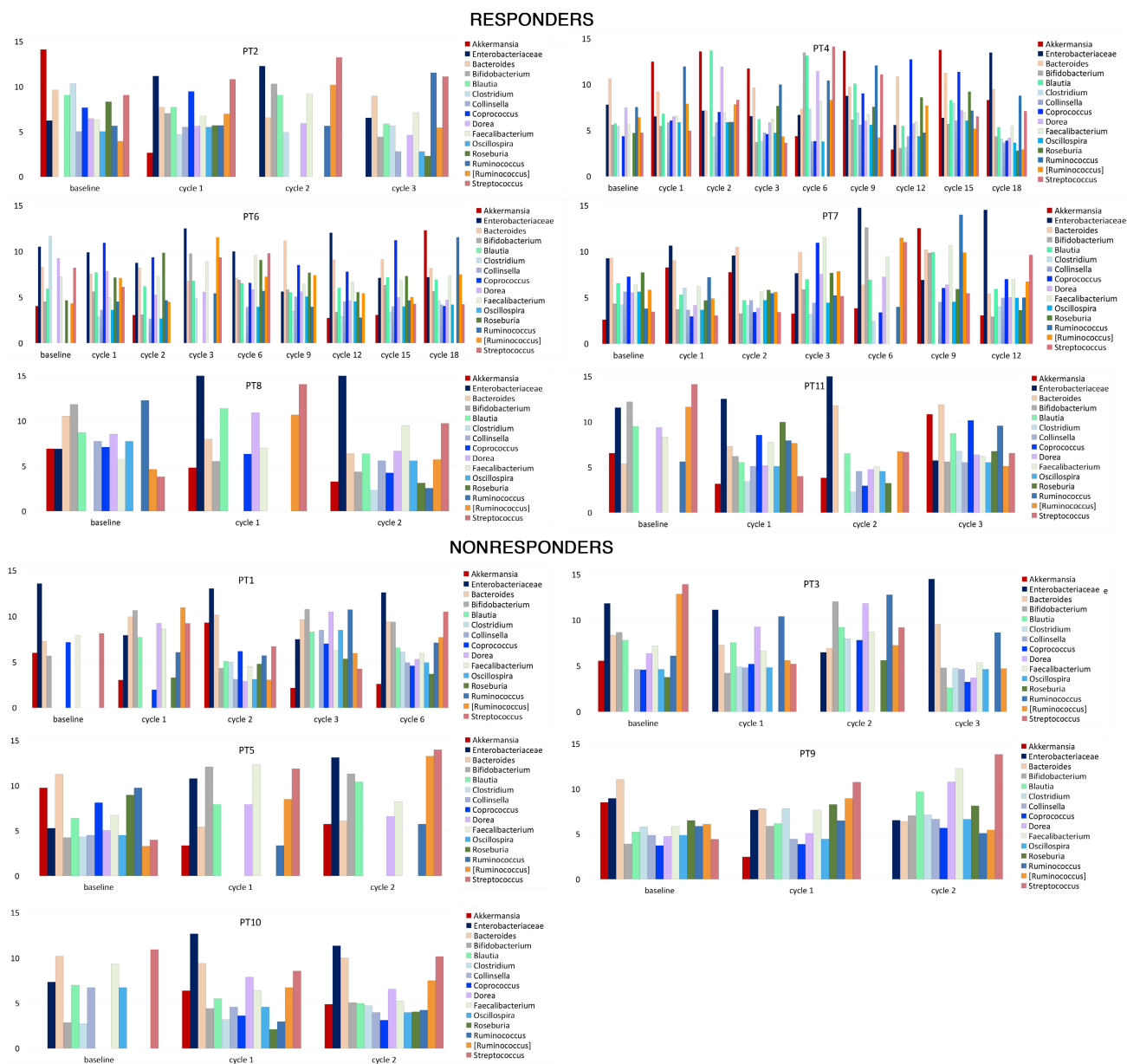
FIG. 2. Evolution of gut microbiota composition during treatment. Bar plots show gut microbiota temporal trend; normalized bacterial abundance (variance-stabilizing transformation) is plotted on the *y* axis.

family; *Akkermansia*, on the other hand, showed mainly nonstationary and variable behavior (Supporting Table S1). *Bifidobacterium* was a core or persistent component in all four participants with the longest duration of treatment. Longitudinal analysis of temporal connections revealed that *Enterobacteriaceae* followed a temporal pattern similar to that of *Methanobacteriaceae* and different from that of *Bifidobacteriaceae* and *Verrucomicrobiaceae* (Fig. 4; Supporting Table S2). A

close causal connection between *Enterobacteriaceae* and *Methanobacteriaceae* was generally observed; in addition, changes in *Enterobacteriaceae* were caused by *Bifidobacteriaceae* and *Verrucomicrobiaceae*. *Bifidobacterium* and *Akkermansia* belonged to clusters of bacteria with similar temporal patterns and causal relationships, including *Prevotella*, *Veillonella*, *Ruminococcus*, *Roseburia*, *Lachnospira*, *Faecalibacterium*, and *Clostridium*.
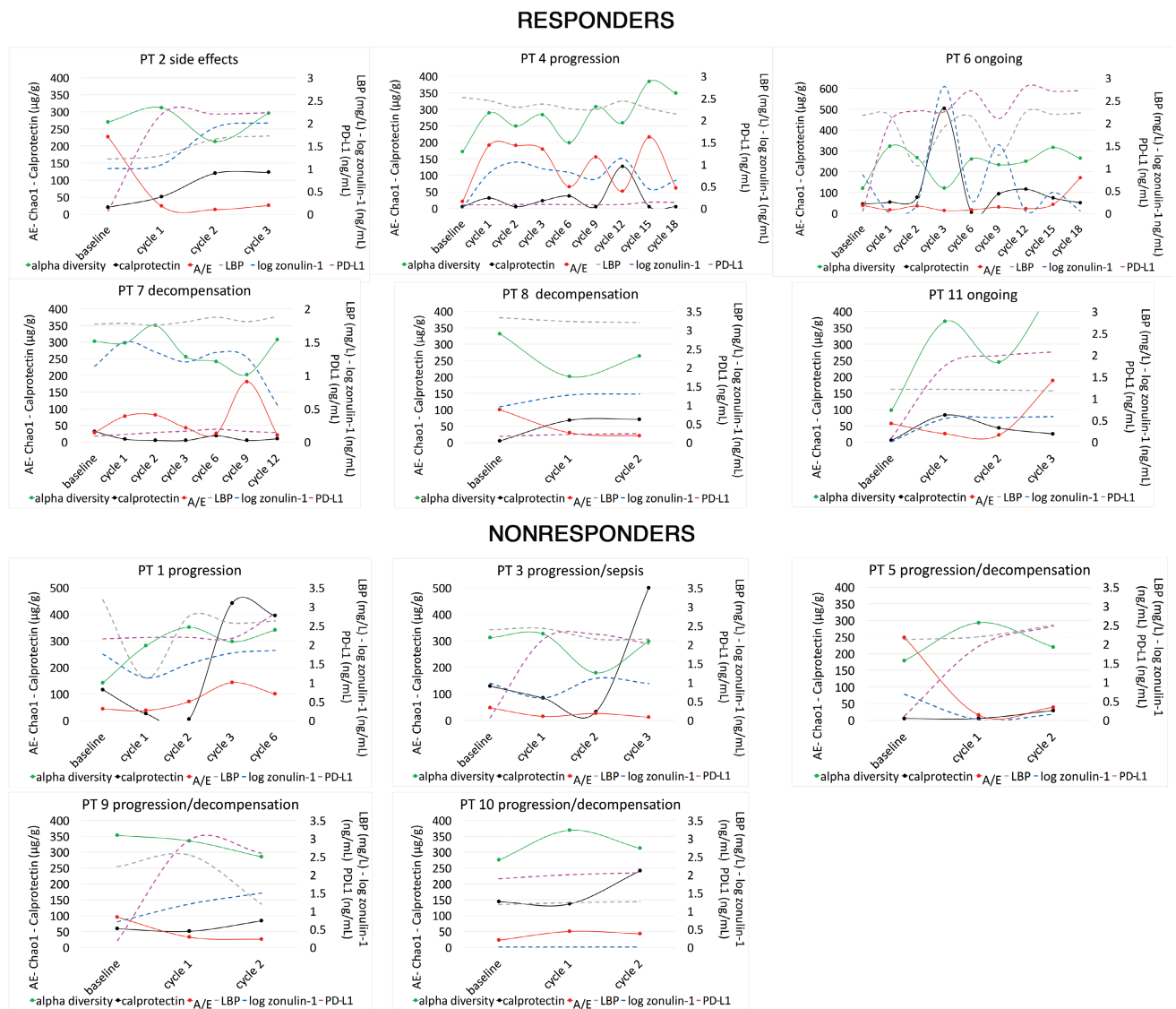
RESPONDERS

NONRESPONDERS

FIG. 3. Changes in markers of gut dysbiosis (AE ratio), intestinal inflammation, and permeability (calprotectin, zonulin-1, LBP) and PD-L1 serum levels over the treatment period. Abbreviations: AE, *Akkermansia* to *Enterobacteriaceae* ratio; LBP, lipopolysaccharide binding protein; PD-L1, programmed death ligand 1.

Discussion

ICIs represent a new treatment option for a large number of solid tumors, with several data pointing to a role for the gut microbiota in the modulation of response. Our study reports the first evidence supporting a prognostic role of intestinal inflammation-associated parameters, such as calprotectin, in patients with liver cirrhosis and HCC receiving ICIs, and demonstrates that the gut microbiota changes dynamically during the treatment period. These modifications are mainly represented by fluctuations in the ratio of beneficial to harmful bacteria, and are related to changes in intestinal inflammation and permeability.

In our case series, pretreatment abundance of *Akkermansia* and low fecal calprotectin concentration were associated with DC. This confirms the association of *Akkermansia* with a better response reported in patients with other solid tumors treated with ICIs, and is consistent with the anti-inflammatory properties of *Akkermansia*.[9] In agreement with these findings, patients who achieved DC had a lower
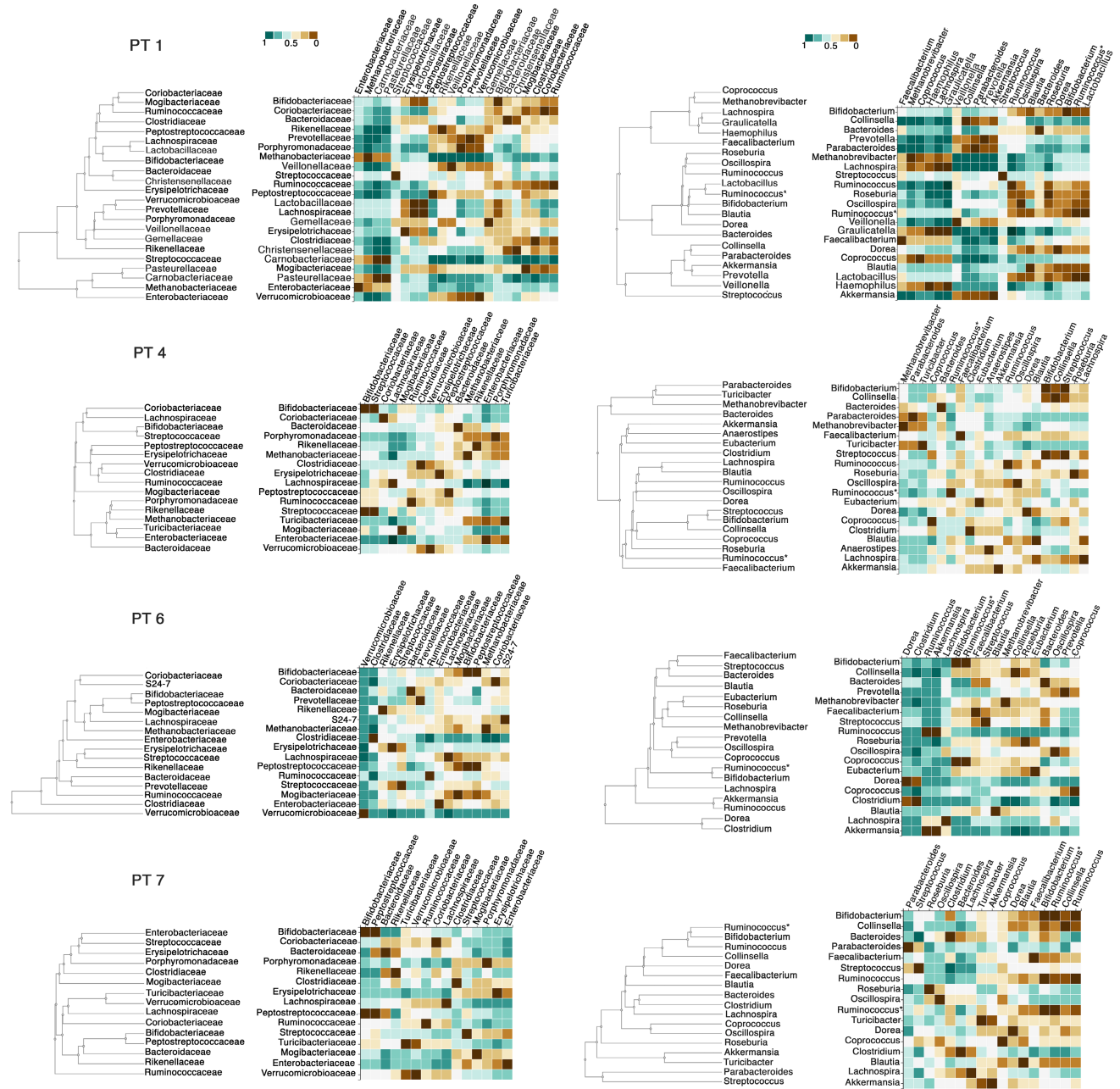
FIG. 4. Similarities of temporal patterns of gut microbiota families (left side) and genera (right side) of PT1, PT4, PT6, and PT7. Hierarchical clustering and heatmaps based on pairwise modified dynamic time warping (DTW) distance show bacteria with a similar temporal variation (DTW near 0, brown color) or different temporal trends (DTW near 1, green color). *Ruminococcus* is *Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Ruminococcus. Ruminococcus** is *Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Ruminococcus.*

fecal calprotectin concentration at baseline, which is indicative of a lower degree of intestinal inflammation. Persistent gut-derived inflammation in patients with cirrhosis leads to exhaustion and paralysis of the immune system, and is an additional factor triggering tumor immune escape.[10] DC was also associated with low baseline serum levels of PD-L1, as already reported for other cancers,[11] as an expression of a milder suppression of the immune response.

During treatment, the gut microbiota changed in all study participants, and the AE ratio, which we used as a marker of dysbiosis, showed an inverse trend

compared with fecal calprotectin, markers of intestinal permeability and bacterial translocation. Furthermore, AE decreased at the time of treatment discontinuation, as if an unfavorable shift in the composition of the gut microbiota was associated with a loss of response to immunotherapy or unfavorable clinical events, such as decompensation. Although the exact immunomodulatory effects of *Akkermansia muciniphila* are still unclear and probably correlated with its anti-inflammatory action and protective role on the intestinal barrier, it has been shown that its supplementation can enhance the effects of ICIs therapy.[12] This can be of paramount importance in some categories of patients, such as those with NAFLD; indeed, a recent study showed a suboptimal response to ICIs in patients with NAFLD-related HCC,[2] in whom a profound derangement of the gut microbiota was demonstrated.[4] Therefore, we think that the role of *Akkermansia* administration as adjuvant therapy to enhance the response to ICIs in patients with HCC deserves further evaluation in future studies.

Our longitudinal analysis also highlighted that *Bifidobacterium* was a core element in patients with the longest follow-up, whereas *Akkermansia* had a more variable behavior. *Akkermansia* and *Bifidobacterium* are usually depleted in patients with cirrhosis and HCC.[4] In addition to *Akkermansia*, *Bifidobacterium* can enhance the effects of ICIs exerting a protective effect on the liver and reinforcing the intestinal barrier.[9] The temporal and causal connections between *Akkermansia*, *Bifidobacterium*, and other members of the gut microbiota, such as *Roseburia*, *Lachnospira*, *Ruminococcus*, and *Faecalibacterium*, could be explained by cross-feeding relationships through the production and use of short chain fatty acids (SCFAs), which are key regulators of the immune system function and have been associated with increased likelihood of response during treatment with ICIs.[13] In contrast, the connections of *Enterobacteriaceae* with *Methanobacteriaceae* could be explained by the implementation of resistance mechanisms based on bacterial metabolism (e.g., bile salt hydrolysis, iron metabolism).

This study suffers from the limitations of case series because it consists mainly of a small number of patients included, due to restrictive selection criteria; furthermore, we cannot exclude the influence of comorbidities on the baseline gut microbiota composition. However, to our knowledge, this is the first Western study focusing on the prognostic role of gut microbiota, intestinal permeability, and inflammation in patients with cirrhosis and HCC receiving ICIs and on their longitudinal evolution. A previous study analyzed the gut microbiota of 8 Asian patients with HCC treated with anti-PD-L1 monoclonal antibodies, and demonstrated that *Akkermansia* among other bacteria was associated with treatment response.[14] However, the study population was not well characterized, and the paper did not report how many patients were affected by liver cirrhosis. In addition, differences in lifestyle and etiology of liver disease make it difficult to apply those findings to Western patients.

In conclusion, cirrhotic patients with HCC who respond to ICIs appear to have a favorable composition of the gut microbiota and a low degree of intestinal inflammation. Dynamic fluctuations in the ratio between beneficial and harmful bacteria are related to changes in intestinal inflammation and permeability during treatment. Our data should prompt further studies in larger cohorts, as precise characterization and modulation of the intestinal environment could allow to identify a subgroup of patients with high probability of a response to ICIs therapy.

REFERENCES

1) Hiam-Galvez KJ, Allen BM, Spitzer MH. Systemic immunity in cancer. Nat Rev Cancer 2021;21:345-359.

2) Pfister D, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, et al. NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450-456.

3) Daillère R, Routy B, Goubet A-G, Cogdill A, Ferrere G, Alves-Costa Silva C, et al. Elucidating the gut microbiota composition and the bioactivity of immunostimulatory commensals for the optimization of immune checkpoint inhibitors. OncoImmunology 2020;9:1794423.

4) **Ponziani FR, Bhoori S**, Castelli C, Putignani L, Rivoltini L, Del Chierico F, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. Hepatology 2019;69:107-120.

5) Abou-Alfa GK, Chan SL, Kudo M, Lau G, Kelley RK, Furuse J, et al. Phase 3 randomized, open-label, multicenter study of tremelimumab (T) and durvalumab (D) as first-line therapy in patients (pts) with unresectable hepatocellular carcinoma (uHCC): HIMALAYA. J Clin Oncol 2022;40:379-379.

6) **Addolorato G, Ponziani FR**, Dionisi T, Mosoni C, Vassallo GA, Sestito L, et al. Gut microbiota compositional and functional fingerprint in patients with alcohol use disorder and alcohol-associated liver disease. Liver Int 2020;40:878–888.

7) Ponziani FR, Putignani L, Paroni Sterbini F, Petito V, Picca A, Del Chierico F, et al. Influence of hepatitis C virus eradication with direct-acting antivirals on the gut microbiota in patients with cirrhosis. Aliment Pharmacol Ther 2018;48:1301-1311.

8) Baksi KD, Kuntal BK, Mande SS. "TIME": a web application for obtaining insights into microbial ecology using longitudinal microbiome data. Front Microbiol 2018;9:36. Erratum in: Front Microbiol 2020;11:605295.

9) Yi M, Yu S, Qin S, Liu Q, Xu H, Zhao W, et al. Gut microbiome modulates efficacy of immune checkpoint inhibitors. J Hematol Oncol 2018;11:47.

10) Ponziani FR, Nicoletti A, Gasbarrini A, Pompili M. Diagnostic and therapeutic potential of the gut microbiota in patients with early hepatocellular carcinoma. Ther Adv Med Oncol 2019;11:1758835919848184.

11) Wei W, Xu B, Wang Y, Wu C, Jiang J, Wu C. Prognostic significance of circulating soluble programmed death ligand-1 in patients with solid tumors: a meta-analysis. Medicine (Baltimore) 2018;97:e9617.

12) Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillère R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018;359:91-97.

13) Malczewski AB, Navarro S, Coward JI, Ketheesan N. Microbiome-derived metabolome as a potential predictor of response to cancer immunotherapy. J Immunother Cancer 2020;8:e001383.

14) Zheng YI, Wang T, Tu X, Huang Y, Zhang H, Tan DI, et al. Gut microbiome affects the response to anti-PD-1 immunotherapy in patients with hepatocellular carcinoma. J Immunother Cancer 2019;7:193.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1905/suppinfo.