

Effects of Immunonutrition in Advanced Human Immunodeficiency Virus Disease: A Randomized Placebo-controlled Clinical Trial (Promaltia Study)

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Background. While nutritional interventions with prebiotics and probiotics seem to exert immunological effects, their clinical implications in human immunodeficiency virus (HIV)-infected subjects initiating antiretroviral therapy (ART) at advanced HIV disease remain unclear.

Methods. This was a pilot multicenter randomized, placebo-controlled, double-blind study in which 78 HIV-infected, ART-naïve subjects with <350 CD4 T cells/μL or AIDS were randomized to either daily PMT25341 (a mixture of synbiotics, omega-3/6 fatty acids and amino acids) or placebo for 48 weeks, each in combination with first-line ART. Primary endpoints were changes in CD4 T-cell counts and CD4/CD8 ratio from baseline to week 48 and safety. Secondary endpoints were changes in markers of T-cell activation, bacterial translocation, inflammation, and α and β microbiota diversity.

Results. Fifty-nine participants completed the follow-up with a mean CD4⁺ T-cell count of 221 ± 108 cells/μL and mean CD4/CD8 ratio of 0.26 ± 0.19. PMT25341 was well tolerated, without grade 3–4 adverse effects attributable to the intervention. While most of the assessed biomarkers improved during the follow-up in both arms, PMT25341-treated subjects did not experience any significant change, compared to placebo-treated subjects, in mean CD4⁺ T-cell count change (278 vs 250 cells/μL, *P* = .474) or CD4/CD8 ratio change (0.30 vs 0.32, *P* = .854). Similarly, we did not detect differences between treatment arms in secondary endpoints.

Conclusions. In HIV-infected patients initiating ART at advanced disease, the clear immunological benefits of ART were not enhanced by this nutritional intervention targeting the gut-associated lymphoid tissue and microbiota.

Clinical Trials Registration. NCT00870363.

Keywords. microbiota; CD4 T cells; probiotics; immunoactivation; inflammation.

Despite global efforts to identify human immunodeficiency virus (HIV) infection at an early stage, HIV is diagnosed late in approximately 50% of cases in high-income countries [1, 2]. According to the consensus definition from the HIV in Europe Study Group, late presenters are defined as those presenting to an antiretroviral therapy (ART) clinic with a CD4 count of <350 cells/μL or an AIDS-defining illness [3]. Low CD4⁺ T-cell counts at ART initiation have been associated with shorter life expectancy [4]. Hence, while early diagnosis and ART initiation must be prioritized in public health strategies, novel interventions

are required for a more successful management of HIV in late-presenter cases.

An increased level of inflammation, immune activation, and low CD4⁺ T-cell nadir is seen in late presenters, along with an impaired immunological recovery during ART [5]. Starting early in HIV infection, the gut-associated lymphoid tissue (GALT) serves as a sanctuary for HIV replication and a portal of systemic inflammation, likely contributing to residual morbidity [6]. A greater decline of circulating CD4⁺ T cells also limits ART-mediated GALT restoration by impairing the number of T cells trafficking to the gut [7, 8], allowing for compositional [9, 10] and functional [11] changes in the microbiota. Indeed, low CD4⁺ T-cell counts in peripheral blood are associated with impaired epithelial proliferation, increased neutrophil infiltration, and mucosal apoptosis in colorectal biopsies, which correlate with the independent predictors of mortality [12, 13].

Different strategies targeting the multifaceted HIV-associated GALT defects have been investigated in an attempt to reduce

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the long-term consequences of chronic inflammation [14–19]. Multiple studies have assessed dietary supplementation in HIV patients with various nutritional products, such as prebiotics and probiotics, among others [10, 14–16, 20], which collectively suggest that this strategy may exert some beneficial immunological effects. Therefore, we developed PMT25341, a product including several nutritional compounds designed to target the different gastrointestinal defects associated with HIV immunopathogenesis. We conducted a double-blind, randomized, placebo-controlled study to evaluate whether PMT25341 administered for 48 weeks in late presenters initiating ART may enhance CD4⁺ T-cell and CD4/CD8 ratio recovery and affect markers of immune activation, inflammation, and bacterial translocation, as well as the gut microbiota composition.

METHODS

Study Design, Participants, Setting, and Eligibility

We conducted a randomized, double-blind, multicenter, placebo-controlled study. Participants were recruited at the HIV units of 7 university hospitals in Spain—Hospital Universitario Ramón y Cajal, Hospital Universitario Clínico San Carlos, Hospital Universitario Doce de Octubre, Hospital Universitario La Paz, Fundación Jiménez Díaz (Madrid), Hospital del Mar (Barcelona), and Hospital San Pedro (Logroño). Participants were HIV-infected, late-presenting (<350 CD4 T cells/μL or AIDS at diagnosis [3]) adults, who initiated ART according to the Spanish Grupo de estudio del SIDA (GESIDA) National Guidelines [21]. Exclusion criteria were age <18 years, pregnancy, type 1 or 2 diabetes, end-stage renal disease, lactose intolerance, use of immunomodulatory drugs, and a neutrophil count of <750 cells/μL. The composition of PMT25341 is detailed in [Supplementary Table 1](#); the placebo was skimmed milk powder. The product and the placebo were prepared by Nutrición Médica, S.L.

The study was approved by the ethics committee of all participating centers and all participants signed an informed consent prior to the initiation of study procedures. This study was registered at ClinicalTrials.gov (identifier NCT03009032).

Randomization

The study participants were randomly assigned to PMT25341 or placebo by a computer-generated randomized number system in blocks. PMT25341 and the placebo were packaged in identically appearing sachets. The clinicians who saw the study participants, the laboratory personnel who handled patient specimens, and the study participants were blinded to the assigned patient group.

Study Outcomes

After screening for eligibility, study visits were scheduled at baseline, and at 4, 24, and 48 weeks. At each visit, a clinical evaluation was carried out, blood samples were collected, and adherence to the intervention and any adverse events were registered. Fecal samples were collected at baseline and week 48. Our

primary outcomes were safety and tolerability, and between-arm changes in CD4⁺ and CD4/CD8 ratio from baseline to week 48. Gastrointestinal (GI) tolerance was also assessed using a questionnaire and scoring the severity of GI symptoms on a 4-point scale.

Our secondary outcomes were changes in plasma markers (interleukin 6 [IL-6], high-sensitivity C-reactive protein [hs-CRP], tumor necrosis factor alpha [TNF-α], soluble CD163 [sCD163], soluble CD14 [sCD14], interferon-gamma-inducible protein 10 [IP-10], lipoteichoic acid [LTA], interleukin 7 [IL-7], interleukin 10 [IL-10], and interleukin 17 [IL-17]), T-cell activation markers (percentage of HLA-DR⁺CD38⁺ CD4⁺ and CD8⁺ T cells), T-cell senescence markers (percentage of CD28[−] T cells), and variations in the microbiota composition (α- and β-diversity metrics) from baseline to week 48. Adherence was assessed using patient diaries that were checked against the returned sachets.

Nucleic Acid Purification, Amplification of the 16S Ribosomal RNA Gene, Sequencing, and Bioinformatics Analysis

Nucleic Acid Purification

Fecal samples were stored in RNAlater (Life Technologies) at −80°C until use. Total DNA was quantified using Qubit fluorometry.

Amplification of the 16S Ribosomal RNA Gene

For each sample, the V3–V4 regions of the 16S ribosomal RNA (rRNA) gene were amplified by polymerase chain reaction (PCR) using the 16S amplicon PCR forward primer (5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 16S Amplicon PCR Reverse Primer (5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). The pooled PCR products were sequenced using the kit V3 (2 × 300 cycles) with MiSeq sequencer (Illumina) at FISABIO Sequencing and Bioinformatics Service, Valencia, Spain. We obtained an average of 62939 16S rRNA joined sequences per sample.

16S RNA Gene Analysis. Biodiversity, and Clustering

Amplicon data from the 16S rRNA gene was analyzed following the recommendations of the metagenomic state-of-the-art pipeline QIIME version 1.9 [22]. Taxonomic information on the 16S rDNA sequences were obtained using the Ribosomal Database Project II [23] and the Greengenes database available in QIIME version 1.8 software. Operational taxonomic units (OTUs) were created using the QIIME version 1.9 script `pick_open_reference_otus.py`, which iteratively uses Uclust [24], and were used to classify different clusters of species with a 97% similarity. OTUs were created using Uclust [25], applying the 97% similarity criterion. Representative sequences were aligned with Pynast [24] against the clustered version of the Greengenes database (database core_set_aligned.fasta.imputed), to use as input to reconstruct the phylogenetic tree using the FastTree software [26]. Distance analysis between samples was performed by the weighted normalized

UniFrac using the script `assign_taxonomybeta_diversity.py`. Sequences were rarefacted to 16590 sequences per sample to standardize α - and β -diversity analyses. A detailed description of the methods is provided in the [Supplementary Materials](#).

Systemic Biomarkers of Disease Progression

Markers of Innate Immune Activation and Bacterial Translocation

A sample of fasting venous blood was obtained to determine the concentrations of glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides using standard

enzymatic methods. Plasma HIV RNA was measured using the Cobas TaqMan HIV-1 assay (Roche Diagnostics Systems, Branchburg, New Jersey). Cryopreserved plasma was assessed by immunoassay in duplicate for plasma levels of the inflammatory markers hs-CRP (Labor Diagnostika, Nordhorn, Germany), sCD163 (Quantikine, R&D Systems, Minneapolis, Minnesota), and the bacterial translocation markers sCD14 (Quantikine, R&D Systems) and LTA (Abbexa, Cambridge, United Kingdom), according to the manufacturers' recommendations. Luminex was used to measure the concentrations

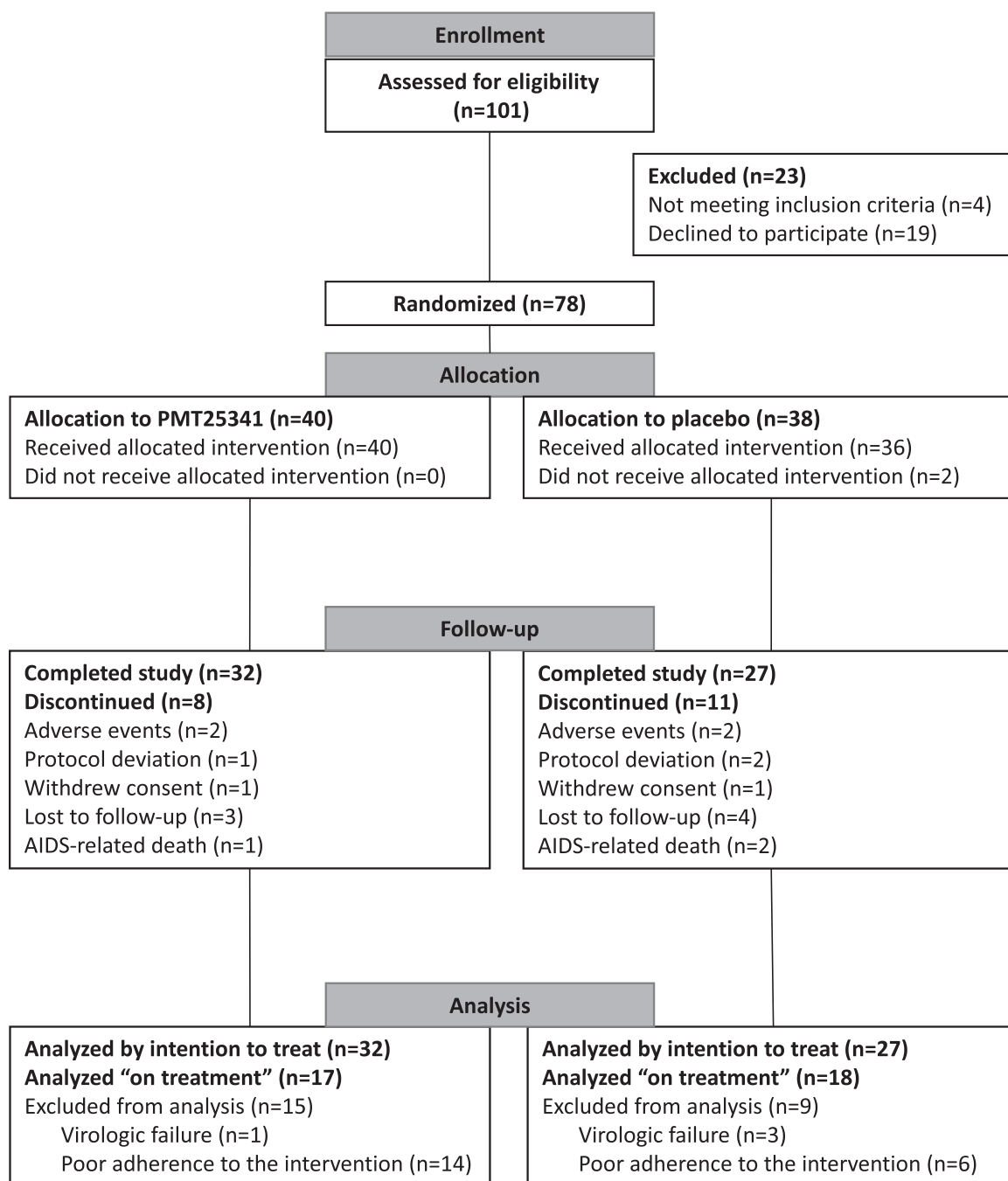


Figure 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram.

of 6 cytokines: IL-6, IL-7, IL-10, IL-17 α , IP-10, and TNF- α (Luminex, R&D Systems), according to the manufacturers' instructions.

Markers of Adaptive Immune Activation

For T-cell immunophenotyping, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque gradient centrifugation and immediately stored in liquid nitrogen. T-cell immunophenotyping from thawed PBMCs was performed with the following antibody combination: CD3-VioBlue, CD4-fluorescein isothiocyanate, CD8-VioGreen, CD28-phycoerythrin, CD38-APC, and HLA-DR-APC-Vio770. Antibodies were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany), and isotype controls were carried out. In brief, PBMCs were incubated with the antibodies for 20 minutes at 4°C, washed, and resuspended in phosphate-buffered saline containing 1% azide. Cells were analyzed using a Gallios flow cytometer (Beckman-Coulter, Brea, California). After initially gating lymphocytes according to morphological parameters, at least 30 000 CD3⁺ T cells were collected for each sample and analyzed with Kaluza software (Beckman-Coulter).

Statistical Methods

To estimate the required sample size, a power calculation was performed based on published CD4⁺ T-cell count longitudinal data from a meta-analysis indicating a mean increase of CD4⁺ T cells after 48 weeks of ART of 160 cells/ μ L [27]. Combined with an anticipated dropout rate of 20%, the ability to detect a 20% difference in the mean CD4⁺ T-cell increase at week 48, with 80% power and an α error of <.05, the sample size estimation was 60 subjects.

Qualitative variables were reported as a frequency distribution whereas quantitative variables were described as median with interquartile range (IQR). Cross-sectional pairwise comparisons between groups were performed using the χ^2 test for categorical variables. Since the distribution of all the assessed variables departed from normality after Shapiro-Wilk test, we used the Mann-Whitney *U* test for the between-group comparisons of continuous variables and Wilcoxon signed-rank matched-pairs test to evaluate differences in numerical outcomes between time-points. In addition, for the primary outcome of changes in CD4⁺ T cells, CD8⁺ T cells, and CD4/CD8 ratio, we used linear mixed models with a random effect for each patient to allow for correlations caused by repeated observations. A robust variance estimator was used given the deviations from normality. Continuous outcome variables were log-transformed to satisfy model assumptions. Statistical analysis was performed using Stata version 15.0 (StataCorp LP, College Station, Texas). Figures were generated using Prism version 7.0 (GraphPad, La Jolla, California).

RESULTS

General Characteristics of the Study Population and Safety Data

Between 2013 and 2016, 101 participants were screened to participate in the study; 23 were not eligible and the remaining 78 were randomized into treatment or placebo groups. A total of 59 subjects completed the 48-week follow-up (Figure 1). Their mean age was 38 years, 91% were male, and 83% were men who have sex with men. The median CD4⁺ T-cell counts were 225 (IQR, 117–288) cells/ μ L and the median CD4/CD8 ratio was 0.27 (IQR, 0.13–0.34). No statistically significant differences were observed among the groups in dietary habits (Supplementary Figure 1). All initiated triple ART. The general characteristics were well balanced between groups (Table 1). Of the participants who completed the follow-up, 14 (43.8%) in the active group and 9 (33.3%) in the placebo group reported adherence to the nutritional intervention <50% (*P* = .716). The lack of adherence was justified by bad taste in 3 subjects in the active group and 1 in the placebo group, and by nausea in 1 subject in the active group; the remaining cases did not provide a justification. No serious adverse events attributable to the intervention were reported.

Table 1. Baseline Characteristics

	PMT25341	Placebo
Characteristic	(n = 32)	(n = 27)
Age, y, mean (SD)	36 (8)	38 (14)
Male sex	28 (88)	26 (96)
Body mass index, kg/m ² , mean (SD)	23.6 (3.1)	22.7 (3.3)
Time since HIV diagnosis, mo, median (P25–P75)	3.1 (0.9–6.9)	1.9 (0.9–11.5)
Risk factor		
IDU	0 (0)	1 (3.7)
MSM	24 (75)	25 (92.6)
Heterosexual	5 (15.6)	1 (3.7)
Unknown	3 (9)	0 (0)
AIDS diagnosis	4 (12.5)	2 (7.4)
HIV RNA, copies/mL, median (P25–P75)	4.9 (4.5–5.4)	4.4 (4–5.2)
CD4 ⁺ T-cell counts, cells/ μ L, median (P25–P75)	226 (117–283)	221 (111–311)
CD8 ⁺ T cell count, cells/ μ L, median (P25–P75)	915 (532–1428)	966 (658–1273)
CD4/CD8 ratio	0.21 (0.13–0.34)	0.22 (0.12–0.41)
HCV coinfection	1 (3.1)	3 (11.1)
Use of antibiotic prophylaxis	5 (18.5)	9 (28.1)
First-line ART used		
INSTI-based	19 (59.4)	17 (63.0)
PI-based	5 (15.6)	4 (14.8)
NNRTI-based	8 (25.0)	6 (22.2)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, injection drug user; INSTI, integrase strand transfer inhibitor; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; P25, 25th percentile; P75, 75th percentile; PI, protease inhibitor; SD, standard deviation.

Effects of the Nutritional Intervention on CD4⁺ T-Cell Count, CD8⁺ T-Cell Count, and CD4/CD8 Ratio Recovery

We sought to compare the extent of CD4⁺ and CD8⁺ T-cell count and CD4/CD8 ratio recovery between the treatment groups. Both CD4⁺ T-cell counts and the CD4/CD8 ratio clearly and significantly increased with ART treatment in all groups over the 48 weeks ($P < .0001$), but there were no significant differences detected between groups (intent-to-treat and on-treatment analysis). CD8⁺ T-cell counts remained stable over the 48-week follow-up, and no significant differences between groups were detected (Figure 2 and Table 2).

Changes in T-Cell Activation, T-Cell Senescence, Inflammation, and Bacterial Translocation Parameters

We analyzed the differences in markers of T-cell activation and inflammation after 48 weeks. Whereas the percentage of CD4⁺HLA-DR⁺CD38⁺ CD8⁺HLA-DR⁺CD38⁺ and CD8⁺CD28⁻ T cells significantly decreased through the 48 weeks in the overall population (P values of $< .0001$, $< .0001$, and $.01$, respectively) (Supplementary Table 2), no significant differences were detected between treatment groups (Table 3). Similarly, although we observed significant decreases through the 48 weeks in the grouped analyses in IL-6, sCD163, sCD14, and IP-10 (all P values $< .0001$) in the overall population, no

consistent effects attributable to the nutritional intervention were detected.

Microbial Composition Analysis

After 48 weeks of ART, subjects did not show significant changes in α -diversity markers, including the number of species, in the Shannon, Pielou indexes, Chao1, or abundance-based coverage (ACE) estimators (Figure 3 and Table 4) or in the relative abundance of the most abundant genera in the overall population (Figure 4A). Nonmetric multidimensional scaling analysis of the composition distribution at OTU level based on the weighted UniFrac distance matrix did not reveal significant clustering of the subject's microbiota related to the study group (Figure 4B). However, linear discriminant effect size (LEfSe) analysis did indicate that those in the PMT25341 group showed an enrichment of unclassified bacteria from the Lachnospiraceae and Victivallaceae families and depletion of *Blautia* species compared with placebo (Figure 4C).

DISCUSSION

We show that among HIV-infected adults diagnosed at advanced stages of the disease, CD4⁺ T-cell counts and CD4/CD8 ratios improved after 48 weeks of ART, with a reduction in the

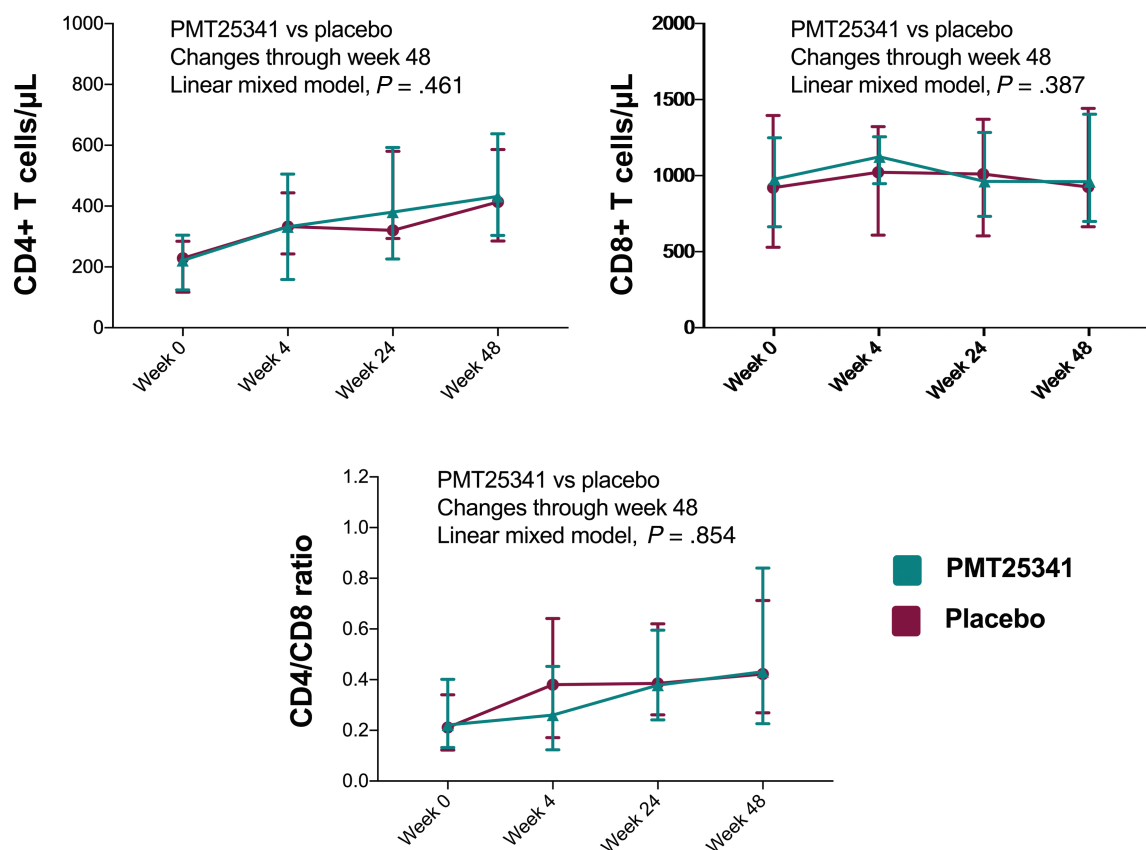


Figure 2. Effects of PMT25341 on CD4⁺ T-cell counts, CD8⁺ T-cell counts, and CD4/CD8 ratio (intent-to-treat population).

Table 2. Linear Mixed Models for Differences in CD4⁺ T Cells, CD8⁺ T Cells, and CD4/CD8 Ratio Trajectories Between Treatment Arms

Model	Coefficient	Standard Error	P Value
CD4⁺ T cells			
Intercept	2.28	0.07	<.0001
Duration (wk)	0.006	0.0009	<.0001
Treatment (PMT25341)	0.0009	0.093	.939
Interaction between treatment and duration	0.008	0.001	.474
CD8⁺ T cells			
Intercept	2.94	0.038	<.0001
Duration (wk)	−0.0003	0.0008	.975
Treatment (PMT25341)	0.017	0.050	.725
Interaction between treatment and duration	0.001	0.001	.387
CD4/CD8 ratio			
Intercept	−0.660	0.060	<.0001
Duration (wk)	0.005	0.0007	<.0001
Treatment (PMT25341)	−0.009	0.088	.913
Interaction between treatment and duration	−0.0003	0.001	.854

level of inflammatory markers, bacterial translocation, and T-cell activation. However, supplementation with PMT25341, a nutritional product specifically designed to target the different GI defects associated with HIV immunopathogenesis, did not enhance this immune reconstitution.

Strategies targeting the GALT and the microbiota in HIV-infected models have yielded inconsistent results. After preliminary observations in cohort studies suggesting a benefit of probiotics on CD4⁺ T-cell dynamics in Africa [28], 2 controlled studies in ART-naïve patients failed to demonstrate this benefit

[29, 30]. Thereafter, the effects of probiotics on other outcomes has been evaluated in pilot studies in ART-treated individuals. For example, daily probiotics for 8 weeks led to a significant decrease in D-dimers, but not-CRP or IL-6 in 24 HIV-infected patients on ART [31]. A further study showed that supplementing ART-treated HIV-infected individuals with the probiotic *Saccharomyces boulardii* led to a slight decrease of a bacterial translocation marker but not other inflammatory markers [20]. Prebiotics are metabolized by gut microorganisms and the subsequent modulation of microbiota composition and/or activity is assumed to be beneficial [32]. Their use has been evaluated in HIV infection, with a significant decline of the immune activation marker sCD14 and in CD4⁺CD25⁺ T cells observed in ART-naïve individuals [14]. As with our current study, others have tried to combine prebiotics, probiotics, and other compounds in an attempt to target the multiple immunological deficits in the gut. For example, Cahn et al showed that a supplement containing prebiotics, omega-3 and -6, bovine colostrum, and cysteine was associated with a slower CD4⁺ T-cell decline in ART-naïve, HIV-infected individuals [15]. We have also noted, using a combination of several prebiotics and glutamine in a pilot-controlled study, a potential beneficial effect on T-cell activation, particularly in ART-naïve individuals [19].

PMT25341 included components previously associated with the enhancement of gut epithelial barrier integrity and decreased bacterial translocation. The components were as follows: prebiotics [14]; the probiotic yeast *S. boulardii* [20, 33]; the essential amino acids glutamine and arginine [34, 35]; a mixture of long-chain fatty acids with known anti-inflammatory properties [36]; vitamin D, as a deficiency predicts impaired CD4 recovery

Table 3. Comparison of Changes in T-Cell Activation, T-Cell Senescence, Inflammatory, and Bacterial Translocation Markers Between Treatment Arms

Parameter	Fold Change Through Week 48		P Value	
	PMT25341 (n = 14)	Placebo (n = 14)	ITT	OT
T-cell activation and senescence				
%CD4 ⁺ HLA-DR ⁺ CD38 ⁺ T cells	0.28 (0.31–0.37)	0.29 (0.21–0.37)	.818	.591
%CD8 ⁺ HLA-DR ⁺ CD38 ⁺ T cells	0.40 (0.24–0.61)	0.33 (0.24–0.50)	.645	.879
%CD4 ⁺ CD28 ⁺ T cells	1.27 (0.77–1.82)	0.70 (0.38–1.30)	.168	.706
%CD8 ⁺ CD28 ⁺ T cells	0.97 (0.92–1.01)	0.95 (0.89–0.99)	.358	.173
Soluble biomarkers				
IL-6, pg/mL	1 (0.50–1.10)	1 (0.25–1.41)	.716	.901
hs-CRP, ng/mL	0.75 (0.37–1.29)	0.72 (0.25–1.50)	.597	.549
TNF- α , pg/mL	0.97 (0.75–4.27)	1 (0.92–4.18)	.577	.224
sCD14, ng/mL	0.85 (0.78–0.93)	0.86 (0.76–0.95)	1	.680
sCD163, ng/mL	0.58 (0.48–0.68)	0.59 (0.47–0.70)	.584	.199
IP-10, pg/mL	0.56 (0.38–0.92)	0.37 (0.24–0.52)	.036	.303
LTA, ng/mL	0.52 (0–1.36)	0.52 (0.34–1.27)	.864	.155
IL-7, pg/mL	0.45 (0.20–1.11)	0.98 (0.30–2.03)	.172	.328
IL-10, pg/mL	0.59 (0.31–1.11)	0.44 (0.26–0.65)	.338	.898
IL-17, pg/mL	0.83 (0.06–1.09)	1.12 (0.83–1.79)	.112	.261

Data are presented as XXX (XXX) unless otherwise indicated.

Abbreviations: hs-CRP, high-sensitivity C-reactive protein; IL, interleukin; IP-10, interferon-gamma-inducible protein 10; ITT, intent-to-treat analysis; LTA, lipoteichoic acid; OT, on-treatment analysis; sCD14, soluble CD14; sCD163, soluble CD163; TNF- α , tumor necrosis factor alpha.

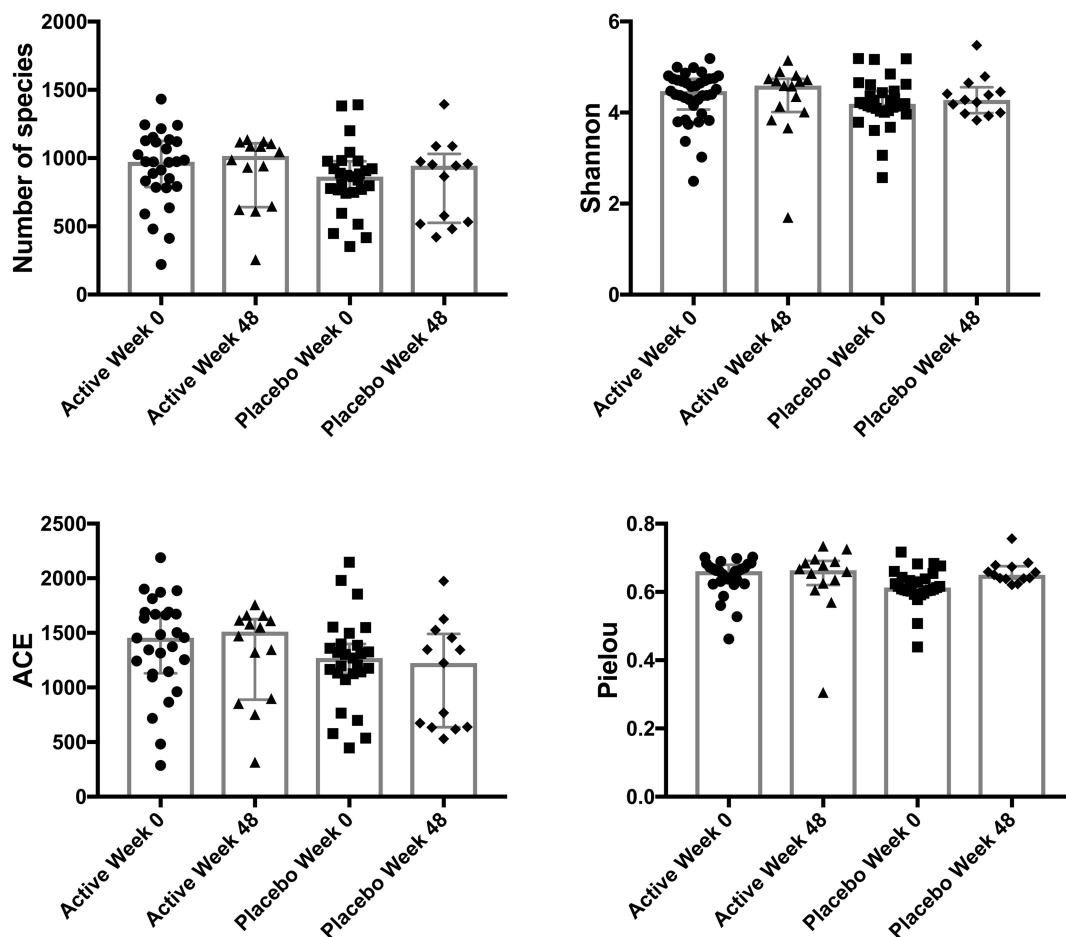


Figure 3. Alpha-diversity of taxonomic data from patients with advanced human immunodeficiency virus disease at baseline and after 48 weeks of antiretroviral therapy and PMT25341 supplementation or placebo. The α -diversity metrics represent the relative abundance and evenness of bacterial species in a community. Higher values indicate communities with greater diversity of species. We did not detect within-group differences in α -diversity metrics. Data are represented as median (P25–P75). Abbreviation: ACE, abundance-based coverage.

[37]; and AM3, an immunomodulatory glycopeptide produced by *Ricinus communis*, which induces the antiviral response of mononuclear cells and attenuates lipopolysaccharide-induced inflammation [38]. Of note, we used a high-dose combination of these compounds, which was administered daily over the 48 weeks in the late-presenting subjects, who could potentially

benefit most from novel strategies boosting immune reconstitution. In contrast with some previous reports in recently diagnosed ART-naïve or stable triple-ART patients [14, 15, 19, 20, 31] in whom nutritional interventions have shown a modest impact on some immunological outcomes, we did not detect any beneficial effect on these outcomes in late presenters. This could suggest that the long-term immunological consequences of a low nadir CD4⁺ cell count could overshadow the success of immunomodulatory interventions, or that the effects of ART on immune restoration are so large that they overshadow any smaller effects that might be observed with immunonutrition. However, the different nature of the interventions assessed so far in HIV-infected individuals (eg, different prebiotics, different probiotic strains) and the differences in the study populations (eg, ART-naïve or ART-treated individuals) might also explain the divergent results across studies.

In this study, our supplementation did not have a clear impact on gut microbiota composition, and only a few taxa from the Lachnospiraceae and Victivallaceae families were significantly

Table 4. Changes in α -Diversity Metrics of Fecal Microbiota, According to Study Group

Parameter	Fold Change Through Week 48		P Value	
	PMT25341	Placebo	ITT	OT
No. of species	0.87 (0.77–1.10)	0.79 (0.60–1.09)	.593	.795
Shannon	0.98 (0.87–1.05)	0.99 (0.96–1.03)	.662	.449
Pielou	1 (0.92–1.03)	1.05 (1.01–1.07)	.19	.131
Chao1	0.75 (0.62–0.99)	0.69 (0.48–0.96)	.56	.545
ACE	0.83 (0.72–1.05)	0.72 (0.51–1.02)	.497	.597

Data are presented as median (P50–P75).

Abbreviations: ACE, abundance-based coverage; ITT, intent-to-treat analysis; OT, on-treatment analysis.

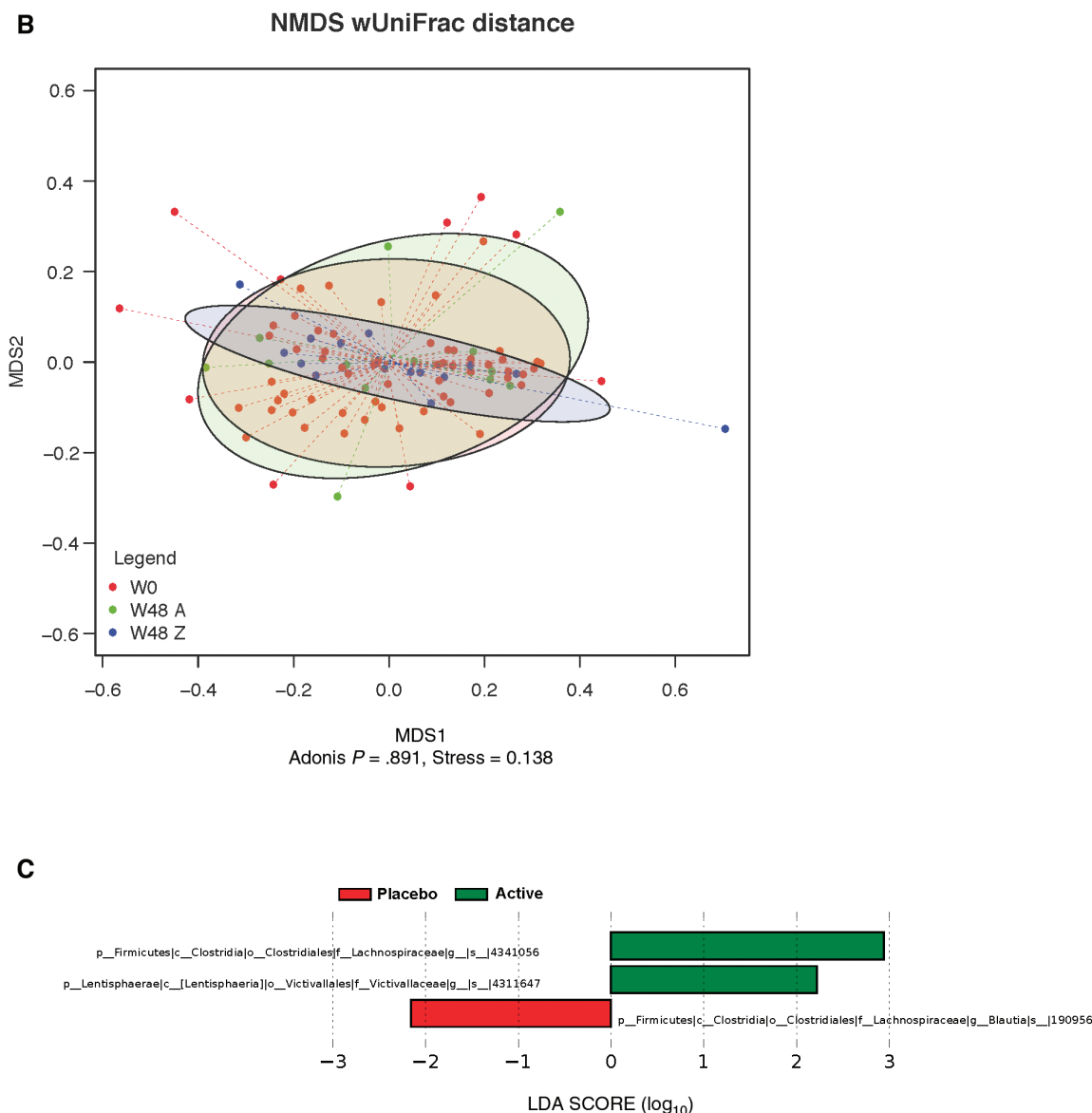


Figure 4. (Continued)

individual ingredients of PMT25341 may have proved efficacious if administered at higher doses than in the tested formulation.

In conclusion, in HIV-infected patients with a CD4 count of <350 cells/ μ L at diagnosis, a 48-week supplementation with a combination of compounds previously shown to exert immunomodulatory effects in HIV did not improve circulating T-cell numbers, inflammation, or immunoactivation or affected gut microbiota structure. In HIV-infected patients initiating ART at advanced disease, the clear immunological benefits of ART were not enhanced by this nutritional intervention targeting the GALT and microbiota.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted

materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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