Effects of Immunonutrition in Advanced HIV Disease: a Randomized Placebo Controlled Clinical Trial (Promaltia Study)

Sergio Serrano-Villar[1*], María de Lagarde[2], Jorge Vázquez-Castellanos[3], Alejandro Vallejo[1], J. Ignacio Bernadino[4], Nadia Madrid[1], Mariano Matarranz[2], Alberto Díaz-Santiago[1], Carolina Gutiérrez[1], Alfonso Cabello[5], Judit Villar-García[6], José Ramón Blanco[7], Otilia Bisbal[2], Talía Sainz[4], Andrés Moya[2], Santiago Moreno[2], María José Gosalbes[2], Vicente Estrada[8]

Affiliations

[1]Department of Infectious Diseases, Hospital Universitario Ramon y Cajal, Facultad de Medicina, Universidad de Alcalá, IRYCIS, Madrid, Spain

[2]HIV Unit, Hospital Universitario Doce de Octubre, Madrid, Spain

[3]Area of Genomics and Health, FISABIO-Salud Pública, Valencia, Spain

[4]HIV Unit, Hospital Universitario La Paz, Madrid, Spain

[5]HIV Unit, Fundación Jiménez Díaz, Madrid, Spain

[6]HIV Unit, Hospital del Mar, IMIM, Barcelona, Spain

[7]Department of Internal Medicine, Hospital San Pedro, Logroño, Spain

[8]HIV Unit, Hospital Clínico San Carlos, Madrid, Spain

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25 ****Corresponding author.*** Sergio Serrano-Villar, MD, PhD

26 Department of Infectious Diseases, Hospital Universitario Ramon y Cajal,

27 Facultad de Medicina, Universidad de Alcalá (IRYCIS)

28 Carretera de Colmenar Viejo, Km 9.100, 28034 Madrid, Spain.

29 Tel: +34 913 368 975;

30 E-mail: sergio.serrano@salud.madrid.org

31

32 ***Summary***. After 48 weeks of immunonutrition in HIV-infected individuals starting

33 ART at advanced disease, most of T cell, inflammation and bacterial translocation

34 markers improved similarly in the active and control groups.

35

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ABSTRACT

Background: While nutritional interventions with prebiotics and probiotics seem to exert immunological effects, their clinical implications in HIV-infected subjects initiating ART at advanced HIV disease remain unclear.

Methodology: Pilot multicenter randomized, placebo-controlled, double-blind study in which 78 HIV-infected, ART-naive subjects with <350 CD4 T-cells/µl or AIDS were randomized to either daily PMT25341 (a mixture of prebiotics, probiotics, oligonutrients, DHA, EPA, GLA, and aminoacids) 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 microbiota composition (Clinicaltrials.gov: NCT00870363).

Results: Fifty-nine participants completed the follow-up with a mean CD4+ T-cell count of 221±108/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 median CD4+ T-cell count change (226 cells/µl vs. 414, P=0.461) or CD4/CD8 ratio change (0.21 vs. 0.48, P=0.854). Similarly, we did not detect differences between treatment arms in the variations of %HLADR+CD38+ or %CD28- T-cells, sCD14, LTA, IL-6, CRP, TNF-α, sCD163, IP-10, IL-7, IL-10, or IL-17 or alpha and beta microbiota diversity.

60 **Conclusion:** In HIV-infected patients initiating ART at advanced disease, the clear
61 immunological benefits of ART were not enhanced by this nutritional intervention
62 targeting the GALT and microbiota.
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66 # INTRODUCTION

67 Despite global efforts to identify HIV infection at an early stage, HIV is diagnosed
68 late in approximately 50% of cases in high-income countries [1,2]. According to
69 the consensus definition from the HIV in Europe study group, late presenters are
70 defined as those presenting to an antiretroviral therapy (ART) clinic with a CD4
71 count of less than 350 cells/µl or an AIDS defining illness [3]. Low CD4+ T-cell
72 counts at ART initiation have been associated with shorter life expectancy [4].
73 Hence, while early diagnosis and ART initiation must be prioritized in public health
74 strategies, novel interventions are required for a more successful management of
75 HIV in late presenter cases.

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

87 Different strategies targeting the multifaceted HIV-associated GALT defects have
88 been investigated in an attempt to reduce the long-term consequences of chronic
89 inflammation [14–19]. Multiple studies have assessed dietary supplementation in

90 HIV patients with various nutritional products, such as prebiotics and probiotics
91 among others [10,14–16,20] , which collectively suggest that this strategy may exert
92 some beneficial immunological effects. Therefore, we developed PMT25341, a
93 product including several nutritional compounds designed to target the different
94 gastrointestinal defects associated with HIV immunopathogenesis. We designed a
95 double blind, randomized, placebo-controlled study to evaluate whether
96 PMT25341 administered for 48 weeks in late presenters initiating ART may
97 enhance CD4+ T-cell and CD4/CD8 ratio recovery and affect markers of immune
98 activation, inflammation and bacterial translocation, as well as the gut microbiota
99 composition.
100

101 # METHODS

102 **Study design, participants, setting and eligibility**

103 We conducted a randomized, double-blind, multicenter, placebo controlled study.
104 Participants were recruited at the HIV units of 7 university hospitals in Spain —
105 Hospital Universitario Ramón y Cajal, Hospital Universitario Clínico San Carlos,
106 Hospital Universitario Doce de Octubre, Hospital Universitario La Paz, Fundación
107 Jiménez Díaz (Madrid), Hospital del Mar (Barcelona) and Hospital San Pedro
108 (Logroño). Participants were HIV-infected, late presenting (<350 CD4 T-cells/ µl or
109 AIDS at diagnosis [3]) adults, who initiated ART according to the Spanish GESIDA
110 National Guidelines [21]. Exclusion Criteria were age <18 years, pregnancy, type
111 1 or 2 diabetes, end-stage renal disease, lactose intolerance, use of
112 immunomodulatory drugs and a neutrophil count of <750 cells/µL. The

113 composition or PMT25341 is detailed in **Table S1**, the placebo was skimmed milk
114 powder. The product and the placebo were prepared by Nutricion Médica, S.L.
115 The study was approved by the Ethics Committee of all participating centers and all
116 participants signed an informed consent prior to the initiation of study procedures.
117 Clinical Trials Registry Identification Number Identifier (clinicaltrials.gov):
118 NCT03009032.
119

120 **Randomization**

121 The study participants were randomly assigned to PMT25341 or placebo by a
122 computer-generated randomized number system in blocks. PMT25341 and the
123 placebo were packaged in identically appearing sachets. The clinicians who saw
124 the study participants, the laboratory personnel, who handled patient specimens,
125 and the study participants were blind to the assigned patient group.
126

127 **Study Outcomes**

128 After screening for eligibility, study visits were scheduled at baseline, and at 4, 24
129 and 48 weeks. At each visit, a clinical evaluation was carried out, blood samples
130 were collected, and adherence to the intervention and any adverse events were
131 registered. Fecal samples were collected at baseline and week 48. Our primary
132 outcomes were safety and tolerability, and between-arm changes in CD4+ and
133 CD4/CD8 ratio from baseline to week 48. GI tolerance was also assessed using a
134 questionnaire and scoring the severity of GI symptoms on a 4-point scale.
135 Our secondary outcomes were changes in plasma markers (IL-6, hs-CRP, TNF-α,
136 sCD163, sCD14, IP-10, LTA, IL-7, IL-10 and IL17), T-cell activation markers (% of

137 HLADR+CD38+ CD4+ and CD8+ T-cells), T-cell senescence markers (% of CD28-
138 T-cells) and variations in the microbiota composition (alpha and beta diversity
139 metrics) from baseline to week 48. Adherence was assessed using patient diaries
140 that were checked against the returned sachets.

141

142 **Nucleic acid purification, amplification of the 16S rRNA gene, sequencing and**
143 **bioinformatics analysis**

144 *Nucleic acid purification*. Fecal samples were stored in RNAlater (Life
145 Technologies) at -80ºC until use. Total DNA was quantified using Qubit
146 fluorometry.

147 *Amplification of the 16S rRNA gene*. For each sample, the V3-V4 regions of the 16S
148 rRNA gene were amplified by polymerase chain reaction (PCR) using 16S Amplicon
149 PCR Forward Primer (5'
150 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG)
151 and 16S Amplicon PCR Reverse Primer (5'
152 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAA
153 TCC). The pooled PCR products were sequenced using the kit V3 (2x300 cycles)
154 with MiSeq sequencer (Illumina) at FISABIO Sequencing and Bioinformatics
155 Service, Valencia, Spain. We obtained an average of 62939 16S rRNA joined
156 sequences per sample.

157 *16S RNA gene analysis. Biodiversity and clustering***.** Amplicon data from the 16S
158 rRNA gene was analyzed following the recommendations of the metagenomic
159 state-of-the-art pipeline QIIME v1.9[22]. Taxonomic information on the 16S rDNA
160 sequences were obtained using the Ribosomal Database Project-II (RDP)[23] and

161 the Greengenes database available in the QIIME v1.8 software. The Operational
162 Taxonomic Units (OTUs) were created using the Qiime v1.9 script
163 pick_open_reference_otus.py, which iterative use UCLUST [25], and were used to
164 classify different clusters of species with a 97% of similarity. OTUs were created
165 using Uclust [24], applying the 97% similarity criterion. Representative sequences
166 were aligned with Pynast [25] against the clustered version of the Greengenes
167 database (database core_set_aligned.fasta.imputed), to use as input to reconstruct
168 the phylogenetic tree using the FastTree software [26]. Distances analysis between
169 samples were performed by the weighted normalized Unifrac using the script
170 assign_taxonomybeta_diversity.py. Sequences were rarefacted to 16590 sequences
171 per sample to standardize Alpha and Beta diversity analyses. A detailed description
172 of the methods is provided in the **supplemental materials.**
173

174 **Systemic biomarkers of disease progression**

175 *Markers of innate immune activation and bacterial translocation*

176 A sample of fasting venous blood was obtained to determine the concentrations of
177 glucose, total cholesterol, high-density lipoprotein cholesterol, and triglycerides
178 using standard enzymatic methods. Plasma HIV RNA was measured using the
179 Cobas Taq-Man HIV-1 assay (Roche Diagnostics Systems, Inc., Branchburg, NJ,
180 USA). Cryopreserved plasma was assessed by immunoassay in duplicate for plasma
181 levels of the inflammatory markers high-sensitivity C-reactive protein (hs-CRP)
182 (Labor Diagnostika, Nordhorn, Germany), sCD163 (Quantikine, R&D,
183 Minneapolis, MD, USA), LTA (Abbexa, Cambridge, UK), and the bacterial
184 translocation markers sCD14 (Quantikine, R&D, Minneapolis, MD, USA) and LTA

185 (Abbexa, Cambridge, UK), according to the manufacturers' recommendations.
186 Luminex was used to measure the concentrations of 6 cytokines: IL-6, IL-7, IL-10,
187 IL-17α, IP-10 and TNFα (Luminex, R&D, Minneapolis, MD, USA), according to the
188 manufacturers' instructions.

189 *Markers of adaptive immune activation*

190 T-cell immunophenotyping. Peripheral blood mononuclear cells (PBMCs) were
191 isolated by Ficoll-Hypaque gradient-centrifugation and immediately stored in liquid
192 nitrogen. T-cell immunophenotyping from thawed PBMCs was performed with the
193 following antibody combination: CD3-VioBlue, CD4-Fluorescein isothiocyanate
194 (FITC), CD8-VioGreen, CD28-Phycoerythrin (PE), CD38-APC and HLA-DR-APC-
195 Vio770. Antibodies were purchased from Myltenyi Biotec (Bergisch Gladbach,
196 Germany), and isotype controls were carried out. Briefly, PBMCs were incubated
197 with the antibodies for 20 min at 4°C, washed and resuspended in PBS containing
198 1% azide. Cells were analyzed using a Gallios flow cytometer (Beckman-Coulter,
199 CA, USA). After initially gating lymphocytes according to morphological
200 parameters, at least 30000 CD3+ T-cells were collected for each sample and
201 analyzed with Kaluza software (Beckman-Coulter).

202

203 **Statistical methods**

204 To estimate the required sample size, a power calculation was performed based on
205 published CD4+ T-cell count longitudinal data from a meta-analysis indicating a
206 mean increase of CD4+ T-cells after 48 weeks of ART of 160 cells/ μL [27].
207 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 alpha error of <0.05, the sample size estimation was 60 subjects.

Qualitative variables were reported as a frequency distribution whereas quantitative variables were described as medians with their interquartile ranges (IQRs). Cross-sectional pairwise comparisons between groups were performed using the Chi square test for categorical variables. Since the distribution of all the assessed variables departed from normality after Shapiro Wilk tests, 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 v15.0 (StataCorp LP, College Station, TX). Figures were generated using Prism v.7.0, GraphPad, Inc., La Jolla, CA)

RESULTS

General characteristics of the study population and safety data

Between 2013 and 2016, 101 participants were screened to participate in the study; 29 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

232 with men. The median CD4+ T-cell counts were 225 (117-288) and the median
233 CD4/CD8 ratio was 0.27 (0.13-0.34). No statistically significant differences were
234 observed among the groups in dietary habits (**Figure S1**). All initiated triple ART.
235 The general characteristics were well balanced between groups (**Table 1**). Of the
236 participants who completed the follow-up, 14 (43.8%) in the active group and 9
237 (33.3%) in the placebo group reported adherence to the nutritional intervention
238 below 50% (p=0.716). The lack of adherence was justified by bad taste in 3 subjects
239 in the active group and 1 in the placebo group, and by nausea in 1 subject in the
240 active group, the remaining cases did not provide a justification. No serious adverse
241 events attributable to the intervention were reported.

242

243 **Effects of the nutritional intervention on CD4+ T-cell count, CD8+ T-cell count**
244 **and CD4/CD8 ratio recovery**

245 We sought to compare the extent of CD4+ and CD8+ T-cell count and CD4/CD8
246 ratio recovery between the treatment groups. Both CD4+ T-cell counts and the
247 CD4/CD8 ratio clearly and significantly increased with ART treatment in all groups
248 over the 48 weeks (p<0.0001), but there were no significant differences detected
249 between groups (ITT and OT analysis). CD8+ T cell counts remained stable over
250 the 48-week follow-up, and no significant differences between groups were
251 detected (**Figure 2** and **Table 2**).

252

253 **Changes in T-cell activation, T-cell senescence, inflammation and bacterial**
254 **translocation parameters.**

255 We analyzed the differences in markers of T-cell activation and inflammation after
256 48 weeks. Whereas the percentage of CD4+HLADR+CD38+
257 CD8+HLADR+CD38+ and CD8+CD28- T-cells significantly decreased through the
258 48 weeks in the overall population (p<0.0001, <0.0001 and 0.01, respectively)
259 (**Table S2**), no significant differences were detected between treatment groups
260 (**Table 3**). Similarly, although we observed significant decreases through the 48
261 weeks in the grouped analyses in IL-6, sCD163, sCD14 and IP-10 (all p values
262 <0.0001) in the overall population, no consistent effects attributable to the
263 nutritional intervention were detected.
264
265 **Microbial composition analysis**
266 After 48 weeks of ART, subjects did not show significant changes in alpha diversity
267 markers, including the number of species, in the Shannon, Pielou indexes, Chao1
268 or ACE estimators **(Figure 3** and **Table 4)** or in the relative abundance of the most
269 abundant genera in the overall population (**Figure 4a**). Nonmetric Multidimensional
270 Scaling (NMDS) analysis of the composition distribution at OTU level based on the
271 weighted Unifrac distance matrix did not reveal significant clustering of the
272 subject's microbiota related to the study group (**Figure 4b**). However, LEfSe analysis
273 did indicate that those in the PMT25341 group showed an enrichment of
274 unclassified bacteria from the Lachnospiraceae and Victivallaceae families and
275 depletion of *Blautia spp.* compared to placebo (**Figure 4c**).
276
277
278

279 # DISCUSSION

280 We show that among HIV-infected adults diagnosed at advanced stages of the
281 disease, CD4+ T-cell counts and CD4/CD8 ratios improved after 48 weeks of ART,
282 with a reduction in the level of inflammatory markers, bacterial translocation and
283 T-cell activation. However, the supplementation with PMT25341, a nutritional
284 product specifically designed to target the different GI defects associated with HIV
285 immunopathogenesis, did not enhance this immune reconstitution.
286 Strategies targeting the GALT and the microbiota in HIV-infected models have
287 yielded inconsistent results. After preliminary observations in cohort studies
288 suggesting a benefit of probiotics on CD4+ T-cell dynamics in Africa [28], two
289 controlled studies in ART naïve patients failed to demonstrate this benefit [29,30].
290 Thereafter, the effects of probiotics on other outcomes has been evaluated in pilot
291 studies in ART treated individuals. For example, daily probiotics for 8 weeks lead
292 to a significant decrease in D-dimers, but not-CRP or IL-6 in 24 HIV-infected
293 patients on ART [31]. A further study showed that supplementing ART-treated HIV-
294 infected individuals with the probiotic *Saccharomyces boulardii* lead to a slight
295 decrease of a bacterial translocation marker but not in other inflammatory markers
296 [20]. Prebiotics are metabolized by gut microorganisms and the subsequent
297 modulation of microbiota composition and/or activity is assumed to be beneficial
298 [32]. Their use has been evaluated in HIV infection, with a significant decline of
299 the immune activation marker sCD14 and in CD4+CD25+T-cells observed in ART-
300 naïve individuals [14]. As with our current study, others have tried to combine
301 prebiotics, probiotics and other compounds in an attempt to target the multiple
302 immunological deficits in the gut. For example, Cahn. et al showed that a

303 supplement containing prebiotics, omega 3 and 6, bovine colostrum, and cysteine
304 was associated with a slower CD4+ T-cell decline in ART-naïve HIV-infected
305 individuals [15]. We have also noted, using a combination of several prebiotics and
306 glutamine in a pilot-controlled study, a potential beneficial effect on T-cell
307 activation, particularly in ART naïve individuals [19].
308 PMT25341 included components previously associated with the enhancement of
309 gut epithelial barrier integrity and decreased bacterial translocation. The
310 components were: prebiotics [14]; the probiotic yeast *Saccharomyces boulardii*
311 [20,33]; the essential aminoacids glutamine and arginine [34,35]; a mixture of long-
312 chain fatty acids with known anti-inflammatory properties [36]; vitamin D, as a
313 deficiency predicts impaired CD4 recovery; [37] and AM3, an immunomodulatory
314 glycopeptide produced by *Ricinus communis,* which induces the antiviral response
315 of mononuclear cells and attenuates LPS-induced inflammation [38]. Of note, we
316 used a high-dose combination of these compounds, which was administered daily
317 over the 48 weeks in the late presenting subjects, who could potentially benefit
318 most from novel strategies boosting immune reconstitution. In contrast with some
319 previous reports in recently diagnosed ART-naïve or stable triple ART patients
320 [14,15,19,20,31] where nutritional interventions have shown a modest impact on
321 some immunological outcomes, we did not detect any beneficial effect on these
322 outcomes in late presenters. This could suggest that the long-term immunological
323 consequences of a low nadir CD4+ could overshadow the success of
324 immunomodulatory interventions, or that the effects of ART on immune restoration
325 are so large that they overshadow any smaller effects that might be observed with
326 immunonutrition. However, the different nature of the interventions assessed so far

327 in HIV-infected individuals (e.g., different prebiotics, different probiotic strains) and
328 the differences in the study populations (e.g., naïve or ART-treated individuals)
329 might also explain the divergent results across studies.
330 In this study, our supplementation did not have a clear impact on gut microbiota
331 composition, and only a few taxa from the Lachnospiraceae and Victivallaceae
332 families were significantly affected by the intervention. Species from the
333 Lachnospiraceae family are considered beneficial as they produce butyrate from
334 the digestion of dietary fiber [39] and have previously been shown to be depleted
335 in HIV-infected subjects [9,40,41]. However, despite its depletion at the
336 compositional level, we have previously shown that the Lachnospiraceae family is
337 among those who become most transcriptionally active during HIV infection [11],
338 and that increases in their abundance with prebiotics correlated with decreases in
339 inflammatory markers [19]. Only one study has studied the Victivallaceae family in
340 HIV-infected subjects, where its depletion was observed [42]. So, while PMT25341
341 exerted only minor effects on the microbiota structure, this could be beneficial in
342 the long term, although given the absence of any major impact here the gut
343 microbiota of ART-treated subjects appears more resilient than that of ART-naïve
344 subjects, as previously noted [11,19].
345 As main strengths, our study was double blind, randomized, placebo controlled
346 and assessed an intervention over 48 weeks, which should have allowed for the
347 detection of small effect sizes, and included a comprehensive analysis with an
348 assessment of compositional changes in the microbiome. However, there were
349 some limitations: Firstly, a considerable number of subjects (N=19) discontinued
350 the study, which can be explained by the difficulties of evaluating a 48-week

351 intervention in a population prone to experiencing adverse outcomes [43];
352 secondly, the adherence to the intervention was poorer in the active group, which
353 was due to a fishy flavor produced by the omega 3 and 6 fatty acids of the active
354 treatment. Finally, it is unknown whether any of the individual ingredients of
355 PMT25341 may have proved efficacious if administered at higher doses than in the
356 tested formulation.
357 In conclusion, in HIV-infected patients with less than 350 CD4/ µL at diagnosis, a
358 48-week supplementation with a combination of compounds previously shown to
359 exert immunomodulatory effects in HIV, did not improve circulating T cell
360 numbers, inflammation or immunoactivation of affected gut microbiota structure.
361 In HIV-infected patients initiating ART at advanced disease, the clear
362 immunological benefits of ART were not enhanced by this nutritional intervention
363 targeting the GALT and microbiota.
364

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381

382 CONFLICTS OF INTEREST

383 No competing interests exist.
384

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519

520 **TABLES**

521

Table 1. Baseline characteristics

	PMT25341 n=32	Placebo n=27
Age, mean (SD), years	36 (8)	38 (14)
Male, n (%)	28 (88)	26 (96)
Body mass index, mean (SD), kg/m2	23.6 (3.1)	22.7 (3.3)
Time since HIV diagnosis, median (P25-P75), months	3.1 (0.9-6.9)	1.9 (0.9-11.5)
Risk factor, n (%)		
IDU	0 (0)	1 (3.7)
MSM	24 (75)	25 (92.6)
HTX	5 (15.6)	1 (3.7)
Unknown	3 (9)	0 0
AIDS diagnosis, n (%)	4 (12.5)	2 (7.4)
HIV RNA, median (P25-P75), copies/mL	4.9 (4.5-5.4)	4.4 (4-5.2)
CD4+ T cell counts, median (P25-P75), cells/uL	226 (117-283)	221 (111-311)
CD8+ T cell counts, median (P25-P75), cells/uL	915 (532-1428)	966 (658-1273)
CD4/CD8 ratio	0.21 (0.13-0.34)	0.22 (0.12-0.41)
HCV coinfection, n (%)	1 (3.1)	3 (11.1)
Use of antibiotic prophylaxis, n (%)	5 (18.5)	9 (28.1)
First-line ART used, n (%)		
INSTI-based	19 (59.4)	17 (63.0)
PI-based	5 (15.6)	4 (14.8)
NNRTI-based	8 (25.0)	6 (22.2)

Abbreviations: IDU, injection drug use; MSM, men who have sex with men; HTX, heterosexual; INSTI, integrase strand transfer inhibitor; PI, protease inhibitor; NNRTI, non-nucleoside retrotranscriptase inhibitor.

522

Table 2. Linear mixed models for differences in CD4+ T cells, CD8+ T cells and CD4/CD8 ratio trajectories between treatment arms

		Coefficient	Standard Error	P value
CD4+ T cells	**Intercept**	2.28	0.07	<0.0001
	Duration (weeks)	0.006	0.0009	<0.0001
	Treatment (PMT25341)	0.0009	0.093	0.939
	Interaction treatment#week	0.008	0.001	0.474
CD8+ T cells	**Intercept**	2.94	0.038	<0.0001
	Duration (weeks)	-0.0003	0.0008	0.975
	Treatment (PMT25341)	0.017	0.050	0.725
	Interaction treatment#week	0.001	0.001	0.387
CD4/CD8 ratio	**Intercept**	-0.660	0.060	<0.0001
	Duration (weeks)	0.005	0.0007	<0.0001
	Treatment (PMT25341)	-0.009	0.088	0.913
	Interaction treatment#week	-0.0003	0.001	0.854

523

Table 3. Comparison of changes in T cell activation, T cell senescence, inflammatory and bacterial translocacion markers between treatment arms

Parameter	Fold change through week 48		p value	
	PMT25341	Placebo	ITT	OT
T cell activation and senescence	n=14	n=14		
%CD4+ HLADR+CD38+ T cells	0.28 (0.31-0.37)	0.29 (0.21-0.37)	0.818	0.591
%CD8+ HLADR+CD38+ T cells	0.40 (0.24-0.61)	0.33 (0.24-0.50)	0.645	0.879
%CD4+ CD28- T cells	1.27 (0.77-1.82)	0.70 (0.38-1.30)	0.168	0.706
%CD8+ CD28- T cells	0.97 (0.92-1.01)	0.95 (0.89-0.99)	0.358	0.173
Soluble biomarkers	n=30	n=26		
IL-6, pg/mL	1 (0.50-1.10)	1 (0.25-1.41)	0.716	0.901
hs-CRP, ng/mL	0.75 (0.37-1.29)	0.72 (0.25-1.50)	0.597	0.549
TNF-α, pg/mL	0.97 (0.75-4.27)	1 (0.92-4.18)	0.577	0.224
sCD14, ng/mL	0.85 (0.78-0.93	0.86 (0.76-0.95)	1	0.680
sCD163, ng/mL	0.58 (0.48-0.68)	0.59 (0.47-0.70)	0.584	0.199
IP-10, pg/mL	0.56 (0.38-0.92)	0.37 (0.24-0.52)	0.036	0.303
LTA, ng/mL	0.52 (0-1.36)	0.52 (0.34-1.27)	0.864	0.155
IL-7, pg/mL	0.45 (0.20-1.11)	0.98 (0.30-2.03)	0.172	0.328
IL-10, pg/mL	0.59 (0.31-1.11)	0.44 (0.26-0.65)	0.338	0.898
IL-17, pg/mL	0.83 (0.06-1.09)	1.12 (0.83-1.79)	0.112	0.261

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525

Table 4. Changes in alpha diversity metrics of fecal microbiota according to the study group

Parameter	Fold change through week 48		p value	
	PMT25341	Placebo	ITT	OT
Number of species	0.87 (0-77-1.10)	0.79 (0.60-1.09)	0.593	0.795
Shannon	0.98 (0.87-1.05)	0.99 (0.96-1.03)	0.662	0.449
Pielou	1 (0.92-1.03)	1.05 (1.01-1.07)	0.19	0.131
Chao1	0.75 (0.62-0.99)	0.69 (0.48-0.96)	0.56	0.545
ACE	0.83 (0.72-1.05)	0.72 (0.51-1.02)	0.497	0.597

ITT, intention-to-treat analysis. OT, on treatment analysis.

526

527 ## FIGURE LEGENDS

528 **Figure 1. Consort flow diagram.**

529 **Figure 2. Effects of PMT25341 on CD4+ T-cell counts, CD8+ T-cell counts and**
530 **CD4/CD8 ratio (ITT population).**

531 **Figure 3. Alpha diversity of taxonomic data from patients with advanced HIV**
532 **disease at baseline and after 48 weeks of ART and PMT25341 supplementation or**
533 **placebo.** The alpha diversity metrics represent the relative abundance and evenness
534 of bacterial species in a community. Higher values indicate communities with
535 greater diversity of species. We did not detect within-group differences in alpha
536 diversity metrics.

537 **Figure 4 Beta diversity analysis. a. Heatmap of the abundance of most abundant**
538 **genera in the study population.** Each row represents an individual genus, relative
539 values are denoted by color below each subject column. In the first row, blue
540 represent the abundance of these genera at baseline in the PMT25341 and placebo
541 groups. Green represents the placebo group at week 48, blue represents the
542 PMT25341 group. Error bars represent the median, P25 and P75 values. **b.**
543 **Nonmetric Multidimensional Scaling (NMDS) analysis of the composition**
544 **distribution at OTU level based on the weighted Unifrac distance matrix.** NMDS
545 analysis is a robust method to visualize similarities or dissimilarities in high-
546 dimensional data. In this case, it assigns each patient's microbial composition to a
547 location in a 2-dimensional graph where the distance between any 2 samples is a
548 measure of their similarity (smaller distance for higher similarity). The microbial
549 composition of patients at baseline (red circles and triangles) was not significantly
550 different from patients at week 48 treated with PMT25341 (blue) or with placebo

551 (green). **c. LEfSe analysis of taxonomic data from patients with advanced HIV**
552 **disease at baseline and after 48 weeks of ART + PMT25341 supplementation or**
553 **placebo.** LEfSe is a tool to identify biomarkers from high dimensional data of 2 or
554 more groups using, in this case, relative abundances of bacterial taxa. This
555 approach revealed that after 48 weeks, subjects treated with PMT25341 were
556 significantly enriched with unclassified bacteria from the Lachnospiraceae and
557 Victivallaceae families, but depleted in *Blautia spp*.
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