The Breast 88 (2026) 104830

Contents lists available at ScienceDirect

The Breast

journal homepage: www.journals.elsevier.com/the-breast

A mixed prebiotic/probiotic intervention (MBR-01) for the management of diarrhea during abemaciclib treatment of early breast cancer in postmenopausal patients: A single-center prospective case–control pilot study

Daniele Generali [a,b,*,1], Alexandro Membrino [b,1], Alessandra Fontana [c], Federica Gattazzo [b], Carla Strina [b], Manuela Milani [b], Valeria Cervoni [b], Aldo Caltavituro [d,e], Andrea Castagnetti [f], Sergio Del Bianco [g], Francesco Schettini [e,h,**]

[a] Multidisciplinary Unit of Breast Pathology and Translational Research, ASST of Cremona, Cremona, Italy
[b] Department of Medicine, Surgery and Health Sciences, University of Trieste, Cattinara Hospital, Trieste, 34128, Italy
[c] Department for Sustainable Food Process-DiSTAS, Università Cattolica Del Sacro Cuore, Piacenza, Italy
[d] Clinical and Translational Oncology, Scuola Superiore Meridionale, Naples, Italy
[e] Translational Genomics and Targeted Therapies in Solid Tumors, August Pi i Sunyer Biomedical Research Institute (IDIBAPS), Barcelona, Spain
[f] Wellmicro Srl, Via Bruno Rizzi 1, Bussolengo, VR, 37012, Italy
[g] Precision Oncology Department, Sanatrix Clinic, Rome, Italy
[h] Clinic Barcelona Comprehensive Cancer Center, Hospital Clinic Barcelona, Barcelona, Spain

ARTICLE INFO

Keywords:
Probiotics
Prebiotics
Microbiome
Abemaciclib
Diarrhea
Breast cancer

ABSTRACT

Background: Adjuvant abemaciclib + endocrine therapy (ET) improves long-term outcomes in high-risk, hormone receptor–positive (HR+)/HER2-negative early breast cancer (eBC). However, treatment is frequently complicated by diarrhea, affecting adherence and quality-of-life (QoL). Increasing evidence suggests that abemaciclib-induced gastrointestinal toxicity may involve gut microbiota alterations. We conducted a prospective case-control pilot study evaluating the efficacy of MBR-01, a standardized prebiotic/probiotic formulation, in mitigating abemaciclib-induced diarrhea.

Methods: We enrolled 20 postmenopausal patients with high-risk HR+/HER2-negative eBC considered unfit for adjuvant chemotherapy. Patients received abemaciclib + letrozole (control, n = 10) or abemaciclib + letrozole + MBR-01 (experimental, n = 10). The primary endpoint was the incidence and severity of diarrhea; secondary endpoints included treatment adherence, QoL assessments and exploratory baseline/week-12 microbiota characterization according to treatment arm. Trial registration number: ISRCTN11948182.

Results: Diarrhea occurred in all patients. In the control group, diarrhea was predominantly grade 1 (50%) or grade 2 (40%), with one grade 3 event (10%). In the MBR-01 group, diarrhea frequency and severity were reduced by ~70% at the end of week-12; 90% of patients experienced only grade 1 diarrhea or none by week-12, and no grade ≥3 events. Dose modification was only required in one control. Decrease in alpha-diversity and *F. prausnitzii* were associated with earlier diarrhea onset and longer duration; increase in *E.coli* correlated with higher grade events. MBR-01 supplementation seemed to preserve microbial diversity and limited *E.coli* expansion. QoL was significantly improved with MBR-01.

Conclusion: MBR-01 may effectively mitigate abemaciclib-induced diarrhea, likely through the achievement of stabilization of gut microbiota composition. Larger prospective studies are warranted to validate these preliminary findings.

* Corresponding author. Multidisciplinary Unit of Breast Pathology and Translational Research, Cremona Hospital, Viale Concordia 1, 26100, Cremona, Italy.
** Corresponding author. Clinic Barcelona Comprehensive Cancer Center, Hospital Clinic Barcelona, C. Villaroel 170, 08036, Barcelona, Spain.
E-mail addresses: daniele.generali@asst-cremona.it (D. Generali), schettini@clinic.cat (F. Schettini).
[1] co-first authors.

https://doi.org/10.1016/j.breast.2026.104830
Received 18 December 2025; Received in revised form 9 April 2026; Accepted 1 June 2026
Available online 1 June 2026
0960-9776/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Hormone receptor–positive (HR+), human epidermal growth factor receptor 2-negative (HER2-) breast cancer represents the most common breast tumor subtype [1]. Adjuvant endocrine therapy (ET) has significantly reduced recurrence risk and mortality in this group. Still, patients with high-risk features continue to experience substantial rates of relapse [2].

Cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors have transformed the management of advanced HR+/HER2-breast cancer by prolonging progression-free (PFS) and overall survival (OS) in combination with ET [3,4]. Building on this success, their role in the adjuvant setting has been actively explored. Among available agents, abemaciclib is unique for its continuous dosing schedule and broader kinase inhibition profile [5]. The pivotal monarchE trial demonstrated that adding abemaciclib to ET significantly improved invasive disease-free survival (iDFS) and OS in patients with high-risk early breast cancer [6,7]. On the basis of these results, abemaciclib has become the first CDK4/6 inhibitor (CDK4/6i) approved for adjuvant use. However, the clinical benefits of abemaciclib are tempered by its distinct toxicity profile. Diarrhea occurs in up to 80% of treated patients, with approximately 10–20% experiencing grade ≥3 events [8,9]. While usually manageable with antidiarrheal therapy and dose interruptions or reductions, gastrointestinal toxicity remains the leading cause of treatment modification and can significantly impair adherence and quality of life (QoL). Maintaining dose intensity is critical, given post hoc analyses of monarchE suggesting a correlation between treatment adherence and clinical benefit [10]. The mechanisms underlying abemaciclib-induced diarrhea are not fully understood, but off-target activity on cyclin-dependent kinase 9 (CDK9), glycogen synthase kinase-3β (GSK3β), and calcium/calmodulin-dependent protein kinase II (CAMKII) have been hypothesized to disrupt the balance between epithelial proliferation and differentiation, compromising barrier function and promoting diarrhea [11–13].

In parallel, research has increasingly highlighted the role of the gut microbiota in modulating treatment efficacy and toxicity across different solid tumors. In fact, gut microbiota is central to immune regulation, drug metabolism, and epithelial integrity [14], and dysbiosis, characterized by loss of microbial diversity and depletion of protective taxa, has been linked to systemic inflammation, carcinogenesis, and altered responses to systemic therapy [15,16], including CDK4/6i + ET [17].

Emerging evidence suggests that abemaciclib interacts with the gut microbiota, differently from other CDK4/6i. Indeed, a recent prospective study showed that abemaciclib treatment, but not ribociclib, was associated with reduced alpha-diversity and distinct taxonomic shifts, which may contribute to diarrhea onset and severity [18].

Probiotics, prebiotics, and postbiotics have shown potential to enhance barrier function, reduce mucosal inflammation, and stabilize microbial ecosystems [19]. De Sanctis et al. showed promising results in controlling abemaciclib-induced diarrhea with a postbiotic microbiota stabilizer [20], supporting the concept that microbiota-targeted interventions may help mitigating this relevant side effect. Building on these findings, we designed a prospective pilot study to evaluate whether MBR-01, a standardized probiotic + prebiotic formulation, could reduce diarrhea in patients receiving adjuvant abemaciclib + ET for high-risk early breast cancer and possible correlations between baseline microbiota composition and diarrhea occurrence. The protocol included a butyrate-producing probiotic, the *Clostridium Butyrricum,* which may enhance gut mucosal health, water and electrolyte absorption, and intestinal barrier integrity [21,22], together with a second probiotic formulation providing *Enterococcus faecium L3*, whose bacteriocins inhibit enteric pathogens and strengthen epithelial barrier function [23,24], and *Bifidobacterium animalis subsp. Lactis BB-12*, which reinforces mucosal integrity and supports microbiota recovery after dysbiosis [25]. These probiotics were complemented by a prebiotic fiber mixture that supports beneficial gut microbes and helps maintain intestinal microbiota equilibrium [26].

This study aims to provide early clinical evidence supporting microbiota-targeted supportive care strategies in oncology and to inform future randomized trials.

2. Materials and methods

2.1. Study design and ethical considerations

This prospective, single-center, case–control pilot study was conducted at the Cremona Hospital (Italy), in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was reviewed and approved by the institutional ethics committee (approval number: 34,326 - 18/10/2019- EC/ATS Val Padana). All patients provided written informed consent prior to enrollment. The trial was registered in the World Health Organization-recognized registry ISRCTN with study registration number ISRCTN11948182 [27].

2.2. Patient selection

Eligible patients were women aged ≥18 years with histologically confirmed HR+/HER2– early breast cancer at high-risk of recurrence. High-risk was defined as ≥4 positive axillary lymph nodes (N2), or 1–3 positive nodes (N1) with additional features such as tumor size ≥5 cm (T3), histologic grade 3 (G3), or Ki-67 ≥ 20%. Patients were deemed unsuitable for adjuvant chemotherapy due to comorbidities frailty, or patient preference. Additional inclusion criteria were: Eastern Cooperative Oncology Group (ECOG) performance status 0–2, adequate hematologic and organ function, and ability to provide fecal samples. Exclusion criteria included: prior exposure to CDK4/6i, chronic diarrheal disorders, inflammatory bowel disease, intestinal resection affecting absorption, systemic antibiotic, proton pump inhibitors or probiotic use within 4 weeks prior to enrollment, immunosuppressive treatment, or concurrent participation in another interventional study.

2.3. Treatment groups and interventions

Patients who refused chemotherapy due to the presence of relevant comorbidities such as reduced left ventricular ejection fraction (LVEF) or overall frailty and personal circumstances, including preference to avoid alopecia and the need to maintain work or caregiving responsibilities were enrolled into the study. Patients were assigned to the control or intervention group based on their willingness to receive prophylactic MBR-01, a standardized probiotic and prebiotic protocol designed by the Multidisciplinary Oncology Unit at the Cremona Hospital. Treatment allocation was therefore patient-driven in the context of this pilot case-controlled design. The use of over-the-counter probiotics, prebiotics, or fiber supplements during the study period was an exclusion criterion for all participants, minimizing the risk of confounding from uncontrolled co-interventions. Dietary fiber intake was not formally restricted but was monitored *via* patient diaries. Patients in the experimental arm received the MBR-01. The control group received abemaciclib 150 mg twice daily + letrozole 2.5 mg daily. The experimental group received the same regimen together with MBR-01. Treatment was administered for 12 consecutive weeks. No prophylactic anti-diarrheal medication was permitted, although loperamide could be used at the discretion of the treating physician if clinically required or indicated. Loperamide use was documented prospectively.

2.4. Study endpoints

The primary endpoint was the incidence and severity of abemaciclib-induced diarrhea, graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version

5.0 at week 12. Secondary endpoints included: the need for dose reductions, treatment interruptions or discontinuations due to gastrointestinal toxicity, and patient-reported stool frequency and consistency recorded through daily electronic diaries. Health-related QoL (HRQoL) was also a secondary endpoint and was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30 and QLQ-BR23, focusing on predefined functional domains from both instruments, namely QLQ-C30's emotional, role, and physical functioning and QLQ-BR23's body image and sexual functioning. An exploratory QoL interference score (custom composite) and diary-based stool frequency were analyzed as additional patient reported outcomes (PROMs). An exploratory endpoint assessed changes in gut microbiota composition and diversity from baseline to week 12, together with their potential association with diarrhea occurrence and severity.

2.5. Clinical assessments

Clinical evaluations were performed at baseline and every 28 days. At each visit, the incidence, grade, onset and duration of diarrhea were recorded and cross-checked with daily electronic diaries (care@you®), which also captured patient-reported stool frequency and consistency. Treatment adherence was assessed using pill counts and patient self-report. Laboratory monitoring, including hematology and biochemistry, was performed at each visit according to standard practice.

2.6. Stool sample collection and processing, DNA extraction and purification

Fecal samples were collected at baseline and at day 84 (week 12). Patients were instructed to collect mid-stool aliquots using the standardized collection kits SMART-eNAT® (Copan Italia S.p.a., Brescia, Italy). Samples were immediately stabilized in collection medium, stored at ambient temperature, and transported within 24 h to the study site, where they were frozen at −80 °C until analysis. Negative controls (blank collection kits) and positive controls (mock microbial community standards) were processed in parallel to monitor potential contamination. Fecal samples were mechanically lysed using inert beads and the FastPrep® homogenizer (MP Biomedicals, Irvine, CA, USA). DNA extraction and purification was performed using the Mag-Bind® Universal Pathogen Kit (Omega Bio-Tek, Norcross, GA, USA) on the automated NIMBUS Presto platform (Hamilton Company, Reno, NV, USA). Microbial DNA was quantified using the Qubit™ 1X dsDNA Broad Range Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) on the Synergy Instrument (Agilent Technologies, Santa Clara, CA, USA) and normalized to a concentration of 13.3 ng/μl.

2.7. Bioinformatic analysis

Metagenomic libraries were generated with the Illumina® DNA Prep kit (Illumina, San Diego, CA, USA) following the manufacturer's protocol adapted for the VANTAGE automated platform (Hamilton Company). Samples were subjected to capillary electrophoresis using the Qiaxcel™ instrument (Qiagen, Hilden, Germany). The libraries were quantified with the Qubit™ 1X dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific) and normalized to a concentration of 4 nM and sequenced on the NovaSeq6000Dx system (Illumina). Quality control of raw reads was performed using FastQC. Taxonomic profiling was carried out using MetaPhlAn4, enabling species-level resolution.

2.8. Statistical analyses

Descriptive statistics were used to summarize baseline characteristics and clinical variables. Proportions between treatment groups were compared using Fisher's exact test. Continuous variables, including patient-reported outcomes (PROs) and HRQoL scores, were analyzed using the Mann–Whitney *U* test for between-group comparisons and the Wilcoxon signed-rank test for within-group changes from baseline to week 12. For microbiome analysis, alpha diversity (Shannon, Simpson and Chao1 indices) was compared within and between groups using non-parametric tests as appropriate. Beta diversity was assessed using Bray–Curtis and weighted UniFrac distances and compared by PERMANOVA. Principal coordinate analysis (PCoA) was used to visualize differences in microbial community structure between groups and timepoints. Differential taxonomic analyses were performed using Linear Discriminant Analysis Effect Size (LEfSe), including Kruskal–Wallis testing, with false discovery rate (FDR) correction applied and a linear discriminant analysis (LDA) score threshold >3.0 used to define a meaningful effect size. Taxa with the highest LDA scores in the 'depleted in control' and 'enriched in control' categories were selected for subsequent exploratory analyses, based on comparisons between baseline and week 12 samples. Correlations between microbial features and clinical variables, including diarrhea onset, duration and grade, were assessed using Spearman's rank correlation, focusing on taxa showing the most pronounced and statistically significant shifts in the overall comparative analysis. An exploratory multivariable logistic regression model was used to evaluate whether relevant clinical and microbial baseline features independently predicted the occurrence of grade ≥ 2 diarrhea. The study was hypothesis-generating, with no formal sample size calculation. All statistical tests were two-sided, and p-values <0.05 were reported descriptively to identify preliminary signals of activity of the MBR-01 combination. As the study was purely exploratory, numerical trends were prioritized over formal statistical significance, and no correction for multiplicity was applied. All analyses were performed in R version 4.3.2 for MacOSX.

3. Results

3.1. Patient characteristics

Between January 2023 and April 2025, 20 postmenopausal patients with high-risk HR+/HER2– early breast cancer were enrolled: 10 in the control group and 10 in the intervention group (Table 1). Baseline demographic and clinical characteristics were similar between the two

Table 1
Baseline characteristics of the study population.

VARIABLES	Control	Experimental	*P value*
	N (%)	N (%)	
	10 (100%)	N (100%)	
Median age, years (min-max)	73 (68-79)	68 (65-72)	-
Menopausal status			
Postmenopausal	10 (100)	10 (100)	-
Pre/perimenopausal	0 (0)	0 (0)	
ECOG			
0	4 (40)	8 (80)	0.170
1	6 (60)	2 (20)	
2	0 (0)	0 (0)	
T			
<5 cm	6 (60)	7 (70)	1.00
≥ 5 cm	4 (40)	3 (30)	
N			
1-3 positive lymphnodes	8 (80)	5 (50)	0.350
≥ 4 positive lymphnodes	2 (20)	5 (50)	
Endocrine therapy			
Aromatase inhibitor	10 (100)	10 (100)	-
Tamoxifen	0 (0)	0 (0)	
Loperamide use			
Yes	7 (70)	2 (20)	0.070
No	3 (30)	8 (80)	
Median loperamide doses in mg (IQR)	6 (4–9)	2 (1–3)	0.041

Legend and footnotes. ECOG: Eastern Cooperative Oncology Group performance status; T/N: tumor size/nodal status; p values from Mann–Whitney *U* test for doses; Fisher's exact test for proportions.

groups. No significant differences were observed in comorbidities. The overall median age was 72 years (range, 65-79 years), with 100% of patients being postmenopausal, 70% with ECOG performance status 0 and 30% with ECOG performance status 1. As per inclusion criteria, patients had not been exposed to systemic antibiotics, probiotics and proton pump inhibitors before study treatment start.

3.2. Incidence and severity of diarrhea

Diarrhea was reported in all patients receiving abemaciclib in the control group, with five patients (50%) experiencing grade 1, four patients (40%) grade 2 and one patient (10%) grade 3 diarrhea after the first cycle. In the MBR-01 group, 8 (80%) patients reported diarrhea after the first cycle, half of them of grade 1 and the rest of grade 2. By week 12, the proportion of patients with grade 1-3 diarrhea remained stable in the control group, whereas 90% of patients in the MBR-01 group reported either grade 1 (50%) or no (40%) diarrhea and one patient (10%) reported a grade 2 event. No patient in the MBR-01 group experienced grade ≥3 diarrhea at any timepoint. Dose reductions due to gastrointestinal toxicity were required in one patient (10%) in the control group, whereas none were needed in the MBR-01 group. All patients in both arms completed the planned 12 weeks of therapy. Although the difference between groups at weeks 12 did not reach statistical significance ($p = 0.141$), the observed trend favored the MBR-01 regimen (Fig. 1). Median time to diarrhea onset was 8 days (range, 4-14 days), and the median duration of episodes was 9-10 days (interquartile range [IQR], 7 - 12 days) for grade 2 and 6-7 days (IQR 4 - 9 days) for grade 1. Loperamide use was numerically higher in the control arm (70% vs. 20%; $p = 0.071$), with a significantly greater median dose compared to the experimental arm (6 mg vs. 2 mg; $p = 0.041$) (Table 1).

3.3. Patient-reported outcomes, QoL and treatment adherence

Daily electronic diaries (care@you®) confirmed investigator-reported diarrhea events and provided additional granularity on stool frequency and consistency. At baseline, stool frequency and patient-reported outcomes were comparable between groups (Fig. 2A). The experimental group reported a significantly lower median of 2 (IQR 1 - 2.8) stools/day as compared to controls ($p = 0.022$) (Fig. 2B), without any significant increase in frequency ($p = 0.484$) during the 12 weeks of abemaciclib supported by MBR-01 treatment (Fig. 2C). Conversely, the control group patients reported a median of 4.0 (IQR 3.3 – 4.8) stools/day during diarrheal episodes, representing a significant increase ($p = 0.013$) from the pre-treatment median of 1.0 (IQR 1.0 - 1.8) stool/day (Fig. 2D).

By the end of the 12-week treatment, QoL interference scores were significantly lower in the experimental group compared to control ($p = 0.002$) (Fig. 2B), with a slight reduction from baseline in the former group ($p = 0.037$) (Fig. 2C) and a significant increase in the latter ($p = 0.014$) (Fig. 2D). Sexual functioning ($p = 0.673$), emotional functioning ($p = 0.270$), and body image ($p = 0.545$) scores were not significantly different between groups (Fig. 2B). In contrast, role functioning ($p < 0.001$) and physical functioning ($p = 0.021$) scores were significantly higher in the experimental arm after 12 weeks compared to controls (Fig. 2B), without significant changes from baseline ($p = 0.286$ and $p = 1.00$, respectively) (Fig. 2C). In the control group, emotional, role, and physical functioning scores decreased significantly over time (Fig. 2D).

The treatment adherence was numerically higher in the experimental group compared with controls. All patients in the interventional group maintained full abemaciclib dosing throughout the 12-week period, whereas one patient (10%) in the control arm required a dose reduction due to persistent grade 3 diarrhea ($p = 0.305$). No permanent discontinuations occurred in either group.

3.4. Exploratory microbiome analysis

At baseline, alpha-diversity indices (Shannon, Simpson, Chao1) were comparable between groups (all $p > 0.05$). Longitudinal analyses focused on the Shannon index, given its robustness and widespread use for capturing within-sample diversity changes. By day 84, patients in the control group exhibited a significant reduction in microbial alpha-diversity (median Shannon index 3.7 at baseline vs. 2.7 at day 84, $p = 0.010$). In contrast, MBR-01 supplementation preserved microbial diversity, with only a modest, non-significant decline observed (median Shannon index 3.8 vs. 3.5, $p = 0.210$) (Fig. 3A). Beta-diversity analysis using Bray-Curtis dissimilarity and weighted UniFrac showed no significant differences between groups at baseline (PERMANOVA $p > 0.05$), confirming comparable starting microbial compositions. By week 12, distinct clustering of microbial communities was observed between control and MBR-01 groups (PERMANOVA $p = 0.030$), suggesting that MBR-01 modulated the trajectory of abemaciclib-associated microbial shifts. These findings are illustrated in the PCoA plots, showing overlapping 95% confidence ellipses at baseline and non-overlapping clustering at week 12 (Fig. 3B and C).

3.5. Taxonomic shifts associated with diarrhea

Taxonomic profiling revealed significant shifts in gut microbiota composition after 12 weeks. LEfSe analysis (LDA >3.0, FDR <0.05) identified *Faecalibacterium prausnitzii* and *Escherichia coli* as the taxa with the largest effect sizes between baseline and week 12. Among

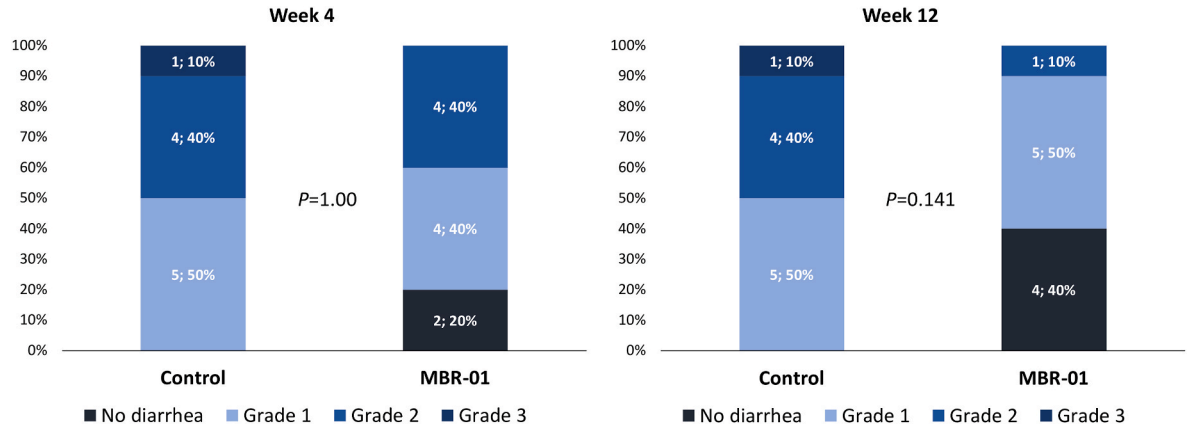

Fig. 1. Incidence and severity of diarrhea graded according to CTCAE v5.0 criteria at week 4 and 12
Legend: CTCAE: Common Terminology Criteria for Adverse Events. P-values are referred to Fisher's exact tests comparing the proportions of no diarrhea/grade 1 diarrhea and grade ≥2 diarrhea in the control vs. experimental cohort.

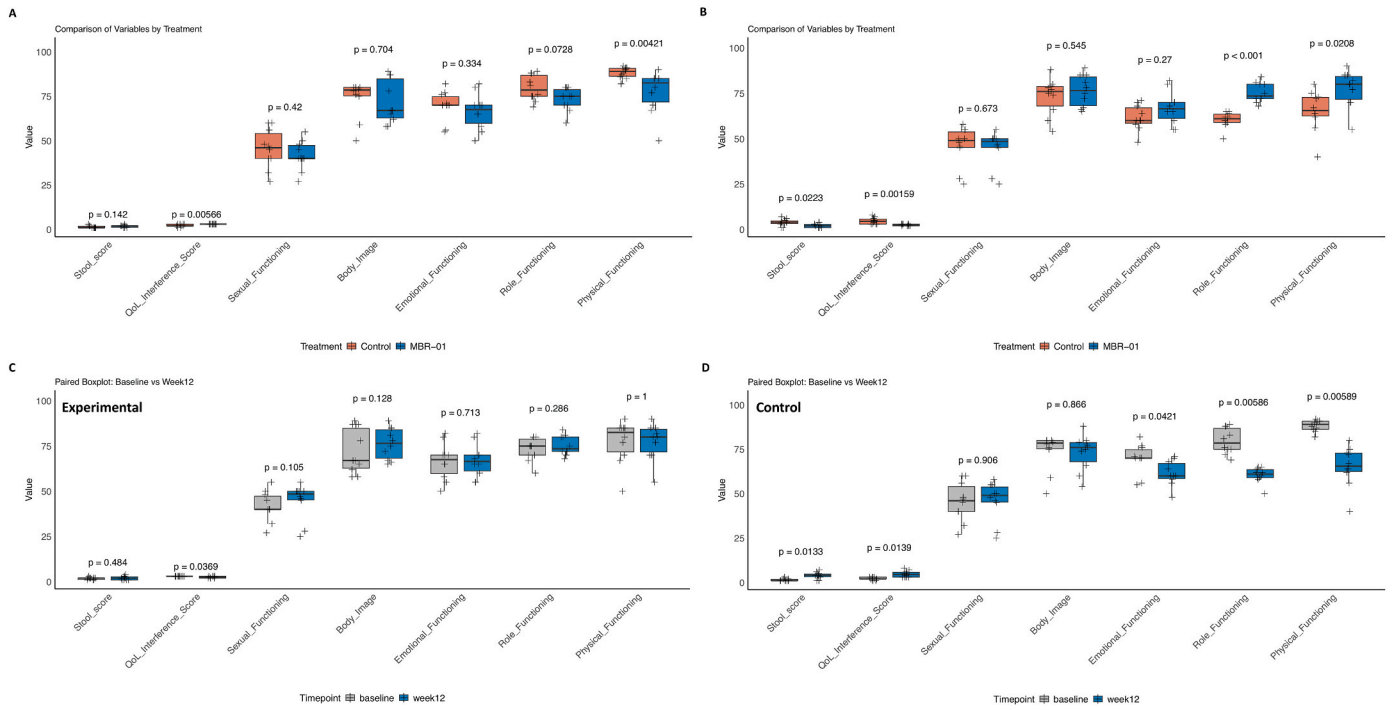

Fig. 2. Paired and unpaired comparisons among QoL scores of interest

Legend. A: Scores' distribution at baseline according to treatment group. **B:** Scores' distribution at week 12 according to treatment group. **C:** Scores' distribution at baseline and week 12 in the experimental group. **D:** Scores' distribution at baseline and week 12 in the control group. *P values* in A and B are referred to Mann Whitney U tests for unpaired samples, while in C and D are referred to Wilcoxon signed rank tests for paired samples; QoL = quality of life.

significantly different taxa (FDR <0.05), *F. prausnitzii* showed the highest LDA score among taxa depleted in the control group and enriched in the MBR-01 group, whereas *E. coli* ranked highest among taxa enriched in the control group and depleted in the MBR-01 group (Fig. 4A), providing the rationale for subsequent focused analyses.

In the control group, *F. prausnitzii* declined from a mean of 12% at baseline to 4% at week 12 ($p < 0.010$). Concurrently, *E. coli* expanded from 3% to 9% ($p = 0.040$), correlating with diarrhea onset and severity (Fig. 3D). Correlation analyses showed that lower baseline *F. prausnitzii* abundance correlated with earlier diarrhea onset (Spearman $r = -0.62$, $p = 0.020$) and longer episode duration, while *E. coli* enrichment correlated with higher maximum diarrhea grade ($r = 0.55$, $p = 0.040$). In the MBR-01 group, *F. prausnitzii* levels were preserved (13% at baseline vs. 10% at week 12, $p = 0.210$), and no significant *E. coli* expansion was observed (2% to 3%, $p = 0.180$) (Fig. 3D).

We attempted to perform a targeted analysis of the relative abundance of the probiotic strains contained in MBR-01 at baseline and week 12. However, the shallow sequencing depth achieved in this pilot study, an inherent limitation of the sample size and the exploratory nature of the protocol, did not provide sufficient reads for reliable species-level quantification of these low-abundance commensal strains against the background of the full fecal metagenome.

3.6. Clinical–microbiota correlations

An exploratory multivariate logistic regression incorporating baseline microbial diversity, age, body mass index (BMI), ECOG performance status, and treatment group identified two independent predictors of grade ≥2 diarrhea: the reduced microbial alpha-diversity (OR 3.8, 95% CI 1.2–11.5, $p = 0.02$) and low baseline abundance of *F. prausnitzii* (OR 4.1, 95% CI 1.3–12.8, $p = 0.01$), while the MBR-01 intervention was independently associated with a reduced risk of grade ≥2 diarrhea (OR 0.25, 95% CI 0.07–0.88, $p = 0.03$). No significant associations were observed for age, BMI, or ECOG status (Fig. 4B). These findings should be interpreted in light of the limited sample size.

4. Discussion

This prospective, single-center pilot study provides preliminary evidence that MBR-01, a standardized probiotic/prebiotic formulation, may mitigate abemaciclib-induced diarrhea, likely preserving gut microbiota diversity in postmenopausal patients with high-risk HR+/HER2-early breast cancer. Adjuvant abemaciclib in combination with ET represents a significant advance for patients with high-risk HR+/HER2– early breast cancer [6,7]. However, its therapeutic benefit is counterbalanced by a high incidence of diarrhea, including grade 3 or higher in 1/5 of the cases [8,9]. Our study confirmed that diarrhea was nearly universal among abemaciclib-treated patients. Although generally manageable with antidiarrheal medications and dose modifications, diarrhea is the most common reason for treatment interruptions and can negatively impact adherence, QoL, and potentially, treatment efficacy [10]. In fact, in our study, the control group experienced a significant decline in emotional, role and physical functioning, alongside an increased stool frequency and a clear interference with their QoL, with 10% patients requiring abemaciclib dose reduction. None of this happened in the experimental group where, in turn, a slight reduction of the QoL interference score was observed alongside reduction in diarrhea CTCAE grade and preservation of abemaciclib full dose, with MBR-01 supplementation. Notably, no grade ≥3 diarrhea occurred in the MBR-01 group. These findings suggest that microbiota-targeted strategies may help overcome one of the most clinically relevant barriers to sustained abemaciclib exposure.

The pathogenesis of abemaciclib-induced diarrhea is multifactorial. Preclinical studies have shown abemaciclib uniquely causes crypt hyperplasia, villous blunting, and mucosal inflammation in rodent models [13]. These types of changes have not been observed with palbociclib or ribociclib. In addition to CDK4/6 inhibition, abemaciclib targets CDK9, GSK3β, and CAMKII, integral kinases of the intestinal epithelial homeostasis [12]. Disruption of these pathways likely contributes to epithelial barrier dysfunction and increased susceptibility to diarrhea. Our microbiota analysis further supports the hypothesis that gut

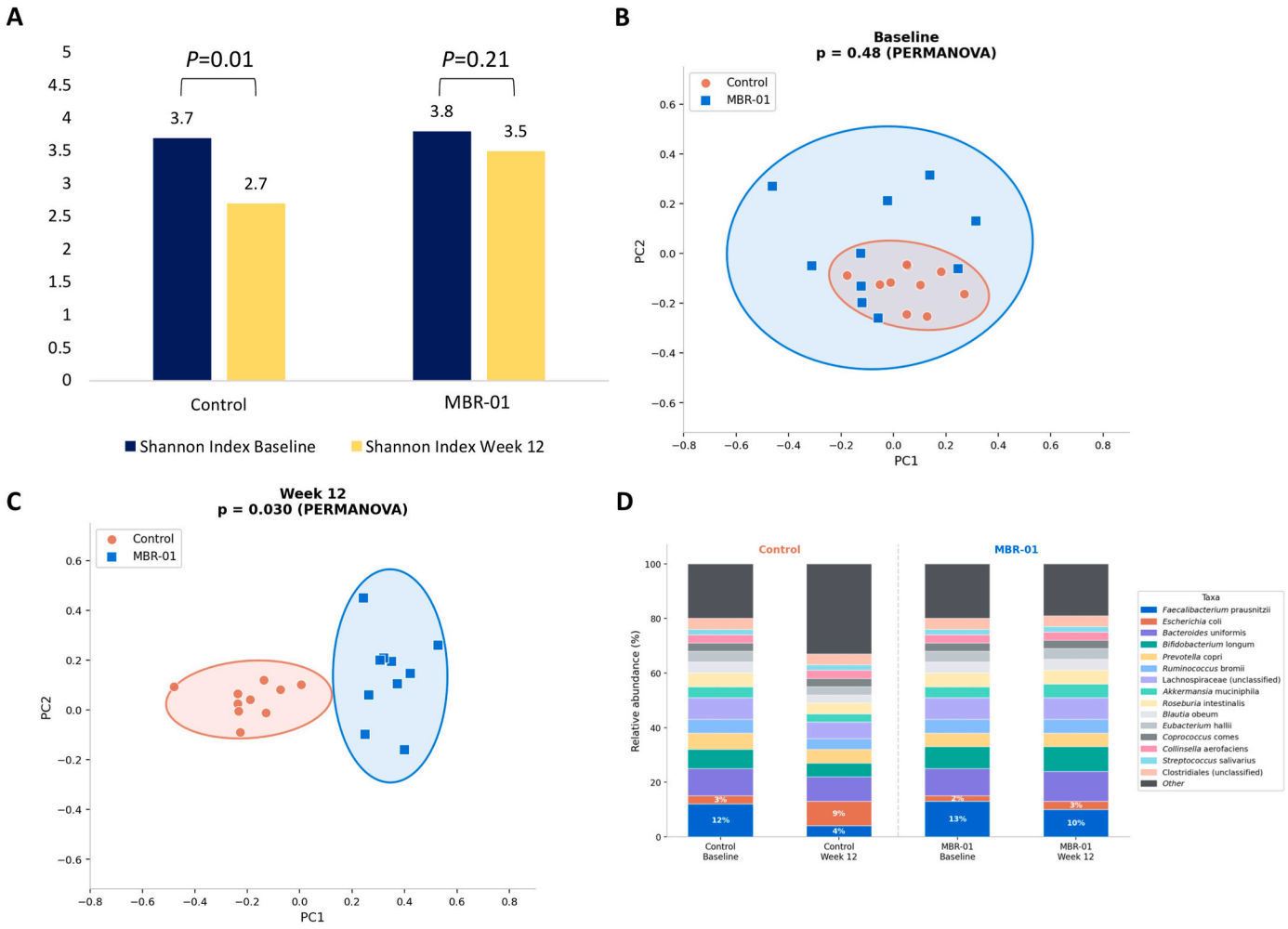

Fig. 3. Alpha and beta diversity and taxonomic shifts in selected fecal microbiota key taxa

Legend. A: Shannon Index median values (alpha diversity) at baseline and week 12 for control and MBR-01 groups. Higher values indicate greater microbial diversity. Statistical comparisons (Wilcoxon signed-rank test, p-values annotated) are shown for within-group changes. **B:** PCoA plot based on Bray–Curtis dissimilarity at baseline. Each point represents one patient. The two groups show overlapping centroids and 95% confidence ellipses, indicating comparable baseline microbial composition. **C:** PCoA plot based on Bray–Curtis dissimilarity at week 12. A separation between groups is observed, consistent with a divergence in microbial community structure over time, potentially associated with MBR-01 treatment. **D**: relative abundance of bacterial taxa, including *Faecalibacterium prausnitzii* and *Escherichia coli*, expressed as normalized proportions of total reads according to treatment arm and timepoint. Data illustrate a reduction in diversity and beneficial taxa of interest in the Control group over time, while MBR-01 maintains a more stable microbial profile. PCoA: principal coordinate analysis.

dysbiosis amplifies gastrointestinal toxicity. Control patients demonstrated a significant decline in alpha-diversity during abemaciclib therapy, alongside depletion of *F. prausnitzii*, a key butyrate producer essential for barrier integrity [28,29], and expansion of *E. coli*, a pathobiont often associated with inflammation [30]. Both features correlated with earlier onset, longer duration, and higher severity of diarrhea. Conversely, MBR-01 supplementation preserved microbial diversity, seemed to attenuate *F. prausnitzii* loss and limit *E. coli* overgrowth, suggesting that stabilization of microbial ecosystems may be protective against toxicity. These findings are consistent with prior reports linking microbiota diversity to resilience against treatment-related toxicity [16]. Together, these data reinforce the notion that drug–microbiota interactions are bidirectional, i.e. abemaciclib perturbs the microbiota, which in turn may exacerbate gastrointestinal toxicity.

Conventional strategies for managing abemaciclib-induced diarrhea, mainly loperamide and dose modifications, are often reactive and do not address underlying biological mechanisms. Our results provide preliminary evidence that a mixed probiotic/prebiotic intervention can reduce gastrointestinal toxicity while favorably modulating microbiota composition. Mechanistically, prebiotics may act through multiple, complementary pathways: restoration of epithelial barrier integrity, stimulation of short-chain fatty acid (SCFA) production (notably butyrate), suppression of pro-inflammatory signalling, and ecological stabilization of microbial communities [31,32]. Clinical data support a heterogeneous picture in practice. A recent pragmatic 2-group study using a commercially-available postbiotic reported fewer severe diarrhea events and fewer dose reductions during abemaciclib therapy compared with historical controls, suggesting benefit from microbiota stabilizers in this setting [20]. By contrast, the randomized MERMAID trial testing a probiotic (*Bifidobacterium*) ± trimebutine maleate did not reduce the incidence of grade ≥2 diarrhea compared with historical control, although grade 3 events were infrequent and some reduction in highest-grade events was observed, underscoring that not all microbiota-modulating strategies produce the same clinical effect [33]. Taken together, these data suggest two practical implications: first, supplementation with a defined combination of probiotics (e.g., *Clostridium butyricum*, *Enterococcus faecium L3* and *Bifidobacterium animalis subsp. Lactis BB-12*) together with complementary prebiotic fibers, as done in our study, may provide broader translational benefits than single-strain probiotics, by promoting gut microbial community balance, enhancing SCFA production, and supporting mucosal barrier function; second, a precision approach in selecting live-microbe

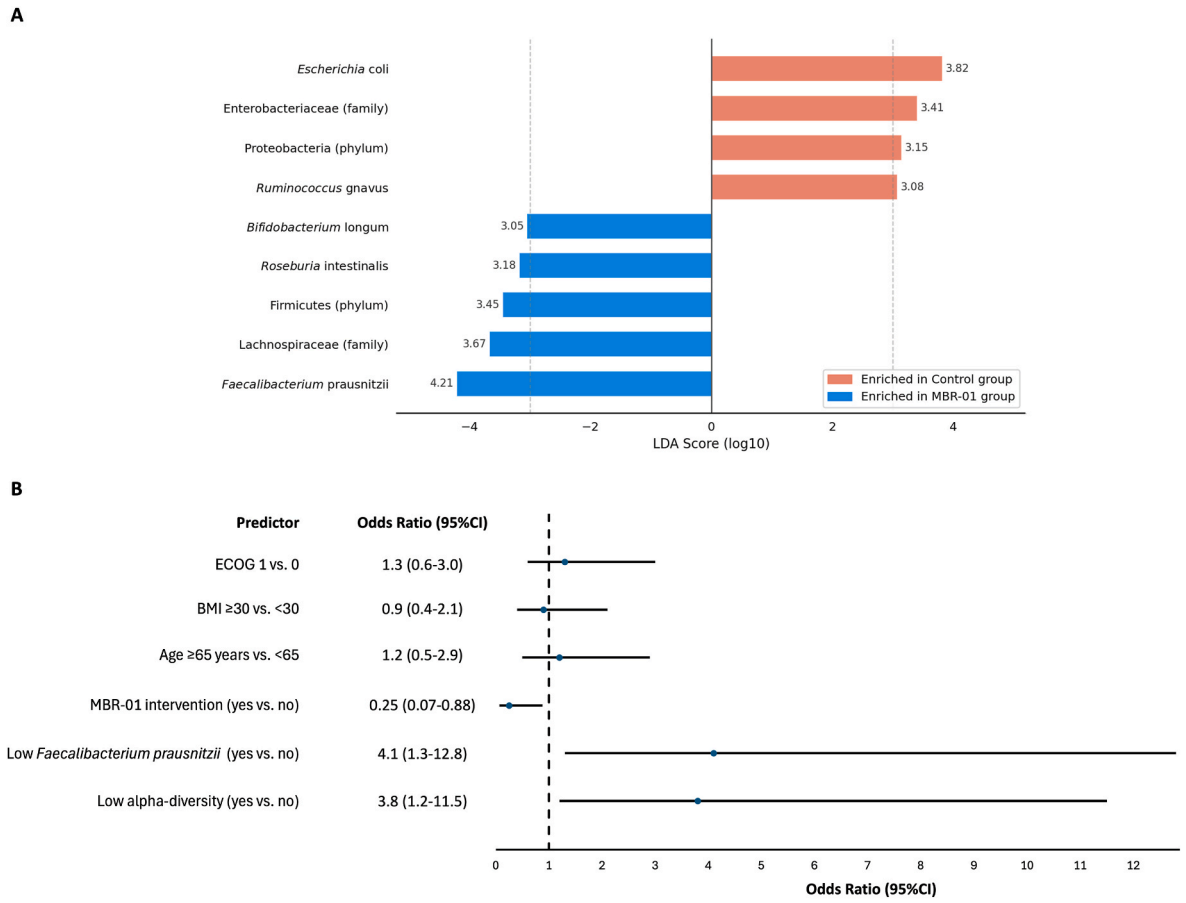

Fig. 4. LEfSe analysis and multivariable logistic regression

Legend and notes. A. LEfSe analysis identifying differentially abundant taxa between baseline and week 12. Horizontal bars represent the LDA score (log 10 scale) for each taxon showing differences between baseline and week 12 (LDA threshold >3.0, FDR <0.05). Orange bars indicate taxa enriched in the Control group at week 12, and blue bars indicate taxa enriched in the MBR-01 group at week 12. *F. prausnitzii* showed the highest LDA score among taxa enriched in the MBR-01 group (LDA = 4.21), whereas *E. coli* showed the highest LDA score among taxa enriched in the Control group (LDA = 3.82), providing the rationale for their selection as primary taxa of interest in the main analyses. **B.** Forest plot of multivariable logistic regression exploring the clinical–microbiota associations with reduced risk of grade ≥2 diarrhea, including microbiota alpha-diversity at baseline and clinical patients characteristics. Cut-off for low alpha-diversity was Shannon index<3.5. Cut-off for low relative abundance of *Faecalibacterium*<5%. Cut-offs were defined based on the distribution of baseline values in the study cohort, using the threshold corresponding to the lower tertile (33rd percentile). These thresholds were used to dichotomize variables for exploratory analyses. Given the limited sample size, these thresholds should be interpreted as exploratory. BMI: body mass index; CI: confidence intervals; LDA: linear discriminant analysis; LEfSe: Linear Discriminant Analysis Effect Size; FDR: false discovery rate.

products, tailoring probiotic or symbiotic choices to an individual's baseline microbiota, is biologically plausible and may increase the likelihood of clinical benefit.

Our study has several limitations: 1) it is a pilot study designed to detect preliminary signals of efficacy, without power to draw any definitive conclusion; 2) the sample size was small and the study was conducted at a single center, limiting generalizability; 3) the intervention was not randomized, introducing potential selection bias; 4) follow-up was limited to 12 weeks, and longitudinal changes in microbiota beyond this timeframe remain unknown; 5) all enrolled patients were postmenopausal, and our findings cannot be directly generalized to premenopausal women, in whom the hormonal milieu and baseline gut microbiome composition may differ meaningfully from our cohort; 6) while correlations between microbial features and diarrhea were observed, causality cannot be definitively established; 7) we were unable to draw conclusions regarding engraftment of the strains contained in the MBR-01 from the present dataset. We acknowledge that if engraftment is absent, the prebiotic component, rather than the probiotic strains *per se*, may be the primary driver of the observed community-level stabilization. Disentangling these contributions will require future studies with deeper metagenomic sequencing and strain-level tracking.

Our findings have several clinical implications worthy of further exploration. Firstly, microbiota-targeted prophylaxis with MBR-01 reduced diarrhea severity and frequency, potentially improving adherence to abemaciclib, a factor directly associated with therapeutic benefit in the adjuvant setting [10]. Secondly, baseline microbial features such as low alpha-diversity and reduced *F. prausnitzii* abundance in patients experiencing more diarrhea onset and higher grade, suggest that microbiota profiling could help identify patients most likely to benefit from prophylactic interventions. Thirdly, the increase in *E. coli* in the group experiencing more diarrhea suggests the potential to dynamically monitoring fecal microbiota composition to proactively detect patients without baseline dysbiosis who could develop it during treatment and may potentially benefit from the introduction of on-treatment microbiota-modulating strategies. Fourth, the preservation of microbial diversity in the MBR-01 group was associated with a benefit in several domains related to QoL, suggesting a broader health benefit beyond diarrhea mitigation.

Overall, this pilot study demonstrates that MBR-01, a mixed prebiotic/probiotic intervention, may effectively reduce abemaciclib-induced diarrhea and preserve gut microbial diversity in postmenopausal

patients with high-risk HR+/HER2– early breast cancer. A larger, randomized trial is warranted to confirm these results, to elucidate microbiota-mediated mechanisms, and to define the role of microbiota modulation in supportive oncology care. Future studies should also incorporate comprehensive longitudinal microbiota profiling, functional analyses, and biomarker discovery to clarify mechanistic pathways to potentially extend microbiota-based interventions beyond abemaciclib and optimize cancer therapy outcomes.

Funding

MEDnoTE srl with the support of care@you app for the monitoring PROMs. Copan Italia S.p.a. - Brescia – Italy provided the kits for fecal samples collection.

CRediT authorship contribution statement

Daniele Generali: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Alexandro Membrino:** Investigation, Writing – original draft, Writing – review & editing. **Alessandra Fontana:** Formal analysis, Writing – review & editing. **Federica Gattazzo:** Investigation, Writing – review & editing. **Carla Strina:** Investigation, Writing – review & editing. **Manuela Milani:** Investigation, Writing – review & editing. **Valeria Cervoni:** Investigation, Writing – review & editing. **Aldo Caltavituro:** Investigation, Writing – review & editing. **Andrea Castagnetti:** Formal analysis, Investigation, Writing – review & editing. **Sergio Del Bianco:** Investigation, Writing – review & editing. **Francesco Schettini:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

F. Schettini reports honoraria from Novartis, Astra-Zeneca, Gilead, Veracyte and Daiichy-Sankyo for educational events/materials, advisory fees from Astra-Zeneca, Pfizer, Daiichy-Sankyo and Veracyte, and travel expenses from Novartis, Gilead and Daiichy-Sankyo. D. Generali reports honoraria from Novartis, Astra-Zeneca, Pfizer, Lilly, Roche and Daiichy-Sankyo for educational events/materials, advisory fees from Novartis, Astra-Zeneca, Pfizer, Lilly and Roche, travel expenses from Roche and research grants from Novartis, Pfizer and Myriad. All remaining authors have no relevant financial or non-financial interests to disclose.

Acknowledgements

We are grateful to all the patients and their families who participated in this study. Dr. F. Schettini is supported by a Juan Rodés clinician scientist contract from the Instituto de Salud Carlos III (JR24/00024).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

References

[1] Schettini F, Giuliano M, Giudici F, Conte B, De Placido P, Venturini S, et al. Endocrine-based treatments in clinically-relevant subgroups of hormone Receptor-Positive/HER2-Negative metastatic breast cancer: systematic review and meta-analysis. Cancers March 22, 2021;13(6):1458. Basel.
[2] Braybrooke J, Bradley R, Hills RK, Peto R, Kerr A, Pan H, et al. Extending the duration of endocrine treatment for early breast cancer: patient-level meta-analysis of 12 randomised trials of aromatase inhibitors in 22 031 postmenopausal women already treated with at least 5 years of endocrine therapy. Lancet 2025;406 (10503):603–14.
[3] Gao JJ, Cheng J, Bloomquist E, Sanchez J, Wedam SB, Singh H, et al. CDK4/6 inhibitor treatment for patients with hormone receptor-positive, HER2-negative, advanced or metastatic breast cancer: a US food and drug administration pooled analysis. Lancet Oncol February 2020;21(2):250–60.
[4] Schettini F, Giudici F, Giuliano M, Cristofanilli M, Arpino G, Del Mastro L, et al. Overall survival of CDK4/6-inhibitors-based treatments in clinically relevant subgroups of metastatic breast cancer: systematic review and meta-analysis. J Natl Cancer Inst May 14, 2020;112(11):1089–97.
[5] Schettini F, De Santo I, Rea CG, De Placido P, Formisano L, Giuliano M, et al. CDK 4/6 inhibitors as single agent in advanced solid tumors. Front Oncol 2018;8:608.
[6] Johnston SRD, Toi M, O'Shaughnessy J, Rastogi P, Campone M, Neven P, et al. Abemaciclib plus endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer (monarchE): results from a preplanned interim analysis of a randomised, open-label, phase 3 trial. Lancet Oncol 2023;24(1):77–90.
[7] Johnston S, Martin M, O'Shaughnessy J, Hegg R, Tolaney SM, Guarneri V, et al. Overall survival with abemaciclib in early breast cancer. Ann Oncol 2026;37(2): 155–65.
[8] Rugo HS, Huober J, García-Sáenz JA, Masuda N, Sohn JH, Andre VAM, et al. Management of abemaciclib-associated adverse events in patients with hormone receptor-positive, human epidermal growth factor receptor 2-Negative advanced breast cancer: safety analysis of MONARCH 2 and MONARCH 3. Oncologist 2021; 26(3):e522. March 1.
[9] Johnston SRD, Harbeck N, Hegg R, Toi M, Martin M, Shao ZM, et al. Abemaciclib combined with endocrine therapy for the adjuvant treatment of HR+, HER2-, node-positive, high-risk, early breast cancer (monarchE). J Clin Oncol 2020;38(34): 3987–98. December 1.
[10] Goetz MP, Cicin I, Testa L, Tolaney SM, Huober J, Guarneri V, et al. Impact of dose reductions on adjuvant abemaciclib efficacy for patients with high-risk early breast cancer: analyses from the monarchE study. npj Breast Cancer April 26, 2024;10(1): 34.
[11] Hafner M, Mills CE, Subramanian K, Chen C, Chung M, Boswell SA, et al. Multiomics profiling establishes the polypharmacology of FDA approved CDK4/6 inhibitors and the potential for differential clinical activity. Cell Chem Biol August 15, 2019;26(8):1067–1080.e8.
[12] Cousins EM, Goldfarb D, Yan F, Roques J, Darr D, Johnson GL, et al. Competitive kinase enrichment proteomics reveals that abemaciclib inhibits GSK3β and activates WNT signaling. Mol Cancer Res February 1, 2018;16(2):333–44.
[13] Thibault S, Hu W, Hirakawa B, Kalabat D, Franks T, Sung T, et al. Intestinal toxicity in rats following administration of CDK4/6 inhibitors is independent of primary pharmacology. Mol Cancer Therapeut February 4, 2019;18(2):257–66.
[14] Ding G, Yang X, Li Y, Wang Y, Du Y, Wang M, et al. Gut microbiota regulates gut homeostasis, mucosal immunity and influences immune-related diseases. Mol Cell Biochem 2025;480(4):1969–81.
[15] Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. Microb Ecol Health Dis 2015;26:26191.
[16] Schettini F, Gattazzo F, Nucera S, Rubio Garcia E, López-Aladid, Morelli L, et al. Navigating the complex relationship between human gut microbiota and breast cancer: physiopathological, prognostic and therapeutic implications. Cancer Treat Rev 2024;130:102816.
[17] Schettini F, Fontana A, Gattazzo F, Strina C, Milani M, Cappelletti MR, et al. Faecal microbiota composition is related to response to CDK4/6-inhibitors in metastatic breast cancer: a prospective cross-sectional exploratory study. Eur J Cancer September 2023;191:112948.
[18] Donato M, Fogolari M, Iuliani M, Simonetti S, Cavaliere S, Scafetta R, et al. 113P dynamic changes in gut microbiota in patients with ER+/HER2- breast cancer treated with cyclin-dependent kinase 4/6 inhibitors. ESMO Open 2025;10(4): 104667. Supplement.
[19] Liu Y, Wang J, Wu C. Modulation of gut microbiota and immune system by probiotics, pre-biotics, and post-biotics. Front Nutr 2022;8:634897. January 3.
[20] De Sanctis R, Tiberio P, Jacobs F, Gaudio M, Benvenuti C, Giordano L, et al. Optimizing abemaciclib-induced diarrhea management in patients with breast cancer: a pragmatic 2-group study using a postbiotic microbiota stabilizer. Oncologist 2024;29(9):e1113–9. September 1.
[21] Hagihara M, Kuroki Y, Ariyoshi T, Higashi S, Fukuda K, Yamashita R, et al. Clostridium butyricum modulates the microbiome to protect intestinal barrier function in mice with antibiotic-induced dysbiosis. iScience 2019;23(1):100772. December 13.
[22] Hagihara M, Ariyoshi T, Kuroki Y, Eguchi S, Higashi S, Mori T, et al. Clostridium butyricum enhances colonization resistance against Clostridioides difficile by metabolic and immune modulation. Sci Rep 2021;11(1):15007. July 22.
[23] Huang S, Rong X, Liu M, Liang Z, Geng Y, Wang X, et al. Intestinal mucosal immunity-mediated modulation of the gut microbiome by oral delivery of Enterococcus faecium against salmonella enteritidis pathogenesis in a laying hen model. Front Immunol 2022;13:853954.
[24] Klingspor S, Bondzio A, Martens H, Aschenbach JR, Bratz K, Tedin K, et al. Enterococcus faecium NCIMB 10415 modulates epithelial integrity, heat shock protein, and proinflammatory cytokine response in intestinal cells. Mediat Inflamm 2015;2015(1):304149.
[25] West N, Horn P, Db D, Gebski V, Lahtinen S, Fricker P, et al. Probiotic supplementation for respiratory and gastrointestinal illness symptoms in healthy physically active individuals. Clin Nutr 2014;33(4):581–7.
[26] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: the international scientific association for probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol August 2017;14(8):491–502.
[27] Using a mixed probiotic/prebiotic supplement (MBR-01) to help prevent diarrhea in patients taking abemaciclib for early breast cancer [Internet]. ISRCTN Registry. [cited December 11, 2025]. Available at: https://www.isrctn.com/ISRCTN11948182.

[28] Lopez-Siles M, Duncan SH, Garcia-Gil LJ, Martinez-Medina M. Faecalibacterium prausnitzii: from microbiology to diagnostics and prognostics. ISME J April 2017; 11(4):841–52.
[29] Lenoir M, Martín R, Torres-Maravilla E, Chadi S, González-Dávila P, Sokol H, et al. Butyrate mediates anti-inflammatory effects of Faecalibacterium prausnitzii in intestinal epithelial cells through Dact3. Gut Microbes. 12(1):1–16..
[30] Kittana H, Gomes-Neto J, Heck K, Geis A, Segura Muñoz R, Cody L, et al. Commensal Escherichia coli strains can promote intestinal inflammation via differential Interleukin-6 production. Front Immunol 2018;9:2318.
[31] Smolinska S, Popescu Zemelka-Wiacek M. A review of the influence of prebiotics, probiotics, synbiotics, and postbiotics on the human gut microbiome and intestinal integrity. J Clin Med 2025;14(11):3673.
[32] Sun J, Chen S, Zang D, Sun H, Sun Y, Chen J. Butyrate as a promising therapeutic target in cancer: from pathogenesis to clinic. Int J Oncol February 29, 2024;64(4): 44. https://doi.org/10.3892/ijo.2024.5632 (Review).
[33] Masuda H, Tanabe Y, Sakai H, Matsumoto K, Shimomura A, Doi M, et al. Efficacy of probiotics and trimebutine maleate for abemaciclib-induced diarrhea: a randomized, open-label phase II trial (MERMAID, WJOG11318B). Breast October 1, 2023;71:22–8.