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Fecal microbiota transplantation plus pembrolizumab and axitinib in metastatic renal cell carcinoma: the randomized phase 2 TACITO trial

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Renal cell carcinoma (RCC) is a common malignancy with limited durable responses to first-line immune checkpoint inhibitor (ICI)-based therapies. Emerging evidence implicates the gut microbiome in modulating ICI efficacy. In the investigator-initiated, randomized, double-blind placebo-controlled phase 2a TACITO trial, we evaluated whether fecal microbiota transplantation (FMT) from complete ICI responders enhances clinical outcomes in treatment-naive patients with metastatic RCC (mRCC) receiving pembrolizumab + axitinib. The primary endpoint was the rate of patients free from disease progression at 12 months after randomization (12-month progression-free survival (PFS)). Secondary endpoints were median PFS and median overall survival, objective response rate (ORR), safety and microbiome changes, after randomization. Forty-five patients randomly received donor FMT (d-FMT) or placebo FMT (p-FMT). Although the primary endpoint was not met (70% versus 41% for d-FMT versus p-FMT, respectively, $P = 0.053$), the secondary endpoint of median PFS was significantly longer with d-FMT (24.0 months in the d-FMT arm versus 9.0 months in the p-FMT arm; hazard ratio = 0.50, $P = 0.035$). The ORR was 52% of patients in the d-FMT arm and 32% of patients receiving placebo. Microbiome analysis confirmed donor strain engraftment and increased α-diversity and larger microbiome shifts (β-diversity) compared with baseline composition in the d-FMT treatment group. Acquisition or loss of specific strains, but not total engraftment, was associated with the primary endpoint. Our findings support the safety and potential efficacy of selected donor FMT to enhance ICI-based treatment in mRCC, which deserves further investigations. ClinicalTrials.gov identifier: NCT04758507.

Immune checkpoint inhibitor (ICIs) have changed the management of different cancers, including melanoma, non-small cell lung cancer and renal cell carcinoma (RCC). The combination of pembrolizumab (a programmed cell death protein 1 (PD-1) inhibitor monoclonal antibody) with axitinib (a vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor) has significantly improved overall survival, progression-free survival (PFS) and objective response rate (ORR) in patients with metastatic RCC (mRCC)[1], and it is currently used as first-line therapy in this population. However, most patients experience disease progression within 16 months, with limited therapeutic options thereafter.

A growing body of evidence suggests that the composition of the gut microbiome may affect the efficacy of ICIs in several epithelial tumors, including RCC[2]. First, the use of antibiotics, which are known to disrupt the gut microbiome[3], has been associated with worse clinical response to ICIs in different types of cancer[4], including RCC[5]. Also, several microbial taxa and/or ecological communities have been associated with response to ICIs in different epithelial cancers[2], and the

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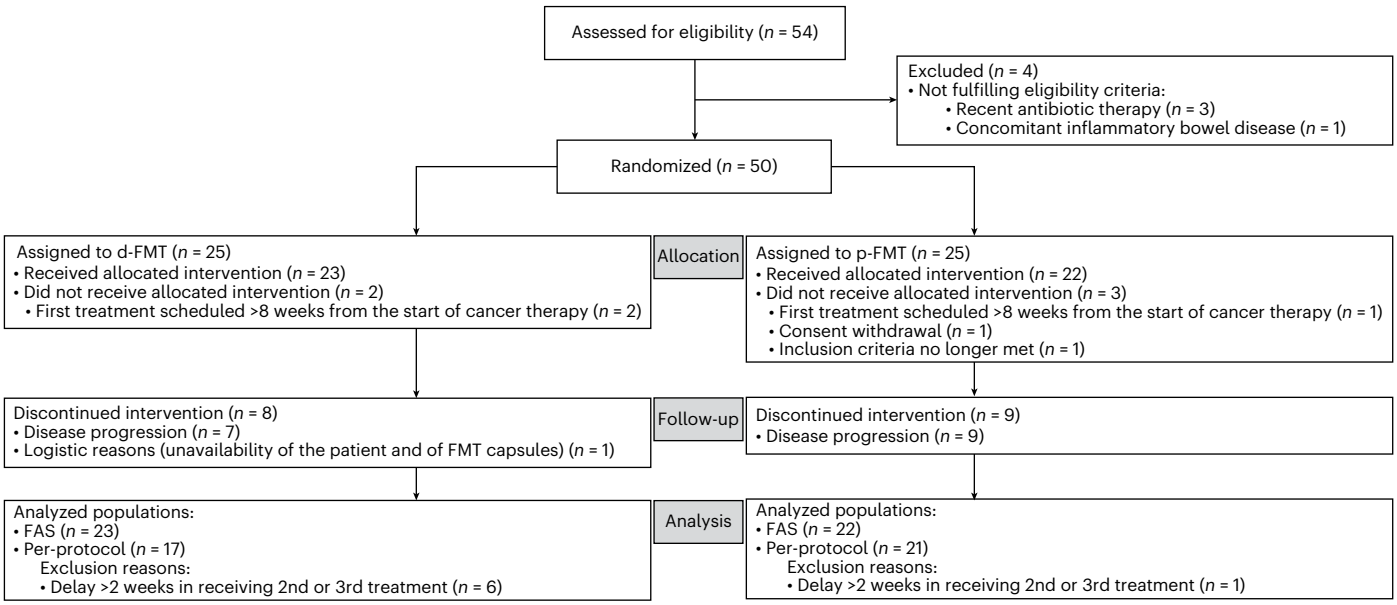

Fig. 1 | CONSORT flow diagram. The FAS included all patients who had received at least one treatment. The per-protocol population included all patients who had received treatments without significant delay (≤2 weeks).

transfer of gut microbiome from humans to mice was proven to influence the response to ICIs in cancer mouse models[6]. Fecal microbiota transplantation (FMT) provided clinical benefit in ICI-refractory[7–9] or treatment-naive[10] patients with advanced solid tumors, mostly melanoma, without serious adverse events (SAEs). However, thus far, no randomized controlled trials (RCTs) have demonstrated the efficacy of FMT in mRCC. Our phase 2a placebo-controlled RCT aims to evaluate whether FMT from patients with mRCC with complete response to ICIs was effective in improving response to combined first-line therapy with pembrolizumab and axitinib in patients with mRCC.

Results

Study design and participant characteristics

In this double-blind, placebo-controlled, investigator-initiated phase 2a RCT, we enrolled patients with metastatic, histologically confirmed RCC who were eligible to receive pembrolizumab and axitinib as first-line therapy. We excluded patients unable to undergo FMT, with a history of concomitant gastrointestinal or autoimmune disorders, with prior exposure to systemic immunomodulators or with ongoing or recent (<4 weeks prior to enrollment) antibiotic use. Full eligibility criteria are described in Methods. The primary endpoint was the rate of patients free from disease progression at 12 months after randomization (12-month PFS). Secondary endpoints were median PFS and median overall survival, ORR, safety and microbiome changes, after randomization.

After a baseline visit, patients were randomized 1:1 to receive donor FMT (d-FMT) or placebo FMT (p-FMT) three times in 6 months, unless disease progression occurred. Both groups underwent the first procedure by colonoscopy, followed by capsulized FMT after 12 weeks and 24 weeks, respectively. Due to the potential delay in treatments depending on donor feces availability, we reevaluated patients 2–5 days before the first treatment to collect a stool sample closer to treatments and check their eligibility based on the following predefined withdrawal criteria: actual provision of the first treatment more than 8 weeks from the start of ICI therapy and actual changes in the eligibility criteria evaluated at the time of enrollment.

Fifty-four patients were assessed for eligibility, and 50 patients were enrolled and randomized to treatments between 11 February 2021 and 11 November 2023 (Fig. 1).

The 45 patients who received at least the first allocated treatment (23 in the d-FMT arm and 22 in the p-FMT arm) were included in the full analysis set (FAS). Thirty-eight patients (17/23 in the d-FMT arm and 21/22 in the p-FMT arm, overall 84%) received further treatments, until they were eligible, within or no later than 2 weeks after the planned schedule and were included in the per-protocol population. In the d-FMT arm, three patients received the second FMT with a delay of 16 weeks each. Moreover, one patient received the second treatment with a 7-week delay and the third treatment with a delay of 26 weeks, and, for another patient, the second treatment was delayed by 4 weeks and the third treatment by 8 weeks. Reasons for delay were the unavailability of FMT capsules and/or the unavailability of patients to attend procedures timely. One patient in the p-FMT arm received the second treatment with a 8-week delay because of their inability to travel.

In the FAS population, 35 patients received two FMTs (19 in the d-FMT arm and 15 in the p-FMT arm), and 28 of them received three FMTs (15 in the d-FMT arm and 13 in the p-FMT arm). Disease progression was the most frequent reason for treatment discontinuation. One patient in the d-FMT arm did not receive the third FMT, despite being free from disease progression, because of logistic reasons, as initially FMT capsules were unavailable (with a delay also in the second treatment), and then the patient was not able to reach our center. Thirteen months after randomization, the patient experienced disease progression and was then not eligible to receive treatments anymore.

The baseline characteristics of the two treatment groups in the FAS population were well balanced (Table 1). Most participants were male (73%), and the median age at the time of treatment initiation was 62 years (range, 41–79 years). According to the International Metastatic RCC Database Consortium (IMDC) risk model, 69% of participants had intermediate-prognosis and poor-prognosis disease. Concerning the tumor histology, most patients had clear cell RCC ($n = 40$, 89%), whereas a slight minority had papillary ($n = 4$, 9%) and chromophobe ($n = 1$, 2%) RCC. The most common sites of metastasis include lung ($n = 33$, 73%), lymph nodes ($n = 18$, 40%) and liver ($n = 8$, 18%).

Donors

We identified five patients with long-term complete response to ICI as candidate donors. Two of these individuals—one 57-year-old man and one 52-year-old woman with RCC, both experiencing complete response

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Table 1 | Baseline characteristics of participants included in the FAS

	d-FMT *n*=23 (%)	p-FMT *n*=22 (%)
Median age (min–max)	62 (47–75)	61 (41–79)
Male sex	17 (74%)	16 (73%)
Nephrectomy	14 (61%)	13 (59%)
Histology		
Clear cell	21 (91%)	19 (86%)
Papillary	1 (4%)	3 (14%)
Chromophobe	1 (4%)	0 (0%)
Interval from nephrectomy to therapy <1 year	13 (56%)	13 (59%)
Sites of metastases		
Lung	17 (74%)	16 (73%)
Lymph nodes	9 (39%)	14 (64%)
Pancreas	5 (22%)	2 (9%)
Bone	5 (22%)	5 (23%)
Liver	2 (9%)	6 (27%)
Adrenal gland	4 (17%)	4 (18%)
Soft tissue	4 (17%)	3 (14%)
IMDC prognostic class		
Favorable	7 (30%)	7 (32%)
Intermediate	14 (61%)	10 (45%)
Poor	2 (9%)	5 (23%)

after nivolumab + ipilimumab and nivolumab alone, respectively—passed the screening process (Methods) and were eligible to donate.

Primary and secondary efficacy outcomes

At the data cutoff on 6 February 2025 (2 months after the last study visit), the median follow-up time was 32 months (interquartile range (IQR), 22–37 months). Overall, 31 of 45 patients (69%) in the FAS population experienced disease progression. A PFS event occurred in 14 of 23 patients in the d-FMT arm (61%) and in 17 of 22 patients in the p-FMT arm (77%). Median PFS was significantly improved in the d-FMT arm (24.0 months, 95% confidence interval (CI): 8.0–40.0 months) compared with the p-FMT arm (9.0 months, 95% CI: 2.2–15.2 months) (hazard ratio = 0.50, 90% CI: 0.27–0.92, $P = 0.035$; Fig. 2a). As hypothesized for the primary efficacy outcome, the proportion of patients without progression or death 12 months after randomization was higher in the d-FMT arm than in the p-FMT arm (d-FMT: 16/23 patients, 70%; p-FMT: 9/22 patients, 41%; $P = 0.053$). After adjusting for established prognostic factors in mRCC—that is, IMDC criteria and presence of liver metastases—findings were similar to those arising from the unadjusted analysis (hazard ratio = 0.48, 95% CI: 0.23–0.99, $P = 0.048$).

Seventeen of 45 patients (38%) died by the data cutoff, specifically six of 23 patients in the d-FMT arm (26%) and 11 of 22 patients in the p-FMT arm (50%). Median overall survival was longer in the d-FMT arm (41.0 months) than in the p-FMT arm (28.3 months) although not significantly (hazard ratio = 0.36 (95% CI: 0.13–0.99), $P = 0.167$; Fig. 2b).

The ORR in the FAS population was 52% (12/23 patients, 95% CI: 0.33–0.71) in the d-FMT arm and 32% (7/22 patients, 95% CI: 0.14–0.55) in the p-FMT arm. Complete response was observed in two patients (9%) in the p-FMT arm and in none of the patients in the d-FMT arm. Five patients (23%) in the p-FMT arm and 12 patients (52%) in the d-FMT arm experienced a partial response. Stable disease was found in nine patients (39%) in the d-FMT arm and in eight patients (36%) in the p-FMT arm, and two patients (9%) in the d-FMT arm and seven patients (32%) in the p-FMT arm had progressive disease.

Safety assessment

Overall, 49 patients (all randomized patients except one patient who withdrew his consent) were included in the safety analysis (Extended Data Table 1). Generally, experimental treatments were well tolerated. Adverse events strictly related to the experimental procedures occurred rarely. One patient experienced grade 2 diarrhea after colonoscopic p-FMT, but this symptom was self-limited and disappeared in few days. Only one patient in the p-FMT arm experienced a grade 3 treatment-related adverse event (TRAE) (oral mucositis) during the intake of frozen capsules, which led to the discontinuation of their ingestion. No deaths related to experimental treatments were reported. No transmission of any infectious agent after d-FMT was observed.

Grade 3 or higher adverse events related to axitinib and/or pembrolizumab occurred in 28% (7/25) of the patients in the d-FMT group and in 16% (4/24) of the patients in the p-FMT group and led to the interruption of treatments in 8% and 6%, dose reduction in 12% and 12% and treatment discontinuation in 8% and 0% of patients in the d-FMT arm and in the p-FMT arm, respectively.

Among adverse events of special interest (which included gastrointestinal adverse events), the most common of all grades were diarrhea (d-FMT: 52% versus p-FMT: 44%) and aspartate aminotransferase/alanine aminotransferase (AST/ALT) increase (d-FMT: 20% versus p-FMT: 28%), and the most common high-grade (grade 3 and grade 4) events were again diarrhea (d-FMT: 12% versus p-FMT: 4%) and AST/ALT increase (d-FMT: 12% versus p-FMT: 8%), all non-significantly different.

Assessment of microbiome changes after treatments

We collected stool samples throughout the study from the 45 patients in the FAS population ($n = 246$ samples, collected at baseline and, respectively, 1 week, 4 weeks, 12 weeks, 24 weeks, 36 weeks and 52 weeks after the first treatment in both study arms) and the two donors ($n = 9$ samples, with collection of all donor stool aliquots administered to the patients). Samples were metagenomically sequenced at high depth and profiled with the computational tools in the bioBakery suite[11] (Methods).

As a first step, taxonomic profiles obtained via MetaPhlAn 4 (ref. 12) were used to compare the baseline microbiome and the microbiome effects of the treatments in each arm. As expected, given the randomized study design, the overall baseline microbiome compositions (Extended Data Fig. 1) did not differ between treatment groups in terms of Shannon α-diversity ($P = 0.15$; Fig. 3a), species richness ($P = 0.66$; Fig. 3a) and Bray–Curtis dissimilarity ($P = 0.92$; Fig. 3b) before treatments. Donors had similar diversity levels to patients (Fig. 3a), but their microbiomes stood out as outliers in terms of composition (Fig. 3b), which was likely linked to the most abundant species in donors being *Bifidobacterium adolescentis* (SGB17244) (average 12.5%), whereas this was only the 30th most abundant species in patients (average 0.7% relative abundance). We also noticed that the donor providing 98% of the stool aliquots administered to the d-FMT group lacked metagenomically detectable *Akkermansia muciniphila* (SGB9226), a 50% prevalence species at the patient's baseline, having, instead, the rarer (9% prevalence in patients) related clade recently proposed for reclassification as *Akkermansia massiliensis* (SGB9228)[13,14]. *Candidatus Cibionibacter quicibialis* (SGB15286) (average 9.2% relative abundance), *Ruminococcus bromii* (SGB4285) (5.2%) and *Bifidobacterium longum* (SGB17248) (4.7%) also composed relevant fractions of the donors' microbiome (Extended Data Fig. 1).

After treatments, we observed an increase in Shannon α-diversity versus baseline at week 1 ($P = 0.05$), week 4 ($P < 0.001$), week 12 ($P = 0.02$) and week 24 ($P = 0.048$) follow-ups (Fig. 3c) and significantly increased species richness versus baseline at week 4 follow-up ($P = 0.001$; Extended Data Fig. 2) in the d-FMT group. Notably, such longitudinal increase in α-diversity was not observed in any of the follow-up timepoints of the p-FMT group (Fig. 3c and Extended Data Fig. 2). We also observed a significantly higher number of species acquired after

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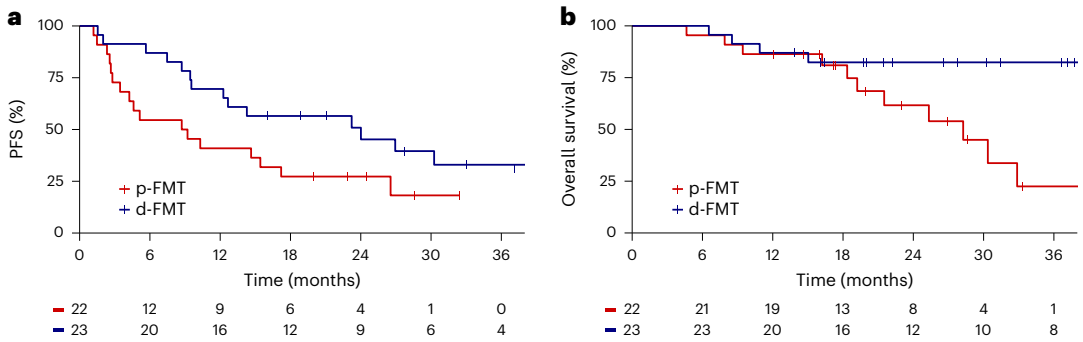

Fig. 2 | Efficacy outcomes in the overall FAS population. a, Median PFS. Median PFS was significantly improved in the d-FMT arm (24.0 months, 95% CI: 8.0–40.0 months) compared with the p-FMT arm (9.0 months, 95% CI: 2.2–15.2 months) (hazard ratio = 0.50, 90% CI: 0.27–0.92, $P = 0.035$). **b**, Median overall survival. Median overall survival was longer in the d-FMT arm than in the p-FMT arm (41.0 months versus 28.3 months, hazard ratio = 0.36, 95% CI: 0.13–0.99, $P = 0.167$). The two-sided Breslow test was applied to assess statistical significance.

treatments (that is, absent at baseline but present in follow-up samples) among patients receiving d-FMT versus those in the p-FMT arm, corroborating the hypothesis that their microbiome enrichment was driven by FMT ($P < 0.006$ across all timepoints; Fig. 3d). Despite such α-diversity increase, the d-FMT arm also showed a higher percentage of baseline species lost compared with the p-FMT arm, with a significant difference between arms only at week 4 follow-up (median 19.9% versus 14.8%, $P = 0.045$) but with a clear trend across other follow-up timepoints (Fig. 3e).

FMT had a considerable modulatory effect of FMT also on microbiome composition, with significantly higher Bray–Curtis dissimilarity between posttreatment and baseline microbiomes in d-FMT versus p-FMT patients at the week 4 follow-up ($P = 0.015$; Fig. 3f). As both absolute α-diversity and β-diversity deviation to the baseline was increased in the d-FMT arm compared with the p-FMT arm after treatments, the preplanned microbiome-related secondary outcomes were achieved.

We next evaluated whether these broad microbiome changes were associated with the primary efficacy outcome. We focused on the week 1 and week 4 follow-ups as the only timepoints allowing for statistical comparisons—that is, the ones with five or more individuals in each group (PFS > 12 months versus PFS > 12 months) of the d-FMT arm. In both arms, the number of acquired species and the species-level compositional shift after baseline were not associated with the primary outcome (Extended Data Fig. 3). Notably, the percentage of baseline species lost at follow-up was higher among individuals with PFS > 12 months in the d-FMT arm (week 1 median 28% versus 20%, $P = 0.056$; week 4 median 24.3% versus 18.2%, $P = 0.078$; Fig. 3g) but not in the p-FMT arm (week 1 median 19% versus 18%, $P = 1$; week 4 median 12.9% versus 15.5%, $P = 0.64$; Fig. 3g). This effect of donor FMT in depleting baseline species might be linked to a rewiring of the recipient microbiome that favors immunotherapy clinical response.

Quantification of donor microbiome engraftment

To directly associate the quantitative level of donor FMT success with the effect of donor strains on the recipient microbiome, we used a StrainPhlAn-based pipeline to infer the donor strain engraftment rate (DoSER), defined as the number of strains of the donor that are present in each recipient's sample after the FMT.

Only a small proportion of donor strains were already present in patients at baseline, possibly as a consequence of previous direct or indirect contact between individuals or, more likely, of the presence of shared clonal strains in the local population, and, as expected, this fraction did not differ significantly between arms (median DoSER: d-FMT 2.2% versus p-FMT 2.2%, $P = 0.73$; Fig. 4a). The DoSER in patients receiving d-FMT was consistently higher than in the p-FMT arm throughout the whole study period ($P < 0.001$ across all timepoints starting from week 1 follow-up; Fig. 5a). Specifically, the p-FMT DoSER remained as low as the baseline one (one-sided Wilcoxon signed-rank test $P > 0.22$ across all timepoints versus baseline, with medians ranging between 1.1% and 2.2%). As expected, individuals in the d-FMT arm showed very high DoSER (median at week 1 follow-up: 18.1%; Fig. 4a) already after the first treatment, confirming a microbiologically successful FMT. High engraftment rates were sustained throughout the study with the aid of the additional treatments (week 4: 14.6%; week 12: 13.9%; week 24: 20.5%; week 36: 24.0; week 52: 17.4%), with a positive correlation observed between strain abundances in the donor aliquots and strain engraftment frequency (Extended Data Fig. 4).

The effect of d-FMT was also recapitulated by the strain replacement rate with respect to the baseline (Methods), which increased over time in both arms, because of expected longitudinal changes in the gut microbiome ($P = 0.002$ and $P = 0.004$ across the longitudinal comparison week 1 versus week 52 follow-ups for the d-FMT arm and the p-FMT arm, respectively) but was significantly higher in the d-FMT arm ($P < 0.003$ across all cross-sectional comparisons between arms; Fig. 4b).

Donor microbiome engraftment effects on clinical outcomes

We next evaluated whether the DoSER was associated with PFS at the 12-month follow-up after randomization. We found that patients in the d-FMT arm who experienced PFS > 12 months showed similar engraftment rates to patients with PFS < 12 months both at week 1 (median DoSER: 12.0% versus 20.0%, respectively, $P = 0.15$; Fig. 4c) and at week 4 (median DoSER: 14.5% versus 14.8%, respectively, $P = 0.53$; Fig. 4c) follow-ups. These findings suggest that, despite the clear clinical benefit of d-FMT, the absolute number of donor strains engrafting in patients does not appear to be relevant in influencing it.

We then set out to test whether the engraftment success or failure of particular strains was linked with outcomes. This was done by evaluating the fraction of individuals acquiring the donor strain of each species in each group (progression free versus disease progression at the 12-month follow up after randomization) at week 1 (Fig. 5d) or week 4 (Extended Data Fig. 5a) follow-ups. The acquisition of the *Blautia wexlerae* (SGB4837) strain from the donor at week 1 was positively associated with 12-month PFS (percentage of recipients acquiring strain with versus without PFS > 12 months: 50% versus 0%, $P = 0.047$; Fig. 5d). On the other hand, acquisition of the donor strain of a yet-to-be-described species (SGB14845) of the family Oscillospiraceae (percentage of recipients acquiring strain with versus without PFS > 12 months: 7% versus 71%, $P = 0.006$; Fig. 4d) was inversely associated with 12-month PFS. The same pattern was observed for the acquisition of the donor strain of the rarer—as opposed to the prevalent *A. muciniphila* (SGB9226)—*A. massiliensis* sp. nov. (SGB9228) (percentage of recipients acquiring strain with versus without PFS > 12 months: 0% versus 57%, $P = 0.006$; Fig. 4d) at the week 1 follow-up.

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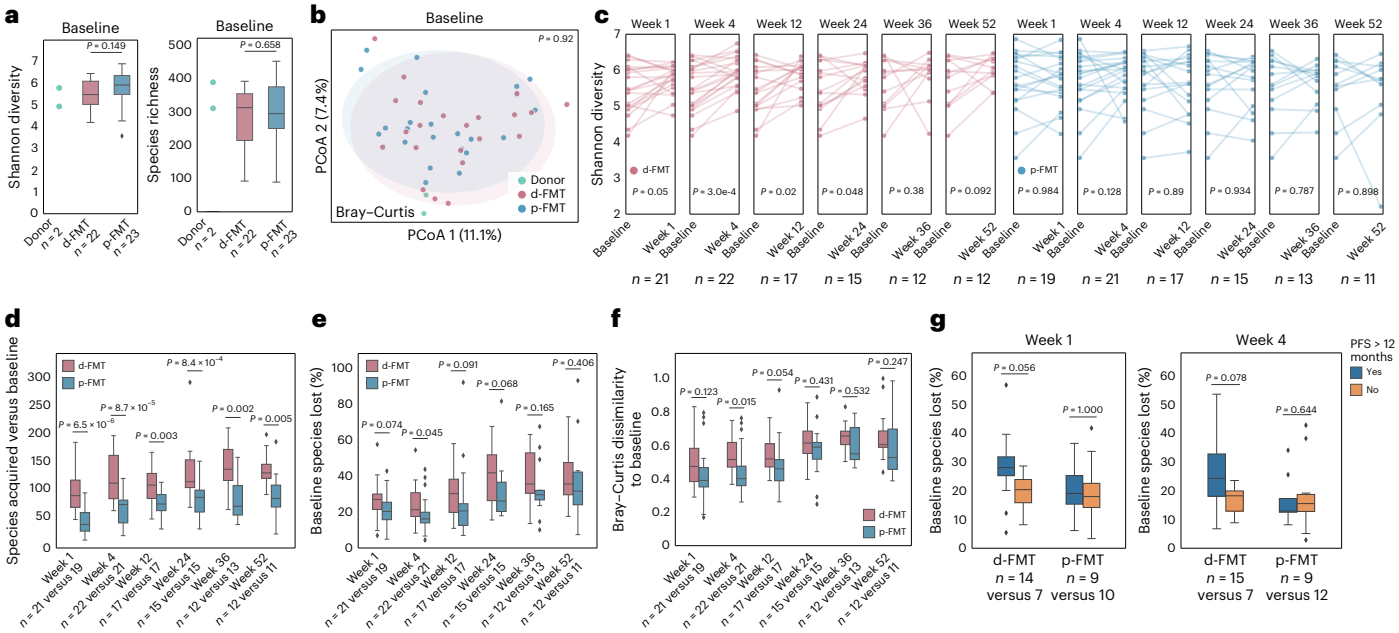

Fig. 3 | Microbiome differences at baseline and after treatments between the two treatment arms. a, Shannon diversity (left) and species richness (right) box plots comparing arms at baseline. The two-sided Mann–Whitney test was applied to assess statistical significance. Donor diversity levels in their first provided aliquot are included for reference. **b**, Principal coordinate analyses (PCoAs) on Bray–Curtis dissimilarities comparing microbiome compositions between arms at baseline. The two-sided PERMANOVA test was applied to assess statistical significance. Donor microbiome compositions at their first provided aliquot are included for reference. **c**, Shannon diversity change compared with baseline for the d-FMT arm (left) and the p-FMT arm (right) at each follow-up sample collection (week 1, week 4, week 12, week 24, week 36 and week 52). The two-sided Wilcoxon signed-rank test was applied to assess statistical significance. **d**, Box plots with the number of species acquired as compared with baseline comparing each arm at each follow-up sample collection. The two-sided Mann–Whitney test was applied to assess statistical significance. **e**, Box plots with the percentage of baseline species lost comparing each arm at each follow-up sample collection. The two-sided Mann–Whitney test was applied to assess statistical significance. **f**, Box plots with the Bray–Curtis dissimilarity with respect to the baseline comparing each arm at each follow-up sample collection. The two-sided Mann–Whitney test was applied to assess statistical significance. **g**, Box plots with the percentage of baseline species lost at week 1 (left) or week 4 (right) for each arm comparing patients with or without PFS > 12 months. The two-sided Mann–Whitney test was applied to assess statistical significance. Across all box plots, box edges represent the lower and upper quartiles, the center line indicates the median, and whiskers extend to the most extreme data point that is no farther than 1.5 times the IQR.

Besides assessing acquisition of donor strains, we also evaluated at week 1 (Fig. 4e) and week 4 (Extended Data Fig. 5b) the loss of strains present in the recipients at baseline. The loss of the *Ruminococcus bromii* (SGB4290) baseline strain at the week 1 follow-up was associated with a shortened PFS (percentage of recipients acquiring strain with or without PFS > 12 months: 7% versus 57%, $P = 0.025$; Fig. 4d). On the other hand, loss of the baseline *Escherichia coli* (SGB10068) strain or the baseline strain of an uncharacterized *Clostridium* species (SGB6179) at the week 1 follow-up was associated with 12-month PFS. All patients in the d-FMT arm who experienced disease progression before 12 months did not lose the baseline strain of those two species, whereas, respectively, 36% and 43% of the patients who had a PFS longer than 12 months did so, although these associations were not significant ($P = 0.12$ and $P = 0.06$, respectively; Fig. 4e).

Sensitivity analyses

At the data cutoff on 6 February 2025, 27 of 38 patients (71%) in the per-protocol population experienced disease progression—specifically, seven of 17 (41%) in the d-FMT arm and 17 of 21 (81%) in the p-FMT arm. Median PFS was significantly improved in the d-FMT arm (26.9 months, 95% CI: 7.5–not evaluable) versus the p-FMT arm (8.7 months, 95% CI: 2.8–15.4 months) (hazard ratio = 0.43, 90% CI: 0.20–0.92, $P = 0.028$; Extended Data Fig. 6a).

The proportion of patients without progression or death 12 months after randomization was significantly higher in the d-FMT arm than in the p-FMT arm (d-FMT: 12/17, 71%; p-FMT: 8/21, 38%, $P = 0.046$).

Sixteen of 38 patients (42%) died by the data cutoff: five of 17 (29%) in the d-FMT arm and 11 of 21 (52%) in the p-FMT arm. Median overall survival was not significantly longer in the d-FMT arm (41 months) versus the p-FMT arm (28.3 months) (hazard ratio = 0.36 (95% CI: 0.13–0.99), $P = 0.146$; Extended Data Fig. 6b), probably because of the insufficient number of events.

The ORR in the per-protocol population was 59% (10/17 patients, 95% CI: 0.36–0.78) in the d-FMT arm and 33% (7/21 patients, 95% CI: 0.17–0.55) in the p-FMT arm. Complete response was observed in two patients (9%) in the p-FMT arm versus none of the patients in the d-FMT arm. Five patients (24%) in the p-FMT arm and 10 patients (59%) in the d-FMT arm experienced a partial response. Stable disease was found in five patients (29%) in the d-FMT arm and in seven patients (33%) in the p-FMT arm. Two patients (12%) in the d-FMT arm and seven patients (33%) in the p-FMT arm had progressive disease.

In the intention-to-treat (ITT) population at data cutoff, 33 of 50 patients (67%) experienced disease progression (d-FMT arm: 16/25, 64%; p-FMT arm: 17/25, 68%). Median PFS was not significantly longer in the d-FMT arm (23.2 months, 95% CI: 7.4–39.1 months) than in the p-FMT arm (9.2 months, 95% CI: 0–20.5 months) (hazard ratio = 0.66, 95% CI: 0.33–1.35, $P = 0.18$; Extended Data Fig. 7a). Sixteen patients in the d-FMT arm and 10 patients in the p-FMT arm achieved 12-month PFS (d-FMT 64% versus p-FMT 48%, $P = 0.20$).

Nineteen of 50 patients (38%) died by the data cutoff (d-FMT arm: 8/25, 32%; p-FMT arm: 11/25, 44%). Median overall survival was non-significantly longer in the d-FMT arm (40.9 months, 95% CI: 39.2–42.7 months) than in the p-FMT arm (28.2 months, 95% CI: 20.1–36.5 months) (hazard ratio = 0.51, 95% CI: 0.20–1.30, $P = 0.146$; Extended Data Fig. 7b).

The ORR in the ITT population was 52% (13/25 patients, 95% CI: 0.33–0.70) in the d-FMT arm and 28% (7/22 patients, 95% CI: 0.14–0.55) in the p-FMT arm. We observed a complete response in two patients

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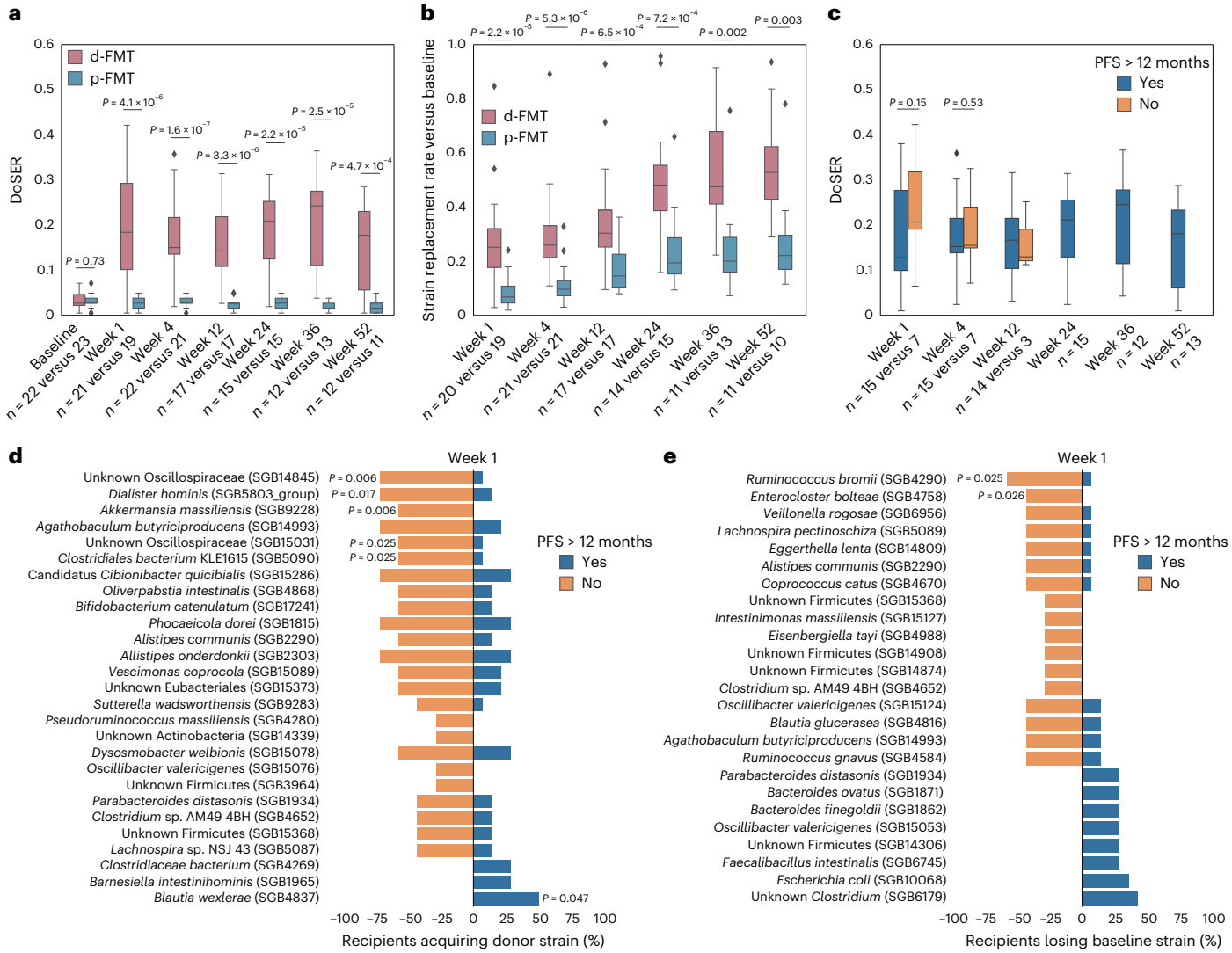

Fig. 4 | High microbiological success of the d-FMT treatment and links between strain-level modifications and the primary outcome. a, Box plots with the DoSER (Methods) comparing each arm at each follow-up sample collection (week 1, week 4, week 12, week 24, week 36 and week 52). The two-sided Mann–Whitney test was applied to assess statistical significance. **b**, Box plots with the donor strain replacement rate (Methods) with respect to the baseline comparing each arm at each follow-up sample collection. The two-sided Mann–Whitney test was applied to assess statistical significance. **c**, Box plots with the DoSER in the d-FMT arm comparing patients with or without PFS > 12 months at each follow-up sample collection. The two-sided Mann–Whitney test was applied to assess statistical significance only at week 1 and week 4 follow-ups. Across all box plots, box edges represent the lower and upper quartiles, the center line indicates the median, and whiskers extend to the most extreme data point that is no farther than 1.5 times the IQR. **d**, Bar plots with the percentage of patients in the d-FMT arm at week 1 follow-up acquiring the donor strain of each donor species according to presence (blue) or absence (orange) of PFS > 12 months. Species are ordered by the prevalence difference between groups (no − yes), with only species differing by at least 25% between groups depicted. A two-sided Fisher's exact test was applied to assess statistical significance, with only *P* values reaching significance ($P < 0.05$) shown. **e**, Bar plots with the percentage of patients in the d-FMT arm at week 1 follow-up acquiring the donor strain of each donor species according to presence (blue) or absence (orange) of PFS > 12 months. Species are ordered by the prevalence difference between groups (no − yes), with only species differing by at least 25% between groups depicted. A two-sided Fisher's exact test was applied to assess statistical significance, with only *P* values reaching significance ($P < 0.05$) shown.

(8%) in the p-FMT arm and in none of the patients in the d-FMT arm. Five patients (20%) in the p-FMT arm and 13 patients (52%) in the d-FMT arm experienced partial response. The disease was stable in 11 patients in the p-FMT arm (44%) and in nine patients in the d-FMT arm (36%), respectively, whereas two patients (8%) in the d-FMT arm and seven patients (28%) in the p-FMT arm had disease progression as best response.

Post hoc analyses

As a post hoc analysis, we evaluated efficacy outcomes in patients with intermediate-prognosis or poor-prognosis disease based on IMDC classification, who typically benefit from pembrolizumab + axitinib combination more than those with favorable-prognosis disease[1]. In this subpopulation, differences between the two treatment groups became larger in all evaluated efficacy outcomes. The median PFS was 18.8 months in the 16 patients of the d-FMT arm and 5.1 months in the 15 patients of the p-FMT arm ($P = 0.033$; Fig. 5a). The 12-month PFS was achieved by 10 of 16 patients (63%) in the d-FMT arm and by four of 15 patients (27%) in the p-FMT arm ($P = 0.045$). The median overall survival was 41.0 months in the d-FMT arm, similar to the overall population, versus 21.5 months in the p-FMT arm ($P = 0.164$; Fig. 5b). The ORR in this subset was 50% (8/16 patients, 95% CI: 0.28–0.72) in the d-FMT arm and 8% (1/13 patients, 95% CI: 0.0–0.35) in the p-FMT arm.

Discussion

To our knowledge, the TACITO trial is the first RCT to investigate the effect of targeted FMT in patients with mRCC undergoing first-line

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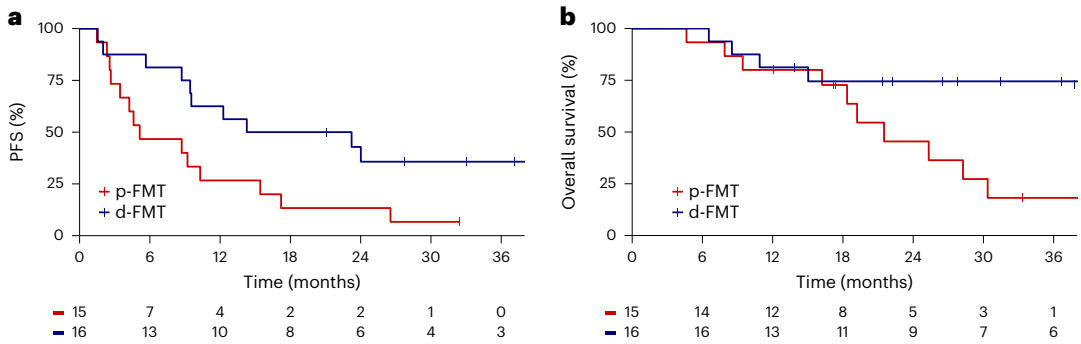

Fig. 5 | Efficacy outcomes in the IMDC-based intermediate-prognosis or poor-prognosis disease population. a, Median PFS. The median PFS was 18.8 months in the d-FMT arm and 5.1 months in the p-FMT arm ($P = 0.033$). **b**, Median overall survival. The median overall survival was 41 months in the d-FMT arm, similar to the overall population, versus 21.5 months in the p-FMT arm ($P = 0.164$). The two-sided Breslow test was applied to assess statistical significance.

combination therapy with pembrolizumab + axitinib as well as the first study to evaluate PFS and overall survival as clinical endpoints, with several intriguing findings.

First, donor FMT was superior to placebo in significantly improving the median PFS, with a considerable difference in its duration (24.0 months in the d-FMT arm versus 9.0 months in the p-FMT arm; hazard ratio = 0.50, $P = 0.035$). However, when evaluating the 12-month PFS (the primary outcome), the statistical difference between arms was only close to the pre-set significance threshold (70% versus 41% for d-FMT versus p-FMT, respectively, $P = 0.053$). This difference may suggest that stronger effects of donor FMT might be appreciated in a longer run than 1 year, both for clinical reasons (because the main cancer therapy in the control arm remains active, effects of FMT on PFS are expected to be more impactful after its median value) and for microbiological reasons (the donor microbiome may exert its effect over 12 months, especially in those patients who received all treatments).

We observed a notable but non-significant difference in the overall survival between the two groups (41 months in the d-FMT arm versus 28.3 months in the p-FMT arm). We are continuing to follow-up our cohort to confirm this hypothesis. Finally, we observed a 52% response in the d-FMT arm compared with 32% in the p-FMT arm. In the control arm, response rate was lower than previously reported for this combination in the KEYNOTE-426 phase 3 trial[1], which may be related to the inclusion of patients with non-clear cell histology as well as the evaluation of the PFS from the point of randomization during the study.

The post hoc analysis of patients with intermediate or poor prognosis based on IMDC criteria showed stronger results of d-FMT compared with p-FMT.

First, for the primary outcome, the absolute difference between the two arms was larger and significant for patients in this population (64% in the d-FMT arm versus 27% in the p-FMT arm, $P = 0.045$) than in the overall population. Moreover, the ORR of these patients was similar to that of the overall population in the d-FMT arm (52% versus 50%) but dropped considerably in the p-FMT arm (32% versus 8%). These results suggest that patients with intermediate or good prognosis at baseline might benefit more from donor FMT as an add-on to cancer immunotherapy. Further studies on this specific subgroup are warranted. Notably, as already observed in studies coming from our center and other groups[15–21], donor FMT is safe, with no FMT-related SAEs reported in the study period.

These clinical results were associated with interesting microbiological findings.

First, we observed a significant increase in both α-diversity and β-diversity in the d-FMT arm compared with the p-FMT arm after treatments (secondary outcomes of our study). These findings, together with the finding that the DoSER was consistently higher in patients receiving donor FMT than in the p-FMT group throughout the whole study period, confirm that the donor FMT was microbiologically successful in our study.

After assessing the microbiological validity of our intervention, we investigated potential associations between posttreatment microbiome changes and clinical results. Neither α-diversity and β-diversity nor the DoSER was associated with 12-month PFS, suggesting that qualitative rather than quantitative dynamics of donor FMT might be relevant in determining clinical outcomes.

Then, we found that immediate post-FMT (week 1 follow-up) acquisition of the *B. wexlerae* donor strain was significantly associated with 12-month PFS. *B. wexlerae* is a beneficial anarerobic bacterium, which produces short-chain fatty acids (SCFAs), already associated with response to neoadjuvant chemotherapy in patients with rectal cancer[22]. Moreover, its oral administration had anti-inflammatory effects and ameliorated obesity and diabetes in mice[23].

On the other hand, we found an inverse association between the engraftment of *A. massiliensis* (SGB9228) and the primary outcome. This finding may have several explanations.

A. massiliensis is a novel species defined from the recent proposed reclassification of *A. muciniphila*. Previous studies found an association between the abundance of *A. muciniphila* and response to ICIs[24–26], but deeper analyses support this association only for *A. muciniphila sensu stricto* rather than for *A. massiliensis* (SGB9228)[27]. Notably, an overabundance of *A. muciniphila* was previously associated with resistance to ICIs and was highlighted as an indirect sign of dysbiosis (as observed in patients with a history of antibiotic exposure)[24,27]. Moreover, direct experimental evidence shows that *Akkermansia* strains can be hardly replaced by other external *Akkermansia* strains, due to their high genomic similarity[28]. Therefore, patients with adequate baseline abundance of *A. muciniphila* could have not been colonized by *A. massiliensis*, and the combination of these beneficial strains and of the donor strains could have contributed to the clinical success of the d-FMT. Overall, these results support further investigations.

Moreover, we observed that the d-FMT group experienced over time not only a significantly higher acquisition of new species but also a significantly higher loss of baseline species compared with the p-FMT group. We also found a borderline significant association between the proportion of species immediately lost after treatment (week 1 follow-up) and the 12-month PFS in the d-FMT group but not in the p-FMT group. We found that the loss of an *E. coli* strain (SGB10068) in recipients was associated with PFS > 12 months, whereas the inverse association was found for an *R. bromii* strain (SGB4290). These results might be explained by the well-known inflammatory features of several *E. coli* strains[29] and by the ability of *R. bromii* to hydrolize resistant starch and to support, consequently, the growth of beneficial SCFA-producing bacteria[30]. Although the detected loss can also be the effect of falling below the level of detection as a consequence of

relative abundance increase of other species, this finding suggests an effect of donor FMT in displacing, or at least depleting, originally present patient species besides the more expected enrichment with donor microbiome components. Also here, further investigations on the taxonomy and functional potential of these microbes are advocated.

Notably, we did not use antibiotic preconditioning, as this trial was started before our discovery of its association with microbiome engraftment[31] and the publication of relevant studies[7,8]. Although we did not observe any association between quantitative engraftment and clinical response, we acknowledge that the engraftment rate immediately after donor FMT was 18%, which is much lower than that observed in patients with *Clostridioides difficile* infection (where FMT is mostly successful), who usually undergo an antibiotic preconditioning before FMT and have a history of previous antibiotic therapies. In addition, it remains unclear whether healthy donor FMT or patient donor FMT (as used here) is the best approach.

This study has some limitations. The sample size was relatively small, with only 45 patients evaluated for the primary endpoint. Some baseline characteristics could not be completely well balanced due to the reduced sample size. Finally, most stool aliquots were provided by a single donor, whose microbiome was rich in Bifidobacteria, *A. massiliensis* (associated with PFS < 12 months if acquired) and *R. bromii* (the loss of which in patients was associated with negative response). We also identified other donors, but they were not willing to donate for logistical reasons. Although there are advantages to relying on a single donor, the lack of immediate stool availability led to delays in treatment for nine patients. Although the effects of this protocol deviation are unclear, findings from the per-protocol sensitivity analysis suggest that timely provision of donor FMT might be associated with improved clinical outcomes. The recruitment of stool donors is a critical step for an academic FMT center[32], and the difficulties in its consistent fulfillment is one of the factors that limit the testing of FMT worldwide[33]. The investigation of further approaches to microbiome modulation, such as microbiome consortia/live biotherapeutic products, could overcome the issue of stool shortage and is also supported by the promising results coming from already available studies[34–36].

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-025-04189-2.

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Methods

Study design and approvals

The TACITO trial was an investigator-initiated, randomized, double-blind, placebo-controlled phase 2a clinical trial that aimed to evaluate whether donor FMT from complete ICI responders is effective in improving response to combined first-line therapy with pembrolizumab and axitinib in patients with mRCC.

The study was conducted in accordance with the Declaration of Helsinki and International Conference on the Harmonization of Good Clinical Practice guidelines as well as in compliance with local and institutional regulations. The study was approved by the institutional review board (IRB)/local ethics committee (ID: 2664) and was prospectively registered at ClinicalTrials.gov (registration identifier: NCT04758507, registration date: 11 February 2021). All enrolled patients gave their written informed consent to participate in the study. This study was conducted by following CONSORT guidelines[37], and a CONSORT checklist is provided in Supplementary Table 1.

The full study protocol is available in the Supplementary Information file.

Study population

Patients were enrolled in the Fondazione Policlinico Gemelli IRCCS, a tertiary care center and academic hospital in Rome, Italy, and were either screened from our oncology outpatient clinic or referred to our center from seven other Italian hospitals, all referral centers for RCC. The inclusion criteria were as follows: histologically confirmed RCC; metastatic disease; radiological assessment within 8 weeks before enrollment; patient eligible for therapy with ICIs for mRCC or started within 8 weeks; ability to provide written informed consent; and ability to be compliant with the scheduled procedures.

We excluded patients for the following reasons: major comorbidities; concomitant gastrointestinal or autoimmune disorders or HIV, HBV or HCV infection; continuative corticosteroid therapy; previous treatment with systemic immunosuppressants or immunomodulatory drugs; and antibiotic therapy within 4 weeks prior to enrollment.

Predefined withdrawal criteria before the start of treatments (checked at the pretreatment evaluation visit, described below) were as follows: voluntary withdrawal of the patient; absence of the planned eligibility criteria at the pretreatment evaluation visit; and actual provision of the first treatment (FMT or placebo) later than 8 weeks from the start of ICI therapy. We predefined this criterion as a late provision of the first treatment that could affect the efficacy of FMT. The delay of further treatments was not considered a criterion to withdraw patients from the study as the donor microbiome can engraft the recipient gut up to 6 months, based on available evidence[38]. Patients who experienced a disease progression during the study procedures were discontinued from further treatments. Moreover, patients who withdrew their consent to participate in the study at any time were discontinued from the trial.

The last enrolled patient received his first treatment on 16 December 2023 and had his last follow-up visit on 5 December 2024.

Two of the 25 patients randomized in the d-FMT arm did not receive the first treatment as the timespan between the scheduled procedure and the start of their cancer therapy was longer than 8 weeks due to unavailability of donor stools. Three of the 25 patients randomized in the p-FMT arm did not receive the first treatment for the following reasons: one patient withdrew consent before the scheduled procedure; one patient had no metastatic disease at internal reevaluation of the baseline computed tomography scan; and one patient was not treated within 8 weeks from the start of cancer therapy, because of his logistical unavailability to reach our center.

Analysis sets

ITT set. This set was used for sensitivity analyses and included all randomized patients, regardless of study treatment.

FAS. This set was used for efficacy analyses (primary and secondary efficacy outcomes) and included all randomized patients who received at least the first treatment (regardless of completion status).

Per-protocol set. This set was used for sensitivity analyses and included all randomized patients who received all assigned treatments for which they were eligible (so, unless disease progression, consent withrawal or other conditions for study discontinuation occurred) within the planned schedule (regardless of their number), without delays.

Safety set. This set includes all randomized patients, regardless of study treatment. Only the TRAEs and the adverse events of special interest, which included gastrontestinal toxicities, have been collected prospectively. Incidence of treatment interruption or discontinuation related to the axitinib and/or pembrolizumab as well as the incidence of grade 3–5 adverse events in each treatment arm have been collected retrospectively.

Moreover, we conducted a post hoc analysis by excluding patients with favorable-prognosis disease based on the IMDC score.

Study endpoints

The primary efficacy endpoint was 12-month PFS, defined as the number of participants free from tumor progression, as assessed by Response Evaluation Criteria in Solids Tumors (RECIST) version 1.1, 12 months after randomization[39], in the FAS population.

Secondary efficacy endpoints were median PFS, median overall survival and ORR in the FAS population, safety and microbiome changes after treatments.

Safety was evaluated in the per-protocol and FAS populations.

PFS and ORR were determined using RECIST version 1.1, as assessed by local investigators. PFS was defined as the time from randomization until disease progression or death, whichever occurred first. Overall survival was evaluated from the date of randomization to death from any cause or the last contact.

ORR was defined as the proportion of patients with a partial or complete response.

Safety was assessed as the incidence, nature and severity of TRAEs, recorded and classified according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Microbiome changes after treatments were defined as the number of participants with significant increase in α-diversity (assessed by Shannon index) and β-diversity (assessed by Bray–Curtis dissimilarity) of their gut microbiota after treatments, compared with baseline.

Study procedures

Screening, baseline and pretreatment assessments. Screening, baseline and pretreatment assessments were all conducted in combination by physicians from the Gastroenterology Unit and from the Oncology Unit.

During the screening visit, after a thorough discussion of the study details with the investigators, potentially eligible patients who were willing to participate in the study signed the informed consent. Then, we evaluated the patient and reviewed inclusion and exclusion criteria to ensure the eligibility of the patient for the trial.

The baseline assessment included the collection of patient medical history, including the following data: age, gender, date of cancer diagnosis, nephrectomy status and date, histology, Fuhrman grade, diagnosis of metastatic disease and site of metastases, Karnofsky scale, IMDC score, complete blood count and starting date of cancer therapy. Moreover, all patients underwent a full clinical examination. At the end of the baseline visit, enrolled patients were randomized to study treatments.

The pretreatment assessment occurred within 2–5 days before the first scheduled experimental procedure (FMT or placebo). Here, investigators reviewed the eligibility criteria to check their actual validity as well as the time distance between the start of ICI therapy and the first treatment (to be no longer than 8 weeks).

Selection of stool donors. Stool donors were selected within the historical cohort of our Oncology Unit among patients with advanced RCC who had experienced a complete response to PD-1 inhibitors (pembrolizumab or nivolumab).

Once identified, available patients from this cohort were screened according to protocols recommended by international guidelines, based on a multilevel framework[32,40,41]. First, donor candidates underwent a specific questionnaire aimed at addressing the following: known history or lifestyle-related risk factors for potentially communicable diseases (for example, drug addiction or promiscuous sexual behavior); recent (<6 months) use of specific drugs (for example, antibiotics); a family history of gastrointestinal cancer or inflammatory bowel disease; systemic diseases; and the use of drugs that could be excreted in feces with potential risk for the recipients. Subsequently, selected patients underwent blood and stool examinations to exclude potentially transmittable diseases. Candidates who passed these two steps were qualified as eligible for donating and repeated this screening procedure every 2 months[32,40,41]. At the time of each donation, the chosen patients underwent a further questionnaire to screen for any recent acute digestive disease, newly contracted infectious diseases or other potentially harmful situations (for example, risky sexual contacts) and a nasopharyngeal swab for SARS-CoV-2 (after March 2020)[32,40,41]. Moreover, each stool donation provided by qualified donors underwent direct stool testing, which included a culture assay for detection of multidrug-resistant bacteria, a rapid molecular assay for common intestinal pathogens (Seegene, RT–PCR Allplex Gastrointestinal Panel Assay) and a stool molecular assay for SARS-CoV2 (since March 2020)[32,40,41]. An extended description of the donor screening is available in the study protocol. Selected donors were also asked to provide stool samples for microbiome analysis every 6 months.

Five patients with long-term complete response to ICI were identified as candidate donors.

Three donors did not pass the screening process (two for chronic use of proton pump inhibitors and another one for history of previous colorectal surgery for diverticulitis), whereas two of them were eligible to donate.

Donor 1 was a 57-year-old man who underwent radical nephrectomy and retrocaval lymph node resection for a clear cell RCC at original pT3a pN1 M0 disease stage. After 4 months, for disease recurrence with more than 60 bilateral pulmonary metastases (the largest of 14 mm in size), he started first-line systemic therapy with nivolumab + ipilimumab, achieving a complete response that is still lasting after 6 years of therapy (that is, May 2025). He provided the vast majority (n = 56/57, 98%) of stool aliquots. The other donor who passed the screening process (donor 2) was a 52-year-old woman who developed lung and pancreatic metastases 2 years after nephrectomy for a clear cell RCC (pT2, grade 2) and received third-line therapy with nivolumab after disease progression to sunitinib for 3 years and everolimus for subsequent 3 years. Nivolumab led to a complete disease response, and, as of today (that is, May 2025, 9 years after), she is still under therapy with no evidence of target lesions.

Manufacturing of fecal aliquots and FMT capsules. For each donation, feces was collected by the donor in the morning and rapidly transported to our hospital in a refrigerated bag (within 4 hours from defecation). After the completion of the questionnaire already described, stool donations were brought to the FMT laboratory of our center (Microbiology Unit) for direct stool testing. Immediately after this step, they were manufactured as fecal aliquots for colonoscopic FMT and/or as FMT capsules, based on specific needs related to planned treatments, following recommendations by international guidelines[32,40,41]. All manufactured aliquots or capsules were quarantined for 24–48 hours while waiting for results of the direct stool testing, and positive donations were then discarded. Also, stool donations less than 60 g were considered insufficient to prepare a stool aliquot or a capsule set and were discarded[42]. All procedures were performed within a biological safety cabinet (Biosafety Level 2). Using specific strainer bags (Seward), the fecal material was first diluted with nearly 200 ml of sterile saline (0.9%) for fecal aliquots and nearly 100 ml of sterile saline for FMT capsules, and glycerol (Monico S.p.A.) was added up to a final concentration of 10% to cryopreserve bacteria at −80 °C. Then, this suspension was homogenized automatically through a Stomacher 400 homogenizer (Seward), which allowed a simultaneous filtration of the solution. The homogenization program chosen was 260 rev min^{-1} for 1 minute[43]. Further filtration and purification of the fecal suspension were performed using sterile gauzes and a funnel. The deriving solution was blended and, after the supernatant was strained, transferred into a sterile flask.

For the manufacturing of colonoscopic FMT aliquots, the fecal suspension was divided, in ready-to-use aliquots of at least 60 g and nearly 200 ml, as previously described[43], and aliquots were frozen at −80 °C up to 6 months. Eligible aliquots were thawed in a warm (37 °C) water bath on the day of fecal infusion.

For the manufacturing of FMT capsules, the fecal suspension was poured into a sterile container ready for capsule production. Specifically, size 1 capsules were filled with 0.420 ml of fecal suspension and, then, encapsulated with size 00 capsules (Farmalabor), with nearly 150 capsules for each stool donation[44,45]. Capsules were quickly stored at −80 °C, in plastic boxes, for up to 6 months.

Each suspension bottle or capsule box was labeled with a unique barcode of the corresponding donor, and with the date of collection/manipulation, to assure its complete traceability, as recommended by international guidelines[40,41]. The personnel involved in the manufacturing of FMT were not involved in any part of the clinical trial and had no contact with patients.

Colonoscopic FMT. All colonoscopy procedures were performed in the morning, under sedation. Before colonoscopy, patients in both groups underwent bowel cleansing with 4 l of Macrogol (SELG ESSE) the day before the procedure. All procedures were performed by two expert endoscopists (G.I. and G.C.) using pediatric colonoscopes and carbon dioxide insufflation. Both fecal aliquots and placebo aliquots were delivered through the operative channel of the scope after reaching the cecum or the more proximal point of the large bowel, using 50-ml syringes filled with the infusate during colonoscopy. After the procedures, patients were monitored in the recovery room of our endoscopy center for nearly 3 hours. Patients were requested to fast for at least 6 hours after the procedure and were allowed to have a light meal thereafter.

Capsulized FMT. Patients were scheduled to receive capsulized FMT, respectively, 12 weeks (±2) and 24 weeks (±2) after the first treatment. Delay in receiving the scheduled treatments longer than 2 weeks was considered a protocol deviation. Patients received a styrofoam box, filled with dry ice and 10 capsule containers. Each container included 15 capsules. Patients were instructed to put capsule containers at −20 °C (the home freezer) and to take five capsules three times a day (at least 1 hour after a meal) for 10 days, for a total of 150 capsules. Originally, we planned to give 120 capsules for each treatment cycle, but we slightly increased the number of capsules as FMT capsules needed to be filled with a slightly lower quantity of fecal material to avoid them becoming damp and then potentially identifiable by the participants. Patients were asked to report the capsule assumption and to bring back capsule containers after the end of therapy, to monitor the assumption of capsules.

Follow-up assessments. Follow-up visits were performed in combination by physicians from the Gastroenterology Unit and from the Oncology Unit. Follow-up visits were scheduled at week 1, week 4, week 12, week 24 and week 52 after the first treatment, respectively. At each

follow-up visit, the investigators evaluated the efficacy and safety of treatments, as described in the 'Study endpoints' subsection. Dates and status of disease progression, as well as date and type of best response, were recorded. Unscheduled follow-up visits were offered if requested by the patients.

Stool collection, DNA extraction and shotgun metagenomic sequencing

Samples were collected during the pretreatment visits and at the planned follow-up visits by using a stool collector with a DNA/RNA Shield buffer (Zymo Research), brought directly by patients to our center in a refrigerated box within 6 hours from collection and then stored at −80 °C for up to 36 months before being shipped in dry ice to the next-generation sequencing facility of the Department of Cellular, Computational and Integrative Biology of the University of Trento (Trento, Italy) for DNA extraction and sequencing. DNA extraction consisted of sample homogenization followed by DNA isolation with the PowerSoil Pro DNA Isolation Kit (Qiagen). Metagenomic sequencing libraries were prepared with the Nextera DNA Library Preparation Kit (Illumina) and sequenced on the Illumina NovaSeq 6000 platform with a target depth of 7.5 Gbp for patient samples (n = 247) and 15 Gbp for donor samples (n = 9).

Metagenome preprocessing and microbiome profiling

Metagenomes underwent quality control with our previously validated preprocessing pipeline (available at https://github.com/SegataLab/preprocessing). This consisted of removal of low-quality reads (Q < 20 or Q > 2 ambiguous bases or <75 bp) with TrimGalore (version 0.6.6) and removal of reads mapping against the human genome (hg19) or the Illumina spike-in phiX 174 as identified with Bowtie 2 (version 2.3.4.3). Remaining reads were sorted and split to create standard forward, reverse and unpaired quality-controlled FASTQ files for each metagenome. After this quality control, one metagenome with <3 Gbp was discarded, with the vast majority of the remaining ones (98.8%) having >7.5 Gbp (approximately 50 million reads). These were then used as input in MetaPhlAn 4 (version 4.1)[11,12] using the marker genes database vJun23_202307 to generate taxonomic profiles at species-level genome bin (SGB) resolution for each sample.

Assessment of donor strain engraftment

DoSER was defined as the number of strains (that is, specific genomic variants within species that can be profiled at the single-nucleotide level from metagenomes) of the donor that are present in each recipient's sample after the FMT. In the case of d-FMT samples of individuals who had experienced donations from multiple aliquots, the pool of strains present in any of the received aliquots is considered in this estimation. For example, a DoSER of 50% in a sample means that half of the strains that are detectable in the administered donor's fecal samples were also observed in that specific post-FMT recipient sample. As the fraction of a patient's microbiome strain pool is also present in a different patient's microbiome, the DoSER inference can be applied to any pair of samples, including samples from unrelated individuals not linked by FMT. The DoSER on unrelated individuals (for example, the baseline of the d-FMT group) is used here to assess the baseline strain sharing in a target population (as some strains might be clonally spreading in a population, and there can be direct or indirect social interactions within populations leading to strain transmission[46]); it can be applied also in the case of p-FMT patients to verify the absence of a donor's effects in this arm. To detect engraftment of donor strains, we rely on our strain-sharing inference method previously reported[31] plus recent improvements to the pipeline[46,47]. In brief, this consists of building strain-level phylogenies for all SGBs detected in the aforementioned taxonomic profiles using StrainPhlAn 4 (version 4.1)[11,12]. Those phylogenetic trees depict the relationship between conspecific strains detected in each sample of our cohort, with their phylogenetic distance being able to provide information on whether the same strain is observed in two samples. To reliably identify those strain-sharing events, we define SGB-specific strain boundaries. This is done by augmenting the phylogenetic trees with strains from publicly available longitudinal metagenomic datasets (n = 16 datasets including 4,322 stool metagenomes from 1,334 healthy individuals[48–64]) and by leveraging previous observations that, in close longitudinal samples (<6 months apart) of the same individual both having a given SGB, the same strain of that SGB will likely be observed, whereas, in unrelated (for example, from different cohorts/populations) samples having the same SGB, different strains of that SGB will likely be observed. That considered, the SGB-specific strain boundary (or strain-sharing threshold) is defined as the threshold that best separates pairwise phylogenetic distance distributions from longitudinal samples from those of unrelated samples, following criteria defined previously[46]. After this step, a strain-sharing matrix indicating how many strains are shared between each pair of samples in our cohort is generated. This is used to compute the DoSER, defined as the number of donor strains detected in the patients (considering all donor samples administered to the recipient up until that point) over the total number of SGBs typed at strain level in the donor. To allow comparison of the DoSER between arms, the main donor baseline aliquot administered to the d-FMT arm is used as reference for the p-FMT arm. The strain replacement rate was defined as the number of different strains profiled in longitudinal samples of the same individual over the common SGBs typed at strain level in both samples.

Randomization and masking

An online random number generator software (https://www.sealedenvelope.com) was used to provide random permuted blocks with a block size of four and an equal allocation ratio; the sequence was hidden until the interventions were assigned. Blocked randomization of patients was performed by an external individual not involved in the study.

To mask treatments to recipients, both infusate bottles and syringes were covered with dark-colored paper before the infusion, and the patients were sedated. Placebo capsules were made of cellulose and were identical in appearance to the FMT capsules, to ensure the blinding of patients and study staff. Moreover, both placebo suspension bottles and placebo capsule boxes were labeled with similar barcodes as FMT ones. Physicians who evaluated patients at follow-up (clinical outcome assessors) were blinded to administered treatments, as were authors involved in outcome analysis.

Sample size

Sample size calculation was based on the hypothesis of the superiority of FMT + standard of care (SOC) over SOC alone. The 1-year PFS rate for SOC has been reported to be nearly 60%. The alternative hypothesis is that FMT can improve the 1-year PFS rate from 60% to 80% when associated with SOC. A total of 50 patients is required to enter this two-treatment, parallel-design study. The probability is 80% that the study detects a treatment difference at a one-sided 5.0% significance level, if the true hazard ratio is 0.436. This is based on the assumption that the accrual period will be 36 months, the follow-up period will be 36 months and the median survival is 15.1 months. The total number of events is expected to be 36.

Statistical analysis

Data were summarized as median and range when referred to quantitative variables and as absolute counts and percentages if related to categorical items and reported according to the randomized arm. Survival curves were estimated with the Kaplan–Meier method and compared with the Breslow test. We chose this test as it gives more relevance to initial events. In our study, early disease progression would be more important than late disease progression, as the patient who experiences progression is not allowed to receive further FMTs,

which are expected to strengthen the donor effect. Therefore, early events would be more important than late events in reducing the global efficacy of the donor FMT.

Median survival times were reported with their 95% CIs. A Cox proportional hazard model was implemented after checking the proportionality assumptions, and the hazard ratio with its 90% CI was presented. Differences between proportions were assessed with the chi-square test.

A non-parametric approach was followed to analyze microbiome features based on Wilcoxon signed-rank or Mann–Whitney test when applied to quantitative variables either paired or unpaired, respectively, and Fisher's exact test for categorical items. The PERMANOVA test evaluates whether the average microbiome structure or the compositional variability differs significantly between groups[65]. This test was applied on Bray–Curtis dissimilarity matrices to evaluate compositional microbiome differences between arms at baseline.

IBM-SPSS version 28.0 statistical software, GraphPad Prism version 10, R version 4.4.2 ('survival' and 'survminer' packages) and Python version 3.10.12 ('scikit-bio' and 'scipy' packages) were used for the analyses.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The shotgun metagenomic data generated in this study are available at the European Nucleotide Archive under accession number PRJEB94043. All requests for data access should be sent to the corresponding author, G.I. The minimum dataset, without individual patient data, used for the primary, secondary and post hoc analyses, may be shared under a data use agreement for IRB-approved research. Requests will be considered and responded to within 2 months of receipt. Patient-related data not included in the paper were generated as part of a clinical trial and are subject to patient confidentiality.

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Author contributions

G.I. conceived the study. G.I., R.I. and N.S. designed the study. G.I. and R.I. supervised the conduct of the clinical trial. N.S. supervised the microbiome analyses. S.P., C.C., A.S., D.A. and R.I. carried out the study visits. S.P. carried out the clinical screening of donors. D.R. coordinated the organization of patient visits, the screening of donors, the collection of stool donations and the collection of stool samples. G.Q. and L.M. carried out the laboratory screening of donors and manufactured donor and placebo aliquots and capsules. C.C., D.A., S.B., G.F., F. Primi, L.S., G.C.G., A.D., J.R.G.B., G.S. and R.I. recruited the study patients. G.C. and G.I. performed the colonoscopic procedures. S.P. managed the capsulized FMT procedures. V.H., D.G., R.I. and G.I. performed the statistical analyses. F. Pinto and F. Armanini managed the stool samples and DNA extraction and microbiome sequencing. V.H., M.P., T.B.B., G.P. and F. Asnicar performed the microbiome analyses. L.D., G.K. and L.Z. advised on taxonomy analytic strategy and biomarker discovery. S.P., C.C., V.H., G.Q., D.A., D.G., N.S., R.I. and G.I. interpreted the data and wrote the initial draft of the paper. L.D., G.K., M.S., L.M., A.G., G.T., G.C. and L.Z. contributed to the editing of the paper. All authors provided critical revision of the paper and approved the final version for submission.

Competing interests

S.P. has served as a speaker for WellMicro. G.K. has been holding research contracts with Daiichi-Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sutro, Tollys and Vascage. G.K. is on the Board of Directors of Bristol Myers Squibb Foundation France. G.K. is also a scientific co-founder of everImmune, Osasuna Therapeutics, Samsara Therapeutics and Therafast Bio; is on the scientific advisory boards of Hevolution, Institut Servier and Rejuveron Life Sciences/Centenara Labs AG; and is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis and metabolic disorders. A close family member of G.K. was an employee of Sanofi and now consults for Boehringer Ingelheim. A.G. reports personal fees for consultancy for Eisai S.r.l., 3PSolutions, Real Time Meeting, Fondazione Istituto Danone, Sinergie S.r.l. Board MRGE and Sanofi S.p.A.; personal fees for acting as a speaker for Takeda S.p.A., AbbVie and Sandoz S.p.A.; and personal fees for acting on advisory boards for VSL3 and Eisai. L.Z. is a co-founder of everImmune and its scientific advisory board president; received a research contract from Kaleido and 9 Meters/Innovate Pharma; and is sponsored by Pileje. G.C. has received personal fees for acting as an advisor for Ferring Therapeutics. G.I. has received personal fees for acting as a speaker for Biocodex and Illumina and for acting as a consultant/advisor for Ferring Therapeutics. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-025-04189-2.

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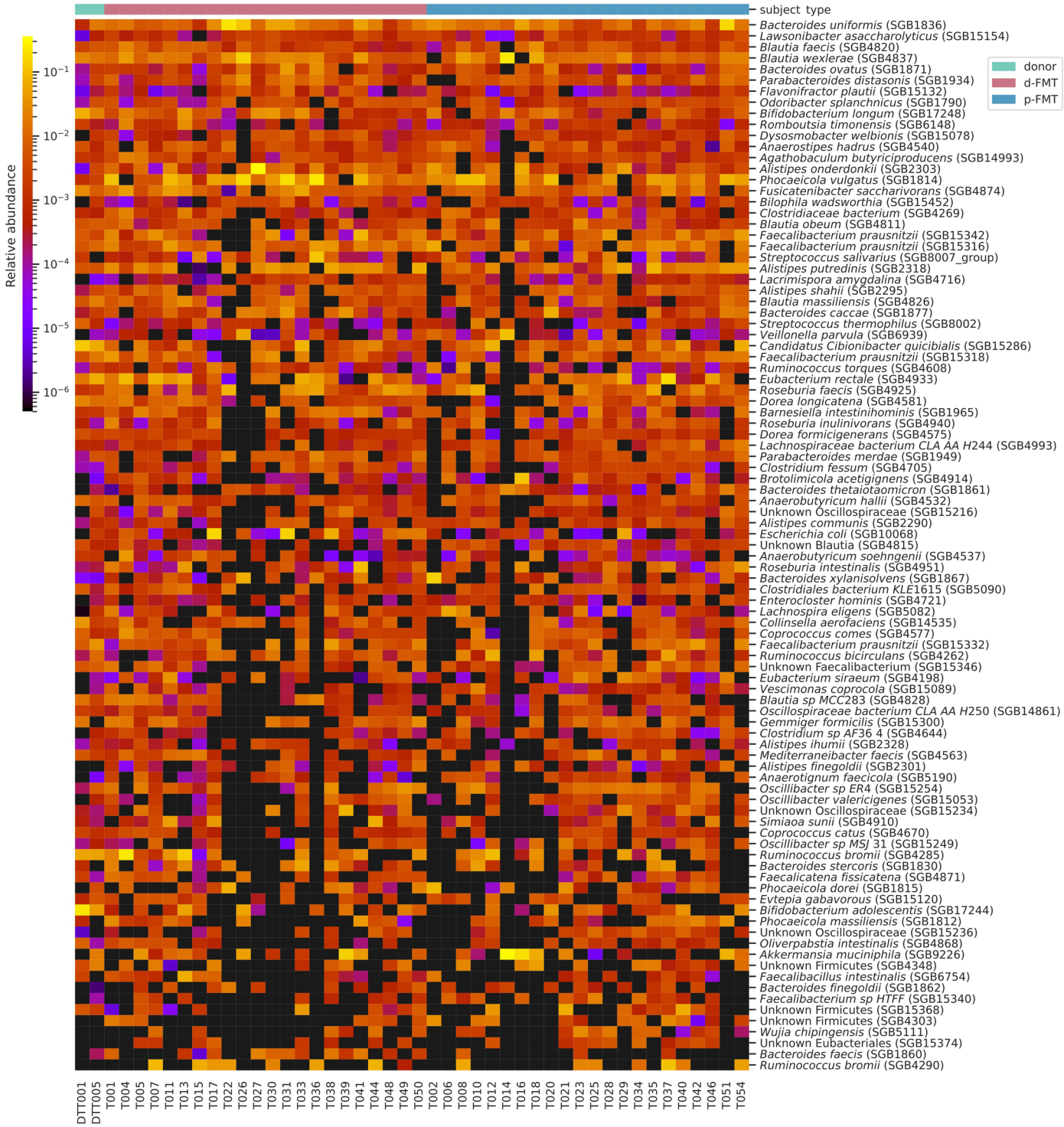

Extended Data Fig. 1 | Gut microbiome compositions at baseline. Heatmap with relative abundances of baseline patient samples. The initial donated aliquot of each donor are also shown. Species shown are the ones present at > 0.1% relative abundance in at least 30% of samples.

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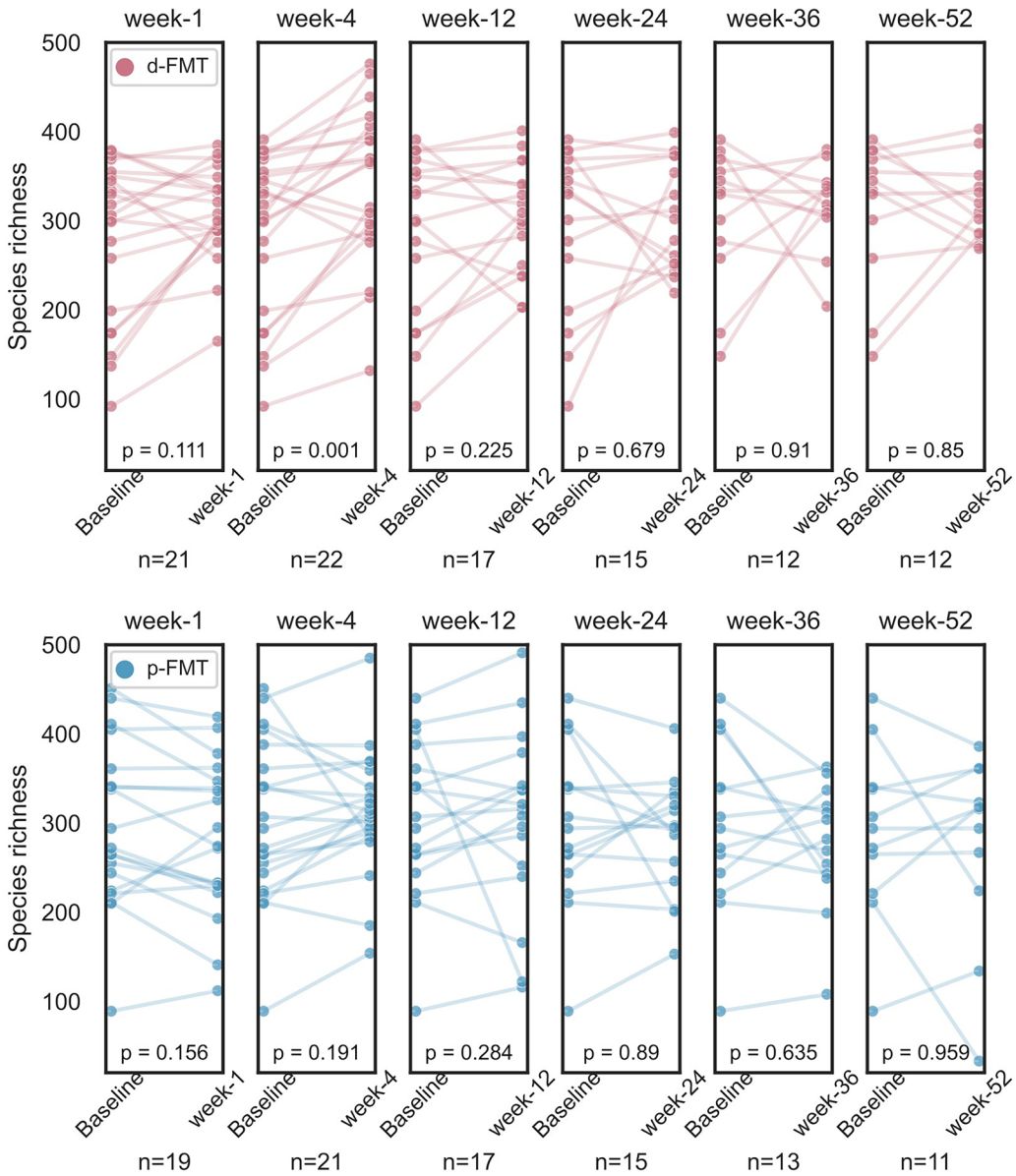

Extended Data Fig. 2 | Species richness changes following treatments. Species richness changes compared to baseline for the d-FMT arm (top panel) and p-FMT arm (bottom panel) at each follow-up sample collection (week-1, week-4, week-12, week-24, week-36, week-52). The Wilcoxon signed-rank test was applied to assess statistical significance.

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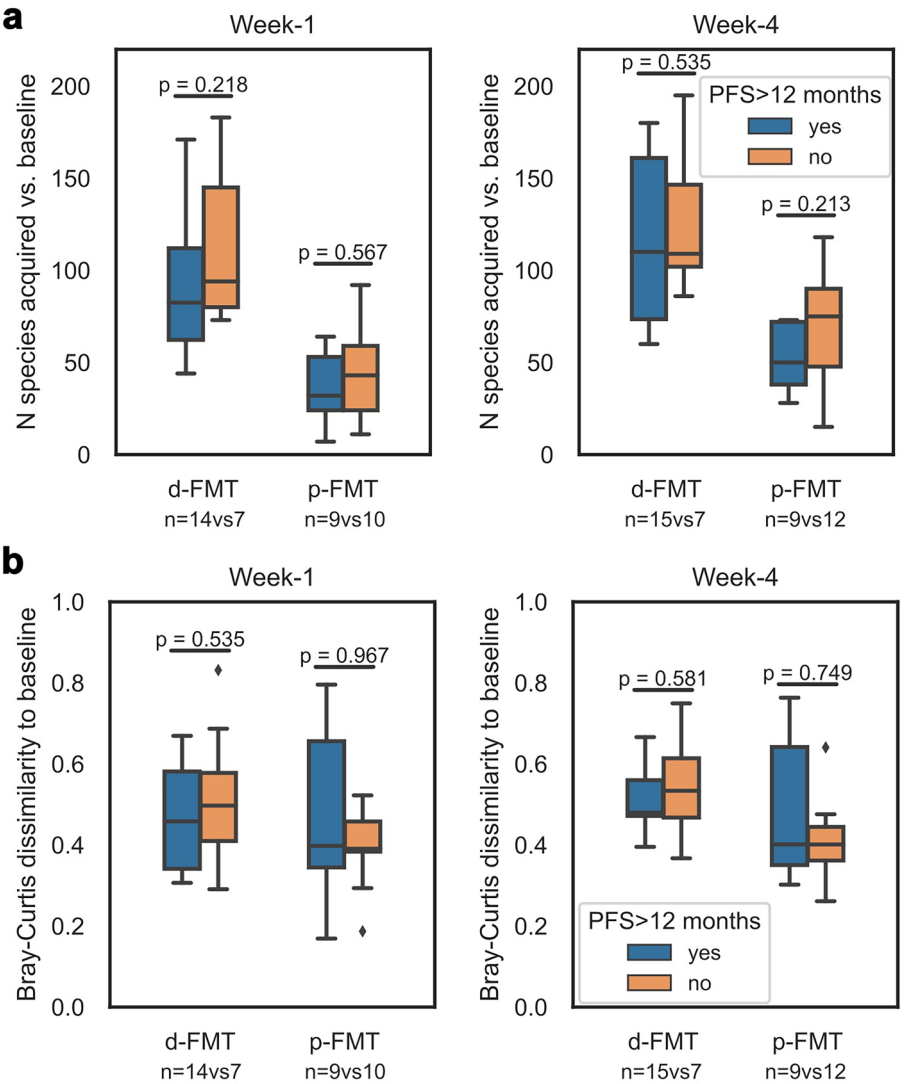

Extended Data Fig. 3 | Association between species-level microbiome changes and the primary outcome. a) Boxplots with the number of species acquired as compared to baseline at week-1 (left panel) or week-4 (right panel) for each arm comparing patients with or without PFS > 12 months. The Mann-Whitney test was applied to assess statistical significance. **b**) Boxplots with the Bray-Curtis dissimilarity to baseline at week-1 (left panel) or week-4 (right panel) for each arm comparing patients with or without PFS > 12 months. The Mann-Whitney test was applied to assess statistical significance.

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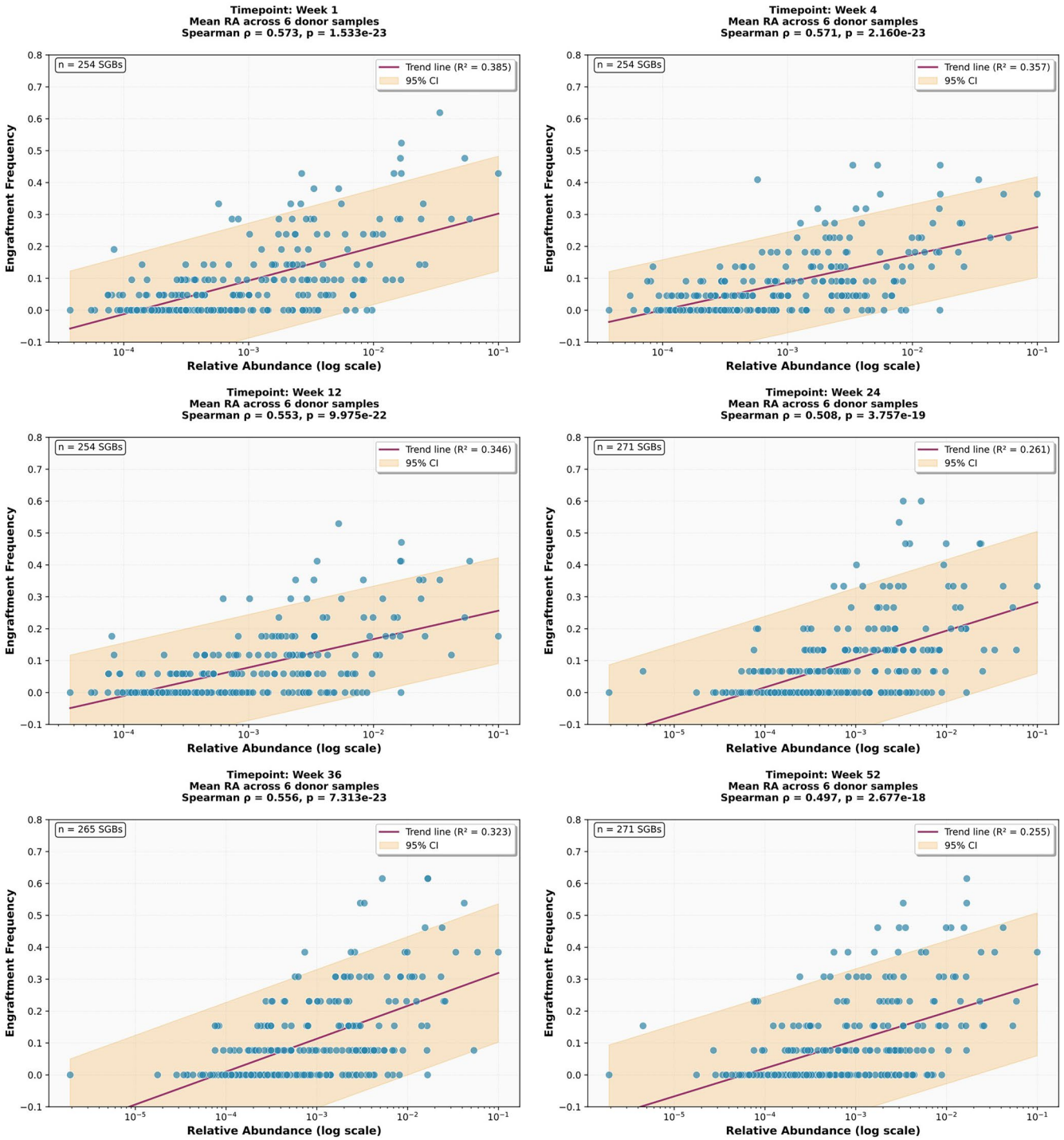

Extended Data Fig. 4 | Relationship between donor species abundance and engraftment frequency across time. Scatterplots show the association between the mean relative abundance (log scale) of gut microbiome species in donor samples (x-axis) and their strain-level engraftment frequency (y-axis) across patients in the d-FMT arm at six post-treatment follow-ups (week-1, week-4, week-12, week-24, week-36, and week-52). Engraftment frequency was calculated as the proportion of recipients in whom a given donor strain successfully engrafted. Donor species abundances are calculated as the mean relative abundances across all donor aliquots administered to recipients up to a given timepoint. Only patients receiving donations from the main donor in the trial are included in this analysis. Statistical significance was evaluated with the Spearman's rank correlation test, with trendline and 95% confidence intervals also shown.

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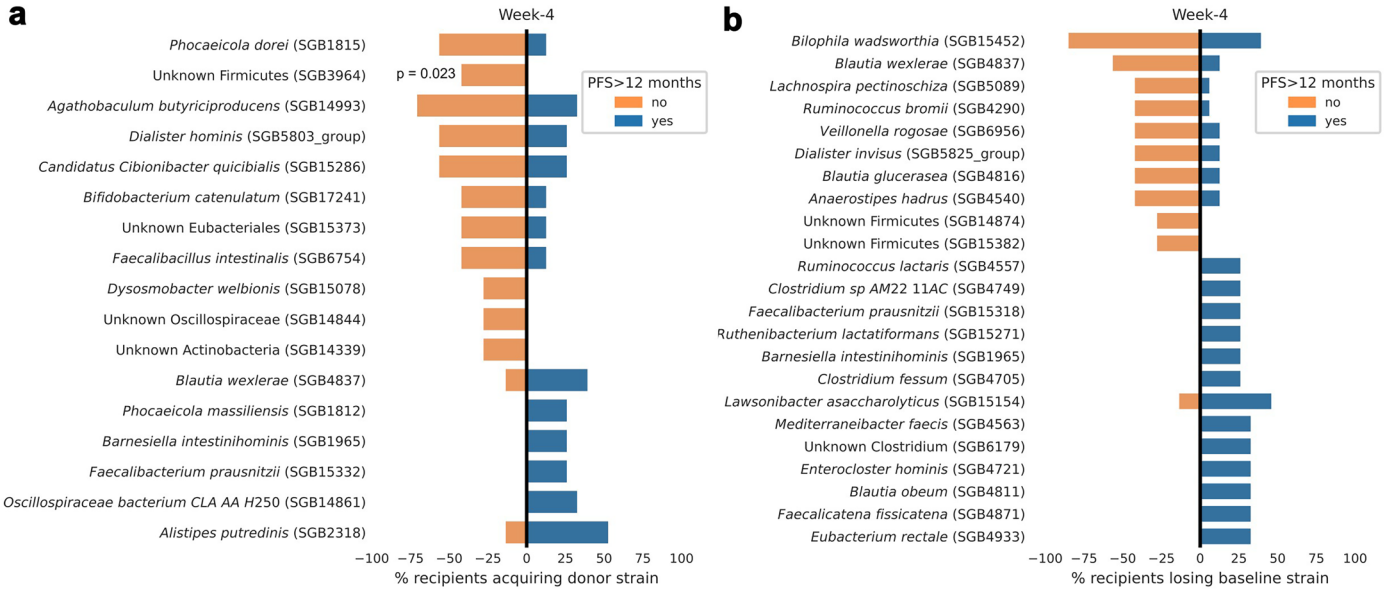
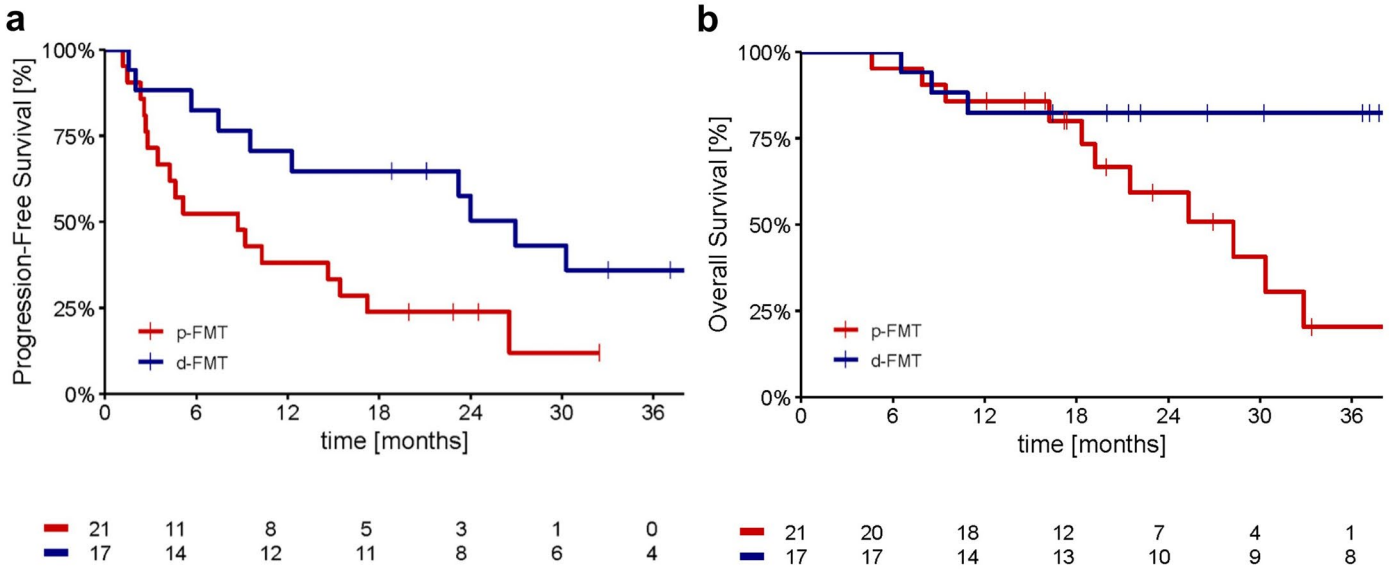


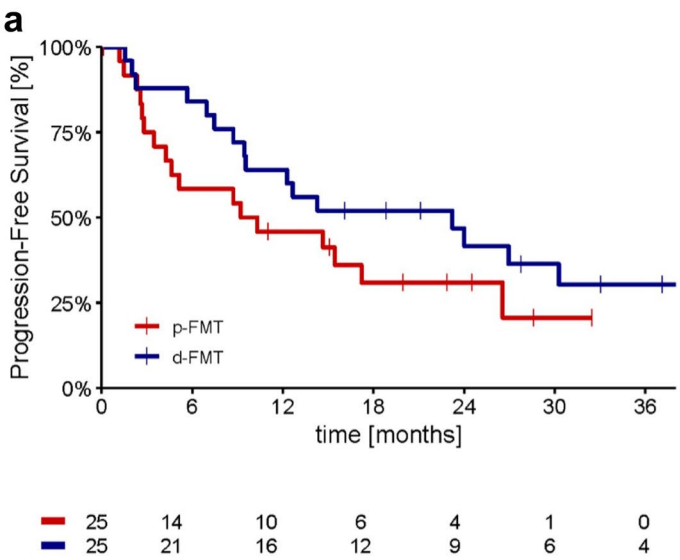
Extended Data Fig. 5 | Association between strain-level modifications at week-4 and the primary outcome. a) Barplots with the percentage of patients in the d-FMT arm at week-4 follow-up acquiring the donor strain of each donor species according to presence (blue) or absence (orange) of PFS > 12 months. Only species differing by at least 25% between groups are depicted. A Fisher's exact test was applied to assess statistical significance. **b**) Barplots with the percentage of patients in the d-FMT arm at week-4 follow-up acquiring the donor strain of each donor species according to presence (blue) or absence (orange) of PFS > 12 months. Species are ordered by the prevalence difference between groups (no - yes), with only species differing by at least 25% between groups are depicted. A Fisher's exact test was applied to assess statistical significance. Across all panels statistical significance is represented as *: $p < 0.05$.

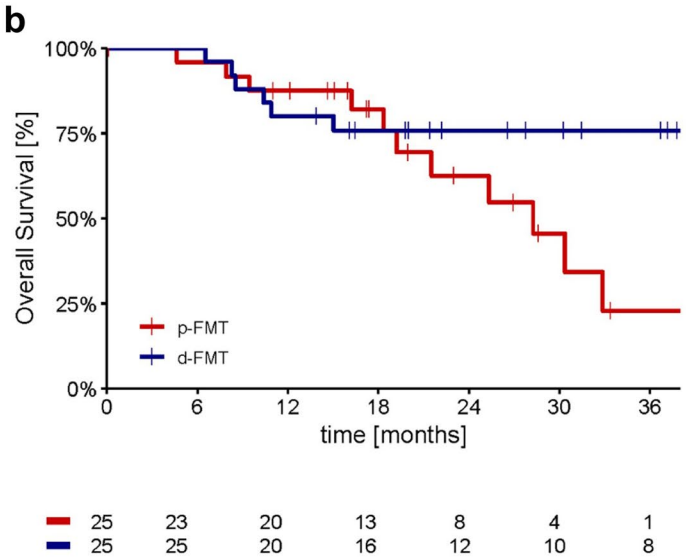
https://doi.org/10.1038/s41591-025-04189-2



Extended Data Fig. 6 | PFS and OS in the PP population. a) *Median PFS*. In the PP population, seven of 17 patients in the d-FMT arm (41%) and 17 of 21 subjects in the p-FMT arm (81%) experienced a disease progression. Median PFS was significantly improved in the d-FMT arm (26.9 months, 95% CI 7.5-NE months) compared with the p-FMT arm (8.7 months, 95% CI 2.8-15.4 months) (HR: 0.43, 90% CI: 0.20-0.92, p = 0.028). **b**) *Median OS*. In the PP population, five of 17 patients in the d-FMT arm (29%) and 11 of 21 patients in the p-FMT arm (52%) died. Median OS was nonsignificantly longer in the d-FMT arm (41 months) than in the p-FMT arm (28.3 months) although not significantly (41 months in the d-FMT arm vs 28.3 months in the p-FMT arm, HR 0.36, 95% CI 0.13-0.99, p = 0.146). The Breslow test was used for the statistical analyses.

https://doi.org/10.1038/s41591-025-04189-2





Extended Data Fig. 7 | PFS and OS in the ITT population. a) *Median PFS*. In the ITT population, median PFS was longer in the d-FMT arm (23.2 months, 95% CI: 7.4-39.1 months) than in the p-FMT arm (9.2 months, 95% CI: 0-20.5 months), although nonsignificantly (HR: 0.66, 95% CI: 0.33-1.35, p = 0.18; **b**) *Median OS*. Median OS was nonsignificantly longer in the d-FMT arm (40.9 months, 95% CI: 39.2-42.7) than in the p-FMT arm (28.2 months, 95% CI: 20.1-36.5) (HR 0.51, 95% CI: 0.20-1.30, p = 0.146). The Breslow test was used for the statistical analyses.

https://doi.org/10.1038/s41591-025-04189-2

Extended Data Table 1 | Treatment-related adverse events

	D-FMT N=25 (%)	P-FMT N=24 (%)
AEs related to d-FMT/p-FMT		
- Any grade	0	2 (8%)
- Grade 3 or 4	0	1 (4%)
- AEs leading to treatment discontinuation	0	1 (4%)*
Overall AEs related to pembrolizumab/axitinib		
- Grade 3 or 4	7 (28%)	4 (16%)
AEs of special interest related to pembrolizumab/axitinib**		
Diarrhea		
- Any grade	13 (52%)	11 (44%)
- Grade 3-4	3 (12%)	1 (4%)
Colitis	3 (12%)	2 (8%)
- Any grade	0 (0%)	1 (4%)
- Grade 3-4		
Nausea	3 (12%)	1 (4%)
- Any grade	1 (4%)	0 (0%)
- Grade 3-4		
AST/ALT increase	5 (20%)	7 (28%)
- Any grade	3 (12%)	2 (8%)
- Grade 3-4		

*The patient discontinued capsules assumption due to grade 3 oral mucositis
**Including gastrointestinal AEs.

nature portfolio

Corresponding author(s): Gianluca Ianiro

Last updated by author(s): Dec, 1, 2025

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microsoft Excel for Mac (Microsoft Excel. Redmond, Washington: Microsoft, 2011) was used for clinical data collection.
Data analysis	IBM-SPSS v.28.0 statistical software, GraphPad Prism v. 10, R v.4.4.2 ('survival' and 'survminer' packages), and Python v3.10.12 ('scikit-bio' and 'scipy' packages) for the statistical analyses; MetaPhlAn v. 4 and StrainPhlAn v. 4.1 for the taxonomical analyses

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The shotgun metagenomic data generated in this study are available at the European Nucleotide Archive under accession number PRJEB94043. Clinical data from this study will be made available upon reasonable request to the corresponding author from a qualified medical or scientific professional for the specific purpose

laid out in that request and might include de-identified individual participant data. The data for this request will be available after a data access agreement has been signed. Patient-related data not included in the paper were generated as part of a clinical trial and are subject to patient confidentiality.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	The sex of the study participants is reported in Table 1. Sex stratification was not used for any of our analyses
Reporting on race, ethnicity, or other socially relevant groupings	We did not perform any analysis, nor reported, race, ethnicity, or any social relevant grouping in our paper
Population characteristics	We enrolled patients with metastastic renal cell carcinoma. Demographics and disease-related characteristics of this population are desribed in Table 1.
Recruitment	Subjects were enrolled in the Fondazione Policlinico Gemelli IRCCS, a tertiary care center and academic hospital in Rome, Italy and were either screened from our oncology outpatient clinic or referred to our center from other seven Italian hospitals, all referral centers for renal cell carcinoma
Ethics oversight	The study was performed in compliance with the Declaration of Helsinki. Ethical approval was granted by Ethics Committee of the Fondazione Policlinico Gemelli IRCCS (ID #2664). Written informed consent was obtained from all participants

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size calculation was based on the hypothesis of the superiority of FMT+standard of care (SOC) over SOC alone. The 1-year PFS rate for SOC has been reported to be nearly 60%. The alternative hypothesis is that FMT can improve the 1-year PFS rate from 60% to 80% when associated with SOC. A total of 50 patients is required to enter this two-treatment parallel-design study. The probability is 80 percent that the study detects a treatment difference at a one-sided 5.0 percent significance level, if the true hazard ratio is 0.436. This is based on the assumption that the accrual period will be 36 months and the follow up period will be 36 months and the median survival is 15.1 months. The total number of events are expected to be 36.
Data exclusions	One metagenome with subpar sequencing depth (<3Gbp) was discarded
Replication	Technical replication on metagenomic sequencing is not necessary and not performed in the metagenomic field. Biological replication would involve including new patients which is not appropriate given the sample size power calculation discussed above
Randomization	An online random number generator software (https://www.sealedenvelope.com) was used to provide random permuted blocks with a block size of four and an equal allocation ratio; the sequence was hidden until the interventions were assigned. Blocked randomization of subjects was performed by an external individual not involved in the study.
Blinding	To mask treatments to recipients, both infusate bottles and syringes were covered with dark-colored paper before the infusion, and the patients were sedated. Placebo capsules were made of cellulose and were identical in appearance to the FMT capsules, to ensure the blinding of patients and study staff. Moreover, both placebo suspension bottles and placebo capsule boxes were labelled with similar barcodes than FMT ones. Physicians who evaluated patients at follow-up (clinical outcome assessors) were blinded to administered treatments, as well as authors involved in outcome analysis.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic*

Research sample	*information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*

Disturbance | *Describe any disturbance caused by the study and how it was minimized.*

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☐	☒ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used | *Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.*

Validation | *Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.*

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s) | *State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.*

Authentication | *Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination | *Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*

Commonly misidentified lines (See ICLAC register) | *Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Palaeontology and Archaeology

Specimen provenance | *Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.*

Specimen deposition | *Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods | *If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight | *Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	*For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ClinicalTrials.gov identifier: NCT04758507
Study protocol	The study protocol is available as supplementary information
Data collection	All patients were enrolled between February 11, 2021 and November 11, 2023. The last enrolled patient received his first treatment on December 16, 2023, and had his last follow-up visit on December 5, 2024. All procedures have been carried out at the Fondazione Policlinico Gemelli IRCCS, Rome, Italy
Outcomes	The primary efficacy endpoint was the 12-month PFS, defined as the number of participants free from tumor progression, as assessed by RECIST version 1.1 criteria, 12 months after randomization44, in the FAS population. Secondary efficacy endpoints were median PFS, median OS, and ORR in the FAS population, safety, microbiome changes after treatments. Safety was evaluated in the PP and FAS populations. PFS and ORR were determined using RECIST version 1.1, as assessed by local investigators. PFS was defined as the time from randomization until disease progression or death, whichever occurred first. OS was evaluated from the date of randomization to death from any cause or the last contact. ORR was defined as the proportion of patients with a partial or complete response. Safety was assessed as the incidence, nature and severity of treatment-related AEs, recorded and classified according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Microbiome changes after treatments were defined as the number of participants with significant increase in alpha-diversity (assessed by Shannon index) and beta-diversity (assessed by Bray-Curtis dissimilarity) of their gut microbiota after treatments, compared with baseline.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
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☒	☐	National security
☒	☐	Crops and/or livestock
☒	☐	Ecosystems
☒	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
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☒	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☒	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☒	☐	Increase transmissibility of a pathogen
☒	☐	Alter the host range of a pathogen
☒	☐	Enable evasion of diagnostic/detection modalities
☒	☐	Enable the weaponization of a biological agent or toxin
☒	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N.A.
Novel plant genotypes	N.A.
Authentication	N.A.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*
Software	*Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.*
Cell population abundance	*Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.*
Gating strategy	*Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.*

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.