A functional immune-based platform for donor-recipient matching in faecal microbiota transplantation for inflammatory bowel disease

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Summary

Background Faecal microbiota transplantation (FMT) shows variable efficacy in inflammatory bowel disease (IBD), and current donor selection strategies rely primarily on microbiome characteristics, while host immune responses to donor microbiota remain largely unexplored. Here, we investigated whether recipient-specific immune responses to donor microbiota could be leveraged to develop a personalised donor-recipient matching strategy for FMT in IBD.

Methods We developed a proof-of-concept (POC) assay, termed Gut Microbiota–Leukocyte Reaction (GMLR), to assess immune compatibility between donor microbiota and recipients with IBD. Lamina propria mononuclear cells isolated from intestinal biopsies were exposed *ex vivo* to microbiota from healthy donors, and cytokines relevant to IBD pathophysiology were measured.

Findings Donor microbiota clustered into distinct groups associated with differential immune signatures, including significant IL-22 induction ($p = 0.033$), whereas IL-17 showed a non-significant trend toward reduction ($p = 0.054$) that was not consistently observed across immune cell subsets. However, immune responses were highly individualised across recipients, with substantial inter-patient variability. Based on these responses, we developed an algorithm to generate donor-recipient compatibility scores, providing a framework to prioritise potential donor-recipient pairs.

Interpretation Our findings suggest that donor-recipient immune compatibility is highly personalised and may represent a key determinant of FMT efficacy, challenging the "super-donor" paradigm. This *ex vivo* proof-of-concept platform may help prioritise donor-recipient pairs and should be prospectively validated against clinical FMT outcomes.

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Keywords: Faecal microbiota transplantation; Inflammatory bowel disease; Donor-recipient matching; Host–microbiome interaction; Immune profiling; Precision medicine

Introduction

Faecal microbiota transplantation (FMT) is highly effective for treating recurrent *Clostridioides difficile infection* (rCDI), achieving cure rates of nearly 90%,[1,2] and is currently recommended by international clinical guidelines.[3] Following its success in rCDI, FMT has been explored as a potential therapeutic strategy for chronic disorders associated with gut dysbiosis,

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Research in context

Evidence before this study

Faecal microbiota transplantation has shown variable efficacy in inflammatory bowel disease, with donor-related microbiome features only partially explaining clinical outcomes. Previous studies have explored donor selection based on microbial composition, diversity, and functional traits, but these approaches have shown inconsistent predictive value. More recent efforts have attempted to incorporate immune-related features, including donor-induced regulatory responses in preclinical models or *ex vivo* stimulation assays; however, these strategies remain limited in scope, lack standardisation, and do not directly assess patient-specific immune responses to donor microbiota. The literature search was conducted in PubMed and ClinicalTrials.gov from January 1, 2000 to April 12, 2026, using the search terms "faecal microbiota transplantation", "inflammatory bowel disease", "ulcerative colitis", "Crohn's disease", "donor selection", "donor microbiome", "donor-recipient matching", "immune response", and "immunomodulation", alone and in combination.

Added value of this study

We developed a proof-of-concept, *ex vivo* assay (Gut Microbiota–Leukocyte Reaction) that directly measures patient-specific immune responses to donor microbiota using human intestinal immune cells. This approach reveals substantial inter-individual variability in immune responses and enables the generation of a quantitative compatibility index to support donor-recipient matching. Our findings provide a functional framework to move beyond donor-centric microbiome profiling toward personalised donor selection.

Implications of all the available evidence

These findings support a shift toward integrating host immune responses into donor selection strategies. If validated in clinical settings, this approach could improve the predictability and efficacy of microbiota-based therapies in inflammatory bowel disease and contribute to the development of precision microbiome-based approaches.

including inflammatory bowel disease (IBD).[4–6] However, unlike rCDI, FMT efficacy in IBD remains variable, with clinical remission rates ranging from approximately 25%–50% in ulcerative colitis (UC) and Crohn's disease (CD).[7–11]

Several factors have been proposed to influence FMT outcomes in IBD, encompassing procedural variables, such as antibiotic pre-treatment, bowel cleansing, route of delivery, the number and volume of fecal infusions, as well as donor- and recipient-related characteristics.[12,13] Current clinical practice primarily focuses on donor screening to ensure safety and prevent the transmission of infectious agents,[14] while additional donor features such as microbiota diversity, taxonomic composition, and functional capacity have been investigated as potential determinants of treatment success.[13] In nonrandomised cohorts of IBD patients, higher microbial richness and donor strain engraftment have been associated with improved outcomes,[9,15,16] as well as the presence of specific taxa, such as *Lachnospiraceae*, *Akkermansia*, *Bacteroides*, and *Ruminococcaceae*, or microbial functions, including bile acid metabolism and short-chain fatty acid production.[17–19] However, these largely donor-centric, microbiome-focused approaches have shown inconsistent predictive value, suggesting that donor microbiota composition alone may not fully explain FMT efficacy in immune-mediated diseases, where host–microbiome interactions are likely to play a critical role.

IBD is characterised by a disruption of host–microbiota interactions driven by defects in immune regulation and microbial sensing pathways.[20,21] Genetic variants affecting pathways such as NOD2, CARD9, and IL-10 signalling highlight the importance of immune tolerance toward commensal microorganisms in maintaining intestinal homoeostasis.[22–24] In this context, the therapeutic effect of FMT may depend not only on donor microbiota characteristics but critically on how the recipient's immune system responds to the introduced microbial community. However, this host–microbiome interaction is rarely accounted for in current donor selection strategies, and functional approaches to assess donor-recipient compatibility remain largely unexplored. A major unmet need in this field is the lack of functional tools to guide donor selection, resulting in unpredictable clinical outcomes and limiting the scalability of this therapeutic approach.

Inspired by the mixed lymphocyte reaction (MLR), a classical assay used to evaluate donor-recipient compatibility in organ transplantation,[25] we developed a preclinical, proof-of-concept (POC) tool to investigate immune responses of IBD patients to potential donor microbiota. In this study, lamina propria mononuclear cells (LPMCs) isolated from intestinal biopsies of UC and CD patients were exposed *ex vivo* to microbiota derived from healthy FMT donors. Immune responses were assessed by measuring cytokines relevant to IBD pathophysiology,[26] including TNF-α, IFN-γ, and IL-17, which drive pathogenic Th1/Th17 responses,[27] as well as IL-10 and IL-22, associated with anti-inflammatory and mucosal healing functions.[28,29] These data were integrated into an algorithm to generate donor-recipient compatibility scores.

This preclinical, translational approach aims to explore whether functional immune responses to donor microbiota could inform future strategies for donor selection in FMT, providing a platform for validation in prospective clinical studies.

Methods

Human subjects

This single-centre study was conducted at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy). Ethical approval for this study was obtained from the CE Milano Area 2, number 557_2018. All participants provided written informed consent prior to inclusion in the study. Participants were recruited and biological specimens were collected between May 2021 and September 2023. Intestinal biopsies for the isolation of LPMCs were obtained from endoscopically inflamed colonic areas of patients affected by CD and UC (Table 1); inclusion and exclusion criteria for the enrolled IBD patients are summarised in Tables 2–5. Potential healthy donors were initially pre-screened for clinical suitability using a preliminary questionnaire among blood donors at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy). Stool and serology screening was performed for bacterial, parasitic, and viral pathogens according to Italian CNT (National Transplant Centre) guidelines for FMT donor screening (Table 6).[30] Donors had not used antibiotics within 8 weeks before screening. The characteristics of enrolled donors are reported in Table 1. Descriptive statistics for baseline characteristics are reported in Table 1. Categorical data are presented as counts and percentages. Continuous variables are presented as median and interquartile range (IQR) where individual-level data were available, and as mean ± standard deviation otherwise (as indicated in Table 1). Sex was recorded as biological sex (male or female) based on participants' clinical records. Gender identity was not collected as a separate variable.

Given the exploratory, proof-of-concept nature of the study, no formal sample size or power calculation was performed. The sample size was determined by the availability of eligible IBD participants and healthy donors during the study period.

LPMCs isolation

LPMCs were isolated from intestinal biopsies. Biopsies were cleared of mucus and epithelial cells in sequential steps with DTT (0.1 mmol/l) and EDTA (1 mmol/l) (Sigma–Aldrich) and then digested with collagenase D (100 U/mL) and 150 μg/ml DNase I at 37 °C for 1 h with vigorous shaking. The digested tissue was filtered through a 100 μm cell strainer and LPMCs were then separated with a Percoll gradient.

Healthy donor fecal microbiome preparation

Stool samples from healthy donors were collected in collaboration with the faecal FMT biobank of the Policlinico Hospital, Milan.[30] All donors included in the faecal biobank were used in the current study. No bowel cleansing preparations were allowed before donation. Once collected material was stored at –80 °C. At the time of analyses, material was thawed and resuspended 1:10 (w/v) in pre-reduced PBS + 10% heat-inactivated foetal bovine serum (FBS) and filtered through a 0.75 μm filter to remove large debris; microbiota cell density was quantified by qPCR using the primers F_Bact1369: CGGTGAATACGTTCCCGG and R_Prok1492: TACGGCTACCTTGTTACGACTT.[31]

Briefly, 1 ml of resuspended samples was used for total gDNA extraction using the FastDNA™ SPIN Kit for Faeces. For bacterial load quantification, a seven-point standard curve was constructed using 10-fold serial dilutions (from 10^3 to 10^9) of gDNA extracted from a pure culture of *Escherichia coli* at known concentration, to convert threshold cycle (Ct) values into estimates of bacterial genomes per mL of sample. Amplification specificity of target gene was checked by melting curve analysis. Upon quantification,

Clinical parameter	UC patients (n = 6)	CD patients (n = 10)	Healthy donors (n = 8)
Male/Female. n (%)	3/3 (50)	7/3 (70)	3/5 (37.5)
Age. median [IQR] (years)	50 (31–65.5)	45 (32–54.5)	27 (25–35)
Disease duration. median [IQR] (years)	10.5 (6.4–14.6)	15.3 (9.2–21.4)	
BMI. median [IQR] (kg/m^2)	22.5 (20.4–24.6)	20.7 (17.9–23.5)	22.3 (20.3–24.3)
UC location. n (%)			
E1 proctitis	0 (0)		
E2 left sided	6 (100)		
E3 pancolitis	0 (0)		
CD location. n			
L1 ileal		0 (0)	
L2 colonic		5 (50)	
L3 ileocolonic		5 (50)	

Table 1: **Baseline clinical characteristics of the enrolled subjects.**

Patients must meet all the following INCLUSION CRITERIA at enrolment to participate:
1. Males and females aged 18–75 years. inclusive
2. Clinically and endoscopically active UC (i.e., extent of more than 15 cm and less or to the lienal flexure) of >3 months duration. a total Mayo score of 4–10. and endoscopy subscore ≥2.
3. Use of oral 5-aminosalicylates (stable dose for 8 weeks. maximal dose 4 g). was allowed
4. Provide written informed consent to participate as shown by a signature on the consent form

Table 2: **Study participant inclusion criteria for UC patients.**

resuspended samples were adjusted to the concentration of 1×10^9 cell·mL–1 and heat-killed at 95 °C by performing three cycles of incubation at 95 °C for 5 min followed by –20 °C for 5 min, followed by a final incubation at 95 °C for 10 min before being stored at –80 °C until use in downstream experimentation.

Ex vivo LPMC stimulation assay

Human LPMCs were plated at a density of 2×10^5 in RPMI-1640 supplemented with 10% FBS and exposed to 1×10^9 cell·mL^{-1} of the faecal microbiome suspension for 24 h as previously described.[32] For intracellular cytokine labelling, cells were incubated for 3 h at 37 °C in RPMI-1640 + 10% FBS with phorbol myristate acetate (PMA) (50 ng/ml, Merck, IT), ionomycin (1 μg/ml, Merck), and brefeldin A (10 μg/ml, Merck, IT). At the end of the experiment, cells were analysed with flow cytometry. Viability was assessed by Zombie (Biolegend) staining.

Each donor-recipient GMLR stimulation was performed as a single measurement, without technical replicates, and experiments were not independently repeated.

Patients must not meet any of the following EXCLUSION CRITERIA at enrolment to participate:
1. Patients defined as in remission by the investigator
2. Evidence or history of toxic megacolon
3. A diagnosis of Crohn's Disease or indeterminate colitis
4. Patients with perianal disease (e.g. fistulae. pre-existing fissures)
5. Severe anaemia. leucopoenia or granulocytopenia
6. Any other significant GI condition e.g. Irritable bowel syndrome. diverticulitis. neoplasm etc
7. Significant gastrointestinal surgery e.g. colon resection. colectomy
8. Patients taking probiotics or antimicrobials (antibiotics. antifungals. antivirals) for any reason. including antibiotics for UC. in the preceding 4 weeks
9. Anti-TNF medication and other biologic therapy or JAK inhibitors in the previous 12 weeks
10. Corticosteroids in the previous 4 weeks
11. Calcineurin inhibitors in the previous 12 weeks
12. Thiopurines or methotrexate in the previous 8 weeks
13. Positive stool culture (*Salmonella. Shigella. Yersinia. Campylobacter.* pathogenic *Escherichia coli*)
14. Cytomegalovirus infection. *Clostridium difficile* infection
15. History of bowel cancer
16. Females who are pregnant or actively trying to fall pregnant
17. Patients unwilling to practice an effective method of contraception throughout the study period
18. Consent not obtained or unable to give informed consent
19. Unable to communicate with the investigators and comply with the study requirements
20. Age >75 years

Table 3: **Study participant exclusion criteria for UC patients.**

Flow cytometry

Human LPMCs were stained with labelled antibodies diluted in PBS +1%FBS for 30 min at 4 °C. For intracellular cytokine labelling, cells were incubated for 3 h at 37 °C in RPMI-164 supplemented with 10% FBS with PMA (50 ng/ml, Merck), Ionomycin (1 μg/ml, Merck) and Brefeldin A (10 μg/ml, Merck). Before intracellular staining, cells were fixed and permeabilized using Cytofix/Cytoperm (BD). Samples were analysed with a FACSLyric™ flow cytometer (BD Biosciences, Franklin Lakes NJ, USA). The antibodies used in the study are reported in Supplementary Table S1. Data were analysed using the FlowJo software (Version 10.8, TreeStar, Ashland, OR, USA).

16S rRNA gene sequencing and data analysis

Fecal samples were stored at –80 °C until DNA extraction. DNA extraction, 16S rRNA gene amplification, purification, library preparation and pair-end sequencing on the Illumina MiSeq platform were performed as previously described (V3–V4 regions, 300 bp paired end).[32] Sequencing generated 251-bp paired-end reads, with a mean sequencing depth of approximately 143,000 read pairs per sample and a minimum depth of 84,964 read pairs per sample. Relative abundance-based methods were applied; no quantitative microbiome profiling, cell-count-based quantification, or spike-in standards were used. Reads were pre-processed using the MICCA pipeline (v.1.7.2) (ht://micca.readthedocs.io/en/latest/#).[33] A microbial mock community (Zymo Research, ZYD6311) and water were included as positive and negative controls, respectively. Forward and reverse primers trimming and quality filtering were performed using micca trim and micca filter, respectively. Filtered sequences were denoised using the UNOISE[34] algorithm implemented in micca otu to determine true biological sequences at the single nucleotide resolution by generating amplicon sequence variants (ASV). Bacterial ASVs were taxonomically classified using micca classify and the Ribosomal Database Project (RDP) Classifier v2.13.[35] Multiple sequence alignment (MSA) of 16S rRNA gene sequences was performed using the Nearest Alignment Space Termination (NAST) algorithm implemented in micca msa.[36] Phylogenetic trees were inferred using micca tree.[36,37] Sampling heterogeneity was reduced rarefying samples at the depth of the less abundant sample using micca tablerare. Alpha (within-sample richness) and beta-diversity (between-sample dissimilarity) estimates were computed using the phyloseq R package.[38] To assess whether the identified donor microbiome clusters (C1 and C2) differed significantly in their overall community composition, a

permutational multivariate analysis of variance (PERMANOVA) was performed on Bray–Curtis dissimilarities using the adonis 2 function in the vegan R package with 999 permutations. ASVs differential abundance testing was carried out using the R package DESeq2 using the non-rarefied data.[38,39]

Whole metagenome shotgun (WMS) sequencing and data analysis

Sequencing libraries were prepared using the TruSeq® Nano DNA Library Prep (Illumina), following the manufacturer's guidelines. Sequencing was performed on the Illumina NovaSeq 6000 platform following manufacturer's protocols. Sequencing generated 61-bp paired-end reads, with a mean sequencing depth of 38.2 million reads per sample and a minimum depth of 27.6 million reads per sample. Relative abundance-based methods were applied; no quantitative microbiome profiling, cell-count-based quantification, or spike-in standards were used.

The quality of the reads was evaluated running FastQC (v0.12.1) on fastq.gz files. Trimmomatic (v0.39)[40] was run to remove low quality reads and adaptor sequences. Host DNA reads were removed mapping input reads on T2T-CHM13v2.0 human genome using Bowtie 2 (v2.5.4)[41] and Samtools (v1.21). Taxonomic profiling of the filtered reads was done using MetaPhlAn (v4.1.1)[42] on mpa_vJun23_CHOCOPhlAnSGB_202403 database. Functional profiling was performed by HUMAnN3 (v3.9)[43] on uniref90_201901b_full database. Gut metabolic modules (GMMs)[44] abundances were computed using Omixer-RPM (v.1.1) (https://github.com/raeslab/omixer-rpm). Post-processing analysis was executed in R environment (v4.4.1). Differential abundance analysis of species, gene families and pathways between conditions was evaluated by Welch's t-test with the rstatix R package (v0.7.2). The correlation of the significantly differentially abundant species and pathways with the enriched cytokine levels was computed using the corr.test function from psych R package (v2.4.12).

Statistical analysis

Microbiome alpha- and beta-diversity were computed using the phyloseq R package. Beta-diversity was visualised by Principal Coordinate Analysis (PCoA) based on Bray–Curtis dissimilarities. PERMANOVA was performed on Bray–Curtis dissimilarities using the adonis 2 function (vegan R package, 999 permutations) to test whether donor microbiome clusters (C1 and C2) differed in overall community composition. Differential abundance of amplicon sequence variants (ASVs) was assessed using DESeq2 on non-rarefied data. Differential abundance of genera, bacterial species, gene families and functional pathways between clusters was evaluated using Welch's t-test (rstatix R package), chosen for its robustness to unequal variances and unequal group sizes in this exploratory setting; p-values were FDR-corrected where indicated. Correlations between significantly differentially abundant species or functional pathways and cytokine levels were assessed using Spearman's correlation (corr.test function, psych R package).

For the immunological analyses, differences in cytokine responses (IL-10, IL-22, IL-17, IFN-γ, TNF-α) induced by C1 versus C2 donor microbiomes were assessed using the Mann–Whitney U test. Within-recipient paired comparisons of cytokine responses to C1 and C2 microbiomes, performed on $CD45^+$, $CD3^+$, $CD3^-$ and $CD4^+$ cell populations, were evaluated using the Wilcoxon matched-pairs signed-rank test. Cytokine responses were expressed as $\log_2$ fold-change relative to unstimulated LPMCs from the same patient.

Descriptive statistics of baseline characteristics are reported in Table 1: categorical data as counts and percentages, and continuous variables as median and

Patients must meet all the following INCLUSION CRITERIA at enrolment to participate:
1. Males and females aged 18–75 years. inclusive
2. Eligible subjects required documentation of typical histopathology for CD of the colon. or colon and ileum. and ulcers ≥0.5 cm > 3 months duration of disease. and a CDAI of ≥150
3. A 12-week washout period was required for patients exposed to thiopurines. methotrexate. corticosteroids. cyclosporine. tacrolimus. antiTNF. vedolizumab. ustekinumab. risankizumab. upadacitinib
4. Provide written informed consent to participate as shown by a signature on the consent form

Table 4: **Study participant inclusion criteria for CD patients.**

Patients must not meet any of the following EXCLUSION CRITERIA at enrolment to participate:
1. Patients defined as in remission by the investigator
2. Active fistulizing disease
3. Perianal or abdominal abscesses
4. Complications requiring surgical treatment
5. Treatment with biological drugs or upacacitinib (ongoing or stopped in the month preceding randomisation); immunosuppressant treatment started or stopped in the 3 months preceding randomisation; intake of nonsteroidal anti-inflammatory drugs (NSAIDs) in the 4 weeks preceding randomisation
6. Antibiotic. probiotic or antifungal treatment in the 4 weeks preceding colonoscopy
7. A diagnosis of Ulcerative colitis
8. Any other significant GI condition e.g. Irritable bowel syndrome. diverticulitis. neoplasm etc
9. Significant gastrointestinal surgery e.g. colon resection. colectomy
10. Positive stool culture (*Salmonella*. *Shigella*. *Yersinia*. *Campylobacter*. pathogenic *Escherichia coli*)
11. Cytomegalovirus infection. *Clostridium difficile* infection
12. History of bowel cancer
13. Females who are pregnant or actively trying to fall pregnant
14. Patients unwilling to practice an effective method of contraception throughout the study period
15. Consent not obtained or unable to give informed consent
16. Unable to communicate with the investigators and comply with the study requirements
17. Age >75 years

Table 5: **Study participant exclusion criteria for CD patients.**

Blood test: CMV (IgG + IgM). EBV(IgG + IgM). Hep B(HBsAg). Hep C (hepatitis C virus antibody). HIV (1 + 2 antibodies). *Treponema pallidum*
Stool tests: *Salmonella. Shigella. Yersinia. Campylobacter. Clostridium difficile toxin. Helicobacter pylori.* Rotavirus. norovirus. enterovirus parechovirus. sapovirus Adenovirus 40/41/52. astrovirus
Parasitological examination

Table 6: **Donor selection criteria.**

interquartile range (IQR) where individual-level data were available, or as mean ± standard deviation otherwise. Statistical analyses were performed in R (v4.4.1). Statistical significance was set at $p < 0.05$.

Experimental procedures were not randomised, and investigators were not blinded during sample processing, flow cytometry acquisition, or data analysis. No samples, assays, or data were missing or excluded from the analyses.

Role of Funders

The funding sources had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The authors were not precluded from accessing the data in the study and accept responsibility for the decision to submit the manuscript for publication.

Ethics

The study was approved by the Ethics Committee Milano Area 2 (approval no. 557_2018) and was conducted in accordance with the ethical principles of the 2024 Declaration of Helsinki. All participants provided written informed consent for participation in the study prior to enrolment.

Results

Donor microbiomes modulate Th17-related responses of IBD recipients

Microbiome-immune system interactions are often overlooked in IBD patients undergoing FMT. To investigate how microbiome-related factors shape immune responses in IBD patients potentially eligible for FMT (Tables 1–5), we enrolled 16 IBD patients (CD, n = 10; UC, n = 6) and 8 healthy donors, whose baseline clinical characteristics are summarised in Table 1 (inclusion and exclusion criteria are reported in Tables 2–5; donor screening criteria in Table 6).

We first characterised the fecal microbiome of the eight enrolled healthy volunteers using 16S rRNA gene sequencing. Based on the Bray–Curtis dissimilarity index, donor gut microbiomes clustered into two distinct community structures, defined as Cluster 1 (C1) and Cluster 2 (C2) (Fig. 1A–B). Both clusters exhibited significantly higher alpha diversity compared with IBD patients (used as dysbiotic controls), with a more pronounced increase in C1 (Fig. 1C).

A detailed taxonomic analysis showed that C1 was enriched in genera such as *Alistipes*, *Pseudoflavonifractor*, as well as unclassified members of the *Ruminococcaceae* and *Oxalobacteraceae* families (Fig. 1D), alongside Amplicon Sequence Variants (ASVs) belonging to *Akkermansia*, *Barnesiella*, *Dorea*, and *Phascolarctobacterium* (Fig. 1E–F; Supplementary Table S2). Conversely, the C2 microbiome was enriched in *Bacteroides*, *Lachnospiraceae incertae sedis*, and *Parabacteroides* (Fig. 1D), together with ASVs from SCFA-producing taxa including *Bifidobacterium*, *Blautia*, Butyricicoccus, *Clostridium* spp., *Coprococcus*, and *Ruminococcus 2* (Fig. 1E–F; Supplementary Table S2).

We next investigated whether donor microbiota differentially modulate recipient immune responses. LPMCs isolated from intestinal biopsies of IBD patients were exposed *ex vivo* to donor-derived microbiota (Supplementary Fig. S1). We found that the C1 microbiome preferentially induced IL-22 production in $CD45^+$ immune cells ($p = 0.033$, Fig. 2A), whereas IL-17 showed a non-significant trend toward reduced expression in response to the C2 microbiome ($p = 0.054$, Fig. 2B). A within-recipient paired analysis confirmed that the C1 microbiome consistently induced stronger IL-22 responses than the C2 microbiome in the same patient ($p = 0.001$, Supplementary Fig. S2). No significant differences were observed in TNF-α, IFN-γ, or IL-10 responses (Fig. 2C–E), suggesting that the donor microbiome composition mainly influences Th17-like immune responses. To address whether this modulation involved specific immune cell subsets, we analysed cytokine responses across $CD45^+$, $CD3^+$, $CD3^-$ and $CD4^+$ compartments. The C1-driven enhancement of IL-22 was consistently detected across all compartments, including $CD45^+$ ($p < 0.001$), $CD3^+$ ($p < 0.001$), $CD3^-$ ($p < 0.001$) and $CD4^+$ cells ($p = 0.001$), whereas IL-17 responses showed no consistent cluster-dependent modulation in any subset (Supplementary Fig. S3).

To identify microbial features associated with these immune responses, we performed whole metagenome shotgun sequencing and correlated taxonomic and functional profiles with cytokine production. We observed that the C1 microbiome was enriched in species such as *Lentihominibacter faecis*, *Pseudoflavonifractor capillosus* and *Alistipes spp.* (Fig. 2F), positively correlating with IL-22 responses (Fig. 2G). Conversely, the C2 microbiome was enriched by different species among which *Parabacteroides merdae*, *Holdemania filiformis*, *Faecalibacillus intestinalis* and *Phocaeicola sartorii* (Fig. 2F), with the latter two negatively correlating with IL-22 responses (Fig. 2G).

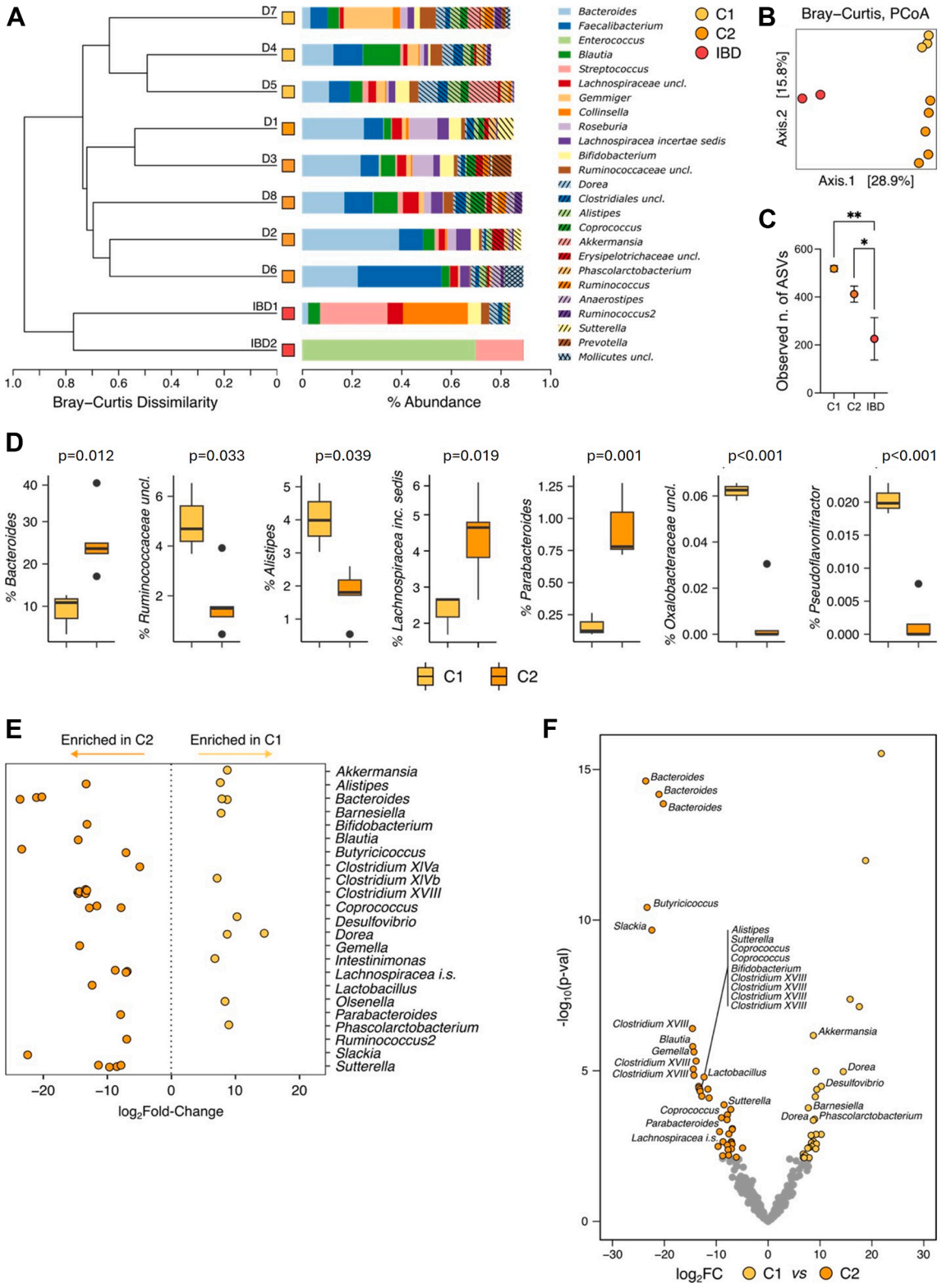

Fig. 1: **Metataxonomy of the donor gut microbiota: A)** Hierarchical clustering analysis of Bray–Curtis distances shows two major microbiome clusters between donors (n = 8). Reported also the mean relative abundance of the top 25 most abundant genera from the analysed

As microbial engraftment alone may not fully explain FMT efficacy, we investigated whether donor microbiome functions are associated with specific recipient immune responses. C1 metagenomes contained a higher proportion of genes involved in the biosynthesis of 1,3-propanediol (Fig. 2H), a product of anaerobic glycerol fermentation,[45] which positively correlated with IL-22 responses (Fig. 2I). In contrast, the C2 microbiome was enriched in pathways related to amino acid and vitamin metabolism, as well as coenzyme A (CoA) biosynthesis (Fig. 2H), which negatively correlated with IL-22 responses (Fig. 2I). Consistently, analysis of gut metabolic modules (GMMs)[44] revealed increased representation of glycolysis, amino acid degradation, and short-chain fatty acid production pathways in C2 microbiomes (Fig. 2J), the majority of which were negatively associated with IL-22 responses (Fig. 2K).

Inter-individual immune variability supports donor-recipient matching in FMT

Although IL-22 and IL-17 expression were more consistently modulated according to donor microbiome composition (Fig. 2A–B), we observed substantial variability in cytokine responses within individual recipients exposed to different donor-derived microbiomes (Fig. 3). This suggests that immune responses to FMT may be highly personalised, reinforcing the need for tailored donor selection. Indeed, some donors selectively enhanced anti-inflammatory and tolerogenic responses (i.e., IL-10 and IL-22; Fig. 3A–B) while suppressing pro-inflammatory cytokines (i.e., IL-17 and IFN-γ; Fig. 3C–D), representing a potentially desirable profile for donor-recipient matching in the autoimmune setting. In contrast, other donors induced mixed or predominantly pro-inflammatory responses, which could be detrimental to FMT success. These distinct immunomodulatory effects highlight the complexity of host–microbiome interactions, challenging the 'super-donor' paradigm in IBD and the one-size-fits-all approach to FMT.[46] To further challenge the GMLR platform with disease-associated microbial communities, a subset of recipients (R8-R15) was additionally stimulated with microbiomes derived from two patients with IBD. Similar to healthy donor microbiomes, IBD-derived microbiomes (Fig. 3A–E, red triangles) elicited heterogeneous recipient-specific responses. However, compared with the range of responses observed for healthy donor microbiomes, IBD-derived microbiomes generally induced lower IL-10 and IL-22 responses, whereas no consistent pattern was observed for IL-17, IFN-γ, or TNF-α. These findings further support the concept that immune responses measured by the GMLR assay emerge from the interaction between a given microbial community and the recipient immune system.

To optimise donor-recipient matching, we developed a simple algorithm to calculate a compatibility index based on the interactions between recipient immune responses and donor microbiota. Since TNF-α expression was uniformly modulated across all donor stimulations and provided limited discriminatory value (Fig. 3E), we excluded this cytokine from further algorithmic steps.

We then assessed whether each donor (S_i) met the following conditions:

Step 1: Cytokine response quantification upon donor microbiome stimulation

For each recipient, cytokine responses upon stimulation with each donor microbiome were quantified as fold-change relative to unstimulated LPMCs and expressed as $\log_2$ fold-change ($\log_2$FC):

$$\log_2 FC\left(\text{Cytokine}_j, S_i\right) = \log_2\left(\frac{E_j(S_i)}{B_j}\right)$$

where:

- $E_j(S_i)$ is the expression level of cytokine j after stimulation with donor S_i microbiome
- B_j is the basal expression level of cytokine j in unstimulated LPMCs

These $\log_2$FC values are then used for downstream donor selection process.

Step 2: Selection of suitable donors for FMT

Each donor (S_i) was evaluated based on predefined criteria reflecting a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory responses:

$$\begin{aligned}\text{condition}\,(S_i) = &\left(\log_2 FC\left(IFN_{-\gamma}, S_i\right) < 0\right)\\ &\left(\log_2 FC\,(IL-17, S_i) < 0\right)\\ &\left(\log_2 FC\,(IL-10, S_i) > 0\right)\\ &\left(\log_2 FC\,(IL-22, S_i) > 0\right)\end{aligned}$$

samples. **B)** Principal Coordinate Analysis (PCoA) of microbial beta-diversity as measured by Bray–Curtis dissimilarity index (PERMANOVA, R^2 = 0.43579, F-stat = 2.7033, p < 0.001). **C)** Observed number of amplicon sequence variants (ASVs) from Cluster 1 (C1), Cluster 2 (C2) donors and IBD patient microbiomes (n = 2) (ordinary one-way ANOVA; *FDR-corrected p < 0.05). **D)** Relative abundance of the differentially enriched genera identified by pairwise comparisons between C1 and C2 (exact FDR-corrected p-values are shown). **E)** Genus vs log2FC plot and **F)** volcano plot showing the significantly enriched ASVs (FDR p < 0.05) by DEseq2 analysis. The names of the significantly enriched ASVs classified to the genus level with FDR p < 0.01 and $\log_2$FC > 2 are also reported.

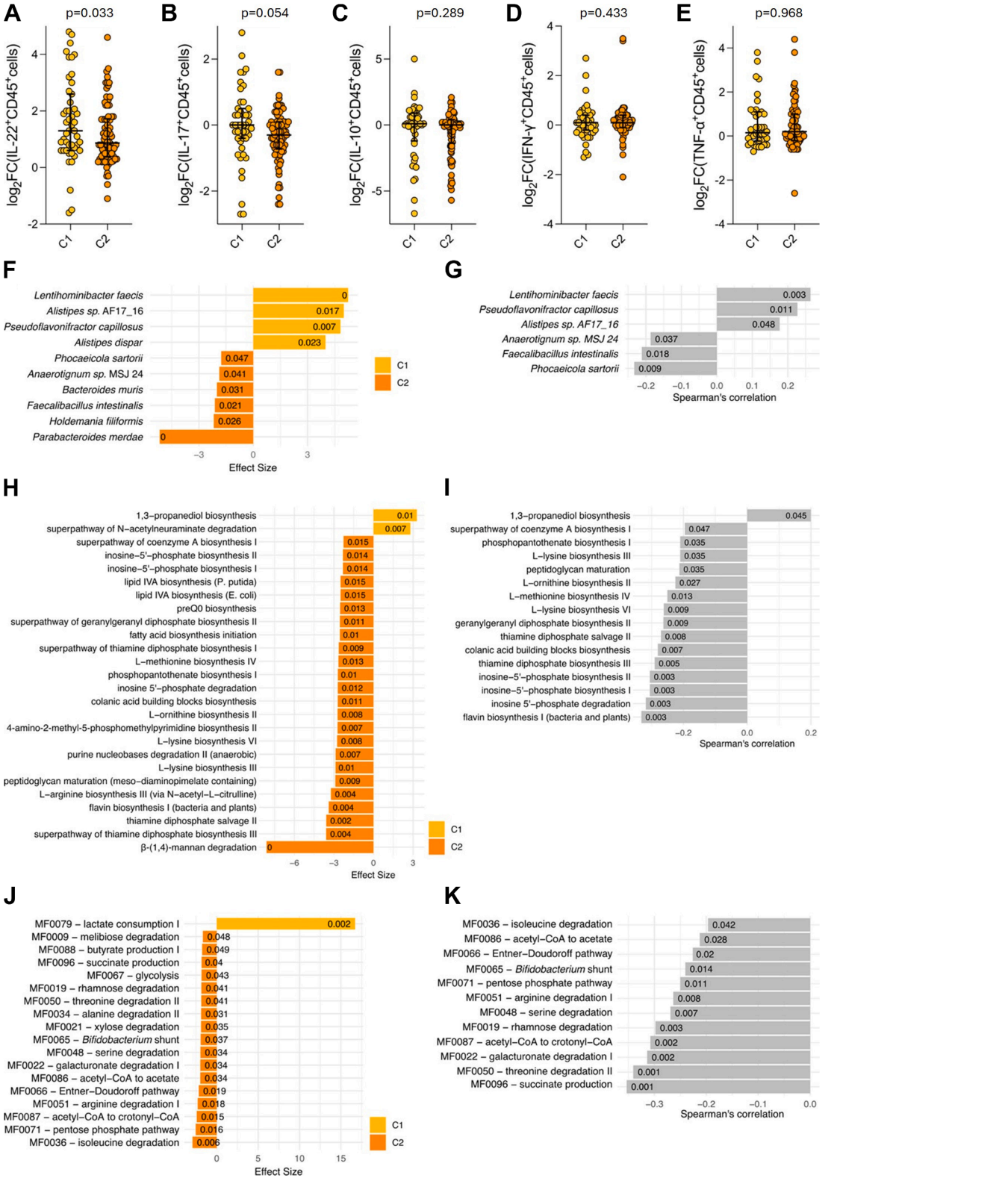

***Fig. 2:* Donor gut microbiomes principally modulate IL-22-mediated responses.** Log_2FC of the frequency for **A)** IL-22$^+$, **B)** IL-17$^+$, **C)** IL-10$^+$, **D)** IFN-γ$^+$ and **E)** TNF-α$^+$ CD45$^+$ immune cells from LPMCs of potential IBD recipients (n = 16) stimulated with the C1 and C2 microbiomes

Only donors meeting these criteria were retained for further analysis.

Step 3: Compatibility index calculation
For each donor (S_i), the **compatibility index** $C(S_i)$ was calculated as:

$$C(S_i) = \sum_j \left| log_2\ FC\left(\text{Cytokine}_j, S_i\right)\right|$$

where j represents the cytokines considered in the analysis.

Step 4: Selection of the most compatible donor
When multiple donors met the selection criteria for a given recipient, the donor with the **highest compatibility index** was selected:

$$S^* = \arg\max_{S_i} C(S_i)$$

This approach prioritises donors inducing the most pronounced and favourable cytokine modulation for each individual recipient.

Using this algorithm, we identified zero, one, or multiple compatible donors for each recipient (Fig. 4A). In cases where multiple donors were suitable for a given recipient, the donor with the highest compatibility index represented the optimal match (Fig. 4B).

Discussion

FMT outcomes in IBD patients are influenced by several factors, including donor microbiota composition and recipient immune status.[13] We propose a functional immune-based approach to guide donor-recipient matching. Current protocols often adopt a one-size-fits-all approach, which does not account for disease heterogeneity and the individualised nature of host–microbiome interactions.[46] Our findings challenge this paradigm, indicating that, beyond the donor microbiota composition, the way in which the host immune system senses and responds to the transplanted microbiome is likely a critical determinant of outcome, conceptually analogous to donor-recipient compatibility in organ transplantation.[25] To address this, we developed a preclinical, POC tool to assess compatibility between donor microbiota and the recipient immune system. This assay, which we named Gut Microbiome–Leukocyte Reaction (GMLR), is inspired by the mixed lymphocyte reaction (MLR) assay used to assess donor-recipient compatibility for organ transplantation. Rather than focussing solely on microbiota composition, this approach evaluates recipient immune responses, including IL-10, IL-22, IL-17, and IFN-γ cytokines that are crucial for IBD pathophysiology.[26]

In this study, we also characterised donor microbiomes to better understand their immunomodulatory potential. Donors clustered into two distinct groups, each characterised by unique microbial profiles. Cluster 1 (C1) was enriched in *Akkermansia, Dorea, Ruminococcaceae, Alistipes*, and *Pseudoflavonifractor*, genera previously associated with anti-inflammatory properties.[47,48] Notably, *Akkermansia* is known for its role in maintaining mucosal barrier integrity and its potential anti-inflammatory effects, which may be particularly relevant for IBD patients with disrupted mucosal function.[49] Cluster 2 (C2) exhibited a higher abundance of *Bacteroides, Lachnospiraceae, Parabacteroides, Clostridium, Blautia, Lactobacillus*, and *Coprococcus*, many of which are known SCFA producers and play crucial roles in gut homoeostasis (Fig. 1).[47,48]

Despite these compositional differences, donor microbiome clusters exerted only partial and pathway-specific immune effects (Fig. 2). C1 promoted IL-22 expression, a cytokine essential for mucosal healing,[50] whereas IL-17 showed only a non-significant trend toward reduction in response to C2, which was not consistently observed across immune cell subsets.[51] Notably, specific bacterial species, including *L. faecis, P. capillosus* and *A. spp.*, correlated positively with IL-22 responses, while microbial metabolic functions, SCFA-related pathways,[52] showed inverse associations (Fig. 2). These findings suggest that microbial composition and function may influence specific immune pathways, although not in a uniform or predictive manner.

A central observation of our study is the marked inter-individual variability in immune responses to donor microbiota. The ability of the recipient's immune system to tolerate or reject the introduced microbiota is critical, as an overly aggressive immune response could lead to inflammation, counteracting the potential benefits of FMT.[53] This is particularly relevant in IBD,

from FMT donors (n = 8). Log_2FC were calculated compared to the baseline frequency of each cytokine analysed in unstimulated LPMCs from IBD patients. All individual samples are shown together with median and IQR; (Mann–Whitney test; exact p-values are shown) **F)** Bacterial species identified by WMS significantly enriched in C1 and C2 microbiomes (Welch t-test; exact p-values are shown). A positive effect size indicates enrichment in C1 while a negative effect size indicates enrichment in C2. **G)** The significantly enriched species from FMT donors correlate with IL-22 responses in $CD45^+$ immune cells (Spearman's correlation test; exact p-values are shown). **H)** MetaCyc functional pathways significantly enriched in C1 and C2 microbiome (Welch t-test; FDR-corrected p-values are shown). **I)** Correlation analysis of the significantly enriched MetaCyc functional pathways with IL-22 responses in $CD45^+$ immune cells (Spearman's correlation test; exact p-values are shown). **J)** Gut Metabolic Modules (GMM) significantly enriched in C1 and C2 microbiomes (Welch t-test; FDR-corrected p-values are shown). **K)** Correlation analysis of the significantly enriched GMM with IL-22 responses in $CD45^+$ immune cells (Spearman's correlation test; exact p-values are shown).

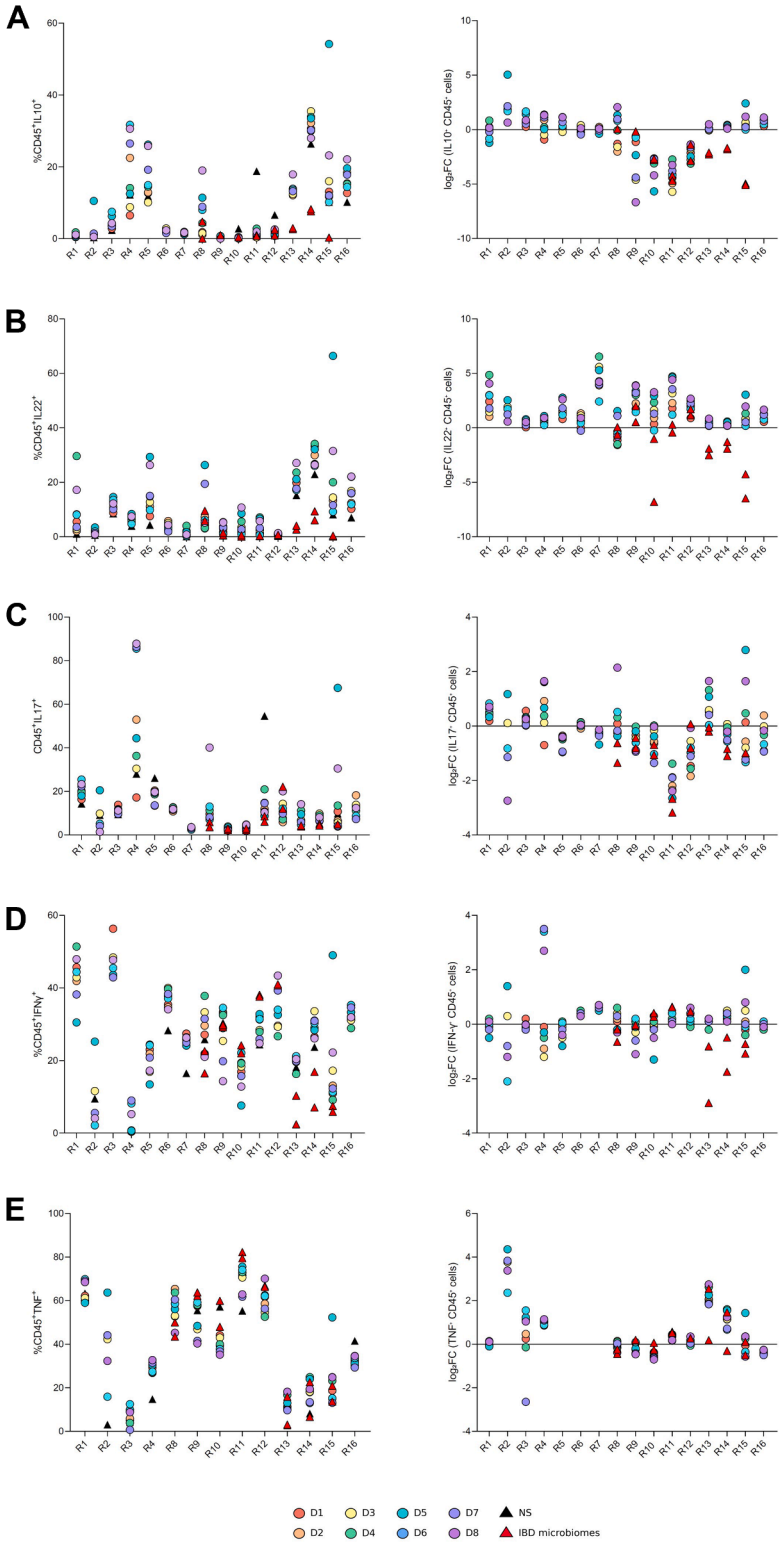

Fig. 3: **Immunological reactivity of recipient-derived LPMCs following stimulation with healthy donor and IBD-associated microbiomes.** Frequency and log_2 fold change (log_2FC) of **A**) IL-10$^+$, **B**) IL-22$^+$, **C**) IL-17$^+$, **D**) IFN-γ$^+$ and **E**) TNF-α$^+$ CD45$^+$ cells from potential IBD recipients (n = 16) following stimulation with faecal microbiota from healthy donors (D1-D8) or IBD patients (IBD1 and IBD2; red triangles). Left panels show the frequency of cytokine-positive cells, while right panels report the corresponding log_2FC relative to unstimulated recipient-derived LPMCs. Black triangles indicate unstimulated recipient-derived LPMCs (NS). Log_2FC values were calculated relative to the baseline frequency of each cytokine measured in unstimulated recipient-derived LPMCs.

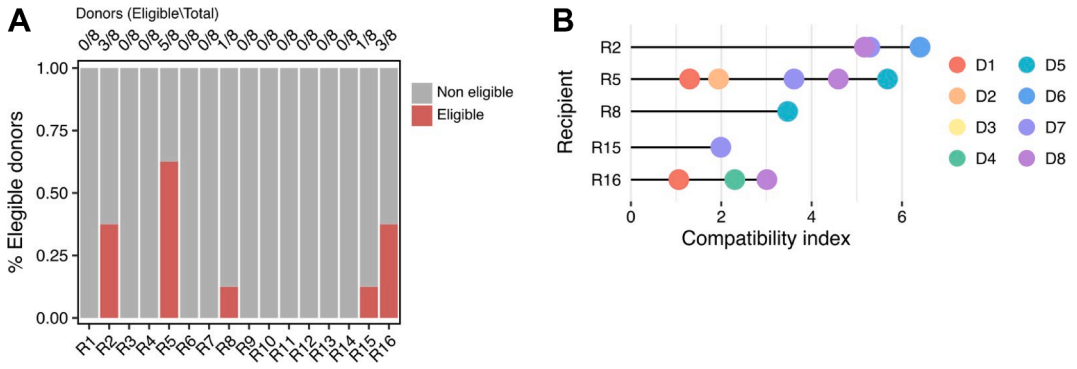

Fig. 4: **Selection of compatible FMT donors following GMLR algorithm use. A**) Frequency of eligible donors identified for each recipient (n = 16). **B**) Compatibility indexes of eligible donor-recipient matches; when multiple donors fulfilled the eligibility criteria for the same recipient, the donor with the highest compatibility index was considered the optimal match.

where immune dysregulation is a central feature of disease pathology.[53] Accordingly, we observed significant inter-individual variability in cytokine responses across recipients, underscoring the personalised nature of immune modulation in FMT. While some donors promoted beneficial immune shifts by enhancing anti-inflammatory and suppressing pro-inflammatory cytokines, others induced mixed or predominantly pro-inflammatory responses, which could be detrimental to FMT success (Fig. 3). This variability underscores that donor effects are not universally transferable across recipients and challenges the concept of a universal "super-donor".[46] Instead, our data support a recipient-specific model of donor selection based on functional immune compatibility.

To address the complexity of donor–recipient interactions, we developed a compatibility index based on recipient cytokine responses to donor microbiota. This algorithm provides a structured platform to support donor-recipient matches based on functional immune readouts (Fig. 4). Previous attempts were made to rationally select the best microbiome donors. In the RESTORE-UC trial, donors were chosen based on microbial load, enterotype, and specific genera abundance.[54] Similarly, in the FMT-AID trial, rural donors with greater microbial richness and healthier microbiome profiles were selected among the healthy population for the donor's pool.[55] However, in both cases donor microbiome composition alone was insufficient to predict efficacy, as host–microbiome interactions were probably overlooked.

Few recent studies introduced the concept that an effective FMT microbiome should promote immune regulation in the host.[56,57] In the first study, FMT donors were selected for their ability to induce Treg expansion and SCFA production *in vivo* by using humanised preclinical models as proxy; however, these features did not translate into clinical efficacy in humans.[56] A Spanish study proposed instead a pre-screening system of donor microbiomes, evaluating the immunomodulatory potential before FMT administration.[57] Donor stool supernatants were tested for activation of cells from recipient rectal biopsies. Microbiomes eliciting weaker pro-inflammatory responses were used for FMT in four UC patients, but no clear clinical benefit emerged. A possible drawback of this approach was probably the use of cell free supernatants rather than of the whole microbiome, limiting breadth in the immune system stimulation. Our approach has several strengths, including the evaluation of intracellular pro- and anti-inflammatory cytokines linked to pathogenic and tolerogenic immune functions in IBD, the use of the whole donor microbiome, and the generation of a quantitative score.

Nevertheless, this study has several limitations. First, the proposed compatibility index has not been clinically validated. While our *ex vivo* data demonstrate that different donor microbiomes can differentially modulate recipient immune responses in a personalised manner, we have not yet assessed whether these immunomodulatory effects translate into improved FMT outcomes in patients. Second, we have yet to establish a definitive threshold for compatibility that determines whether a donor microbiome sufficiently modulates the recipient's immune system to achieve therapeutic success. Establishing such a threshold is essential for integrating this approach into clinical practice, ensuring that donor selection is based not only on relative compatibility but also on validated criteria linked to patient outcomes. Our study moreover underscores that identifying the optimal donor-recipient match is not always guaranteed, as only five of our recipients found a predicted suitable donor. However, expanding the size and diversity of the microbiome biobank could increase the likelihood of successful matches. Future work should focus on correlating immune modulation with clinical response data to refine the predictive power of the compatibility index and define actionable thresholds for donor selection in FMT. A further limitation of this proof-of-concept study

is that longitudinal samples were not available, precluding assessment of the temporal stability of GMLR responses within the same recipient. As mucosal immune responses may vary according to disease activity and treatment exposure,[58] the GMLR assay should be regarded as a functional matching test performed close to the planned FMT procedure. Future prospective studies are needed to evaluate its temporal stability and reproducibility. The limited sample size also precluded meaningful sex-stratified analyses; therefore, potential sex-related differences in microbiome–immune interactions could not be assessed and should be investigated in larger cohorts. Finally, given emerging evidence that host genetic factors, including HLA genotype, may influence microbiota composition and immune–microbial interactions,[59] future prospective studies should integrate HLA profiling with GMLR readouts to assess whether genetic background contributes to donor-recipient compatibility and could complement functional immune-based donor selection.

In conclusion, our study supports a shift from donor-centric to recipient-informed strategies in FMT for IBD. By demonstrating that immune responses to donor microbiota are highly individualised, we provide a rationale for personalised donor selection. While our compatibility index represents a preclinical tool that requires further validation, it offers a scalable and biologically grounded platform for integrating immune profiling into FMT strategies. Future studies should aim to validate this approach in prospective clinical settings and to define actionable criteria for donor–recipient matching in microbiome-based therapies.

Contributors

FF and FC conceived the study. FF, FC, CA, FS designed the experiments. FF, FC and FS supervised the experiments. CA, PM, BC, DN, MG, MM, SF, FP performed the experiments. FF, FC, MV contributed with reagents and resources. FS and PM performed microbiome data analyses. FS and CA wrote the first draft of the manuscript. All authors reviewed and critically edited the manuscript. FF and FC had access to and verified the underlying data. All authors contributed to the article and approved the submitted version.

Data sharing statement

16S rRNA gene sequencing and whole-metagenome shotgun sequencing data are available in the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena) under accession numbers PRJEB88602 and PRJEB101484, respectively. The underlying immune-response data generated from the GMLR experiments and used to calculate the donor-recipient compatibility index are available from the corresponding authors upon reasonable request for research purposes, following submission and approval of a scientifically justified request.

Declaration of interests

The authors have declared that no conflict of interest exists.

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During the preparation of this work, the authors used ChatGPT (OpenAI) solely to improve the readability and language of the manuscript. The authors reviewed and confirmed the validity of the text and take full responsibility for the content of the publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2026.106475.

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