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# Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients

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The gut microbiome has been shown to influence the response of tumors to anti-PD-1 immunotherapy in pre-clinical mouse models and observational patient cohorts. However, modulation of gut microbiota in cancer patients has not been investigated in clinical trials. Here we performed a phase I clinical trial to assess the safety and feasibility of fecal microbiota transplantation (FMT) and re-induction of anti-PD-1 immunotherapy in ten patients with anti-PD-1-refractory metastatic melanoma. We observed clinical responses in three patients, including two partial responses and one complete response. Notably, treatment with FMT was associated with favorable changes in immune cell infiltrates and gene expression profiles in both the gut lamina propria and the tumor microenvironment. Together, these early findings have important implications for modulating the gut microbiota in cancer treatment.

Immunotherapy to inhibit the programmed cell death (PD)-1 checkpoint protein in metastatic melanoma patients has demonstrated durable complete response (CR) rates of 10–20% (1). However, the majority of patients do not respond to PD-1 blockade, and many of the partially responding patients eventually progress (1). Extensive research efforts have been undertaken to overcome resistance to anti-PD-1 therapy. One of the most promising leads involves modulation of the gut microbiota (2–4), which has been shown to have a profound effect on the development and function of the immune system (5). While no specific bacterial taxa have been consistently associated with clinical response to immunotherapy (6), fecal microbiota transplantation (FMT) - which transfers the entire gut microbiota from one host to another - has demonstrated promising results in pre-clinical

models (2–4). Compared to mice that received FMT from melanoma patients not responding to anti-PD-1 therapy, mice that received FMT from responders demonstrated increased intra-tumoral CD8<sup>+</sup> T-cell infiltration and enhanced overall effectiveness of anti-PD-1 therapy (2, 3). Based on these data, we designed a phase I clinical trial (NCT03353402) to assess the safety, feasibility and immune cell impact of FMT and re-induction of anti-PD-1 immunotherapy in patients with refractory metastatic melanoma.

The trial included two FMT donors who had previously been treated with anti-PD-1 monotherapy for metastatic melanoma and achieved a CR for at least one year (table S1 and materials and methods). Patients were considered eligible FMT recipients if they had a diagnosis of metastatic

melanoma and had progressed on at least one line of anti-PD-1 therapy. Recipients harboring a BRAF-V600E mutation must have also progressed on BRAF-targeted therapy. As part of the trial's protocol, recipients underwent an initial "native microbiota depletion" phase in which they were administered with orally ingested antibiotics (vancomycin and neomycin) for 72 hours (Fig. 1A). FMT was then performed via both colonoscopy (protocol day 0) and administration of oral stool capsules followed by re-induction of anti-PD-1 therapy (nivolumab). Six combined treatment cycles composed of anti-PD-1 infusions (nivolumab at standard dose) and additional stool capsules (maintenance FMT) were administered every 14 days until day 90. Each recipient underwent positron emission tomography combined with computed tomography (PET-CT) imaging before the trial and on day 65. Response to treatment was defined as an objective tumor regression per imaging according to iRECIST criteria (7). Objective responders and recipients who demonstrated a clinical benefit to the treatment continued anti-PD-1 beyond day 90 as monotherapy and underwent consecutive PET-CTs in intervals of 6-8 weeks until disease progression.

Correlative studies included stool, gut and tumor analyses (see materials and methods). 16S rRNA gene and metagenomics sequencing were conducted on stool samples which were collected from recipients up to one week before the native microbiota depletion phase (defined as pre-treatment), and on stool samples collected on days 7, 31, and 65. Donor stool samples were collected during the fecal donation period. Gut and tumor biopsies were collected pre-treatment and at days 31 and 70, respectively. Infiltration and activity of immune cells in the tissue samples were assessed using immunohistochemical (IHC) and bulk RNA sequencing (RNA-seq). In cases where no specific gene differed in a statistically significant manner, gene set testing was conducted using the Gene Ontology (GO) dataset. Recipient #2 refused to undergo post-treatment biopsies and withdrew consent immediately after the day 65 imaging assessment, leaving nine available recipients for gut and tumor tissue assessment.

Ten recipients with confirmed progression on anti-PD-1 therapy were enrolled and treated between June 2018 and March 2019 (Table 1 and table S2). Recipients were assigned to receive FMT from one of the two available donors, alternating between Donor #1 and Donor #2. The median recipient age was 66 years (IQR 49-68), the majority were males (70%), and the median elapsed time from the last previous dose of anti-PD-1 to the first dose of the current trial was 113 days (IQR 59-183). The most common PD-L1 expression category in pre-treatment tumor biopsies was  $\geq 5\%$ . This expression category was noted in four recipients, while three recipients had no pre-treatment PD-L1 expression (table S2). In terms of safety results, the only observed FMT-related adverse event was mild bloating between days 3 to 15 in one recipient.

Several mild (grade 1) immune-related Adverse Events (irAEs) were observed, mainly arthralgia (table S3). No moderate to severe irAEs (grade 2-4) were observed, although five recipients had developed such irAEs during their previous anti-PD-1 treatment lines (table S4).

Objective responses to treatment were demonstrated by three recipients, all of them from the Donor #1-group: Recipient #3 achieved a complete response, and Recipients #5 and #7 achieved partial responses (Fig. 1B, Table 1, and table S5). All responders crossed the progression-free survival milestone of six months. Both Recipients #3 and #5 demonstrated an initial increase in their metastases size, followed by regression (Fig. 1, B and C, and fig. S1). This phenomenon is known as pseudo-progression (8), because the increment in metastasis radiological volumes is not caused by tumor proliferation; but rather caused by an influx of anti-tumoral immune cells into the tumor. Recipient #1 (Donor #1-group) demonstrated an initial mix response with regression of some of lesions, but overall disease had progressed (fig. S2).

Stool 16S rRNA gene sequencing analysis demonstrated that post-treatment gut microbiota composition of all recipients significantly differed from their baseline ( $\beta$ -diversity, Unweighted UniFrac,  $p=0.02$ , FDR=0.05, Fig. 2A and figs. S3 and S4). There was no statistically significant difference between the pre-treatment microbiota composition of recipients from the Donor #1-group and those of the Donor #2-group ( $p=0.36$ , FDR=0.45). However, post-treatment microbiota compositions of the Donor #1-group recipients differed from those of the Donor #2-group ( $p=0.001$ , FDR=0.003, Fig. 2A). Donor #2 had a higher microbiota richness ( $\alpha$ -diversity, Faith's Phylogenetic Diversity) in comparison to Donor #1. In accordance, despite similar richness in the pre-treatment compositions ( $p=0.60$ , FDR=0.77), post-treatment compositions of the Donor #2-group demonstrated higher richness than that of the Donor #1-group ( $p<0.001$ , FDR=0.001, fig. S5). Metagenomic sequencing was used to identify specific taxa and functional pathways that differed between the trial's groups. Overall comparison between recipient pre- and post-treatment microbiota compositions (ANCOM test) showed that post-treatment compositions had a higher relative abundance of the immunotherapy-favorable *Veillonellaceae* family (3) and a lower relative abundance of *Bifidobacterium bifidum*, which was reported to promote immune tolerance via T-regulatory cells (9) (figs. S6 and S7). Both donors had previously reportable immunotherapy-favorable features (fig. S8 and table S6) such as high relative abundance of *Lachnospiraceae* (both donors) *Veillonellaceae* (Donor #1) and *Ruminococcaceae* (Donor #2) (fig. S8) (2-4). Comparison of post-treatment recipient microbiota compositions by their assigned donors demonstrated that the Donor #1-group was characterized by higher relative abundance of taxa like *Bifidobacterium adolescentis* (Fig. 2B), while the Donor #2-

group had high relative abundance of taxa like *Ruminococcus bromii* (table S7) - both were previously described as immunotherapy-favorable (2, 3). The pre-treatment microbiota compositions of the three responding patients (Recipients #3, 5 and 7) did not differ from the pre-treatment microbiota compositions of rest of the cohort. When the responders' post-treatment compositions were compared to post-treatment compositions of the other two non-responders from the Donor #1-group (Recipients #1 and #9), four taxa differed in a statistically significant manner (fig. S9A). The responders had a higher relative abundance of *Enterococcaceae*, *Enterococcus*, and *Streptococcus australis*, and a lower relative abundance of *Veillonella atypica*. However, when the abundance of these specific taxa was assessed in the entire patient cohort (fig. S9B), there were some non-responders and even pre-treatment samples with similar dynamics. Hence, no clear association between those taxa and clinical response to therapy were established. Functional metabolic data were based on annotation of genes to the MetaCyc database (direct measurements metabolite levels were not conducted). The functional metabolic data demonstrated that the Donor #1-group up-regulated the lactose and galactose degradation I pathway (logFC=1, FDR=0.015) while the Donor #2-group up-regulated the formaldehyde assimilation II (logFC=2.2, FDR=3.93e<sup>-6</sup>), formaldehyde oxidation I (logFC=2.4, FDR=0.001) and creatinine degradation I (log FC=1.4, FDR=0.014) pathways. Metagenomics GO gene sets which significantly differed between the microbiota of the two donor groups were illustrated in Fig. 2C (table S8). Comparison between the post-treatment microbiota composition of the responding recipient patients #3, #5, and #7 with the other two non-responding patients among the Donor #1-group (#1 and #9) showed no significant functional or metabolic differences.

Gut sample analysis of all available FMT recipient patients demonstrated a post-treatment up-regulation of gene sets which were related to the presentation of peptides by antigen presenting cells (APCs) via Major Histocompatibility Complex (MHC) class I, and interleukin-1 mediated signaling (FDR=0.014 and FDR=0.038, respectively, table S9). Analysis per donor-group demonstrated that the Donor #1-group recipients up-regulated additional gene sets related to APCs activity, innate immunity, and interleukin-12 (table S10). In contrast, the Donor #2-group recipients did not up-regulate any immune-related gene sets (table S11). Per patient analysis demonstrated an increased lamina propria infiltration of CD68<sup>+</sup> cells, representing APCs, from an overall pre-treatment median of 353 cells/mm<sup>2</sup> to 569 cells/mm<sup>2</sup> post-treatment (p=0.05, Fig. 3, A to C, and fig. S10). The CD68<sup>+</sup> infiltration was concentrated in the sub-epithelial area, where the proximity to the gut lumen is the highest. All available recipients increased the post-treatment CD68<sup>+</sup>

infiltration except for Recipient #6 (Donor #2-group, non-responder patient). Notably, gut sample analysis did not demonstrate a statistically significant increase in T cell infiltration in the gut lamina propria (fig. S11), nor T cell related gene set enrichment.

Tumor sample analysis of all available recipients demonstrated post-treatment up-regulation of multiple immune-related gene sets (Fig. 3D and table S12), such as Interferon- $\gamma$  mediated signaling pathway (FDR=1.65e<sup>-13</sup>), T cell activation (FDR=3.27e<sup>-12</sup>), MHC Class II protein complex (FDR=9.31e<sup>-13</sup>), dendritic cell differentiation (FDR=5.15e<sup>-9</sup>) and T helper 1 type immune response (FDR=1.06e<sup>-6</sup>). While these immune-related gene sets remained enriched in the Donor #1 group only analysis (table S13), no immune-related gene sets were statistically significant enriched among tumor samples of the Donor #2-group (table S14). Per patient analysis demonstrated increased post-treatment intra-tumoral CD8<sup>+</sup> T cell infiltration among five patients (#1, #3, #4 #7, and #10) with an overall pre-treatment median of 89 cells/mm<sup>2</sup> versus 282 cells/mm<sup>2</sup> post-treatment (p=0.06, Fig. 3, E to G, and fig. S12). Recipient #5 achieved a near-pathological complete response, as post-treatment viable tumor tissue composed <1% of the entire biopsy, and Recipients #3 and #7 achieved partial pathological response (Table 1). Assessment of commonly investigated genes related to intra-tumoral immune activity demonstrated that the post-treatment tumors of Recipients #1, #3, #5 and #7 up-regulated effector-related genes with some reciprocal exhaustion responses (Fig. 3H). Recipient #10, however, up-regulated exhaustion-related genes without an effector response.

This study demonstrated that the combination of FMT from a CR donor and re-induction of anti-PD-1 therapy in refractory metastatic melanoma patients was safe, feasible and potentially effective. FMT is considered common treatment for recurrent *Clostridioides difficile* colitis, with a well-established safety profile (10), and its safety was demonstrated even in immunocompromised patients (11). Still, the lack of FMT-related complications in the current study among immunocompetent metastatic patients treated with repeated FMTs was reassuring. Interestingly, the combination of FMT and re-induction of anti-PD-1 therapy appeared safe and also resulted in some objective clinical responses. Out of ten anti-PD-1 refractory recipients, three demonstrated clinical responses including one CR. A similar trial of FMT and anti-PD-1 re-induction in refractory melanoma patients reported preliminary results of one objective partial response and one stable disease among the first three patients (12). Since the FMT recipient patients were not treatment naïve, there is a possibility that these clinical outcomes are due to delayed responses to previous anti-PD-1 treatments. However, this possibility is unlikely as Ribas *et al.* reported that delayed response rates in metastatic melanoma patients who

continued anti-PD-1 therapy beyond RECIST-confirmed disease progression were <8% (13). Similarly, Betof Warner *et al.* reported that response rates of metastatic melanoma patients who were re-induced with anti-PD-1 monotherapy were 5/34 (<15%) (14). These results were possibly an over-estimation since 3/5 responders in that report had an elapsed previous-to-re-induction dose time of at least 12 months (14). Such a prolonged time period might enable the re-emergence of immunotherapy-susceptible tumor clones. In our study, the median previous-to-re-induction dose time was only 113 days among the entire cohort and 119 days among the three responders. Moreover, the inclusion criterion of our trial was disease progression on previous anti-PD-1 lines based on iRECIST. According to RECIST 1.1, partial or complete responses may be deemed “unconfirmed” pending follow-up, but the classification of progressive disease is always considered final (15). However, immunotherapies might sometime lead to pseudo-progression (8), as seen in Recipients #3 and #5. iRECIST was designed to distinguish between unconfirmed and confirmed disease progression (7) (table S5). Hence, it is possible that the use of iRECIST in those previous publications would have resulted in even lower-post-failure response rates.

This higher than expected clinical response rate can be explained by the correlative immunological data. Tumor infiltrating DCs have a crucial role in trafficking T cells into tumors (16, 17). Multiple reports from mouse models studies have demonstrated that microbiota modulation promoted infiltration of DCs into remote tumors, which resulted in activation of both T-helper 1 cells via interleukin-12 (4, 18) and cytotoxic CD8<sup>+</sup> T cells (19–21). The same findings were demonstrated in our human FMT trial. Since the donors’ microbiota were transplanted into the recipients’ gut, it is plausible to assume that the immune activation cascade started in the gut. Indeed, the Donor #1-group recipients demonstrated increased post-treatment gut infiltration and activity of APCs. Geva-Zatorsky *et al.* assessed the immune response to colonization of different commensal gut microbes and demonstrated that the local effect of microbes in the gut was mostly on the innate immunity cells (22), which could later migrate into the lymphatic system (23). Notably, some of the Donor #2-group recipients also increased their post-treatment gut APC infiltration, although as a group their RNA-seq findings were not statistically significant. Overall, the recipients who increased their post-treatment intra-tumoral CD8<sup>+</sup> T cell infiltration had also increased their APC gut infiltration. It is unlikely that the increment in CD8<sup>+</sup> T cell infiltration was due to the mere anti-PD-1 administration, since Chen *et al.* used pre- and on-treatment tumor biopsies to demonstrate that non-responding patients undergoing anti-PD-1 therapy did not increase their intra-tumoral CD8<sup>+</sup> infiltration (24). However, microbiota-driven gut APC activation

would not necessarily yield enhanced intra-tumoral CD8<sup>+</sup> activity. Impaired antigen presentation machinery within the tumor cells themselves is a well-known anti-PD-1 resistance mechanism and usually results in lack of intra-tumoral CD8<sup>+</sup> T cell infiltration (25). Indeed, the tumor from Recipient #9 demonstrated such an antigen presentation impairment (fig. S13). Moreover, even the presence of high intra-tumoral infiltration of CD8<sup>+</sup> T cells sometimes fails to translate into a clinical response. Tumors with high CD8<sup>+</sup> T cell infiltration can be refractory if the T cell infiltration is ineffective, for example due to CD8<sup>+</sup> T cell exhaustion after exposure to additional immune checkpoints (26). Recipient #10 had overexpression of these molecules, such as IDO-1 (Fig. 3H). Recipient #1, who demonstrated increased intra-tumoral CD8<sup>+</sup> T cell activity, had an initial regression in some metastases but eventually progressed due to an unknown cause. These tumor characteristics of different patients emphasize the wide context of clinical responses to immunotherapy, and that beneficial microbiota composition is not the only factor in treatment response.

The microbiota composition of the two donors and the post-treatment recipients from both donor-groups were characterized by high relative abundance of taxa that were previously associated with response to immunotherapy. Yet, the three responding recipients were solely part of the Donor #1-group. The reason for this dissonance is unclear. However, this study was statistically powered to assess safety, and was not designed to compare efficiency between donors. Lack of clinical responders among Donor #2-group does not necessarily implicate that clinical responses could not be observed in a larger cohort. Moreover, our inability to pinpoint specific “response-inducer” microbiota characteristics echoes the inconsistency among previous observational reports (6). As the characteristics of optimal microbiota compositions of donors and recipients remain elusive, the design and implementation of future microbiome modulation clinical trials must be carefully considered. Numerous considerations must be taken into account when contemplating strategies to modulate gut microbes, including diet (27). Studies in pre-clinical models incorporating microbiota into germ-free mice “avatars” may yield insight into both microbe and host factors. Nonetheless, in light of the decades-based safety profile of FMTs (10), promising results in pre-clinical models (2–4, 18, 19, 21) and findings suggesting treatment effectiveness in our current clinical trial, clinical institutions should not be deterred by the lack of sufficient mechanistic knowledge to examine the clinical potential of FMTs in the setting of well-designed and supervised human trials. This is especially true for refractory patients, in whom the risk-benefit ratio of FMTs appears favorable.

One limitation of this clinical trial arises from the use of antibiotics as part of the pre-FMT preparation. Antibiotic



preparation was adopted since it enhanced the FMT ability to modulate microbiota composition in reported murine models (28). The vancomycin-neomycin protocol was reported as an effective pre-FMT protocol in humans (29). Since all of our recipients underwent the exact same pre-FMT protocol, we believe that the use of antibiotic did not affect the observed immune and clinical outcomes. However, this possibility cannot be ruled out in the current study design.

In conclusion, FMT from CR donors and re-induction of anti-PD-1 therapy in refractory metastatic melanoma patients was safe and feasible. In some patients, this treatment increased the intra-tumoral immune activity, which was translated into objective clinical responses. These findings support the concept of overcoming resistance to immunotherapy by modulating the gut microbiota.

## REFERENCES AND NOTES

1. P. A. Ascierto, G. V. Long, C. Robert, B. Brady, C. Dutriaux, A. M. Di Giacomo, L. Mortier, J. C. Hassel, P. Rutkowski, C. McNeil, E. Kalinka-Warchoła, K. J. Savage, M. M. Hernberg, C. Lebbé, J. Charles, C. Mihalciou, V. Chiarion-Sileni, C. Mauch, F. Cognetti, L. Ny, A. Arance, I. M. Svane, D. Schadendorf, H. Gogas, A. Sazi, J. Jiang, J. Rizzo, V. Atkinson, Survival Outcomes in Patients With Previously Untreated BRAF Wild-Type Advanced Melanoma Treated With Nivolumab Therapy: Three-Year Follow-up of a Randomized Phase 3 Trial. *JAMA Oncol.* **5**, 187–194 (2019). [doi:10.1001/jamaoncol.2018.4514](https://doi.org/10.1001/jamaoncol.2018.4514) Medline
2. V. Gopalakrishnan, C. N. Spencer, L. Nezi, A. Reuben, M. C. Andrews, T. V. Karpinets, P. A. Prieto, D. Vicente, K. Hoffman, S. C. Wei, A. P. Cogdill, L. Zhao, C. W. Hudgens, D. S. Hutchinson, T. Manzo, M. P. de Macedo, T. Cotechini, T. Kumar, W. S. Chen, S. M. Reddy, R. S. Sloane, J. Galloway-Pena, H. Jiang, P. L. Chen, E. J. Shpall, K. Rezvani, A. M. Alousi, R. F. Chemaly, S. Shelburne, L. M. Vence, P. C. Okhuysen, V. B. Jensen, A. G. Swennes, F. McAllister, E. M. Riquelme Sanchez, Y. Zhang, E. Le Chatelier, L. Zitvogel, N. Pons, J. L. Austin-Breneman, L. E. Haydu, E. M. Burton, J. M. Gardner, E. Sirmans, J. Hu, A. J. Lazar, T. Tsujikawa, A. Diab, H. Tawbi, I. C. Glitza, W. J. Hwu, S. P. Patel, S. E. Woodman, R. N. Amaria, M. A. Davies, J. E. Gershenwald, P. Hwu, J. E. Lee, J. Zhang, L. M. Coussens, Z. A. Cooper, P. A. Futreal, C. R. Daniel, N. J. Ajami, J. F. Petrosino, M. T. Tetzlaff, P. Sharma, J. P. Allison, R. R. Jenq, J. A. Wargo, Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **359**, 97–103 (2017). [doi:10.1126/science.aan4236](https://doi.org/10.1126/science.aan4236) Medline
3. V. Matson, J. Fessler, R. Bao, T. Chongswat, Y. Zha, M.-L. Alegre, J. J. Luke, T. F. Gajewski, The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science* **359**, 104–108 (2018). [doi:10.1126/science.aao3290](https://doi.org/10.1126/science.aao3290) Medline
4. B. Routy, E. Le Chatelier, L. Derosa, C. P. M. Duong, M. T. Alou, R. Daillère, A. Fluckiger, M. Messaoudene, C. Rauber, M. P. Roberti, M. Fidelle, C. Flament, V. Poirier-Colame, P. Opolon, C. Klein, K. Iribarren, L. Mondragón, N. Jacquolot, B. Qu, G. Ferrere, C. Clémenson, L. Mezquita, J. R. Masip, C. Naltet, S. Brosseau, C. Kaderbhai, C. Richard, H. Rizvi, F. Levenez, N. Galleron, B. Quinquis, N. Pons, B. Ryffel, V. Minard-Colin, P. Gonin, J.-C. Soria, E. Deutsch, Y. Loriot, F. Ghiringhelli, G. Zalcman, F. Goldwasser, B. Escudier, M. D. Hellmann, A. Eggermont, D. Raoult, L. Albige, G. Kroemer, L. Zitvogel, Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91–97 (2017). [doi:10.1126/science.aan3706](https://doi.org/10.1126/science.aan3706) Medline
5. Y. Belkaid, T. W. Hand, Role of the microbiota in immunity and inflammation. *Cell* **157**, 121–141 (2014). [doi:10.1016/j.cell.2014.03.011](https://doi.org/10.1016/j.cell.2014.03.011) Medline
6. V. Gopalakrishnan, B. A. Helmink, C. N. Spencer, A. Reuben, J. A. Wargo, The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell* **33**, 570–580 (2018). [doi:10.1016/j.ccell.2018.03.015](https://doi.org/10.1016/j.ccell.2018.03.015) Medline
7. L. Seymour, J. Bogaerts, A. Perrone, R. Ford, L. H. Schwartz, S. Mandrekas, N. U. Lin, S. Litière, J. Dancey, A. Chen, F. S. Hodi, P. Therasse, O. S. Hoekstra, L. K. Shankar, J. D. Wolchok, M. Ballinger, C. Caramella, E. G. E. de Vries, RECIST working group, iRECIST: Guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol.* **18**, e143–e152 (2017). [doi:10.1016/S1470-2045\(17\)30074-8](https://doi.org/10.1016/S1470-2045(17)30074-8) Medline
8. E. Borcoman, Y. Kanjanapan, S. Champiat, S. Kato, V. Servoir, R. Kurzrock, S. Goel, P. Bedard, C. Le Tourneau, Novel patterns of response under immunotherapy. *Ann. Oncol.* **30**, 385–396 (2019). [doi:10.1093/annonc/mdz003](https://doi.org/10.1093/annonc/mdz003) Medline
9. R. Verma, C. Lee, E.-J. Jeun, J. Yi, K. S. Kim, A. Ghosh, S. Byun, C.-G. Lee, H.-J. Kang, G.-C. Kim, C.-D. Jun, G. Jan, C.-H. Suh, J.-Y. Jung, J. Sprent, D. Rudra, C. De Castro, A. Molinaro, C. D. Surh, S.-H. Im, Cell surface polysaccharides of *Bifidobacterium bifidum* induce the generation of Foxp3<sup>+</sup> regulatory T cells. *Sci. Immunol.* **3**, eaat6975 (2018). [doi:10.1126/sciimmunol.aat6975](https://doi.org/10.1126/sciimmunol.aat6975) Medline
10. U. Iqbal, H. Anwar, M. A. Karim, Safety and efficacy of encapsulated fecal microbiota transplantation for recurrent *Clostridium difficile* infection: A systematic review. *Eur. J. Gastroenterol. Hepatol.* **30**, 730–734 (2018). [doi:10.1097/MFG.0000000000001147](https://doi.org/10.1097/MFG.0000000000001147) Medline
11. S. C. Lin, C. D. Alonso, A. C. Moss, Fecal microbiota transplantation for recurrent *Clostridium difficile* infection in patients with solid organ transplants: An institutional experience and review of the literature. *Transpl. Infect. Dis.* **20**, e12967 (2018). [doi:10.1111/tid.12967](https://doi.org/10.1111/tid.12967) Medline
12. G. Trinchieri, Abstract IA28: Targeting the microbiome in cancer immunotherapy. *Cancer Res.* **80**, IA28 (2020). [doi:10.1158/1538-7445.MVC2020-IA28](https://doi.org/10.1158/1538-7445.MVC2020-IA28)
13. A. Ribas, J. M. Kirkwood, K. T. Flaherty, Anti-PD-1 antibody treatment for melanoma. *Lancet Oncol.* **19**, e219 (2018). [doi:10.1016/S1470-2045\(18\)30202-X](https://doi.org/10.1016/S1470-2045(18)30202-X) Medline
14. A. Betof Warner, J. S. Palmer, A. N. Shoushtari, D. A. Goldman, K. S. Panageas, S. A. Hayes, R. Bajwa, P. Momtaz, M. K. Callahan, J. D. Wolchok, M. A. Postow, P. B. Chapman, Long-Term Outcomes and Responses to Retreatment in Patients With Melanoma Treated With PD-1 Blockade. *J. Clin. Oncol.* **38**, 1655–1663 (2020). [doi:10.1200/JCO.19.01464](https://doi.org/10.1200/JCO.19.01464) Medline
15. E. A. Eisenhauer, P. Therasse, J. Bogaerts, L. H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij, New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur. J. Cancer* **45**, 228–247 (2009). [doi:10.1016/j.ejca.2008.10.026](https://doi.org/10.1016/j.ejca.2008.10.026) Medline
16. S. Spranger, D. Dai, B. Horton, T. F. Gajewski, Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. *Cancer Cell* **31**, 711–723.e4 (2017). [doi:10.1016/j.ccell.2017.04.003](https://doi.org/10.1016/j.ccell.2017.04.003) Medline
17. S. Spranger, T. F. Gajewski, Tumor-intrinsic oncogene pathways mediating immune avoidance. *Oncotarget* **5**, e1086862 (2016). [doi:10.1080/2162402X.2015.1086862](https://doi.org/10.1080/2162402X.2015.1086862) Medline
18. M. Vétizou, J. M. Pitt, R. Daillère, P. Lepage, N. Waldschmitt, C. Flament, S. Rusakiewicz, B. Routy, M. P. Roberti, C. P. M. Duong, V. Poirier-Colame, A. Roux, S. Becharef, S. Formenti, E. Golden, S. Cording, G. Eberl, A. Schlitzer, F. Ginhoux, S. Mani, T. Yamazaki, N. Jacquolot, D. P. Enot, M. Bérard, J. Nigou, P. Opolon, A. Eggermont, P.-L. Woerther, E. Chachaty, N. Chaput, C. Robert, C. Mateus, G. Kroemer, D. Raoult, I. G. Boneca, F. Carbonnel, M. Chamailard, L. Zitvogel, Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science* **350**, 1079–1084 (2015). [doi:10.1126/science.aad1329](https://doi.org/10.1126/science.aad1329) Medline
19. A. Sivan, L. Corrales, N. Hubert, J. B. Williams, K. Aquino-Michaels, Z. M. Earley, F. W. Benyamin, Y. M. Lei, B. Jabri, M.-L. Alegre, E. B. Chang, T. F. Gajewski, Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084–1089 (2015). [doi:10.1126/science.aac4255](https://doi.org/10.1126/science.aac4255) Medline
20. T. Tanoue, S. Morita, D. R. Plichta, A. N. Skelly, W. Suda, Y. Sugiura, S. Narushima, H. Vlamakis, I. Motoo, K. Sugita, A. Shiota, K. Takeshita, K. Yasuma-Mitobe, D. Riethmacher, T. Kaisho, J. M. Norman, D. Mucida, M. Suematsu, T. Yaguchi, V. Bucci, T. Inoue, Y. Kawakami, B. Olle, B. Roberts, M. Hattori, R. J. Xavier, K. Atarashi, K. Honda, A defined commensal consortium elicits CD8 T cells and anti-cancer immunity. *Nature* **565**, 600–605 (2019). [doi:10.1038/s41586-019-0878-z](https://doi.org/10.1038/s41586-019-0878-z) Medline
21. M. Uribe-Herranz, K. Bittinger, S. Rafail, S. Guedan, S. Pierini, C. Tanes, A. Ganetsky, M. A. Morgan, S. Gill, J. L. Tanyi, F. D. Bushman, C. H. June, A. Facciabene, Gut microbiota modulates adoptive cell therapy via CD8 $\alpha$  dendritic cells and IL-12. *JCI Insight* **3**, e94952 (2018). [doi:10.1172/jci.insight.94952](https://doi.org/10.1172/jci.insight.94952) Medline

22. N. Geva-Zatorsky, E. Sefik, L. Kua, L. Pasman, T. G. Tan, A. Ortiz-Lopez, T. B. Yanortsang, L. Yang, R. Jupp, D. Mathis, C. Benoist, D. L. Kasper, Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell* **168**, 928–943.e11 (2017). [doi:10.1016/j.cell.2017.01.022](https://doi.org/10.1016/j.cell.2017.01.022) [Medline](#)
23. Z. Zhang, J. Li, W. Zheng, G. Zhao, H. Zhang, X. Wang, Y. Guo, C. Qin, Y. Shi, Peripheral Lymphoid Volume Expansion and Maintenance Are Controlled by Gut Microbiota via RALDH<sup>+</sup> Dendritic Cells. *Immunity* **44**, 330–342 (2016). [doi:10.1016/j.immuni.2016.01.004](https://doi.org/10.1016/j.immuni.2016.01.004) [Medline](#)
24. P. L. Chen, W. Roh, A. Reuben, Z. A. Cooper, C. N. Spencer, P. A. Prieto, J. P. Miller, R. L. Bassett, V. Gopalakrishnan, K. Wani, M. P. De Macedo, J. L. Austin-Breneman, H. Jiang, Q. Chang, S. M. Reddy, W.-S. Chen, M. T. Tetzlaff, R. J. Broadus, M. A. Davies, J. E. Gershenwald, L. Haydu, A. J. Lazar, S. P. Patel, P. Hwu, W.-J. Hwu, A. Diab, I. C. Glitza, S. E. Woodman, L. M. Vence, I. I. Wistuba, R. N. Amaria, L. N. Kwong, V. Prieto, R. E. Davis, W. Ma, W. W. Overwijk, A. H. Sharpe, J. Hu, P. A. Futreal, J. Blando, P. Sharma, J. P. Allison, L. Chin, J. A. Wargo, Analysis of Immune Signatures in Longitudinal Tumor Samples Yields Insight into Biomarkers of Response and Mechanisms of Resistance to Immune Checkpoint Blockade. *Cancer Discov.* **6**, 827–837 (2016). [doi:10.1158/2159-8290.CD-15-1545](https://doi.org/10.1158/2159-8290.CD-15-1545) [Medline](#)
25. M. Sade-Feldman, Y. J. Jiao, J. H. Chen, M. S. Rooney, M. Barzily-Rokni, J.-P. Eliane, S. L. Bjorgaard, M. R. Hammond, H. Vitzthum, S. M. Blackmon, D. T. Frederick, M. Hazar-Rethinam, B. A. Nadres, E. E. Van Severter, S. A. Shukla, K. Yizhak, J. P. Ray, D. Rosebrock, D. Livitz, V. Adalsteinsson, G. Getz, L. M. Duncan, B. Li, R. B. Corcoran, D. P. Lawrence, A. Stemmer-Rachamimov, G. M. Boland, D. A. Landau, K. T. Flaherty, R. J. Sullivan, N. Hacohen, Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat. Commun.* **8**, 1136 (2017). [doi:10.1038/s41467-017-01062-w](https://doi.org/10.1038/s41467-017-01062-w) [Medline](#)
26. J. A. Trujillo, R. F. Sweis, R. Bao, J. J. Luke, T Cell-Inflamed versus Non-T Cell-Inflamed Tumors: A Conceptual Framework for Cancer Immunotherapy Drug Development and Combination Therapy Selection. *Cancer Immunol. Res.* **6**, 990–1000 (2018). [doi:10.1158/2326-6066.CIR-18-0277](https://doi.org/10.1158/2326-6066.CIR-18-0277) [Medline](#)
27. J. L. McQuade, C. R. Daniel, B. A. Helmink, J. A. Wargo, Modulating the microbiome to improve therapeutic response in cancer. *Lancet Oncol.* **20**, e77–e91 (2019). [doi:10.1016/S1470-2045\(18\)30952-5](https://doi.org/10.1016/S1470-2045(18)30952-5) [Medline](#)
28. S. K. Ji, H. Yan, T. Jiang, C. Y. Guo, J. J. Liu, S. Z. Dong, K. L. Yang, Y. J. Wang, Z. J. Cao, S. L. Li, Preparing the Gut with Antibiotics Enhances Gut Microbiota Reprogramming Efficiency by Promoting Xenomicrobiota Colonization. *Front. Microbiol.* **8**, 1208 (2017). [doi:10.3389/fmicb.2017.01208](https://doi.org/10.3389/fmicb.2017.01208) [Medline](#)
29. J. Ni, T. D. Shen, E. Z. Chen, K. Bittiger, A. Bailey, M. Roggiani, A. Sirota-Madi, E. S. Friedman, L. Chau, A. Lin, I. Nissim, J. Scott, A. Lauder, C. Hoffmann, G. Rivas, L. Albenberg, R. N. Baldassano, J. Braun, R. J. Xavier, C. B. Clish, M. Yudkoff, H. Li, M. Goulian, F. D. Bushman, J. D. Lewis, G. D. Wu, A role for bacterial urease in gut dysbiosis and Crohn's disease. *Sci. Transl. Med.* **9**, eaah6888 (2017). [doi:10.1126/scitranslmed.aah6888](https://doi.org/10.1126/scitranslmed.aah6888) [Medline](#)
30. J. S. Bakken, T. Borody, L. J. Brandt, J. V. Brill, D. C. Demarco, M. A. Franzos, C. Kelly, A. Khoruts, T. Louie, L. P. Martinielli, T. A. Moore, G. Russell, C. Surawicz, Fecal Microbiota Transplantation Workgroup, Treating *Clostridium difficile* infection with fecal microbiota transplantation. *Clinical Gastroenterol. Hepatol.* **9**, 1044–1049 (2011). [doi:10.1016/j.cgh.2011.08.014](https://doi.org/10.1016/j.cgh.2011.08.014) [Medline](#)
31. G. Cammarota, G. Ianiro, H. Tilg, M. Rajilić-Stojanović, P. Kump, R. Satokari, H. Sokol, P. Arkkila, C. Pintus, A. Hart, J. Segal, M. Aloï, L. Masucci, A. Molinaro, F. Scadaferri, G. Gasbarrini, A. Lopez-Sanroman, A. Link, P. de Groot, W. M. de Vos, C. Högenauer, P. Malfertheiner, E. Mattila, T. Milosavljević, M. Nieuwdorp, M. Sanguinetti, M. Simren, A. Gasbarrini, European FMT Working Group, European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* **66**, 569–580 (2017). [doi:10.1136/gutjnl-2016-313017](https://doi.org/10.1136/gutjnl-2016-313017) [Medline](#)
32. A. Pataer, N. Kalhor, A. M. Correa, M. G. Raso, J. J. Erasmus, E. S. Kim, C. Behrens, J. J. Lee, J. A. Roth, D. J. Stewart, A. A. Vaporciyan, I. I. Wistuba, S. G. Swisher, University of Texas M. D. Anderson Lung Cancer Collaborative Research Group, Histopathologic response criteria predict survival of patients with resected lung cancer after neoadjuvant chemotherapy. *J. Thorac. Oncol.* **7**, 825–832 (2012). [doi:10.1097/JTO.0b013e318247504a](https://doi.org/10.1097/JTO.0b013e318247504a) [Medline](#)
33. I. Youngster, J. Mahabamunuge, H. K. Systrom, J. Sauk, H. Khalili, J. Levin, J. L. Kaplan, E. L. Hohmann, Oral, frozen fecal microbiota transplant (FMT) capsules for recurrent *Clostridium difficile* infection. *BMC Med.* **14**, 134 (2016). [doi:10.1186/s12916-016-0680-9](https://doi.org/10.1186/s12916-016-0680-9) [Medline](#)
34. National Cancer Institute, “Common Technology Criteria for Adverse Events (CTCAE),” version 5.0 (National Institutes of Health, 2017); [https://ctep.cancer.gov/protocoldevelopment/electronic\\_applications/ctc.htm#ctc\\_50](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50).
35. M. Wlodarska, A. D. Kostic, R. J. Xavier, An integrative view of microbiome-host interactions in inflammatory bowel diseases. *Cell Host Microbe* **17**, 577–591 (2015). [doi:10.1016/j.chom.2015.04.008](https://doi.org/10.1016/j.chom.2015.04.008) [Medline](#)
36. N. Zmora, G. Zilberman-Schapira, J. Suez, U. Mor, M. Dori-Bachash, S. Bashardes, E. Kotler, M. Zur, D. Regev-Lehavi, R. B.-Z. Brik, S. Federici, Y. Cohen, R. Linevsky, D. Rothschild, A. E. Moor, S. Ben-Moshe, A. Harmelin, S. Itzkovitz, N. Maharshak, O. Shibolet, H. Shapiro, M. Pevsner-Fischer, I. Sharon, Z. Halpern, E. Segal, E. Elinav, Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell* **174**, 1388–1405.e21 (2018). [doi:10.1016/j.cell.2018.08.041](https://doi.org/10.1016/j.cell.2018.08.041) [Medline](#)
37. S. P. Costello, P. A. Hughes, O. Waters, R. V. Bryant, A. D. Vincent, P. Blatchford, R. Katsikeros, J. Makanyanga, M. A. Campaniello, C. Mavangelos, C. P. Rosewarne, C. Bickley, C. Peters, M. N. Schoeman, M. A. Conlon, I. C. Roberts-Thomson, J. M. Andrews, Effect of Fecal Microbiota Transplantation on 8-Week Remission in Patients With Ulcerative Colitis: A Randomized Clinical Trial. *JAMA* **321**, 156–164 (2019). [doi:10.1001/jama.2018.20046](https://doi.org/10.1001/jama.2018.20046) [Medline](#)
38. S. Paramsothy, S. Nielsen, M. A. Kamm, N. P. Deshpande, J. J. Faith, J. C. Clemente, R. Paramsothy, A. J. Walsh, J. van den Bogaerde, D. Samuel, R. W. L. Leong, S. Connor, W. Ng, E. Lin, T. J. Borody, M. R. Wilkins, J.-F. Colombel, H. M. Mitchell, N. O. Kaakoush, Specific Bacteria and Metabolites Associated With Response to Fecal Microbiota Transplantation in Patients With Ulcerative Colitis. *Gastroenterology* **156**, 1440–1454.e2 (2019). [doi:10.1053/j.gastro.2018.12.001](https://doi.org/10.1053/j.gastro.2018.12.001) [Medline](#)
39. C. S. Smillie, J. Sauk, D. Gevers, J. Friedman, J. Sung, I. Youngster, E. L. Hohmann, C. Staley, A. Khoruts, M. J. Sadowsky, J. R. Allegretti, M. B. Smith, R. J. Xavier, E. J. Alm, Strain Tracking Reveals the Determinants of Bacterial Engraftment in the Human Gut Following Fecal Microbiota Transplantation. *Cell Host Microbe* **23**, 229–240.e5 (2018). [doi:10.1016/j.chom.2018.01.003](https://doi.org/10.1016/j.chom.2018.01.003) [Medline](#)
40. E. Bolyen, J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwards, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciorek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y.-X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Priesse, L. B. Rasmussen, A. Rivers, M. S. Robeson II, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hoof, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, J. G. Caporaso, Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **37**, 852–857 (2019). [doi:10.1038/s41587-019-0209-9](https://doi.org/10.1038/s41587-019-0209-9)
41. T. Z. DeSantis, P. Hugenholtz, N. Larsen, M. Rojas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, G. L. Andersen, Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72**, 5069–5072 (2006). [doi:10.1128/AEM.03006-05](https://doi.org/10.1128/AEM.03006-05) [Medline](#)
42. C. Lozupone, R. Knight, UniFrac: A new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228–8235 (2005). [doi:10.1128/AEM.71.12.8228-8235.2005](https://doi.org/10.1128/AEM.71.12.8228-8235.2005) [Medline](#)
43. D. P. Faith, Conservation evaluation and phylogenetic diversity. *Biol. Conserv.* **61**, 1–10 (1992). [doi:10.1016/0006-3207\(92\)91201-3](https://doi.org/10.1016/0006-3207(92)91201-3)
44. N. A. Hasan, B. A. Young, A. T. Minard-Smith, K. Saeed, H. Li, E. M. Heizer, N. J. McMillan, R. Isom, A. S. Abdullah, D. M. Bornman, S. A. Faith, S. Y. Choi, M. L. Dickens, T. A. Cebula, R. R. Colwell, Microbial community profiling of human saliva using shotgun metagenomic sequencing. *PLOS ONE* **9**, e97699 (2014).

- [doi:10.1371/journal.pone.0097699](https://doi.org/10.1371/journal.pone.0097699) Medline
45. A. Ottesen, P. Ramachandran, E. Reed, J. R. White, N. Hasan, P. Subramanian, G. Ryan, K. Jarvis, C. Grim, N. Daquigan, D. Hanes, M. Allard, R. Colwell, E. Brown, Y. Chen, Enrichment dynamics of *Listeria monocytogenes* and the associated microbiome from naturally contaminated ice cream linked to a listeriosis outbreak. *BMC Microbiol.* **16**, 275 (2016). [doi:10.1186/s12866-016-0894-1](https://doi.org/10.1186/s12866-016-0894-1) Medline
  46. D. Ponnusamy, E. V. Kozlova, J. Sha, T. E. Erova, S. R. Azar, E. C. Fitts, M. L. Kirtley, B. L. Tiner, J. A. Andersson, C. J. Grim, R. P. Isom, N. A. Hasan, R. R. Colwell, A. K. Chopra, Cross-talk among flesh-eating *Aeromonas hydrophila* strains in mixed infection leading to necrotizing fasciitis. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 722–727 (2016). [doi:10.1073/pnas.1523817113](https://doi.org/10.1073/pnas.1523817113) Medline
  47. N. Segata, L. Waldron, A. Ballarini, V. Narasimhan, O. Jousson, C. Huttenhower, Metagenomic microbial community profiling using unique clade-specific marker genes. *Nat. Methods* **9**, 811–814 (2012). [doi:10.1038/nmeth.2066](https://doi.org/10.1038/nmeth.2066) Medline
  48. S. Mandal, W. Van Treuren, R. A. White, M. Eggesbø, R. Knight, S. D. Peddada, Analysis of composition of microbiomes: A novel method for studying microbial composition. *Microb. Ecol. Health Dis.* **26**, 27663 (2015). [doi:10.3402/mehd.v26.27663](https://doi.org/10.3402/mehd.v26.27663) Medline
  49. Y. Benjamini, Y. Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J. Roy. Stat. Soc. B* **57**, 289–300 (1995). [doi:10.1111/j.2517-6161.1995.tb02031.x](https://doi.org/10.1111/j.2517-6161.1995.tb02031.x)
  50. H. Wickham, *ggplot2: Elegant Graphics for Data Analysis* (Springer, 2016).
  51. E. A. Franzosa, L. J. McIver, G. Rahnard, L. R. Thompson, M. Schirmer, G. Weingart, K. S. Lipson, R. Knight, J. G. Caporaso, N. Segata, C. Huttenhower, Species-level functional profiling of metagenomes and metatranscriptomes. *Nat. Methods* **15**, 962–968 (2018). [doi:10.1038/s41592-018-0176-y](https://doi.org/10.1038/s41592-018-0176-y) Medline
  52. A. D. Fernandes, J. M. Macklaim, T. G. Linn, G. Reid, G. B. Gloor, ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLOS ONE* **8**, e67019 (2013). [doi:10.1371/journal.pone.0067019](https://doi.org/10.1371/journal.pone.0067019) Medline
  53. W. Liebermeister, E. Noor, A. Flamholz, D. Davidi, J. Bernhardt, R. Milo, Visual account of protein investment in cellular functions. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 8488–8493 (2014). [doi:10.1073/pnas.1314810111](https://doi.org/10.1073/pnas.1314810111) Medline
  54. T. Neuman, M. London, J. Kania-Almog, A. Litvin, Y. Zohar, L. Fridel, J. Sandbank, I. Barshak, G. W. Vainer, A Harmonization Study for the Use of 22C3 PD-L1 Immunohistochemical Staining on Ventana's Platform. *J. Thorac. Oncol.* **11**, 1863–1868 (2016). [doi:10.1016/j.jtho.2016.08.146](https://doi.org/10.1016/j.jtho.2016.08.146) Medline
  55. E. R. Parra, C. Behrens, J. Rodriguez-Canales, H. Lin, B. Mino, J. Blando, J. Zhang, D. L. Gibbons, J. V. Heymach, B. Sepesi, S. G. Swisher, A. Weissferdt, N. Kalhor, J. Izzo, H. Kadara, C. Moran, J. J. Lee, I. I. Wistuba, Image Analysis-based Assessment of PD-L1 and Tumor-Associated Immune Cells Density Supports Distinct Intratumoral Microenvironment Groups in Non-small Cell Lung Carcinoma Patients. *Clin. Cancer Res.* **22**, 6278–6289 (2016). [doi:10.1158/1078-0432.CCR-15-2443](https://doi.org/10.1158/1078-0432.CCR-15-2443) Medline
  56. R. Büttner, J. R. Gosney, B. G. Skov, J. Adam, N. Motoi, K. J. Bloom, M. Dietel, J. W. Longshore, F. López-Ríos, F. Penault-Llorca, G. Viale, A. C. Wotherspoon, K. M. Kerr, M.-S. Tsao, Programmed Death-Ligand 1 Immunohistochemistry Testing: A Review of Analytical Assays and Clinical Implementation in Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* **35**, 3867–3876 (2017). [doi:10.1200/JCO.2017.74.7642](https://doi.org/10.1200/JCO.2017.74.7642) Medline
  57. C. W. Law, M. Alhamdoosh, S. Su, X. Dong, L. Tian, G. K. Smyth, M. E. Ritchie, RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. *F1000 Res.* **5**, 1408 (2016). [doi:10.12688/f1000research.9005.1](https://doi.org/10.12688/f1000research.9005.1) Medline
  58. P. Danaher, S. Warren, R. Lu, J. Samayoa, A. Sullivan, I. Pekker, B. Wallden, F. M. Marincola, A. Cesano, Pan-cancer adaptive immune resistance as defined by the Tumor Inflammation Signature (TIS): Results from The Cancer Genome Atlas (TCGA). *J. Immunother. Cancer* **6**, 63 (2018). [doi:10.1186/s40425-018-0367-1](https://doi.org/10.1186/s40425-018-0367-1) Medline
  59. G. P. Mognol, R. Spreafico, V. Wong, J. P. Scott-Browne, S. Togher, A. Hoffmann, P. G. Hogan, A. Rao, S. Trifari, Exhaustion-associated regulatory regions in CD8<sup>+</sup> tumor-infiltrating T cells. *Proc. Natl. Acad. Sci. U.S.A.* **114**, E2776–E2785 (2017). [doi:10.1073/pnas.1620498114](https://doi.org/10.1073/pnas.1620498114) Medline
  60. K. Woroniecka, P. Chongsathidkiet, K. Rhodin, H. Kemeny, C. Dechant, S. H. Farber, A. A. Elsamadicy, X. Cui, S. Koyama, C. Jackson, L. J. Hansen, T. M. Johanns, L. Sanchez-Perez, V. Chandramohan, Y. A. Yu, D. D. Bigner, A. Giles, P. Healy, G. Dranoff, K. J. Weinhold, G. P. Dunn, P. E. Fecci, T-Cell Exhaustion Signatures Vary with Tumor Type and Are Severe in Glioblastoma. *Clin. Cancer Res.* **24**, 4175–4186 (2018). [doi:10.1158/1078-0432.CCR-17-1846](https://doi.org/10.1158/1078-0432.CCR-17-1846) Medline
  61. P. Sharma, S. Hu-Lieskovan, J. A. Wargo, A. Ribas, Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell* **168**, 707–723 (2017). [doi:10.1016/j.cell.2017.01.017](https://doi.org/10.1016/j.cell.2017.01.017) Medline
  62. D. A. Schaer, R. P. Beckmann, J. A. Dempsey, L. Huber, A. Forest, N. Amaladas, Y. Li, Y. C. Wang, E. R. Rasmussen, D. Chin, A. Capen, C. Carpenito, K. A. Staschke, L. A. Chung, L. M. Litchfield, F. F. Merzoug, X. Gong, P. W. Iversen, S. Buchanan, A. de Dios, R. D. Novosiadly, M. Kalos, The CDK4/6 Inhibitor Abemaciclib Induces a T Cell Inflamed Tumor Microenvironment and Enhances the Efficacy of PD-L1 Checkpoint Blockade. *Cell Rep.* **22**, 2978–2994 (2018). [doi:10.1016/j.celrep.2018.02.053](https://doi.org/10.1016/j.celrep.2018.02.053) Medline
  63. G. Abril-Rodriguez, D. Y. Torrejon, W. Liu, J. M. Zaretsky, T. S. Nowicki, J. Tsoi, C. Puig-Saus, I. Baselga-Carretero, E. Medina, M. J. Quist, A. J. Garcia, W. Senapedis, E. Baloglu, A. Kalbasi, G. Cheung-Lau, B. Berent-Maoz, B. Comin-Anduix, S. Hu-Lieskovan, C.-Y. Wang, C. S. Grasso, A. Ribas, PAK4 inhibition improves PD-1 blockade immunotherapy. *Nat. Cancer* **1**, 46–58 (2020). [doi:10.1038/s43018-019-0003-0](https://doi.org/10.1038/s43018-019-0003-0)
  64. J. E. Gershenwald, R. A. Scolyer, Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. *Ann. Surg. Oncol.* **25**, 2105–2110 (2018). [doi:10.1245/s10434-018-6513-7](https://doi.org/10.1245/s10434-018-6513-7) Medline

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declare no competing interests. **Data and materials availability:** Microbiome 16S rRNA and metagenomics sequencing data have been deposited in NCBI's Sequence Read Archive (SRA) under BioProject ID PRJNA678737. The human gut and tumor RNA sequencing data have been deposited at NCBI's Gene Expression Omnibus (GEO) under BioProject ID GSE162436.

#### **SUPPLEMENTARY MATERIALS**

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Materials and Methods

Supplementary Text

Figs. S1 to S13

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References (30–64)

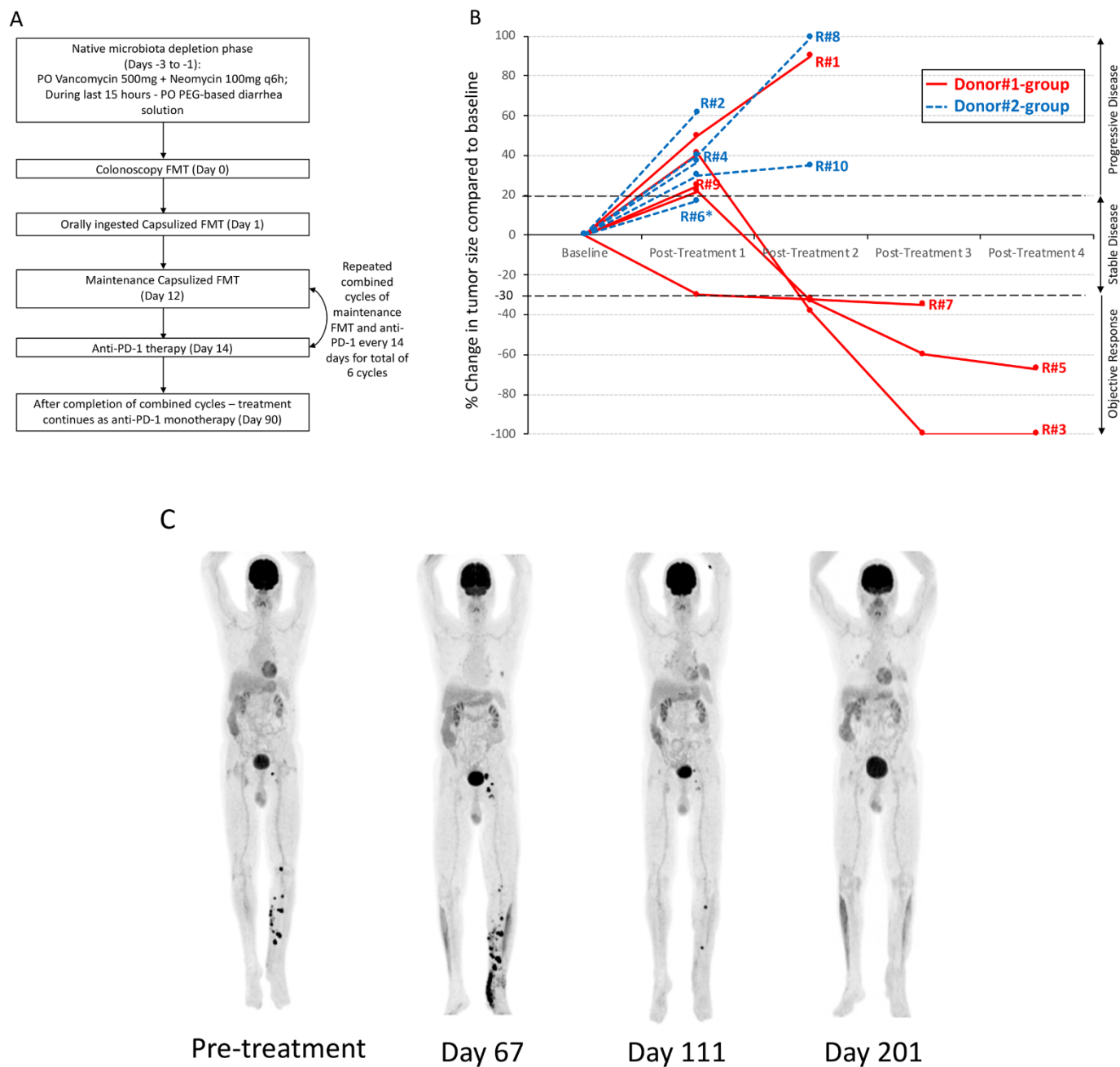
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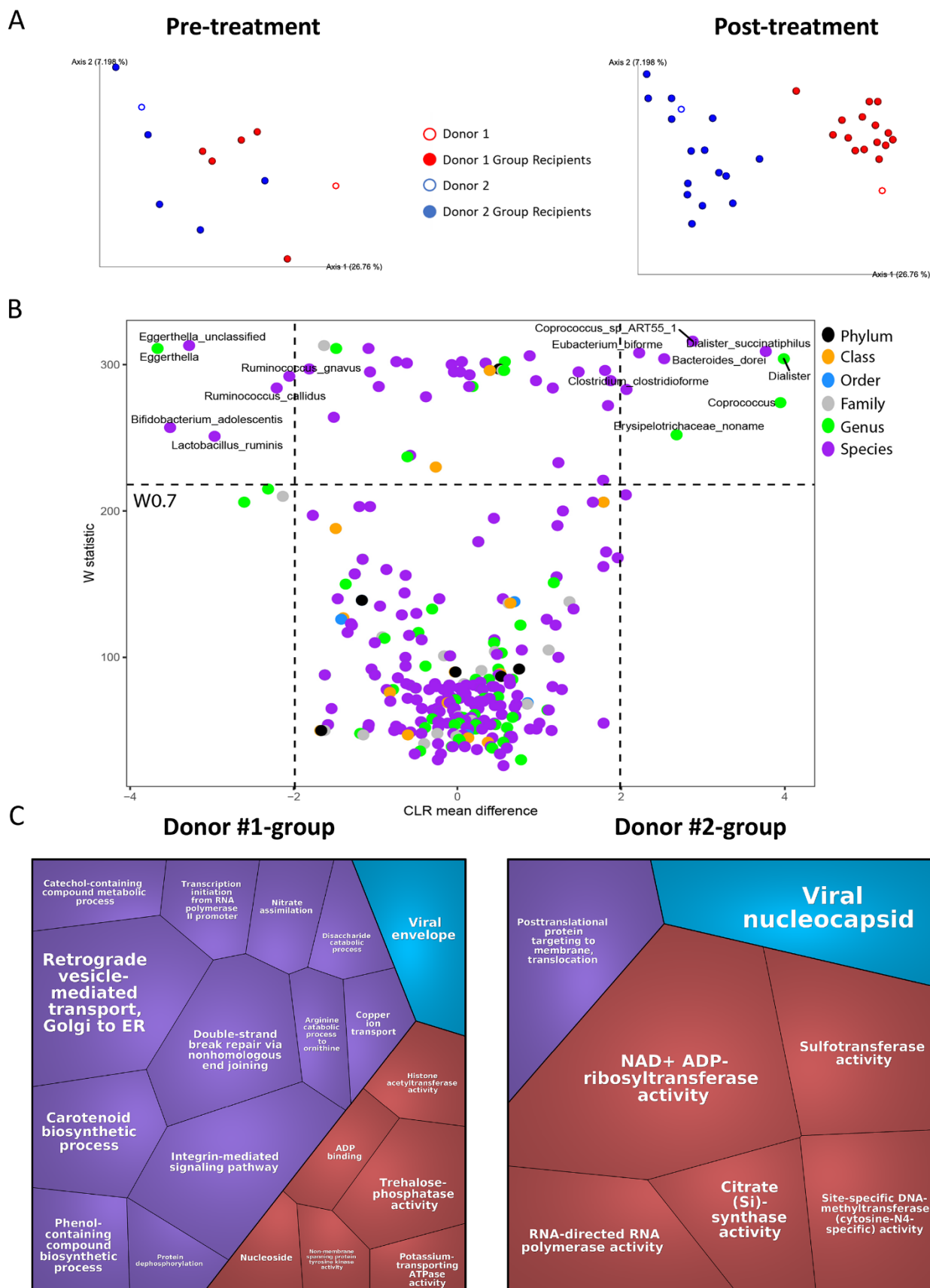
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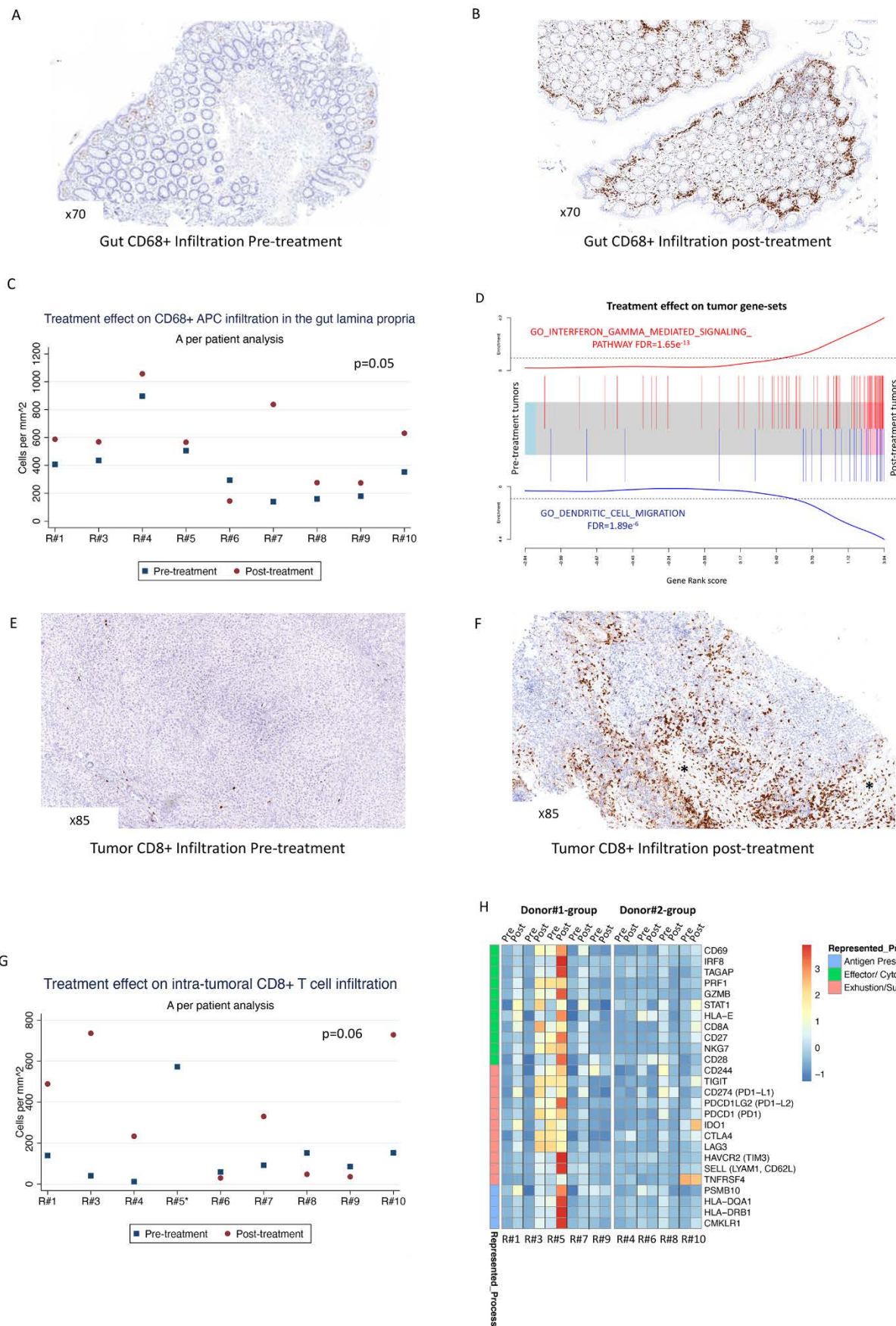


**Fig. 1. The clinical trial protocol and the effect of the FMT and re-induction of immunotherapy on recipient patient tumor size.** (A) Flow chart describing the clinical trial protocol. Fecal microbiota transplantation (FMT) recipients underwent a 72-hour “native microbiota depletion” phase which included a combination of orally-administered vancomycin, neomycin and a polyethylene-glycol (PEG) solution. Recipients underwent FMT by both colonoscopy and orally ingested stool capsules (capsulized FMT). Maintenance FMT (mFMT) was performed by giving patients capsulized FMT on day 12, followed two days later by the first anti-PD-1 dose (nivolumab). This mFMT + anti-PD-1 combination was repeated every 14 days for a total of six cycles. Responder and recipient patients with a clinical benefit to the treatment, continued anti-PD-1 as monotherapy until disease progression. (B) Spider plot demonstrating the change in radiological tumor size of all ten recipients. Recipients were colored according to their donor-group: the Donor #1-group recipients were marked by red full lines while the Donor #2-group recipients were marked by blue dashed lines. Recipient #3 demonstrated a complete response to treatment, and Recipients #5 and #7 demonstrating partial responses. Radiological assessment was conducted in accordance with the immune Response Evaluation Criteria in Solid Tumors (iRECIST) (7) and included measurements of target and new target lesions. \* - Recipient #6 was excluded from the trial after the first post-treatment imaging study due to unstable metastatic brain disease (hemorrhage into a brain metastasis that was known prior to inclusion into the trial). (C) Recipient #3 positron emission tomography combined with computed tomography (PET-CT) imaging. The metastatic lesions, represented as black emission areas, were concentrated in the left leg and groin (inguinal). Due to the treatment, the metastases had initially increased in size and new lesions appeared (Day 67). However, a complete resolution of all lesion was demonstrated in consecutive follow-up imaging studies. The initial tumor size increment was likely due to the substantial increase in CD8<sup>+</sup> T cell intra-tumoral infiltration which was observed in this patient (14 cells/mm<sup>2</sup> pre-treatment versus 736 cells/mm<sup>2</sup> on day 70, see below Fig. 3, E to G), a phenomenon known as pseudo-progression (8).



**Fig. 2. The effect of FMT on gut microbiota composition in metastatic melanoma recipient patients.** (A) Principal Component Analysis (PCA) plots of patient gut microbiota compositions based on stool 16S rRNA gene sequencing dissimilarity test (beta-diversity, Unweighted UniFrac). The distance between samples on the plot represents their dissimilarity – the greater the distance between two samples, the higher the dissimilarity of their composition. Recipient patients were grouped according to their donors – those who receive FMT implants from Donor #1 were colored in red while those who received implants from Donor #2 were colored in blue. The plots demonstrated no difference between the pre-treatment recipient compositions of the two donor-groups (FDR=0.45), in contrast to a clear post-treatment donor-based division (FDR=0.003). (B) A volcano plot based on the Analysis of Composition of Microbiomes (ANCOM) test. The plot compared the relative abundance of specific taxa between the Donor #1-group (negative X axis) and the Donor #2-group (positive X axis). Each donor-group was composed of post-treatment samples of the relevant recipients and the donor sample. Taxa which differed between the groups with FDR  $q \leq 0.05$  were presented above the horizontal dash line. The center log transformation (CLR) mean difference on the X-axis is an ANCOM calculation which is used to determine compositional differences in microbial communities. For convenience, only taxa with a mean difference above an absolute value of two were labeled with text. The full list of taxa that significantly differed between the two donor-groups was detailed in table S7. (C) Voronoi treemap plots of microbiota GO gene sets that were enriched among the different donor-group's microbiotas. The abundance of gene sets was compared across donors and post-treatment recipient samples. Gene sets that showed statistically significant differences between the Donor #1-group and the Donor #2-group and had a  $\log_2$  differential abundance  $>1$  (table S8) were plotted. The polygon size represents the scale of the  $\log_2$  abundance difference – a bigger polygon represents a more abundant pathway. The GO gene sets were also colored in accordance to their GO category: purple for biological processes, light blue for cellular components, and red for molecular functions.





**Fig. 3. The effect of FMT and re-induction of immunotherapy on immune activity in the gut and in the tumor microenvironment of metastatic melanoma recipient patients.** (A) Immunohistochemical (IHC) staining of CD68, representing antigen presenting cells (APC), conducted on pre-treatment sigmoid colon biopsies of Recipient #7. (B) IHC staining of CD68<sup>+</sup> cells conducted on the post-treatment (day 31) sigmoid colon biopsy of Recipient #7, demonstrating a clear increase in CD68<sup>+</sup> cells infiltration in the gut lamina propria. This infiltration was especially prominent in the sub-epithelial area, which is physically closer to the gut. (C) An image analysis algorithm was used to quantify the number of CD68<sup>+</sup> APC within the gut lamina propria of each recipient patient. A post-treatment increment in CD68<sup>+</sup> cell infiltration was demonstrated in most recipients ( $p=0.05$ ). (D) A barcode plot of gene set enrichment among tumor samples. Each bar represented a single gene out of the entire gene set. The plot demonstrated the up-regulation of APC and T cell related gene sets among post-treatment tumor samples. The full list of enriched gene sets is detailed in table S12. (E) IHC staining of CD8, representing cytotoxic T cells, conducted on pre-treatment tumor metastasis (subcutaneous, left leg) of Recipient #3. (F) IHC staining of CD8<sup>+</sup> T cells conducted on post-treatment biopsy from another subcutaneous metastasis in the left leg of Recipient #3, demonstrating a clear increase in intra-tumoral CD8<sup>+</sup> T cell infiltration and immune-induced tumor necrosis (marked by asterisks). (G) An image analysis algorithm was used to quantify the number of CD8<sup>+</sup> stained T cells within viable tumor tissue for each remote tumor metastasis biopsy. Post-treatment tumor biopsies were preferably taken from the same metastasis used for the pre-treatment biopsy, or from another metastasis at the same organ. Five recipient patients had increased their intra-tumoral CD8<sup>+</sup> T cell infiltration in post-treatment biopsies ( $p=0.06$ ). \* - Recipient #5 achieved a near-pathological complete response (<1% viable tumor), and hence their post-treatment CD8<sup>+</sup> infiltration could not be accurately assessed. (H) Heatmap of tumor immune gene expression. The heatmap illustrated expression dynamics before and after treatment across three representative immune processes – anti-tumoral effector activity, suppression/exhaustion activity, and antigen presenting cells activity/abundance. Note that only members of Donor #1-group demonstrated a post-treatment up-regulation of effector T cell response. Recipient #10 demonstrated a post-treatment up-regulation of the immune checkpoints IDO-1 and TIGIT without an effector response. Scale represents the Z-score of gene counts.

**Table 1. Clinical characteristics of patients receiving Fecal microbiota transplantation (FMT) and re-induction of anti-PD-1 treatment.** The time from previous anti-PD-1 treatment dose to the first trial dose was calculated from the most recent anti-PD-1 treatment dose to the day of the first anti-PD-1 treatment on the clinical trial. The percentage of viable tumor was calculated as the percentage of viable tumor out of the entire tumor tissue which was examined in a hematoxylin and eosin (H&E) slide of the tumor biopsy (see materials and methods). Clinical responses were based on the immune Response Evaluation Criteria in Solid Tumors (iRECIST) (7). Response category “None” represented iRECIST-confirmed progressive disease. Recipient #2 did not consent to undergo repeated tumor and gut biopsies, and hence the percentage of viable tumor was presented as “Not available (N/A)”. Additional clinical data per donor and recipient can be found in the supplementary materials (tables S1 and S2, respectively). PD-1 – Programmed cell Death -1; D – Dabrafenib; T – Trametinib; Nivo – Nivolumab; Pembro –Pembrolizumab; Ipi- Ipilimumab; T-VEC - Talimogene laherparepvec; TIL – Adoptive cell therapy composed of tumor infiltrating lymphocytes.

FMT donor-group	Recipient	Previous treatment lines (in chronological order)	Best response during all previous anti-PD-1 lines	Time from previous anti-PD-1 dose to first trial dose (days)	Percentage of viable tumor during the current trial		Clinical response in the current trial
					Pre-treatment	Post-treatment	
Donor #1	1	D+T; Nivo; D+T re-induction; Ipi+Nivo	None	57	100	95	None
	3	Pembro	None	66	100	30	Complete
	5	Ipi+Nivo	Partial	119	100	<1	Partial
	7	Pembro; D+T	Complete	204	80	30	Partial
	9	Nivo (adjuvant); Ipi; Carboplatin + Paclitaxel	None	209	80	90	None
Donor #2	2	Pembro; Ipi; Pembro re-induction	Stable disease	114	N/A	N/A	None
	4	Nivo (adjuvant) Ipi; Pembro; D+T; Nivo, T-VEC + Nivo; TIL; D+T re-induction; Palbociclib; Carboplatin + Paclitaxel	None	112	85	90	None
	6		Partial	322	100	85	None
	8	Ipi+Nivo	Mixed	42	90	100	None
	10	Ipi+Nivo	Stable disease	57	100	90	None

## Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients

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