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Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients

V. Gopalakrishnan,^{1,2*} C. N. Spencer,^{2,3*} L. Nezi,^{3*} A. Reuben,¹ M. C. Andrews,¹ T. V. Karpinets,³ P. A. Prieto,^{1†} D. Vicente,¹ K. Hoffman,⁴ S. C. Wei,⁵ A. P. Cogdill,^{1,5} L. Zhao,³ C. W. Hudgens,⁶ D. S. Hutchinson,⁷ T. Manzo,³ M. Petaccia de Macedo,^{6‡} T. Cotechini,⁸ T. Kumar,³ W. S. Chen,⁹ S. M. Reddy,¹⁰ R. Szczepaniak Sloane,¹ J. Galloway-Pena,¹¹ H. Jiang,¹ P. L. Chen,^{9§} E. J. Shpall,¹² K. Rezvani,¹² A. M. Alousi,¹² R. F. Chemaly,¹¹ S. Shelburne,^{3,11} L. M. Vence,⁵ P. C. Okhuysen,¹¹ V. B. Jensen,¹³ A. G. Swennes,⁷ F. McAllister,¹⁴ E. Marcelo Riquelme Sanchez,¹⁴ Y. Zhang,¹⁴ E. Le Chatelier,¹⁵ L. Zitvogel,¹⁶ N. Pons,¹⁵ J. L. Austin-Breneman,^{1||} L. E. Haydu,^{1†} E. M. Burton,¹ J. M. Gardner,¹ E. Sirmans,¹⁷ J. Hu,¹⁸ A. J. Lazar,^{6,9} T. Tsujikawa,⁸ A. Diab,¹⁷ H. Tawbi,¹⁷ I. C. Glitza,¹⁷ W. J. Hwu,¹⁷ S. P. Patel,¹⁷ S. E. Woodman,¹⁷ R. N. Amaria,¹⁷ M. A. Davies,¹⁷ J. E. Gershenwald,¹ P. Hwu,¹⁷ J. E. Lee,¹ J. Zhang,³ L. M. Coussens,⁸ Z. A. Cooper,^{1,3¶} P. A. Futreal,³ C. R. Daniel,^{4,2} N. J. Ajami,⁷ J. F. Petrosino,⁷ M. T. Tetzlaff,^{6,9} P. Sharma,^{5,19} J. P. Allison,⁵ R. R. Jenq,^{3#} J. A. Wargo,^{1,3#**}

¹Department of Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ²Department of Epidemiology, Human Genetics and Environmental Sciences, University of Texas School of Public Health, Houston, TX 77030, USA. ³Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ⁴Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ⁵Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ⁶Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ⁷Department of Molecular Virology and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA. ⁸Department of Cell, Developmental and Cell Biology, Oregon Health and Sciences University, Portland, OR 97239, USA. ⁹Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹⁰Department of Breast Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹¹Department of Infectious Diseases, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹²Department of Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹³Department of Veterinary Medicine and Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹⁴Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹⁵Centre de Recherche de Jouy-en-Josas, Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas, France. ¹⁶Centre d'Investigation Clinique Biothérapie, Institut Gustave-Roussy, 94805 Villejuif Cedex, France. ¹⁷Department of Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹⁸Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. ¹⁹Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA.

*These authors contributed equally to this work. †Present address: University of Rochester James P. Wilmot Cancer Center, Rochester, NY 14642, USA. ‡Present address: A.C. Camargo Cancer Center, São Paulo, Brazil. §Present address: Moffitt Cancer Center, Tampa, FL 33612, USA. ||Present address: Harvard University, Cambridge, MA 02138, USA. ¶Present address: MedImmune, Gaithersburg, MD 20878, USA. #These authors contributed equally to this work.

**Corresponding author. Email: jwargo@mdanderson.org

Pre-clinical mouse models suggest that the gut microbiome modulates tumor response to checkpoint blockade immunotherapy; however, this has not been well-characterized in human cancer patients. Here we examined the oral and gut microbiome of melanoma patients undergoing anti-PD-1 immunotherapy (n=112). Significant differences were observed in the diversity and composition of the patient gut microbiome of responders (R) versus non-responders (NR). Analysis of patient fecal microbiome samples (n=43, 30R, 13NR) showed significantly higher alpha diversity (p<0.01) and relative abundance of Ruminococcaceae bacteria (p<0.01) in responding patients. Metagenomic studies revealed functional differences in gut bacteria in R including enrichment of anabolic pathways. Immune profiling suggested enhanced systemic and anti-tumor immunity in responding patients with a favorable gut microbiome, as well as in germ-free mice receiving fecal transplants from responding patients. Together, these data have important implications for the treatment of melanoma patients with immune checkpoint inhibitors.

Tremendous advances have been made in the treatment of melanoma and other cancers using immune checkpoint inhibitors targeting the cytotoxic T-lymphocyte-associated antigen (CTLA-4) and the programmed death 1 (PD-1) protein, however responses to these therapies are often heterogeneous and not durable (1–3). It has recently emerged that factors beyond tumor genomics influence cancer development

and therapeutic responses (4–7), including host factors such as the gastrointestinal (gut) microbiome (8–10). A number of studies have shown that the gut microbiome may influence anti-tumor immune responses via innate and adaptive immunity (11, 12), and that therapeutic responses may be improved via its modulation (13, 14), however this has not been extensively studied in cancer patients.

To better understand the role of the microbiome in response to immune checkpoint blockade, we prospectively collected microbiome samples from patients with metastatic melanoma starting treatment with anti-PD-1 therapy (n=112 patients) (fig. S1 and table S1). Oral (buccal) and gut (fecal) microbiome samples were collected at treatment initiation, and tumor biopsies and blood samples were collected at matched pre-treatment time points when possible to assess for genomic alterations, as well as the density and phenotype of tumor-infiltrating and circulating immune cell subsets (Fig. 1A and fig. S2). Taxonomic profiling via 16S rRNA gene sequencing was performed on all available oral and gut samples, with metagenomic whole genome shotgun (WGS) sequencing on a subset (n=25). Eligible patients (n=89) were classified as responders (R; n=54) versus non-responders (NR; n=35) based on radiographic assessment using the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) criteria (15) at 6 months after treatment initiation. Patients were classified as R if they achieved an objective response (complete or partial response or stable disease lasting at least 6 months), versus NR if they progressed on therapy or had stable disease lasting less than 6 months. This classification accounts for the subset of patients who may derive long-term disease benefit despite not achieving a bona fide RECIST response, and has been employed in numerous published studies of patients on checkpoint blockade (16–19). Of note, patients in R versus NR groups were similar with respect to age, gender, primary type, prior therapy, concurrent systemic therapy and serum lactate dehydrogenase (LDH) (table S2). Prior genomic analyses have demonstrated that patients with tumors having a higher mutational load are more likely to respond to anti-CTLA-4 (16, 20, 21) or anti-PD-1 therapy (21–24), however a high mutational load alone appears neither sufficient nor essential for response. In this cohort, the total number and specific melanoma driver mutations were within comparable parameters between R and NR following anti-PD-1 therapy (fig. S3), though the number of tumors available for sequencing (n=10, R=7, NR=3) was limited and may have reduced our ability to detect a significant association between mutational burden and response.

We first assessed the landscape of the oral and gut microbiome in all available samples in patients (n=112) with metastatic melanoma via 16S sequencing, noting that both communities were relatively diverse, with a high abundance of Lactobacillales in the oral microbiome and Bacteroidales in the fecal microbiome (Fig. 1B). Bipartite network analysis (25) demonstrated a clear separation of community structure between the oral and fecal microbiomes in terms of both matched and aggregate samples (fig. S4), suggesting that these communities are distinct. Loss of microbial diversity (dysbiosis) is associated with chronic health conditions (26–

28) and cancer (8–10), and is also associated with poor outcomes to certain forms of cancer therapy including allogeneic stem cell transplant (29). Based on these data, we examined the diversity of the oral and gut microbiomes in eligible patients on anti-PD1 therapy, and found that alpha diversity of the gut microbiome was significantly higher in R (n=30) compared to NR (n=13) using several indices ($p<0.01$, Fig. 1C and fig. S5). No significant differences were observed in the oral microbiome (R=54, NR=32, $p=0.11$, fig. S6). We then tested the relationship of diversity and progression-free survival (PFS) in our cohort by stratifying patients based on tertiles of Inverse Simpson scores, demonstrating that patients with a high diversity in the fecal microbiome had significantly prolonged PFS compared to those with intermediate or low diversity ($p=0.02$ and 0.04 , respectively; Fig. 1, D and E, and fig. S7). No differences in PFS were noted when comparing diversity of the oral microbiome (fig. S8). Importantly, upon visualizing beta diversity weighted UniFrac distances (30) by principal coordinate analysis, we found a notable clustering effect by response status in the gut microbiome of these patients, which was not observed in the oral microbiome (Fig. 1F and fig. S8E).

Since compositional differences in the microbiome may also influence cancer development and response to therapy (12, 14, 15, 23), we sought to determine if differences existed in the oral or gut microbiomes of R and NR to anti-PD-1 therapy. To test this, we first compared an enrichment of operational taxonomic units (OTUs) in R versus NR, demonstrating that distinct sets of rare low abundance OTUs were associated with response to anti-PD-1 therapy, with enrichment of Clostridiales in R and Bacteroidales in NR in the gut microbiome ($p<0.01$, Fig. 2, A and B, and fig. S9, A and C). No significant differences in enrichment were noted in the oral microbiome of R versus NR (fig. S9, B and D, and fig. S10). To further explore these findings, we performed high dimensional class comparisons via linear discriminant analysis of effect size (LEfSe) (31), which again demonstrated differentially abundant bacteria in the fecal microbiome of R versus NR to anti-PD-1 therapy, with Clostridiales/Ruminococcaceae enriched in R and Bacteroidales enriched in NR (Fig. 2, C and D). No major differences were observed in the oral microbiome between R and NR, with the exception of higher Bacteroidales in NR to anti-PD-1 therapy (fig. S11). Pairwise comparisons were then performed for bacterial taxa at all levels by response. In addition to confirming the previous taxonomic differences, these analyses identified the *Faecalibacterium* genus as significantly enriched in R (Fig. 2E and table S3). Metagenomic WGS further confirmed enrichment of *Faecalibacterium* species in addition to others in R, while *Bacteroides thetaiotaomicron*, *Escherichia coli*, and *Anaerotruncus colihominis* were enriched in NR (Fig. 2F and

table S4). Importantly, the gut microbiome was shown to be relatively stable over time in a limited number of longitudinal samples tested (fig. S12).

We next asked whether bacterial composition and abundances within the gut and/or oral microbiomes of patients were associated with a specific treatment outcome to anti-PD-1 therapy. We grouped all identified OTUs into clusters of related OTUs (crOTUs) via construction of a phylogenetic tree from sequence alignment data (32). This technique involves comparison of abundances of different potential groupings of bacteria based on 16S sequence similarity and helps address the sparse distribution of OTU abundances observed in the absence of this approach (fig. S13). Unsupervised hierarchical clustering of crOTU abundances within the gut and oral microbiomes was then performed without input of response data. We found that patients segregated into 2 distinct community types. Type 1 comprised entirely of R and was enriched for Clostridiales, whereas Type 2 comprised a mixture of R and NR ($p=0.02$) and was enriched for Bacteroidales (Fig. 3A). To better understand compositional differences between these crOTU community types, we again performed pairwise comparisons of the gut microbiota, and identified a pattern very similar to that seen when clustering by response, with Clostridiales/Ruminococcaceae enriched in Type 1, and Bacteroidales enriched in Type 2 (fig. S14A and table S5). Further, these communities clustered distinctly using principal coordinate analysis of weighted Unifrac distances (fig. S14B). Analysis of crOTUs in the oral microbiome revealed no apparent relationship to treatment response (fig. S15, A and B).

To explore how specific bacterial taxa impact patient treatment response, we compared PFS following anti-PD-1 therapy as it related to the “top hits” consistently observed across our analyses. From the Ruminococcaceae family of the Clostridiales order, we focused on the *Faecalibacterium* genus in R, and Bacteroidales order in NR, and stratified patients into high versus low categories based on the median relative abundance of these taxa in the gut microbiome. Patients with high *Faecalibacterium* abundance had a significantly prolonged PFS versus those with a low abundance ($p=0.03$). Conversely, patients with a high abundance of Bacteroidales had a shortened PFS compared to those with a low abundance ($p=0.05$, Fig. 3D). This is in line with recently published data in a small cohort of patients on CTLA-4 blockade, where patients with a higher abundance of *Faecalibacterium* had a prolonged PFS compared to those with a higher abundance of Bacteroidales in the gut microbiome (33). In addition, univariate Cox proportional hazards analyses demonstrated that the strongest microbial predictors of response to anti-PD-1 therapy were alpha diversity [Intermediate hazard ratio (HR)=3.60, 95% C.I.=1.02-12.74; Low HR=3.57, 95% confidence interval (C.I.)=1.02-12.52], and

abundance of *Faecalibacterium* (HR=2.92, 95% C.I.=1.08-7.89) and Bacteroidales (HR= 0.39, 95% CI=0.15-1.03) in the fecal microbiome. There was no association found between PFS and stage in our cohort. Our final multivariate model was selected by forward stepwise selection and included *Faecalibacterium* abundance (HR=2.95, 95% C.I.=1.31-7.29, $p=0.03$) and prior immunotherapy (HR=2.87, 95% C.I.=1.10-7.89, $p=0.03$) (table S6). Abundance of *Faecalibacterium* and Bacteroidales also outperformed relevant clinical variables in receiver operating characteristic curve (ROC) analysis (fig. S16).

Next, we sought to gain insight into the mechanism through which the gut microbiome may influence response to anti-PD-1 therapy, and first conducted functional genomic profiling of gut microbiome samples via metagenomic WGS sequencing ($n=25$) in R ($n=14$) vs NR ($n=11$). Organism-specific gene hits were assigned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO), and based on these annotations, metagenomes for each sample were reconstructed into metabolic pathways using the MetaCyc hierarchy of pathway classifications (34, 35). Unsupervised hierarchical clustering of predicted pathway enrichment identified two groups of patient samples, with response rates of 69.2% and 41.7% (Fig. 3E). A similar pattern was also noted for KO-abundances with 70.6% and 37.5% response rates (fig. S17). Comparisons of pathway enrichment across these groups showed changes in metabolic functions, with anabolic functions predominating in R including amino acid biosynthesis (Fig. 3E), which may promote host immunity (36), whereas catabolic functions predominated in NR (Fig. 3E, fig. S16, and table S7).

There is clear evidence in pre-clinical models that differential composition of the gut microbiome may influence therapeutic responses to anti-PD-1 therapy at the level of the tumor microenvironment (12), thus we next examined the relationship between the gut microbiota and systemic and anti-tumor immune responses in our cohort of patients on anti-PD-1 therapy. We compared the tumor-associated immune infiltrates via multi-parameter immunohistochemistry (IHC) and observed a higher density of CD8+ T cells in baseline samples of R versus NR ($p=0.04$), consistent with prior reports (Fig. 4A and fig. S18) (18, 37). Pairwise comparisons using Spearman rank correlations were then performed between specific bacterial taxa enriched in the gut microbiome of R and NR and immune markers in the tumor microenvironment, demonstrating a statistically significant positive correlation between the CD8+ T cell infiltrate in the tumor and abundance of the *Faecalibacterium* genus, the Ruminococcaceae family and the Clostridiales order in the gut and a non-significant but negative correlation with Bacteroidales (Fig. 4, B and C, and figs. S19 and S20). No associations were seen between CD8+ T cell density and diversity or crOTU community type membership (fig. S21). Analysis of

systemic immune responses via flow cytometry and cytokine assays revealed that patients with a high abundance of Clostridiales, Ruminococcaceae or *Faecalibacterium* in the gut had higher levels of effector CD4+ and CD8+ T cells in the systemic circulation with a preserved cytokine response to anti-PD-1 therapy, whereas patients with a higher abundance of Bacteroidales in the gut microbiome had higher levels of regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC) in the systemic circulation, with a blunted cytokine response (Fig. 4D and figs. S22 and S23). To better understand the influence of compositional differences in the gut microbiome on antigen processing and presentation within the tumor microenvironment, we next performed multiplex IHC targeting the myeloid compartment (38). In these studies, patients with a high abundance of *Faecalibacterium* in the gut microbiome had a higher density of immune cells and markers of antigen processing and presentation compared to those with a high abundance of Bacteroidales (Fig. 4, E and F, and figs. S24 and S25), suggesting a possible mechanism through which the gut microbiome may modulate anti-tumor immune responses (12), though this must be validated in a larger cohort.

To investigate a causal link between a “favorable” gut microbiome and response to immune checkpoint blockade, we performed Fecal Microbiome Transplantation (FMT) experiments in germ-free recipient mice (Fig. 4G). In these studies, mice that were transplanted with stool from R to anti-PD-1 therapy (R-FMT) had significantly reduced tumor growth ($p=0.04$, Fig. 4H and fig. S26A) by day 14 compared to those transplanted with stool from NR (NR-FMT). Importantly, mice transplanted with R-FMT also exhibited improved responses to anti-PD-L1 therapy (Fig. 4I) in contrast to mice that were transplanted with stool from NR (NR-FMT). Next we performed 16S sequencing on fecal samples collected from mice treated with FMT, demonstrating that R-FMT mice also had significantly higher abundance of *Faecalibacterium* in their gut microbiome ($p<0.01$) (fig. S27). We also wanted to better understand the mechanism through which the gut microbiome may influence systemic and anti-tumor immune responses, and performed correlative studies on tumors, peripheral blood and spleens from these mice. These studies demonstrated that tumors of mice receiving R-FMT had a higher density of CD8+ T cells than mice receiving NR-FMT, consistent with human data (Fig. 4J and fig. S26B, top series). Analysis of CD45+ myeloid and lymphoid tumor infiltrating cells by flow cytometry confirmed this result (fig. S26C). Moreover, FMT from R locally increased the number of CD45+ immune and CD8+ T cells in the gut compared to NR-FMT (Fig. 4K and fig. S26B, bottom series). Mass cytometry analysis using t-SNE dimension reduction was performed on tumors from mice, and demonstrated up-regulation of PD-L1

in the tumor microenvironment of mice receiving R-FMT versus NR-FMT (fig. S26D), suggesting the development of a “hot” tumor microenvironment. Further phenotypic studies of tumor immune infiltrates revealed a significant enrichment of innate effector cells (expressing CD45+CD11b+Ly6G+) in mice receiving R-FMT (fig. S26E). A lower frequency of suppressive myeloid cells (expressing CD11b+CD11c+) was observed in mice receiving R-FMT compared to mice receiving NR-FMT (fig. S26F). Finally, an increase in the frequency of ROR γ T+ Th17 cells in the tumor was also detected in NR-FMT mice (fig. S26G), in line with what we observed in tumors from patients who failed to respond to anti-PD-1 therapy. Mice receiving NR-FMT also had higher levels of regulatory CD4+ FoxP3+ T cells (fig. S26H) and CD4+ IL-17+ (fig. S26I) cells in the spleen, suggesting impaired host immune responses.

Our results indicate that the gut microbiome may modulate responses to anti PD-1 immunotherapy in melanoma patients. We propose that patients with a “favorable” gut microbiome (e.g., high diversity and abundance of Ruminococcaceae/*Faecalibacterium*) have enhanced systemic and anti-tumor immune responses mediated by increased antigen presentation, and improved effector T cell function in the periphery and the tumor microenvironment. In contrast, patients with an “unfavorable” gut microbiome (e.g., low diversity and high relative abundance of Bacteroidales) have impaired systemic and anti-tumor immune responses mediated by limited intratumoral lymphoid and myeloid infiltration and weakened antigen presentation capacity. These findings highlight the therapeutic potential of modulating the gut microbiome in patients receiving checkpoint blockade immunotherapy, and warrant prompt evaluation in cancer patients through clinical trials.

REFERENCES AND NOTES

1. D. Schadendorf, F. S. Hodi, C. Robert, J. S. Weber, K. Margolin, O. Hamid, D. Patt, T.-T. Chen, D. M. Berman, J. D. Wolchok, Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J. Clin. Oncol.* **33**, 1889–1894 (2015). doi:10.1200/JCO.2014.56.2736 [Medline](#)
2. C. Robert *et al.*, KEYNOTE-006 investigators, Pembrolizumab versus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **372**, 2521–2532 (2015). doi:10.1056/NEJMoa1503093 [Medline](#)
3. C. Robert, G. V. Long, B. Brady, C. Dutriaux, M. Maio, L. Mortier, J. C. Hassel, P. Rutkowski, C. McNeil, E. Kalinka-Warzocho, K. J. Savage, M. M. Hernberg, C. Lebbé, J. Charles, C. Mihalciou, V. Chiarion-Sileni, C. Mauch, F. Cognetti, A. Arance, H. Schmidt, D. Schadendorf, H. Gogas, L. Lundgren-Eriksson, C. Horak, B. Sharkey, I. M. Waxman, V. Atkinson, P. A. Ascierto, Nivolumab in previously untreated melanoma without BRAF mutation. *N. Engl. J. Med.* **372**, 320–330 (2015). doi:10.1056/NEJMoa1412082 [Medline](#)
4. M. D. Vesely, R. D. Schreiber, Cancer immunoeediting: Antigens, mechanisms, and implications to cancer immunotherapy. *Ann. N. Y. Acad. Sci.* **1284**, 1–5 (2013). doi:10.1111/nyas.12105 [Medline](#)
5. S. L. Topalian, C. G. Drake, D. M. Pardoll, Immune checkpoint blockade: A common denominator approach to cancer therapy. *Cancer Cell* **27**, 450–461 (2015). doi:10.1016/j.ccell.2015.03.001 [Medline](#)

6. E. Tran, P. F. Robbins, S. A. Rosenberg, 'Final common pathway' of human cancer immunotherapy: Targeting random somatic mutations. *Nat. Immunol.* **18**, 255–262 (2017). [doi:10.1038/ni.3682](https://doi.org/10.1038/ni.3682) [Medline](#)
7. P. Bachireddy, U. E. Burkhardt, M. Rajasagi, C. J. Wu, Haematological malignancies: At the forefront of immunotherapeutic innovation. *Nat. Rev. Cancer* **15**, 201–215 (2015). [doi:10.1038/nrc3907](https://doi.org/10.1038/nrc3907) [Medline](#)
8. W. S. Garrett, Cancer and the microbiota. *Science* **348**, 80–86 (2015). [doi:10.1126/science.aaa4972](https://doi.org/10.1126/science.aaa4972) [Medline](#)
9. J. A. Segre, Microbial growth dynamics and human disease. *Science* **349**, 1058–1059 (2015). [doi:10.1126/science.aad0781](https://doi.org/10.1126/science.aad0781) [Medline](#)
10. J. L. Drewes, F. Housseau, C. L. Sears, Sporadic colorectal cancer: Microbial contributors to disease prevention, development and therapy. *Br. J. Cancer* **115**, 273–280 (2016). [doi:10.1038/bjc.2016.189](https://doi.org/10.1038/bjc.2016.189) [Medline](#)
11. C. M. Paulos, C. Wrzesinski, A. Kaiser, C. S. Hinrichs, M. Chieppa, L. Cassard, D. C. Palmer, A. Boni, P. Muranski, Z. Yu, L. Gattinoni, P. A. Antony, S. A. Rosenberg, N. P. Restifo, Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8⁺ T cells via TLR4-signaling. *J. Clin. Invest.* **117**, 2197–2204 (2007). [doi:10.1172/JCI32205](https://doi.org/10.1172/JCI32205) [Medline](#)
12. 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](#)
13. N. Iida, A. Dzutsev, C. A. Stewart, L. Smith, N. Bouladoux, R. A. Weingarten, D. A. Molina, R. Salcedo, T. Back, S. Cramer, R.-M. Dai, H. Kiu, M. Cardone, S. Naik, A. K. Patri, E. Wang, F. M. Marincola, K. M. Frank, Y. Belkaid, G. Trinchieri, R. S. Goldszmid, Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013). [doi:10.1126/science.1240527](https://doi.org/10.1126/science.1240527) [Medline](#)
14. S. Viaud, F. Saccheri, G. Mignot, T. Yamazaki, R. Daillère, D. Hannani, D. P. Enot, C. Pfirschke, C. Engblom, M. J. Pittet, A. Schlitzer, F. Ginhoux, L. Apetoh, E. Chachaty, P.-L. Woerther, G. Eberl, M. Bérard, C. Ecobichon, D. Clermont, C. Bizet, V. Gaboriau-Routhiau, N. Cerf-Bensussan, P. Opolon, N. Yessaad, E. Vivier, B. Ryffel, C. O. Elson, J. Doré, G. Kroemer, P. Lepage, I. G. Boneca, F. Ghiringhelli, L. Zitvogel, The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013). [doi:10.1126/science.1240537](https://doi.org/10.1126/science.1240537) [Medline](#)
15. L. H. Schwartz, S. Litière, E. de Vries, R. Ford, S. Gwyther, S. Mandrekar, L. Shankar, J. Bogaerts, A. Chen, J. Dancy, W. Hayes, F. S. Hodi, O. S. Hoekstra, E. P. Huang, N. Lin, Y. Liu, P. Therasse, J. D. Wolchok, L. Seymour, RECIST 1.1-Update and clarification: From the RECIST committee. *Eur. J. Cancer* **62**, 132–137 (2016). [doi:10.1016/j.ejca.2016.03.081](https://doi.org/10.1016/j.ejca.2016.03.081) [Medline](#)
16. A. Snyder, V. Makarov, T. Merghoub, J. Yuan, J. M. Zaretsky, A. Desrichard, L. A. Walsh, M. A. Postow, P. Wong, T. S. Ho, T. J. Hollmann, C. Bruggeman, K. Kannan, Y. Li, C. Elipenahli, C. Liu, C. T. Harbison, L. Wang, A. Ribas, J. D. Wolchok, T. A. Chan, Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N. Engl. J. Med.* **371**, 2189–2199 (2014). [doi:10.1056/NEJMoa1406498](https://doi.org/10.1056/NEJMoa1406498) [Medline](#)
17. P. Sharma, M. Retz, A. Siefker-Radtke, A. Baron, A. Necchi, J. Bedke, E. R. Plimack, D. Vaena, M.-O. Grimm, S. Bracarda, J. Á. Arranz, S. Pal, C. Ohyama, A. Saci, X. Qu, A. Lambert, S. Krishnan, A. Azrilovich, M. D. Galsky, Nivolumab in metastatic urothelial carcinoma after platinum therapy (CheckMate 275): A multicentre, single-arm, phase 2 trial. *Lancet Oncol.* **18**, 312–322 (2017). [doi:10.1016/S1470-2045\(17\)30065-7](https://doi.org/10.1016/S1470-2045(17)30065-7) [Medline](#)
18. 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. Broaddus, 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](#)
19. W. Roh, P.-L. Chen, A. Reuben, C. N. Spencer, P. A. Prieto, J. P. Miller, V. Gopalakrishnan, F. Wang, Z. A. Cooper, S. M. Reddy, C. Gumbs, L. Little, Q. Chang, W.-S. Chen, K. Wani, M. P. De Macedo, E. Chen, J. L. Austin-Breneman, H. Jiang, J. Roszik, M. T. Tetzlaff, M. A. Davies, J. E. Gershenwald, H. Tawbi, A. J. Lazar, P. Hwu, W.-J. Hwu, A. Diab, I. C. Glitza, S. P. Patel, S. E. Woodman, R. N. Amaria, V. G. Prieto, J. Hu, P. Sharma, J. P. Allison, L. Chin, J. Zhang, J. A. Wargo, P. A. Futreal, Integrated molecular analysis of tumor biopsies on sequential CTLA-4 and PD-1 blockade reveals markers of response and resistance. *Sci. Transl. Med.* **9**, eaah3560 (2017). [doi:10.1126/scitranslmed.aah3560](https://doi.org/10.1126/scitranslmed.aah3560) [Medline](#)
20. E. M. Van Allen, D. Miao, B. Schilling, S. A. Shukla, C. Blank, L. Zimmer, A. Sucker, U. Hillen, M. H. G. Foppen, S. M. Goldinger, J. Utikal, J. C. Hassel, B. Weide, K. C. Kaehler, C. Loquai, P. Mohr, R. Gutzmer, R. Dummer, S. Gabriel, C. J. Wu, D. Schadendorf, L. A. Garraway, Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207–211 (2015). [doi:10.1126/science.aad0095](https://doi.org/10.1126/science.aad0095) [Medline](#)
21. N. McGranahan, A. J. S. Furness, R. Rosenthal, S. Ramskov, R. Lyngaa, S. K. Saini, M. Jamal-Hanjani, G. A. Wilson, N. J. Birkbak, C. T. Hiley, T. B. K. Watkins, S. Shafi, N. Murugaesu, R. Mitter, A. U. Akarca, J. Linares, T. Marafioti, J. Y. Henry, E. M. Van Allen, D. Miao, B. Schilling, D. Schadendorf, L. A. Garraway, V. Makarov, N. A. Rizvi, A. Snyder, M. D. Hellmann, T. Merghoub, J. D. Wolchok, S. A. Shukla, C. J. Wu, K. S. Peggs, T. A. Chan, S. R. Hadrup, S. A. Quezada, C. Swanton, Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463–1469 (2016). [doi:10.1126/science.aaf1490](https://doi.org/10.1126/science.aaf1490) [Medline](#)
22. W. Hugo, J. M. Zaretsky, L. Sun, C. Song, B. H. Moreno, S. Hu-Lieskovan, B. Berent-Maoz, J. Pang, B. Chmielowski, G. Cherry, E. Seja, S. Lomeli, X. Kong, M. C. Kelley, J. A. Sosman, D. B. Johnson, A. Ribas, R. S. Lo, Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell* **165**, 35–44 (2016). [doi:10.1016/j.cell.2016.02.065](https://doi.org/10.1016/j.cell.2016.02.065) [Medline](#)
23. D. B. Johnson, G. M. Frampton, M. J. Rioto, E. Yusko, Y. Xu, X. Guo, R. C. Ennis, D. Fabrizio, Z. R. Chalmers, J. Greenbowe, S. M. Ali, S. Balasubramanian, J. X. Sun, Y. He, D. T. Frederick, I. Puzanov, J. M. Balko, J. M. Cates, J. S. Ross, C. Sanders, H. Robins, Y. Shyr, V. A. Miller, P. J. Stephens, R. J. Sullivan, J. A. Sosman, C. M. Lovly, Targeted next generation sequencing identifies markers of response to PD-1 blockade. *Cancer Immunol. Res.* **4**, 959–967 (2016). [doi:10.1158/2326-6066.CCR-16-0143](https://doi.org/10.1158/2326-6066.CCR-16-0143) [Medline](#)
24. N. A. Rizvi, M. D. Hellmann, A. Snyder, P. Kvistborg, V. Makarov, J. J. Havel, W. Lee, J. Yuan, P. Wong, T. S. Ho, M. L. Miller, N. Rekhtman, A. L. Moreira, F. Ibrahim, C. Bruggeman, B. Gasmir, R. Zappasodi, Y. Maeda, C. Sander, E. B. Garon, T. Merghoub, J. D. Wolchok, T. N. Schumacher, T. A. Chan, Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124–128 (2015). [doi:10.1126/science.aaa1348](https://doi.org/10.1126/science.aaa1348) [Medline](#)
25. B. D. Muegge, J. Kuczynski, D. Knights, J. C. Clemente, A. González, L. Fontana, B. Henrissat, R. Knight, J. I. Gordon, Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. *Science* **332**, 970–974 (2011). [doi:10.1126/science.1198719](https://doi.org/10.1126/science.1198719) [Medline](#)
26. Human Microbiome Project Consortium, Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207–214 (2012). [doi:10.1038/nature11234](https://doi.org/10.1038/nature11234) [Medline](#)
27. P. J. Turnbaugh, F. Backhed, L. Fulton, J. I. Gordon, Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* **3**, 213–223 (2008). [doi:10.1016/j.chom.2008.02.015](https://doi.org/10.1016/j.chom.2008.02.015) [Medline](#)
28. J. Qin et al., MetaHIT Consortium, A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010). [doi:10.1038/nature08821](https://doi.org/10.1038/nature08821) [Medline](#)
29. Y. Taur, R. R. Jenq, M.-A. Perales, E. R. Littmann, S. Morjaria, L. Ling, D. No, A. Gobourne, A. Viale, P. B. Dahi, D. M. Ponce, J. N. Barker, S. Giralto, M. van den Brink, E. G. Pamer, The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* **124**, 1174–1182 (2014). [doi:10.1182/blood-2014-02-554725](https://doi.org/10.1182/blood-2014-02-554725) [Medline](#)
30. C. Lozupone, M. E. Lladser, D. Knights, J. Stombaugh, R. Knight, UniFrac: An effective distance metric for microbial community comparison. *ISME J.* **5**, 169–172 (2011). [doi:10.1038/ismej.2010.133](https://doi.org/10.1038/ismej.2010.133) [Medline](#)
31. N. Segata, J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W. S. Garrett, C. Huttenhower, Metagenomic biomarker discovery and explanation. *Genome Biol.* **12**, R60 (2011). [doi:10.1186/gb-2011-12-6-r60](https://doi.org/10.1186/gb-2011-12-6-r60) [Medline](#)
32. J. U. Peled, S. M. Devlin, A. Staffas, M. Lumish, R. Khanin, E. R. Littmann, L. Ling, S. Kosuri, M. Maloy, J. B. Slingerland, K. F. Ahr, K. A. Porosnicu Rodriguez, Y.

- Shono, A. E. Slingerland, M. D. Docampo, A. D. Sung, D. Weber, A. M. Alousi, B. Gyurkocza, D. M. Ponce, J. N. Barker, M.-A. Perales, S. A. Giral, Y. Taur, E. G. Pamer, R. R. Jenq, M. R. M. van den Brink, Intestinal microbiota and relapse after hematopoietic-cell transplantation. *J. Clin. Oncol.* **35**, 1650–1659 (2017). [doi:10.1200/JCO.2016.70.3348](https://doi.org/10.1200/JCO.2016.70.3348) [Medline](#)
33. N. Chaput, P. Lepage, C. Coutzac, E. Soularue, K. Le Roux, C. Monot, L. Boselli, E. Routier, L. Cassard, M. Collins, T. Vaysse, L. Marthey, A. Eggermont, V. Asvatourian, E. Lanoy, C. Mateus, C. Robert, F. Carbonnel, Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann. Oncol.* **28**, 1368–1379 (2017). [doi:10.1093/annonc/mdx108](https://doi.org/10.1093/annonc/mdx108) [Medline](#)
34. R. Caspi, H. Foerster, C. A. Fulcher, P. Kaipa, M. Krummenacker, M. Latendresse, S. Paley, S. Y. Rhee, A. G. Shearer, C. Tissier, T. C. Walk, P. Zhang, P. D. Karp, The MetaCyc Database of metabolic pathways and enzymes and the BioCyc collection of Pathway/Genome Databases. *Nucleic Acids Res.* **36**, D623–D631 (2008). [doi:10.1093/nar/gkm900](https://doi.org/10.1093/nar/gkm900) [Medline](#)
35. M. Kanehisa, S. Goto, KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30 (2000). [doi:10.1093/nar/28.1.27](https://doi.org/10.1093/nar/28.1.27) [Medline](#)
36. E. Blacher, M. Levy, E. Tatrovsky, E. Elinav, Microbiome-modulated metabolites at the interface of host immunity. *J. Immunol.* **198**, 572–580 (2017). [doi:10.4049/jimmunol.1601247](https://doi.org/10.4049/jimmunol.1601247) [Medline](#)
37. P. C. Tumeh, C. L. Harview, J. H. Yearley, I. P. Shintaku, E. J. M. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A. N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A. J. Gutierrez, T. R. Grogan, C. Mateus, G. Tomicic, J. A. Glaspy, R. O. Emerson, H. Robins, R. H. Pierce, D. A. Elashoff, C. Robert, A. Ribas, PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014). [doi:10.1038/nature13954](https://doi.org/10.1038/nature13954) [Medline](#)
38. T. Tsujikawa, S. Kumar, R. N. Borkar, V. Azimi, G. Thibault, Y. H. Chang, A. Balter, R. Kawashima, G. Choe, D. Sauer, E. El Rassi, D. R. Clayburgh, M. F. Kulesz-Martin, E. R. Lutz, L. Zheng, E. M. Jaffee, P. Leyshock, A. A. Margolin, M. Mori, J. W. Gray, P. W. Flint, L. M. Coussens, Quantitative multiplex immunohistochemistry reveals myeloid-inflamed tumor-immune complexity associated with poor prognosis. *Cell Reports* **19**, 203–217 (2017). [doi:10.1016/j.celrep.2017.03.037](https://doi.org/10.1016/j.celrep.2017.03.037) [Medline](#)
39. Human Microbiome Project Consortium, A framework for human microbiome research. *Nature* **486**, 215–221 (2012). [doi:10.1038/nature11209](https://doi.org/10.1038/nature11209) [Medline](#)
40. J. G. Caporaso, C. L. Lauber, W. A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S. M. Owens, J. Betley, L. Fraser, M. Bauer, N. Gormley, J. A. Gilbert, G. Smith, R. Knight, Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **6**, 1621–1624 (2012). [doi:10.1038/ismej.2012.8](https://doi.org/10.1038/ismej.2012.8) [Medline](#)
41. T. Rognes, T. Flouri, B. Nichols, C. Quince, F. Mahé, VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **4**, e2584 (2016). [doi:10.7717/peerj.2584](https://doi.org/10.7717/peerj.2584) [Medline](#)
42. R. C. Edgar, Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460–2461 (2010). [doi:10.1093/bioinformatics/btq461](https://doi.org/10.1093/bioinformatics/btq461) [Medline](#)
43. R. C. Edgar, UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**, 996–998 (2013). [doi:10.1038/nmeth.2604](https://doi.org/10.1038/nmeth.2604) [Medline](#)
44. 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](#)
45. J. R. Cole, Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, J. M. Tiedje, Ribosomal Database Project: Data and tools for high throughput rRNA analysis. *Nucleic Acids Res.* **42**, D633–D642 (2014). [doi:10.1093/nar/gkt1244](https://doi.org/10.1093/nar/gkt1244) [Medline](#)
46. C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F. O. Glöckner, The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–D596 (2013). [doi:10.1093/nar/gks1219](https://doi.org/10.1093/nar/gks1219) [Medline](#)
47. J. G. Caporaso, K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, R. Knight, PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**, 266–267 (2010). [doi:10.1093/bioinformatics/btp636](https://doi.org/10.1093/bioinformatics/btp636) [Medline](#)
48. P. D. Schloss, S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, C. F. Weber, Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**, 7537–7541 (2009). [doi:10.1128/AEM.01541-09](https://doi.org/10.1128/AEM.01541-09) [Medline](#)
49. M. N. Price, P. S. Dehal, A. P. Arkin, FastTree 2—approximately maximum-likelihood trees for large alignments. *PLOS ONE* **5**, e9490 (2010). [doi:10.1371/journal.pone.0009490](https://doi.org/10.1371/journal.pone.0009490) [Medline](#)
50. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, R. Knight, QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **7**, 335–336 (2010). [doi:10.1038/nmeth.f.303](https://doi.org/10.1038/nmeth.f.303) [Medline](#)
51. N. Yutin, M. Y. Galperin, A genomic update on clostridial phylogeny: Gram-negative spore formers and other misplaced clostridia. *Environ. Microbiol.* **15**, 2631–2641 (2013). [Medline](#)
52. 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](#)
53. X. C. Morgan, C. Huttenhower, Chapter 12: Human microbiome analysis. *PLOS Comput. Biol.* **8**, e1002808 (2012). [doi:10.1371/journal.pcbi.1002808](https://doi.org/10.1371/journal.pcbi.1002808) [Medline](#)
54. A. Chao, Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.* **11**, 265–270 (1984).
55. E. H. Simpson, Measurement of diversity. *Nature* **163**, 688–688 (1949). [doi:10.1038/163688a0](https://doi.org/10.1038/163688a0)
56. C. E. Shannon, A mathematical theory of communication. *Mob. Comput. Commun. Rev.* **5**, 3–55 (2001). [doi:10.1145/584091.584093](https://doi.org/10.1145/584091.584093)
57. C. E. Shannon, W. Weaver, A mathematical theory of communication. *Bell Syst. Tech. J.* **27**, 623–656 (1948). [doi:10.1002/j.1538-7305.1948.tb00917.x](https://doi.org/10.1002/j.1538-7305.1948.tb00917.x)
58. P. T. Shannon, M. Grimes, B. Kutlu, J. J. Bot, D. J. Galas, Rcytoscape: Tools for exploratory network analysis. *BMC Bioinformatics* **14**, 217 (2013). [doi:10.1186/1471-2105-14-217](https://doi.org/10.1186/1471-2105-14-217) [Medline](#)
59. J. Li et al., MetaHIT Consortium, An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014). [doi:10.1038/nbt.2942](https://doi.org/10.1038/nbt.2942) [Medline](#)
60. B. Langmead, S. L. Salzberg, Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **9**, 357–359 (2012). [doi:10.1038/nmeth.1923](https://doi.org/10.1038/nmeth.1923) [Medline](#)
61. A. Cotillard et al., ANR MicroObes Consortium, Dietary intervention impact on gut microbial gene richness. *Nature* **500**, 585–588 (2013). [doi:10.1038/nature12480](https://doi.org/10.1038/nature12480) [Medline](#)
62. N. Pons, J.-M. Batto, S. Kennedy, M. Almeida, F. Boumezbuer, paper presented at the Annual Conference of the JOBIM (Journées Ouvertes en Biologie, Informatique et Mathématique), Montpellier, France, 7 to 9 September 2010.
63. H. B. Nielsen et al., MetaHIT Consortium, Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. *Nat. Biotechnol.* **32**, 822–828 (2014). [doi:10.1038/nbt.2939](https://doi.org/10.1038/nbt.2939) [Medline](#)
64. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**, 1754–1760 (2009). [doi:10.1093/bioinformatics/btp324](https://doi.org/10.1093/bioinformatics/btp324) [Medline](#)
65. A. McKenna, M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytksy, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, M. A. DePristo, The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010). [doi:10.1101/gr.107524.110](https://doi.org/10.1101/gr.107524.110) [Medline](#)
66. K. Cibulskis, M. S. Lawrence, S. L. Carter, A. Sivachenko, D. Jaffe, C. Sougnez, S. Gabriel, M. Meyerson, E. S. Lander, G. Getz, Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat. Biotechnol.* **31**, 213–219 (2013). [doi:10.1038/nbt.2514](https://doi.org/10.1038/nbt.2514) [Medline](#)
67. K. Ye, M. H. Schulz, Q. Long, R. Apweiler, Z. Ning, Pindel: A pattern growth approach to detect break points of large deletions and medium sized insertions from paired-end short reads. *Bioinformatics* **25**, 2865–2871 (2009). [doi:10.1093/bioinformatics/btp394](https://doi.org/10.1093/bioinformatics/btp394) [Medline](#)
68. R. Sachidanandam et al., International SNP Map Working Group, A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* **409**, 928–933 (2001). [doi:10.1038/35057149](https://doi.org/10.1038/35057149) [Medline](#)

69. 1000 Genomes Project Consortium, An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56–65 (2012). [doi:10.1038/nature11632](https://doi.org/10.1038/nature11632) [Medline](#)
70. M. Lek *et al.*, Exome Aggregation Consortium, Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285–291 (2016). [doi:10.1038/nature19057](https://doi.org/10.1038/nature19057) [Medline](#)
71. W. Fu, T. D. O'Connor, G. Jun, H. M. Kang, G. Abecasis, S. M. Leal, S. Gabriel, M. J. Rieder, D. Altshuler, J. Shendure, D. A. Nickerson, M. J. Bamshad, J. M. Akey, NHLBI Exome Sequencing Project, Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. *Nature* **493**, 216–220 (2013). [doi:10.1038/nature11690](https://doi.org/10.1038/nature11690) [Medline](#)
72. T. Zhang, K. Dutton-Regester, K. M. Brown, N. K. Hayward, The genomic landscape of cutaneous melanoma. *Pigment Cell Melanoma Res.* **29**, 266–283 (2016). [doi:10.1111/pcmr.12459](https://doi.org/10.1111/pcmr.12459) [Medline](#)
73. Z. A. Cooper, A. Reuben, C. N. Spencer, P. A. Prieto, J. L. Austin-Breneman, H. Jiang, C. Haymaker, V. Gopalakrishnan, M. T. Tetzlaff, D. T. Frederick, R. J. Sullivan, R. N. Amaria, S. P. Patel, P. Hwu, S. E. Woodman, I. C. Glitza, A. Diab, L. M. Vence, J. Rodriguez-Canales, E. R. Parra, I. I. Wistuba, L. M. Coussens, A. H. Sharpe, K. T. Flaherty, J. E. Gershenwald, L. Chin, M. A. Davies, K. Clise-Dwyer, J. P. Allison, P. Sharma, J. A. Wargo, Distinct clinical patterns and immune infiltrates are observed at time of progression on targeted therapy versus immune checkpoint blockade for melanoma. *Oncoimmunology* **5**, e1136044 (2016). [Medline](#)
74. P. Blanche, J. F. Dartigues, H. Jacqmin-Gadda, Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat. Med.* **32**, 5381–5397 (2013). [doi:10.1002/sim.5958](https://doi.org/10.1002/sim.5958) [Medline](#)
75. C. O. Fritz, P. E. Morris, J. J. Richler, Effect size estimates: Current use, calculations, and interpretation. *J. Exp. Psychol. Gen.* **141**, 2–18 (2012). [doi:10.1037/a0024338](https://doi.org/10.1037/a0024338) [Medline](#)
76. R Development Core Team, (R Foundation for Statistical Computing, Vienna, Austria, 2017).
77. National Research Council, *Guide for the Care and Use of Laboratory Animals* (National Academies Press, ed. 8, 2011), pp. 1–220.
78. Z. A. Cooper, V. R. Juneja, P. T. Sage, D. T. Frederick, A. Piris, D. Mitra, J. A. Lo, F. S. Hodi, G. J. Freeman, M. W. Bosenberg, M. McMahon, K. T. Flaherty, D. E. Fisher, A. H. Sharpe, J. A. Wargo, Response to BRAF inhibition in melanoma is enhanced when combined with immune checkpoint blockade. *Cancer Immunol. Res.* **2**, 643–654 (2014). [doi:10.1158/2326-6066.CCR-13-0215](https://doi.org/10.1158/2326-6066.CCR-13-0215) [Medline](#)
79. E. D. Amir, K. L. Davis, M. D. Tadmor, E. F. Simonds, J. H. Levine, S. C. Bendall, D. K. Shenfeld, S. Krishnaswamy, G. P. Nolan, D. Pe'er, viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nat. Biotechnol.* **31**, 545–552 (2013). [doi:10.1038/nbt.2594](https://doi.org/10.1038/nbt.2594) [Medline](#)

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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S28

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References (39–79)

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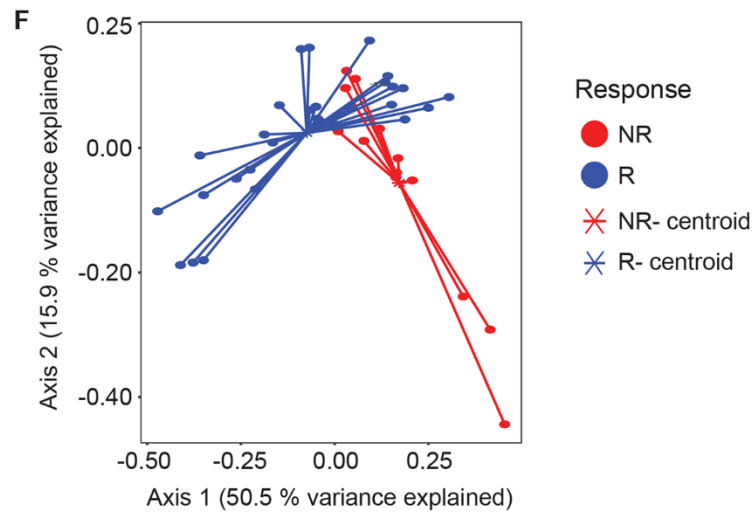
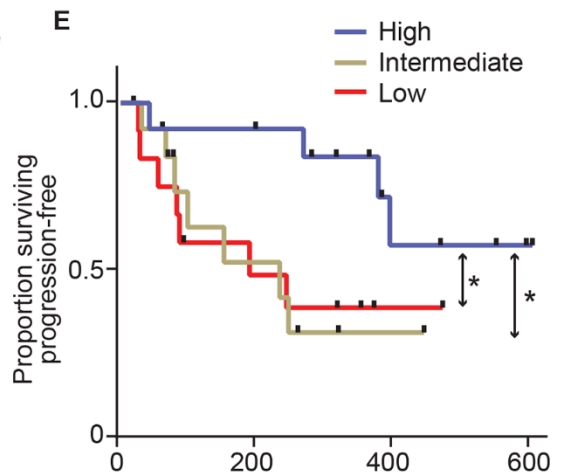
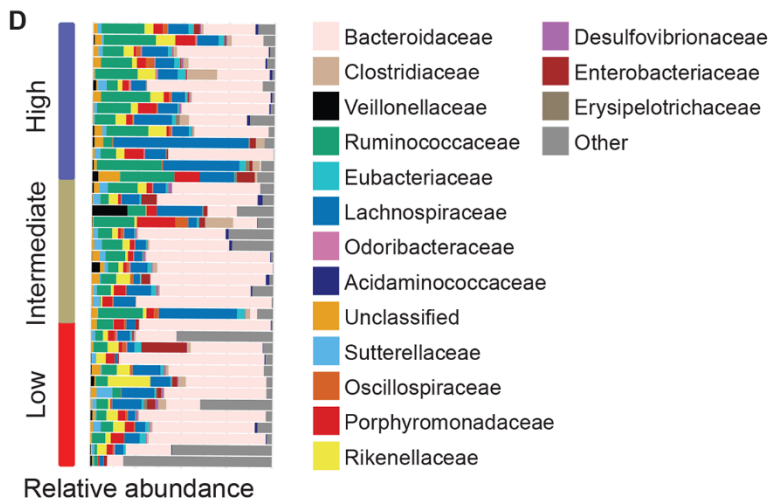
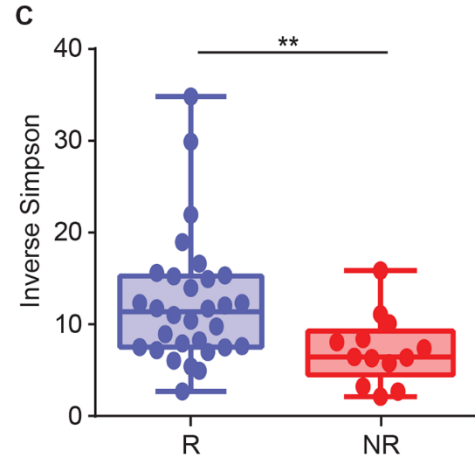
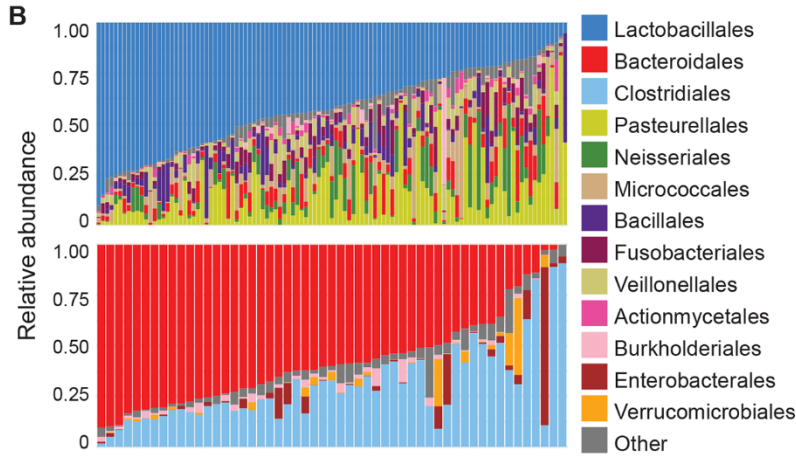
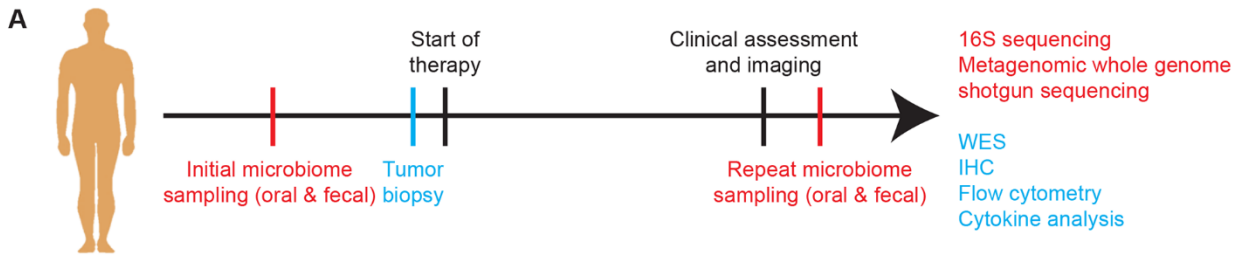


Fig. 1. Enhanced gut microbiome diversity is associated with improved response to anti-PD-1 immunotherapy in patients with metastatic melanoma. (A) Schema of sample collection and analyses. (B) Stacked bar plot of phylogenetic composition of common bacterial taxa (>0.1% abundance) at the order level in oral (n=109, top) and fecal (n=53, bottom) samples by 16S rRNA sequencing. (C) Inverse Simpson diversity scores of the gut microbiome in R (n=30) and NR (n=13) to anti PD-1 immunotherapy by Mann-Whitney (MW) test. Error bars represent the distribution of diversity scores. (D) Phylogenetic composition of fecal samples (n=39) at the family level (>0.1% abundance) at baseline. High (blue) (>11.63, n=13), intermediate (gold) (7.46-11.63, n=13) and low (red) (<7.46, n=13) diversity groups were determined using tertiles of Inverse Simpson scores. (E) Kaplan-Meier (KM) plot of progression-free survival (PFS) by fecal diversity; high (median PFS undefined), intermediate (median PFS=232 days), and low (median PFS=188 days). High vs intermediate diversity (HR 3.60, 95% C.I. 1.02-12.74) and high vs low (HR 3.57, 95% C.I. 1.02-12.52) by univariate Cox model. * $p < 0.05$, ** $p < 0.01$. (F) Principal coordinate analysis of fecal samples (n=43) by response using Weighted UniFrac distances.

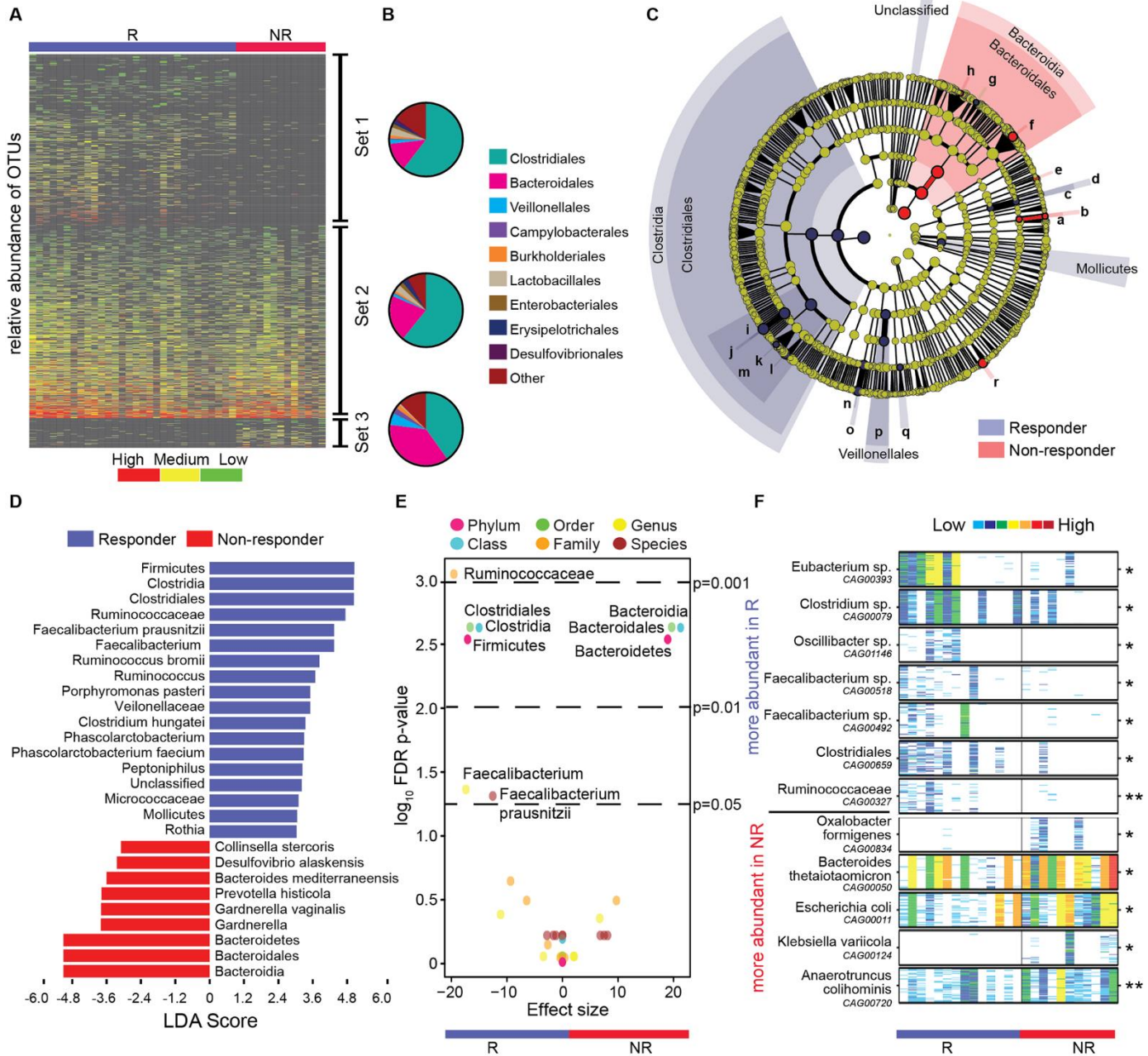


Fig. 2. Compositional differences in the gut microbiome are associated with responses to anti-PD-1 immunotherapy (A) Heatmap of OTU abundances in R (n=30) and NR (n=13). Columns denote patients grouped by response and sorted by diversity within R and NR groups; rows denote bacterial OTUs grouped into 3 sets according to their enrichment/depletion in R versus NR: Set 1 (enriched in R), Set 2 (unenriched), and Set 3 (enriched in NR), and then sorted by mean abundance within each set. (B) Phylogenetic composition of OTUs within each set at the order level. Set 1 (enriched in R); Set 2 (unenriched); Set 3 (enriched in NR). (C) Taxonomic cladogram from LEfSe showing differences in fecal taxa. Dot size is proportional to the abundance of the taxon. Letters correspond to the following taxa: (a) *Gardnerella vaginalis*, (b) Gardnerella, (c) *Rothia*, (d) *Micrococcaceae*, (e) *Collinsella stercoris*, (f) *Bacteroides mediterraneensis*, (g) *Porphyromonas pasteri*, (h) *Prevotella histicola*, (i) *Faecalibacterium prausnitzii*, (j) *Faecalibacterium*, (k) *Clostridium hungatei*, (l) *Ruminococcus bromii*, (m) Ruminococcaceae, (n) *Phascolarctobacterium faecium*, (o) *Phascolarctobacterium*, (p) *Veilonellaceae*, (q) *Peptoniphilus*, (r) *Desulfovibrio alaskensis*. (D) LDA scores computed for differentially-abundant taxa in the fecal microbiomes of R (blue) and NR (red). Length indicates effect size associated with a taxon. $p=0.05$ for the Kruskal-Wallis test; LDA score > 3 . (E) Differentially-abundant gut bacteria in R (blue) vs NR (red) by MW test (FDR-adjusted) within all taxonomic levels. (F) Pairwise comparisons by MW test of abundances of metagenomic species (MGS) identified by metagenomic WGS in fecal samples (n=25): R (n=14, blue), NR (n=11, red). * $p<0.05$, ** $p<0.01$. Colors reflect gene abundances visualized using "barcodes" with the following order of intensity: white(0)<light blue<blue<green<yellow<orange<red for increasing abundance and each color change corresponds to a 4x fold abundance change. In these barcodes, MGS appear as vertical lines (co-abundant genes in a sample) colored according to the gene abundance. blue<blue<green<yellow<orange<red for increasing abundance and each color change corresponds to a 4x fold abundance change. In these barcodes, MGS appear as vertical lines (co-abundant genes in a sample) colored according to the gene abundance.

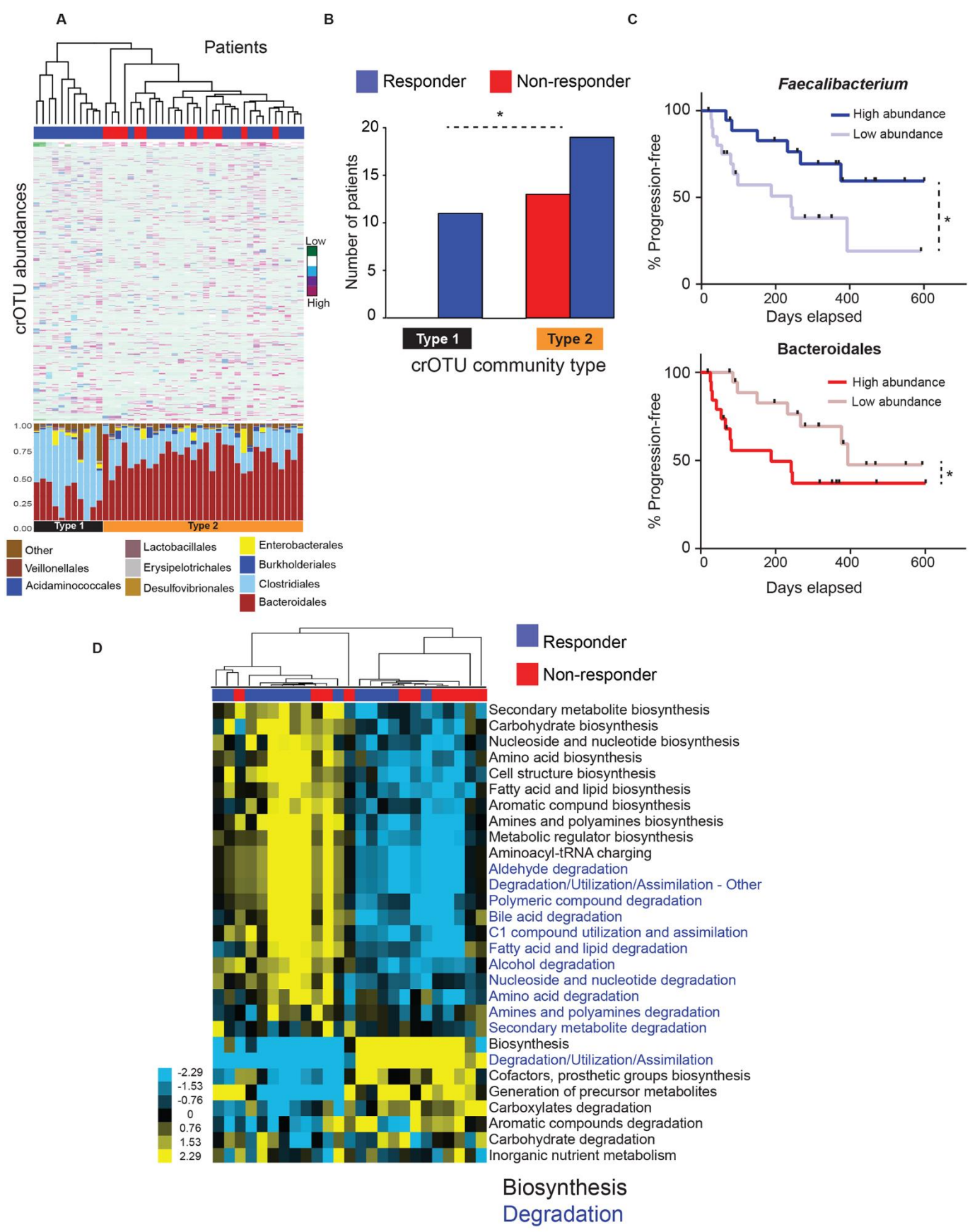


Fig. 3. Abundance of crOTUs within the gut microbiome is predictive of response to anti-PD-1 immunotherapy (A) Top: Unsupervised hierarchical clustering by complete linkage of Euclidean distances of crOTU abundances in 43 fecal samples. Bottom: Stacked bar plot of relative abundances at the order level by crOTU community-type. (B) Association of crOTU community types with response to anti-PD-1 by Fisher's exact test. crOTU community type 1 (black, n=11: R=11, NR=0); crOTU community type 2 (orange, n=32: R=19, NR=13). Blue bars indicate responders, whereas red bars indicate non-responders. (C) Comparison KM PFS curves by long-rank test in patients with high abundance (dark blue, n=19, median PFS=undefined) or low abundance (light blue, n=20, median PFS=242 days) of *Faecalibacterium* (top PFS curve). High abundance (dark red, n=20, median PFS=188 days) or low abundance (light red, n=19, median PFS=393 days) of Bacteroidales (bottom PFS curve). (D) Unsupervised hierarchical clustering of pathway class enrichment calculated as the number of MetaCyc pathways predicted in the metagenomes of fecal samples from 25 patients (R=14, NR=11). Columns represent patient samples (blue=R, red=NR) and rows represent enrichment of predicted MetaCyc pathways (blue=low enrichment, black=medium enrichment, yellow= high enrichment). Black text: biosynthetic pathways, blue text: degradative pathways. * $p < 0.05$.

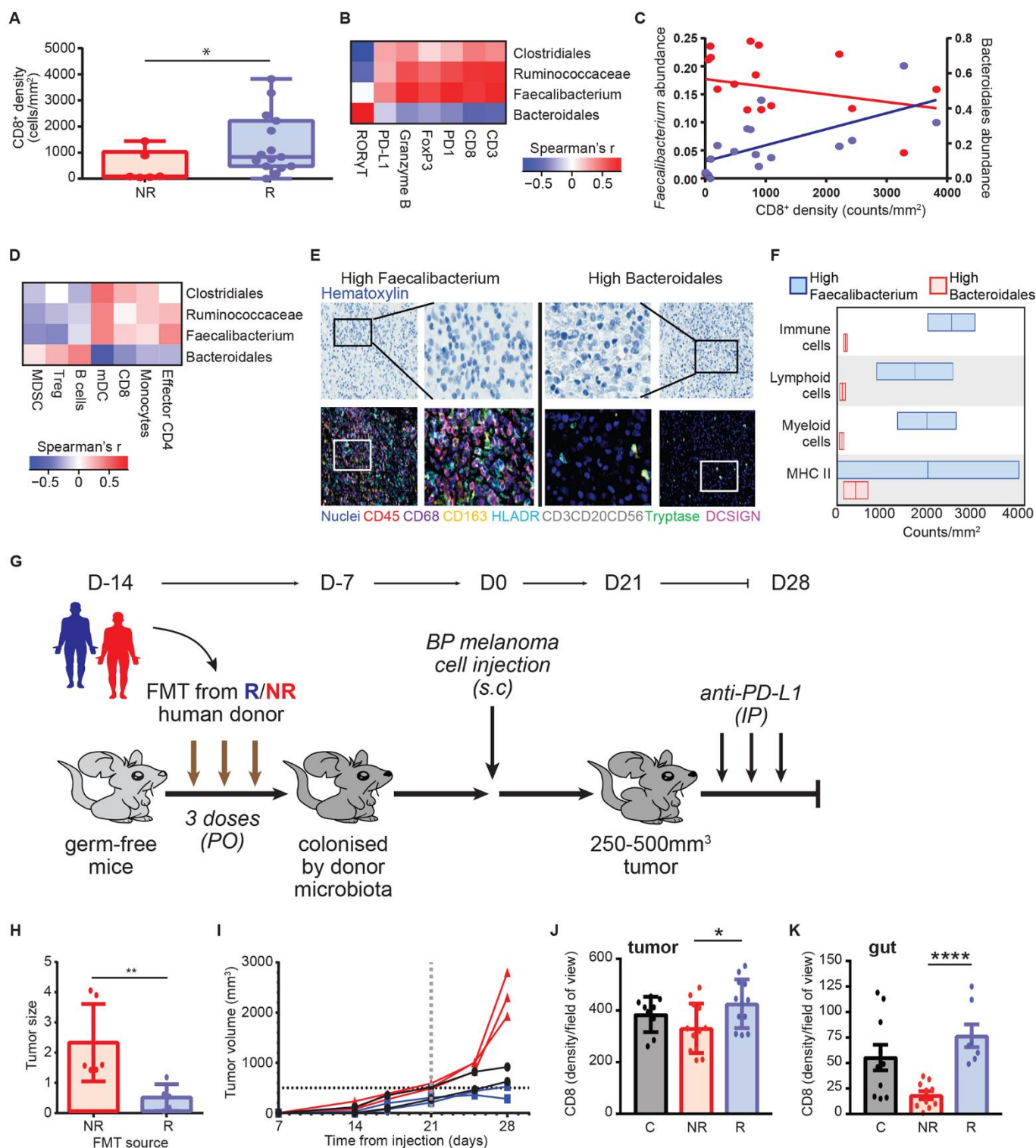


Fig. 4. A favorable gut microbiome is associated with enhanced systemic and anti-tumor immunity. (A) Quantification by IHC of the CD8+ T cell infiltrate at pre-treatment in tumors in R (n=15, blue) and NR (n=6, red) by one-sided MW test. Error bars represent the distribution of CD8+ T cell densities. (B) Pairwise Spearman rank correlation heatmap of significantly different taxa in fecal samples (n=15) at baseline and CD3, CD8, PD-1, FoxP3, Granzyme B, PD-L1 and ROR γ T density by H-score in matched tumors. (C) Univariate linear regression between CD8+ counts/mm² in the tumor versus *Faecalibacterium* (blue, $r^2=0.42$, $p<0.01$) and Bacteroidales (red, $r^2=0.06$, $p=0.38$) abundance in the gut. (D) Pairwise Spearman rank correlation heatmap between significantly different fecal taxa and frequency of indicated cell types by flow cytometry in peripheral blood at baseline. (E) Representative multiplex IHC images and (F) Frequency of various immune cell types in patients having high *Faecalibacterium* (n=2) or Bacteroidales (n=2) in the gut. (G) Experimental design of studies in germ-free (GF) mice. Time in days (indicated as D) relative to tumor injection (2.5×10^5 tumor cells). (H) Difference in size by MW test of tumors at day 14, implanted in R-FMT (blue) and NR-FMT mice (red) expressed as fold change (FC) relative to average tumor volume of Control GF mice. Data from 2 independent FMT experiments (R-FMT, n=5, median FC=0.18; NR-FMT, n=6, median FC=1.52). (I) Representative tumor growth curves for each GF mouse from α -PD-L1 treated R-FMT (blue n=2, median tumor volume=403.7 mm³), NR-FMT (red n=3, median tumor volume=2301 mm³), and Control (black, n=2, median tumor volume=771.35 mm³) mice. Statistics are as follows: $p=0.20$ (R-FMT vs NR-FMT), $p=0.33$ (NR-FMT vs Control) by MW test. Dotted black line marks tumor size cutoff for α -PD-L1 treatment (500mm³). (J) Quantification of CD8+ density in tumor of R-FMT (n=2, median=433.5 cells/HPF across 12 regions), NR-FMT (NR-FMT n=2, median=325 cells/HPF across 12 regions) and Control mice (n=2, median=412 cells/HPF across 9 regions). MW test $p=0.30$ (R-FMT vs Control). (K) Quantification of CD8+ density in gut (R-FMT n=2, median=67 cells/HPF across 7 regions), NR-FMT (n=2, median=24 cells/HPF across in 5 regions), Control n=2 (median=47 cells/HPF across 10 regions). MW test $p=0.17$ (R-FMT vs Control). * $p<0.05$, ** $p<0.01$, **** $p<0.0001$.

Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients

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. Petaccia de Macedo, T. Cotechini, T. Kumar, W. S. Chen, S. M. Reddy, R. Szczepaniak 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. Marcelo 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 and J. A. Wargo

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