From: Ulrich, Gretchen

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Subject: U.S. TRADEMARK APPLICATION NO. 86300247 - ONCOBIOME - 00449 - Request for Reconsideration Denied - Return to TTAB - Message 1 of 18

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Attachment Information:

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Files: oncobiome mirage- full txt_Page_01.jpg, oncobiome mirage- full txt_Page_02.jpg, oncobiome mirage- full txt_Page_03.jpg, oncobiome mirage- full txt_Page_04.jpg, oncobiome mirage- full txt_Page_05.jpg, oncobiome mirage- full txt_Page_06.jpg, oncobiome mirage- full txt_Page_07.jpg, oncobiome mirage- full txt_Page_08.jpg, oncobiome mirage- full txt_Page_09.jpg, oncobiome mirage- full txt_Page_10.jpg, oncobiome mirage- full txt_Page_11.jpg, oncobiome mirage- full txt_Page_12.jpg, ovarian oncobiome_Page_01.jpg, ovarian oncobiome_Page_02.jpg, ovarian oncobiome_Page_03.jpg, ovarian oncobiome_Page_04.jpg, ovarian oncobiome_Page_05.jpg, ovarian oncobiome_Page_06.jpg, ovarian oncobiome_Page_07.jpg, ovarian oncobiome_Page_08.jpg, 86300247.doc

UNITED STATES PATENT AND TRADEMARK OFFICE (USPTO)
OFFICE ACTION (OFFICIAL LETTER) ABOUT APPLICANT'S TRADEMARK APPLICATION

U.S. APPLICATION SERIAL NO. 86300247

MARK: ONCOBIOME

CORRESPONDENT ADDRESS:
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GENERAL TRADEMARK INFORMATION:

http://www.uspto.gov/trademarks/index.jsp

VIEW YOUR APPLICATION FILE

APPLICANT: EVELO THERAPEUTICS, INC.

CORRESPONDENT'S REFERENCE/DOCKET NO:

00449

CORRESPONDENT E-MAIL ADDRESS:

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REQUEST FOR RECONSIDERATION DENIED

ISSUE/MAILING DATE: 7/21/2017

The trademark examining attorney has carefully reviewed applicant's request for reconsideration and is denying the request for the reasons stated below. *See* 37 C.F.R. §2.63(b)(3); TMEP §§715.03(a)(ii)(B), 715.04(a). The following requirement(s) and/or refusal(s) made final in the Office action dated December 29, 2016 are maintained and continue to be final: Section 2(e)(1) refusal. *See* TMEP §§715.03(a)(ii)(B), 715.04(a). The following requirement(s) and/or refusal(s) made final in the Office action are withdrawn: requirement for a definite Class 5 identification of goods. *See* TMEP §§715.03(a)(ii)(B), 715.04(a).

In the present case, applicant's request has not resolved all the outstanding issue(s), nor does it raise a new issue or provide any new or compelling evidence with regard to the outstanding issue(s) in the final Office action. In addition, applicant's analysis and arguments are not persuasive nor do they shed new light on the issues. Accordingly, the request is denied.

If applicant has already filed a timely notice of appeal with the Trademark Trial and Appeal Board, the Board will be notified to resume the appeal. *See* TMEP §715.04(a).

If no appeal has been filed and time remains in the six-month response period to the final Office action, applicant has the remainder of the response period to (1) comply with and/or overcome any outstanding final requirement(s) and/or refusal(s), and/or (2) file a notice of appeal to the Board. TMEP §715.03(a)(ii)(B); *see* 37 C.F.R. §2.63(b)(1)-(3). The filing of a request for reconsideration does not stay or extend the time for filing an appeal. 37 C.F.R. §2.63(b)(3); *see* TMEP §§715.03, 715.03(a)(ii)(B), (c).

FINAL REFUSAL: SECTION 2(e)(1) MERELY DESCRIPTIVE - MAINTAINED & CONTINUED

Registration is refused because the applied-for mark merely describes a feature, characteristic, purpose, intended use and subject matter of applicant's goods and services. Trademark Act Section 2(e)(1), 15 U.S.C. §1052(e)(1); *see* TMEP §§1209.01(b), 1209.03 *et seq.*

A mark is merely descriptive if it describes or immediately conveys knowledge of an ingredient, quality, characteristic, function, feature, purpose, or use of an applicant's goods and/or services. TMEP §1209.01(b); *see, e.g.*, *In re TriVita, Inc.*, 783 F.3d 872, 874, 114 USPQ2d 1574, 1575 (Fed. Cir. 2015) (quoting *In re Oppedahl & Larson LLP*, 373 F.3d 1171, 1173, 71 USPQ2d 1370, 1371 (Fed. Cir. 2004)); *In re Steelbuilding.com*, 415 F.3d 1293, 1297, 75 USPQ2d 1420, 1421 (Fed. Cir. 2005) (citing *Estate of P.D. Beckwith, Inc. v. Comm'r of Patents*, 252 U.S. 538, 543 (1920)). "A mark may be merely descriptive even if it does not describe the 'full scope and extent' of the applicant's goods or services." *In re Oppedahl & Larson LLP*, 373 F.3d 1171, 1173, 71 USPQ2d 1370, 1371 (Fed. Cir. 2004) (citing *In re Dial-A-Mattress Operating Corp.*, 240 F.3d 1341, 1346, 57 USPQ2d 1807, 1812 (Fed. Cir. 2001)); TMEP §1209.01(b). Rather, it is enough if a mark describes only one significant function, attribute, or property. *In re The Chamber of Commerce of the U.S.*, 675 F.3d 1297, 1300, 102 USPQ2d 1217, 1219 (Fed. Cir. 2012); TMEP §1209.01(b); *see In re Oppedahl & Larson LLP*, 373 F.3d at 1173, 71 USPQ2d at 1371.

Determining the descriptiveness of a mark is done in relation to an applicant's goods and/or services, the context in which the mark is being used, and the possible significance the mark would have to the average purchaser because of the manner of its use or intended use. *See In re The Chamber of*

Commerce of the U.S., 675 F.3d 1297, 1300, 102 USPQ2d 1217, 1219 (Fed. Cir. 2012) (citing *In re Bayer Aktiengesellschaft*, 488 F.3d 960, 963-64, 82 USPQ2d 1828, 1831 (Fed. Cir. 2007)); TMEP §1209.01(b). Descriptiveness of a mark is not considered in the abstract. *In re Bayer Aktiengesellschaft*, 488 F.3d at 963-64, 82 USPQ2d at 1831.

Applicant's mark is "ONCOBIOME" for the following goods and services:

Class 5: Microbial preparations for pharmaceutical, veterinary and medical purposes, namely, microbial preparations for use as tumor suppressing agents, immuno-suppressing agents, immuno-stimulating agents, and anti-cancer agents; dietary supplements, nutritional supplements, nutraceuticals for use as dietary supplements, food supplements, nutritionally fortified vegetable-based food, beverages, and nutritional food bars, all of the foregoing containing microorganisms for medical, therapeutic, or preventative purposes; therapeutic pharmaceuticals containing microorganisms for medical and veterinary use for the treatment and prevention of pre-neoplastic lesions, neoplasms, tumors, cancers, and metastases; therapeutic pharmaceuticals containing derivatives of microorganisms for medical and veterinary use for the treatment and prevention of pre-neoplastic lesions, neoplasms, tumors, cancers, and metastases; pharmaceutical, veterinary and medical preparations and substances, namely, anti-microbial preparations and substances, anti-bacterial, anti-fungal, anti-viral, and anti-protist preparations and substances, all for the treatment of cancer, cancer symptoms, cancer-related diseases, and side-effects of cancer therapies; anti-cancer preparations and substances; preparations for inhibiting microbiological decomposition in food, beverages, animal feed and pharmaceuticals, namely, combination preparations containing in majority part antimicrobial preparations and lesser amounts of microbial preparations and anti-cancer preparations for use as a preservative; pharmaceutical preparations and substances for the treatment of cancer, namely, microbial and anti-microbial preparations for veterinary and medical use; anti-cancer preparations for use in treating cancer symptoms, cancer-related diseases, and side-effects of cancer therapies; pharmaceutical preparations for the treatment of infectious diseases and cancer, namely, combination preparations of anti-cancer agents, microbial preparations and anti-microbial preparations; diagnostic kits for diagnosing disease, determination of likelihood of disease, prognosis of responsiveness to therapy and/or patient prognosis comprised of diagnostic reagents and buffers for the detection of microbes, viral, bacterial, fungal, and parasitic or non-parasitic protist infections, inflammation, and biomarkers associated with pre-neoplastic lesions, neoplasms, tumors, cancers, or metastases; preparations for detecting mutations in genes for medical purposes as used in the collection of large-scale genomic, metagenomic, proteomic, transcriptomic, epigenetic, and metabolomic medical data and related clinical data

Class 9: Scientific, analytical, and statistical computer software for creating and managing databases containing genomic, metagenomic, proteomic, transcriptomic, epigenetic, and metabolomic data derived from patient clinical care information and medical research data;

computer software for analyzing and creating statistical models derived from patient clinical care information and medical research data in the nature of genomic, metagenomic, proteomic, transcriptomic, epigenetic, and metabolomic data

Class 10: Medical diagnostic instruments for screening, assessing, and determining appropriate courses of treatment in relation to pre-neoplastic lesions, neoplasms, tumors, cancers and metastases

Class 42: Electronic data storage services, namely, electronic storage of large-scale genomic, metagenomic, proteomic, transcriptomic, epigenetic, and metabolomic data and related clinical data for treatment and diagnostic purposes; database, computer-readable program and software development services, namely, development of scientific, analytical, and statistical software and related databases for use in conjunction with genomic, metagenomic, proteomic, transcriptomic, epigenetic, and metabolomic data gathered from patient clinical care information and medical research data; scientific, chemical, biochemical and microbiological research; scientific research in the field of genetics and genetic engineering of microbes; scientific research in the field of genetic and molecular tests, including tests for microbial populations in vertebrate samples; research and development of therapeutic pharmaceuticals, consumer medical products, diagnostic equipment and prognostic tools consisting of genetically engineered microorganisms, combinations of microorganisms, and individually selected microorganisms for treatment and prevention of pre-neoplastic lesions, neoplasms, tumors, cancers and metastases

Class 44: Medical clinics; veterinary dentistry; behavioural analysis for medical purposes; genetic testing for medical purposes; collection and preservation of human blood for medical purposes; medical diagnostic testing for assessing pre-neoplastic lesions, neoplasms, tumors, cancers, or metastases

The term comprising the applied-for mark "oncobiome" refers to the "microbiota associated with cancer development." *See*, https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-016-0203-0; *see also*, "oncobiome" defined as, the "interplay between the study of the human microbiome and its influence on cancer development." *The Microbiome and Cancer: Is the 'Oncobiome'*, https://www.biomodulation.com/attachments/article/134/PIIS2405803315000060.pdf. As applicant's Class 5 goods are oncobiome medications, they are intended to work with the oncobiome, the applied-for mark describes an intended use, purpose, feature and characteristic of these goods. Applicant's software is oncobiome software that is for use in the oncobiome field and will presumably feature algorithms that accurately profile microorganisms in metagenomic samples; thus, the applied-for mark is descriptive of a feature, characteristic, intended use, purpose and subject matter of applicant's Class 9 and Class 42 software goods and services. Applicant's medical diagnostic instruments will be used to screen, biopsy, test and assessing the oncobiome, the applied-for mark is descriptive of a feature,

characteristic, intended use and purpose of the Class 10 goods. As applicant's electronic data storage services will store oncobiome data, and applicant's research services are oncobiome research services, "oncobiome" is merely descriptive of a feature, characteristic, intended use and purpose of these Class 42 services. As applicant's clinical and testing services are ones that will feature testing and clinical services related to the oncobiome, the applied-for mark is merely descriptive of a feature, characteristic, purpose and intended use of these Class 44 services.

Additionally, as the previously and currently attached internet evidence demonstrates, "oncobiome" is commonly used in a descriptive manner in relation to applicant's goods and services in that "oncobiome" describes an intended use, purpose, feature and characteristic of them. In the article "The Microbiome and Cancer: Is the 'Oncobiome' Mirage Real?" the authors note that "much of oncobiome research is currently focused on colorectal cancer" and that "oncobiome screening could potentially be designed to detect not only individual bacterial species associated with cancer but also…before…cancer has developed." https://www.biomodulation.com/attachments/article/134/PIIS2405803315000060.pdf. This article also provides a schematic entitled "Care of the oncobiome patient" which requires "screening," "treatment" and "surveillance" and a figure entitled "Proposed Oncobiome Studies Necessary to Progress the Field of Research." *See Id*; *see also,* https://www.cancer.gov/about-cancer/screening/screening-tests, https://www.cancer.gov/about-cancer/screening/screening-tests#other-tests. Thus, the term "oncobiome" in this article is used in a descriptive manner- it describes a feature and characteristic of medical testing, medical screenings, medical research, and scientific research, as well as the intended use and purpose of these services.

Additionally, it is worth noting that this article has been cited nine times in other scholarly articles, in multiple journal blogs and in numerous articles accessible via the internet. *See,* https://www.scopus.com/results/citedbyresults.uri?sort=plf-f&cite=2-s2.0-84957795451&src=s&imp=t&sid=282EB2466B5D09CA21FC9DB433B96998.wsnAw8kcdt7IPYLO0V48gA%3a30&sot=cite&sdt=a&sl=0&origin=inward&editSaveSearch=&txGid=282EB2466B5D09CA21FC9DB433B96998.wsnAw8kcdt7IPYLO0V48gA%3a2; http://www.blogsearchengine.org/search.html?cx=partner-pub-9634067433254658%3A5laonibews6&cof=FORID%3A10&ie=ISO-8859-1&q=%22the+microbiome+and+cancer+is+the+oncobiome+mirage+real%3F%22&sa.x=48&sa.y=14; https://www.google.com/?gws_rd=ssl#q=%22the+microbiome+and+cancer+is+the+oncobiome+mirage+real?%22&spf=1500648572897. Thus, this descriptive use of the term "oncobiome" in relation to applicant's services has been widely viewed.

Additional recent articles also use "oncobiome" descriptively. In the newly published article entitled "The ovarian cancer oncobiome" the authors note that the medical diagnostic element used in the study employed "probes." *See,* http://www.impactjournals.com/oncotarget/index.php?journal=oncotarget&page=article&op=download&path%5B%5D=16717&path%5B%5D=53459. Thus, "oncobiome" is descriptive of the intended use and purpose of these medical diagnostic elements, as well as a feature and characteristic of them.

Despite the recent release of this publication, this article is widely available and accessible via the internet. See, https://www.google.com/?gws_rd=ssl#q=%22The+ovarian+cancer+oncobiome%22&spf=1500652543703. In "The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next?" the authors note that "All four studies in patients with CIN [54, 55, 56, 57] are observational studies, and with lack of longitudinal data, it is only possible to demonstrate association with disease states rather than causality. This has been acknowledged as one of the current limitations of ongoing research into the 'oncobiome'; that is the microbiota associated with cancer development." https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-016-0203-0.

Oncobiome research employs software. Specifically, oncobiome research is performed using a proprietary platform, the applied-for mark is descriptive in relation to software goods and services. *See,* https://news.uchicago.edu/article/2016/04/21/uchicago-evelo-biosciences-develop-microbiome-based-cancer-therapy. "Platform" is defined as, "support software for a particular activity." See, http://foldoc.org//platform. Thus, oncobiome describes the intended use, feature, purpose and subject matter of applicant's software goods and services.

The oncobiome is also the subject matter of lectures, seminars, and PowerPoint presentations, and forums within the scientific and medical communities. *See,* seminar, University of Arizona, "Microbial Genomics and Colorectal Cancer: The Birth of the Oncobiome" http://cbc.arizona.edu/events/microbial-genomics-and-colorectal-cancer-birth-oncobiome; Surgery Grans Rounds – Dr. Ryan M. Thomas Presents 'Evidence for the Oncobiome: Can the Human Microbiota Regulate Carcinogenesis?' https://ufhealth.org/events/surgery-grand-rounds-dr-ryan-m-thomas-presents-evidence-oncobiome-can-human-microbiota; "Microbiome and cancer (oncobiome), https://cancercenter-facca.sites.medinfo.ufl.edu/files/2016/04/Topic-1-Viruses-Bacteria-and-the-Microbiome-Dr.-Jobin.pdf; Microbiome World Congress "Exploring the Cancer Microbiome or 'Oncobiome', and Whether it Will Offer a New Horizon in Colon Treatment and Diagnosis?" http://www.terrapinn.com/conference/microbiome-americas/agenda.stm; "It's the oncobiome, the interplay between the human microbiome and cancer development, which researchers feel may be important factor to consider in response to therapy, including immunotherapies and existing anti-neoplastic agents." http://worldmedicalinnovation.org/wp-content/uploads/2016/04/Partners-FORUM-2016-BROCHURE-D12-Cancer-160422_0942-FREV1-WEB-X3-SM-SPREADS.pdf.

The knowledge of the oncobiome and oncobiome research extends beyond the scientific and medical communities in to the public sphere. The term "oncobiome" is used descriptively in multiple news outlets, thus ensuring its perception by consumers as a descriptive term. A University of Chicago press release entitled "UChicago, Evelo Biosciences to develop microbiome-based cancer therapy" discusses its partnership with the applicant and contains the following quote: "Immunotherapy is a rapidly growing field with huge potential, and the University is at the forefront of oncobiome research." While the quotation mentions applicant, this usage of "oncobiome" indicates that it is not only a field of

research, but that it is used to descriptively in relation to cancer therapy and therapies. *See,* https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/oral-chemotherapy.html, https://www.cancer.gov/about-cancer/treatment/types. This news was also covered by Biotechnology Calendar in its "Science Market Update" section, and contains the same quote. *See*, http://info.biotech-calendar.com/uchicago-and-evelo-biosciences-partner-to-develop-new-cancer-immunotherapy. In "Big deal: a first real shot at the oncobiome" the author notes that "a bacterial pill based on bioinformatics that treats severe and deadly disease by reestablishing bacterial composition of the gut" and that "The oncobiome seems to suppress the immune response to the tumor effectively shielding it from harm and supporting it with nutrients." https://medium.com/@deal_by_deal/big-deal-a-first-real-shot-at-the-oncobiome-67b3dff1389d#.y46o0og84. The press release entitled "COSMOSID ANNOUNCES ONCOBIOME PARTNERSHIP WITH THE WHITE HOUSE CANCER MOONSHOT INITIATIVE" indicates that its bioinformatics platform (algorithms that accurately profile microorganisms in metagenomic samples) will be used for cancer microbiome, or oncobiome, research studies. See, http://www.cosmosid.com/blog-cosmosid/2016/10/17/cosmosid-announces-oncobiome-partnership-with-the-white-house-cancer-moonshot-initiative. This press release was republished by PR Newswire and Bioportfolio. *See,* http://www.prnewswire.com/news-releases/cosmosid-announces-oncobiome-partnership-with-the-white-house-cancer-moonshot-initiative-300345759.html, http://www.bioportfolio.com/news/article/2874663/CosmosID-Announces-Oncobiome-Partnership-with-the-White-House-Cancer-Moonshot-Initiative.html. Cathy Biase, a nutrition consultant provides the following article on her website: "Oncobiome: The New Frontier of Cancer Research?" See, http://www.cathybiase.com/oncobiome-the-new-frontier-of-cancer-research/?doing_wp_cron=1500642432.5371088981628417968750. The Ride for Roswell, a cancer charity race sponsored by Roswell Park Cancer Institute, had participants under Team "OncoBiome – Microbes Against Cancer" http://give.roswellpark.org/site/TR/Events/General?fr_id=1060&pg=team&team_id=1708; https://rideforroswell.org/about/; *see also,* "Now, to be clear, the research is still emerging and many questions remain to be answered, but the potential breakthroughs of research into the relationship between cancer and the microbiome, or "oncobiome" [12], may finally help us end our quest for the Holy Grail." https://rebelhealthtribe.com/microbiome-holy-grail/; "Not to get too much out in left field.....the (that) field of the microbiome & cancer (new term is "oncobiome")studies are in their relative infancy." https://www.inspire.com/fighterm/journal/why-chemotherapy-works-for-some-but-not-for-all-alternative-treatments/?page=9&s_item=post&s_type=ahpfr-; LinkedIn, "Or is it possible that a merging of two completely different disease-fighting strategies such as immuno-oncology and microbiome will lead to a new one? Perhaps an 'Oncobiome' is just around the corner. I'm excited about such innovative possibilities." https://www.linkedin.com/pulse/innovation-through-adversity-stepping-towards-solutions-singh; Facebook, https://www.facebook.com/cosmosid/; Twitter, https://twitter.com/hashtag/oncobiome?lang=en.

Please note that the two major reasons for not protecting descriptive marks are (1) to prevent the owner of a descriptive mark from inhibiting competition in the marketplace and (2) to avoid the possibility of costly infringement suits brought by the trademark or service mark owner. *In re Abcor Dev. Corp.*, 588 F.2d 811, 813, 200 USPQ 215, 217 (C.C.P.A. 1978); TMEP §1209. Businesses and competitors

should be free to use descriptive language when describing their own goods and/or services to the public in advertising and marketing materials. *See In re Styleclick.com Inc.*, 58 USPQ2d 1523, 1527 (TTAB 2001). The term "oncobiome" is already used widely and descriptively.

These noted uses of "oncobiome" not only demonstrate that oncobiome describes the intended use, the purpose, a feature and characteristic of applicant's goods and services, but that the widespread dissemination and usage of oncobiome in a descriptive manner will cause consumers to view the applied-for mark merely as a descriptive term. Therefore, registration is denied for the applied-for mark. The final refusal is maintained and continued.

Applicant's Arguments Against the Refusal

Applicant provides several arguments against the refusal. For the reasons noted, below, these arguments are unpersuasive.

The applicant argues that the applied-for mark ONCOBIOME "is suggestive, not descriptive, as applied to the applicant's goods and services" and that "one would need to reflect a bit on the mark in order to have an idea of the goods and services offered under it." Applicant's argument is unpersuasive. Determining the descriptiveness of a mark is done in relation to an applicant's goods and services, the context in which the mark is being used, and the possible significance the mark would have to the average purchaser because of the manner of its use or intended use. *See In re The Chamber of Commerce of the U.S.*, 675 F.3d 1297, 1300, 102 USPQ2d 1217, 1219 (Fed. Cir. 2012) (citing *In re Bayer Aktiengesellschaft*, 488 F.3d 960, 963-64, 82 USPQ2d 1828, 1831 (Fed. Cir. 2007)); TMEP §1209.01(b). "Whether consumers could guess what the product [or service] is from consideration of the mark alone is not the test." *In re Am. Greetings Corp.*, 226 USPQ 365, 366 (TTAB 1985). The question is not whether someone presented only with the mark could guess what the goods and/or services are, but "whether someone who knows what the goods and[/or] services are will understand the mark to convey information about them." *DuoProSS Meditech Corp. v. Inviro Med. Devices, Ltd.*, 695 F.3d 1247, 1254, 103 USPQ2d 1753, 1757 (Fed. Cir. 2012) (quoting *In re Tower Tech, Inc.*, 64 USPQ2d 1314, 1316-17 (TTAB 2002)); *In re Franklin Cnty. Historical Soc'y*, 104 USPQ2d 1085, 1087 (TTAB 2012). Thus, descriptiveness of a mark is not considered in the abstract. *In re Bayer Aktiengesellschaft*, 488 F.3d at 963-64, 82 USPQ2d at 1831. In this case, someone who knows what the goods and services are will understand the mark to convey information about them, namely that their purpose and intended use is with the oncobiome, that the oncobiome is a feature and characteristic of them, and that applicant's research is oncobiome research. Please note that "[a] mark may be merely descriptive even if it does not describe the 'full scope and extent' of the applicant's goods or services." *In re Oppedahl & Larson LLP*, 373 F.3d 1171, 1173, 71 USPQ2d 1370, 1371 (Fed. Cir. 2004) (citing *In re Dial-A-Mattress Operating Corp.*, 240 F.3d 1341, 1346, 57 USPQ2d 1807, 1812 (Fed. Cir. 2001)); TMEP §1209.01(b). It is enough if a mark describes only one significant function, attribute, or property. *In re The Chamber of Commerce of the*

U.S., 675 F.3d 1297, 1300, 102 USPQ2d 1217, 1219 (Fed. Cir. 2012); TMEP §1209.01(b); *see In re Oppedahl & Larson LLP*, 373 F.3d at 1173, 71 USPQ2d at 1371.

Applicant argues that because other marks that contain the suffix "BIOME" – CARDIOBIOME, PROBIOME and µBIOME- its mark should also be able to register. Applicant's argument is unpersuasive. The fact that third-party registrations exist for marks allegedly similar to applicant's mark is not conclusive on the issue of descriptiveness. *See In re Scholastic Testing Serv., Inc.*, 196 USPQ 517, 519 (TTAB 1977); TMEP §1209.03(a). An applied-for mark that is merely descriptive does not become registrable simply because other seemingly similar marks appear on the register. *In re Scholastic Testing Serv., Inc.*, 196 USPQ at 519; TMEP §1209.03(a). It is well settled that each case must be decided on its own facts and the Trademark Trial and Appeal Board is not bound by prior decisions involving different records. *See In re Nett Designs, Inc.*, 236 F. 3d 1339, 1342, 57 USPQ2d 1564, 1566 (Fed. Cir. 2001); *In re Datapipe, Inc.*, 111 USPQ2d 1330, 1336 (TTAB 2014); TMEP §1209.03(a). The question of whether a mark is merely descriptive is determined based on the evidence of record at the time each registration is sought. *In re theDot Commc'ns Network LLC*, 101 USPQ2d 1062, 1064 (TTAB 2011); TMEP §1209.03(a); *see In re Nett Designs, Inc.*, 236 F.3d at 1342, 57 USPQ2d at 1566.

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Trends in Cancer

CellPress

Opinion

The Microbiome and Cancer: Is the 'Oncobiome' Mirage Real?

Ryan M. Thomas[1,2,*] and Christian Jobin[3,*]

Investigations focused on the interplay between the human microbiome and cancer development, herein termed the 'oncobiome', have been growing at a rapid rate. However, these studies to date have primarily demonstrated associative relationships rather than causative ones. We pose the question of whether this emerging field of research is a 'mirage' without a clear picture, or truly represents a paradigm shift for cancer research. We propose the necessary steps needed to answer crucial questions and push the field forward to bring the mirage into a tangible reality.

The Oncobiome Mirage Appears

Of all human maladies, nothing strikes fear into our hearts, minds, and souls as cancer. A diagnosis of hypertension, diabetes, or any other litany of chronic diseases that can be controlled with medication will produce a very different response than that of cancer. Researchers therefore press forward, attempting to uncover the smoking gun to explain tumor susceptibility, initiation, and progression. This search has been tried countless times with similar, often discouraging, results. What then makes investigators think that work involving the host microbiota and cancer will be any different, or is it all only a mirage?

The microbiota encompass a wide variety of microorganisms (bacteria, viruses, protozoa, fungi, and archea) and this eclectic ecosystem shares the body space of every individual, creating a **commensal**, symbiotic, and **pathobiont** relationship that has garnered increasing attention regarding its role in carcinogenesis (see Glossary) [1–5]. Of all the body surface, the gastrointestinal tract harbors the greatest number and diversity of microbes in the human body, with bacteria representing the bulk of the microbiota (10^{12} bacteria/gm feces) [6]. Although the oncogenic role of viruses has been recognized [5], bacteria represent the chief member of the microbiota and will be the focus of this discussion. Perhaps the most recognized link between bacteria and cancer is the case of *Helicobacter pylori* and non-cardia gastric carcinoma [7,8]. This bacterium has been shown to secrete several virulence factors such as *CagA* (cytotoxin-associated gene A), *VacA* (vacuolating cytotoxin A), urease, and *NapA* (neutrophil-activating protein A) that result in oxidative stress, chronic inflammation, and host DNA damage that can lead to carcinoma [9–11]. Considering that *H. pylori* has been designated a type I carcinogen by the World Health Organization [12,13], several clinical trials have been performed to modulate the risk of gastric cancer by eradicating the bacteria in infected individuals [14,15]. A recent meta-analysis of six randomized controlled trials demonstrates a slight risk reduction of gastric cancer with *H. pylori* elimination [16]. Despite this link between a pathogenic organism and carcinogenesis, there still has been little direct evidence that the symbiotic microbiota modulates carcinogenesis in humans. The relationship between cancer and the host microbiota, to be termed the '**oncobiome**', could be a mirage: we have an idea of an image in the distance but are uncertain of its true reality or significance. What is known is that more people are taking notice of this mirage – but is it real?

Trends

The 'oncobiome' is the expanding field of research investigating the role of the microbiota and associated microbiome on human cancer development.

While particular bacteria, such as *Escherichia coli*, *Bacteroides*, and *Fusobacterium*, as well as associated toxins/genotoxins, have been associated with colon cancer development in mouse and human studies, there is no evidence that these microbes or metabolites directly cause cancer.

The oncobiome field is currently limited by studies of microbiota association with cancer, rather than with causation of cancer.

Should the influence of the oncobiome be confirmed, it can be envisaged that the screening, treatment, and surveillance of cancer patients will one day incorporate this research.

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For our interpretation of the oncobiome to become clearer, we have much more to uncover. Much of oncobiome research is currently focused on colorectal cancer (CRC), which has been considered the ideal malignancy to study the effects of the host–microbe relationship on carcinogenesis and will serve as the model for most discussion points in this article. This focus is for obvious reasons as the large intestine harbors the greatest number and diversity of microbes in the human body (10^{12} bacteria/gm feces) [6]. Multiple studies using advanced genomic approaches have expanded the relationship between intestinal microbes and CRC development [17–26]. However, these investigations have mostly yielded circumstantial evidence implicating bacteria in CRC, and should give pause to those delving into the field because the mirage may be deceiving.

What Does the Mirage Look Like?

Our current interest and vision of the oncobiome developed over a century ago with the identification of bacteria in cancer specimens [27]. Since that time this association has been further explored [28] as well as the differences in fecal bacterial composition in populations at risk for CRC development [29]. Carcinogenesis is inherently a process of inflammation, with many proinflammatory and immunosuppressive pathways acting along the neoplastic process (Box 1) [30–32]. These immunological pathways have been functionally investigated in humans and mouse models of cancer, including CRC [33–40]. With a well-established impact of microbial products on the innate and adaptive immune system [41–45], one could speculate that bacteria could influence carcinogenesis through immune responses. The concept is clearly illustrated in a mouse model of impaired intestinal barrier function where the exposure of immune cells to the microbial product lipopolysaccharide (LPS) favors intestinal tumor growth through the action of interleukin (IL)-23/IL17 [46]. Although the link between microbial products, inflammation, and carcinogenesis is firmly established, the role of microbes acting as a consortium on neoplasia is less clear. Using genomic approaches, multiple studies have compared the intraluminal and mucosal surface microbiota between healthy patients and those with CRC [19,20,23,47,48]. Although no consensus 'cancer-biota' has emerged from these studies, it appears that the abundance of taxa associated with a protective function (e.g., *Roseburia*) decreased while taxa with potential deleterious effects (e.g., *Escherichia/Shigella*, *Klebsiella*, *Fusobacterium*)

Box 1. The Interplay Between Microbes, Inflammation, and Cancer

Although the majority of data for the interplay between host microbiota, inflammation, and carcinogenesis involve investigations on CRC, many of these same pathways may be applicable to other malignancies, particularly those that have a direct communication to the gastrointestinal tract. Bacteria may exert deleterious effects on their host in several ways, including metabolism of ingested material into toxic metabolites, direct secretion of toxic substances, and promotion of inflammatory pathways. For example, microorganism-associated molecular patterns (MAMPs) are components of the microbe such as lipopolysaccharide (LPS), flagellin, and nucleic acids that are recognized by the host immune system via pattern recognition receptors (PRRs) [154–156]. The best-characterized of these PRRs include Toll-like receptors (TLRs) and Nod-like receptors (NLRs) family [157,158]. Upon binding of MAMPs to these PRRs, various host responses occur that modify immune status. For example, LPS, present as part of the outer membrane on Gram-negative bacteria [154,159], binds to TLR4 [160,161] which upregulates IL-6 [162] and tumor necrosis factor (TNF) production, with subsequent recruitment of mononuclear cells, inhibition of T cell apoptosis, and inhibition of regulatory T cell (Treg) differentiation [32,163]. These events lead to persistent and unchecked inflammation. Furthermore, MAMPs serve to activate Th17 with subsequent upregulation of the proinflammatory cytokine, IL-23, and inhibition of IL-10, an anti-inflammatory cytokine [32]. These proinflammatory cytokines foster the neoplastic cascade by promoting cellular proliferation and inhibiting apoptosis [164,165]. Based on dietary carbohydrate consumption, byproducts of bacterial fermentation lead to the production of short-chain fatty acids (SCFA) which, through various G protein-coupled receptors located on colonic epithelium, activate $CD4^+$ T cells with differentiation into regulatory T cells and the production of the anti-inflammatory cytokines IL-10 and transforming growth factor (TGF)-β [69]. These mediators also serve to inhibit the proinflammatory cytokines IL-6 and TNF. Therefore, in a state of dysbiosis whereby decreased fermentation of carbohydrates into SCFAs leads to a relative decrease in the anti-inflammatory signaling pathways, potentially leaving proinflammatory pathways unchecked. This may further lead to disruption of normal epithelial barriers, resulting in bacterial translocation and further aberrant signaling. Such dysregulation would potentially lead to increased host cellular proliferation, decreased apoptosis, and anchorage-independent growth – all hallmarks of malignant transformation [166].

Glossary

Commensal: describes the relationship between two organisms in which one benefits without affecting, or itself being beneficial, to the second organism.
Dysbiosis: a state whereby the microbial composition of the host is unbalanced or skewed toward particular microorganisms as compared to the composition of a 'healthy' host.
Eubiosis: a state whereby the microbial composition of the host is of a normal proportion that is typically found in 'healthy' individuals.
Germ-free (GF): refers to animals conceived, born, and raised in a sterile environment and thus lack any microorganisms (except endogenous viruses).
Metabolomic: the study of the specific metabolites produced by microbes, either individually or collectively as part of the host microbiota.
Oncobiome: the intricate interplay and study of the human microbiome and its influence on cancer development.
Pathobiont: microorganisms that normally behave in a symbiotic manner with their host but exhibit pathogenic potential based on changes in their abundance or environmental conditions.
Planktonic: microbes that exist as single cells in a free-floating environment that are typically fast-growing and susceptible to environmental influences/drugs, as opposed to microbes in a biofilm which are slower-growing communities of adherent bacteria that are more tolerant of environmental influences.

increased in either stool or mucosal location [49]. These studies therefore suggest that microbial **dysbiosis** is associated with CRC development. Whether these associations are causative and can therefore modulate initiation, progression, or metastasis remains unclear.

Nevertheless, the concept that dysbiosis could be linked to CRC pushed investigators to test whether microbial genes could serve as cancer biomarkers [50,51]. These studies showed that the detection of non-invasive, early-stage CRC could be feasible by using taxonomic microbial markers. Although microbial biomarkers do not need to be functionally linked to CRC to be clinically useful, the study of microbial genomics in relation to CRC pathogenesis must push beyond the associative phase to significantly contribute to disease knowledge. Adding complexity to this issue, microbiome data are not informative of the organizational level of microbial communities in a given niche. A recent study has reinforced the notion that genomic analysis of fecal samples alone may provide limited information on host–microbiota interaction in CRC [52]. Indeed, right-sided colon tumors are more likely associated with biofilm-producing bacteria because they were present in 89% of samples versus only 12% of left-sided tumors associated with biofilm-producing bacteria. In addition, subjects with biofilm-positive tumors possessed biofilm aggregates that were distant from their tumors and that were associated with normal mucosa, perhaps indicating susceptibility to such colonization. Clearly, microbes living in a **planktonic** state exhibit a different phenotypic and **metabolomic** profile than those organized in a biofilm community [53–56], and this must be accounted for in future investigations.

Even assuming a single causative organism, which is unlikely to be the case, difficulties culturing specific microbes to fulfill Koch's postulate have created barriers to establishing cancer causation. Nevertheless, evidence gathered from preclinical models hint at a functional role of microbes in CRC. For example, **germ-free** (GF) Fischer rats demonstrated decreased spontaneous tumor formation compared to conventionally housed rats, as well as decreased intestinal tumors in a 1,2-dimethylhydrazine-induced model of carcinogenesis [57,58]. In addition, utilizing the adenomatous polyposis coli (APC) multiple intestinal neoplasia (Min) murine model of colon carcinogenesis, which possesses a point mutation in the murine homolog of the human *APC* tumor-suppressor gene that results in multiple (>100) intestinal adenomas [59], a reduction in colon tumors was noted in GF $Apc^{Min/+}$ mice compared to conventionally housed controls [60]. Finally, increased carcinogenesis was noted after the enteral introduction of *Fusobacterium nucleatum* or enterotoxigenic *Bacteroides fragilis*, in $Apc^{Min/+}$ mice confirming the effect of bacteria on cancer formation *in vivo* [47,61]. However, as opposed to IBD where antibiotic usage has shown some clinical effectiveness [62–64], no such clinical studies are available for CRC. Despite these data in favor of the oncobiome, the microbiota may also prevent carcinogenesis through protective mechanisms, detoxification, or anticancer metabolites [65–67]. Precedence has nevertheless been established that supports a potential modulatory role for microbes in carcinogenesis [57,58,60,68]. However, these models do not replicate clinical reality, and CRC-causing bacteria in mouse models have not been confirmed by observational studies in humans.

The Ever-Changing Mirage

While the oncobiome mirage has not revealed the oasis of desired answers, the image is beginning to morph from bacterial association to causative pathways. Despite the fact that prospective/longitudinal studies have not been able to assess CRC risk in patients based on changes in their microbiota, animal studies have begun to interrogate the mechanistic details of bacteria-associated carcinogenesis. Current studies are focusing on the links between CRC and toxic bacterial metabolites, diet-induced changes, and bacteria-derived genotoxic substances, albeit unproven in human studies. For example, under eubiotic conditions the microflora ferments ethanol into acetaldehyde and carbohydrates into the three primary short-chain fatty acids, acetate, propionate, and butyrate [69,70]. It has been demonstrated that a correlation may exist between low-butyrate and high-acetate levels in patients with adenomatous polyp

formation and colon cancer [71,72]. Although no difference in the overall bacterial community was demonstrated, titers of the butyrate-producing species *Ruminococcus* and *Pseudobutyrivibrio ruminis* were lower in CRC stool specimens, which correlated with lower butyrate levels [72]. While preclinical models have demonstrated a role of microbe-derived butyrate in dampening colitis-associated CRC development, similar studies have shown the opposite effect [73–77]. This may be secondary to host genetics or possible implications of dietary fiber [78], thus setting the stage for microbial activities being central to diet-induced carcinogenesis. The role of butyrate and other diet-induced metabolites in carcinogenesis likely requires further investigation.

In addition to the products of carbohydrate metabolism, toxins from bacterial metabolism have likewise been implicated in CRC. For example, a variety of ingested compounds and nutritional components are metabolized by host microbes into potentially pro- and anticarcinogenic metabolites [69,79,80], such as the metabolism of proteins into *N*-nitroso compounds, ammonia, polyamines, and hydrogen sulfide. Colonic epithelial exposure to these metabolites results in chronic inflammation [69,81–86]. The role of these compounds in CRC development is in many ways still hypothetical, and may be related to direct dietary ingestion rather than to byproducts of bacterial metabolism [87]. However, these studies are limited in that they rely on the local effects of microbiota-produced toxins, such as inflammation and epithelial cell damage in the case of CRC. Although potentially important, studies have not taken into consideration carcinogenic mediators that may act from distant sites [88–91]. It is currently unknown if systemic absorption of such metabolites infers the same potential cancer susceptibility as seen experimentally at the local (epithelial) level. While the production of pro- and anti-inflammatory metabolites by the commensal system has been implicated in CRC initiation and progression, the data regarding an actual causal relationship still do not exist [21,24,92]. Furthermore, fecal samples alone should not be relied upon for investigating the influence of microbial metabolites on carcinogenesis because metabolites from various sources can be detected in serum and urine samples, and may correlate with gastrointestinal dysbiosis or CRC risk. Metabolomics of serum and urine samples should therefore be undertaken to detect dysbiosis and cancer risk because fecal samples alone may not account for metabolites that have been absorbed by the host [93–97]. It is these metabolites, and their resultant influence on the host, that will potentially play a large role, if one exists, in cancer development and demands further investigation. The development of computational algorithms capable of integrating the vast amounts of heterogeneous biomedical data (metabolites, GWAS, etc.) may help to generate an interacting map between microbial metabolites and host cancer susceptibility, and foster design of hypothesis-driven experiments.

Moreover, bacteria-derived genotoxic substances have garnered attention for their direct ability to impart DNA damage, which is distinct from genotoxicity from byproducts of bacterial metabolism such as hydrogen sulfide and reactive oxygen species [69,82,85,86,92]. An example of such a genotoxin is colibactin, encoded by the polyketide synthase (*pks*) genotoxicity island, which is found primarily in the Enterobacteriaceae family of bacteria, of which *E. coli* of the B2 group represents the main carrier [98]. The genotoxic effect of *pks*-positive strains of *E. coli* is likely secondary to the induction of double-strand DNA breaks with subsequent cell cycle arrest and genomic instability [66,99,100]. Previous studies showed that colonic mucosal samples from patients with CRC had a higher prevalence of *pks*-positive *E. coli* compared to controls [99,101,102]. Although preclinical models showed that *pks*-positive *E. coli* strains promote CRC [99,101,103], the link between high *E. coli* prevalence, genotoxicity, and neoplasia in human CRC has not been demonstrated. Therefore, the microbiota-mediated mechanisms of cancer initiation and progression, at least as it currently stands for CRC, are potentially multifold. As the mirage changes, further details regarding a potential role for specific microbes, microbial metabolites, and/or genotoxic agents will be necessary to maintain a clear image.

How to Make the Mirage Clearer

To pass the correlative threshold of oncobiome research and move into causative territory, a variety of studies using human-derived, cancer-associated microbes are necessary (Figure 1). For example, it would be important to determine the oncogenic potential of both human biofilm-positive and luminal microbial communities in preclinical models and to define their carcinogenic activities. This is especially important given that studies using stools from either healthy subjects or CRC patients have provided surprising results on the relationship between luminal bacteria and CRC, such that CRC development was more prominent in GF mice transplanted with stools from healthy subjects than those from CRC patients [104]. Therefore, although CRC is communicable between mice [105], the transfer of carcinogenesis between human and mouse remains to be established. Preclinical models using transmission of these microbes would therefore help to define the natural history of both sporadic and hereditary forms of carcinogenesis, as has been used to study the impact of early *E. coli pks*$^{+}$ colonization on intestinal mucosa [106].

Moreover, it is important to understand if the acquisition of microbes with carcinogenic potential at birth influences CRC development later in life? It is known from kindred data and population

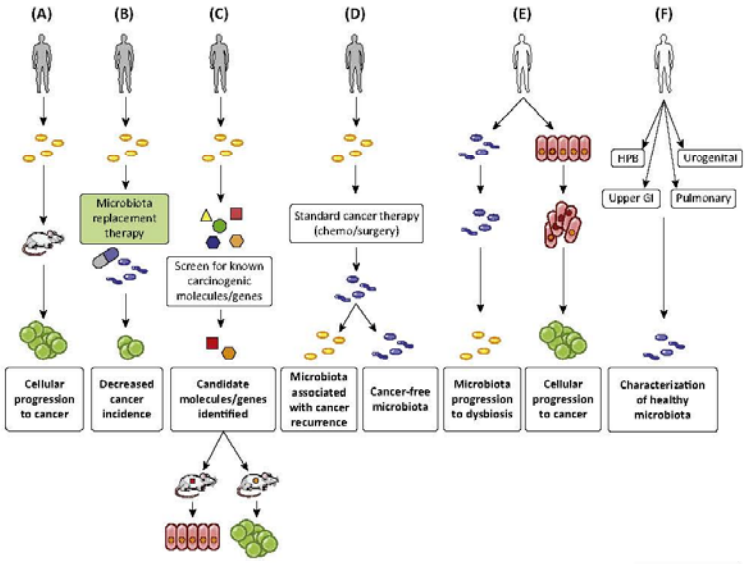

Figure 1. Proposed Oncobiome Studies Necessary to Progress the Field of Research. Cancer subjects (gray) or healthy subjects (white) are presented for each area of needed investigation. (A) Cancer-associated microbiota (yellow) is transmitted to mice with humanized immune systems to investigate their interaction with the human immune system and their ability to cause cancer. (B) Patients at high risk or with a genetic predisposition to cancer are treated with microbiota replacement therapy to restore eubiosis (blue). Patients are compared to the general population and control subjects not treated with microbiota replacement therapy for differences in cancer incidence. (C) The microbiota of cancer patients are screened for known carcinogenic molecules and genes. Candidates are identified and tested *in vivo* for their ability to cause cancer formation. (D) The microbiota of cancer patients are determined before and after standard cancer treatment. Determination is made regarding restoration of eubiosis and if continued or recurrent dysbiosis is associated with cancer recurrence. (E) The microbiota of healthy subjects are determined prospectively and correlated with the development of pre-cancerous and cancerous lesions. (F) The microbiota of healthy individuals are determined for various organ systems and body fluids. This will prove crucial for future investigations and to determine if body fluids/specimens from one site can act as a surrogate for a different disease site.

studies that the age of onset for people with predispositions to cancers, such as hereditary breast or gastric, or those associated with chronic inflammatory states such as CRC with ulcerative colitis, is younger than for sporadic forms ([107–112]; National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) Program Fact Sheets: Breast Cancer, http://seer.cancer.gov/statfacts/html/breast.html; Colon and Rectum Cancer, http://seer.cancer.gov/statfacts/html/colorect.html; Stomach Cancer, http://seer.cancer.gov/statfacts/html/stomach.html). This demonstrates a potential lead-time between genetically induced and microbe-induced cancers, and likely represents different timelines of progression. Regardless, the question remains: is microbial carcinogenic activity triggered early upon colonization or is it under the influence of external factors (diet, inflammation, or environment)? Evidence exists that our microbiota are established *in utero* and develop rapidly, but with stable diversity during the first year of life, and continue to increase in abundance throughout the first decade [113–115]. This may suggest that alteration of the microbiota during life via external factors (diet, environment) alters cancer susceptibility. The finding that the *E. coli pks*$^{+}$ strain fails to promote CRC in a model of colitis-associated CRC highlights the complex interaction between microbes and host [116]. In this study, the authors showed, using *Il10*$^{-/-}$ mice defective in mature T and B lymphocytes, that development of colitis-carcinogenesis led to transcriptional changes in *E. coli* gene repertoire, including genes present in the *pks* genotoxic island.

Furthermore, could microbe-derived carcinogenic molecules and genes be detected in CRC patients and do they correlate with malignancies? For example, expression of the *F. nucleatum* virulence factor FadA (*Fusobacterium* adhesin A), which is implicated in bacterial attachment and invasion, is increased in carcinoma tissues of CRC subjects and correlates with oncogenic and inflammatory host gene expression (Box 1) [117]. Similarly, the *bft* (*Bacteroides fragilis* toxin) gene responsible for the enterotoxic properties of enterotoxigenic *Bacteroides fragilis* and CRC development in *Apc*$^{Min/+}$ mice [61] was found in a greater proportion of colon cancer specimens than healthy mucosal controls, suggesting a role for this bacterial toxin in carcinogenesis [118]. Finally, as the data on the interaction of the microbiota with the host immune system and tumor microenvironment mature [6,47,66,119], utilizing mice with humanized immune systems in conjunction with human microbes will prove crucial in dissecting the interaction between host microbes and the immune system in carcinogenesis [120,121].

As insight is gained regarding the oncobiome, focus will be placed on treatment of human diseases and mitigating cancer risk. Growing research has demonstrated the influence of the host microbiota on various chemotherapeutics [119,122,123]. Because not all chemotherapy trials result in drug efficacy against their targeted cancer, it can be hypothesized that this may be secondary to intestinal dysbiosis, which was not accounted for during trial design [124]. As such, it would be advantageous if these trials incorporated microbiota studies to correlate drug efficacy with microbial composition.

Finally, although the oncobiome in CRC is presently the most mature area, other malignancies demand attention. Gastrointestinal tumors (e.g., esophageal, gastric, hepatobiliary, and pancreatic) seem to be natural starting points, and the findings of CRC-microbiota research are potentially directly applicable to these malignancies as well. However, it is currently unknown what fluid/tissue sample(s) from these organs best reflect their unique microbiota. For example, is the microbiota of the bile, pancreatic fluid, or duodenum most reflective of hepato-pancreato-biliary (HPB) malignancies, or are these malignancies not influenced by their local microbiota but instead by microbiota from distant sites? The difficulty then becomes sample acquisition because obtaining such samples requires invasive procedures. However, one solution could be to recruit patients for trials who require upper endoscopy as part of their cancer workup. With these samples, future studies can initially focus on understanding the normal biota and later its possible connection between microorganisms and carcinogenesis.

Have We Reached the Mirage?

That the microbiota can cause cancer is a unique and potentially paradigm-shifting event. What then is to be done if particular bacterial species are confirmed to cause cancer? Hypothetical interventions would be based on the considerations of screening, treatment, and surveillance for each particular cancer (Figure 2). Using CRC as an example, screening begins at the age of 50 years for patients at average risk, and oncobiome tests could potentially augment or replace current screening modalities. For example, one National Comprehensive Cancer Network recommendation is that stool-based high-sensitivity guaiac or immunohistochemical testing be performed annually. This testing aims to detect occult blood and has been shown to reduce CRC mortality [125–127]. Because adenomatous (pre-malignant) polyps and early cancers may bleed only intermittently, if at all, this testing has the disadvantage of not being able to detect these lesions, prompting the recommendation to test three successive specimens. Emerging technologies rely on the detection of mutated *APC* or *KRAS* genes, or vimentin methylation in tumor cells sloughed in the stool. Overall, these tests have demonstrated poor sensitivity and specificity, and only one is currently available in the USA [128–130]. This method of screening is costly and, similarly to other screening methods, it relies on the detection of early signs or symptoms of cancer and thus is not necessarily preventative. However, oncobiome screening could potentially be designed to detect not only individual bacterial species associated with cancer but also dysbiosis long before adenomatous polyps or cancer have developed. Indeed a recent study characterized the microbiota from 'healthy' subjects and those with adenomas or CRC as confirmed by colonoscopy [51,131], and found that, when combined with known clinical risk factors for CRC (age, race, body mass index), combining six specific operational taxonomic

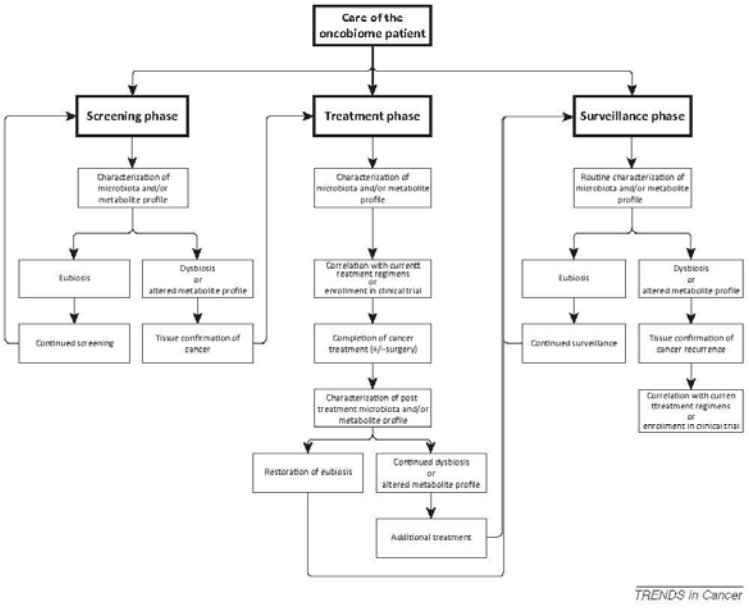

Figure 2. Hypothetical Integration of the Oncobiome into the Care of Cancer Patients. Each area is divided into screening, treatment, and surveillance phases. Each phase characterizes the microbiota of the patient based on the cancer to be screened/treated (i.e., feces for CRC). Treatment is based upon tested regimens that have demonstrated efficacy with a particular microbiota or metabolite profile. It can be envisaged that patients who have restoration of eubiosis will have improved cancer survival compared to those who maintain or relapse to dysbiosis after treatment.

units (OTU) of gut microbiota in stool samples significantly increased the ability to differentiate healthy subjects from those with adenomas or CRC. Likewise, taxonomic markers were identified through metagenomic sequencing of fecal samples to distinguish CRC patients from tumor-free patients [50]. While the sensitivity and specificity of the taxonomic markers was similar to the currently used screening method of fecal occult blood testing (FOBT: sensitivity 58% vs 49%, respectively; specificity 92%), their combined use with FOBT increased the sensitivity of CRC detection by more than 45% compared to FOBT alone (72% vs 49%, respectively), while maintaining the specificity (92%). While these data are limited, they demonstrate the possibility of utilizing microbiota composition to predict disease. It may therefore be envisaged that a proactive, as opposed to reactive, screening strategy could be implemented to prevent cancer formation, possibly through dietary modification as one example. However, while diet has previously been shown to alter the host microbiota, the direct effect of dietary modification on carcinogenesis is currently unresolved [132–134]. This screening strategy may also be useful for other malignancies as more evidence emerges on the oncobiome: saliva for oropharyngeal cancers, sputum for lung cancer, vaginal secretions for ovarian/uterine/cervical cancers, urine for renal and urinary bladder malignancies, and potentially feces for other gastrointestinal malignancies.

In the field of oncology, it is the hope that with the screening and diagnosis of cancer will come treatment options. Much effort has focused on individualized medicine, as evidenced by the use of gene arrays and patient-derived tumor xenografts to help guide patient discussions on cancer treatment and recurrence risk [135–140]. Assuming that the host microbiota plays a role in cancer, it too will provide an individualized approach to treatment. The potential influence of the microbiota on drug efficacy has been highlighted, and may have a great impact on future chemotherapy trials [119,123,141,142]. A situation can be envisaged whereby the microbiota of each patient is tested, before starting a chemotherapeutic treatment strategy, to choose the agents that will offer the greatest benefit. In this manner, the oncobiome will enter the arena of personalized medicine for cancer care with limitless possibilities.

Finally, as we gain a greater understanding of host **eubiosis** and the dysbiosis that occurs in various diseases, it is intriguing to think about restoration of eubiosis after cancer treatment. Typical cancer surveillance involves radiographic imaging which incurs a large financial burden to the patient and the medical community. However, if the curative treatment of cancer results in the restoration of eubiosis, this can be used to the advantage of the medical community for the purpose of cancer surveillance while limiting the use of current modalities. Much in the same way as the postoperative rise of serum carcinoembryonic antigen (CEA) levels may indicate CRC recurrence [143–145], a state of dysbiosis after postoperative restoration of eubiosis may indicate cancer recurrence or risk of recurrence. Tests for dysbiosis could potentially improve or augment the sensitivity and specificity of currently-available serum tumor markers, especially when one considers the fact that commonly used tumor markers have variable sensitivity and specificity for the diseases they aim to detect, are not produced in every clinical scenario by specific tumor types, and can have false positives even in the setting of curative surgery [146–152]. Cancer survival could be modified significantly by identifying patients who 'relapse' into a dysbiotic state. Lifestyle, dietary, or pharmacologic modifications could therefore be made to restore eubiosis and mitigate this risk. Alternatively, introduction of an entirely new microbiota could be envisaged, similarly to patients with recurrent *Clostridium difficile* infection [153].

Concluding Remarks

Despite the fact that there is, as yet, no direct evidence linking the human microbiota to cancer development and progression, the oncobiome field continues to grow rapidly with many unanswered questions (see Outstanding questions). The appeal of microbiota as an active component of diseases and health is too great to ignore, and intense investigation in this field of

Outstanding Questions

What cancer-associated microbiota profiles result in cancer initiation in mice with humanized immune systems?

Does the acquisition of microbes with carcinogenic potential early in life influence cancer development later in life?

Is the carcinogenic activity of particular microbes triggered upon colonization, or is a second 'hit' from external factors (diet, environment, chronic inflammation) required?

What is the lead time between colonization with cancer-associated microbes and cancer development?

Can microbe-derived carcinogenic molecules and/or genes be detected in human specimens (stool, urine, saliva), and do they correlate with cancer presence, stage of disease, or response to chemotherapy?

What patient samples are most reflective of the microbiota of a particular organ or cancer? For example, is bile most reflective of hepato-pancreato-biliary malignancies or is stool an appropriate surrogate?

Is dysbiosis a hallmark of particular cancers compared to healthy controls, and is restoration of eubiosis after treatment for cancer an indicator of improved survival?

Can alteration of the microbiota of individuals at high-risk for cancer development mitigate their risk?

research, especially cancer, would likely shed light onto this novel paradigm. While some studies demonstrate the association of microbial populations with various cancers, others have begun to interrogate the intricate relationship between the host, its immune system, and its microbiota. Placing added focus on the oncobiome in the context of clinical chemotherapy trials will undoubtedly yield important information with regards to drug metabolism and efficacy. Finally, should elements of the host microbiota prove to have a direct role in cancer development, the implications for cancer screening, treatment, and surveillance are limitless. This dynamic field is only in its infancy, and advancing it will require a concerted effort between the medical and scientific communities to view the mirage as reality.

References

1. Gill, S.R. *et al.* (2006) Metagenomic analysis of the human distal gut microbiome. *Science* 312, 1355–1359
2. Qin, J. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65
3. Rajilic-Stojanovic, M. *et al.* (2007) Diversity of the human gastrointestinal tract microbiota revisited. *Environ. Microbiol.* 9, 2125–2136
4. Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214
5. Moore, P.S. and Chang, Y. (2010) Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat. Rev. Cancer* 10, 878–889
6. Sommer, F. and Backhed, F. (2013) The gut microbiota – masters of host development and physiology. *Nat. Rev. Microbiol.* 11, 227–238
7. Marshall, B.J. and Warren, J.R. (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1, 1311–1315
8. Peek, R.M., Jr and Crabtree, J.E. (2006) *Helicobacter* infection and gastric neoplasia. *J. Pathol.* 208, 233–248
9. Hardbower, D.M. *et al.* (2013) Chronic inflammation and oxidative stress: the smoking gun for *Helicobacter pylori*-induced gastric cancer? *Gut Microbes* 4, 475–481
10. Koeppel, M. *et al.* (2015) *Helicobacter pylori* infection causes characteristic DNA damage patterns in human cells. *Cell Rep.* 11, 1703–1713
11. Wroblewski, L.E. and Peek, R.M., Jr (2013) *Helicobacter pylori* in gastric carcinogenesis: mechanisms. *Gastroenterol. Clin. North Am.* 42, 285–298
12. IARC Working Group (1994) *Infection with Helicobacter pylori. In IARC Monographs on the Evaluation of Carcinogenic Risks to Humans (Vol. 61) Schistosomes, Liver Flukes, and Helicobacter pylori*, pp. 177–240, WHO International Agency for Research on Cancer
13. de Martel, C. *et al.* (2012) Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. *Lancet Oncol.* 13, 607–615
14. Wong, B.C. *et al.* (2004) *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 291, 187–194
15. Pan, K.F. *et al.* (2015) A large randomised controlled intervention trial to prevent gastric cancer by eradication of *Helicobacter pylori* in Linqu County, China: baseline results and factors affecting the eradication. *Gut* Published online May 18, 2015. PMID: 25986943.
16. Fordl, A.C. *et al.* (2014) *Helicobacter pylori* eradication therapy to prevent gastric cancer in healthy asymptomatic infected individuals: systematic review and meta-analysis of randomised controlled trials. *BMJ* 348, g3174
17. Ahn, J. *et al.* (2013) Human gut microbiome and risk for colorectal cancer. *J. Natl. Cancer Inst.* 105, 1907–1911
18. Castellarin, M. *et al.* (2012) *Fusobacterium nucleatum* infection is prevalent in human colorectal carcinoma. *Genome Res.* 22, 299–306
19. Chen, W. *et al.* (2012) Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS ONE* 7, e39743
20. Kostic, A.D. *et al.* (2012) Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res.* 22, 292–298
21. Marchesi, J.R. *et al.* (2011) Towards the human colorectal cancer microbiome. *PLoS ONE* 6, e20447
22. McCoy, A.N. *et al.* (2013) *Fusobacterium* is associated with colorectal adenomas. *PLoS ONE* 8, e53653
23. Sanapareddy, N. *et al.* (2012) Increased rectal microbial richness is associated with the presence of colorectal adenomas in humans. *ISME J.* 6, 1858–1868
24. Sobhani, I. *et al.* (2011) Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS ONE* 6, e16393
25. Wang, T. *et al.* (2012) Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J.* 6, 320–329
26. Wu, N. *et al.* (2013) Dysbiosis signature of fecal microbiota in colorectal cancer patients. *Microb. Ecol.* 66, 462–470
27. Russell, W. (1890) An address on a characteristic organism of cancer. *Br. Med. J.* 2, 1356–1360
28. Wuerthele-Caspe, V. *et al.* (1950) Cultural properties and pathogenicity of certain microorganisms obtained from various proliferative and neoplastic diseases. *Am. J. Med. Sci.* 220, 638–646
29. Aries, V. *et al.* (1969) Bacteria and the aetiology of cancer of the large bowel. *Gut* 10, 334–335
30. Crusz, S.M. and Balkwill, F.R. (2015) Inflammation and cancer: advances and new agents. *Nat. Rev. Clin. Oncol.* Published online June 30, 2015. http://dx.doi.org/10.1038/nrclinonc.2015.105
31. Mantovani, A. *et al.* (2008) Cancer-related inflammation. *Nature* 454, 436–444
32. Elinav, E. *et al.* (2013) Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat. Rev. Cancer* 13, 759–771
33. Punkenburg, E. *et al.* (2015) Batf-dependent Th17 cells critically regulate IL-23 driven colitis-associated colon cancer. *Gut* Published online April 2, 2015. http://dx.doi.org/10.1136/gutjnl-2014-308227
34. Tiniakou, I. *et al.* (2015) High-density lipoprotein attenuates Th1 and Th17 autoimmune responses by modulating dendritic cell maturation and function. *J. Immunol.* 194, 4676–4687
35. Hodi, F.S. *et al.* (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363, 711–723
36. Lipson, E.J. and Drake, C.G. (2011) Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma. *Clin. Cancer Res.* 17, 6958–6962
37. Roshani, R. *et al.* (2014) Inflammatory cytokines in human pancreatic cancer. *Cancer Lett.* 345, 157–163
38. Galon, J. *et al.* (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 313, 1960–1964
39. Ebrahimi, B. *et al.* (2004) Cytokines in pancreatic carcinoma: correlation with phenotypic characteristics and prognosis. *Cancer* 101, 2727–2736
40. Grivennikov, S.I. (2013) Inflammation and colorectal cancer: colitis-associated neoplasia. *Semin. Immunopathol.* 35, 229–244

41. Ivanov, I.I. *et al.* (2008) Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 4, 337–349
42. Ivanov, I.I. *et al.* (2009) Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 139, 485–498
43. Hooper, L.V. *et al.* (2012) Interactions between the microbiota and the immune system. *Science* 336, 1268–1273
44. Macpherson, A.J. and Harris, N.L. (2004) Interactions between commensal intestinal bacteria and the immune system. *Nat. Rev. Immunol.* 4, 478–485
45. Hill, D.A. and Artis, D. (2010) Intestinal bacteria and the regulation of immune cell homeostasis. *Annu. Rev. Immunol.* 28, 623–667
46. Grivennikov, S.I. *et al.* (2012) Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 491, 254–258
47. Kostic, A.D. *et al.* (2013) *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 14, 207–215
48. Shen, X.J. *et al.* (2010) Molecular characterization of mucosal adherent bacteria and associations with colorectal adenomas. *Gut Microbes* 1, 138–147
49. Jobin, C. (2013) Colorectal cancer: looking for answers in the microbiota. *Cancer Discov.* 3, 384–387
50. Zeller, G. *et al.* (2014) Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol. Syst. Biol.* 10, 766
51. Zackular, J.P. *et al.* (2014) The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev. Res.* 7, 1112–1121
52. Dejea, C.M. *et al.* (2014) Microbiota organization is a distinct feature of proximal colorectal cancers. *Proc. Natl. Acad. Sci. U.S. A.* 111, 18321–18326
53. Johnson, C.H. *et al.* (2015) Metabolism links bacterial biofilms and colon carcinogenesis. *Cell Metab.* 21, 891–897
54. Sonnenburg, J.L. *et al.* (2004) Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nat. Immunol.* 5, 569–573
55. Villanueva, M.T. (2015) Metabolism: bacterial biofilms may feed colon cancer. *Nat. Rev. Cancer* 15, 320
56. Macfarlane, S. and Dillon, J.F. (2007) Microbial biofilms in the human gastrointestinal tract. *J. Appl. Microbiol.* 102, 1187–1196
57. Reddy, B.S. *et al.* (1975) Colon carcinogenesis with azoxymethane and dimethylhydrazine in germ-free rats. *Cancer Res.* 35, 287–290
58. Sacksteder, M.R. (1976) Occurrence of spontaneous tumors in the germfree F344 rat. *J. Natl. Cancer Inst.* 57, 1371–1373
59. Su, L.K. *et al.* (1992) Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 256, 668–670
60. Li, Y. *et al.* (2012) Gut microbiota accelerate tumor growth via c-jun and STAT3 phosphorylation in $APC^{Min/+}$ mice. *Carcinogenesis* 33, 1231–1238
61. Wu, S. *et al.* (2009) A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.* 15, 1016–1022
62. Singh, S. et al. (2015) Comparative efficacy of pharmacologic interventions in preventing relapse of Crohn's disease after surgery: a systematic review and network meta-analysis. *Gastroenterology* 148, 64–76
63. Kostic, A.D. *et al.* (2014) The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 146, 1489–1499
64. Wu, X.W. *et al.* (2015) Meta-analysis of ciprofloxacin in treatment of Crohn's disease. *Biomed. Rep.* 3, 70–74
65. Zhan, Y. *et al.* (2013) Gut microbiota protects against gastrointestinal tumorigenesis caused by epithelial injury. *Cancer Res.* 73, 7199–7210
66. Schwabe, R.F. and Jobin, C. (2013) The microbiome and cancer. *Nat. Rev. Cancer* 13, 800–812
67. Bolij, A. and Tjalsma, H. (2012) Gut bacteria in health and disease: a survey on the interface between intestinal microbiology and colorectal cancer. *Biol. Rev. Camb. Philos. Soc.* 87, 701–730
68. Chen, G.Y. *et al.* (2008) The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res.* 68, 10060–10067
69. Louis, P. *et al.* (2014) The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* 12, 661–672
70. Macfarlane, G. and Gibson, G. (1997) Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In *Gastrointestinal Microbiology*, pp. 269–318, Chapman and Hall
71. Weaver, G.A. *et al.* (1988) Short chain fatty acid distributions of enema samples from a sigmoidoscopy population: an association of high acetate and low butyrate ratios with adenomatous polyps and colon cancer. *Gut* 29, 1539–1543
72. Weir, T.L. *et al.* (2013) Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS ONE* 8, e70803
73. Singh, N. *et al.* (2014) Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* 40, 128–139
74. Donohoe, D.R. *et al.* (2014) A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discov.* 4, 1387–1397
75. Lupton, J.R. (2004) Microbial degradation products influence colon cancer risk: the butyrate controversy. *J. Nutr.* 134, 479–482
76. Belcheva, A. *et al.* (2014) Gut microbial metabolism drives transformation of MSH2-deficient colon epithelial cells. *Cell* 158, 288–299
77. Ohland, C.L. and Jobin, C. (2014) Bugs and food: a recipe for cancer? *Cell Metab.* 20, 937–938
78. Freeman, H.J. (1986) Effects of differing concentrations of sodium butyrate on 1,2-dimethylhydrazine-induced rat intestinal neoplasia. *Gastroenterology* 91, 596–602
79. Flint, H.J. *et al.* (2012) The role of the gut microbiota in nutrition and health. *Nat. Rev. Gastroenterol. Hepatol.* 9, 577–589
80. Pomare, E.W. *et al.* (1985) Carbohydrate fermentation in the human colon and its relation to acetate concentrations in venous blood. *J. Clin. Invest.* 75, 1448–1454
81. Windey, K. *et al.* (2012) Relevance of protein fermentation to gut health. *Mol. Nutr. Food Res.* 56, 184–196
82. Hughes, R. and Rowland, I.R. (2000) Metabolic activities of the gut microflora in relation to cancer. *Microb. Ecol. Health Dis.* 2000 (Suppl. 2), 179–185
83. Di Martino, M.L. *et al.* (2013) Polyamines: emerging players in bacteria–host interactions. *Int. J. Med. Microbiol.* 303, 484–491
84. Pegg, A.E. (2013) Toxicity of polyamines and their metabolic products. *Chem. Res. Toxicol.* 26, 1782–1800
85. Attene-Ramos, M.S. *et al.* (2007) Hydrogen sulfide induces direct radical-associated DNA damage. *Mol. Cancer Res.* 5, 455–459
86. Magee, E.A. *et al.* (2000) Contribution of dietary protein to sulfide production in the large intestine: an in vitro and a controlled feeding study in humans. *Am. J. Clin. Nutr.* 72, 1488–1494
87. Loh, Y.H. *et al.* (2011) N-Nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am. J. Clin. Nutr.* 93, 1053–1061
88. Khor, B. *et al.* (2011) Genetics and pathogenesis of inflammatory bowel disease. *Nature* 474, 307–317
89. Nestle, F.O. *et al.* (2009) Skin immune sentinels in health and disease. *Nat. Rev. Immunol.* 9, 679–691
90. Pabst, O. (2012) New concepts in the generation and functions of IgA. *Nat. Rev. Immunol.* 12, 821–832
91. Salzman, N.H. *et al.* (2007) Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa. *Semin. Immunol.* 19, 70–83
92. Huycke, M.M. and Gaskins, H.R. (2004) Commensal bacteria, redox stress, and colorectal cancer: mechanisms and models. *Exp. Biol. Med.* 229, 586–597

93. Cross, A.J. *et al.* (2014) A prospective study of serum metabolites and colorectal cancer risk. *Cancer* 120, 3049–3057
94. Cross, A.J. *et al.* (2014) Metabolites of tobacco smoking and colorectal cancer risk. *Carcinogenesis* 35, 1516–1522
95. Guertin, K.A. *et al.* (2015) Serum biomarkers of habitual coffee consumption may provide insight into the mechanism underlying the association between coffee consumption and colorectal cancer. *Am. J. Clin. Nutr.* 101, 1000–1011
96. Marcobal, A. *et al.* (2013) A metabolomic view of how the human gut microbiota impacts the host metabolome using humanized and gnotobiotic mice. *ISME J.* 7, 1933–1943
97. Zhang, Y. *et al.* (2015) Metagenomic and metabolomic analysis of the toxic effects of trichloroacetamide-induced gut microbiome and urine metabolome perturbations in mice. *J. Proteome Res.* 14, 1752–1761
98. Nougayrede, J.P. *et al.* (2006) *Escherichia coli* induces DNA double-strand breaks in eukaryotic cells. *Science* 313, 848–851
99. Arthur, J.C. *et al.* (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338, 120–123
100. Cuevas-Ramos, G. *et al.* (2010) *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11537–11542
101. Bonnet, M. *et al.* (2014) Colonization of the human gut by *E. coli* and colorectal cancer risk. *Clin. Cancer Res.* 20, 859–867
102. Buc, E. *et al.* (2013) High prevalence of mucosa-associated *E. coli* producing cyclomodulin and genotoxin in colon cancer. *PLoS ONE* 8, e56964
103. Cougnoux, A. *et al.* (2015) Small-molecule inhibitors prevent the genotoxic and protumoural effects induced by colibactin-producing bacteria. *Gut* Published online January 14, 2015. http://dx.doi.org/10.1136/gutjnl-2014-307241
104. Baxter, N.T. *et al.* (2014) Structure of the gut microbiome following colonization with human feces determines colonic tumor burden. *Microbiome* 2, 20 PMID: 24967088
105. Couturier-Maillard, A. *et al.* (2013) NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J. Clin. Invest.* 123, 700–711
106. Payros, D. *et al.* (2014) Maternally acquired genotoxic *Escherichia coli* alters offspring's intestinal homeostasis. *Gut Microbes* 5, 313–325
107. Fostira, F. *et al.* (2012) Prevalence of BRCA1 mutations among 403 women with triple-negative breast cancer: implications for genetic screening selection criteria: a Hellenic Cooperative Oncology Group Study. *Breast Cancer Res. Treat.* 134, 353–362
108. Rebbeck, T.R. *et al.* (2011) Modification of BRCA1-associated breast and ovarian cancer risk by BRCA1-interacting genes. *Cancer Res.* 71, 5792–5805
109. van der Post, R.S. *et al.* (2015) Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. *J. Med. Genet.* 52, 361–374
110. Guilford, P. *et al.* (1998) E-cadherin germline mutations in familial gastric cancer. *Nature* 392, 402–405
111. Chun, N. and Ford, J.M. (2012) Genetic testing by cancer site: stomach. *Cancer J.* 18, 355–363
112. Choi, P.M. and Zelig, M.P. (1994) Similarity of colorectal cancer in Crohn's disease and ulcerative colitis: implications for carcinogenesis and prevention. *Gut* 35, 950–954
113. Endo, A. *et al.* (2014) Long-term monitoring of the human intestinal microbiota from the 2nd week to 13 years of age. *Anaerobe* 28, 149–156
114. Funkhouser, L.J. and Bordenstein, S.R. (2013) Mom knows best: the universality of maternal microbial transmission. *PLoS Biol.* 11, e1001631
115. Rautava, S. *et al.* (2012) Microbial contact during pregnancy, intestinal colonization and human disease. *Nat. Rev. Gastroenterol. Hepatol.* 9, 565–576
116. Arthur, J.C. *et al.* (2014) Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. *Nat. Commun.* 5, 4724
117. Rubinstein, M.R. *et al.* (2013) *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe* 14, 195–206
118. Boleij, A. *et al.* (2015) The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin. Infect. Dis.* 60, 208–215
119. Viaud, S. *et al.* (2013) The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 342, 971–976
120. Ando, K. *et al.* (2008) Humanizing bone marrow in immune-deficient mice. *Curr. Top. Microbiol. Immunol.* 324, 77–86
121. Werner-Klein, M. *et al.* (2014) Immune humanization of immune-deficient mice using diagnostic bone marrow aspirates from carcinoma patients. *PLoS ONE* 9, e97860
122. Justino, P.F. *et al.* (2014) Treatment with *Saccharomyces boulardii* reduces the inflammation and dysfunction of the gastrointestinal tract in 5-fluorouracil-induced intestinal mucositis in mice. *Br. J. Nutr.* 111, 1611–1621
123. Iida, N. *et al.* (2013) Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* 342, 967–970
124. Perez-Chanona, E. and Jobin, C. (2014) From promotion to management: the wide impact of bacteria on cancer and its treatment. *Bioessays* 36, 658–664
125. Hardcastle, J.D. *et al.* (1996) Randomised controlled trial of faecal-occult-blood screening for colorectal cancer. *Lancet* 348, 1472–1477
126. Kronborg, O. *et al.* (1996) Randomised study of screening for colorectal cancer with faecal-occult-blood test. *Lancet* 348, 1467–1471
127. Mandel, J.S. *et al.* (1993) Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon Cancer Control Study. *N. Engl. J. Med.* 328, 1365–1371
128. Ned, R.M. *et al.* (2011) Fecal DNA testing for colorectal cancer screening: the ColoSure test. *PLoS Curr.* 3, RRN1220
129. Ahlquist, D.A. *et al.* (2008) Stool DNA and occult blood testing for screen detection of colorectal neoplasia. *Ann. Intern. Med.* 149, 441–450 W481
130. Imperiale, T.F. *et al.* (2014) Multitarget stool DNA testing for colorectal-cancer screening. *N. Engl. J. Med.* 370, 1287–1297
131. Narayanan, V. *et al.* (2014) Human fecal microbiome-based biomarkers for colorectal cancer. *Cancer Prev. Res.* 7, 1108–1111
132. Flint, H.J. (2012) The impact of nutrition on the human microbiome. *Nutr. Rev.* 70 (Suppl. 1), S10–S13
133. Scott, K.P. *et al.* (2013) The influence of diet on the gut microbiota. *Pharmacol. Res.* 69, 52–60
134. Walker, A.W. *et al.* (2011) Dominant and diet-responsive groups of bacteria within the human colonic microbiota. *ISME J.* 5, 220–230
135. Kraus, S. *et al.* (2014) Recent advances in personalized colorectal cancer research. *Cancer Lett.* 347, 15–21
136. Sivanand, S. *et al.* (2012) A validated tumorgraft model reveals activity of dovitinib against renal cell carcinoma. *Sci. Transl. Med.* 4, 137ra175
137. Stebbing, J. *et al.* (2014) Patient-derived xenografts for individualized care in advanced sarcoma. *Cancer* 120, 2006–2015
138. Weroha, S.J. *et al.* (2014) Tumorgrafts as in vivo surrogates for women with ovarian cancer. *Clin. Cancer Res.* 20, 1288–1297
139. You, Y.N. *et al.* (2015) Oncotype DX colon cancer assay for prediction of recurrence risk in patients with stage II and III colon cancer: a review of the evidence. *Surg. Oncol.*
140. Thomas, R.M. *et al.* (2015) The canary in the coal mine: the growth of patient-derived tumorgrafts in mice predicts clinical recurrence after surgical resection of pancreatic ductal adenocarcinoma. *Ann. Surg. Oncol.* 22, 1884–1892
141. Lee, J.R. *et al.* (2015) Gut microbiota and tacrolimus dosing in kidney transplantation. *PLoS ONE* 10, e0122399
142. Catry, E. *et al.* (2015) Ezetimibe and simvastatin modulate gut microbiota and expression of genes related to cholesterol metabolism. *Life Sci.* 132, 77–84

143. Desch, C.E. *et al.* (2005) Colorectal cancer surveillance: 2005 update of an American Society of Clinical Oncology practice guideline. *J. Clin. Oncol.* 23, 8512–8519

144. Primrose, J.N. *et al.* (2014) Effect of 3 to 5 years of scheduled CEA and CT follow-up to detect recurrence of colorectal cancer: the FACS randomized clinical trial. *JAMA* 311, 263–270

145. Locker, G.Y. *et al.* (2006) ASCO 2006 update of recommendations for the use of tumor markers in gastrointestinal cancer. *J. Clin. Oncol.* 24, 5313–5327

146. Palmqvist, R. *et al.* (2003) Prediagnostic levels of carcinoembryonic antigen and CA 242 in colorectal cancer: a matched case-control study. *Dis. Colon Rectum* 46, 1538–1544

147. Sorbye, H. and Dahl, O. (2003) Carcinoembryonic antigen surge in metastatic colorectal cancer patients responding to oxaliplatin combination chemotherapy: implications for tumor marker monitoring and guidelines. *J. Clin. Oncol.* 21, 4466–4467

148. Ballesta, A.M. *et al.* (1995) Carcinoembryonic antigen in staging and follow-up of patients with solid tumors. *Tumour Biol.* 16, 32–41

149. Litvak, A. *et al.* (2014) False-positive elevations of carcinoembryonic antigen in patients with a history of resected colorectal cancer. *J. Natl. Compr. Canc. Netw.* 12, 907–913

150. Tempero, M.A. *et al.* (1987) Relationship of carbohydrate antigen 19-9 and Lewis antigens in pancreatic cancer. *Cancer Res.* 47, 5501–5503

151. Zhang, B. and Yang, B. (1999) Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. *J. Med. Screen.* 6, 108–110

152. Farinati, F. *et al.* (2006) Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am. J. Gastroenterol.* 101, 524–532

153. van Nood, E. *et al.* (2013) Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N. Engl. J. Med.* 368, 407–415

154. Raetz, C.R. and Whitfield, C. (2002) Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* 71, 635–700

155. Hayashi, F. *et al.* (2001) The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410, 1099–1103

156. Alexopoulou, L. *et al.* (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413, 732–738

157. Moresco, E.M. *et al.* (2011) Toll-like receptors. *Curr. Biol.* 21, R488–R493

158. Elinav, E. *et al.* (2011) Regulation of the antimicrobial response by NLR proteins. *Immunity* 34, 665–679

159. Galanos, C. *et al.* (1985) Synthetic and natural *Escherichia coli* free lipid A express identical endotoxic activities. *Eur. J. Biochem.* 148, 1–5

160. Poltorak, A. *et al.* (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085–2088

161. Poltorak, A. *et al.* (1998) Genetic and physical mapping of the Lps locus: identification of the toll-4 receptor as a candidate gene in the critical region. *Blood Cells Mol. Dis.* 24, 340–355

162. Schromm, A.B. *et al.* (1998) The charge of endotoxin molecules influences their conformation and IL-6-inducing capacity. *J. Immunol.* 161, 5464–5471

163. Beutler, B. and Rietschel, E.T. (2003) Innate immune sensing and its roots: the story of endotoxin. *Nat. Rev. Immunol.* 3, 169–176

164. Dranoff, G. (2004) Cytokines in cancer pathogenesis and cancer therapy. *Nat. Rev. Cancer* 4, 11–22

165. Langowski, J.L. *et al.* (2006) IL-23 promotes tumour incidence and growth. *Nature* 442, 461–465

166. Hanahan, D. and Weinberg, R.A. (2011) Hallmarks of cancer: the next generation. *Cell* 144, 646–674

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Research Paper

The ovarian cancer oncobiome

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ABSTRACT

Humans and other mammals are colonized by microbial agents across the kingdom which can represent a unique microbiome pattern. Dysbiosis of the microbiome has been associated with pathology including cancer. We have identified a microbiome signature unique to ovarian cancers, one of the most lethal malignancies of the female reproductive system, primarily because of its asymptomatic nature during the early stages in development. We screened ovarian cancer samples along with matched, and non-matched control samples using our pan-pathogen array (PathoChip), combined with capture-next generation sequencing. The results show a distinct group of viral, bacterial, fungal and parasitic signatures of high significance in ovarian cases. Further analysis shows specific viral integration sites within the host genome of tumor samples, which may contribute to the carcinogenic process. The ovarian cancer microbiome signature provides insights for the development of targeted therapeutics against ovarian cancers.

INTRODUCTION

In the US, ovarian cancer is the second most common and most deadly of the gynecologic cancers, affecting 1 in 70 women, with a mortality rate of 1% of all women (http://www.merckmanuals.com/professional/gynecology-and-obstetrics/gynecologic-tumors/ovarian-cancer). This accounts for its being the 5th leading cause of cancer-related deaths in women, causing an estimated 22,280 new cases (1.3% of all new cancer cases) and 14,240 deaths (2.4% of all cancer deaths) in 2016 (www.cancer.org). Importantly, the incidence is even higher in developed countries (http://www.wcrf.org). Due to the asymptomatic nature of the early stage of the disease most patients go undiagnosed until the cancer reaches an advanced stage [1]. Thus finding specific biomarkers for early diagnosis of the disease is of utmost importance. Many studies have found that DNA of the Human Papillomavirus (HPV)-16 and HPV-18 is associated with ovarian carcinomas [2–5]. However, recent studies from our laboratory and others [6–8] have found that the tumor microbiome may be far more complex. We have defined unique microbial signatures associated with triple negative breast cancer and head and neck cancer [6] (Banerjee *et al.*, unpublished). These signatures potentially provide insight into predisposition, presence or prognosis of the cancer. Such diagnostic data may increase the therapeutic potential for early detection and treatment.

In the present study we used the PathoChip, a microarray-based approach comprised of probes for detection of all known viruses and other human pathogenic microorganisms [6, 9]. The current version of the PathoChip contains 60,000 probes representing all known viruses, 250 helminths, 130 protozoa, 360 fungi and 320 bacteria [6, 9]. In addition to probes that identify specific viruses and micro-organisms, PathoChip also contains family-specific conserved probes which provide a means for detecting previously uncharacterized members

of a family. Using this technique we have previously identified a microbiome signature associated with triple negative breast cancers [6], and oropharyngeal squamous cell carcinomas (Banerjee *et al.*, unpublished).

We have used 99 ovarian cancer samples and 20 matched (tissue adjacent to the tumor deemed non-cancerous by pathological analysis) and 20 unmatched control samples to define a specific ovarian cancer microbiome signature which is distinct from the signature of the controls. To corroborate these results we selected microbial probes across the different organisms detected by the PathoChip screen and used them to capture the signatures from the ovarian cancer samples. This enrichment allowed targeted next generation sequencing to validate the PathoChip screen results and also allowed us to identify microbial insertion sites in the host genome of the ovarian cancer tissues. The data generated in this study suggest a robust and specific microbiome associated with ovarian cancer. Whether or not these organisms contribute as direct drivers to the cancer or simply persist as bystanders or secondary in a supportive tumor microenvironment remains to be determined.

RESULTS

Microbial signatures uniquely associated with ovarian cancer

We used the PathoChip technology to screen ovarian cancer samples, as well as matched and non-matched controls. To establish the microbiome signatures we compared the average hybridization signal for each probe in the cancer samples versus the controls. Those probes that detected significant hybridization signals in the cancer samples (p-value < 0.05, log fold change in hybridization signal > log1), were considered. Additionally, we calculated the percent prevalence of the specific microbial signatures in the cancer samples, these data indicate how prevalent a significant virus or microorganism signature is in the cancer samples regardless of the hybridization intensity. Similarly, we also detected microbiome signatures in the matched and non-matched control samples versus the ovarian cancer samples. The signature of non-matched controls is quite distinct while there is more similarity between the tumor tissue and the matched controls. However, there are distinct viral and microbial signatures in the tumor-specific signature.

Viral signatures associated with ovarian cancer

The viral signatures detected in the ovarian cancer and control samples are shown according to their decreasing hybridization signal along with their prevalence in Figure 1A–1E. By summing all of the hybridization signals for viral families we found that the predominant signatures detected in the ovarian cancers were positive sense single stranded RNA viruses, double stranded DNA viruses and negative sense single stranded RNA viruses (Figure 1A). Among the signatures for viral families detected, 23% were identified as tumorigenic viruses (Figure 1B), and were prevalent on average, in more than 50% of the cancer samples screened (Figure 1C). Signatures of Retroviridae showed the highest hybridization signal, followed by that of Hepadnaviridae, Papillomaviridae, Flaviviridae, Polyomaviridae and Herpesviridae (Figure 1C). Notably, Papillomaviridae family members have previously been shown to be associated with ovarian cancer [2, 10]. Interestingly, we found papilloma virus signatures in the cancer samples and in the non-matched controls, but not at significant levels in the matched controls. The papilloma virus signatures in the ovarian cancer samples screened included not only HPV16 and 18 but also other HPVs (HPV-2, 4, 5, 6b, 7, 10, 32, 48, 49, 50, 60, 54, 92, 96, 101, 128, 129, 131, 132) (Figure 1F). However the HPV signatures in matched controls that showed significantly high hybridization signal intensity over those in cancer samples, were HPV 41, 88, 53 and 103 (Figure 1F). We also found an abundance of other viral signatures in the ovarian cancer samples (Table 1, Figure 1F, and Supplementary Figure 1), including Herpesviridae (HHV4, HHV8, HHV5, HHV6a, HHV 6b), Poxviridae (both pox and parapoxvirus), Polyomaviridae (Merkel cell polyomavirus, JC polyomavirus, Simian virus 40), Retroviridae (Simian foamy virus, Mouse mammary tumor virus).

In the adjacent matched controls and in non-matched control samples, we also detected signatures of tumorigenic viral families, along with other viral signatures (Figure 1D and 1E). Figure 1G and Table 1 shows the common as well as unique viral signatures detected in ovarian cancer, when compared to the matched and non-matched controls.

The data suggest a substantial perturbation of the virome in ovarian cancer. First, the average hybridization signal for the viral families detected in the cancer is actually lower compared to the control samples (compare Supplementary Figure 1 with Figure 1C–1E); Second, despite lower hybridization signal for many viruses in the cancer samples, the viral families present are quite different from controls; for example, signatures of Ancelloviridae, Astroviridae, Birnaviridae, Bornaviridae, Caliciviridae, Hepadnaviridae, Iridoviridae, Paramyxoviridae, Rhabdoviridae and Togaviridae were detected at significant levels only in the cancer samples (Supplementary Figure 1, Table 1). Third, among the viral families detected in both cancer and control samples, specific members of a virus family differed between cancer and controls. For example, specific molecular signatures of the high risk HPV16 and 18 were detected only in the cancer samples and not in the matched or non-matched control group.

Table 1. Microbial signatures detected in ovarian cancer and control samples

	Cancer	MC	NC	Cancer/MC	Cancer/NC	Cancer/MC/NC
Viral signatures	Anelloviridae Astroviridae Birnaviridae Bornaviridae Caliciviridae Hepadnaviridae Iridoviridae Paramyxoviridae Rhabdoviridae Togaviridae			Nodaviridae Parvoviridae	Arenaviridae Circoviridae Flaviviridae Orthomyxoviridae Papillomaviridae	Adenoviridae Bunyaviridae Coronaviridae Herpesviridae Picornaviridae Polyomaviridae Poxviridae Reoviridae Retroviridae
Bacterial signatures	**Proteobacteria:** *Aeromonas* *Aerobacterium* *Anaplasma* *Arcobacter* *Bartonella* *Brucella* *Burkholderia* *Campylobacter* *Coxiella* *Francisella* *Helicobacter* *Klebsiella* *Legionella* *Methylobacterium* *Neisseria* *Orientia* *Pasteurella* *Proteus* *Pseudomonas* *Rickettsia* *Shewanella* *Shigella* *Sphingomonas* *Stenotrophomonas* *Vibrio* *Wolbachia* *Yersinia* **Firmicutes:** *Abiotrophia* *Bacillus* *Enterococcus* *Erysipelothrix* *Geobacillus* *Lactobacillus* *Lactococcus* *Listeria* *Pediococcus* *Peptoniphilus* *Staphylococcus* **Bacteroidetes:** *Bacteroides* *Flavobacterium* *Porphyromonas* *Prevotella* *Actinobacteria:* *Corynebacterium* *Propionibacterium* **Chlamydiae:** *Chlamydia* *Chlamydophila* *Fusobacteria:* *Fusobacterium* *Streptobacillus* **Spirochaetes:** *Leptospira* *Treponema* **Tenericutes:** *Mycoplasma* *Ureaplasma*	**Proteobacteria:** *Morganella*	**Proteobacteria:** *Brevundimonas* *Campylobacter*	**Proteobacteria:** *Azorhizobium* *Escherichia* *Firmicutes:* *Clostridium*	**Proteobacteria:** *Bordetella* *Salmonella* *Bacteroidetes:* *Chryseobacterium* *Actinobacteria:* *Mycobacterium* *Firmicutes:* *Streptococcus*	

Fungal signatures	*Acremonium* *Ajellomyces* *Aspergillus* *Candida* *Cladosporium* *Coccidioides* *Cryptococcus* *Cunninghamella* *Issatchenkia* *Nosema* *Paracoccidioides* *Penicillium* *Pleistophora* *Pneumocystis* *Rhizomucor* *Rhizopus* *Rhodotorula* *Trichophyton*	*Exophiala* *Phialophora*		*Alternaria* *Malassezia* *Mucor* *Trichosporon*	*Absidia* *Cladophialophora* *Fusarium*	*Geotrichum*
Parasitic signatures	*Ancylostoma* *Anisakis* *Armillifer* *Ascaris* *Babesia* *Balantidium* *Bipolaris* *Blastocystis* *Capillaria* *Dicrocoelium* *Dipylidium* *Echinococcus* *Echinostoma* *Entamoeba* *Enterobius* *Hartmannella* *Heteroconium* *Hymenolepis* *Leishmania* *Loa* *Metagonimus* *Necator* *Onchocerca* *Plasmodium* *Sarcocystis* *Schistosoma* *Strongyloides* *Toxascaris* *Toxocara* *Trichomonas* *Trichuris* *Wuchereria*	*Prosthodendrium*		*Acanthamoeba* *Naegleria* *Taenia* *Trichinella*	*Contracaecum* *Diphyllobothrium*	

MC: Matched Control, NC: Non-matched Control.

Instead the non-matched control samples showed significant detection of molecular signatures of the L1 major capsid gene of HPV 41, 88, 53, and E1 gene of HPV 103 (Figure 1F and Supplementary Table 2). A similar situation was detected with the poxviridae. While signatures of poxviridae that are conserved across the family were significantly detected in cancer as well as the controls (both matched and non-matched) (Figure 1F, Supplementary Table 2), highly specific signatures of certain poxviruses [Monkeypox virus, Myxoma virus, Yaba monkey tumor virus (YMTV), Yaba-like disease virus (YLDV)] and parapoxviruses [(Pseudocowpox virus (PCP), Orf virus (Orf), Bovine papular stomatitis virus (BPSV)] were detected only in the ovarian cancer samples (Figure 1F, Supplementary Table 2). The specific parapoxvirus signatures detected were that of IL-10 encoded by Orf virus and Bovine papular stomatitis virus, and the A-type inclusion protein of Pseudocowpox virus and Orf virus, as well as the glycoprotein of Orf virus (Supplementary Table 2). Specific signatures of poxviruses detected were sequences of thymidine kinase (66R) and ankyrin repeat (147R) of the tumorigenic Yaba monkey tumor virus, 3-beta-hydroxysteroid dehydrogenase of Yaba-like disease virus (Supplementary Table 2). Also, the majority of the Polyomavirus probes significantly detected in the ovarian cancers were that of Merkel cell Polyomaviruses which were undetectable in the controls, whereas the majority of the Polyomavirus probes detected in the controls were that of SV40, traces of which were also detected in the cancers (Figure 1F, Supplementary Table 2). Among the retroviral probes detected in the majority of cancers were

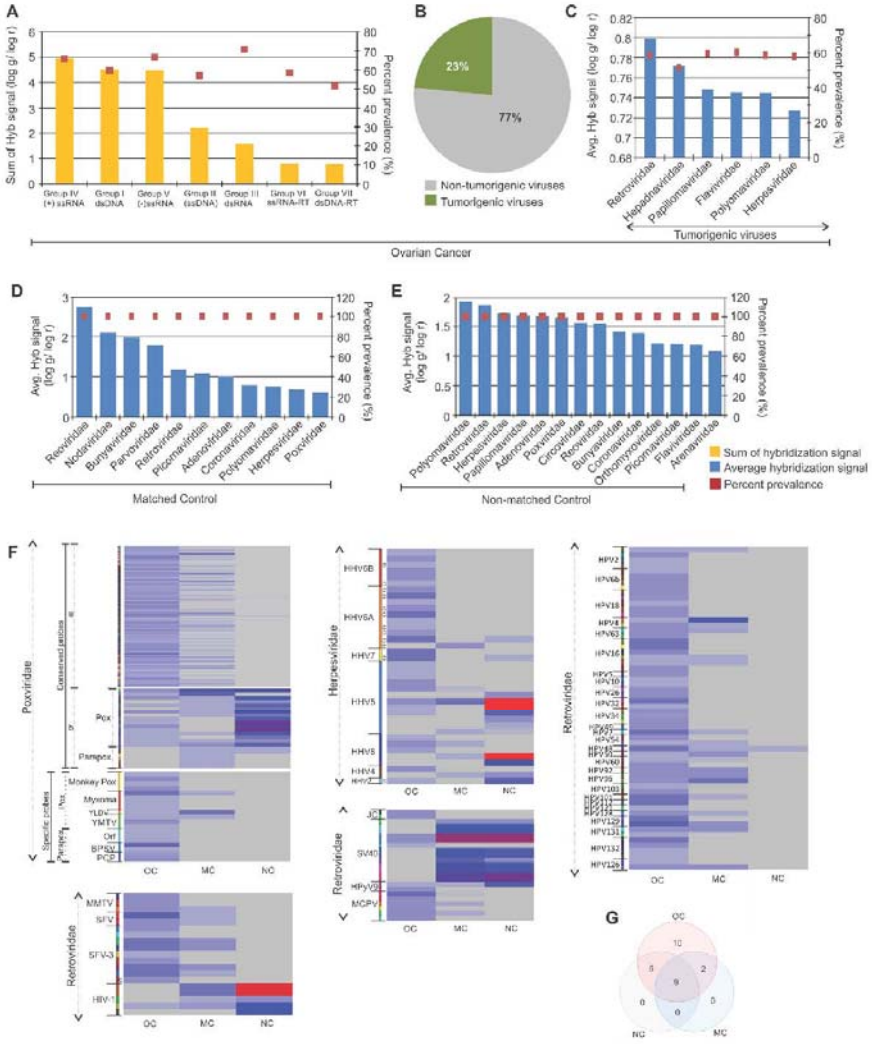

Figure 1: Viral signatures detected in ovarian, matched and non-matched controls. (**A**) Molecular signatures of viral groups detected in ovarian cancer, with the total hybridization signal for each viral groups (sum of average hybridization signal for all the representative families in the group) represented according to descending order as a bar graph and prevalence of the same as dots. (**B**) The percentage of tumorigenic viral signatures detected in the ovarian cancers are represented in a pie chart. (**C**) The average hybridization signal of the tumorigenic viral signatures detected in the ovarian cancers are represented in the decreasing order as a bar graph, whereas their respective prevalence are represented as dots. (**D** and **E**) The signatures of viral families detected in matched (D) and non-matched (E) controls are represented according to decreasing average hybridization signals as bar graphs, and their respective prevalence as dots. (**F**) Heat map of average hybridization signals for probes of Poxviruses, Retroviruses, Herpesviruses, Polyomaviruses and Papillomaviruses detected in ovarian cancers (OC), matched (MC) and non-matched (NC) controls. Heat map of average hybridization signal of both conserved and specific probes of Poxviridae are shown. Among the conserved poxviridae probes mentioned, (a) comprises the conserved probes detected significantly in the ovarian cancer versus the controls, and (b) comprises the conserved probes detected significantly in the controls versus the ovarian cancers screened. In the heat map with Herpesviridae probes, those mentioned (c) are conserved probes. All other probes in these heat maps are specific probes. (**G**) Venn diagram showing the number of viral families common or unique to the ovarian cancer and control samples.

specific probes of Mammary Tumor Virus (MMTV) and Foamy Virus (SFV), whereas, the majority of Retroviral probes detected in the controls were specific probes for the lentivirus subgroup of retroviruses (Figure 1E, Supplementary Table 2). Interestingly, the detection of Herpesviridae probes identified HHV2 with high significance in the non-matched control compared to the cancers. However, the cancer samples showed detection for conserved and specific probes of HHV6A and HHV6B which were undetectable in the controls. Other herpesviridae probes of HHV4, HHV5 and HHV8 were detected in both cancer and non-matched control samples (Figure 1F, Supplementary Table 2).

The data as a whole suggest that specific viral signatures are dramatically altered in the cancer tissue. Some signatures appear only in the cancer or have significantly increased hybridization intensity, while others are decreased compared to the surrounding tissue. Several points must be kept in mind when considering these data: 1) the tumor microenvironment may provide advantages for the persistence of some viruses, thus promoting their presence in the cancer. Hence, their presence need not be related to the cause of the cancer. Similarly, the appearance of a virus in the matched control and not the cancer may suggest that the tumor microenvironment is inhibitory for persistence of the virus. 2) The probes may also be detecting relatives or variants of known viruses from which the probes were derived. For example, specific probes for lentiviruses including HIV-1 were positive in the analysis of control samples. These are de-identified samples; however we doubt that these patients were HIV positive but suspect that the probes are likely detecting the presence of a related, uncharacterized human lentivirus.

Identification of bacterial signatures associated with ovarian cancer

Similar to that seen with the viruses, the bacterial signatures of the tumor tissue were dramatically altered from those of matched and non-matched controls. The specific bacterial signatures detected in the cancer and the matched and non-matched samples are shown in Figure 2A according to their decreasing prevalence. Two predominant bacterial phyla were detected in the ovarian cancer samples screened. They were Proteobacteria (52%), followed by Firmicutes (22%) (Figure 2B). We also detected other phyla at lower percentages including Bacteroidetes, Actinobacteria, Chlamydiae, Fusobacteria, Spirochaetes and Tenericutes in the cancer samples. Signatures of Proteobacteria and Firmicutes were also detected significantly in the matched control samples screened, and that of Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes were detected significantly in the non-matched control samples (Figure 2B). Many more bacterial signatures were significantly detected in the cancer samples compared to the controls. The signatures associated only with the ovarian cancer samples are listed Table 1). The different bacterial signatures, unique or common to the control and ovarian cancer samples are listed in Table 1 and represented in Figure 2C.

While signatures of Pediococcus was detected with the highest hybridization signal in the ovarian cancer samples screened, followed closely by that of Burkholderia, Sphingomonas, Chryseobacterium, Enterococcus, Staphylococcus, Treponema and Francisella [(log g/log r) > 1], Shewanella signatures were detected with the highest prevalence in 91% of the cancers (Figure 2A). The majority of the bacterial signatures detected in the cancers had high prevalence, except for signatures of Escherichia, Legionella, Streptobacillus, Ureaplasma, Clostridium, Geobacillus which were detected in less than 50 percent of the cancer samples screened (Figure 2A). Interestingly, there are no common bacteria between all 3 types of samples (Figure 2C, Table 1). However, 5 agents were shared between the cancer and non-matched controls, and 3 agents between the cancer and matched controls (Figure 2C, Table 1). 52 unique bacterial agents were detected predominantly in only the cancer (Figure 2C, Table 1).

Identification of fungal signatures associated with ovarian cancer

Our pathogen screen for fungal signatures again suggests a significant perturbation of the microbiome in the tumor. The fungal signatures detected in the ovarian cancer and controls are shown according to their decreasing prevalence in Figure 3A. The 18 fungal signatures that were detected only in the ovarian cancer samples and interestingly not found associated with the controls are listed (Table 1, Figure 3B). 18S rRNA signatures of *Cladosporium* were detected in all the ovarian cancer samples with the highest hybridization signal (Figure 3A). Signatures of *Pneumocystis*, *Acremonium*, *Cladophialophora*, *Malassezia* and Microsporidia *Pleistophora* were also detected significantly in all the ovarian cancer samples screened (Figure 3A). Signatures of *Rhizomucor*, *Rhodotorula*, *Alternaria*, *Geotrichum* were found to be associated with more than 95% of the ovarian cancer samples screened (Figure 3A). It should be noted that the signature of *Geotrichum* was also detected in all the control samples (Table 1 and Figure 3A). Therefore the associated fungal agents appear to be dominant in the ovarian cancer with only *Geotrichum* common among the cancer and controls. This suggests that the fungal signatures may be more tightly associated in this particular microenvironment than previously predicted.

Identification of parasitic signatures associated with ovarian cancer

The parasitic signatures detected in the ovarian cancer and controls are shown (Figure 4A), according to their decreasing prevalence. The parasitic signature significantly detected in cancer samples was far more complex than the matched and, especially, the non-matched controls, once again suggesting a marked perturbation of the tumor microbiome. The parasitic signatures detected only in the ovarian cancer samples are listed (Figure 4B, Table 1). All of the tumor samples showed a high hybridization signal (log g/log r > 2) for the 28S rRNA signature of *Dipylidium*. A high hybridization signal for the 18S rRNA signatures of *Trichuris* and *Leishmania* was also found in all of the ovarian cancer samples (Figure 4A). The 18S rRNA signatures of *Babesia* were also significantly detected in all the ovarian cancer samples, although with a relatively moderate hybridization signal (log g/log r > 1, < 2) (Figure 4A). 18S rRNA signatures of *Trichinella*, *Ascaris*, and *Trichomonas* were detected in >95% of the ovarian cancer samples screened, also with a moderate hybridization signal intensity (log g/log r > 1, < 2) (Figure 4A). The other parasitic signatures detected in the ovarian cancer listed in Figure 4A were detected with lower hybridization signal intensity (log g/log r < 1), although with high prevalence except for signatures of *Loa loa*, *Acanthamoeba*, *Taenia*, *Dicrocoelium*, Wuchereria which were detected in less than 45% of the ovarian cancer samples screened. Signatures of 4 parasites that were detected in the cancer samples were also found in the adjacent matched control samples; these include *Acanthamoeba*, *Naegleria*, *Taenia* and *Trichinella* (Figure 4A, Table 1). However, they were not detected in the non-matched controls (Figure 4A).

Hierarchical clustering of the ovarian cancer samples

Hierarchical clustering analysis compares the similarity of the overall microbiome signatures detected in each ovarian cancer sample and clusters the samples together based on common microbiome similarity (Figure 5A–5B). While some samples did not group into a cluster (namely un-grouped 1 and 2) (Figure 5B), majority of the samples grouped into three distinct clusters, namely cluster 1, 2 and 3 (Figure 5A and 5B), with cluster 3 samples showing significant differences in detection of several viral and other microbial signatures compared to the samples of cluster 1 and 2. Supplementary Table 3 shows the significant differences in microbial detection between the clusters. Ovarian cancer samples of cluster 1

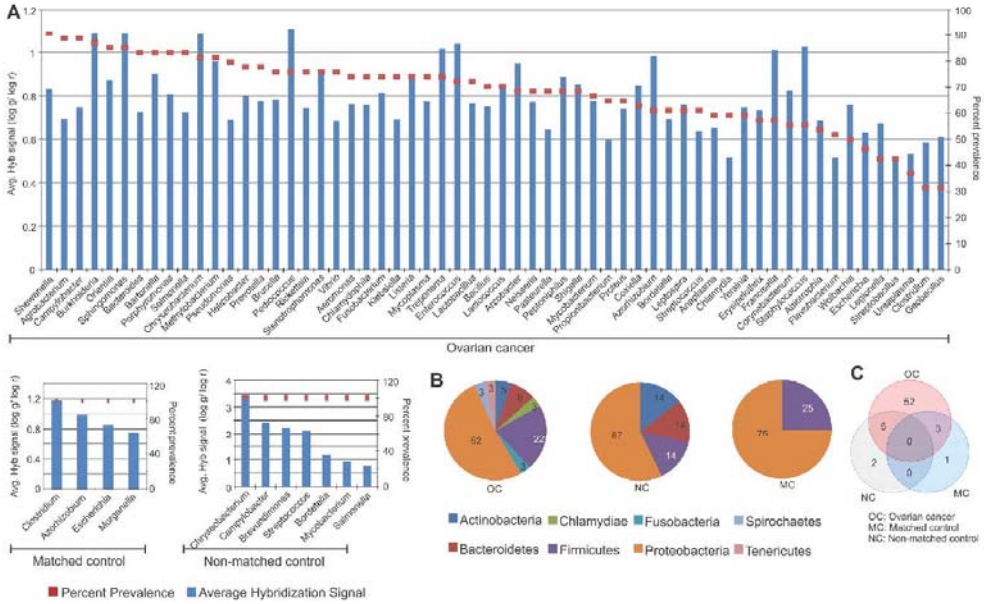

Figure 2: Bacterial signatures detected in ovarian, matched and non-matched controls. (**A**) Bacterial signatures detected in ovarian cancers, matched and non-matched controls. The prevalence of those signatures are represented in the decreasing order as dots, and their average hybridization signal being represented as a bar graph. (**B**) Distribution of bacterial phyla detected in ovarian cancer, matched and non-matched controls. (**C**) Venn diagram showing the number of bacteria common or unique to the ovarian cancer and control samples.

and 2 showed significant differences in the detection of 2 viral agents (Arenaviridae and Flaviviridae) and bacterial agents (*Coxiella* and *Listeria*) signatures, and few fungal (*Acremonium*, *Cladosporium*, *Mucor*, *Pleistophora*, *Pneumocystis* and *Rhodotorula*) and parasitic (*Babesia*, *Dipylidium*, *Leishmania*, *Toxocara*, *Trichinella*, *Trichomonas* and *Trichuris*) signatures. These signatures are all of higher intensities in cluster 2 than 1. On the other hand, ovarian cancer samples of cluster 3 had significantly less detection of almost all the viral and several microbial signatures mentioned in Supplementary Table 3.

Based on topological analysis, the ovarian cancer samples clustered into 3 groups (A, B and C), while some could not be grouped together (singletons) (Figure 5C). Supplementary Table 4 shows significant differences in microbial detection in each groups. Group B had significantly higher detection of the following signatures compared to Group A: viral signatures of Coronaviridae, Astroviridae, Togaviridae, Reoviridae, Papillomaviridae, Poxviridae, Bunyaviridae, Picornaviridae, Paramyxoviridae, Bornaviridae, Birnaviridae, Rhabdoviridae, Caliciviridae, Arenaviridae and Flaviviridae; along with certain bacterial signatures of *Porphyromonas*, *Anaplasma*, *Azorhizobium*, *Corynebacterium*, *Arcobacter*, *Lactococcus*, *Methylobacterium*, *Shigella*, *Proteus*, *Brucella*, *Ureaplasma* and *Prevotella*; fungal signatures of *Absidia*, *Trichophyton*, *Ajellomyces*, *Geotrichum* and *Candida*;and parasitic signatures of *Ascaris*, *Bipolaris*, *Acanthamoeba*, *Sarcocystis*, *Balantidium*, *Echinostoma*, *Dicrocoelium* and *Wolbachia*. Group C differed from group B in having significantly higher signatures of mainly viral families of Poxviridae, Papillomaviridae, Coronaviridae, Bunyaviridae, Retroviridae, Herpesviridae, Reoviridae, Anelloviridae and Togaviridae and bacterial signatures of *Rickettsia* and *Legionella* compared to Group B. Group C differed from Group A in having significantly higher detection of the viral signatures of Poxviridae, Togaviridae, Papillomaviridae, Coronaviridae, Bunyaviridae, Herpesviridae, Anelloviridae, Retroviridae, Reoviridae, Parvoviridae, Rhabdoviridae, Paramyxoviridae, Arenaviridae, Picornaviridae, Circoviridae, Flaviviridae, Adenoviridae, Birnaviridae, Caliciviridae, Polyomaviridae, Orthomyxoviridae, Iridoviridae, Bornaviridae, Astroviridae; bacterial signatures of *Legionella*,

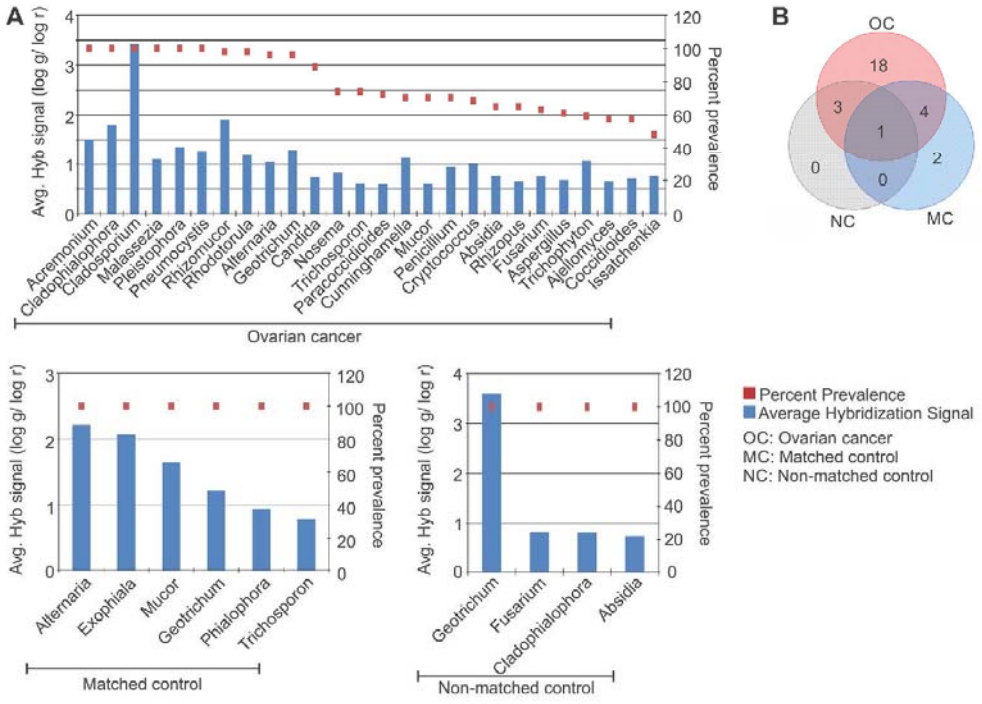

Figure 3: Fungal signatures detected in ovarian, matched and non-matched controls. (**A**) Fungal signatures detected in ovarian cancer, matched and non-matched controls. The prevalence of those signatures are represented in the decreasing order as dots, and their average hybridization signal being represented as a bar graph. (**B**) Venn diagram showing the number of fungi common or unique to the ovarian cancer and control samples.