



Cancer's second genome: Microbial cancer diagnostics and redefining clonal evolution as a multispecies process

Gregory Sepich-Poore, Caitlin Guccione, Lucie Laplane, Thomas Pradeu, Kit Curtius, Rob Knight

► To cite this version:

Gregory Sepich-Poore, Caitlin Guccione, Lucie Laplane, Thomas Pradeu, Kit Curtius, et al.. Cancer's second genome: Microbial cancer diagnostics and redefining clonal evolution as a multispecies process. *BioEssays*, 2022, 44 (5), pp.2100252. <10.1002/bies.202100252>. <hal-03883528>

HAL Id: hal-03883528

<https://hal.science/hal-03883528v1>

Submitted on 3 Dec 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Solicited special issue: "Advances in Cancer Diagnostics"

Submission type: Review essay

Manuscript title: Impact of the cancer metagenome on clinical care and multispecies clonal evolution

Authors & Affiliations: Gregory D. Sepich-Poore^{1,†}, Caitlin Guccione^{2,3,4,†}, Lucie Laplane^{5,6,‡}, Thomas Pradeu^{7,‡}, Kit Curtius^{2,3}, Rob Knight^{1,4,8,*}

¹Department of Bioengineering, University of California San Diego, La Jolla, CA 92093, USA.

²Division of Biomedical Informatics, Department of Medicine, University of California San Diego, La Jolla, CA 92093, USA.

³Bioinformatics and Systems Biology Program, University of California San Diego, La Jolla, CA 92093, USA.

⁴Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA

⁵Institut d'histoire et de philosophie des sciences et des techniques (UMR8590), CNRS & Panthéon-Sorbonne University, 75006 Paris, France.

⁶Hematopoietic stem cells and the development of myeloid malignancies (UMR1287), Gustave Roussy Cancer Campus, 94800 Villejuif, France.

⁷ImmunoConcept (UMR5164), CNRS & University of Bordeaux, 33076 Bordeaux Cedex, France.

⁸Department of Computer Science and Engineering, University of California San Diego, La Jolla, CA 92093, USA.

[†]These authors contributed equally.

[‡]These authors contributed equally.

*Corresponding author: robknight@eng.ucsd.edu

Keywords: cancer microbiome, clonal evolution, diagnostics, therapeutic modulation, prognostics

SUBTITLE

Humans and their tumors are not aseptic, and the multispecies nature of cancer modulates clinical care and clonal evolution.

ABSTRACT

The presence and role of microbes in human cancers has come full circle in the last century. Tumors are no longer considered aseptic, but implications for cancer biology and oncology remain underappreciated. Opportunities to identify and build translational diagnostics, prognostics, and therapeutics that exploit cancer's second genome—the metagenome—are manifold, but require careful consideration of microbial experimental idiosyncrasies that are distinct from host-centric methods. Furthermore, the discoveries of intracellular and intra-metastatic cancer bacteria necessitate fundamental changes in describing clonal evolution and selection, reflecting bidirectional interactions with non-human residents. Reconsidering cancer clonality as a multispecies process similarly holds key implications for understanding metastasis and prognosing therapeutic resistance while providing rational guidance for the next generation of bacterial cancer therapies. Guided by the above opportunities and challenges, this Review describes opportunities to exploit cancer's metagenome in oncology and proposes an evolutionary framework as a first step towards modeling multispecies cancer clonality.

INTRODUCTION

A long and rich history exists between microbes and cancer. As early as 1550 BCE, Egyptian writings suggested a crude therapy for tumors through incision and application of a poultice, thereby inciting an infection.^[1–3] Nearly three millennia later, Saint Peregrine Laziosi (c. 1265–1345) documented spontaneous regression of a septic sarcoma on his leg large enough to pierce through skin.^[2] Although these accounts predated modern germ theory, they presciently associated acute infections and the retrogression of cancer, which would be independently re-discovered by three physicians between 1868-1893: Wilhelm Busch, Friedrich Fehleisen, and William Coley.^[4–6]

Many spontaneous tumor regressions described by these three physicians were tied to the skin pathogen *Streptococcus pyogenes*, and its concomitant infectious syndrome, erysipelas. However, only Coley seriously considered treating new patients—usually with late-stage or inoperable cancers—by administering live bacteria (which carried serious clinical sequelae), and, later, heat-killed microbial (*Streptococcus* and *Serratia*) toxins. These clinical experiments revealed >10-year disease-free survival in 60 of 210 patients across multiple cancer types, roughly one-third of all patients treated—a statistic only matched by modern immunotherapy.^[7] Nonetheless, an unknown mechanism and severe flu-like side effects made ‘Coley’s toxins’ unpalatable to oncology, especially when compared to the burgeoning radiotherapy and chemotherapy fields.^[8,9] It would take another century for scientists to realize that Coley’s approach comprised the first intentional application of immunotherapy, and accurately predicted a causal relationship between immunotherapy efficacy and an individual’s endogenous or exogenously-administered microbiome.^[10–16]

Viruses have also been crucial for understanding cancer and its genetic material. Peyton Rous's seminal 1911 discovery of his eponymous, transmissible, oncogenic, RNA virus galvanized investigation of the viral origins of cancer, leading to key links between Epstein-Barr, human papilloma (HPV), hepatitis, and most recently Merkel cell polyomavirus and carcinogenesis.^[17–19] Although several decades of laborious research led to the conclusion that viruses cause only a minority of cancers, the pursuit of oncogenic viruses indirectly led to the definition of and search for 'oncogenes' capable of transforming benign tissue into malignant tissue.^[19] One particularly important oncogene was *src*, a protein kinase identified in transforming-only strains of Rous's Sarcoma Virus (RSV), but found by Michael Bishop and Harold Varmus to exist in cells of non-infected, phylogenetically-divergent birds.^[20] Their data suggested a non-viral, cellular origin of *src*: hosts normally contain oncogenes, and transforming strains of RSV had acquired one. This discovery earned them the 1989 Nobel Prize in Medicine.^[19,20] Realizing oncogenes were *internal* to cancer motivated characterization of all possible oncogenes in the human cancer genome by sequencing the normal human genome as a reference.^[21] Modern cancer genomics thus had its roots in tumor virology.

The story of RSV and its hijacking of *src* showed how genetic information could transfer between tumors, microbes, and their hosts over evolutionary time and under various selection pressures. After Rous's initial discovery, successive passaging of RSV enabled researchers to evolve the chicken-specific virus to induce tumors in ducks and pigeons, then rats, rabbits, and mice, presumably by activating similar kinase-related oncogenic pathways.^[19,22,23] This process represented early examples of intentional transfection and directed evolution, whereby recipient cells received potent genetic cargo capable of being expressed to change cellular fitness. Decades later, a similar ability of bacteria to transfect genetic material, either microbial or human in origin,^[24–28] to cells—including cancer cells^[29]—with subsequent protein expression would be

demonstrated and coined “bactofection.”^[30] Bactofection was primarily sought after as an alternative to conventional gene therapy or vaccination, but has received little attention.^[27,30,31]

Since Bishop and Varmus’s discovery shifted attention to factors internal to the cancer cell, the last 30 years of cancer research has primarily focused on characterizing all major coding, noncoding, structural, and copy number aberrations in the cancer genome.^[32–36] Substantial study of the tumor microenvironment (TME) has further elucidated the impacts of heterogeneous tumor architecture, spatial organization, and multifaceted cellular agents (e.g., immune and stromal cells) on cancer evolution, clonality, antitumor immunity, and metastasis.^[37–39] Further work has revealed similarities between microbial and cancer evolution. For example, the ubiquitous presence of plasmid-like, extrachromosomal DNA (ecDNA) segments and their unequal segregation during cancer cell division is analogous to unequal segregation of high copy plasmids during bacterial replication.^[40–44] Hybrid viral-human sequences on ecDNA segments in HPV-infected cancers even contribute to immune evasion and carcinogenesis.^[45,46] Nonetheless, most cancer ‘omic’ studies have portrayed tumors as sterile entities, and microbial constituents as being unrelated to tumor evolution or clinical care.

The last five years have persuasively unveiled metabolically-active, immunoreactive, intracellular, cancer type-specific communities of bacteria (and viruses) living within tumor tissues, several of which modulate cancer therapies.^[47–60] These microbes may move during metastasis from one bodily location to another and facilitate leaving and/or seeding of metastatic cancer cells.^[53,54,61–63] Critically, intratumoral and gut microbes can create chemo-, radio-, and hormonal therapeutic resistance without any genetic or non-genetic changes within the cancer genome.^[47,64,65] Conversely, microbiota may render other chemo-, radio-, hormonal, and immunotherapies possible and/or effective without any intervention from cancer cells.^[12–14,64,66–68] Trace amounts of cancer type-specific bacterial DNA have also been identified in the circulation of cancer patients,

suggesting a novel class of microbial cancer diagnostics.^[58,69] Most, if not all, human cancers lack sterility, and their microbes are clinically relevant.

Towards building a microbially-conscious framework of cancer, we posit cancer-bearing humans as meta-organisms colonized by numerous and diverse microbial constituents (see **Box 1**—“Quantifying the cancer microbiome”).^[70,71] We propose the clinical utility of microbial information as cancer diagnostics, prognostics, and therapeutics and consider (intracellular) microbes as live, mobile agents within tumors that encounter selection pressures alongside/within cancer cells. Finally, we hypothesize that fundamental ecological rules governing microbial activity and spatial placement (e.g., redox, chemotactic, oxygen gradients)^[72] outside tumors also govern them inside tumors. This Review details the study of cancer’s “second genome” and its use to advance patient care and models of cancer clonal evolution.

BOX 1—Quantifying the cancer microbiome

Broadly speaking, the human body microbiota include $\sim 4 \times 10^3$ species accounting for $\sim 4 \times 10^{13}$ total microorganisms, with $\sim 97\%$ of those cells comprising colonic bacteria and $\sim 2\text{--}3\%$ comprising extra-colonic bacteria while archaea and eukarya—including fungi—comprise smaller populations around $\sim 0.1\text{--}1\%$ of the total microbial abundance.^[70,73] Human virus and phage abundance estimates remain undercharacterized but likely have even greater diversity than bacteria.^[74] The human gut microbiome contains the largest bodily microbial biomass by far—roughly 0.2 kilograms^[70,75]—with substantial effects on host antitumor immunity.^[3] Biomass estimates of other body sites or tissues remain unknown.

Intratumoral microbiome diversity estimates with stringent decontamination controls ($\sim 1\text{--}2$

control to sample ratio) suggest that at least 500 distinct bacterial species inhabit tumors.^[57] Intratumoral microbiome abundance estimates have been inferred using shotgun read quantification and quantitative polymerase chain reaction (qPCR) of 16S rRNA.^[57,58] Microbial profiling of all whole genome and transcriptome studies from The Cancer Genome Atlas (TCGA) revealed an average of 2.5% of total sequencing reads to be microbial and an average of 0.9% of total reads that were resolvable at the genus-level.^[58] Given the difference between typical microbial and human genome sizes—often 10^3 -fold smaller—it is possible that these percentages underestimate true microbial density. To quantitate abundance, bootstrapping 16S rRNA qPCR data by Nejman *et al.* revealed a heterogeneous average number of bacteria per cancer type, ranging from ~13 to ~70 per 40 nanograms (ng) of DNA, among seven major human cancers (**Table 1**).^[57] The pan-cancer average was 34.19 bacteria per 40 ng of DNA (**Table 1**). To translate these values to percent tumor composition, it is necessary to first estimate the number of tumor cells per 40 ng of DNA. One way to estimate this for haploid cells is as follows:

$$\begin{aligned}
 \text{DNA mass (haploid)} &\approx (3.2 \times 10^9 \text{ bp/cell}) \left(\frac{1 \text{ mole}}{6.022 \times 10^{23} \text{ bp}} \right) \left(\frac{660 \text{ g}}{1 \text{ mole base pair}} \right) \\
 &\approx 3.5 \text{ picograms / haploid cell}
 \end{aligned}$$

To translate from haploid cell to tumor cell, an estimate of ploidy is needed, which can be derived from the most recent Pan-Cancer Analysis of Whole Genomes (PCAWG) dataset.^[32] The mean estimated ploidy in PCAWG across all human cancers is 2.36 and ranges from a low of 1.28 to a high of 6.22. If we assume average cancer ploidy, the average DNA mass per cancer cell is thus:

$$\begin{aligned}
 \text{DNA mass (cancer cell)} &\approx (3.5 \text{ picograms / haploid cell}) \times (2.36 \text{ avg. ploidy}) \\
 &\approx \mathbf{8.26 \text{ pg / cancer cell}}
 \end{aligned}$$

Similarly, the range of DNA masses per cancer cell based on ploidy would be 4.48 pg to 21.77 pg. For simplicity, one can round the average mass value to 8 pg/cancer cell. Assuming that the DNA mass of microbes is negligible compared to that of the host, since its genome is roughly 10^3 -fold smaller and there are fewer of them expected, then the estimated percent composition is as follows:

$$\begin{aligned}
 \text{Pure tumor bacterial composition} &\approx \left(\frac{34.19 \text{ bacteria}}{40 \text{ ng DNA}} \right) \left(\frac{0.008 \text{ ng}}{1 \text{ cancer cell}} \right) (100\%) \\
 &= 0.68\% \text{ bacterial}
 \end{aligned}$$

This estimate, however, assumes 100% tumor purity. Fortunately, PCAWG estimated tumor purity across the same samples, showing an average tumor purity of 63.8%.^[32] Instead of 5000 cancer cells per 40 ng of DNA, assuming 8 pg per cancer cell, average tumor purity suggests 3190 cancer cells with the remaining cells comprising the TME. While this does not change the percent bacterial composition of the tumor, it does change the ratio of bacteria to cancer cells to approximately ~1:100 or ~1% (i.e. 34.19 bacteria:3190 cancer cells; **Table 1**). Using the 95% confidence interval bounds of the pan-cancer mean number of bacteria per tumor (**Table 1**) gives a range of 0.75% to 1.46% bacterial.

In the case of high tumor ploidy and low tumor purity, it may become important to weigh the contributions between tumor (aneuploid) and stroma (diploid) to the number of cells within 40

ng of DNA. This may be done as follows, for example using a tumor ploidy of 6.0 and 20% purity:

$$\begin{aligned} \text{Composition} &\approx \left(\frac{34.19 \text{ bacteria}}{40 \text{ ng DNA}} \right) \left[\frac{20}{100} \left(\frac{0.02177 \text{ ng DNA}}{1 \text{ cancer cell}} \right) + \frac{80}{100} \left(\frac{0.007 \text{ ng DNA}}{1 \text{ stromal cell}} \right) \right] (100\%) \\ &= 0.85\% \text{ bacterial} \end{aligned}$$

whereas a tumor of 100% purity at a ploidy of 6.0 would provide an average tumor bacterial composition of 1.86%. It is noted that cases with high ploidy and high purity will maximize this percentage value, in addition to when there is more observed bacteria.

To compare these bacterial abundances to intratumor immune cell populations, which are usually reported as densities of immune cell counts per square millimeter, it is necessary to first estimate the total number of cells per square millimeter in a tumor. While a handful of density estimates exist in the literature, such as a mean of 5,558 cells (SD 1,980) per mm² in metastatic melanoma,^[76] it can be inferred directly from circle packing theory.^[77] Specifically, given the average diameter of cells in a tissue, then the number of possible cells within the 1 mm² square can be calculated. In one way, this can be interpreted as a conservative estimate since cells are often compressed and non-circular in real tissues; conversely, it may overestimate cell density in regions with dense blood or lymphatic vessels. The typical diameter of lymphocytes approximates 6-7 µm in diameter^[78] while the diameter of cancer cells vary by type and are approximately ~20 µm in diameter across many cancer cell lines.^[79] Using average cell diameters of 12 µm, 15 µm, and 18 µm, circle packing theory predicts the following total cell abundances per 1 mm²: 8213 cells, 5208 cells, and 3589 cells.

Then, using the previously calculated average pan-cancer tumor bacterial composition of 0.68% (assuming tumor homogeneity), the estimated number of bacteria inferred as the following: 56, 35, 24 bacteria/mm² (assuming 12 μ m, 15 μ m, and 18 μ m average diameter cells, respectively). Notably, these bacterial abundance estimates are similar to the proportion of PD1⁺ cells identified in a recent pan-cancer imaging dataset (~22 PD1⁺ cells/mm²) and roughly one-tenth of CD8⁺ T-cell density (~385 cells/mm²).^[80] Overall, the values reflected in this analysis may vary from tumor to tumor, depending on the assumptions made above—tumor ploidy, purity, homogeneity—but the analysis provides a rough approximation and analogy of intratumor bacterial abundances to immune cell abundances.

To summarize, these calculations estimate an average pan-cancer bacterial composition of ~0.68% with two- and three-dimensional estimates of ~35 bacteria/mm² (assuming 5200 cells/mm²) and approximately 6×10⁵ to 6×10⁶ bacteria per palpable 1 cm³ tumor (assuming 10⁸-10⁹ cells/cm³).^[81] Notably, these estimates can vary between patients by three orders of magnitude and require further examination in additional cohorts.

TABLE 1. Abundance estimates of intratumoral bacteria among seven major human cancers profiled by Nejman *et al.* (data shared via private communication with Ravid Straussman).^[57] One thousand iteration-bootstraps of the mean approximated the average number of bacteria per 40 nanograms of DNA on a per-cancer and pan-cancer basis. Conversions and assumptions of tumor ploidy, purity, and homogeneity are detailed in **Box 1**. Area density estimates assume 5200 total cells/mm² and volume density estimates assume 10⁹ total cells/cm³.

Cancer type in Nejman <i>et al.</i>	qPCR sample size (<i>n</i>)	Absolute range (bacteria/40ng) (min, max)	Bootstrapped estimate of average bacteria per 40 ng	Area density estimate	Volume density estimate
-------------------------------------	-------------------------------	---	---	-----------------------	-------------------------

<i>al. 2020</i> ^[57]			DNA (mean, 95% CI) (1000 iterations)	(bacteria /mm ²)	(bacteria/ cm ³)
Melanoma	200	(0.85, 3023)	31.69 (9.71, 71.20)	~33	~6.3×10 ⁶
Lung	274	(1.2, 3663)	22.50 (7.90, 50.35)	~23	~4.5×10 ⁶
Ovarian	57	(1.84, 73.2)	12.72 (10.25, 16.00)	~13	~2.5×10 ⁶
GBM	37	(3.41, 77.4)	15.55 (10.89, 20.85)	~16	~3.1×10 ⁶
Pancreatic	66	(3.82, 2147)	70.43 (26.19, 147.78)	~73	~14×10 ⁶
Bone	30	(1.62, 76.4)	19.33 (13.97, 25.51)	~20	~3.9×10 ⁶
Breast	352	(0.765, 1723)	44.63 (31.41, 59.83)	~46	~8.9×10 ⁶
Pan-cancer	1016	(0.765, 3663)	34.19 (24.04, 46.56)	~35	~6.8×10 ⁶

CANCER MICROBIOME DIAGNOSTICS AND PROGNOSTICS

The concept of “strength in numbers” applies to cancer diagnostics, especially for low-biomass material. For instance, liquid biopsies in cancer rely on detecting minute quantities of analytes (DNA, RNA, proteins, or modifications thereof) shed from the tumor to diagnose the presence and/or type of cancer.^[82] The low-biomass, limited unique number, and limits of detection of these analytes usually restricts utility of liquid biopsies to tumors on the scale of multiple cubic centimeters, corresponding to later stage cancers.^[82,83] Critically, more analytes or modifications, even if rare, increase the overall sensitivity of the test sigmoidally.^[84] Cristiano *et al.* demonstrated this principle using Monte Carlo simulations of liquid biopsies, showing that a test examining DNA modifications comprising $\leq 0.001\%$ of total plasma material could still have near-perfect sensitivity if at least 256 alterations were interrogated.^[84]

166

167 These conclusions from cancer genomics suggest that the inherent diversity of the intratumoral

168 microbiome (≥ 500 unique bacterial species)^[57] and the gut microbiome ($\sim 4 \times 10^3$ bacterial

169 species)^[73] provide strong rationale for creating microbiome-focused cancer diagnostics, even if

170 any individual microbe is rare or lowly abundant. Two alternative ways of phrasing this idea is that

171 (i) high microbial diversity provides “many shots on goal” for making a single diagnosis and (ii),

172 using machine learning syntax, interrogating the microbiome is analogous to employing an

173 ensemble of many weak learners that collectively provide strong prediction performance (i.e., the

174 conceptual basis of boosting). We further note that for diagnostic purposes detected microbes do

175 not need to be causally associated with carcinogenesis but only consistently correlated with

176 cancer presence, absence, and/or growth. These microbial-informed or augmented diagnostics

177 and prognostics hold much potential to improve clinical cancer care (**Figure 1**).

178

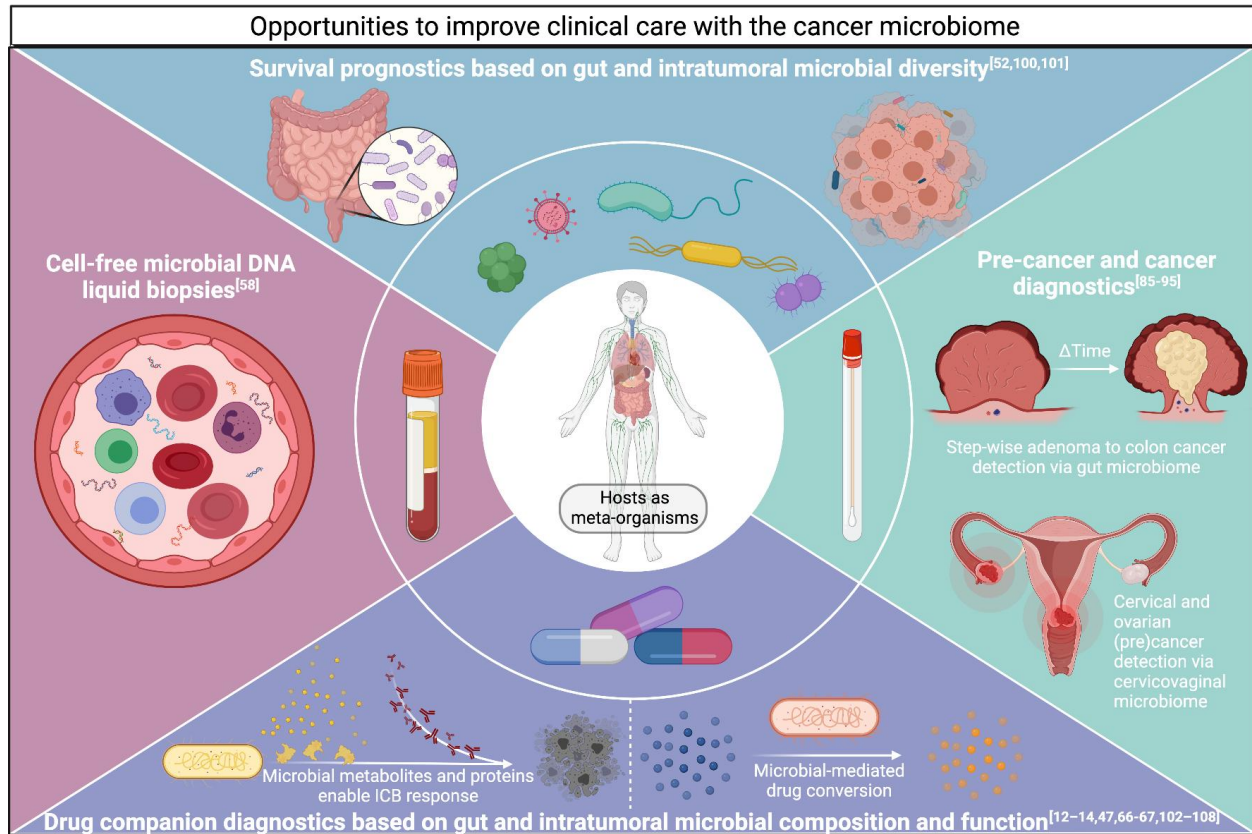


FIGURE 1. Illustration of opportunities to enhance clinical cancer diagnostics and prognostics using the cancer microbiome. Relevant references are listed in the title of each quadrant.

Pre-cancer and cancer microbiome diagnostics

Pre-cancer diagnostics identify lesions that are likely to progress to cancer but otherwise do not meet the criteria for malignant tissue, most commonly including cervical and colorectal cancer (CRC) precursors. With a focus on the gut microbiota, metagenomic studies have identified distinct fecal microbial compositions between colonic adenoma-bearing hosts and healthy individuals, often but not always with increases in *Proteobacteria* abundance.^[85-88] Yachida *et al.* further characterized shotgun metagenomic and metabolomic shifts in the guts of healthy individuals, those with polypoid adenomas, and those with stage 0 to stage IV CRCs, revealing

distinct stage-wise microbial and metabolic compositions sufficient to build fecal stage-specific classifiers.^[85] Other studies of the vaginal microbiome have revealed distinguishable microbial compositions and functions between healthy patients, those with cervical intraepithelial neoplasia or cervical cancer, and modifying effects of HPV or HIV status.^[89–91] In a longitudinal trial, Usyk *et al.* found that women presenting for high-risk HPV infection with abundant vaginal *Lactobacillus* were more likely to clear the infection by their second visit (average 1.5 years later); conversely, those with abundant vaginal *Gardnerella* upon presentation were much more likely to show disease progression by the second visit.^[89] These studies suggest the opportunity for minimally-invasive, swab-based stool and vaginal microbiome diagnostics that detect precursor cancer lesions and/or forecast risk of cancer conversion.

Pre-cancerous syndromes are also pertinent for microbiome diagnostics, such as genetically-driven familial adenomatous polyposis (FAP), pre-leukaemic myeloproliferation (PMP), and *BRCA1* status, for they augur subsequent carcinogenesis in ways not fully predicted by host genomics. For example, PMP is highly associated with *Tet2* mutations, but only a fraction of people with germline *Tet2* mutations develop PMP or bona fide myeloid malignancies.^[92] Comparing gut microbiota from patients with and without FAP, Dejea *et al.* elucidated that FAP encourages biofilm formation comprising genotoxic strains of *Escherichia coli* and *Bacteroides fragilis* with greater expression of their colibactin and *B. fragilis* toxins, thereby increasing IL-17-dependent inflammation, DNA damage, and faster cancer conversion.^[93] Meisel and colleagues then demonstrated that microbial translocation from the gut to systemic circulation with resultant IL-6 production mechanistically drives conversion from predisposing *Tet2* germline mutations to PMP.^[94] Nené *et al.* also reported significant cervicovaginal microbiome changes—absence of *Lactobacillus* spp.—among *BRCA1*-positive, non-cancer-carrying women that were shared among women with ovarian cancer, suggesting that germline mutations can affect microbial composition and may show continuity with subsequent cancer conversion.^[95] Collectively, these

studies argue that pre-cancerous syndromes indeed modify and interact with microbiota, suggesting an opportunity to develop diagnostic tools tracking their presence, and interventions that reduce cancer conversion rates.

For solid tumor and blood microbiome diagnostics, Nejman *et al.* and Poore *et al.* provide the most comprehensive analyses to date, demonstrating cancer type-specific microbial signatures among >30 cancer types, showing their diagnostic applicability to human plasma samples, and providing evidence of intracellular microbial localization in tumors.^[57,58] Nejman and colleagues combined imaging, cultivation, qPCR, and a multi-region 16S rRNA sequencing strategy to thoroughly characterize intratumoral bacteria among breast, bone, pancreas, brain, ovarian, lung, melanoma, and colon cancers. Inclusion of 811 experimental contamination controls (i.e., DNA extraction controls, no-template PCR controls, paraffin controls) for 1010 tumor samples enabled stringent decontamination that removed 94.5% of detected bacterial species, leaving 528 confident species-level calls. Poore and colleagues used an alternative approach by mining all whole genome and transcriptome sequencing data in TCGA (n=18,116 samples) and using shotgun metagenomic strategies to derive ~2000 genus-level calls.^[58] *In silico* decontamination based on sample DNA or RNA concentrations^[96] removed up to 92.3% of microbial information, but machine learning performance to distinguish between cancer types and tumor versus adjacent normal tissue remained strong. Based on historical and epidemiological data associating bacteremias with subsequent CRC diagnosis,^[69,97] they then tested whether blood-derived, genus-level microbes in TCGA were capable of distinguishing CRC from other cancer types. Finding this to be true, they next tested whether blood-derived microbiomes could discriminate between ~20 other cancer types, as well as when restricting samples to early cancer stages (stages 1-2) and tumors without any canonical mutations on two commercial cell-free tumor DNA (ctDNA) panels. Application of the same approach to 100 plasma samples from three cancer types (lung, prostate, melanoma) and 69 HIV-negative, non-cancer controls suggested that cell-

free microbial DNA (cf-mbDNA) was capable of distinguishing between healthy and cancer patients and between cancer types.^[58] Although the origin of cf-mbDNA remains unknown, we speculate based on the literature a multiplicity of sources including the oral, gut, and intratumoral microbiomes.^[53,61,62,98,99] We also speculate that the strength of the cf-mbDNA test derives from the quantity of microbial biomarkers assayed rather than the absolute amount of microbial DNA present in plasma, as analogously shown in fragmentomic-based liquid biopsies.^[84] Both of these studies lay the foundation for multiple cancer detection tests using the cancer microbiome.

Prognostics and companion diagnostics

The impact of gut and intratumoral microbiomes on local and systemic immune tone and host metabolites makes them versatile prognostics and companion diagnostics.^[3] Higher alpha diversity of intratumoral or gut microbiomes prognoses long-term survivors in pancreatic and cervical cancers, as well as in patients undergoing hematopoietic stem cell transplantation for cancer therapy.^[52,100,101] Additionally, colorectal cancer stages reflect successive microbial changes in the fecal microbiome,^[85,88] and early versus late stage lung cancer can be distinguished through lower airway microbiota compositions.^[51] Intratumoral microbiomes can similarly distinguish stage I from stage IV tumors in multiple gastrointestinal cancers (stomach, colon, rectal) and renal cell cancer.^[58]

Therapeutically, numerous studies demonstrate how the efficacies of anti-CTLA-4 and anti-PD-(L)1 immune checkpoint blockade (ICB) are predicted by and mechanistically tied to gut microbiome composition and function,^[12–14,67,102–106] and recently the intratumoral microbiome has shown a similar capacity.^[49,57] Similarly, the efficacy and host toxicity of cyclophosphamide,^[66,107] gemcitabine^[47], and platinum-based^[67,108] chemotherapy depend on the composition and metabolic capacity of gut and intratumoral microbiota.^[109] In specific cases, bacterial enzymes

directly degrade chemotherapy compounds into non-functional byproducts (e.g., gemcitabine degradation by cytidine deaminase),^[47] suggesting colonized patients would have no drug response or quickly develop therapeutic resistance. In HER2-positive breast cancer, antibiotic administration impairs trastuzumab efficacy and fecal microbiota transplant from non-responders to responders improves outcomes, implicating gut microbiota as critical agents for therapeutic response.^[68]

Gut microbiota also affect hormonal therapies. Administration of abiraterone acetate (AA) in the setting of castrate-resistant prostate cancer promoted outgrowth of *Akkermansia muciniphila* and appeared to aid overall AA therapeutic efficacy.^[110] However, androgen deprivation therapy also increases gut-residing *Ruminococcus* species containing CYP17A1-like enzymes that catalyze pregnenolone conversion to the sex hormone precursor dehydroepiandrosterone (DHEA) and testosterone, thereby enhancing progression to castration-resistant prostate cancer.^[65] Thus, targeted longitudinal profiling of implicated gut microbes may provide an early indicator of failing androgen deprivation therapy while also substantiating their timed targeted removal. It has been speculated, albeit unproven, that estrogen-receptor-positive breast cancer may similarly be affected by microbial hormone metabolism.^[111,112] It further remains unknown if or how intratumoral microbes affect hormonal metabolism. Altogether, the myriad of gut and intratumoral microbiome effects on virtually every domain of cancer therapy and predictive associations with patient survival enforce their clinical utility as prognostic indicators and companion diagnostics.

Challenges for cancer microbiome diagnostics and prognostics

Low-biomass microbial sampling creates analysis challenges that necessitate careful consideration and removal of contamination.^[113,114] While less impactful in gut microbiome studies or large-scale meta-analyses, external (e.g., environmental) and internal (e.g., cross-seeding

between samples) contamination can skew small-to-moderate scale profiling of the cancer microbiome.^[113,114] Standardized experimental contamination controls (**Figure 2**) alongside *in silico* decontamination methods^[96,114] can enable more robust and reproducible results, especially for assaying intratumoral and blood-borne microbes, thereby enabling better microbiome-augmented cancer diagnostics and prognostics. Notably, very few cancer genomics studies implement any of these contamination controls and basic usage thereof would allow broad utilization of “cancer-specific” data for simultaneous interrogation of microbial analytes, although this may be overcomable by integrating many thousands of samples.

Other challenges with microbiome studies include (i) the degree to which results vary with sample and bioinformatics processing choices;^[115] (ii) fundamental differences in data properties and appropriate statistics when using relative abundances compared to host ‘omic data;^[116–118] and (iii) compositional differences as a function of geography and ethnicity, particularly when assaying gut microbiota.^[119–121] One or more of these factors have, for example, resulted in three major microbiome studies^[12–14] concluding that different gut microbes predict anti-PD(L)1 immunotherapy response—a fact that has remained irreconcilable despite analyses that reprocessed all the data equally or instead examined their microbial functions.^[104] Large meta-analyses can surmount some of these problems, with two key studies identifying conserved gut microbial signatures predictive of colorectal cancer across diverse cohorts and geographies.^[122,123]

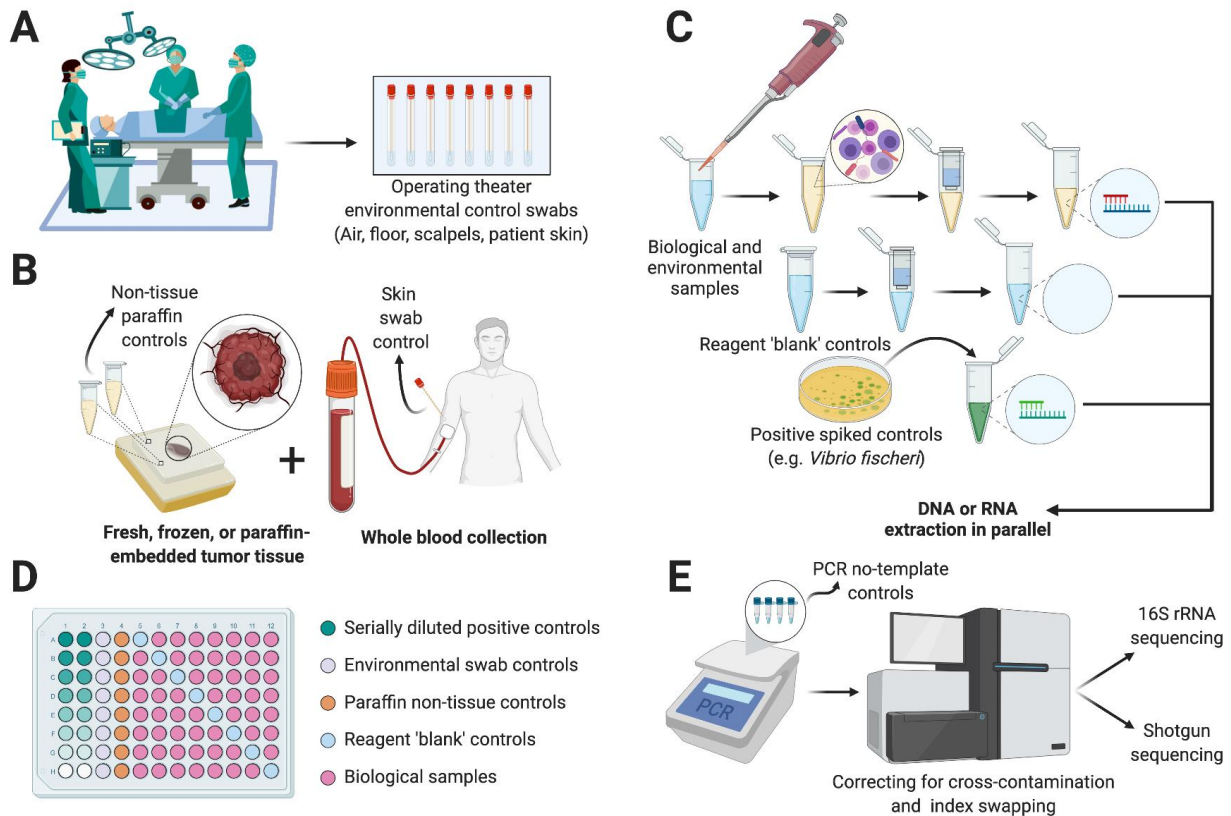


FIGURE 2. Extracting and analyzing low-biomass microbiomes requires special care to control external and internal contamination.^[96,113,114] **(A)** Collection of environmental controls ideally begins in the operating room to account for non-patient environmental sources. **(B)** Post-operative tissues, if paraffin embedded, can have non-tissue paraffin controls taken to ensure the embedding process is not contaminated. Whole blood should ideally be collected with a skin swab to account for peri-needle contamination. **(C)** Negative reagent-only 'blank' controls and positive titrated controls should be processed simultaneously alongside nucleic acid extraction from biological and environmental samples. **(D)** Plating strategies should be considered to reduce cross-contamination; controls may include up to 40% of total samples. **(E)** Amplification steps may include PCR no-template controls and sequencing may include correction for cross-contamination or index swapping, although the latter remains challenging.

REDEFINING CANCER CLONALITY AS MULTISPECIES

Redefining traditional meaning of clonal evolution and selection in cancer

Cancer cells evolve through space and time. Although the traditional view of clonal evolution has historically centered on genetic alterations,^[37,124,125] it is increasingly recognized that non-genetic alterations such as epimutations also contribute.^[126–128] The emergence of single-cell multi-omics and longitudinal studies offers opportunities for a more inclusive, multi-analyte view of intratumor heterogeneity and clonal evolution.^[129,130] Recognition of the role of multi-omics in functional clonal diversity advocates for broader definitions beyond cancer genomics.^[38]

Research demonstrating effects of extracellular and intracellular microbes on the cancer cell genome,^[93,131] transcriptome,^[48,50,51,132] proteome,^[49] and metabolome^[47,65] strongly justify their inclusion in any multi-omic model of clonal evolution (**Figure 3**). Additional microbial functions that enable or abolish chemo-, radio-, and/or immunotherapy efficacy without *any* interventional cancer genomic changes provide further rationale for their inclusion.^[3,11,133] Intracellular localization of metabolically active, immunogenic cancer microbes that shape cancer immunoediting—evolutionary processes and selection pressures previously privileged to cancer clonal selection—also provides justification.^[49,57,63] Identification of hybrid microbial-human reads involved in carcinogenesis on plasmid-like ecDNA segments intimately links cancer and microbial fitness.^[45,46] Microbial mechanisms that modify immunosurveillance also impact when and where tumors grow and/or metastasize.^[49,53,54,61,62,134] Negatively, ignorance of microbial information in clonal evolution precludes accurate identification of cancer dynamics, therapeutic resistance, and metastasis. However, as distinct agents from cancer cells with separate genetic material that is, at times, under discordant selection pressure(s) from the cancer genome (e.g., antibiotic therapy for bacteria, targeted kinase therapies for cancer cells), there must be nuance. Cancer microbes

359 cannot merely be added as another “-ome.” Simultaneously, studies examining the roles of cancer
360 microbiota have not seriously considered the clonality of these microbes or their impacts on
361 cancer cell clonality. Thus, there is a persisting theoretical gap between the microbiota in cancer
362 and clonal evolution modelling that we propose bridging.

363

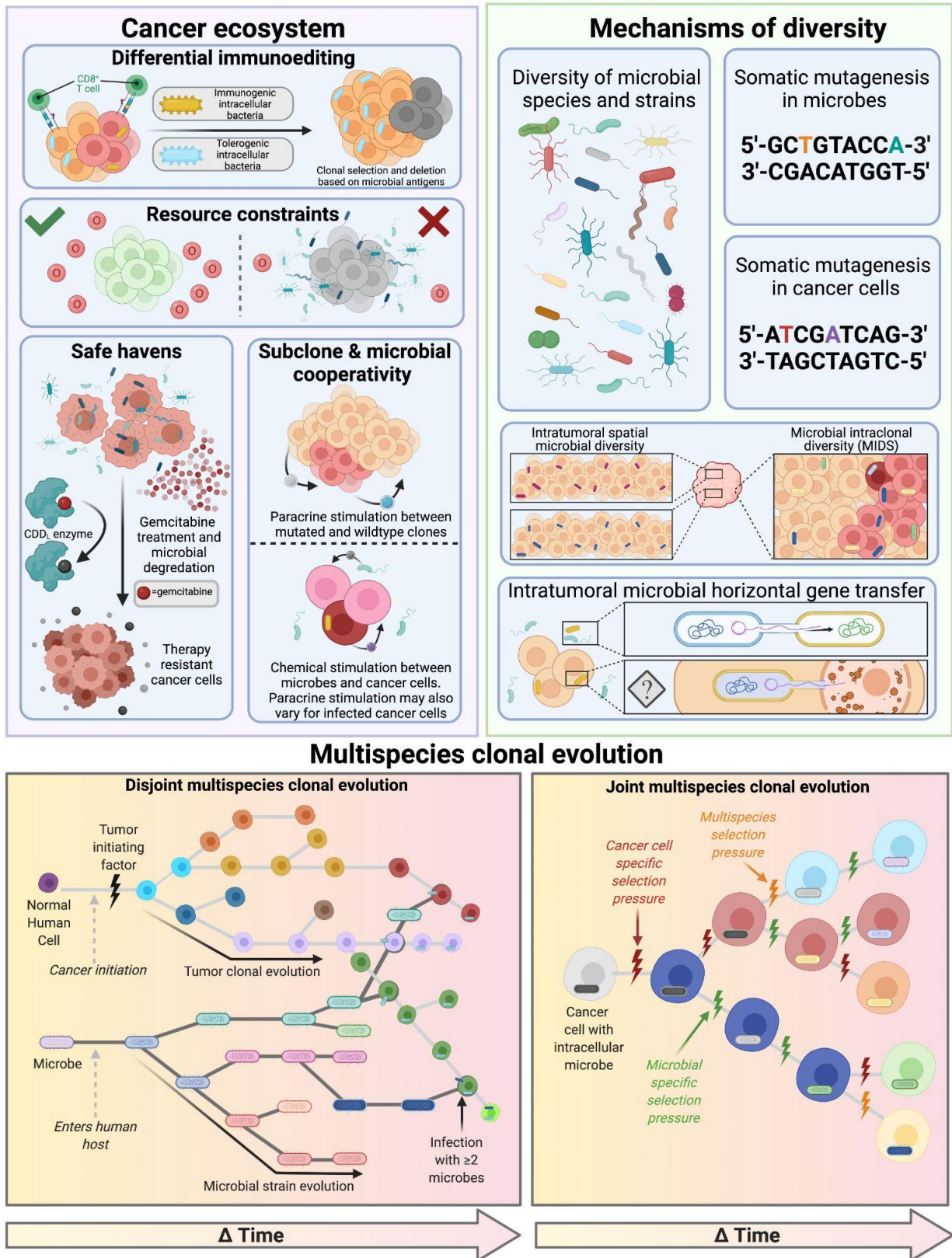


FIGURE 3. Impacts of intratumoral microbes on cancer evolution and arguments for multispecies clonal evolution modelling. Effects are summarized into three major categories: modulation ecosystem effects, mechanisms of clonal diversity, and example disjoint and joint phylogenetic clonal evolution.

Key evidence that argues cancer clonality is multispecies

Decoupling of therapeutic efficacy from host and cancer cell genetic changes

The genetic model of clonal evolution provides explanations for relapses caused by mutagenesis. For example, mutations in cancer cell epidermal growth factor receptor (EGFR) induce resistance to various generations of EGFR tyrosine kinase inhibitors while simultaneously creating favorable selection pressures for mutated cells over non-mutated counterparts.^[135,136] As a result, EGFR-mutated cells outcompete their neighbors and clonally expand.

However, this model fails to always explain treatment efficacy or failure, both for conventional cancer and microbial-modulated therapies. For example, isocitrate dehydrogenase (IDH1/IDH2)-mutated acute myeloid leukemia patients treated with IDH1/2 inhibitors can show complete and sustainable responses to treatment without eliminating mutated cells.^[137–139] The same is observed in chronic myelomonocytic leukemia treated with hypomethylating agents. Patient responses, even when complete, demonstrate no decrease in the mutational load and no specific selection events explaining secondary escape.^[140] Moreover, despite a clear reduction in cancer cell burden, thereby generating a selective bottleneck, relapse can occur without genetic

selection. For example, in childhood B-cell precursor acute lymphoblastic leukemia, a recent study by Turati *et al.* demonstrated how treatment with vincristine and dexamethasone drastically reduced the leukemic burden but induced very little change, if any, in clonal composition.^[141] Conversely, a transcriptional bottleneck was observed in single-cell RNA-Seq, with a major loss in transcriptomic intratumor heterogeneity. A similar resistant transcriptomic profile was found in the leukemic cells before treatment, suggesting positive selection of these rare pre-existing resistant cells rather than induction of that phenotype under treatment exposure. These resistant cells comprised a subfraction of low cycling cells and have been associated with a distinct metabolic program.^[141,142] Several hypotheses are currently discussed with regard to this non-genetic resistance to therapies,^[143] which mostly focus on transcriptomic and epigenetic properties.

Classic genetic clonal evolution also fails to account for microbial-mediated treatment efficacy or failure of (i) cyclophosphamide,^[66,107] gemcitabine^[47], and platinum-based^[67,108] chemotherapy; (ii) anti-CTLA-4 and anti-PD-(L)1 ICB efficacy;^[12–14,102–106] (iii) androgen deprivation therapy in prostate cancer;^[65] and (iv) trastuzumab in HER-2-positive breast cancer.^[68] Notably, some of these examples (e.g., gemcitabine resistance) rely on *microbial* genetic content (e.g., cytidine deaminase long (CDD_L) isoforms),^[47] which further may be shared among multiple species through conventional horizontal gene transfer and may also be intracellular. Similarly, cancer clonal selection may entirely occur on CDD_L-containing microbes by providing growth advantages to those that can metabolize it as a concentrated carbon source, and cancer cell survival is tied to CDD_L⁺-microbe proximity. Yet, cancer genome-centric evolutionary models miss all of these effects and fail to accurately forecast evolutionary changes.

Impact of intracellular bacteria on cancer cell properties and fitness

Immunohistochemistry, immunofluorescence, and electron microscopy data document the intracellular localization of intratumoral bacteria.^[49,57,61,63] Bacteria inside cancer cells modify their properties—transcriptional state,^[63] proteome,^[49] and metabolic repertoire^[47,57]—in ways that are intrinsically tied to clonal evolution. Extracellular bacteria also modulate these properties and cause cancer cell genomic mutations.^[93,131,144] Key affected clonal aspects comprise cancer cell metabolism, immunoediting, clonal expansion and metastasis, and mutagenesis.

First, intracellular microbes change host cell metabolism, including degradation of exogenous chemotherapy^[47] and xenobiotic D-alanine.^[57] Geller *et al.* originally identified microbial gemcitabine resistance through incidentally discovering *Mycoplasma* contamination of cell cultures and concomitant drug resistance.^[47] Isolation of the responsible enzyme and its drug-degrading isoform (CDDL) followed by bioinformatic searches revealed >300 CDDL⁺ species, 98.4% within *Gammaproteobacteria*. Imaging, sequencing, and cultivation from gemcitabine-associated pancreatic cancer patient biopsies indeed revealed CDDL⁺ bacteria in most samples that conferred gemcitabine resistance in subsequent co-cultures with cancer cell lines.^[47]

Second, intratumoral microbes modulate the immune response, favoring immune escape, or, conversely, recognition. *Fusobacterium nucleatum* inhibits natural killer cell (NK)-dependent tumor killing through Fap2 interaction with TIGIT, constituting a bacterium-dependent mechanism of tumor-immune evasion.^[145] Pancreatic cancer bacteria also induce innate and adaptive immune suppression, including via selective Toll-like receptor ligation leading to T-cell anergy.^[48] Another metastatic melanoma study elucidated immunogenic, MHC I and II-bound bacterial peptides presented on cancer and immune cells that putatively shape cancer immunoediting and posit gut-tumor antigenic overlap.^[49] Moreover, an uneven partitioning of microbes among cancer cells can result in the differential elimination or maintenance thereof. Such a perspective enriches the

traditional “3Es” of elimination, equilibrium, and escape^[146] and documents how cancer cell fitness is decoupled from its own genome.

Third, intratumoral microbes can favor cancer cell expansion and metastases. Bullman *et al.* demonstrated *Fusobacterium* persistence in colorectal cancers through successive mouse xenografts and similar bacterial compositions in matched primary-metastasis (colorectal-liver) patient samples.^[61] Metronidazole treatment reduced tumor growth, implying greater fitness conferred by *Fusobacterium* colonization.^[61] Bertocchi *et al.* later showed that colorectal bacteria stepwise enter tumor tissue, modify the gut vascular barrier, migrate to the liver, and foster the formation of a premetastatic niche favoring metachronous metastasis.^[62] Parhi *et al.* noted how *Fusobacterium*-seeded breast cancers metastasized earlier. Hence, intratumoral bacteria enhance metastatic formation and seeding.

Fourth, microbes cause genotoxin-mediated mutagenesis.^[93,131] Pleguezuelos-Manzano *et al.* showed how *pks*⁺ *E. coli* generates mutational signatures in head and neck, colorectal, and urinary tract cancers. Moreover, various gut-residing *Proteobacteria* species produce cytolethal distending toxin (CDT) capable of inducing single- and double-stranded DNA breaks.^[144] Collectively, all of these mechanisms shape cancer cell properties and fitness.

Implications and hypotheses if cancer clonality is multispecies

Imaging data portray intracellular bacteria as unevenly distributed among cancer cells and tumor regions,^[57,61] suggesting differential fitness at the single cell level that may not correspond with mutational status. This challenges the definition of cancer clones as private lineages of mutated cells stemming from common ancestors and violates modelling assumptions whereby clonal lineages comprise homogeneous cell populations. Although no two cancer cells are equal in every

respect, the primary assertion of clonality is that individual differences between two cancer cells of the same clone are negligible.^[147] However, if intracellular bacteria alter phenotypes, behaviors, and fitness of spatially-adjacent cancer cells, then they create major intraclonal heterogeneity, which we define as “microbial intraclonal diversity” (MIDS). MIDS questions clonal lineage homogeneity and motivates revising clonal boundaries, most simplistically through further subsetting (e.g., *KRAS*-mutated, *Fusobacterium*-infected cells versus *KRAS*-mutated uninfected cells) or more accurately through revised modelling approaches that account for discordant microbe-cancer selection pressures. MIDS also includes mimicry between microbial and cancer antigens.^[148,149] Should genetic cargo be shared between intracellular bacteria and host cells, as biotechnology already shows is possible^[24] and cancer virology affirms,^[45,46] MIDS must account for DNA and RNA from multiple species.

Beyond challenging clonal boundaries, intracellular bacteria may require revision of the evolutionary tree. Typical clonal evolution model depicts an evolutionary tree with one trunk and several branches, relying on the assumption of vertically transmitted traits from mother cells to daughter cells at each round of cell division. If future research affirms horizontal/lateral gene transfers between intracellular bacteria and host cancer cells, multiple tree trunks and connexions between branches would be required. A similar debate has taken place in evolutionary biology, challenging the traditional Darwinian view about “tree of life.”^[150–152] Clonal evolution may then be better articulated as a case of “reticulated evolution,” wherein horizontal/lateral transfers change the fitness, function, or/phenotype of host cancer cells.

Considerations for cancer microbiome therapeutics under multispecies clonality

Multispecies cancer clonality offers new therapeutic strategies that neither human nor microbial clonality alone propose. For instance, Byndloss *et al.* demonstrated an interplay between

fastidious anaerobic gut bacteria and butyrate-mediated, PPAR- γ -dependent host signaling that maintained low oxygen levels in the gut and prevented outgrowth of facultative pathogens.^[153] Conversely, antibiotics increased gut oxygen concentration and pathogen outgrowth.^[153] Analogously, there may be opportunities in cancer to target host processes that facilitate microbial homeostasis as a means to mitigate microbial-mediated carcinogenesis in favor of blunted antibiotics. Butler *et al.* provide another example whereby administration of a bacterial protease depleted cellular MYC in colon and bladder cancers.^[154] Similarly, identification of anticancer microbial enzymes or metabolites may provide effective host-modulating cancer therapies or improve the efficacy of existing therapies—a strategy that several groups have already taken with immunotherapy.^[105,106]

EVOLUTIONARY MODELING OF THE CANCER MICROBIOME

Example of *Helicobacter pylori*

Incorporating intratumoral microbes into evolutionary models requires nuance because selection pressures may be discordant with those experienced by cancer cells. A long-standing and well-studied example of microbes in the cancer environment is *Helicobacter pylori*,^[155] which has adapted to thrive in more than half of the human population long enough to trace human migration events.^[156] *H. pylori* has co-evolved protective and pathogenic roles within humans: protective in gastric cardia and esophageal adenocarcinoma^[157,158] and pathogenic in noncardiac gastric cancer.^[155] Most *H. pylori*-positive patients carry multiple strains, including at least one strain unique to their body along with more common strains such as VacA, CagA, and BabA.^[156] This extreme genetic diversity stems from slipped-strand mispairing in multiple genes and *H. pylori*'s lack of DNA repair genes unlike most bacteria.^[156] High strain diversity across individual human hosts also enhances *H. pylori*'s population-wide resilience, allowing it to adapt to many diverse

environments by expanding upon the strain with the highest fitness in that setting. Collectively, high diversity and concomitant mutagenesis of *H. pylori* combined with human immune selection pressures and pathological impacts on noncardiac gastric carcinogenesis help portray an exemplary “big picture” of multispecies cancer evolution. Building on this idea, we describe how existing clonal evolution modeling may take intratumoral microbes into account.

Common constraints of the tumor microenvironment

As detailed above, the TME contains intracellular and extracellular microbes that affect cancer clonality and comprise independent clonal agents. Importantly, the TME contexture applies simultaneous, shared selective pressures and environmental constraints on co-located cancer cells and microbes. Shared resources necessitate cooperative use and/or competition, which may further limit their abundance. For instance, oxygen availability drives spatial organization and metabolic capacities of cancer cells^[159] and is known to similarly affect microbes in environmental contexts and model systems (e.g., Winogradsky columns).^[160–162] Common selection pressures may in turn drive common evolutionary solutions, such as metabolic symbiosis between cancer cells^[163] or between microbes positioned along the oxygen gradient.^[162] Gradients of pH are tied to oxygen and common in tumors,^[159] and they shape microbial compositions in environmental contexts.^[164] Hence, multispecies evolutionary models should take into account joint environmental constraints.

Anderson and colleagues have presented compatible multiscale mathematical models of cancer growth that take into account both cellular biophysical properties and TME factors.^[165–167] Their model determined that aggressive cancer clones were established under the harshest TME conditions (e.g., hypoxia, heterogenous extracellular matrix) but that their impact on overall tumor invasiveness was blunted under milder TME conditions. Hence, microenvironmentally harsh

chemotherapy may worsen long-term cancer invasiveness. Incorporating microbes into their multiscale model equations—their reliance and impact on TME chemical gradients and cancer metabolism—could inform multispecies clonal dynamics and ideal TME conditions that in turn would inform multispecies therapeutic strategies.

Microbes affect clonal fitness

As detailed above, intratumoral microbes affect cancer cell fitness, justifying their inclusion to accurately model clonal evolution. Current models typically account for factors like probabilities of cell division and cell death alongside inferred mutation rates and human driver genes. However, intracellular microbes likely need to be included in these equations as well in certain scenarios, particularly their mutational, division, and death rates. In circumstances of microbial enzymatic degradation, such as CDD_L-mediated gemcitabine metabolism,^[47] transcriptional rates and enzymatic efficiencies may be useful variables to include. In circumstances of genotoxin-mediated mutagenesis, such as from *pks*⁺ *E. coli*,^[131] the base-pair motif and rate of mutations may also be instructive to include.

Likelihood of clone development, treatment resistance, and fitness are all major parts of clonal evolution models and are related to extracellular and intracellular microbes, but these have not been typically considered in models of cancer evolution thus far. In common population genetics models of clonal evolution, including Wright-Fisher diffusion type models^[168] and Moran type models,^[169] it may be helpful to consider clonal fitness as a function of time-dependent fluctuations in microbial abundances or presence/absence of particular species. Additionally, branching process stochastic models of tumor growth that parameterize evolution in terms of proliferation and mutation rates^[170–172] may also benefit from considering microbial colonization rates and species-specific transcriptional effects. Furthermore, cancer microbes individually (and likely

jointly) undergo somatic clonal evolution, as described in the case of *H. pylori*. Phylogenetic tree reconstructions of clonal evolution^[173] may thus need to include multispecies lineages, but specialized methods likely need to be created for this purpose. To summarize, we have created a table of major clonal evolution models and suggested strategies for incorporating microbial information into them (**Table 2**).

CONCLUSIONS

Rigorous studies provide extensive evidence for the existence and functionality of cancer-associated gut and intratumoral microbes while echoing ancient historical narratives of microbial-mediated recovery. Drawing from cancer genomics, the inherently high diversity of the cancer microbiome substantiates its strong predictive power, even when any individual microbe is rare or lowly abundant. Cancer microbiota can distinguish healthy, pre-cancer, and cancerous tissues across multiple cancer and sample types, although most diagnostics remain unvalidated in large, multi-national, prospective cohorts. Cancer microbiota also demonstrate stage-specific differences that may enable simultaneous identification and prognostication of tumors. Nonetheless, contamination challenges in low-biomass settings and analytic idiosyncrasies of microbiomic data have hitherto complicated routine clinical application of cancer microbial diagnostics or prognostics.

Numerous microbial mechanisms affect the cancer genome, transcriptome, proteome, and metabolome, advocating for their inclusion in models of cancer evolution. Extracellular and intracellular microbes affect virtually every cancer medication class and may drive therapeutic efficacy or resistance without any cancer cell(s) interventions. Negatively, it is not possible to accurately model cancer-drug dynamics, clonality, or fitness without accounting for microbes. Serious consideration of multispecies clonality, however, is complex and necessitates reworking

cancer evolution models since microbes carry distinct, although plausibly shareable, genetic cargo that may undergo discordant selection pressures from the cancer genome. Flexible evolutionary models treating intratumoral microbes as independent, albeit rule-abiding agents within the TME may be appropriate. Multispecies clonality also informs treatments, such that intentional modifications of cancer pathways may comprise more effective ways to restore healthy microbial ecologies than targeted antimicrobials, and vice versa. Multispecies treatment strategies may further benefit from target selectivity, for targeting microbial genes generally carries fewer side effects than targeting the host's. Altogether, understanding cancer's metagenome carries key ramifications for cancer care and clonal evolution for the benefit of patients worldwide.

TABLE 2. Microbial integration into mathematical models of evolution. Overview of each model's characteristics and references provided with modeling examples, as well as suggested ways that microbes could be potentially incorporated into model structure. Hybrid models that include aspects of more than one model type are also utilized in practice.

Model Type	Overview of Model	Examples of Potential Incorporation of Microbes	Literature
Agent-based model	<ul style="list-style-type: none"> Define 'agents' : individuals or members of the microenvironment with specific properties and actions on a structured grid or 3-D space Can have stochastic and deterministic components with spatial constraints Define environmental rules and presence of factors in space such as signalling proteins like VEGF Define agent-agent interaction rules 	<ul style="list-style-type: none"> Create microbe as one agent type and cancer cell as another agent type Allow clonal evolution of cancer cells and separate evolution of microbes in equations Create biophysical rules accounting for spatial movement of microbes and effect of microbes on evolutionary rates such as proliferation and survival of cancer cells Introduce complexity with microbes as agents within the 	[174,175]

		microenvironment	
Wright-Fisher type model	<ul style="list-style-type: none"> Population size remains constant over time (can be extended to growing populations) Considers finite number of population species/k-alleles To create the next non-overlapping generation, alleles are randomly sampled with replacement Allele frequency in new generation is combination of random sampling of population and the fitness of alleles Captures genetic drift and natural selection if included 	<ul style="list-style-type: none"> Microbes will likely not be directly considered in the population species, but instead the effects of microbes will be interwoven into fitness Fitness parameter of certain genotypes may depend on metabolites, proteins, and antigens from intracellular bacteria, which in certain cases may drive differential immunoediting between cancer cell-bearing bacteria 	[168,176]
Moran-type model	<ul style="list-style-type: none"> Two or more species considered in a population Asexual reproduction, overlapping generations Simultaneous birth and death events occur As in the Wright-Fisher model, can be formulated as a diffusion approximation 	<ul style="list-style-type: none"> Similar to the Wright-Fisher type model, microbes will likely not be directly considered in the population species, but instead the effects of microbes will be interwoven into fitness Fitness parameter of certain genotypes may depend on metabolites, proteins, and antigens from intracellular bacteria, which in certain cases may drive differential immunoediting between cancer cell-bearing bacteria 	[169,177]
Birth-death stochastic process	<ul style="list-style-type: none"> Continuous time Markov model (branching process) where 'birth' or 'death' events can change the state/population size A 'birth' increases the state by one A 'death' decreases the state by one Allows for multiple cell types (e.g., with/without driver mutations), fluctuations in total population size, stochastic extinction of cells, and mutation to other types 	<ul style="list-style-type: none"> Option 1 <ul style="list-style-type: none"> Birth and death defined in terms of human cancer cells with: <ul style="list-style-type: none"> Probability of birth and/or death affected by microenvironment Probability of birth and/or death dependent on a function of the population of intratumoral microbes present Option 2 	[178,179]

		<ul style="list-style-type: none"> ○ Birth and death events defined in terms of both human cancer cells and microbial populations 	
Evolutionary game theory model	<ul style="list-style-type: none"> ● Includes density-dependent fitness with cell-cell interactions ● Models cooperation, e.g., between tumor and stromal cells ● Fitness landscapes in non-cancer models have been central to understanding microbial evolution such as <i>E. coli</i> 	<ul style="list-style-type: none"> ● Include microbes as a type of “player” in the modeled ecosystem including tumor cells for limited chemicals and nutrients (e.g., oxygen, sugars, etc.) ● “Public good” produced by tumor cells, such as glycolytic acid and vascular endothelial growth factor, included in a game as competing resources with microbial populations 	[180–183]

615
616
617
618

REFERENCES

- 619 1. Ebbell, B. (1937). *The Papyrus Ebers: the greatest Egyptian medical document*. Levin &
620 Munksgaard.
- 621 2. Hoption Cann, S. A., van Netten, J. P., & van Netten, C. (2003). Dr William Coley and tumour
622 regression: a place in history or in the future. *Postgraduate Medical Journal*, 79(938), 672–
623 680. <https://www.ncbi.nlm.nih.gov/pubmed/14707241>
- 624 3. Sepich-Poore, G. D., Zitvogel, L., Straussman, R., Hasty, J., Wargo, J. A., & Knight, R.
625 (2021). The microbiome and human cancer. *Science*, 371(6536).
626 <https://doi.org/10.1126/science.abc4552>
- 627 4. Busch, W. (1868). Aus der Sitzung der medicinischen Section vom 13 November 1867. *Berl*
628 *Klin Wochenschr*, 5, 137.
- 629 5. Fehleisen, F. (1882). Ueber die Züchtung der Erysipelkokken auf künstlichem Nährboden und
630 ihre Übertragbarkeit auf den Menschen. *Deutsche Medizinische Wochenschrift*, 8(31),
631 553–554.
- 632 6. Coley, W. B. (1893). The treatment of malignant tumors by repeated inoculations of

erysipelas: With a report of ten original cases. 1. *The American Journal of the Medical Sciences*, 105(6), 487.

<https://search.proquest.com/openview/09fb106c24157c028c895edfa8049551/1?pq-origsite=gscholar&cbl=41361>

7. Starnes, C. O. (1992). Coley's toxins in perspective. *Nature*, 357(6373), 11–12.

<https://doi.org/10.1038/357011a0>

8. Hobohm, U. (2001). Fever and cancer in perspective. *Cancer Immunology, Immunotherapy: CII*, 50(8), 391–396. <https://doi.org/10.1007/s002620100216>

9. Unproven Methods of Cancer Treatment. (1961). *CA: A Cancer Journal for Clinicians*, 11(5), 191–193. <https://doi.org/10.3322/canjclin.11.5.191>

10. Gopalakrishnan, V., Helmink, B. A., Spencer, C. N., Reuben, A., & Wargo, J. A. (2018). The Influence of the Gut Microbiome on Cancer, Immunity, and Cancer Immunotherapy. *Cancer Cell*, 33(4), 570–580. <https://doi.org/10.1016/j.ccell.2018.03.015>

11. Zitvogel, L., Ma, Y., Raoult, D., Kroemer, G., & Gajewski, T. F. (2018). The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. *Science*, 359(6382), 1366–1370. <https://doi.org/10.1126/science.aar6918>

12. Gopalakrishnan, V., Spencer, C. N., Nezi, L., Reuben, A., Andrews, M. C., Karpnits, T. V., Prieto, P. A., Vicente, D., Hoffman, K., Wei, S. C., Cogdill, A. P., Zhao, L., Hudgens, C. W., Hutchinson, D. S., Manzo, T., Petaccia de Macedo, M., Cotechini, T., Kumar, T., Chen, W. S., ... Wargo, J. A. (2018). Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science*, 359(6371), 97–103. <https://doi.org/10.1126/science.aan4236>

13. Routy, B., Le Chatelier, E., Derosa, L., Duong, C. P. M., Alou, M. T., Daillère, R., Fluckiger, A., Messaoudene, M., Rauber, C., Roberti, M. P., Fidelle, M., Flament, C., Poirier-Colame, V., Opolon, P., Klein, C., Iribarren, K., Mondragón, L., Jacquelot, N., Qu, B., ... Zitvogel, L. (2018). Gut microbiome influences efficacy of PD-1-based immunotherapy against

- epithelial tumors. *Science*, 359(6371), 91–97. <https://doi.org/10.1126/science.aan3706>
14. Matson, V., Fessler, J., Bao, R., Chongsuwat, T., Zha, Y., Alegre, M.-L., Luke, J. J., & Gajewski, T. F. (2018). The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. *Science*, 359(6371), 104–108. <https://doi.org/10.1126/science.aao3290>
15. Davar, D., Dzutsev, A. K., McCulloch, J. A., Rodrigues, R. R., Chauvin, J.-M., Morrison, R. M., Deblasio, R. N., Menna, C., Ding, Q., Pagliano, O., Zidi, B., Zhang, S., Badger, J. H., Vetizou, M., Cole, A. M., Fernandes, M. R., Prescott, S., Costa, R. G. F., Balaji, A. K., ... Zarour, H. M. (2021). Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*, 371(6529), 595–602. <https://doi.org/10.1126/science.abf3363>
16. Baruch, E. N., Youngster, I., Ben-Betzalel, G., Ortenberg, R., Lahat, A., Katz, L., Adler, K., Dick-Necula, D., Raskin, S., Bloch, N., Rotin, D., Anafi, L., Avivi, C., Melnichenko, J., Steinberg-Silman, Y., Mamtani, R., Harati, H., Asher, N., Shapira-Frommer, R., ... Boursi, B. (2020). Fecal microbiota transplant promotes response in immunotherapy-refractory melanoma patients. *Science*. <https://doi.org/10.1126/science.abb5920>
17. White, M. K., Pagano, J. S., & Khalili, K. (2014). Viruses and human cancers: a long road of discovery of molecular paradigms. *Clinical Microbiology Reviews*, 27(3), 463–481. <https://doi.org/10.1128/CMR.00124-13>
18. Rous, P. (1911). A SARCOMA OF THE FOWL TRANSMISSIBLE BY AN AGENT SEPARABLE FROM THE TUMOR CELLS. *The Journal of Experimental Medicine*, 13(4), 397–411. <https://doi.org/10.1084/jem.13.4.397>
19. Martin, G. S. (2004). The road to Src. *Oncogene*, 23(48), 7910–7917. <https://doi.org/10.1038/sj.onc.1208077>
20. Stehelin, D., Varmus, H. E., Bishop, J. M., & Vogt, P. K. (1976). DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature*,

- 260(5547), 170–173. <https://doi.org/10.1038/260170a0>
21. Dulbecco, R. (1986). A turning point in cancer research: sequencing the human genome. *Science*, 231(4742), 1055–1056. <https://doi.org/10.1126/science.3945817>
22. Simons, P. J., & Dougherty, R. M. (1963). ANTIGENIC CHARACTERISTICS OF THREE VARIANTS OF ROUS SARCOMA VIRUS. *Journal of the National Cancer Institute*, 31, 1275–1283. <https://www.ncbi.nlm.nih.gov/pubmed/14071832>
23. Svet-Moldavsky, G. J. (1957). Development of multiple cysts and of haemorrhagic affections of internal organs in albino rats treated during the embryonic or new-born period with Rous sarcoma virus. *Nature*, 180(4597), 1299–1300. <https://www.nature.com/articles/1801299a0>
24. Mulligan, R. C., & Berg, P. (1980). Expression of a bacterial gene in mammalian cells. *Science*, 209(4463), 1422–1427. <https://doi.org/10.1126/science.6251549>
25. Grillot-Courvalin, C., Goussard, S., Huetz, F., Ojcius, D. M., & Courvalin, P. (1998). Functional gene transfer from intracellular bacteria to mammalian cells. *Nature Biotechnology*, 16(9), 862–866. <https://doi.org/10.1038/nbt0998-862>
26. Laner, A., Goussard, S., Ramalho, A. S., Schwarz, T., Amaral, M. D., Courvalin, P., Schindelhauer, D., & Grillot-Courvalin, C. (2005). Bacterial transfer of large functional genomic DNA into human cells. *Gene Therapy*, 12(21), 1559–1572. <https://doi.org/10.1038/sj.gt.3302576>
27. Darji, A., Guzmán, C. A., Gerstel, B., Wachholz, P., Timmis, K. N., Wehland, J., Chakraborty, T., & Weiss, S. (1997). Oral somatic transgene vaccination using attenuated *S. typhimurium*. *Cell*, 91(6), 765–775. [https://doi.org/10.1016/s0092-8674\(00\)80465-1](https://doi.org/10.1016/s0092-8674(00)80465-1)
28. Sizemore, D. R., Branstrom, A. A., & Sadoff, J. C. (1995). Attenuated *Shigella* as a DNA delivery vehicle for DNA-mediated immunization. *Science*, 270(5234), 299–302. <https://doi.org/10.1126/science.270.5234.299>
29. Johnson, S. A., Ormsby, M. J., McIntosh, A., Tait, S. W. G., Blyth, K., & Wall, D. M. (2019). Increasing the bactofection capacity of a mammalian expression vector by removal of the f1

- ori. *Cancer Gene Therapy*, 26(7-8), 183–194. <https://doi.org/10.1038/s41417-018-0039-9>
30. Pálffy, R., Gardlík, R., Hodosy, J., Behuliak, M., Resko, P., Radvánský, J., & Celec, P. (2006). Bacteria in gene therapy: bactofection versus alternative gene therapy. *Gene Therapy*, 13(2), 101–105. <https://doi.org/10.1038/sj.gt.3302635>
31. Baban, C. K., Cronin, M., O'Hanlon, D., O'Sullivan, G. C., & Tangney, M. (2010). Bacteria as vectors for gene therapy of cancer. *Bioengineered Bugs*, 1(6), 385–394. <https://doi.org/10.4161/bbug.1.6.13146>
32. ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. (2020). Pan-cancer analysis of whole genomes. *Nature*, 578(7793), 82–93. <https://doi.org/10.1038/s41586-020-1969-6>
33. Li, Y., Roberts, N. D., Wala, J. A., Shapira, O., Schumacher, S. E., Kumar, K., Khurana, E., Waszak, S., Korb, J. O., Haber, J. E., Imielinski, M., PCAWG Structural Variation Working Group, Weischenfeldt, J., Beroukhim, R., Campbell, P. J., & PCAWG Consortium. (2020). Patterns of somatic structural variation in human cancer genomes. *Nature*, 578(7793), 112–121. <https://doi.org/10.1038/s41586-019-1913-9>
34. PCAWG Transcriptome Core Group, Calabrese, C., Davidson, N. R., Demircioğlu, D., Fonseca, N. A., He, Y., Kahles, A., Lehmann, K.-V., Liu, F., Shiraishi, Y., Soulette, C. M., Urban, L., Greger, L., Li, S., Liu, D., Perry, M. D., Xiang, Q., Zhang, F., Zhang, J., ... PCAWG Consortium. (2020). Genomic basis for RNA alterations in cancer. *Nature*, 578(7793), 129–136. <https://doi.org/10.1038/s41586-020-1970-0>
35. Rheinbay, E., Nielsen, M. M., Abascal, F., Wala, J. A., Shapira, O., Tiao, G., Hornshøj, H., Hess, J. M., Juul, R. I., Lin, Z., Feuerbach, L., Sabarinathan, R., Madsen, T., Kim, J., Mularoni, L., Shuai, S., Lanzós, A., Herrmann, C., Maruvka, Y. E., ... PCAWG Consortium. (2020). Analyses of non-coding somatic drivers in 2,658 cancer whole genomes. *Nature*, 578(7793), 102–111. <https://doi.org/10.1038/s41586-020-1965-x>
36. Cortés-Ciriano, I., Lee, J. J.-K., Xi, R., Jain, D., Jung, Y. L., Yang, L., Gordenin, D.,

- Klimczak, L. J., Zhang, C.-Z., Pellman, D. S., PCAWG Structural Variation Working Group, Park, P. J., & PCAWG Consortium. (2020). Comprehensive analysis of chromothripsis in 2,658 human cancers using whole-genome sequencing. *Nature Genetics*, 52(3), 331–341. <https://doi.org/10.1038/s41588-019-0576-7>
37. McGranahan, N., & Swanton, C. (2017). Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. *Cell*, 168(4), 613–628. <https://doi.org/10.1016/j.cell.2017.01.018>
38. Black, J. R. M., & McGranahan, N. (2021). Genetic and non-genetic clonal diversity in cancer evolution. *Nature Reviews. Cancer*, 21(6), 379–392. <https://doi.org/10.1038/s41568-021-00336-2>
39. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell*, 144(5), 646–674. <https://doi.org/10.1016/j.cell.2011.02.013>
40. Turner, K. M., Deshpande, V., Beyter, D., Koga, T., Rusert, J., Lee, C., Li, B., Arden, K., Ren, B., Nathanson, D. A., Kornblum, H. I., Taylor, M. D., Kaushal, S., Cavenee, W. K., Wechsler-Reya, R., Furnari, F. B., Vandenberg, S. R., Rao, P. N., Wahl, G. M., ... Mischel, P. S. (2017). Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. *Nature*, 543(7643), 122–125. <https://doi.org/10.1038/nature21356>
41. Wu, S., Turner, K. M., Nguyen, N., Raviram, R., Erb, M., Santini, J., Luebeck, J., Rajkumar, U., Diao, Y., Li, B., Zhang, W., Jameson, N., Corces, M. R., Granja, J. M., Chen, X., Coruh, C., Abnoui, A., Houston, J., Ye, Z., ... Mischel, P. S. (2019). Circular ecDNA promotes accessible chromatin and high oncogene expression. *Nature*, 575(7784), 699–703. <https://doi.org/10.1038/s41586-019-1763-5>
42. Kim, H., Nguyen, N.-P., Turner, K., Wu, S., Gujar, A. D., Luebeck, J., Liu, J., Deshpande, V., Rajkumar, U., Namburi, S., & Others. (2020). Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers. *Nature Genetics*, 52(9), 891–897. <https://www.nature.com/articles/s41588-020-0678-2>
43. Verhaak, R. G. W., Bafna, V., & Mischel, P. S. (2019). Extrachromosomal oncogene

- amplification in tumour pathogenesis and evolution. *Nature Reviews. Cancer*, 19(5), 283–288. <https://doi.org/10.1038/s41568-019-0128-6>
44. Münch, K., Münch, R., Biedendieck, R., Jahn, D., & Müller, J. (2019). Evolutionary model for the unequal segregation of high copy plasmids. *PLoS Computational Biology*, 15(3), e1006724. <https://doi.org/10.1371/journal.pcbi.1006724>
45. Deshpande, V., Luebeck, J., Nguyen, N.-P. D., Bakhtiari, M., Turner, K. M., Schwab, R., Carter, H., Mischel, P. S., & Bafna, V. (2019). Exploring the landscape of focal amplifications in cancer using AmpliconArchitect. *Nature Communications*, 10(1), 392. <https://doi.org/10.1038/s41467-018-08200-y>
46. Pang, J., Nguyen, N.-P., Luebeck, J., Ball, L., Finegersh, A., Ren, S., Nakagawa, T., Flagg, M., Sadat, S., Mischel, P. S., Xu, G., Fisch, K., Guo, T., Cahill, G., Panuganti, B., Bafna, V., & Califano, J. (2021). Extrachromosomal DNA in HPV mediated oropharyngeal cancer drives diverse oncogene transcription. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*. <https://doi.org/10.1158/1078-0432.CCR-21-2484>
47. Geller, L. T., Barzily-Rokni, M., Danino, T., Jonas, O. H., Shental, N., Nejman, D., Gavert, N., Zwang, Y., Cooper, Z. A., Shee, K., Thaiss, C. A., Reuben, A., Livny, J., Avraham, R., Frederick, D. T., Ligorio, M., Chatman, K., Johnston, S. E., Mosher, C. M., ... Straussman, R. (2017). Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. *Science*, 357(6356), 1156–1160. <https://doi.org/10.1126/science.aah5043>
48. Pushalkar, S., Hundeyin, M., Daley, D., Zambirinis, C. P., Kurz, E., Mishra, A., Mohan, N., Aykut, B., Usyk, M., Torres, L. E., Werba, G., Zhang, K., Guo, Y., Li, Q., Akkad, N., Lall, S., Wadowski, B., Gutierrez, J., Kochen Rossi, J. A., ... Miller, G. (2018). The Pancreatic Cancer Microbiome Promotes Oncogenesis by Induction of Innate and Adaptive Immune Suppression. *Cancer Discovery*, 8(4), 403–416. <https://doi.org/10.1158/2159-8290.CD-17->

1134

49. Kalaora, S., Nagler, A., Nejman, D., Alon, M., Barbolin, C., Barnea, E., Ketelaars, S. L. C., Cheng, K., Vervier, K., Shental, N., Bussi, Y., Rotkopf, R., Levy, R., Benedek, G., Trabish, S., Dadosh, T., Levin-Zaidman, S., Geller, L. T., Wang, K., ... Samuels, Y. (2021). Identification of bacteria-derived HLA-bound peptides in melanoma. *Nature*, 592(7852), 138–143. <https://doi.org/10.1038/s41586-021-03368-8>
50. Jin, C., Lagoudas, G. K., Zhao, C., Bullman, S., Bhutkar, A., Hu, B., Ameh, S., Sandel, D., Liang, X. S., Mazzilli, S., Whary, M. T., Meyerson, M., Germain, R., Blainey, P. C., Fox, J. G., & Jacks, T. (2019). Commensal Microbiota Promote Lung Cancer Development via $\gamma\delta$ T Cells. *Cell*, 176(5), 998–1013.e16. <https://doi.org/10.1016/j.cell.2018.12.040>
51. Tsay, J.-C. J., Wu, B. G., Sulaiman, I., Gershner, K., Schluger, R., Li, Y., Yie, T.-A., Meyn, P., Olsen, E., Perez, L., Franca, B., Carpenito, J., Iizumi, T., El-Ashmawy, M., Badri, M., Morton, J. T., Shen, N., He, L., Michaud, G., ... Segal, L. N. (2021). Lower Airway Dysbiosis Affects Lung Cancer Progression. *Cancer Discovery*, 11(2), 293–307. <https://doi.org/10.1158/2159-8290.CD-20-0263>
52. Riquelme, E., Zhang, Y., Zhang, L., Montiel, M., Zoltan, M., Dong, W., Quesada, P., Sahin, I., Chandra, V., San Lucas, A., Scheet, P., Xu, H., Hanash, S. M., Feng, L., Burks, J. K., Do, K.-A., Peterson, C. B., Nejman, D., Tzeng, C.-W. D., ... McAllister, F. (2019). Tumor Microbiome Diversity and Composition Influence Pancreatic Cancer Outcomes. *Cell*, 178(4), 795–806.e12. <https://doi.org/10.1016/j.cell.2019.07.008>
53. Parhi, L., Alon-Maimon, T., Sol, A., Nejman, D., Shhadeh, A., Fainsod-Levi, T., Yajuk, O., Isaacson, B., Abed, J., Maalouf, N., Nissan, A., Sandbank, J., Yehuda-Shnaidman, E., Ponath, F., Vogel, J., Mandelboim, O., Granot, Z., Straussman, R., & Bachrach, G. (2020). Breast cancer colonization by *Fusobacterium nucleatum* accelerates tumor growth and metastatic progression. *Nature Communications*, 11(1), 3259. <https://doi.org/10.1038/s41467-020-16967-2>

54. Le Noci, V., Guglielmetti, S., Arioli, S., Camisaschi, C., Bianchi, F., Sommariva, M., Storti, C., Triulzi, T., Castelli, C., Balsari, A., Tagliabue, E., & Sfondrini, L. (2018). Modulation of Pulmonary Microbiota by Antibiotic or Probiotic Aerosol Therapy: A Strategy to Promote Immunosurveillance against Lung Metastases. *Cell Reports*, 24(13), 3528–3538. <https://doi.org/10.1016/j.celrep.2018.08.090>
55. Shi, Y., Zheng, W., Yang, K., Harris, K. G., Ni, K., Xue, L., Lin, W., Chang, E. B., Weichselbaum, R. R., & Fu, Y.-X. (2020). Intratumoral accumulation of gut microbiota facilitates CD47-based immunotherapy via STING signaling. *The Journal of Experimental Medicine*, 217(5). <https://doi.org/10.1084/jem.20192282>
56. Mima, K., Sukawa, Y., Nishihara, R., Qian, Z. R., Yamauchi, M., Inamura, K., Kim, S. A., Masuda, A., Nowak, J. A., Nosho, K., Kostic, A. D., Giannakis, M., Watanabe, H., Bullman, S., Milner, D. A., Harris, C. C., Giovannucci, E., Garraway, L. A., Freeman, G. J., ... Ogino, S. (2015). *Fusobacterium nucleatum* and T Cells in Colorectal Carcinoma. *JAMA Oncology*, 1(5), 653–661. <https://doi.org/10.1001/jamaoncol.2015.1377>
57. Nejman, D., Livyatan, I., Fuks, G., Gavert, N., Zwang, Y., Geller, L. T., Rotter-Maskowitz, A., Weiser, R., Mallel, G., Gigi, E., Meltser, A., Douglas, G. M., Kamer, I., Gopalakrishnan, V., Dadosh, T., Levin-Zaidman, S., Avnet, S., Atlan, T., Cooper, Z. A., ... Straussman, R. (2020). The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science*, 368(6494), 973–980. <https://doi.org/10.1126/science.aay9189>
58. Poore, G. D., Kopylova, E., Zhu, Q., Carpenter, C., Fraraccio, S., Wandro, S., Kosciulek, T., Janssen, S., Metcalf, J., Song, S. J., Kanbar, J., Miller-Montgomery, S., Heaton, R., McKay, R., Patel, S. P., Swafford, A. D., & Knight, R. (2020). Microbiome analyses of blood and tissues suggest cancer diagnostic approach. *Nature*, 579(7800), 567–574. <https://doi.org/10.1038/s41586-020-2095-1>
59. Wieland, A., Patel, M. R., Cardenas, M. A., Eberhardt, C. S., Hudson, W. H., Obeng, R. C., Griffith, C. C., Wang, X., Chen, Z. G., Kissick, H. T., Saba, N. F., & Ahmed, R. (2021).

- Defining HPV-specific B cell responses in patients with head and neck cancer. *Nature*, 597(7875), 274–278. <https://doi.org/10.1038/s41586-020-2931-3>
60. Zapatka, M., Borozan, I., Brewer, D. S., Iskar, M., Grundhoff, A., Alawi, M., Desai, N., Sültmann, H., Moch, H., PCAWG Pathogens, Cooper, C. S., Eils, R., Ferretti, V., Lichter, P., & PCAWG Consortium. (2020). The landscape of viral associations in human cancers. *Nature Genetics*, 52(3), 320–330. <https://doi.org/10.1038/s41588-019-0558-9>
61. Bullman, S., Pedamallu, C. S., Sicinska, E., Clancy, T. E., Zhang, X., Cai, D., Neuberg, D., Huang, K., Guevara, F., Nelson, T., Chipashvili, O., Hagan, T., Walker, M., Ramachandran, A., Diosdado, B., Serna, G., Mulet, N., Landolfi, S., Ramon Y Cajal, S., ... Meyerson, M. (2017). Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. *Science*, 358(6369), 1443–1448. <https://doi.org/10.1126/science.aal5240>
62. Bertocchi, A., Carloni, S., Ravenda, P. S., Bertalot, G., Spadoni, I., Lo Cascio, A., Gandini, S., Lizier, M., Braga, D., Asnicar, F., Segata, N., Klaver, C., Brescia, P., Rossi, E., Anselmo, A., Guglietta, S., Maroli, A., Spaggiari, P., Tarazona, N., ... Rescigno, M. (2021). Gut vascular barrier impairment leads to intestinal bacteria dissemination and colorectal cancer metastasis to liver. *Cancer Cell*, 39(5), 708–724.e11. <https://doi.org/10.1016/j.ccell.2021.03.004>
63. Casasanta, M. A., Yoo, C. C., Udayasuryan, B., Sanders, B. E., Umaña, A., Zhang, Y., Peng, H., Duncan, A. J., Wang, Y., Li, L., Verbridge, S. S., & Slade, D. J. (2020). *Fusobacterium nucleatum* host-cell binding and invasion induces IL-8 and CXCL1 secretion that drives colorectal cancer cell migration. In *Science Signaling* (Vol. 13, Issue 641, p. eaba9157). <https://doi.org/10.1126/scisignal.aba9157>
64. Shiao, S. L., Kershaw, K. M., Limon, J. J., You, S., Yoon, J., Ko, E. Y., Guarnerio, J., Potdar, A. A., McGovern, D. P. B., Bose, S., Dar, T. B., Noe, P., Lee, J., Kubota, Y., Maymi, V. I., Davis, M. J., Henson, R. M., Choi, R. Y., Yang, W., ... Underhill, D. M. (2021). Commensal bacteria and fungi differentially regulate tumor responses to radiation therapy. *Cancer Cell*,

- 39(9), 1202–1213.e6. <https://doi.org/10.1016/j.ccell.2021.07.002>
65. Pernigoni, N., Zagato, E., Calcinotto, A., Troiani, M., Mestre, R. P., Cali, B., Attanasio, G., Troisi, J., Minini, M., Mosole, S., Revandkar, A., Pasquini, E., Elia, A. R., Bossi, D., Rinaldi, A., Rescigno, P., Flohr, P., Hunt, J., Neeb, A., ... Alimonti, A. (2021). Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. *Science*, 374(6564), 216–224. <https://doi.org/10.1126/science.abf8403>
66. Viaud, S., Saccheri, F., Mignot, G., Yamazaki, T., Daillère, R., Hannani, D., Enot, D. P., Pfirschke, C., Engblom, C., Pittet, M. J., Schlitzer, A., Ginhoux, F., Apetoh, L., Chachaty, E., Woerther, P.-L., Eberl, G., Bérard, M., Ecobichon, C., Clermont, D., ... Zitvogel, L. (2013). The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science*, 342(6161), 971–976. <https://doi.org/10.1126/science.1240537>
67. Iida, N., Dzutsev, A., Stewart, C. A., Smith, L., Bouladoux, N., Weingarten, R. A., Molina, D. A., Salcedo, R., Back, T., Cramer, S., Dai, R.-M., Kiu, H., Cardone, M., Naik, S., Patri, A. K., Wang, E., Marincola, F. M., Frank, K. M., Belkaid, Y., ... Goldszmid, R. S. (2013). Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science*, 342(6161), 967–970. <https://doi.org/10.1126/science.1240527>
68. Di Modica, M., Gargari, G., Regondi, V., Bonizzi, A., Arioli, S., Belmonte, B., De Cecco, L., Fasano, E., Bianchi, F., Bertolotti, A., Tripodo, C., Villani, L., Corsi, F., Guglielmetti, S., Balsari, A., Triulzi, T., & Tagliabue, E. (2021). Gut Microbiota Condition the Therapeutic Efficacy of Trastuzumab in HER2-Positive Breast Cancer. *Cancer Research*, 81(8), 2195–2206. <https://doi.org/10.1158/0008-5472.CAN-20-1659>
69. Kwong, T. N. Y., Wang, X., Nakatsu, G., Chow, T. C., Tipoe, T., Dai, R. Z. W., Tsoi, K. K. K., Wong, M. C. S., Tse, G., Chan, M. T. V., Chan, F. K. L., Ng, S. C., Wu, J. C. Y., Wu, W. K. K., Yu, J., Sung, J. J. Y., & Wong, S. H. (2018). Association Between Bacteremia From Specific Microbes and Subsequent Diagnosis of Colorectal Cancer. *Gastroenterology*, 155(2), 383–390.e8. <https://doi.org/10.1053/j.gastro.2018.04.028>

70. Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, 14(8), e1002533.
<https://doi.org/10.1371/journal.pbio.1002533>
71. Belkaid, Y., & Naik, S. (2013). Compartmentalized and systemic control of tissue immunity by commensals. *Nature Immunology*, 14(7), 646–653. <https://doi.org/10.1038/ni.2604>
72. Ramírez-Flandes, S., González, B., & Ulloa, O. (2019). Redox traits characterize the organization of global microbial communities. *Proceedings of the National Academy of Sciences of the United States of America*, 116(9), 3630–3635.
<https://doi.org/10.1073/pnas.1817554116>
73. Almeida, A., Nayfach, S., Boland, M., Strozzi, F., Beracochea, M., Shi, Z. J., Pollard, K. S., Sakharova, E., Parks, D. H., Hugenholtz, P., Segata, N., Kyrpides, N. C., & Finn, R. D. (2021). A unified catalog of 204,938 reference genomes from the human gut microbiome. *Nature Biotechnology*, 39(1), 105–114. <https://doi.org/10.1038/s41587-020-0603-3>
74. Dion, M. B., Oechslin, F., & Moineau, S. (2020). Phage diversity, genomics and phylogeny. *Nature Reviews. Microbiology*, 18(3), 125–138. <https://doi.org/10.1038/s41579-019-0311-5>
75. Sender, R., Fuchs, S., & Milo, R. (2016). Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell*, 164(3), 337–340.
<https://doi.org/10.1016/j.cell.2016.01.013>
76. Erdag, G., Schaefer, J. T., Smolkin, M. E., Deacon, D. H., Shea, S. M., Dengel, L. T., Patterson, J. W., & Slingluff, C. L., Jr. (2012). Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Research*, 72(5), 1070–1080. <https://doi.org/10.1158/0008-5472.CAN-11-3218>
77. Stephenson, K. (2005). *Introduction to Circle Packing: The Theory of Discrete Analytic Functions*. Cambridge University Press.
<https://play.google.com/store/books/details?id=38PxEmKKhysC>

- 919 78. Downey, G. P., Doherty, D. E., Schwab, B., 3rd, Elson, E. L., Henson, P. M., & Worthen, G.
 920 S. (1990). Retention of leukocytes in capillaries: role of cell size and deformability. *Journal*
 921 *of Applied Physiology*, 69(5), 1767–1778. <https://doi.org/10.1152/jappl.1990.69.5.1767>
- 922 79. Shashni, B., Ariyasu, S., Takeda, R., Suzuki, T., Shiina, S., Akimoto, K., Maeda, T., Aikawa,
 923 N., Abe, R., Osaki, T., Itoh, N., & Aoki, S. (2018). Size-Based Differentiation of Cancer and
 924 Normal Cells by a Particle Size Analyzer Assisted by a Cell-Recognition PC Software.
 925 *Biological & Pharmaceutical Bulletin*, 41(4), 487–503. [https://doi.org/10.1248/bpb.b17-](https://doi.org/10.1248/bpb.b17-00776)
 926 00776
- 927 80. Kather, J. N., Suarez-Carmona, M., Charoentong, P., Weis, C.-A., Hirsch, D., Bankhead, P.,
 928 Horning, M., Ferber, D., Kel, I., Herpel, E., Schott, S., Zörnig, I., Utikal, J., Marx, A., Gaiser,
 929 T., Brenner, H., Chang-Claude, J., Hoffmeister, M., Jäger, D., & Halama, N. (2018).
 930 Topography of cancer-associated immune cells in human solid tumors. *eLife*, 7.
 931 <https://doi.org/10.7554/eLife.36967>
- 932 81. Monte, U. D., & Del Monte, U. (2009). Does the cell number 10⁹ still really fit one gram of
 933 tumor tissue? In *Cell Cycle* (Vol. 8, Issue 3, pp. 505–506).
 934 <https://doi.org/10.4161/cc.8.3.7608>
- 935 82. Avanzini, S., Kurtz, D. M., Chabon, J. J., Moding, E. J., Hori, S. S., Gambhir, S. S., Alizadeh,
 936 A. A., Diehn, M., & Reiter, J. G. (2020). A mathematical model of ctDNA shedding predicts
 937 tumor detection size. *Science Advances*, 6(50). <https://doi.org/10.1126/sciadv.abc4308>
- 938 83. Abbosh, C., Birkbak, N. J., Wilson, G. A., Jamal-Hanjani, M., Constantin, T., Salari, R., Le
 939 Quesne, J., Moore, D. A., Veeriah, S., Rosenthal, R., Marafioti, T., Kirkizlar, E., Watkins, T.,
 940 B. K., McGranahan, N., Ward, S., Martinson, L., Riley, J., Fraioli, F., Al Bakir, M., ...
 941 Swanton, C. (2017). Phylogenetic ctDNA analysis depicts early-stage lung cancer
 942 evolution. *Nature*, 545(7655), 446–451. <https://doi.org/10.1038/nature22364>
- 943 84. Cristiano, S., Leal, A., Phallen, J., Fiksel, J., Adleff, V., Bruhm, D. C., Jensen, S. Ø., Medina,
 944 J. E., Hruban, C., White, J. R., Palsgrove, D. N., Niknafs, N., Anagnostou, V., Forde, P.,

- Naidoo, J., Marrone, K., Brahmer, J., Woodward, B. D., Husain, H., ... Velculescu, V. E. (2019). Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature*, 570(7761), 385–389. <https://doi.org/10.1038/s41586-019-1272-6>
85. Yachida, S., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., Watanabe, H., Masuda, K., Nishimoto, Y., Kubo, M., Hosoda, F., Rokutan, H., Matsumoto, M., Takamaru, H., Yamada, M., Matsuda, T., Iwasaki, M., Yamaji, T., Yachida, T., ... Yamada, T. (2019). Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nature Medicine*, 25(6), 968–976. <https://doi.org/10.1038/s41591-019-0458-7>
86. Dadkhah, E., Sikaroodi, M., Korman, L., Hardi, R., Baybick, J., Hanzel, D., Kuehn, G., Kuehn, T., & Gillevet, P. M. (2019). Gut microbiome identifies risk for colorectal polyps. *BMJ Open Gastroenterology*, 6(1), e000297. <https://doi.org/10.1136/bmjgast-2019-000297>
87. Peters, B. A., Dominianni, C., Shapiro, J. A., Church, T. R., Wu, J., Miller, G., Yuen, E., Freiman, H., Lustbader, I., Salik, J., Friedlander, C., Hayes, R. B., & Ahn, J. (2016). The gut microbiota in conventional and serrated precursors of colorectal cancer. *Microbiome*, 4(1), 69. <https://doi.org/10.1186/s40168-016-0218-6>
88. Kordahi, M. C., Stanaway, I. B., Avril, M., Chac, D., Blanc, M.-P., Ross, B., Diener, C., Jain, S., McCleary, P., Parker, A., Friedman, V., Huang, J., Burke, W., Gibbons, S. M., Willis, A. D., Darveau, R. P., Grady, W. M., Ko, C. W., & DePaolo, R. W. (2021). Genomic and functional characterization of a mucosal symbiont involved in early-stage colorectal cancer. *Cell Host & Microbe*, 29(10), 1589–1598.e6. <https://doi.org/10.1016/j.chom.2021.08.013>
89. Usyk, M., Zolnik, C. P., Castle, P. E., Porras, C., Herrero, R., Gradissimo, A., Gonzalez, P., Safaeian, M., Schiffman, M., Burk, R. D., & Costa Rica HPV Vaccine Trial (CVT) Group. (2020). Cervicovaginal microbiome and natural history of HPV in a longitudinal study. *PLoS Pathogens*, 16(3), e1008376. <https://doi.org/10.1371/journal.ppat.1008376>
90. Tango, C. N., Seo, S.-S., Kwon, M., Lee, D.-O., Chang, H. K., & Kim, M. K. (2020).

- Taxonomic and Functional Differences in Cervical Microbiome Associated with Cervical Cancer Development. *Scientific Reports*, 10(1), 9720. <https://doi.org/10.1038/s41598-020-66607-4>
91. Klein, C., Gonzalez, D., Samwel, K., Kahesa, C., Mwaiselage, J., Aluthge, N., Fernando, S., West, J. T., Wood, C., & Angeletti, P. C. (2019). Relationship between the Cervical Microbiome, HIV Status, and Precancerous Lesions. *mBio*, 10(1). <https://doi.org/10.1128/mBio.02785-18>
92. Delhommeau, F., Dupont, S., Della Valle, V., James, C., Trannoy, S., Massé, A., Kosmider, O., Le Couedic, J.-P., Robert, F., Alberdi, A., Lécluse, Y., Plo, I., Dreyfus, F. J., Marzac, C., Casadevall, N., Lacombe, C., Romana, S. P., Dessen, P., Soulier, J., ... Bernard, O. A. (2009). Mutation in TET2 in myeloid cancers. *The New England Journal of Medicine*, 360(22), 2289–2301. <https://doi.org/10.1056/NEJMoa0810069>
93. Dejea, C. M., Fathi, P., Craig, J. M., Boleij, A., Taddese, R., Geis, A. L., Wu, X., DeStefano Shields, C. E., Hechenbleikner, E. M., Huso, D. L., Anders, R. A., Giardiello, F. M., Wick, E. C., Wang, H., Wu, S., Pardoll, D. M., Housseau, F., & Sears, C. L. (2018). Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science*, 359(6375), 592–597. <https://doi.org/10.1126/science.aah3648>
94. Meisel, M., Hinterleitner, R., Pacis, A., Chen, L., Earley, Z. M., Mayassi, T., Pierre, J. F., Ernest, J. D., Galipeau, H. J., Thuille, N., Bouziat, R., Buscarlet, M., Ringus, D. L., Wang, Y., Li, Y., Dinh, V., Kim, S. M., McDonald, B. D., Zurenski, M. A., ... Jabri, B. (2018). Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature*, 557(7706), 580–584. <https://doi.org/10.1038/s41586-018-0125-z>
95. Nené, N. R., Reisel, D., Leimbach, A., Franchi, D., Jones, A., Evans, I., Knapp, S., Ryan, A., Ghazali, S., Timms, J. F., Paprotka, T., Bjørge, L., Zikan, M., Cibula, D., Colombo, N., & Widschwendter, M. (2019). Association between the cervicovaginal microbiome, BRCA1 mutation status, and risk of ovarian cancer: a case-control study. *The Lancet Oncology*,

- 20(8), 1171–1182. [https://doi.org/10.1016/S1470-2045\(19\)30340-7](https://doi.org/10.1016/S1470-2045(19)30340-7)
96. Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, 6(1), 226. <https://doi.org/10.1186/s40168-018-0605-2>
97. Klein, R. S., Recco, R. A., Catalano, M. T., Edberg, S. C., Casey, J. I., & Steigbigel, N. H. (1977). Association of *Streptococcus bovis* with carcinoma of the colon. *The New England Journal of Medicine*, 297(15), 800–802. <https://doi.org/10.1056/NEJM197710132971503>
98. Abed, J., Emgård, J. E. M., Zamir, G., Faroja, M., Almogy, G., Grenov, A., Sol, A., Naor, R., Pikarsky, E., Atlan, K. A., Mellul, A., Chaushu, S., Manson, A. L., Earl, A. M., Ou, N., Brennan, C. A., Garrett, W. S., & Bachrach, G. (2016). Fap2 Mediates *Fusobacterium nucleatum* Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. *Cell Host & Microbe*, 20(2), 215–225. <https://doi.org/10.1016/j.chom.2016.07.006>
99. Abed, J., Maalouf, N., Manson, A. L., Earl, A. M., Parhi, L., Emgård, J. E. M., Klutstein, M., Tayeb, S., Almogy, G., Atlan, K. A., Chaushu, S., Israeli, E., Mandelboim, O., Garrett, W. S., & Bachrach, G. (2020). Colon Cancer-Associated *Fusobacterium nucleatum* May Originate From the Oral Cavity and Reach Colon Tumors via the Circulatory System. *Frontiers in Cellular and Infection Microbiology*, 10, 400. <https://doi.org/10.3389/fcimb.2020.00400>
100. Sims, T. T., El Alam, M. B., Karpinets, T. V., Dorta-Estremera, S., Hegde, V. L., Nookala, S., Yoshida-Court, K., Wu, X., Biegert, G. W. G., Delgado Medrano, A. Y., Solley, T., Ahmed-Kaddar, M., Chapman, B. V., Sastry, K. J., Mezzari, M. P., Petrosino, J. F., Lin, L. L., Ramondetta, L., Jhingran, A., ... Klopp, A. H. (2021). Gut microbiome diversity is an independent predictor of survival in cervical cancer patients receiving chemoradiation. *Communications Biology*, 4(1), 237. <https://doi.org/10.1038/s42003-021-01741-x>
101. Peled, J. U., Gomes, A. L. C., Devlin, S. M., Littmann, E. R., Taur, Y., Sung, A. D., Weber, D., Hashimoto, D., Slingerland, A. E., Slingerland, J. B., Maloy, M., Clurman, A. G., Stein-

- 1023 Thoeringer, C. K., Markey, K. A., Docampo, M. D., Burgos da Silva, M., Khan, N., Gessner,
 1024 A., Messina, J. A., ... van den Brink, M. R. M. (2020). Microbiota as Predictor of Mortality in
 1025 Allogeneic Hematopoietic-Cell Transplantation. *The New England Journal of Medicine*,
 1026 382(9), 822–834. <https://doi.org/10.1056/NEJMoa1900623>
- 1027 102. Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz,
 1028 S., Routy, B., Roberti, M. P., Duong, C. P. M., Poirier-Colame, V., Roux, A., Becharef, S.,
 1029 Formenti, S., Golden, E., Cording, S., Eberl, G., Schlitzer, A., Ginhoux, F., ... Zitvogel, L.
 1030 (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.
 1031 *Science*, 350(6264), 1079–1084. <https://doi.org/10.1126/science.aad1329>
- 1032 103. Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M.,
 1033 Benyamin, F. W., Lei, Y. M., Jabri, B., Alegre, M.-L., Chang, E. B., & Gajewski, T. F. (2015).
 1034 Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti-PD-L1
 1035 efficacy. *Science*, 350(6264), 1084–1089. <https://doi.org/10.1126/science.aac4255>
- 1036 104. Limeta, A., Ji, B., Levin, M., Gatto, F., & Nielsen, J. (2020). Meta-analysis of the gut
 1037 microbiota in predicting response to cancer immunotherapy in metastatic melanoma. *JCI*
 1038 *Insight*, 5(23). <https://doi.org/10.1172/jci.insight.140940>
- 1039 105. Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C. A., Brown, K., Ramay, H., Paik, S.,
 1040 Stagg, J., Groves, R. A., Gallo, M., Lewis, I. A., Geuking, M. B., & McCoy, K. D. (2020).
 1041 Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy.
 1042 *Science*, 369(6510), 1481–1489. <https://doi.org/10.1126/science.abc3421>
- 1043 106. Griffin, M. E., Espinosa, J., Becker, J. L., Luo, J.-D., Carroll, T. S., Jha, J. K., Fanger, G.
 1044 R., & Hang, H. C. (2021). Enterococcus peptidoglycan remodeling promotes checkpoint
 1045 inhibitor cancer immunotherapy. *Science*, 373(6558), 1040–1046.
 1046 <https://doi.org/10.1126/science.abc9113>
- 1047 107. Daillère, R., Vétizou, M., Waldschmitt, N., Yamazaki, T., Isnard, C., Poirier-Colame, V.,
 1048 Duong, C. P. M., Flament, C., Lepage, P., Roberti, M. P., Routy, B., Jacquelot, N., Apetoh,

- 1049 L., Becharef, S., Rusakiewicz, S., Langella, P., Sokol, H., Kroemer, G., Enot, D., ...
 1050 Zitvogel, L. (2016). Enterococcus hirae and Barnesiella intestinihominis Facilitate
 1051 Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects. *Immunity*, 45(4), 931–
 1052 943. <https://doi.org/10.1016/j.immuni.2016.09.009>
- 1053 108. Lam, K. C., Araya, R. E., Huang, A., Chen, Q., Di Modica, M., Rodrigues, R. R., Lopès, A.,
 1054 Johnson, S. B., Schwarz, B., Bohrsen, E., Cogdill, A. P., Bosio, C. M., Wargo, J. A., Lee,
 1055 M. P., & Goldszmid, R. S. (2021). Microbiota triggers STING-type I IFN-dependent
 1056 monocyte reprogramming of the tumor microenvironment. *Cell*, 184(21), 5338–5356.e21.
 1057 <https://doi.org/10.1016/j.cell.2021.09.019>
- 1058 109. Alexander, J. L., Wilson, I. D., Teare, J., Marchesi, J. R., Nicholson, J. K., & Kinross, J. M.
 1059 (2017). Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nature Reviews.*
 1060 *Gastroenterology & Hepatology*, 14(6), 356–365. <https://doi.org/10.1038/nrgastro.2017.20>
- 1061 110. Daisley, B. A., Chanyi, R. M., Abdur-Rashid, K., Al, K. F., Gibbons, S., Chmiel, J. A.,
 1062 Wilcox, H., Reid, G., Anderson, A., Dewar, M., Nair, S. M., Chin, J., & Burton, J. P. (2020).
 1063 Abiraterone acetate preferentially enriches for the gut commensal Akkermansia muciniphila
 1064 in castrate-resistant prostate cancer patients. *Nature Communications*, 11(1), 4822.
 1065 <https://doi.org/10.1038/s41467-020-18649-5>
- 1066 111. Kwa, M., Plottel, C. S., Blaser, M. J., & Adams, S. (2016). The Intestinal Microbiome and
 1067 Estrogen Receptor-Positive Female Breast Cancer. *Journal of the National Cancer*
 1068 *Institute*, 108(8). <https://doi.org/10.1093/jnci/djw029>
- 1069 112. Komorowski, A. S., & Pezo, R. C. (2020). Untapped “-omics”: the microbial metagenome,
 1070 estrobolome, and their influence on the development of breast cancer and response to
 1071 treatment. *Breast Cancer Research and Treatment*, 179(2), 287–300.
 1072 <https://doi.org/10.1007/s10549-019-05472-w>
- 1073 113. Eisenhofer, R., Minich, J. J., Marotz, C., Cooper, A., Knight, R., & Weyrich, L. S. (2019).
 1074 Contamination in Low Microbial Biomass Microbiome Studies: Issues and

- 1075 Recommendations. *Trends in Microbiology*, 27(2), 105–117.
 1076 <https://doi.org/10.1016/j.tim.2018.11.003>
- 1077 114. Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner,
 1078 P., Parkhill, J., Loman, N. J., & Walker, A. W. (2014). Reagent and laboratory
 1079 contamination can critically impact sequence-based microbiome analyses. *BMC Biology*,
 1080 12(1), 87. <https://doi.org/10.1186/s12915-014-0087-z>
- 1081 115. Sinha, R., Abu-Ali, G., Vogtmann, E., Fodor, A. A., Ren, B., Amir, A., Schwager, E.,
 1082 Crabtree, J., Ma, S., Microbiome Quality Control Project Consortium, Abnet, C. C., Knight,
 1083 R., White, O., & Huttenhower, C. (2017). Assessment of variation in microbial community
 1084 amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium.
 1085 *Nature Biotechnology*, 35(11), 1077–1086. <https://doi.org/10.1038/nbt.3981>
- 1086 116. Morton, J. T., Marotz, C., Washburne, A., Silverman, J., Zaramela, L. S., Edlund, A.,
 1087 Zengler, K., & Knight, R. (2019). Establishing microbial composition measurement
 1088 standards with reference frames. *Nature Communications*, 10(1), 2719.
 1089 <https://doi.org/10.1038/s41467-019-10656-5>
- 1090 117. Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias
 1091 correction. *Nature Communications*, 11(1), 3514. [https://doi.org/10.1038/s41467-020-](https://doi.org/10.1038/s41467-020-17041-7)
 1092 17041-7
- 1093 118. Lin, H., & Peddada, S. D. (2020). Analysis of microbial compositions: a review of
 1094 normalization and differential abundance analysis. *NPJ Biofilms and Microbiomes*, 6(1), 60.
 1095 <https://doi.org/10.1038/s41522-020-00160-w>
- 1096 119. Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras,
 1097 M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B.,
 1098 Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C.,
 1099 Knights, D., ... Gordon, J. I. (2012). Human gut microbiome viewed across age and
 1100 geography. *Nature*, 486(7402), 222–227. <https://doi.org/10.1038/nature11053>

- 1101 120. He, Y., Wu, W., Zheng, H.-M., Li, P., McDonald, D., Sheng, H.-F., Chen, M.-X., Chen, Z.-
 1102 H., Ji, G.-Y., Zheng, Z.-D.-X., Mujagond, P., Chen, X.-J., Rong, Z.-H., Chen, P., Lyu, L.-Y.,
 1103 Wang, X., Wu, C.-B., Yu, N., Xu, Y.-J., ... Zhou, H.-W. (2018). Regional variation limits
 1104 applications of healthy gut microbiome reference ranges and disease models. *Nature*
 1105 *Medicine*, 24(10), 1532–1535. <https://doi.org/10.1038/s41591-018-0164-x>
- 1106 121. Deschasaux, M., Bouter, K. E., Prodan, A., Levin, E., Groen, A. K., Herrema, H., Tremaroli,
 1107 V., Bakker, G. J., Attaye, I., Pinto-Sietsma, S.-J., van Raalte, D. H., Snijder, M. B.,
 1108 Nicolaou, M., Peters, R., Zwinderman, A. H., Bäckhed, F., & Nieuwdorp, M. (2018).
 1109 Depicting the composition of gut microbiota in a population with varied ethnic origins but
 1110 shared geography. *Nature Medicine*, 24(10), 1526–1531. [https://doi.org/10.1038/s41591-](https://doi.org/10.1038/s41591-018-0160-1)
 1111 018-0160-1
- 1112 122. Wirbel, J., Pyl, P. T., Kartal, E., Zych, K., Kashani, A., Milanese, A., Fleck, J. S., Voigt, A.
 1113 Y., Palleja, A., Ponnudurai, R., Sunagawa, S., Coelho, L. P., Schrotz-King, P., Vogtmann,
 1114 E., Habermann, N., Niméus, E., Thomas, A. M., Manghi, P., Gandini, S., ... Zeller, G.
 1115 (2019). Meta-analysis of fecal metagenomes reveals global microbial signatures that are
 1116 specific for colorectal cancer. *Nature Medicine*, 25(4), 679–689.
 1117 <https://doi.org/10.1038/s41591-019-0406-6>
- 1118 123. Thomas, A. M., Manghi, P., Asnicar, F., Pasolli, E., Armanini, F., Zolfo, M., Beghini, F.,
 1119 Manara, S., Karcher, N., Pozzi, C., Gandini, S., Serrano, D., Tarallo, S., Francavilla, A.,
 1120 Gallo, G., Trompetto, M., Ferrero, G., Mizutani, S., Shiroma, H., ... Segata, N. (2019).
 1121 Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial
 1122 diagnostic signatures and a link with choline degradation. *Nature Medicine*, 25(4), 667–678.
 1123 <https://doi.org/10.1038/s41591-019-0405-7>
- 1124 124. Nowell, P. C. (1976). The clonal evolution of tumor cell populations. *Science*, 194(4260),
 1125 23–28. <https://doi.org/10.1126/science.959840>
- 1126 125. Greaves, M., & Maley, C. C. (2012). Clonal evolution in cancer. *Nature*.

- 1127 <https://www.nature.com/articles/nature10762>
- 1128 126. Mazor, T., Pankov, A., Johnson, B. E., Hong, C., Hamilton, E. G., Bell, R. J. A., Smirnov, I.
 1129 V., Reis, G. F., Phillips, J. J., Barnes, M. J., Idbaih, A., Alentorn, A., Kloezezan, J. J.,
 1130 Lamfers, M. L. M., Bollen, A. W., Taylor, B. S., Molinaro, A. M., Olshen, A. B., Chang, S.
 1131 M., ... Costello, J. F. (2015). DNA Methylation and Somatic Mutations Converge on the Cell
 1132 Cycle and Define Similar Evolutionary Histories in Brain Tumors. *Cancer Cell*, 28(3), 307–
 1133 317. <https://doi.org/10.1016/j.ccell.2015.07.012>
- 1134 127. Li, S., Garrett-Bakelman, F. E., Chung, S. S., Sanders, M. A., Hricik, T., Rapaport, F.,
 1135 Patel, J., Dillon, R., Vijay, P., Brown, A. L., Perl, A. E., Cannon, J., Bullinger, L., Luger, S.,
 1136 Becker, M., Lewis, I. D., To, L. B., Delwel, R., Löwenberg, B., ... Mason, C. E. (2016).
 1137 Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid
 1138 leukemia. *Nature Medicine*, 22(7), 792–799. <https://doi.org/10.1038/nm.4125>
- 1139 128. Vaz, M., Hwang, S. Y., Kagiampakis, I., Phallen, J., Patil, A., O'Hagan, H. M., Murphy, L.,
 1140 Zahnow, C. A., Gabrielson, E., Velculescu, V. E., Easwaran, H. P., & Baylin, S. B. (2017).
 1141 Chronic Cigarette Smoke-Induced Epigenomic Changes Precede Sensitization of Bronchial
 1142 Epithelial Cells to Single-Step Transformation by KRAS Mutations. *Cancer Cell*, 32(3), 360–
 1143 376.e6. <https://doi.org/10.1016/j.ccell.2017.08.006>
- 1144 129. Nam, A. S., Chaligne, R., & Landau, D. A. (2021). Integrating genetic and non-genetic
 1145 determinants of cancer evolution by single-cell multi-omics. *Nature Reviews. Genetics*,
 1146 22(1), 3–18. <https://doi.org/10.1038/s41576-020-0265-5>
- 1147 130. Gutierrez, C., Al'Khafaji, A. M., Brenner, E., Johnson, K. E., Gohil, S. H., Lin, Z.,
 1148 Knisbacher, B. A., Durrett, R. E., Li, S., Parvin, S., Biran, A., Zhang, W., Rassenti, L.,
 1149 Kipps, T. J., Livak, K. J., Neuberg, D., Letai, A., Getz, G., Wu, C. J., & Brock, A. (2021).
 1150 Multifunctional barcoding with ClonMapper enables high-resolution study of clonal
 1151 dynamics during tumor evolution and treatment. *Nature Cancer*, 2(7), 758–772.
 1152 <https://doi.org/10.1038/s43018-021-00222-8>

- 1153 131. Pleguezuelos-Manzano, C., Puschhof, J., Rosendahl Huber, A., van Hoeck, A., Wood, H.
 1154 M., Nomburg, J., Gurjao, C., Manders, F., Dalmaso, G., Stege, P. B., Paganelli, F. L.,
 1155 Geurts, M. H., Beumer, J., Mizutani, T., Miao, Y., van der Linden, R., van der Elst, S.,
 1156 Genomics England Research Consortium, Garcia, K. C., ... Clevers, H. (2020). Mutational
 1157 signature in colorectal cancer caused by genotoxic pks *E. coli*. *Nature*, 580(7802), 269–
 1158 273. <https://doi.org/10.1038/s41586-020-2080-8>
- 1159 132. Yu, T., Guo, F., Yu, Y., Sun, T., Ma, D., Han, J., Qian, Y., Kryczek, I., Sun, D., Nagarsheth,
 1160 N., Chen, Y., Chen, H., Hong, J., Zou, W., & Fang, J.-Y. (2017). *Fusobacterium nucleatum*
 1161 Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell*, 170(3),
 1162 548–563.e16. <https://doi.org/10.1016/j.cell.2017.07.008>
- 1163 133. Garrett, W. S. (2015). Cancer and the microbiota. *Science*, 348(6230), 80–86.
 1164 <https://doi.org/10.1126/science.aaa4972>
- 1165 134. Ma, C., Han, M., Heinrich, B., Fu, Q., Zhang, Q., Sandhu, M., Agdashian, D., Terabe, M.,
 1166 Berzofsky, J. A., Fako, V., Ritz, T., Longerich, T., Theriot, C. M., McCulloch, J. A., Roy, S.,
 1167 Yuan, W., Thovarai, V., Sen, S. K., Ruchirawat, M., ... Greten, T. F. (2018). Gut
 1168 microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science*,
 1169 360(6391). <https://doi.org/10.1126/science.aan5931>
- 1170 135. Goss, G., Tsai, C.-M., Shepherd, F. A., Bazhenova, L., Lee, J. S., Chang, G.-C., Crino, L.,
 1171 Satouchi, M., Chu, Q., Hida, T., Han, J.-Y., Juan, O., Dunphy, F., Nishio, M., Kang, J.-H.,
 1172 Majem, M., Mann, H., Cantarini, M., Ghorghiu, S., & Mitsudomi, T. (2016). Osimertinib for
 1173 pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a
 1174 multicentre, open-label, single-arm, phase 2 study. *The Lancet Oncology*, 17(12), 1643–
 1175 1652. [https://doi.org/10.1016/S1470-2045\(16\)30508-3](https://doi.org/10.1016/S1470-2045(16)30508-3)
- 1176 136. Thress, K. S., Paweletz, C. P., Felip, E., Cho, B. C., Stetson, D., Dougherty, B., Lai, Z.,
 1177 Markovets, A., Vivancos, A., Kuang, Y., Ercan, D., Matthews, S. E., Cantarini, M., Barrett,
 1178 J. C., Jänne, P. A., & Oxnard, G. R. (2015). Acquired EGFR C797S mutation mediates

- 1179 resistance to AZD9291 in non–small cell lung cancer harboring EGFR T790M. *Nature*
 1180 *Medicine*, 21(6), 560–562. <https://doi.org/10.1038/nm.3854>
- 1181 137. DiNardo, C. D., Stein, E. M., de Botton, S., Roboz, G. J., Altman, J. K., Mims, A. S.,
 1182 Swords, R., Collins, R. H., Mannis, G. N., Pollyea, D. A., Donnellan, W., Fathi, A. T.,
 1183 Pigneux, A., Erba, H. P., Prince, G. T., Stein, A. S., Uy, G. L., Foran, J. M., Traer, E., ...
 1184 Kantarjian, H. M. (2018). Durable Remissions with Ivosidenib in IDH1-Mutated Relapsed or
 1185 Refractory AML. *The New England Journal of Medicine*, 378(25), 2386–2398.
 1186 <https://doi.org/10.1056/NEJMoa1716984>
- 1187 138. Quek, L., David, M. D., Kennedy, A., Metzner, M., Amatangelo, M., Shih, A., Stoilova, B.,
 1188 Quivoron, C., Heiblig, M., Willekens, C., Saada, V., Alsafadi, S., Vijayabaskar, M. S.,
 1189 Peniket, A., Bernard, O. A., Agresta, S., Yen, K., MacBeth, K., Stein, E., ... Vyas, P. (2018).
 1190 Clonal heterogeneity of acute myeloid leukemia treated with the IDH2 inhibitor enasidenib.
 1191 *Nature Medicine*, 24(8), 1167–1177. <https://doi.org/10.1038/s41591-018-0115-6>
- 1192 139. Roboz, G. J., DiNardo, C. D., Stein, E. M., de Botton, S., Mims, A. S., Prince, G. T.,
 1193 Altman, J. K., Arellano, M. L., Donnellan, W., Erba, H. P., Mannis, G. N., Pollyea, D. A.,
 1194 Stein, A. S., Uy, G. L., Watts, J. M., Fathi, A. T., Kantarjian, H. M., Tallman, M. S., Choe, S.,
 1195 ... Stone, R. M. (2020). Ivosidenib induces deep durable remissions in patients with newly
 1196 diagnosed IDH1-mutant acute myeloid leukemia. *Blood*, 135(7), 463–471.
 1197 <https://doi.org/10.1182/blood.2019002140>
- 1198 140. Merlevede, J., Droin, N., Qin, T., Meldi, K., Yoshida, K., Morabito, M., Chautard, E.,
 1199 Auboeuf, D., Fenaux, P., Braun, T., Itzykson, R., de Botton, S., Quesnel, B., Commes, T.,
 1200 Jourdan, E., Vainchenker, W., Bernard, O., Pata-Merci, N., Solier, S., ... Solary, E. (2016).
 1201 Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia
 1202 responding to hypomethylating agents. *Nature Communications*, 7, 10767.
 1203 <https://doi.org/10.1038/ncomms10767>
- 1204 141. Turati, V. A., Guerra-Assunção, J. A., Potter, N. E., Gupta, R., Ecker, S., Daneviciute, A.,

- 1205 Tarabichi, M., Webster, A. P., Ding, C., May, G., James, C., Brown, J., Conde, L., Russell,
1206 L. J., Ancliff, P., Inglott, S., Cazzaniga, G., Biondi, A., Hall, G. W., ... Enver, T. (2021).
1207 Chemotherapy induces canalization of cell state in childhood B-cell precursor acute
1208 lymphoblastic leukemia. *Nature Cancer*, 2(8), 835–852. [https://doi.org/10.1038/s43018-](https://doi.org/10.1038/s43018-021-00219-3)
1209 021-00219-3
- 1210 142. Dobson, S. M., García-Prat, L., Vanner, R. J., Wintersinger, J., Waanders, E., Gu, Z.,
1211 McLeod, J., Gan, O. I., Grandal, I., Payne-Turner, D., Edmonson, M. N., Ma, X., Fan, Y.,
1212 Voisin, V., Chan-Seng-Yue, M., Xie, S. Z., Hosseini, M., Abelson, S., Gupta, P., ... Dick, J.
1213 E. (2020). Relapse-Fated Latent Diagnosis Subclones in Acute B Lineage Leukemia Are
1214 Drug Tolerant and Possess Distinct Metabolic Programs. *Cancer Discovery*, 10(4), 568–
1215 587. <https://doi.org/10.1158/2159-8290.CD-19-1059>
- 1216 143. Marine, J.-C., Dawson, S.-J., & Dawson, M. A. (2020). Non-genetic mechanisms of
1217 therapeutic resistance in cancer. *Nature Reviews. Cancer*, 20(12), 743–756.
1218 <https://doi.org/10.1038/s41568-020-00302-4>
- 1219 144. Barrett, M., Hand, C. K., Shanahan, F., Murphy, T., & O'Toole, P. W. (2020). Mutagenesis
1220 by Microbe: the Role of the Microbiota in Shaping the Cancer Genome. *Trends in Cancer*
1221 *Research*, 6(4), 277–287. <https://doi.org/10.1016/j.trecan.2020.01.019>
- 1222 145. Gur, C., Ibrahim, Y., Isaacson, B., Yamin, R., Abed, J., Gamliel, M., Enk, J., Bar-On, Y.,
1223 Stanietsky-Kaynan, N., Copenhagen-Glazer, S., Shussman, N., Almog, G., Cuapio, A.,
1224 Hofer, E., Mevorach, D., Tabib, A., Ortenberg, R., Markel, G., Miklić, K., ... Mandelboim, O.
1225 (2015). Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory
1226 receptor TIGIT protects tumors from immune cell attack. *Immunity*, 42(2), 344–355.
1227 <https://doi.org/10.1016/j.immuni.2015.01.010>
- 1228 146. Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., & Schreiber, R. D. (2002). Cancer
1229 immunoediting: from immunosurveillance to tumor escape. *Nature Immunology*, 3(11),
1230 991–998. <https://doi.org/10.1038/ni1102-991>

- 1231 147. Jourdain, A., Duchmann, M., Willekens, C., Solary, É., Perié, L., & Laplane, L. (2021).
 1232 Évolution clonale: Qu'est-ce qu'un clone. *La Biologie Au Défi de L'histoire; Chapitre, 10*,
 1233 167–188.
 1234 [https://www.cairn.info/load_pdf.php?ID_ARTICLE=EDMAT_LOISC_2021_01_0167&downl](https://www.cairn.info/load_pdf.php?ID_ARTICLE=EDMAT_LOISC_2021_01_0167&download=1)
 1235 [oad=1](https://www.cairn.info/load_pdf.php?ID_ARTICLE=EDMAT_LOISC_2021_01_0167&download=1)
- 1236 148. Fluckiger, A., Daillère, R., Sassi, M., Sixt, B. S., Liu, P., Loos, F., Richard, C., Rabu, C.,
 1237 Alou, M. T., Goubet, A.-G., Lemaitre, F., Ferrere, G., Derosa, L., Duong, C. P. M.,
 1238 Messaoudene, M., Gagné, A., Joubert, P., De Sordi, L., Debarbieux, L., ... Zitvogel, L.
 1239 (2020). Cross-reactivity between tumor MHC class I–restricted antigens and an
 1240 enterococcal bacteriophage. *Science*.
 1241 <https://science.sciencemag.org/content/369/6506/936.abstract>
- 1242 149. Bessell, C. A., Isser, A., Havel, J. J., Lee, S., Bell, D. R., Hickey, J. W., Chaisawangwong,
 1243 W., Glick Bieler, J., Srivastava, R., Kuo, F., Purohit, T., Zhou, R., Chan, T. A., & Schneck,
 1244 J. P. (2020). Commensal bacteria stimulate antitumor responses via T cell cross-reactivity.
 1245 *JCI Insight*, 5(8). <https://doi.org/10.1172/jci.insight.135597>
- 1246 150. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science*,
 1247 284(5423), 2124–2129. <https://doi.org/10.1126/science.284.5423.2124>
- 1248 151. Doolittle, W. F., & Baptiste, E. (2007). Pattern pluralism and the Tree of Life hypothesis.
 1249 *Proceedings of the National Academy of Sciences of the United States of America*, 104(7),
 1250 2043–2049. <https://doi.org/10.1073/pnas.0610699104>
- 1251 152. O'Malley, M. A., & Koonin, E. V. (2011). How stands the Tree of Life a century and a half
 1252 after The Origin? *Biology Direct*, 6(1), 32. <https://doi.org/10.1186/1745-6150-6-32>
- 1253 153. Byndloss, M. X., Olsan, E. E., Rivera-Chávez, F., Tiffany, C. R., Cevallos, S. A., Lokken, K.
 1254 L., Torres, T. P., Byndloss, A. J., Faber, F., Gao, Y., Litvak, Y., Lopez, C. A., Xu, G., Napoli,
 1255 E., Giulivi, C., Tsois, R. M., Revzin, A., Lebrilla, C. B., & Bäumler, A. J. (2017). Microbiota-
 1256 activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science*,

- 1257 357(6351), 570–575. <https://doi.org/10.1126/science.aam9949>
- 1258 154. Butler, D. S. C., Cafaro, C., Putze, J., Wan, M. L. Y., Tran, T. H., Ambite, I., Ahmadi, S.,
 1259 Kjellström, S., Welinder, C., Chao, S. M., Dobrindt, U., & Svanborg, C. (2021). A bacterial
 1260 protease depletes c-MYC and increases survival in mouse models of bladder and colon
 1261 cancer. *Nature Biotechnology*, 39(6), 754–764. <https://doi.org/10.1038/s41587-020-00805-3>
- 1262 155. Kusters, J. G., van Vliet, A. H. M., & Kuipers, E. J. (2006). Pathogenesis of *Helicobacter*
 1263 *pylori* infection. *Clinical Microbiology Reviews*, 19(3), 449–490.
 1264 <https://doi.org/10.1128/CMR.00054-05>
- 1265 156. Suerbaum, S., & Josenhans, C. (2007). *Helicobacter pylori* evolution and phenotypic
 1266 diversification in a changing host. In *Nature Reviews Microbiology* (Vol. 5, Issue 6, pp. 441–
 1267 452). <https://doi.org/10.1038/nrmicro1658>
- 1268 157. Anandasabapathy, S., Jhamb, J., Davila, M., Wei, C., Morris, J., & Bresalier, R. (2007).
 1269 Clinical and endoscopic factors predict higher pathologic grades of Barrett dysplasia.
 1270 *Cancer*, 109(4), 668–674. <https://doi.org/10.1002/cncr.22451>
- 1271 158. Islami, F., & Kamangar, F. (2008). *Helicobacter pylori* and Esophageal Cancer Risk: A
 1272 Meta-analysis. In *Cancer Prevention Research* (Vol. 1, Issue 5, pp. 329–338).
 1273 <https://doi.org/10.1158/1940-6207.capr-08-0109>
- 1274 159. Petrova, V., Annicchiarico-Petruzzelli, M., Melino, G., & Amelio, I. (2018). The hypoxic
 1275 tumour microenvironment. *Oncogenesis*, 7(1), 10. [https://doi.org/10.1038/s41389-017-](https://doi.org/10.1038/s41389-017-0011-9)
 1276 0011-9
- 1277 160. Rundell, E. A., Banta, L. M., Ward, D. V., Watts, C. D., Birren, B., & Esteban, D. J. (2014).
 1278 16S rRNA gene survey of microbial communities in Winogradsky columns. *PloS One*, 9(8),
 1279 e104134. <https://doi.org/10.1371/journal.pone.0104134>
- 1280 161. Hoshino, T., Doi, H., Uramoto, G.-I., Wörmer, L., Adhikari, R. R., Xiao, N., Morono, Y.,
 1281 D'Hondt, S., Hinrichs, K.-U., & Inagaki, F. (2020). Global diversity of microbial communities
 1282 in marine sediment. *Proceedings of the National Academy of Sciences of the United States*

- 1283 *of America*, 117(44), 27587–27597. <https://doi.org/10.1073/pnas.1919139117>
- 1284 162. Fenchel, T., & Finlay, B. (2008). Oxygen and the spatial structure of microbial
1285 communities. *Biological Reviews of the Cambridge Philosophical Society*, 83(4), 553–569.
1286 <https://doi.org/10.1111/j.1469-185X.2008.00054.x>
- 1287 163. Semenza, G. L. (2008). Tumor metabolism: cancer cells give and take lactate [Review of
1288 *Tumor metabolism: cancer cells give and take lactate*]. *The Journal of Clinical Investigation*,
1289 118(12), 3835–3837. <https://doi.org/10.1172/JCI37373>
- 1290 164. Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A.,
1291 Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-
1292 Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Pölme, S.,
1293 Sunagawa, S., Ryberg, M., ... Bork, P. (2018). Structure and function of the global topsoil
1294 microbiome. *Nature*, 560(7717), 233–237. <https://doi.org/10.1038/s41586-018-0386-6>
- 1295 165. Anderson, A. R. A., Weaver, A. M., Cummings, P. T., & Quaranta, V. (2006). Tumor
1296 morphology and phenotypic evolution driven by selective pressure from the
1297 microenvironment. *Cell*, 127(5), 905–915. <https://doi.org/10.1016/j.cell.2006.09.042>
- 1298 166. Damaghi, M., West, J., Robertson-Tessi, M., Xu, L., Ferrall-Fairbanks, M. C., Stewart, P.
1299 A., Persi, E., Fridley, B. L., Altrock, P. M., Gatenby, R. A., Sims, P. A., Anderson, A. R. A.,
1300 & Gillies, R. J. (2021). The harsh microenvironment in early breast cancer selects for a
1301 Warburg phenotype. *Proceedings of the National Academy of Sciences of the United*
1302 *States of America*, 118(3). <https://doi.org/10.1073/pnas.2011342118>
- 1303 167. Frankenstein, Z., Basanta, D., Franco, O. E., Gao, Y., Javier, R. A., Strand, D. W., Lee, M.,
1304 Hayward, S. W., Ayala, G., & Anderson, A. R. A. (2020). Stromal reactivity differentially
1305 drives tumour cell evolution and prostate cancer progression. *Nature Ecology & Evolution*,
1306 4(6), 870–884. <https://doi.org/10.1038/s41559-020-1157-y>
- 1307 168. Salehi, S., Kabeer, F., Ceglia, N., Andronescu, M., Williams, M. J., Campbell, K. R.,
1308 Masud, T., Wang, B., Biele, J., Brimhall, J., Gee, D., Lee, H., Ting, J., Zhang, A. W., Tran,

- 1309 H., O'Flanagan, C., Dorri, F., Rusk, N., de Algara, T. R., ... Shah, S. P. (2021). Clonal
 1310 fitness inferred from time-series modelling of single-cell cancer genomes. *Nature*,
 1311 595(7868), 585–590. <https://doi.org/10.1038/s41586-021-03648-3>
- 1312 169. Temko, D., Cheng, Y.-K., Polyak, K., & Michor, F. (2017). Mathematical Modeling Links
 1313 Pregnancy-Associated Changes and Breast Cancer Risk. *Cancer Research*, 77(11), 2800–
 1314 2809. <https://doi.org/10.1158/0008-5472.CAN-16-2504>
- 1315 170. Williams, M. J., Werner, B., Heide, T., Curtis, C., Barnes, C. P., Sottoriva, A., & Graham, T.
 1316 A. (2018). Quantification of subclonal selection in cancer from bulk sequencing data. *Nature*
 1317 *Genetics*, 50(6), 895–903. <https://doi.org/10.1038/s41588-018-0128-6>
- 1318 171. Avanzini, S., & Antal, T. (2019). Cancer recurrence times from a branching process model.
 1319 *PLoS Computational Biology*, 15(11), e1007423.
 1320 <https://doi.org/10.1371/journal.pcbi.1007423>
- 1321 172. Curtius, K., Dewanji, A., Hazelton, W. D., Rubenstein, J. H., & Luebeck, G. E. (2021).
 1322 Optimal Timing for Cancer Screening and Adaptive Surveillance Using Mathematical
 1323 Modeling. *Cancer Research*, 81(4), 1123–1134. [https://doi.org/10.1158/0008-5472.CAN-](https://doi.org/10.1158/0008-5472.CAN-20-0335)
 1324 20-0335
- 1325 173. Schwartz, R., & Schäffer, A. A. (2017). The evolution of tumour phylogenetics: principles
 1326 and practice. *Nature Reviews. Genetics*, 18(4), 213–229.
 1327 <https://doi.org/10.1038/nrg.2016.170>
- 1328 174. Robertson-Tessi, M., Gillies, R. J., Gatenby, R. A., & Anderson, A. R. A. (2015). Impact of
 1329 metabolic heterogeneity on tumor growth, invasion, and treatment outcomes. *Cancer*
 1330 *Research*, 75(8), 1567–1579. <https://doi.org/10.1158/0008-5472.CAN-14-1428>
- 1331 175. Ghaffarizadeh, A., Heiland, R., Friedman, S. H., Mumenthaler, S. M., & Macklin, P. (2018).
 1332 PhysiCell: An open source physics-based cell simulator for 3-D multicellular systems. *PLoS*
 1333 *Computational Biology*, 14(2), e1005991. <https://doi.org/10.1371/journal.pcbi.1005991>
- 1334 176. Beerenwinkel, N., Antal, T., Dingli, D., Traulsen, A., Kinzler, K. W., Velculescu, V. E.,

- 1335 Vogelstein, B., & Nowak, M. A. (2007). Genetic progression and the waiting time to cancer.
 1336 *PLoS Computational Biology*, 3(11), e225. <https://doi.org/10.1371/journal.pcbi.0030225>
- 1337 177. Tomasetti, C., Vogelstein, B., & Parmigiani, G. (2013). Half or more of the somatic
 1338 mutations in cancers of self-renewing tissues originate prior to tumor initiation. In
 1339 *Proceedings of the National Academy of Sciences* (Vol. 110, Issue 6, pp. 1999–2004).
 1340 <https://doi.org/10.1073/pnas.1221068110>
- 1341 178. Curtius, K., Hazelton, W. D., Jeon, J., & Georg Luebeck, E. (2015). A Multiscale Model
 1342 Evaluates Screening for Neoplasia in Barrett's Esophagus. In *PLOS Computational Biology*
 1343 (Vol. 11, Issue 5, p. e1004272). <https://doi.org/10.1371/journal.pcbi.1004272>
- 1344 179. Reiter, J. G., Makohon-Moore, A. P., Gerold, J. M., Bozic, I., Chatterjee, K., Iacobuzio-
 1345 Donahue, C. A., Vogelstein, B., & Nowak, M. A. (2017). Reconstructing metastatic seeding
 1346 patterns of human cancers. *Nature Communications*, 8, 14114.
 1347 <https://doi.org/10.1038/ncomms14114>
- 1348 180. Archetti, M., Ferraro, D. A., & Christofori, G. (2015). Heterogeneity for IGF-II production
 1349 maintained by public goods dynamics in neuroendocrine pancreatic cancer. *Proceedings of*
 1350 *the National Academy of Sciences of the United States of America*, 112(6), 1833–1838.
 1351 <https://doi.org/10.1073/pnas.1414653112>
- 1352 181. Basanta, D., Scott, J. G., Fishman, M. N., Ayala, G., Hayward, S. W., & Anderson, A. R. A.
 1353 (2012). Investigating prostate cancer tumour-stroma interactions: clinical and biological
 1354 insights from an evolutionary game. *British Journal of Cancer*, 106(1), 174–181.
 1355 <https://doi.org/10.1038/bjc.2011.517>
- 1356 182. Nichol, D., Rutter, J., Bryant, C., Hujer, A. M., Lek, S., Adams, M. D., Jeavons, P.,
 1357 Anderson, A. R. A., Bonomo, R. A., & Scott, J. G. (2019). Antibiotic collateral sensitivity is
 1358 contingent on the repeatability of evolution. *Nature Communications*, 10(1), 334.
 1359 <https://doi.org/10.1038/s41467-018-08098-6>
- 1360 183. Kaznatcheev, A., Vander Velde, R., Scott, J. G., & Basanta, D. (2017). Cancer treatment

scheduling and dynamic heterogeneity in social dilemmas of tumour acidity and
 vasculature. *British Journal of Cancer*, 116(6), 785–792. <https://doi.org/10.1038/bjc.2017.5>

ACKNOWLEDGEMENTS

We thank C. Sepich-Poore for providing critical review and feedback on the manuscript and
 figures. Figures were created with BioRender.com. The authors also wish to acknowledge the
 patients and their families who have helped contribute towards a better understanding of this field.

FUNDING SOURCES

G.D.S-P. is supported by a fellowship from the US National Institutes of Health, National Cancer
 Institute (F30 CA243480). T.P. has received funding from the European Research Council (ERC)
 under the European Union's Horizon 2020 research and innovation programme - grant agreement
 n° 637647 – IDEM (P.I.: T. Pradeu). L.L. is funded by the Cancéropôle Île-de-France (n°2021-1-
 EMERG-54-CNRS DR 5-1) and her team is supported by the Ligue National contre le Cancer
 (P.I.: F. Porteu). R.K. is funded in part by grants from the National Cancer Institute within the
 National Institutes of Health (R01 CA255206, U24 CA248454). R.K. and C.G. are funded by
 Mapping host-microbe-metabolite interactions in 3D to find diet-derived enhancers of immunity
 (NIH 1DP1AT010885); Advancing our Understanding of Cancer and the Human Microbiome with
 QIIME 2 (NIH U24CA248454) and NIH Director's Pioneer Award (NIH DP1AT010885). C.G. and
 K.C. are funded by Moores Cancer Center Support Grant NIH (NCI P30 CA023100).

CONFLICTS OF INTEREST

G.D.S.-P. and R.K. are inventors on a US patent application (PCT/US2019/059647) submitted by

1386 The Regents of the University of California and licensed by Micronoma; that application covers
1387 methods of diagnosing and treating cancer using microbial biomarkers in blood and cancer
1388 tissues. G.D.S.-P. and R.K. are founders of and report stock interest in Micronoma. G.D.S.-P. has
1389 filed several additional US patent applications on cancer microbiome diagnostics that are owned
1390 by The Regents of the University of California. R.K. additionally is a member of the scientific
1391 advisory board for GenCirq, holds an equity interest in GenCirq, and can receive reimbursements
1392 for expenses up to US \$5,000 per year.