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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 ~4×10^3 species accounting for ~4×10^{13} total microorganisms, with ~97% of those cells comprising colonic bacteria and ~2-3% comprising extra-colonic bacteria while archaea and eukarya—including fungi—comprise smaller populations around ~0.1-1% 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 (~1:2

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 16*S* 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 16*S* 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:

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

$$\approx 3.5\ picograms\ /\ haploid\ cell$$

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:

$$DNA\ mass\ (cancer\ cell) \approx (3.5\ picograms\ /\ haploid\ cell) \times (2.36\ avg.ploidy)$$

$$\approx \mathbf{8.26\ pg\ /\ cancer\ cell}$$

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:

$$Pure\ tumor\ bacterial\ composition \approx \left(\frac{34.19\ bacteria}{40\ ng\ DNA}\right)\left(\frac{0.008\ ng}{1\ cancer\ cell}\right)(100\%)$$

$$= 0.68\%\ bacterial$$

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:

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

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 mm^2 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 mm^2 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 mm^2: 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/mm^2 (assuming 12 μm, 15 μm, and 18 μm 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/mm^2) and roughly one-tenth of CD8$^+$ T-cell density (~385 cells/mm^2).[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/mm^2 (assuming 5200 cells/mm^2) and approximately 6×10^5 to 6×10^6 bacteria per palpable 1 cm^3 tumor (assuming 10^8-10^9 cells/cm^3).[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/mm^2 and volume density estimates assume 10^9 total cells/cm^3.

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

***al*. 2020**[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 ≤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 (~4×10^3 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**).

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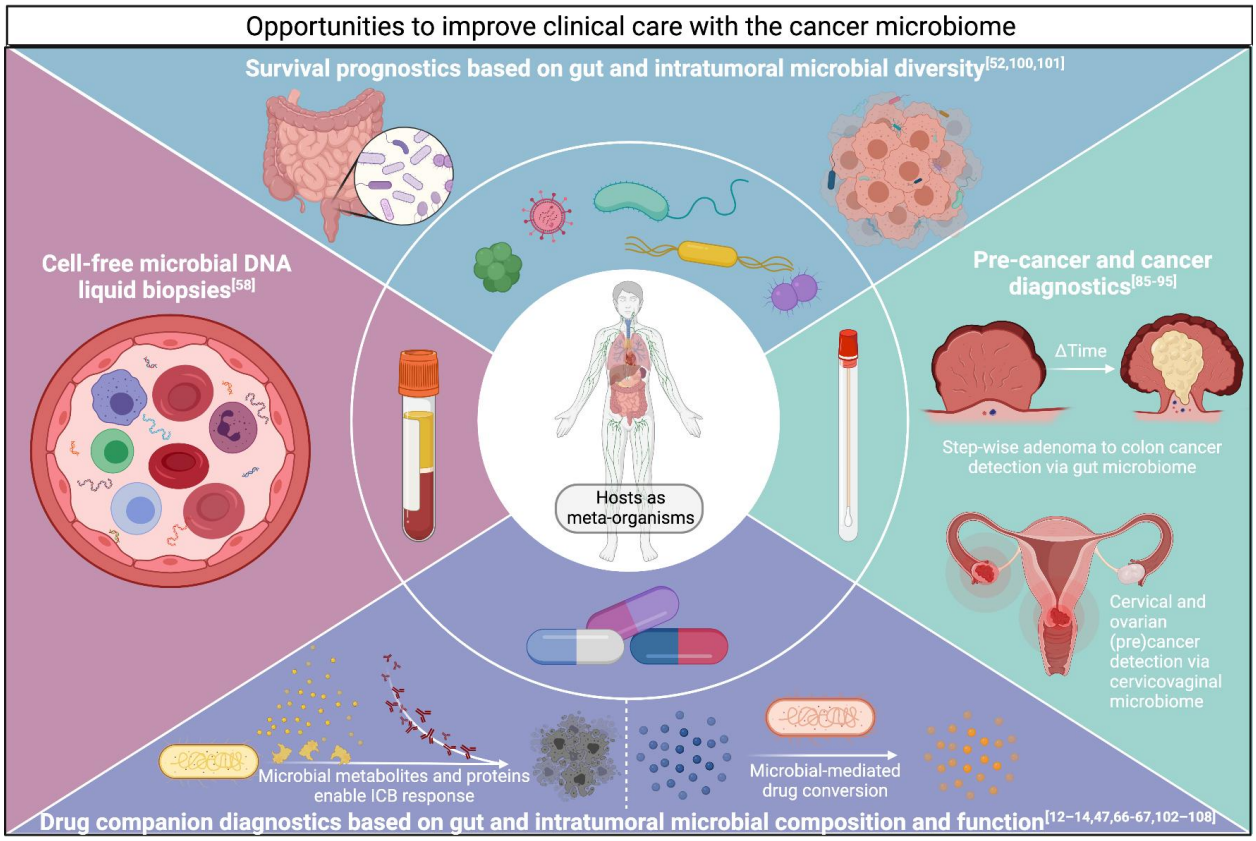


FIGURE 1. Illustration of opportunities to enhance clinical cancer diagnostics and prognostics using the cancer microbiome. Relevant references are listed in the title of each quadrant.

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 16*S* 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]

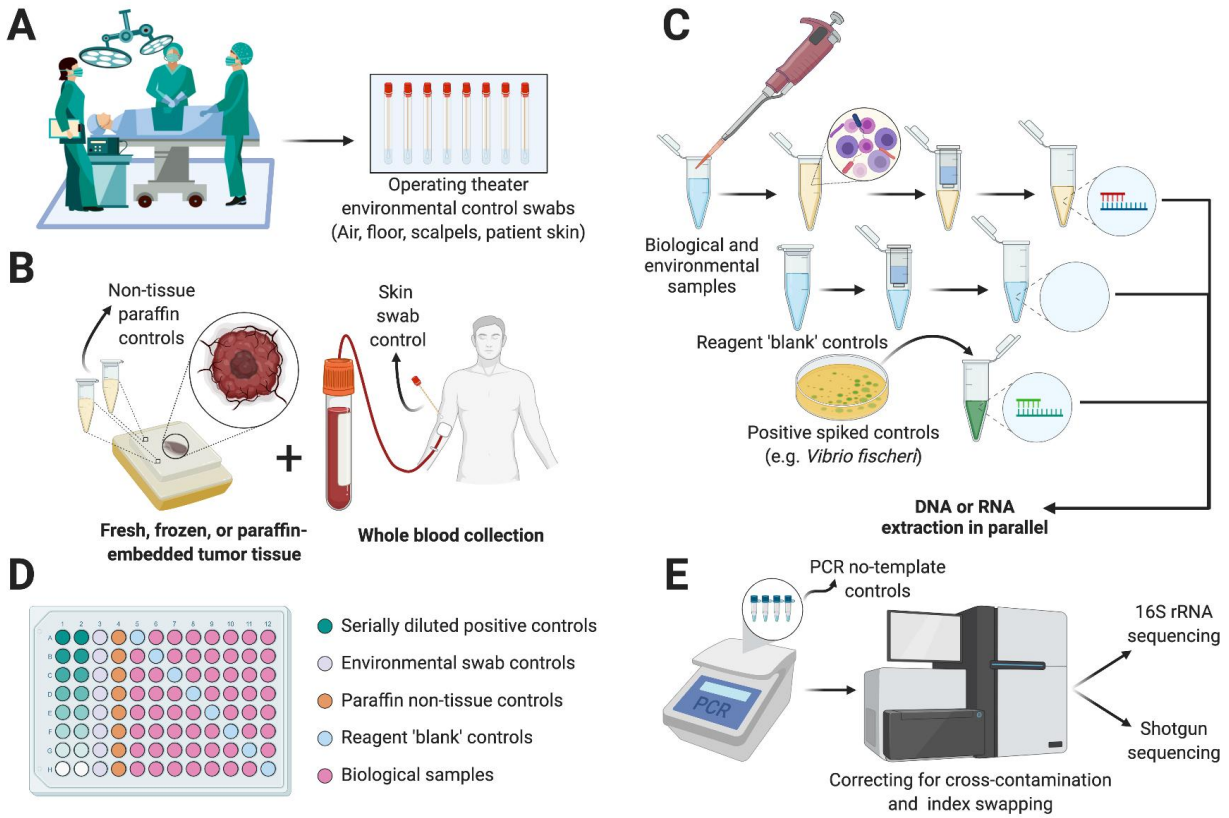


FIGURE 2. Extracting and analyzing low-biomass microbiomes requires special care to control external and internal contamination.[96,113,114] (**A**) Collection of environmental controls ideally begins in the operating room to account for non-patient environmental sources. (**B**) Post-operative tissues, 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.

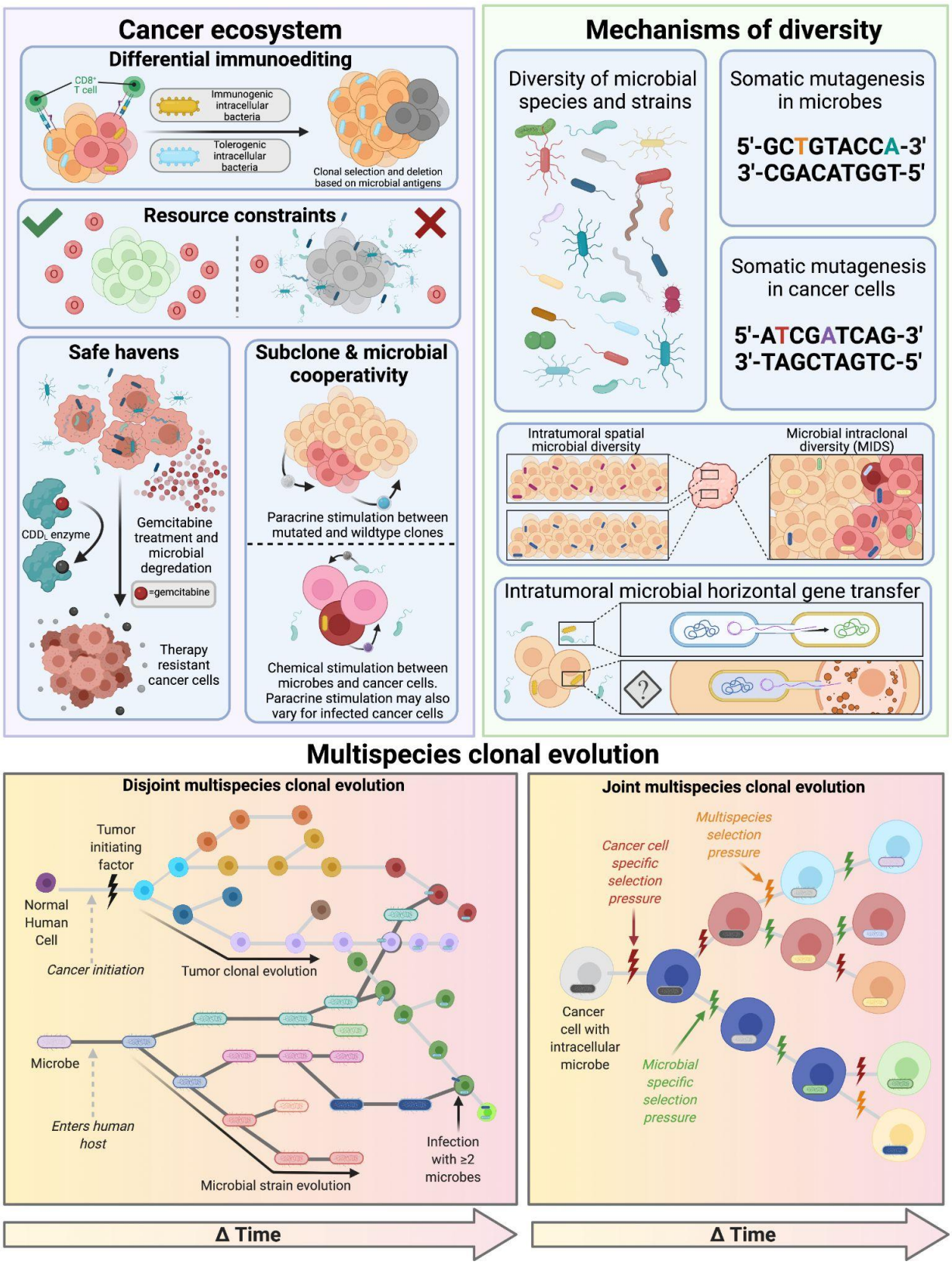
REDEFINING CANCER CLONALITY AS MULTISPECIES

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


Cancer ecosystem
Differential immunoediting
CD8+ T cell
Immunogenic intracellular bacteria
Tolerogenic intracellular bacteria
Clonal selection and deletion based on microbial antigens
Resource constraints
Safe havens
CDD enzyme
Gemcitabine treatment and microbial degredation
=gemcitabine
Therapy resistant cancer cells
Subclone & microbial cooperativity
Paracrine stimulation between mutated and wildtype clones
Chemical stimulation between microbes and cancer cells. Paracrine stimulation may also vary for infected cancer cells
Mechanisms of diversity
Diversity of microbial species and strains
Somatic mutagenesis in microbes
5'-GCTGTACCA-3'
3'-CGACATGGT-5'
Somatic mutagenesis in cancer cells
5'-ATCGATCAG-3'
3'-TAGCTAGTC-5'
Intratumoral spatial microbial diversity
Microbial intraclonal diversity (MIDS)
Intratumoral microbial horizontal gene transfer
Multispecies clonal evolution
Disjoint multispecies clonal evolution
Tumor initiating factor
Normal Human Cell
Cancer initiation
Tumor clonal evolution
Microbe
Enters human host
Infection with ≥2 microbes
Microbial strain evolution
Joint multispecies clonal evolution
Multispecies selection pressure
Cancer cell specific selection pressure
Cancer cell with intracellular microbe
Microbial specific selection pressure
Δ Time
Δ Time

364

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 (CDD_L) followed by bioinformatic searches revealed >300 CDD_L^+ species, 98.4% within *Gammaproteobacteria*. Imaging, sequencing, and cultivation from gemcitabine-associated pancreatic cancer patient biopsies indeed revealed CDD_L^+ 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

468 respect, the primary assertion of clonality is that individual differences between two cancer cells
469 of the same clone are negligible.[147] However, if intracellular bacteria alter phenotypes, behaviors,
470 and fitness of spatially-adjacent cancer cells, then they create major intraclonal heterogeneity,
471 which we define as “microbial intraclonal diversity” (MIDS). MIDS questions clonal lineage
472 homogeneity and motivates revising clonal boundaries, most simplistically through further
473 subsetting (e.g., *KRAS*-mutated, *Fusobacterium*-infected cells versus *KRAS*-mutated uninfected
474 cells) or more accurately through revised modelling approaches that account for discordant
475 microbe-cancer selection pressures. MIDS also includes mimicry between microbial and cancer
476 antigens.[148,149] Should genetic cargo be shared between intracellular bacteria and host cells, as
477 biotechnology already shows is possible[24] and cancer virology affirms,[45,46] MIDS must account
478 for DNA and RNA from multiple species.
479
480 Beyond challenging clonal boundaries, intracellular bacteria may require revision of the
481 evolutionary tree. Typical clonal evolution model depicts an evolutionary tree with one trunk and
482 several branches, relying on the assumption of vertically transmitted traits from mother cells to
483 daughter cells at each round of cell division. If future research affirms horizontal/lateral gene
484 transfers between intracellular bacteria and host cancer cells, multiple tree trunks and connexions
485 between branches would be required. A similar debate has taken place in evolutionary biology,
486 challenging the traditional Darwinian view about “tree of life.”[150–152] Clonal evolution may then be
487 better articulated as a case of “reticulated evolution,” wherein horizontal/lateral transfers change
488 the fitness, function, or/or phenotype of host cancer cells.
489
490 ***Considerations for cancer microbiome therapeutics under multispecies clonality***
491
492 Multispecies cancer clonality offers new therapeutic strategies that neither human nor microbial
493 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

572 jointly) undergo somatic clonal evolution, as described in the case of *H. pylori*. Phylogenetic tree
573 reconstructions of clonal evolution[173] may thus need to include multispecies lineages, but
574 specialized methods likely need to be created for this purpose. To summarize, we have created
575 a table of major clonal evolution models and suggested strategies for incorporating microbial
576 information into them (**Table 2**).
577
578 **CONCLUSIONS**
579
580 Rigorous studies provide extensive evidence for the existence and functionality of cancer-
581 associated gut and intratumoral microbes while echoing ancient historical narratives of microbial-
582 mediated recovery. Drawing from cancer genomics, the inherently high diversity of the cancer
583 microbiome substantiates its strong predictive power, even when any individual microbe is rare
584 or lowly abundant. Cancer microbiota can distinguish healthy, pre-cancer, and cancerous tissues
585 across multiple cancer and sample types, although most diagnostics remain unvalidated in large,
586 multi-national, prospective cohorts. Cancer microbiota also demonstrate stage-specific
587 differences that may enable simultaneous identification and prognostication of tumors.
588 Nonetheless, contamination challenges in low-biomass settings and analytic idiosyncrasies of
589 microbiomic data have hitherto complicated routine clinical application of cancer microbial
590 diagnostics or prognostics.
591
592 Numerous microbial mechanisms affect the cancer genome, transcriptome, proteome, and
593 metabolome, advocating for their inclusion in models of cancer evolution. Extracellular and
594 intracellular microbes affect virtually every cancer medication class and may drive therapeutic
595 efficacy or resistance without any cancer cell(s) interventions. Negatively, it is not possible to
596 accurately model cancer-drug dynamics, clonality, or fitness without accounting for microbes.
597 Serious consideration of multispecies clonality, however, is complex and necessitates reworking

598 cancer evolution models since microbes carry distinct, although plausibly shareable, genetic
599 cargo that may undergo discordant selection pressures from the cancer genome. Flexible
600 evolutionary models treating intratumoral microbes as independent, albeit rule-abiding agents
601 within the TME may be appropriate. Multispecies clonality also informs treatments, such that
602 intentional modifications of cancer pathways may comprise more effective ways to restore healthy
603 microbial ecologies than targeted antimicrobials, and vice versa. Multispecies treatment
604 strategies may further benefit from target selectivity, for targeting microbial genes generally
605 carries fewer side effects than targeting the host's. Altogether, understanding cancer's
606 metagenome carries key ramifications for cancer care and clonal evolution for the benefit of
607 patients worldwide.
608
609
610 **TABLE 2. Microbial integration into mathematical models of evolution.** Overview of each
611 model's characteristics and references provided with modeling examples, as well as suggested
612 ways that microbes could be potentially incorporated into model structure. Hybrid models that
613 include aspects of more than one model type are also utilized in practice.
614

Model Type	Overview of Model	Examples of Potential Incorporation of Microbes	Literature
Agent-based model	● 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	● 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	• 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	• 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	• 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	• 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	• 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
		• Option 1
		  ○ Birth and death defined in terms of human cancer cells with:
		  ■ 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]

		○ Birth and death events defined in terms of both human cancer cells and microbial populations	
Evolutionary game theory model	● 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 *E. coli*	● 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]

REFERENCES

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

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., Korbel, 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., Abnousi, 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

763 amplification in tumour pathogenesis and evolution. *Nature Reviews. Cancer*, *19*(5), 283–
764 288. https://doi.org/10.1038/s41568-019-0128-6
765 44. Münch, K., Münch, R., Biedendieck, R., Jahn, D., & Müller, J. (2019). Evolutionary model for
766 the unequal segregation of high copy plasmids. *PLoS Computational Biology*, *15*(3),
767 e1006724. https://doi.org/10.1371/journal.pcbi.1006724
768 45. Deshpande, V., Luebeck, J., Nguyen, N.-P. D., Bakhtiari, M., Turner, K. M., Schwab, R.,
769 Carter, H., Mischel, P. S., & Bafna, V. (2019). Exploring the landscape of focal
770 amplifications in cancer using AmpliconArchitect. *Nature Communications*, *10*(1), 392.
771 https://doi.org/10.1038/s41467-018-08200-y
772 46. Pang, J., Nguyen, N.-P., Luebeck, J., Ball, L., Finegersh, A., Ren, S., Nakagawa, T., Flagg,
773 M., Sadat, S., Mischel, P. S., Xu, G., Fisch, K., Guo, T., Cahill, G., Panuganti, B., Bafna, V.,
774 & Califano, J. (2021). Extrachromosomal DNA in HPV mediated oropharyngeal cancer
775 drives diverse oncogene transcription. *Clinical Cancer Research: An Official Journal of the*
776 *American Association for Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-21-
777 2484
778 47. Geller, L. T., Barzily-Rokni, M., Danino, T., Jonas, O. H., Shental, N., Nejman, D., Gavert,
779 N., Zwang, Y., Cooper, Z. A., Shee, K., Thaiss, C. A., Reuben, A., Livny, J., Avraham, R.,
780 Frederick, D. T., Ligorio, M., Chatman, K., Johnston, S. E., Mosher, C. M., … Straussman,
781 R. (2017). Potential role of intratumor bacteria in mediating tumor resistance to the
782 chemotherapeutic drug gemcitabine. *Science*, *357*(6356), 1156–1160.
783 https://doi.org/10.1126/science.aah5043
784 48. Pushalkar, S., Hundeyin, M., Daley, D., Zambirinis, C. P., Kurz, E., Mishra, A., Mohan, N.,
785 Aykut, B., Usyk, M., Torres, L. E., Werba, G., Zhang, K., Guo, Y., Li, Q., Akkad, N., Lall, S.,
786 Wadowski, B., Gutierrez, J., Kochen Rossi, J. A., … Miller, G. (2018). The Pancreatic
787 Cancer Microbiome Promotes Oncogenesis by Induction of Innate and Adaptive Immune
788 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 γδ 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., Kosciolek, 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., Calì, 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

78. Downey, G. P., Doherty, D. E., Schwab, B., 3rd, Elson, E. L., Henson, P. M., & Worthen, G. S. (1990). Retention of leukocytes in capillaries: role of cell size and deformability. *Journal of Applied Physiology*, *69*(5), 1767–1778. https://doi.org/10.1152/jappl.1990.69.5.1767

79. Shashni, B., Ariyasu, S., Takeda, R., Suzuki, T., Shiina, S., Akimoto, K., Maeda, T., Aikawa, N., Abe, R., Osaki, T., Itoh, N., & Aoki, S. (2018). Size-Based Differentiation of Cancer and Normal Cells by a Particle Size Analyzer Assisted by a Cell-Recognition PC Software. *Biological & Pharmaceutical Bulletin*, *41*(4), 487–503. https://doi.org/10.1248/bpb.b17-00776

80. Kather, J. N., Suarez-Carmona, M., Charoentong, P., Weis, C.-A., Hirsch, D., Bankhead, P., Horning, M., Ferber, D., Kel, I., Herpel, E., Schott, S., Zörnig, I., Utikal, J., Marx, A., Gaiser, T., Brenner, H., Chang-Claude, J., Hoffmeister, M., Jäger, D., & Halama, N. (2018). Topography of cancer-associated immune cells in human solid tumors. *eLife*, *7*. https://doi.org/10.7554/eLife.36967

81. Monte, U. D., & Del Monte, U. (2009). Does the cell number 109still really fit one gram of tumor tissue? In *Cell Cycle* (Vol. 8, Issue 3, pp. 505–506). https://doi.org/10.4161/cc.8.3.7608

82. Avanzini, S., Kurtz, D. M., Chabon, J. J., Moding, E. J., Hori, S. S., Gambhir, S. S., Alizadeh, A. A., Diehn, M., & Reiter, J. G. (2020). A mathematical model of ctDNA shedding predicts tumor detection size. *Science Advances*, *6*(50). https://doi.org/10.1126/sciadv.abc4308

83. Abbosh, C., Birkbak, N. J., Wilson, G. A., Jamal-Hanjani, M., Constantin, T., Salari, R., Le Quesne, J., Moore, D. A., Veeriah, S., Rosenthal, R., Marafioti, T., Kirkizlar, E., Watkins, T. B. K., McGranahan, N., Ward, S., Martinson, L., Riley, J., Fraioli, F., Al Bakir, M., … Swanton, C. (2017). Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*, *545*(7655), 446–451. https://doi.org/10.1038/nature22364

84. Cristiano, S., Leal, A., Phallen, J., Fiksel, J., Adleff, V., Bruhm, D. C., Jensen, S. Ø., Medina, J. E., Hruban, C., White, J. R., Palsgrove, D. N., Niknafs, N., Anagnostou, V., Forde, P.,

945 Naidoo, J., Marrone, K., Brahmer, J., Woodward, B. D., Husain, H., … Velculescu, V. E.
946 (2019). Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature*,
947 *570*(7761), 385–389. https://doi.org/10.1038/s41586-019-1272-6
948 85. Yachida, S., Mizutani, S., Shiroma, H., Shiba, S., Nakajima, T., Sakamoto, T., Watanabe,
949 H., Masuda, K., Nishimoto, Y., Kubo, M., Hosoda, F., Rokutan, H., Matsumoto, M.,
950 Takamaru, H., Yamada, M., Matsuda, T., Iwasaki, M., Yamaji, T., Yachida, T., … Yamada,
951 T. (2019). Metagenomic and metabolomic analyses reveal distinct stage-specific
952 phenotypes of the gut microbiota in colorectal cancer. *Nature Medicine*, *25*(6), 968–976.
953 https://doi.org/10.1038/s41591-019-0458-7
954 86. Dadkhah, E., Sikaroodi, M., Korman, L., Hardi, R., Baybick, J., Hanzel, D., Kuehn, G.,
955 Kuehn, T., & Gillevet, P. M. (2019). Gut microbiome identifies risk for colorectal polyps.
956 *BMJ Open Gastroenterology*, *6*(1), e000297. https://doi.org/10.1136/bmjgast-2019-000297
957 87. Peters, B. A., Dominianni, C., Shapiro, J. A., Church, T. R., Wu, J., Miller, G., Yuen, E.,
958 Freiman, H., Lustbader, I., Salik, J., Friedlander, C., Hayes, R. B., & Ahn, J. (2016). The gut
959 microbiota in conventional and serrated precursors of colorectal cancer. *Microbiome*, *4*(1),
960 69. https://doi.org/10.1186/s40168-016-0218-6
961 88. Kordahi, M. C., Stanaway, I. B., Avril, M., Chac, D., Blanc, M.-P., Ross, B., Diener, C., Jain,
962 S., McCleary, P., Parker, A., Friedman, V., Huang, J., Burke, W., Gibbons, S. M., Willis, A.
963 D., Darveau, R. P., Grady, W. M., Ko, C. W., & DePaolo, R. W. (2021). Genomic and
964 functional characterization of a mucosal symbiont involved in early-stage colorectal cancer.
965 *Cell Host & Microbe*, *29*(10), 1589–1598.e6. https://doi.org/10.1016/j.chom.2021.08.013
966 89. Usyk, M., Zolnik, C. P., Castle, P. E., Porras, C., Herrero, R., Gradissimo, A., Gonzalez, P.,
967 Safaeian, M., Schiffman, M., Burk, R. D., & Costa Rica HPV Vaccine Trial (CVT) Group.
968 (2020). Cervicovaginal microbiome and natural history of HPV in a longitudinal study. *PLoS*
969 *Pathogens*, *16*(3), e1008376. https://doi.org/10.1371/journal.ppat.1008376
970 90. Tango, C. N., Seo, S.-S., Kwon, M., Lee, D.-O., Chang, H. K., & Kim, M. K. (2020).

971 Taxonomic and Functional Differences in Cervical Microbiome Associated with Cervical
972 Cancer Development. *Scientific Reports*, *10*(1), 9720. https://doi.org/10.1038/s41598-020-
973 66607-4
974 91. Klein, C., Gonzalez, D., Samwel, K., Kahesa, C., Mwaiselage, J., Aluthge, N., Fernando, S.,
975 West, J. T., Wood, C., & Angeletti, P. C. (2019). Relationship between the Cervical
976 Microbiome, HIV Status, and Precancerous Lesions. *mBio*, *10*(1).
977 https://doi.org/10.1128/mBio.02785-18
978 92. Delhommeau, F., Dupont, S., Della Valle, V., James, C., Trannoy, S., Massé, A., Kosmider,
979 O., Le Couedic, J.-P., Robert, F., Alberdi, A., Lécluse, Y., Plo, I., Dreyfus, F. J., Marzac, C.,
980 Casadevall, N., Lacombe, C., Romana, S. P., Dessen, P., Soulier, J., … Bernard, O. A.
981 (2009). Mutation in TET2 in myeloid cancers. *The New England Journal of Medicine*,
982 *360*(22), 2289–2301. https://doi.org/10.1056/NEJMoa0810069
983 93. Dejea, C. M., Fathi, P., Craig, J. M., Boleij, A., Taddese, R., Geis, A. L., Wu, X., DeStefano
984 Shields, C. E., Hechenbleikner, E. M., Huso, D. L., Anders, R. A., Giardiello, F. M., Wick, E.
985 C., Wang, H., Wu, S., Pardoll, D. M., Housseau, F., & Sears, C. L. (2018). Patients with
986 familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria.
987 *Science*, *359*(6375), 592–597. https://doi.org/10.1126/science.aah3648
988 94. Meisel, M., Hinterleitner, R., Pacis, A., Chen, L., Earley, Z. M., Mayassi, T., Pierre, J. F.,
989 Ernest, J. D., Galipeau, H. J., Thuille, N., Bouziat, R., Buscarlet, M., Ringus, D. L., Wang,
990 Y., Li, Y., Dinh, V., Kim, S. M., McDonald, B. D., Zurenski, M. A., … Jabri, B. (2018).
991 Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature*,
992 *557*(7706), 580–584. https://doi.org/10.1038/s41586-018-0125-z
993 95. Nené, N. R., Reisel, D., Leimbach, A., Franchi, D., Jones, A., Evans, I., Knapp, S., Ryan, A.,
994 Ghazali, S., Timms, J. F., Paprotka, T., Bjørge, L., Zikan, M., Cibula, D., Colombo, N., &
995 Widschwendter, M. (2019). Association between the cervicovaginal microbiome, BRCA1
996 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

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-

Thoeringer, C. K., Markey, K. A., Docampo, M. D., Burgos da Silva, M., Khan, N., Gessner, A., Messina, J. A., … van den Brink, M. R. M. (2020). Microbiota as Predictor of Mortality in Allogeneic Hematopoietic-Cell Transplantation. *The New England Journal of Medicine*, *382*(9), 822–834. https://doi.org/10.1056/NEJMoa1900623

102. Vétizou, M., Pitt, J. M., Daillère, R., Lepage, P., Waldschmitt, N., Flament, C., Rusakiewicz, S., Routy, B., Roberti, M. P., Duong, C. P. M., Poirier-Colame, V., Roux, A., Becharef, S., Formenti, S., Golden, E., Cording, S., Eberl, G., Schlitzer, A., Ginhoux, F., … Zitvogel, L. (2015). Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. *Science*, *350*(6264), 1079–1084. https://doi.org/10.1126/science.aad1329

103. Sivan, A., Corrales, L., Hubert, N., Williams, J. B., Aquino-Michaels, K., Earley, Z. M., Benyamin, F. W., Lei, Y. M., Jabri, B., Alegre, M.-L., Chang, E. B., & Gajewski, T. F. (2015). Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science*, *350*(6264), 1084–1089. https://doi.org/10.1126/science.aac4255

104. Limeta, A., Ji, B., Levin, M., Gatto, F., & Nielsen, J. (2020). Meta-analysis of the gut microbiota in predicting response to cancer immunotherapy in metastatic melanoma. *JCI Insight*, *5*(23). https://doi.org/10.1172/jci.insight.140940

105. Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C. A., Brown, K., Ramay, H., Paik, S., Stagg, J., Groves, R. A., Gallo, M., Lewis, I. A., Geuking, M. B., & McCoy, K. D. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Science*, *369*(6510), 1481–1489. https://doi.org/10.1126/science.abc3421

106. Griffin, M. E., Espinosa, J., Becker, J. L., Luo, J.-D., Carroll, T. S., Jha, J. K., Fanger, G. R., & Hang, H. C. (2021). Enterococcus peptidoglycan remodeling promotes checkpoint inhibitor cancer immunotherapy. *Science*, *373*(6558), 1040–1046. https://doi.org/10.1126/science.abc9113

107. Daillère, R., Vétizou, M., Waldschmitt, N., Yamazaki, T., Isnard, C., Poirier-Colame, V., Duong, C. P. M., Flament, C., Lepage, P., Roberti, M. P., Routy, B., Jacquelot, N., Apetoh,

L., Becharef, S., Rusakiewicz, S., Langella, P., Sokol, H., Kroemer, G., Enot, D., … Zitvogel, L. (2016). Enterococcus hirae and Barnesiella intestinihominis Facilitate Cyclophosphamide-Induced Therapeutic Immunomodulatory Effects. *Immunity*, *45*(4), 931–943. https://doi.org/10.1016/j.immuni.2016.09.009

108. Lam, K. C., Araya, R. E., Huang, A., Chen, Q., Di Modica, M., Rodrigues, R. R., Lopès, A., Johnson, S. B., Schwarz, B., Bohrnsen, E., Cogdill, A. P., Bosio, C. M., Wargo, J. A., Lee, M. P., & Goldszmid, R. S. (2021). Microbiota triggers STING-type I IFN-dependent monocyte reprogramming of the tumor microenvironment. *Cell*, *184*(21), 5338–5356.e21. https://doi.org/10.1016/j.cell.2021.09.019

109. Alexander, J. L., Wilson, I. D., Teare, J., Marchesi, J. R., Nicholson, J. K., & Kinross, J. M. (2017). Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nature Reviews. Gastroenterology & Hepatology*, *14*(6), 356–365. https://doi.org/10.1038/nrgastro.2017.20

110. Daisley, B. A., Chanyi, R. M., Abdur-Rashid, K., Al, K. F., Gibbons, S., Chmiel, J. A., Wilcox, H., Reid, G., Anderson, A., Dewar, M., Nair, S. M., Chin, J., & Burton, J. P. (2020). Abiraterone acetate preferentially enriches for the gut commensal Akkermansia muciniphila in castrate-resistant prostate cancer patients. *Nature Communications*, *11*(1), 4822. https://doi.org/10.1038/s41467-020-18649-5

111. Kwa, M., Plottel, C. S., Blaser, M. J., & Adams, S. (2016). The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *Journal of the National Cancer Institute*, *108*(8). https://doi.org/10.1093/jnci/djw029

112. Komorowski, A. S., & Pezo, R. C. (2020). Untapped “-omics”: the microbial metagenome, estrobolome, and their influence on the development of breast cancer and response to treatment. *Breast Cancer Research and Treatment*, *179*(2), 287–300. https://doi.org/10.1007/s10549-019-05472-w

113. Eisenhofer, R., Minich, J. J., Marotz, C., Cooper, A., Knight, R., & Weyrich, L. S. (2019). Contamination in Low Microbial Biomass Microbiome Studies: Issues and

Recommendations. *Trends in Microbiology*, *27*(2), 105–117. https://doi.org/10.1016/j.tim.2018.11.003

114. Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner, P., Parkhill, J., Loman, N. J., & Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*(1), 87. https://doi.org/10.1186/s12915-014-0087-z

115. Sinha, R., Abu-Ali, G., Vogtmann, E., Fodor, A. A., Ren, B., Amir, A., Schwager, E., Crabtree, J., Ma, S., Microbiome Quality Control Project Consortium, Abnet, C. C., Knight, R., White, O., & Huttenhower, C. (2017). Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium. *Nature Biotechnology*, *35*(11), 1077–1086. https://doi.org/10.1038/nbt.3981

116. Morton, J. T., Marotz, C., Washburne, A., Silverman, J., Zaramela, L. S., Edlund, A., Zengler, K., & Knight, R. (2019). Establishing microbial composition measurement standards with reference frames. *Nature Communications*, *10*(1), 2719. https://doi.org/10.1038/s41467-019-10656-5

117. Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature Communications*, *11*(1), 3514. https://doi.org/10.1038/s41467-020-17041-7

118. Lin, H., & Peddada, S. D. (2020). Analysis of microbial compositions: a review of normalization and differential abundance analysis. *NPJ Biofilms and Microbiomes*, *6*(1), 60. https://doi.org/10.1038/s41522-020-00160-w

119. Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R. N., Anokhin, A. P., Heath, A. C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J. G., Lozupone, C. A., Lauber, C., Clemente, J. C., Knights, D., … Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature*, *486*(7402), 222–227. https://doi.org/10.1038/nature11053

120. He, Y., Wu, W., Zheng, H.-M., Li, P., McDonald, D., Sheng, H.-F., Chen, M.-X., Chen, Z.-H., Ji, G.-Y., Zheng, Z.-D.-X., Mujagond, P., Chen, X.-J., Rong, Z.-H., Chen, P., Lyu, L.-Y., Wang, X., Wu, C.-B., Yu, N., Xu, Y.-J., … Zhou, H.-W. (2018). Regional variation limits applications of healthy gut microbiome reference ranges and disease models. *Nature Medicine*, *24*(10), 1532–1535. https://doi.org/10.1038/s41591-018-0164-x

121. Deschasaux, M., Bouter, K. E., Prodan, A., Levin, E., Groen, A. K., Herrema, H., Tremaroli, V., Bakker, G. J., Attaye, I., Pinto-Sietsma, S.-J., van Raalte, D. H., Snijder, M. B., Nicolaou, M., Peters, R., Zwinderman, A. H., Bäckhed, F., & Nieuwdorp, M. (2018). Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. *Nature Medicine*, *24*(10), 1526–1531. https://doi.org/10.1038/s41591-018-0160-1

122. Wirbel, J., Pyl, P. T., Kartal, E., Zych, K., Kashani, A., Milanese, A., Fleck, J. S., Voigt, A. Y., Palleja, A., Ponnudurai, R., Sunagawa, S., Coelho, L. P., Schrotz-King, P., Vogtmann, E., Habermann, N., Niméus, E., Thomas, A. M., Manghi, P., Gandini, S., … Zeller, G. (2019). Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nature Medicine*, *25*(4), 679–689. https://doi.org/10.1038/s41591-019-0406-6

123. Thomas, A. M., Manghi, P., Asnicar, F., Pasolli, E., Armanini, F., Zolfo, M., Beghini, F., Manara, S., Karcher, N., Pozzi, C., Gandini, S., Serrano, D., Tarallo, S., Francavilla, A., Gallo, G., Trompetto, M., Ferrero, G., Mizutani, S., Shiroma, H., … Segata, N. (2019). Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nature Medicine*, *25*(4), 667–678. https://doi.org/10.1038/s41591-019-0405-7

124. Nowell, P. C. (1976). The clonal evolution of tumor cell populations. *Science*, *194*(4260), 23–28. https://doi.org/10.1126/science.959840

125. Greaves, M., & Maley, C. C. (2012). Clonal evolution in cancer. *Nature*.

https://www.nature.com/articles/nature10762

126. Mazor, T., Pankov, A., Johnson, B. E., Hong, C., Hamilton, E. G., Bell, R. J. A., Smirnov, I. V., Reis, G. F., Phillips, J. J., Barnes, M. J., Idbaih, A., Alentorn, A., Kloezeman, J. J., Lamfers, M. L. M., Bollen, A. W., Taylor, B. S., Molinaro, A. M., Olshen, A. B., Chang, S. M., … Costello, J. F. (2015). DNA Methylation and Somatic Mutations Converge on the Cell Cycle and Define Similar Evolutionary Histories in Brain Tumors. *Cancer Cell*, *28*(3), 307–317. https://doi.org/10.1016/j.ccell.2015.07.012

127. Li, S., Garrett-Bakelman, F. E., Chung, S. S., Sanders, M. A., Hricik, T., Rapaport, F., Patel, J., Dillon, R., Vijay, P., Brown, A. L., Perl, A. E., Cannon, J., Bullinger, L., Luger, S., Becker, M., Lewis, I. D., To, L. B., Delwel, R., Löwenberg, B., … Mason, C. E. (2016). Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. *Nature Medicine*, *22*(7), 792–799. https://doi.org/10.1038/nm.4125

128. Vaz, M., Hwang, S. Y., Kagiampakis, I., Phallen, J., Patil, A., O'Hagan, H. M., Murphy, L., Zahnow, C. A., Gabrielson, E., Velculescu, V. E., Easwaran, H. P., & Baylin, S. B. (2017). Chronic Cigarette Smoke-Induced Epigenomic Changes Precede Sensitization of Bronchial Epithelial Cells to Single-Step Transformation by KRAS Mutations. *Cancer Cell*, *32*(3), 360–376.e6. https://doi.org/10.1016/j.ccell.2017.08.006

129. Nam, A. S., Chaligne, R., & Landau, D. A. (2021). Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics. *Nature Reviews. Genetics*, *22*(1), 3–18. https://doi.org/10.1038/s41576-020-0265-5

130. Gutierrez, C., Al'Khafaji, A. M., Brenner, E., Johnson, K. E., Gohil, S. H., Lin, Z., Knisbacher, B. A., Durrett, R. E., Li, S., Parvin, S., Biran, A., Zhang, W., Rassenti, L., Kipps, T. J., Livak, K. J., Neuberg, D., Letai, A., Getz, G., Wu, C. J., & Brock, A. (2021). Multifunctional barcoding with ClonMapper enables high-resolution study of clonal dynamics during tumor evolution and treatment. *Nature Cancer*, *2*(7), 758–772. https://doi.org/10.1038/s43018-021-00222-8

131. Pleguezuelos-Manzano, C., Puschhof, J., Rosendahl Huber, A., van Hoeck, A., Wood, H. M., Nomburg, J., Gurjao, C., Manders, F., Dalmasso, G., Stege, P. B., Paganelli, F. L., Geurts, M. H., Beumer, J., Mizutani, T., Miao, Y., van der Linden, R., van der Elst, S., Genomics England Research Consortium, Garcia, K. C., … Clevers, H. (2020). Mutational signature in colorectal cancer caused by genotoxic pks E. coli. *Nature*, *580*(7802), 269–273. https://doi.org/10.1038/s41586-020-2080-8

132. Yu, T., Guo, F., Yu, Y., Sun, T., Ma, D., Han, J., Qian, Y., Kryczek, I., Sun, D., Nagarsheth, N., Chen, Y., Chen, H., Hong, J., Zou, W., & Fang, J.-Y. (2017). Fusobacterium nucleatum Promotes Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell*, *170*(3), 548–563.e16. https://doi.org/10.1016/j.cell.2017.07.008

133. Garrett, W. S. (2015). Cancer and the microbiota. *Science*, *348*(6230), 80–86. https://doi.org/10.1126/science.aaa4972

134. Ma, C., Han, M., Heinrich, B., Fu, Q., Zhang, Q., Sandhu, M., Agdashian, D., Terabe, M., Berzofsky, J. A., Fako, V., Ritz, T., Longerich, T., Theriot, C. M., McCulloch, J. A., Roy, S., Yuan, W., Thovarai, V., Sen, S. K., Ruchirawat, M., … Greten, T. F. (2018). Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science*, *360*(6391). https://doi.org/10.1126/science.aan5931

135. Goss, G., Tsai, C.-M., Shepherd, F. A., Bazhenova, L., Lee, J. S., Chang, G.-C., Crino, L., Satouchi, M., Chu, Q., Hida, T., Han, J.-Y., Juan, O., Dunphy, F., Nishio, M., Kang, J.-H., Majem, M., Mann, H., Cantarini, M., Ghiorghiu, S., & Mitsudomi, T. (2016). Osimertinib for pretreated EGFR Thr790Met-positive advanced non-small-cell lung cancer (AURA2): a multicentre, open-label, single-arm, phase 2 study. *The Lancet Oncology*, *17*(12), 1643–1652. https://doi.org/10.1016/S1470-2045(16)30508-3

136. Thress, K. S., Paweletz, C. P., Felip, E., Cho, B. C., Stetson, D., Dougherty, B., Lai, Z., Markovets, A., Vivancos, A., Kuang, Y., Ercan, D., Matthews, S. E., Cantarini, M., Barrett, J. C., Jänne, P. A., & Oxnard, G. R. (2015). Acquired EGFR C797S mutation mediates

resistance to AZD9291 in non–small cell lung cancer harboring EGFR T790M. *Nature Medicine*, *21*(6), 560–562. https://doi.org/10.1038/nm.3854

137. DiNardo, C. D., Stein, E. M., de Botton, S., Roboz, G. J., Altman, J. K., Mims, A. S., Swords, R., Collins, R. H., Mannis, G. N., Pollyea, D. A., Donnellan, W., Fathi, A. T., Pigneux, A., Erba, H. P., Prince, G. T., Stein, A. S., Uy, G. L., Foran, J. M., Traer, E., … Kantarjian, H. M. (2018). Durable Remissions with Ivosidenib in IDH1-Mutated Relapsed or Refractory AML. *The New England Journal of Medicine*, *378*(25), 2386–2398. https://doi.org/10.1056/NEJMoa1716984

138. Quek, L., David, M. D., Kennedy, A., Metzner, M., Amatangelo, M., Shih, A., Stoilova, B., Quivoron, C., Heiblig, M., Willekens, C., Saada, V., Alsafadi, S., Vijayabaskar, M. S., Peniket, A., Bernard, O. A., Agresta, S., Yen, K., MacBeth, K., Stein, E., … Vyas, P. (2018). Clonal heterogeneity of acute myeloid leukemia treated with the IDH2 inhibitor enasidenib. *Nature Medicine*, *24*(8), 1167–1177. https://doi.org/10.1038/s41591-018-0115-6

139. Roboz, G. J., DiNardo, C. D., Stein, E. M., de Botton, S., Mims, A. S., Prince, G. T., Altman, J. K., Arellano, M. L., Donnellan, W., Erba, H. P., Mannis, G. N., Pollyea, D. A., Stein, A. S., Uy, G. L., Watts, J. M., Fathi, A. T., Kantarjian, H. M., Tallman, M. S., Choe, S., … Stone, R. M. (2020). Ivosidenib induces deep durable remissions in patients with newly diagnosed IDH1-mutant acute myeloid leukemia. *Blood*, *135*(7), 463–471. https://doi.org/10.1182/blood.2019002140

140. Merlevede, J., Droin, N., Qin, T., Meldi, K., Yoshida, K., Morabito, M., Chautard, E., Auboeuf, D., Fenaux, P., Braun, T., Itzykson, R., de Botton, S., Quesnel, B., Commes, T., Jourdan, E., Vainchenker, W., Bernard, O., Pata-Merci, N., Solier, S., … Solary, E. (2016). Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents. *Nature Communications*, *7*, 10767. https://doi.org/10.1038/ncomms10767

141. Turati, V. A., Guerra-Assunção, J. A., Potter, N. E., Gupta, R., Ecker, S., Daneviciute, A.,

Tarabichi, M., Webster, A. P., Ding, C., May, G., James, C., Brown, J., Conde, L., Russell, L. J., Ancliff, P., Inglott, S., Cazzaniga, G., Biondi, A., Hall, G. W., … Enver, T. (2021). Chemotherapy induces canalization of cell state in childhood B-cell precursor acute lymphoblastic leukemia. *Nature Cancer*, *2*(8), 835–852. https://doi.org/10.1038/s43018-021-00219-3

142. Dobson, S. M., García-Prat, L., Vanner, R. J., Wintersinger, J., Waanders, E., Gu, Z., McLeod, J., Gan, O. I., Grandal, I., Payne-Turner, D., Edmonson, M. N., Ma, X., Fan, Y., Voisin, V., Chan-Seng-Yue, M., Xie, S. Z., Hosseini, M., Abelson, S., Gupta, P., … Dick, J. E. (2020). Relapse-Fated Latent Diagnosis Subclones in Acute B Lineage Leukemia Are Drug Tolerant and Possess Distinct Metabolic Programs. *Cancer Discovery*, *10*(4), 568–587. https://doi.org/10.1158/2159-8290.CD-19-1059

143. Marine, J.-C., Dawson, S.-J., & Dawson, M. A. (2020). Non-genetic mechanisms of therapeutic resistance in cancer. *Nature Reviews. Cancer*, *20*(12), 743–756. https://doi.org/10.1038/s41568-020-00302-4

144. Barrett, M., Hand, C. K., Shanahan, F., Murphy, T., & O'Toole, P. W. (2020). Mutagenesis by Microbe: the Role of the Microbiota in Shaping the Cancer Genome. *Trends in Cancer Research*, *6*(4), 277–287. https://doi.org/10.1016/j.trecan.2020.01.019

145. Gur, C., Ibrahim, Y., Isaacson, B., Yamin, R., Abed, J., Gamliel, M., Enk, J., Bar-On, Y., Stanietsky-Kaynan, N., Coppenhagen-Glazer, S., Shussman, N., Almogy, G., Cuapio, A., Hofer, E., Mevorach, D., Tabib, A., Ortenberg, R., Markel, G., Miklić, K., … Mandelboim, O. (2015). Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity*, *42*(2), 344–355. https://doi.org/10.1016/j.immuni.2015.01.010

146. Dunn, G. P., Bruce, A. T., Ikeda, H., Old, L. J., & Schreiber, R. D. (2002). Cancer immunoediting: from immunosurveillance to tumor escape. *Nature Immunology*, *3*(11), 991–998. https://doi.org/10.1038/ni1102-991

147. Jourdain, A., Duchmann, M., Willekens, C., Solary, É., Perié, L., & Laplane, L. (2021). Évolution clonale: Qu'est-ce qu'un clone. *La Biologie Au Défi de L'histoire; Chapitre*, *10*, 167–188. https://www.cairn.info/load_pdf.php?ID_ARTICLE=EDMAT_LOISC_2021_01_0167&download=1

148. Fluckiger, A., Daillère, R., Sassi, M., Sixt, B. S., Liu, P., Loos, F., Richard, C., Rabu, C., Alou, M. T., Goubet, A.-G., Lemaitre, F., Ferrere, G., Derosa, L., Duong, C. P. M., Messaoudene, M., Gagné, A., Joubert, P., De Sordi, L., Debarbieux, L., … Zitvogel, L. (2020). Cross-reactivity between tumor MHC class I–restricted antigens and an enterococcal bacteriophage. *Science*. https://science.sciencemag.org/content/369/6506/936.abstract

149. Bessell, C. A., Isser, A., Havel, J. J., Lee, S., Bell, D. R., Hickey, J. W., Chaisawangwong, W., Glick Bieler, J., Srivastava, R., Kuo, F., Purohit, T., Zhou, R., Chan, T. A., & Schneck, J. P. (2020). Commensal bacteria stimulate antitumor responses via T cell cross-reactivity. *JCI Insight*, *5*(8). https://doi.org/10.1172/jci.insight.135597

150. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science*, *284*(5423), 2124–2129. https://doi.org/10.1126/science.284.5423.2124

151. Doolittle, W. F., & Bapteste, E. (2007). Pattern pluralism and the Tree of Life hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(7), 2043–2049. https://doi.org/10.1073/pnas.0610699104

152. O'Malley, M. A., & Koonin, E. V. (2011). How stands the Tree of Life a century and a half after The Origin? *Biology Direct*, *6*(1), 32. https://doi.org/10.1186/1745-6150-6-32

153. Byndloss, M. X., Olsan, E. E., Rivera-Chávez, F., Tiffany, C. R., Cevallos, S. A., Lokken, K. L., Torres, T. P., Byndloss, A. J., Faber, F., Gao, Y., Litvak, Y., Lopez, C. A., Xu, G., Napoli, E., Giulivi, C., Tsolis, R. M., Revzin, A., Lebrilla, C. B., & Bäumler, A. J. (2017). Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science*,

357(6351), 570–575. https://doi.org/10.1126/science.aam9949

154. Butler, D. S. C., Cafaro, C., Putze, J., Wan, M. L. Y., Tran, T. H., Ambite, I., Ahmadi, S., Kjellström, S., Welinder, C., Chao, S. M., Dobrindt, U., & Svanborg, C. (2021). A bacterial protease depletes c-MYC and increases survival in mouse models of bladder and colon cancer. *Nature Biotechnology*, *39*(6), 754–764. https://doi.org/10.1038/s41587-020-00805-3

155. Kusters, J. G., van Vliet, A. H. M., & Kuipers, E. J. (2006). Pathogenesis of Helicobacter pylori infection. *Clinical Microbiology Reviews*, *19*(3), 449–490. https://doi.org/10.1128/CMR.00054-05

156. Suerbaum, S., & Josenhans, C. (2007). Helicobacter pylori evolution and phenotypic diversification in a changing host. In *Nature Reviews Microbiology* (Vol. 5, Issue 6, pp. 441–452). https://doi.org/10.1038/nrmicro1658

157. Anandasabapathy, S., Jhamb, J., Davila, M., Wei, C., Morris, J., & Bresalier, R. (2007). Clinical and endoscopic factors predict higher pathologic grades of Barrett dysplasia. *Cancer*, *109*(4), 668–674. https://doi.org/10.1002/cncr.22451

158. Islami, F., & Kamangar, F. (2008). Helicobacter pylori and Esophageal Cancer Risk: A Meta-analysis. In *Cancer Prevention Research* (Vol. 1, Issue 5, pp. 329–338). https://doi.org/10.1158/1940-6207.capr-08-0109

159. Petrova, V., Annicchiarico-Petruzzelli, M., Melino, G., & Amelio, I. (2018). The hypoxic tumour microenvironment. *Oncogenesis*, *7*(1), 10. https://doi.org/10.1038/s41389-017-0011-9

160. Rundell, E. A., Banta, L. M., Ward, D. V., Watts, C. D., Birren, B., & Esteban, D. J. (2014). 16S rRNA gene survey of microbial communities in Winogradsky columns. *PloS One*, *9*(8), e104134. https://doi.org/10.1371/journal.pone.0104134

161. Hoshino, T., Doi, H., Uramoto, G.-I., Wörmer, L., Adhikari, R. R., Xiao, N., Morono, Y., D'Hondt, S., Hinrichs, K.-U., & Inagaki, F. (2020). Global diversity of microbial communities in marine sediment. *Proceedings of the National Academy of Sciences of the United States*

of America, *117*(44), 27587–27597. https://doi.org/10.1073/pnas.1919139117

162. Fenchel, T., & Finlay, B. (2008). Oxygen and the spatial structure of microbial communities. *Biological Reviews of the Cambridge Philosophical Society*, *83*(4), 553–569. https://doi.org/10.1111/j.1469-185X.2008.00054.x

163. Semenza, G. L. (2008). Tumor metabolism: cancer cells give and take lactate [Review of *Tumor metabolism: cancer cells give and take lactate*]. *The Journal of Clinical Investigation*, *118*(12), 3835–3837. https://doi.org/10.1172/JCI37373

164. Bahram, M., Hildebrand, F., Forslund, S. K., Anderson, J. L., Soudzilovskaia, N. A., Bodegom, P. M., Bengtsson-Palme, J., Anslan, S., Coelho, L. P., Harend, H., Huerta-Cepas, J., Medema, M. H., Maltz, M. R., Mundra, S., Olsson, P. A., Pent, M., Põlme, S., Sunagawa, S., Ryberg, M., … Bork, P. (2018). Structure and function of the global topsoil microbiome. *Nature*, *560*(7717), 233–237. https://doi.org/10.1038/s41586-018-0386-6

165. Anderson, A. R. A., Weaver, A. M., Cummings, P. T., & Quaranta, V. (2006). Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. *Cell*, *127*(5), 905–915. https://doi.org/10.1016/j.cell.2006.09.042

166. Damaghi, M., West, J., Robertson-Tessi, M., Xu, L., Ferrall-Fairbanks, M. C., Stewart, P. A., Persi, E., Fridley, B. L., Altrock, P. M., Gatenby, R. A., Sims, P. A., Anderson, A. R. A., & Gillies, R. J. (2021). The harsh microenvironment in early breast cancer selects for a Warburg phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(3). https://doi.org/10.1073/pnas.2011342118

167. Frankenstein, Z., Basanta, D., Franco, O. E., Gao, Y., Javier, R. A., Strand, D. W., Lee, M., Hayward, S. W., Ayala, G., & Anderson, A. R. A. (2020). Stromal reactivity differentially drives tumour cell evolution and prostate cancer progression. *Nature Ecology & Evolution*, *4*(6), 870–884. https://doi.org/10.1038/s41559-020-1157-y

168. Salehi, S., Kabeer, F., Ceglia, N., Andronescu, M., Williams, M. J., Campbell, K. R., Masud, T., Wang, B., Biele, J., Brimhall, J., Gee, D., Lee, H., Ting, J., Zhang, A. W., Tran,

H., O'Flanagan, C., Dorri, F., Rusk, N., de Algara, T. R., … Shah, S. P. (2021). Clonal fitness inferred from time-series modelling of single-cell cancer genomes. *Nature*, *595*(7868), 585–590. https://doi.org/10.1038/s41586-021-03648-3

169. Temko, D., Cheng, Y.-K., Polyak, K., & Michor, F. (2017). Mathematical Modeling Links Pregnancy-Associated Changes and Breast Cancer Risk. *Cancer Research*, *77*(11), 2800–2809. https://doi.org/10.1158/0008-5472.CAN-16-2504

170. Williams, M. J., Werner, B., Heide, T., Curtis, C., Barnes, C. P., Sottoriva, A., & Graham, T. A. (2018). Quantification of subclonal selection in cancer from bulk sequencing data. *Nature Genetics*, *50*(6), 895–903. https://doi.org/10.1038/s41588-018-0128-6

171. Avanzini, S., & Antal, T. (2019). Cancer recurrence times from a branching process model. *PLoS Computational Biology*, *15*(11), e1007423. https://doi.org/10.1371/journal.pcbi.1007423

172. Curtius, K., Dewanji, A., Hazelton, W. D., Rubenstein, J. H., & Luebeck, G. E. (2021). Optimal Timing for Cancer Screening and Adaptive Surveillance Using Mathematical Modeling. *Cancer Research*, *81*(4), 1123–1134. https://doi.org/10.1158/0008-5472.CAN-20-0335

173. Schwartz, R., & Schäffer, A. A. (2017). The evolution of tumour phylogenetics: principles and practice. *Nature Reviews. Genetics*, *18*(4), 213–229. https://doi.org/10.1038/nrg.2016.170

174. Robertson-Tessi, M., Gillies, R. J., Gatenby, R. A., & Anderson, A. R. A. (2015). Impact of metabolic heterogeneity on tumor growth, invasion, and treatment outcomes. *Cancer Research*, *75*(8), 1567–1579. https://doi.org/10.1158/0008-5472.CAN-14-1428

175. Ghaffarizadeh, A., Heiland, R., Friedman, S. H., Mumenthaler, S. M., & Macklin, P. (2018). PhysiCell: An open source physics-based cell simulator for 3-D multicellular systems. *PLoS Computational Biology*, *14*(2), e1005991. https://doi.org/10.1371/journal.pcbi.1005991

176. Beerenwinkel, N., Antal, T., Dingli, D., Traulsen, A., Kinzler, K. W., Velculescu, V. E.,

Vogelstein, B., & Nowak, M. A. (2007). Genetic progression and the waiting time to cancer. *PLoS Computational Biology*, *3*(11), e225. https://doi.org/10.1371/journal.pcbi.0030225

177. Tomasetti, C., Vogelstein, B., & Parmigiani, G. (2013). Half or more of the somatic mutations in cancers of self-renewing tissues originate prior to tumor initiation. In *Proceedings of the National Academy of Sciences* (Vol. 110, Issue 6, pp. 1999–2004). https://doi.org/10.1073/pnas.1221068110

178. Curtius, K., Hazelton, W. D., Jeon, J., & Georg Luebeck, E. (2015). A Multiscale Model Evaluates Screening for Neoplasia in Barrett's Esophagus. In *PLOS Computational Biology* (Vol. 11, Issue 5, p. e1004272). https://doi.org/10.1371/journal.pcbi.1004272

179. Reiter, J. G., Makohon-Moore, A. P., Gerold, J. M., Bozic, I., Chatterjee, K., Iacobuzio-Donahue, C. A., Vogelstein, B., & Nowak, M. A. (2017). Reconstructing metastatic seeding patterns of human cancers. *Nature Communications*, *8*, 14114. https://doi.org/10.1038/ncomms14114

180. Archetti, M., Ferraro, D. A., & Christofori, G. (2015). Heterogeneity for IGF-II production maintained by public goods dynamics in neuroendocrine pancreatic cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(6), 1833–1838. https://doi.org/10.1073/pnas.1414653112

181. Basanta, D., Scott, J. G., Fishman, M. N., Ayala, G., Hayward, S. W., & Anderson, A. R. A. (2012). Investigating prostate cancer tumour-stroma interactions: clinical and biological insights from an evolutionary game. *British Journal of Cancer*, *106*(1), 174–181. https://doi.org/10.1038/bjc.2011.517

182. Nichol, D., Rutter, J., Bryant, C., Hujer, A. M., Lek, S., Adams, M. D., Jeavons, P., Anderson, A. R. A., Bonomo, R. A., & Scott, J. G. (2019). Antibiotic collateral sensitivity is contingent on the repeatability of evolution. *Nature Communications*, *10*(1), 334. https://doi.org/10.1038/s41467-018-08098-6

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

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CONFLICTS OF INTEREST

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

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