Trends

Advances in research and medical practices have made significant inroads towards the treatment of diseases at the single patient level. This paradigm, precision medicine, holds the promise of reducing adverse effects, improving preventative care, and reducing costs by tailoring individual treatment based on highly detailed diagnostics.

Humans harbor trillions of microbes, termed the microbiome, which is now being appreciated as being a hugely substantial facet of health. Immune, metabolic, neurological, and other processes impact and are impacted by the microbiome.

The microbiome not only is a significant factor in health, but it is one that can be both readily assayed through DNA sequencing and directly modified by various targeted interventions. Therefore, the currently genetic information dominated field of precision medicine would be greatly enhanced by the introduction of the microbiome.

Introducing the Microbiome into Precision Medicine

Thomas M. Kuntz[1,2] & Jack A. Gilbert[2,3,4]

[1] Department of Chemistry, University of Chicago, 929 East 57th Street, Chicago, Illinois 60637, USA

[2] The Microbiome Center, Department of Surgery, University of Chicago, 5841 S Maryland Ave, Chicago, IL, U.S.A

[3] Biosciences Division (BIO), Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL, U.S.A

[4] Marine Biological Laboratory, Woods Hole, MA, U.S.A

*Correspondence: gilbertjack@uchicago.edu (J.A. Gilbert)

Abstract

Understanding how individual people respond to medical therapy is a key facet of improving the odd-ratio that interventions will have a positive impact. Reducing the non-responder rate for an intervention or reducing complications associated with a particular treatment or surgery is the next stage of medical advance. The Precision Medicine Initiative, launched in January 2015, set the stage for enhanced collaboration between researchers and medical professionals to develop next-generation techniques to aid patient treatment and recovery, and increased the opportunities for impactful preemptive care. The microbiome plays a crucial role in health and disease, as it influences endocrinology, physiology and even neurology, altering the outcome of many different disease states, and it augments drug responses and tolerance. We review the implications

of the microbiome on precision health initiatives and highlight excellent examples, whereby precision microbiome health has been implemented.

Introduction to Precision Medicine

The sequencing of the human genome [1] in 2001 fostered advances in both our understanding of the genomic basis of disease and in the DNA sequencing technologies required to bring the results of this understanding to patients. This is often referred to as precision genomic medicine, which utilizes a patient's individual genome to inform treatment and care, based on known genomic markers for disease [2]. The broader, inclusive field of precision medicine couples a person's treatment with what is known about their population, life style, and medical history, by matching clinical data and genetic biomarkers. Since the genome is sometimes conceptualized as the core of human individuality, at least in terms of disease, the broader field of precision medicine is often conflated with genomic medicine. Precision medicine, however, includes aspects downstream from the genome, including gene expression and protein expression as well as metabolic markers. Nonetheless, genomic information is the most commonly used and has had great successes [3]. Cancer treatment in particular has been revolutionized by genomic medicine [4], which exemplifies that despite difficulties in implementing precision medicine, it is a deeply important development. In particular, achieving the goals of precision medicine, including diagnosing disease more accurately and reducing the relative risk of treatments, side effects, and non-responses to medications, will revolutionize both treatment courses — ideally at the single patient level [5] — and the structuring of medical care and costs, moving towards cheaper, preventative focused medicine.

The Microbiome as a Precision Medicine Frontier

In this review we focus on a more recent but in many ways analogous development, that of introducing the microbiome into precision medicine. The human microbiome is the "the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space" [6]. These microorganisms, mainly bacteria, fungi, archaea, and viruses in the gastrointestinal tract, are slightly more abundant than the human cells in the body, leading some to classify them as an newly discovered organ [7]. It is important to note however that the microbiome is compositionally and spatiotemporally far more fluid and mutable than human cells and organs. Therefore, the microbial "organ" may be better described as a "cloud" of genetic information accessory to the stable human genome [8]. Certainly, the influences of the microbiome on our physiology are significant and multitudinous, affecting immunology [9], neurology [10,11], endocrinology [12], and, importantly for precision medicine, disease states and clinical outcomes. Because microbiome science is a nascent but quickly developing field, additional important functions of the microbiome are likely still to be discovered. These discoveries are driven by similar sequencing technology as that which has enabled personal genomics, and this technology is decreasing rapidly in price [13], so much so that personal microbiome sequencing is already available to the consumer (e.g. American Gut - americangut.org; uBiome - ubiome.com). Furthermore, the well-developed analysis and statistical techniques of genomic medicine have commonalities with microbiome analysis. Since microbiome states are highly individual even between co-raised identical twins [14], but can be rapidly changed [15] (unlike genetics), there is a profound opportunity for individualized treatments. However, the microbiome, like any ecosystem is also profoundly complex, and so the goals of precision microbial medicine require

considerably more research before they are appropriately realized [16]. Nonetheless, the microbiome, as we shall exemplify here, is primed and ready for precision medicine, and therefore the clinical application of this new therapeutic area is on the immediate horizon. Various complementary routes of assaying and modifying the microbiome have been proposed and tentatively utilized towards this end; these will be laid out here in the following text as well as diagrammatically (Figure 1).

Review of Microbiome Analysis Techniques

How then could microbiome precision medicine be implemented? Currently two complementary analyses, both beginning with the extraction of microbial genomic DNA, are standard in the field: 16S rRNA sequencing and shotgun metagenomics. The 16S rRNA gene has both highly conserved regions, allowing for the usage of extremely bacterially nonspecific primers, and "hypervariable" regions, where base pair differences can often provide species level identification [17]. Thus, 16S rRNA amplicon sequencing provides a robust tool for identification as well as classification and even discovery of bacteria [18]. A typical 16S rRNA study utilizes the differences in observed communities of bacteria between differing samples to obtain statistically significant correlations between bacterial composition and sample description, for example to identify differences in the gut microbiomes of children born to obese mothers [19]. These studies have led to key insights into the human microbiome. While historically the majority of biomedical research on bacteria has focused on eliminating pathogens, many bacteria as well as communities of bacteria are important in both health and disease [6]. Though identifying causative bacteria in disease states will be an important facet of precision

medicine, understanding the overall ecology of the microbiome may be equally or even more vital.

Therefore, to go beyond bacterial identification and subsequent, limited patient stratification, it will be essential to understand the functional potential of the microbiome. Shotgun metagenomics enables the researcher to understand this function potential through analysis of the complete genomic repertoire of the community, by sequencing DNA extracted from that community, rather than relying on amplification of a marker gene. Taxonomy can still be determined from signature genes (including 16S rRNA), but it is also possible to assign phylogeny of the functional genes by comparing the DNA sequence against a library of genomes from close relatives [20]. In addition, metagenomics enables the assembly of genomes from organisms in the microbiome that are resistant to culture, providing a higher resolution exploration of the taxa associated with each person [21]. This enables us to determine the metabolic and signaling capacity of each taxon, to determine how it will interact with the rest of the body [22]. This clearly makes metagenomics of great interest for the development of precision medicine; however, one must be aware of the challenges this technique presents. Metagenomic studies are necessarily more expensive and computationally complex than 16S rRNA based studies. Possible contamination from undesired DNA and biases of analyses towards culturable organisms [23] further complicate matters. Ultimately metagenomics is an extremely useful tool, but the application of this technology to precision medicine will require a better understanding of the implications of these limitations, especially when scaling up to treatments of large patient populations.

Notably, both 16S sequencing and shotgun metagenomics are currently somewhat blunt tools, especially when describing the fluid nature of the microbiome. Evolution of microorganisms, horizontal transfer of genes, and subtleties in the characterization into types of microbiomes [24] problematize the microbiome snapshot style data often acquired. As sequencing costs continue to decrease, however, scientists can sample more densely in time to capture previously unobservable subtleties in microbial interactions and utilize time series techniques to uncover dynamic ecological phenomena [25]. Additionally, the gut microbiome is known to be spatially inhomogeneous, in ways that influence function and disease states [26]. This limitation too might be surpassed in the near future, owing to emerging sampling techniques and protocols (e.g., laser microdissection of colonic crypt mucus [27]).

Avenues Towards Microbiome-Based Precision Therapies

Microbiome-xenobiotic interactions

That gene polymorphisms can drive changes in drug metabolism has been known for some time; it was noted as early as 1957 that atypical forms of serum cholinesterase led to potentially fatal reactions to certain anesthetics [28]. This and other adverse drug reactions are estimated to cost from 30 to 130 billion dollars in the USA annually [29,30] and are a significant source of patient non-compliance and therapy failure [31]. Reducing these adverse reactions is a primary goal of precision medicine. While some interactions are idiosyncratic, a recent survey of adverse drug events observed that about 35% of these events were drug-gene or drug-drug-gene interactions involving cytochrome P450

oxidase (CYP) variants [32]. CYPs are generally considered the body's innate and primary general purpose drug metabolizers; they are involved in about 75% of total human drug modification [33].

However, microbial metabolism in the gut is also a significant factor in biotransformation, especially for low solubility, low permeability compounds [34]. Currently, more than 60 drugs have been identified to have microbiome interactions according to the PharmacoMicrobiomics database [35], and given the vast number of possible unique microbial metabolic transformations [36], many more interactions are likely to be discovered compared with the apparently relatively limited number of human genetic interactions. The plasticity of the microbiome may make these interactions dynamic, necessitating precision medicine that is not only patient specific but temporarily appropriate [37]. Importantly, the primary forms of xenobiotic metabolism are different between human and bacterial cells: oxidation and conjugation dominate in the former case, reduction and hydrolysis in the latter [34]. Metabolism of drugs is actually a key component of many therapies; so-called "prodrugs" are essentially drugs that will be metabolized into a pharmacologically active drug after consumption. Therefore, production of active drug metabolites from prodrugs is sometimes dependent on the microbiome, with the possibility to either improve or worsen outcomes [38]. This often manifests as a modulation of bioavailability to the human, an important consideration for prediction of appropriate dosing in precision medicine. Efficacy and side effects are also altered directly by microbial metabolism. For example, acetaminophen toxicity shows substantial variability within a given human population [39], and the microbiome has

been identified as playing a role in this variability. Members of the genus *Clostridium*, as well as other bacteria can produce *p*-cresol, which competes as a substrate for SULT1A1 (a human liver enzyme) with acetaminophen [40]. A reduction in the breakdown of acetaminophen by SULT1A1 causes a build-up of NAPQI, which leads to hepatotoxicity. This general pattern of competition between bacterial metabolites and drugs for human enzyme modification constitutes a major challenge in pharmacology [41]. Directly harmful substances can also be formed by microbiota, as is the case in bacterial β-Glucuronidase mediated diarrhea in response to an antitumor camphothecin derivative [42]. Strikingly, in some cases even strain level differences can lead to altered metabolism, such as inactivation of digoxin by a non-universal *E. lenta* gene. Digoxin has a narrow therapeutic window, and thus a wrong dosage could lead to significant toxicity, highlighting the need for further study of metagenomic diagnostics and insights to adverse outcomes [43].

Furthermore, alternative mechanisms for xenobiotic-microbiome interaction including immune [9,44,45] and endocrine [12] modulation by bacteria are known to exist, complicating and enlarging the pool of possible drug-microbiome interactions. Lastly, there are possible reciprocal relations: drugs may both be altered by the microbiome and alter the microbiome. For example, antipsychotic medication has been shown to both alter the microbiome and have microbiome-dependent side effects [46]. While this greatly complicates endeavors to understand microbiota-xenobiotic interactions, it also points towards a different microbiome driven approach to precision medicine: directly targeting the microbiome for clinical results.

187

188 *Targeting the microbiome*

189 It is clear that medication is already utilized to have a direct effect on the microbiome;
190 one needs to look no further than antibiotics. While these drugs are utilized for the
191 eradication of pathogenic bacteria, they have widespread effects on the microbiome,
192 possibly leading to adverse outcomes. Secondary infections caused by antibiotics are well
193 known, most saliently *Clostridium difficile* [47], but it is often less appreciated that
194 antibiotics can have side effects on the human, for instance fluoroquinolone associated
195 cardiotoxic [48] and neuropsychiatric [49] reactions. Importantly, consequences of
196 antibiotic usage, such as reduction of inflammation, are possibly not only human off-
197 target drug effects, but also unintended consequences of microbial community disruption
198 [50]. Studies using mouse models suggest that stress induced increases in circulating
199 cytokines were abrogated by broad-spectrum antibiotic treatment [51]. Furthermore,
200 these types of interactions are not limited to drugs classified as antibiotics; many other
201 drugs have antibiotic and other microbial community structure and function modulating
202 properties that are beginning to be appreciated [52,53]. While many of these
203 perturbations to the microbiome are associated with poorer outcomes, some drugs may
204 derive some or all of their beneficial qualities from alteration of the microbiome, thus
205 they could be considered a form of discriminatory antibiotic.

206

207 A precision medicine therapy that leverages microbial community structural modulation
208 could have beneficial clinical impact. Certainly if pathogen-specific antibiotics were
209 developed, the odds ratio could be greatly increased compared to traditional antibiotics. A

clear approach is to design a species-specific enzyme inhibitor or other antimicrobial molecules. For example, a *Streptococcus mutans*-targeted drug based on the fusion of a species-specific targeting peptide domain with a wide-spectrum antimicrobial peptide domain has already been developed [54]. However, the bacterial community was also altered when using this peptide, despite its high specificity [55]. This is likely because the environment of *Streptococcus mutans*, the oral microbiome, presents significant structural and functional complexity [56]. It has been suggested that targeted antibiotics may shift the microbiome into a healthier state, but of course there is also the potential for negative ecological effects, although these may be less than for traditional antibiotics.

An intriguing approach that may largely avoid the problem of system scale changes in microbial community structure, as well as that of increasing antimicrobial resistance, is to non-lethally target specific enzymes in the bacteria. This has been realized at the multi-species level [57] through targeted inhibition of bacterial tri-methyl amine (TMA) formation by 3,3-Dimethyl-1-butanol (DMB, a structural analog of choline) ultimately attenuating atherosclerosis in a high choline diet mouse model. Surprisingly, slight alterations of bacterial composition were still observed, underscoring the extremely dynamic nature of the microbiome. Nonetheless, this study points towards a microbiome-based intervention for a specific (i.e., "Western") diet-driven disease. In this case, a single target approach is undesirable, as reduction of global TMA formation is the goal, but given the availability of single isozyme inhibitors [58], precision, non-lethal drugs likely could be developed. These furthermore have the potential to be minimally bioavailable to the human, limiting side effects, and might be exploited not only to target

pathogens but also to reduce microbiota-drug interactions through selective elimination of problem microbes.

A final approach for targeted antimicrobials has been successfully employed for approximately 100 years, though not as popularly in the western world [59]. Phages were independently discovered in France and England, though developed as a therapy first in the former. Despite great successes in treatment, especially of cholera, commercialization of phage therapy failed due to production problems and other complications and so was subsequently ignored in the US and Europe after the development of antibiotics [60]. Scientists in the Soviet Union (especially Georgia) continued to develop phage therapy, having been cut off from antibiotic advances due to World War II. Here it was effectively used it to control outbreaks of gastrointestinal diseases and refined further during the Cold War and afterwards [61]. The basic premise of this technique is that many bacterial species, and maybe even each strain (sub-species), are predated upon by a unique phage [62]. Phage target bacteria cell-membrane protein and sugar complexes that are unique to each bacterial taxon. Therefore, by identifying the correct phage it should be possible to precisely remove a specific bacterial species from an assemblage. This will enable accurate restructuring of a microbiome so as to precisely augment the functional properties of that consortium. In fact, recent evidence from the commercial sector suggests that the same mechanisms employed by phages to target and penetrate bacterial cells can be programmed into nano-particles that mimic these phage-properties to infect and kill specific cells (Pers. Comm. Jeffrey Miller, UCLA). In this new future, we may have ultimate control over the microbiome.

Prebiotic treatments

Conversely, instead of targeting the microbiome to reduce deleterious bacteria, one could aim to increase the levels of beneficial bacteria or otherwise positively alter the structure or function of the microbiome. Substances applied in this way are often referred to as prebiotics. However, the types of prebiotics currently studied are limited in scope, usually non-digestible fiber compounds that stimulate growth of *Bifidobacterium* and other taxa to produce short chain fatty acids (SCFA) including butyrate and propionate [63]. Though this is promising as a broad treatments for several conditions [64], efforts for precision medicine in this sphere will require the expansion of the scope of prebiotics. Given that metagenomic and metabolomic advances continue to better characterize the metabolic potential of the microbiome, especially across groups with vastly different diets [65], prebiotic compounds that stimulate alternative beneficial bacteria towards useful metabolic endpoints will be discovered [66].

More audaciously, one might aim at fine-tuning the interactions between microbiota of the gut microbiome. The microbiome is a complex, human co-evolved ecosystem that produces many bioactive compounds, often for intercellular communication [26]. These compounds could be mined to find those which modulate the microbiome in a beneficial way, thus unearthing novel prebiotics [67]. While microbial community disruption is the consequence of both xenobiotic and microbiome targeted drug metabolism, these types of prebiotics might provide a more gentle perturbation than possible with the former by harnessing already existing biological pathways. This goal certainly seems distant, but as

dynamical systems approaches to studying the microbiome continue to develop, we may find that treating certain dysbiotic states require perturbations of varying magnitudes or delicate maintenance of the stability of the microbiome, especially in at-risk populations [68].

Precision probiotics

Perhaps the most direct strategy for altering the microbiome is the usage of probiotics, live microbes administered for health benefits. This idea has been employed since at least 1907 when Élie Metchnikoff hypothesized lactic acid producing bacteria could implant in the gastrointestinal tract to enhance longevity [69]. Today the probiotic landscape is still dominated by lactic acid bacteria, specifically genera *Lactobacillus*, though it is now appreciated that their beneficial properties are not limited to the production of a single metabolite and that other potential probiotic bacteria, perhaps isolated from healthy individuals [70], could affect various outcomes through multifarious means [71]. This opens the door to precision probiotic development since application of microorganisms is highly specific with regards to both applied agent and effect. Devices now exist for isolating microorganisms based on metabolic output [72], and work is being done to identify probiotic bacteria that produce particular compounds of therapeutic potential [73]. This may include compounds whose efficacies are contingent on route of administration, for example those that are inactive orally. Furthermore, probiotics are being bioengineered to expand their ranges and modes of actions as well as their robustness and incorporation [74]. However, it is important to keep in mind that interactions with diet, established microbiota, and genetics, are known to modulate

overall health outcomes if not specific effects and mechanisms of probiotics [71]. Therefore effective patient classification and stratification is required for best results. Success of this program will require detailed insights into metagenomic potential and ecological interactions of presumptive probiotic bacteria, making precision probiotic development a task of considerable difficulty but one that has already seen demonstrable results, for example in enhancing resistance to *Clostridium difficile* infection [75] and suppressing hepatocellular carcinoma growth in mice [76].

Regulation and Application

Despite the therapeutic promise of the microbiome, its application to precision medicine requires overcoming considerable hurdles. One may anticipate that failure to successfully apply genomic medicine may lead to delays in the application of the microbiome as a precision therapy. For example, the current legal and R&D model is not well suited for development of genome-informed drugs [77]. Microbiome therapies likewise face difficulties, especially owing to the wide breadth of treatment options, many of which lack analogs in current medical practice. Furthermore, clinicians have been reticent to use the results of genomic information — and thus likely future microbiome data — in treatment due to both uncertainties on its importance and lack of understanding [78]. These problems are highlighted in the case of Plavix® (clopidogrel), whereby despite an FDA box warning [79] indicating serious or fatal risk for those carrying certain CYP2C19 variants, this drug is still routinely used on genetically incompatible patients due to poor coverage by insurance and failure to clinically utilize genetic testing [80]. In the case of the microbiome, fecal transplant treatment for *Clostridium difficile* colitis is

known to be highly effective especially in recurrent infection [81]; however, this procedure still requires a licensed practitioner to have a protocol approved by their local Institutional Review Board, and therefore each patient needs to be consented prior to therapy. For a therapy with >90% success rate this is peculiar. However, it is because we still lack the ability to characterize the microbial community of donor stool appropriately. This means that we do not know the active components of the fecal transplant, and therefore it is very difficult to regulate this using standard legislation under FDA protocols. More importantly, we still don't fully understand the implications for microbiome therapy on a large scale. While fecal transplants are becoming extremely numerous with few legitimate side effects, it is still hard to predict the outcome across a broad population. The same is true for genomic medicine, whereby the interaction of genes with the environment is difficult to predict [82]. This requires enormous sample populations for any investigation to be statistically significant [83]. Though the future is bright for genomic medicine, particular issues currently impede efforts towards its development.

Fortunately, some of the difficulties in genomic medicine research and deployment might be lessened in precision microbiome medicine. Environmental-microbiome interactions are potentially more easily studied because there is a more direct interaction between the two, allowing for simpler identification of sample populations and achievement of statistical power. With the correct experimental design, genetic variation can be sufficiently decoupled from microbiome and environmental factors. In fact, studies of this nature already exist, both on humans [84] and especially on mice, where genetics can

348 be well controlled [85]. This bottom-up approach can then be extended by genomic
349 studies which better account for confounding factors. Even where genetics is a significant
350 factor, such as in mental health disorders, incorporating the microbiome greatly increases
351 understanding and ultimately treatment of diseases [86]. Of course in disease states where
352 the effects of genetic variation are either entirely or nearly absent, the microbiome is a
353 great candidate for investigation. Conditions such as obesity [87] and inflammatory
354 bowel disease [88] can in subsets of patients be driven by dysbiosis, a chronic, systemic
355 maladaptation of the gut microbiome to the host. Unlike genomic medicine, there are
356 possibilities especially for these conditions to do research in an *in vitro* environment,
357 most excitingly in artificial gut paradigms [89]. Microbiome precision medicine also has
358 the opportunity to break free of present R&D and legal hurdles to precision medicine.
359 The regulation and marketing of these treatments will at least pose challenges for
360 traditional models [90,91], as evidenced by the FDA's current stance on probiotics [92],
361 which has led to faster product delivery to the public but also quality control and
362 effectiveness issues [93]. Prioritizing treatments will be an important aspect of achieving
363 R&D, FDA, and ultimately clinician support; unnecessary testing on low risk
364 communities and for low benefit interventions, will only hamper the development of
365 microbiome precision medicine.
366
367 **Notable Application: Medically Underserved Communities**
368 Given the above unique assets of the microbiome modality of precision medicine, a
369 promising potential area for its development is in low socio-economic status (SES) and
370 other under-served communities. Low SES is associated with reduced diversity in the gut

371 microbiome [94]. Numerous factors are also present especially in urban communities that
372 reduce immunoregulation, including reduced exposure to microbes in the natural
373 environment [95] and increased stress [96], and increase obesity prevalence and
374 dysbiosis, including increased density of fast-food restaurants [97] and lack of physical
375 activity [98]. This is likely interrelated with microbiome-associated diseases such as
376 asthma [99] and gastrointestinal symptoms [100]. The vast majority of genomic variants
377 discovered are either rare with large effects or common with small effects, unlike in this
378 situation where there is a possibility of appreciable effect size combined with biomarker
379 occurrence. Therefore, these at-risk communities present a potentially illuminating cohort
380 for microbiome.
381
382 Of course, great care must be taken to not draw inappropriate or invalid associations
383 between microbiome [101] (or genome [102]) variations and minority status. Lack of
384 cultural understanding and disparities in access to services have driven poor research
385 trends in the past and continue to be a deep issue in the development of precision
386 medicine. Access issues in particular have caused demonstrable problems; statistics on
387 epidermal growth factor receptor testing, for example, show associations of lower
388 educational attainment and income with reduced likelihood of testing [103], and studies
389 suggest health insurance coverage alone does not explain this general effect [104,105].
390 For precision medicine to succeed then, under-served populations must be both active
391 participants and beneficiaries of research. Microbiome research in particular could lead to
392 high impact clinical interventions for these communities, hopefully spurring its
393 development. It is both an opportunity and imperative for microbiome precision medicine

394 to address social epidemiological trends, but this is only possible through the combined
395 efforts of researchers, clinicians, the government, and perhaps most importantly the
396 people at large.
397
398 **Concluding Remarks**
399 Here we have presented a collection of potential avenues towards introducing the
400 microbiome into precision medicine. Though it is difficult to know if and when these
401 techniques will ultimately make it to the clinic (see Outstanding Questions), there is
402 substantial evidence that microbiome-based medicine holds great future potential to
403 improve odds-ratios, reduce side effects, stratify patients, and precisely treat previously
404 difficult or untreatable conditions. Ultimately, the microbiome must become an integral
405 part of precision medicine as a whole, since so much of human functioning and
406 metabolism is dependent upon it. If this is to happen in the near future, as it hopefully
407 should, we must better understand the microbiome and its interactions with the human
408 and the environment via a concerted effort and conversation between researchers,
409 clinicians, patients, the government, and most importantly, the broader community.
410
411 **Figure 1**
412 A schematic of methods in precision microbiome medicine and their possible interplay:
413 a) As an example, certain microbes, here represented in red, metabolize the compound
414 cycasin to produce a carcinogenic compound methylazoxymethanol (MAM) [106]. This
415 functional potential of the microbe might be discovered through metagenomic
416 sequencing. b) If targeted removal of the red microorganism — identified in a patient via

16S sequencing — was desired, without harming commensal bacteria, represented in shades of blue, three approaches (green arrows) might be utilized. Direct removal of the deleterious microorganism through targeted antibiotics ideally would not affect commensal bacteria. Probiotic treatment introduces new beneficial microorganisms while prebiotic treatment favors the growth of existing beneficial microorganisms. Note that prebiotic and probiotic treatments do not directly remove the targeted microorganism, but in certain cases may shift the gut ecology such that it does not thrive [107]. In all three cases, the specific circumstances may affect which treatment is best employed and what residual outcomes there are on the microbiome.

References

1 Venter, J.C. *et al*. (2001) The sequence of the human genome. *Science* 291, 1304–51

2 Guttmacher, A.E. *et al*. (2002) Genomic Medicine — A Primer. *N*. *Engl*. *J*. *Med*. 347, 1512–1520

3 McCarthy, J.J. *et al*. (2013) Genomic Medicine: A Decade of Successes, Challenges, and Opportunities. *Sci*. *Transl*. *Med*. 5, 189sr4-189sr4

4 Garraway, L.A. *et al*. (2013) Precision Oncology: An Overview. *J*. *Clin*. *Oncol*. 31, 1803–1805

5 Schork, N.J. (2015) Personalized medicine: Time for one-person trials. *Nature* 520, 609–611

6 Lederberg, J. (2000) Infectious history. *Science* 288, 287–293

7 Sender, R. *et al*. (2016) Are We Really Vastly Outnumbered? Revisiting the Ratio

of Bacterial to Host Cells in Humans. *Cell* 164, 337–340

8 ElRakaiby, M. *et al*. (2014) Pharmacomicrobiomics: the impact of human microbiome variations on systems pharmacology and personalized therapeutics. *OMICS* 18, 402–14

9 Surana, N.K. en Kasper, D.L. (2014) Deciphering the tete-a-tete between the microbiota and the immune system. *J*. *Clin*. *Invest*. 124, 4197–4203

10 Sampson, T.R. en Mazmanian, S.K. (2015) Control of Brain Development, Function, and Behavior by the Microbiome. *Cell Host Microbe* 17, 565–576

11 Dinan, T.G. *et al*. (2015) Collective unconscious: How gut microbes shape human behavior. *J*. *Psychiatr*. *Res*. 63, 1–9

12 Clarke, G. *et al*. (2014) Minireview: Gut Microbiota: The Neglected Endocrine Organ. *Mol*. *Endocrinol*. 28, 1221–1238

13 (2010) Human genome at ten: The sequence explosion. *Nature* 464, 670–671

14 Franzosa, E.A. *et al*. (2015) Identifying personal microbiomes using metagenomic codes. *Proc*. *Natl*. *Acad*. *Sci*. 112, E2930–E2938

15 David, L.A. *et al*. (2013) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563

16 Gilbert, J.A. *et al*. (2016) Microbiome-wide association studies link dynamic microbial consortia to disease. *Nature* 535, 94–103

17 Clarridge, J.E. en Alerts, C. (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin*. *Microbiol*. *Rev*. 17, 840–862

18 Woo, P.C.Y. *et al*. (2008) Then and now: Use of 16S rDNA gene sequencing for

bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clin*. *Microbiol*. *Infect*. 14, 908–934

19 Galley, J.D. *et al*. (2014) Maternal obesity is associated with alterations in the gut microbiome in toddlers. *PLoS One* 9,

20 Sharpton, T.J. (2014) An introduction to the analysis of shotgun metagenomic data. *Front*. *Plant Sci*. 5, 209

21 Sangwan, N. *et al*. (2016) Differential Functional Constraints Cause Strain-Level Endemism in Polynucleobacter Populations. *mSystems* 1, e00003-16

22 Cardona, C. *et al*. (2016) Network-based metabolic analysis and microbial community modeling. *Curr*. *Opin*. *Microbiol*. 31, 124–131

23 Thomas, T. *et al*. (2012) Metagenomics - a guide from sampling to data analysis. *Microb*. *Inform*. *Exp*. 2, 3

24 Jeffery, I.B. *et al*. (2012) Categorization of the gut microbiota: enterotypes or gradients? *Nat*. *Rev*. *Microbiol*. 10, 591–2

25 Faust, K. *et al*. (2015) Metagenomics meets time series analysis: unraveling microbial community dynFaust, K., Lahti, L., Gonze, D., de Vos, W. M., & Raes, J. (2015). Metagenomics meets time series analysis: unraveling microbial community dynamics. Current Opinion in Microbiology. *Curr*. *Opin*. *Microbiol*. 25, 56–66

26 Donaldson, G.P. *et al*. (2015) Gut biogeography of the bacterial microbiota. *Nat*. *Rev*. *Microbiol*. 14, 20–32

27 Rowan, F. *et al*. (2010) Bacterial Colonization of Colonic Crypt Mucous Gel and Disease Activity in Ulcerative Colitis. *Ann*. *Surg*. 252, 869–875

28 Kalow, W. en Genest, K. (1957) A METHOD FOR THE DETECTION OF ATYPICAL FORMS OF HUMAN SERUM CHOLINESTERASE. DETERMINATION OF DIBUCAINE NUMBERS. *Biochem*. *Cell Biol*. 35, 339–346

29 Sultana, J. *et al*. (2013) Clinical and economic burden of adverse drug reactions. *J*. *Pharmacol*. *Pharmacother*. 4, 73

30 Chan, A.L.F. *et al*. (2008) Cost evaluation of adverse drug reactions in hospitalized patients in Taiwan: A prospective, descriptive, observational study. *Curr*. *Ther*. *Res*. 69, 118–129

31 Edwards, I.R. en Aronson, J.K. (2000) Adverse drug reactions: definitions, diagnosis, and management. *Lancet* 356, 1255–1259

32 Verbeurgt, P. *et al*. (2014) How common are drug and gene interactions? Prevalence in a sample of 1143 patients with CYP2C9 , CYP2C19 and CYP2D6 genotyping. *Pharmacogenomics* 15, 655–665

33 Guengerich, F.P. (2008) Cytochrome P450 and Chemical Toxicology. *Chem*. *Res*. *Toxicol*. 21, 70–83

34 Sousa, T. *et al*. (2008) The gastrointestinal microbiota as a site for the biotransformation of drugs. *Int*. *J*. *Pharm*. 363, 1–25

35 R. Rizkallah, M. *et al*. (2012) The PharmacoMicrobiomics Portal: A Database for Drug-Microbiome Interactions. *Curr*. *Pharmacogenomics Person*. *Med*. 10, 195–203

36 Carmody, R.N. en Turnbaugh, P.J. (2014) Host-microbial interactions in the metabolism of therapeutic and diet-derived xenobiotics. *J*. *Clin*. *Invest*. 124, 4173–

4181

37 Wilson, I.D. en Nicholson, J.K. (2015) The Modulation of Drug Efficacy and Toxicity by the Gut Microbiome. bll 323–341

38 Rautio, J. *et al*. (2008) Prodrugs: design and clinical applications. *Nat*. *Rev*. *Drug Discov*. 7, 255–270

39 Watkins, P.B. *et al*. (2006) Aminotransferase Elevations in Healthy Adults Receiving 4 Grams of Acetaminophen Daily. *JAMA* 296, 87

40 Clayton, T.A. *et al*. (2009) Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. *Proc*. *Natl*. *Acad*. *Sci*. *U*. *S*. *A*. 106, 14728–14733

41 Swanson, H.I. (2015) Drug Metabolism by the Host and Gut Microbiota: A Partnership or Rivalry? *Drug Metab*. *Dispos*. 43, 1499–1504

42 Takasuna, K. *et al*. (1996) Involvement of beta-glucuronidase in intestinal microflora in the intestinal toxicity of the antitumor camptothecin derivative irinotecan hydrochloride (CPT-11) in rats. *Cancer Res*. 56, 3752–7

43 Haiser, H.J. en Turnbaugh, P.J. (2013) Developing a metagenomic view of xenobiotic metabolism. *Pharmacol*. *Res*. 69, 21–31

44 Shajib, M.S. en Khan, W.I. (2015) The role of serotonin and its receptors in activation of immune responses and inflammation. *Acta Physiol*. 213, 561–574

45 Viaud, S. *et al*. (2013) The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 342, 971–6

46 Davey, K.J. *et al*. (2013) Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat.

Transl. *Psychiatry* 3, e309

47 Worsley, M. a (1998) Infection control and prevention of Clostridium difficile infection. *J Antimicrob Chemother* 41 Suppl C, 59–66

48 Rubinstein, E. en Camm, J. (2002) Cardiotoxicity of fluoroquinolones. *J*. *Antimicrob*. *Chemother*. 49, 593–6

49 Galatti, L. *et al*. (2005) Neuropsychiatric reactions to drugs: an analysis of spontaneous reports from general practitioners in Italy. *Pharmacol*. *Res*. 51, 211–6

50 Rubin, B.K. en Tamaoki, J., reds (2005) *Antibiotics as Anti-Inflammatory and Immunomodulatory Agents*, Birkhäuser-Verlag.

51 Bailey, M.T. *et al*. (2011) Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain*. *Behav*. *Immun*. 25, 397–407

52 Wallace, B.D. en Redinbo, M.R. (2013) The human microbiome is a source of therapeutic drug targets. *Curr*. *Opin*. *Chem*. *Biol*. 17, 379–384

53 Maurice, C.F. *et al*. (2013) Xenobiotics shape the physiology and gene expression of the active human gut microbiome. *Cell* 152, 39–50

54 Eckert, R. *et al*. (2006) Targeted killing of Streptococcus mutans by a pheromone-guided "smart" antimicrobial peptide. *Antimicrob*. *Agents Chemother*. 50, 3651–7

55 Guo, L. *et al*. (2015) Precision-guided antimicrobial peptide as a targeted modulator of human microbial ecology. *Proc*. *Natl*. *Acad*. *Sci*. *U*. *S*. *A*. 112, 7569–7574

56 Belda-Ferre, P. *et al*. (2015) The human oral metaproteome reveals potential biomarkers for caries disease. *Proteomics* 15, 3497–507

555 57 Wang, Z. *et al*. (2015) Non-lethal Inhibition of Gut Microbial Trimethylamine
556 Production for the Treatment of Atherosclerosis. *Cell* 163, 1585–1595
557 58 Yao, J. *et al*. (2016) A Pathogen-Selective Antibiotic Minimizes Disturbance to the
558 Microbiome. *Antimicrob*. *Agents Chemother*. DOI: 10.1128/AAC.00535-16
559 59 Nobrega, F.L. *et al*. (2015) Revisiting phage therapy: new applications for old
560 resources. *Trends Microbiol*. 23, 185–191
561 60 Kutter, E. *et al*. (2010) Phage Therapy in Clinical Practice: Treatment of Human
562 Infections. *Curr*. *Pharm*. *Biotechnol*. 11, 69–86
563 61 Summers, W.C. (2012) The strange history of phage therapy. *Bacteriophage* 2,
564 130–133
565 62 Koskella, B. en Meaden, S. (2013) Understanding Bacteriophage Specificity in
566 Natural Microbial Communities. *Viruses* 5, 806–823
567 63 Petschow, B. *et al*. (2013) Probiotics, prebiotics, and the host microbiome: The
568 science of translation. *Ann*. *N*. *Y*. *Acad*. *Sci*. 1306, 1–17
569 64 Candela, M. *et al*. (2010) Functional intestinal microbiome, new frontiers in
570 prebiotic design. *Int*. *J*. *Food Microbiol*. 140, 93–101
571 65 O'Keefe, S.J.D. *et al*. (2015) Fat, fibre and cancer risk in African Americans and
572 rural Africans. *Nat*. *Commun*. 6, 6342
573 66 Preidis, G.A. en Versalovic, J. (2009) Targeting the Human Microbiome With
574 Antibiotics, Probiotics, and Prebiotics: Gastroenterology Enters the Metagenomics
575 Era. *Gastroenterology* 136, 2015–2031
576 67 Garber, K. (2015) Drugging the gut microbiome. *Nat*. *Biotechnol*. 33, 228–231
577 68 Gerber, G.K. (2014) The dynamic microbiome. *FEBS Lett*. 588, 4131–4139

578 69 Gordon, S. (2008) Elie Metchnikoff: Father of natural immunity. *Eur*. *J*. *Immunol*.
579 38, 3257–3264
580 70 Belda-Ferre, P. *et al*. (2012) The oral metagenome in health and disease. *ISME J*.
581 6, 46–56
582 71 Bron, P. a. *et al*. (2011) Emerging molecular insights into the interaction between
583 probiotics and the host intestinal mucosa. *Nat*. *Rev*. *Microbiol*. 10, 66–78
584 72 Gavrish, E. *et al*. Devices and methods for the selective isolation of
585 microorganisms. . (2016) , Google Patents
586 73 Strandwitz, P. *et al*. GABA-Modulating Bacteria : Microbiome-Based
587 Therapeutics for Depression? , *RISE*. (2015)
588 74 Amalaradjou, M.A.R. en Bhunia, A.K. (2013) Bioengineered probiotics, a
589 strategic approach to control enteric infections. *Bioengineered* 4, 379–387
590 75 Buffie, C.G. *et al*. (2014) Precision microbiome reconstitution restores bile acid
591 mediated resistance to Clostridium difficile. *Nature* 517, 205–8
592 76 Li, J. *et al*. (2016) Probiotics modulated gut microbiota suppresses hepatocellular
593 carcinoma growth in mice. *Proc*. *Natl*. *Acad*. *Sci*. *U*. *S*. *A*. 113, E1306-15
594 77 Thompson, B.M. en Boiani, J. (2015) The Legal Environment for Precision
595 Medicine. *Clin*. *Pharmacol*. *Ther*. [Accepted, 167–169
596 78 Ginsburg, G.S. (2013) Realizing the opportunities of genomics in health care.
597 *JAMA* 309, 1463–4
598 79 Bristol-Myers Squibb/Sanofi Pharmaceuticals Partnership Plavix [package insert].
599 . (2015) , 1–32
600 80 Johnson, J.A. *et al*. (2012) Clopidogrel: a case for indication-specific

pharmacogenetics. *Clin*. *Pharmacol*. *Ther*. 91, 774–6

81 van Nood Els *et al*. (2013) Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. *N*. *Engl*. *J*. *Med*. 368, 407–415

82 Manuck, S.B. en McCaffery, J.M. (2014) Gene-Environment Interaction. *Annu*. *Rev*. *Psychol*. 65, 41–70

83 Gauderman, W.J. (2002) Sample size requirements for matched case-control studies of gene-environment interaction. *Stat*. *Med*. 21, 35–50

84 Huang, Y.J. en Boushey, H.A. (2015) The microbiome in asthma. *J*. *Allergy Clin*. *Immunol*. 135, 25–30

85 Ellekilde, M. *et al*. (2014) Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. *Sci*. *Rep*. 4,

86 Foster, J. a. en McVey Neufeld, K.A. (2013) Gut-brain axis: How the microbiome influences anxiety and depression. *Trends Neurosci*. 36, 305–312

87 Turnbaugh, P.J. *et al*. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444, 1027–31

88 Frank, D.N. *et al*. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc*. *Natl*. *Acad*. *Sci*. 104, 13780–13785

89 Shah, P. *et al*. (2016) A microfluidics-based in vitro model of the gastrointestinal human–microbe interface. *Nat*. *Commun*. 7, 11535

90 Sun, X. *et al*. (2016) Patent watch: Modulating the human microbiome with live biotherapeutic products: intellectual property landscape. *Nat*. *Rev*. *Drug Discov*. 15, 224–225

91 Morgan, B. (2016) Drug development: A healthy pipeline. *Nature* 533, S116–S117

92 Degnan, F.H. (2008) The US Food and Drug Administration and Probiotics: Regulatory Categorization. *Clin. Infect. Dis.* 46, S133–S136

93 Lewis, Z.T. *et al.* (2016) Validating bifidobacterial species and subspecies identity in commercial probiotic products. *Pediatr. Res.* 79, 445–452

94 Miller, G.E. *et al.* (2016) Lower Neighborhood Socioeconomic Status Associated with Reduced Diversity of the Colonic Microbiota in Healthy Adults. *PLoS One* 11, e0148952

95 Rook, G.A.W. *et al.* (2014) Microbial "old friends", immunoregulation and socioeconomic status. *Clin. Exp. Immunol.* 177, 1–12

96 Bailey, M.T. *et al.* (2011) Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain. Behav. Immun.* 25, 397–407

97 Block, J.P. *et al.* (2004) Fast food, race/ethnicity, and income: A geographic analysis. *Am. J. Prev. Med.* 27, 211–217

98 Powell, L.M. *et al.* (2006) Availability of Physical Activity–Related Facilities and Neighborhood Demographic and Socioeconomic Characteristics: A National Study. *Am. J. Public Health* 96, 1676–1680

99 Almqvist, C. *et al.* (2005) Low socioeconomic status as a risk factor for asthma, rhinitis and sensitization at 4 years in a birth cohort. *Clin. Exp. Allergy* 35, 612–8

100 Bytzer, P. (2001) Low socioeconomic class is a risk factor for upper and lower gastrointestinal symptoms: a population based study in 15 000 Australian adults. *Gut* 49, 66–72

101 McGuire, A.L. *et al*. (2008) Ethical, legal, and social considerations in conducting the Human Microbiome Project. *Genome Res*. 18, 1861–1864

102 Qureshi, N. en Kai, J. (2005) Genomic medicine for underserved minority populations in family medicine. *Am*. *Fam*. *Physician* 72, 386–7

103 Lynch, J.A. *et al*. (2013) Utilization of epidermal growth factor receptor (EGFR) testing in the United States: a case study of T3 translational research. *Genet*. *Med*. 15, 630–638

104 Adler, N.E. (1993) Socioeconomic Inequalities in Health. *JAMA* 269, 3140

105 Penson, D.F. *et al*. (2001) The association between socioeconomic status, health insurance coverage, and quality of life in men with prostate cancer. *J*. *Clin*. *Epidemiol*. 54, 350–358

106 Spatz, M. *et al*. (1967) Role of intestinal microorganisms in determining cycasin toxicity. *Proc*. *Soc*. *Exp*. *Biol*. *Med*. 124, 691–7

107 Schoeni, J.L. en Wong, A.C. (1994) Inhibition of Campylobacter jejuni colonization in chicks by defined competitive exclusion bacteria. *Appl*. *Environ*. *Microbiol*. 60, 1191–7

Outstanding Questions

What is the relative significance of specific microbial actors versus whole microbiome ecology in disease states, and how will drugging specific bacteria affect ecological succession following this perturbation? How will this depend on the milieu in which a species is situated (e.g., presence of different taxa performing a similar ecological role)? Additionally, what roles might phages, fungi, viruses play?

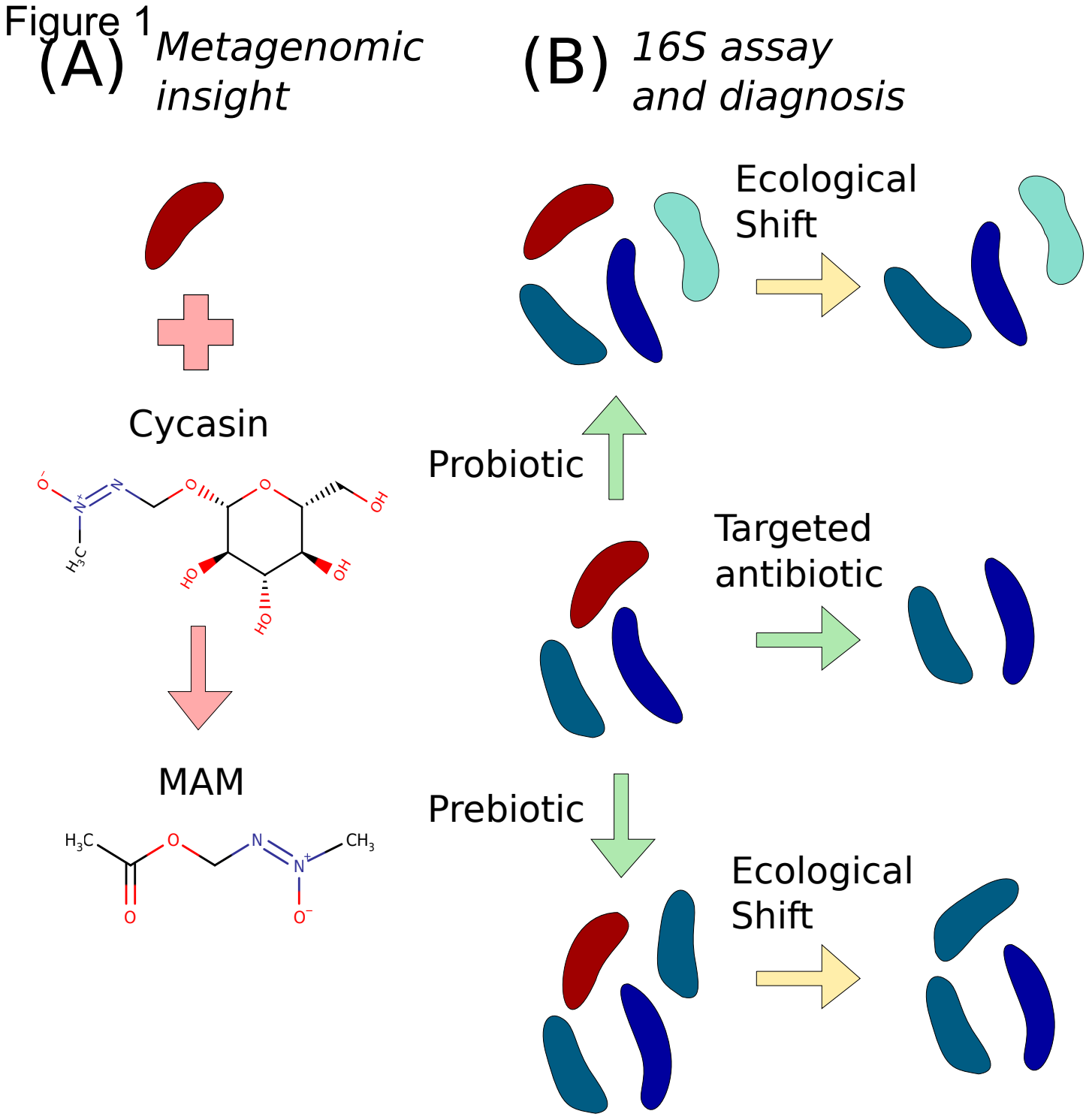
How closely coupled are genetics and the microbiome, and how can these fields be integrated into a unified practice of precision medicine?

Which microbiome-driven disease states can be successfully cured? Which instead require prophylactic or palliative, noncurative therapy?

What is the best way to move precision microbiome medicine results out into the clinic? What changes in regulatory, governmental as well as research and development processes will need to occur for this to happen?

How will the needs of different groups be best addressed across diets, lifestyles, and environments? What interventions will ultimately require social change rather than medical therapy, and what will the interplay between these fields be?

Figure 1


(A) Metagenomic insight
Cycasin
MAM
(B) 16S assay and diagnosis
Ecological Shift
Probiotic
Targeted antibiotic
Prebiotic
Ecological Shift