GIENS WORKSHOPS 2016 / Translational pharmacology

The human gut microbiome as source of innovation for health: Which physiological and therapeutic outcomes could we expect?☆

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Summary From the moment of birth, each human being builds a microbe-host symbiosis which is key for the preservation of its health and well-being. This personal symbiotic coexistence is the result of progressive enrichments in microorganism diversity through external supplies. This diversity is nowadays massively overthrown by drastic changes related to clinical practice in birth management, environmental exposure, nutrition and healthcare behaviors. The last two generations have been the frame of massive modifications in life and food habits, with people being more and more sedentary, overfed and permeated with drugs and pollutants. We are now able to measure the impact of these changes on the gut microbiota diversity. Concomitantly, these modifications of lifestyle were associated with a dramatic increase in incidence of immune-mediated diseases including metabolic, allergic and inflammatory diseases and most likely neurodegenerative and psychiatric disorders. Microbiota is becoming a hot topic in the scientific community and in the mainstream media. The number of scientific publications increased by up to a factor three over the last five years, with gastrointestinal and metabolic diseases being the most productive areas. In the intellectual property landscape, the patent families on microbiota have more than doubled in the meantime. In parallel, funding either from National Institutes (e.g. from NIH which funds research mainly in the field of allergies, infections, cancer and cardiovascular diseases, from the White House which launched the national microbiome initiative) or by pharmaceutical companies follow the same trend, showing a boost and a strong support in the research field on microbiota. All major health players are investing in microbiome research as shown by the number of deals signed and by funding during 2015. The Giens round table addressed how the medicine of tomorrow, considering human beings as a human-microbe symbiotic supraorganism, could leverage microbiome knowledge and tools. The rationale for our working group has been structured around four domains of innovation that could derive from ongoing efforts in deciphering the interactions between human cells and intestinal microbiome as a central component of human health, namely: (1) development of stratification and monitoring tools; (2) identification of new target and drug discovery, as a part of our supra-genome; (4) exploitation of microbiota as a therapeutic target that can be modulated; (4) and finally as a source of live biotherapeutics and adjuvants. These four streams will exemplify how microbiota has changed the way we consider a wide range of chronic and incurable diseases and the consequences of long-lasting dysbiosis. In-depth microbiota analysis is opening one of the broadest fields of investigation for improving human and animal health and will be a source of major therapeutic innovations for tackling today’s medical unmet needs. We thus propose a range of recommendations for basic researchers, care givers as well as for health authorities to gain reliability in microbiome analysis and accelerate discovery processes and their translation into applications for the benefits of the people. Finally, les Ateliers de Giens round table on microbiota benefited from the richness of the French ecosystem. France represents a center of excellence in the microbiota research field, with French institutions as Institut national de la recherche agronomique (INRA [Metagenopolis, Micalis]), Centre national de la recherche scientifique (CNRS), Unité de recherche sur les maladies infectieuses et tropicales emergentes (URMITE), Institut of Cardiometabolism and Nutrition (ICAN), Institut des maladies métaboliques et cardiovasculaires (I2MC), Institut national de la santé et de la recherche médicale (Inserm), Pasteur Institute and Gustave-Roussy being top-players for the number of publications.

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Abbreviations

16S rRNA 16S ribosomal ribonucleic acid
ADD/MS aliments diététiques destinés à des fins médicales spéciales (dietetic foods for special medical purposes)
ANSM Agence nationale de santé et de sécurité du médicament
ANR Agence nationale pour la recherche
Cas9 CRISPR associated protein 9
CNRS Centre national de la recherche scientifique
COPD chronic obstruction pulmonary disease
CRC colorectal cancer
CrispR clustered regularly interposed short palindromic repeats
CTLA-4 cytotoxic T lymphocyte-associated antigen 4
CTP Child-Turcotte-Pugh
DNA deoxyribonucleic acid
ECDC European Center for Diseases Prevention and Control
EDQM European Directorate for Quality of Medicines and Health Care
EFSA European Food Safety Agency
EMA European Medicines Agency
EU European Union
FDA Food and Drug Administration
FMT fecal microbiota transfer
GMP good medical practices
GMO genetically modified organism
HFGP human functional genomics project
HSC hematopoietic stem cells
I2MC Institut des maladies métaboliques et cardiovasculaires
IBD inflammatory bowel disease
IBS irritable bowel syndrome
ICAN Institut of Cardiometabolism and Nutrition
ICH International Conference on Harmonization
IET European Institute of Innovation and Technology
ILSI International Life Sciences Institute
IMI innovative medicine initiative
IND investigational new drug
INRA Institut national de la recherche agronomique
Inserm Institut national de la santé et de la recherche médicale
qPCR quantitative polymerase chain reaction
LBPs live biotherapeutic products
MDR multi drug resistant
MELD model for end-stage liver disease
MetaHit EU-funded consortium
MWAS metagenome wide association studies
NAFLD nonalcoholic fatty liver disease
NASH nonalcoholic steato hepatitis
NO nitric oxide
PD-1 programmed cell death 1
pH potential of hydrogen
PIA Programme des investissements d’avenir
pO2 partial pressure of oxygen
PoC proof of concept
PSA polysaccharide A
R&D research and development
RCT randomized control trial
ROS radical oxygen species
SLE systemic lupus erythematosus
SOPs standard operating procedures
TRL technology readiness level
UK United Kingdom
URMITE Unité de recherche sur les maladies infectieuses et tropicales emergentes (Institut de recherche pour le développement)
US United States
USA United States of America

Introduction

What is the microbiota?

All organisms are living in close interaction with the microbes present in their environment. Humans could thus be considered as supraorganisms composed of human cells and a wide range of associated microorganisms that are located on the skin and at every mucosal interface of the body, linked to the external compartment. This collection of microorganisms is called microbiota. It is now reasonably considered to be our “extended self”, inasmuch as this multicellular organ consumes, stores and redistributes energy harvested from nutrients. It is highly diverse. Its composition is not limited to bacteria, and contains also fungi and other eukaryotes, archaea, viruses and phages [1]. Its structure at the genome level is known as the microbiome — a term introduced by the Nobel laureate Joshua Lederberg to describe the collective genomic content of the gut microbiota. The diversity of the microbiome is huge, with around 10 million non-redundant genes described to date [2], compared to the 24 000 human genes. The 2 kg of microbial biomass in the gut gathers 100 trillion of bacteria which represents in numbers more than there are human cells.

From this reductionist, organ-centered perspective, it is interesting to contemplate the dynamics of symbiosis (defined as an association between organisms of two different species, often to their mutual benefit) throughout the host’s life. Sterile in utero, the newborn gut is immediately colonized at birth by the maternal vaginal microbiota and environmental microbes in contact with the skin. Most influenced by numerous factors such as birth mode, perinatal treatments, early nutrition and overall care, the newborn intestinal microbiota diversifies to reach a climax composition within three years [3]. Thus, recent trends to increase the number of cesarean births and promote a near-sterile environment for babies (as for instance, sterilized milk bottles) are hindering the process of development of host-microbes symbiosis. Obviously, the host genetic is also playing a role in the selection of the composition and diversity of the commensal microorganisms that will be part of this personal ecosystem [4]. A recent study highlighted genome-wide significant associations for overall microbial variation at multiple genetic loci, stressing the VDR gene (encoding vitamin D receptor) as a key player [5]. Moreover, each location in or on the body has its own microbial diversity depending on local microenvironment (oxygen, pH . . .). At birth, microbiota colonization co-occurs with the maturation of the immune system and thus founder microorganisms...
will be considered by the nascent immune system as being part of self [6]. Furthermore, the intestinal epithelial turnover of conventional mice is twice faster when compared to that of germ-free animals, which experience many additional functional defects (e.g. a lowered priming of the immune system and a decreased secretion of mucus and antimicrobial factors).

Once set up, this equilibrium is more or less modified by external events and life styles. Diet, hygiene, travel, diseases and more particularly infections, drugs and especially antibiotics, stress and aging are modulating the composition of the different microbial communities, as well as host genetics and epigenetics. Elderly microbial communities tend to be less diversified and this loss of commensal diversity can be linked to immune-senescence and alteration of a range of physiological processes (Fig. 1).

What are the tools for studying microbiota?

The recent revolution, which has boosted major discoveries in the microbiota field, is next generation sequencing that made it possible to overcome the limitations of in vitro culture and document microbiota diversity including strains unable to grow at the bench. Around 85% of the strains are thus known only through the sequence of their genes [7,8]. Development of meta-analysis tools was also necessary for microbial diversity analysis [9,10]. This breakthrough technology was favored by optimization of deoxyribonucleic acid (DNA) extraction protocols and the high throughput and in-depth sequencing that allows the detection of even sub-dominant DNA sequences. These experiments were mainly carried out from bacterial DNA extracted from feces, the gut being the most densely colonized body compartment. Recent studies have now shown that shotgun sequencing is possible starting from vagina, skin, lung or nose microbiota, thus unveiling for instance the huge diversity of the skin microbiome [11].

Once profiled, the composition of the microbial compartments is analyzed either longitudinally or by comparing microbiome diversity in large populations of unrelated patients/healthy people.

Longitudinal studies aim to follow variation in microbiome diversity in a given donor at different time points (for instance, newborn feces composition from birth onward or patients microbiota variation during onset or progression of chronic diseases) as for example for type 1 diabetes where the drop in abundance for specific bacterial strains precedes the seroconversion of at risk children [12]. Population comparisons of microbiome diversity led to identify signatures associated with diverse pathologies. Such analyses describe associations between presence/absence of a range of bacterial species and the disease, and subsequently it helps to build hypothesis linking dysbiosis and the etiology of a range of pathologies. These associations allowed shedding light on the existing links between all major non-transmissible chronic diseases and dysbiotic signatures [13,14]. In the context of diseases of unknown etiology and with no available prevention or cure, man-microbe symbiosis was shown to be durably altered in conjunction with reciprocal aggravating signals. For instance, altered microbiota could promote intestinal hyperpermeability and inflammation, the latter promoting oxidative stress aggravating microbiota alteration [15–17]).

Pathologies can be linked to a disruption of the ecological balance and overall symbiosis that will favor the risk of opportunistic infections and occurrence of chronic inflammation. However, there is a clear need to understand the underlying mechanisms whereby compositional changes in the gut microbiota could contribute to disease onset and progression. Obviously, pathology-related signatures should
Human gut microbiome as source of innovation for health

Table 1  Some examples of pathologies associated with dysbiosis [18—49].

<table>
<thead>
<tr>
<th>Disease</th>
<th>Analysis tool</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1 diabetes</td>
<td>16S rRNA</td>
<td>[18—20]</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Shotgun seq</td>
<td>[21,22]</td>
</tr>
<tr>
<td>Obesity and metabolic syndrome</td>
<td>Shotgun seq</td>
<td>[23,24]</td>
</tr>
<tr>
<td>Asthma</td>
<td>16S rRNA</td>
<td>[25]</td>
</tr>
<tr>
<td>Food allergy</td>
<td>16S rRNA</td>
<td>[26—28]</td>
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<tr>
<td>Eczema</td>
<td>16S rRNA</td>
<td></td>
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<tr>
<td>Atopy</td>
<td>16S rRNA (skin microbiota)</td>
<td></td>
</tr>
<tr>
<td>IBD/IBS/Crohn’s Disease</td>
<td>16S rRNA and shotgun seq</td>
<td>[29—33]</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celiac disease</td>
<td>16S rRNA</td>
<td>[34—36]</td>
</tr>
<tr>
<td>Autism</td>
<td>Shotgun seq and qPCR</td>
<td>[37,38]</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>Shotgun seq</td>
<td>[39]</td>
</tr>
<tr>
<td>NASH</td>
<td>16S rRNA</td>
<td>[40]</td>
</tr>
<tr>
<td>RRMS</td>
<td>16S rRNA</td>
<td>[41]</td>
</tr>
<tr>
<td>RA</td>
<td>16S rRNA</td>
<td>[42]</td>
</tr>
<tr>
<td>CRC</td>
<td>16S rRNA qPCR</td>
<td>[43—45]</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>16S rRNA</td>
<td>[46]</td>
</tr>
<tr>
<td>Clostridium difficile colitis and antibiotic-associated diarrhea</td>
<td>16S rRNA</td>
<td>[47]</td>
</tr>
<tr>
<td>Colonization by resistant bacteria</td>
<td>16rRNA</td>
<td>[48,49]</td>
</tr>
</tbody>
</table>


be established starting from large populations in order to overcome the individual variability due to patient genetics and numerous other confounding factors. Understanding how a pathological environment in interaction with an evolving microbial ecosystem may bounce man-microbes symbiosis into an alternative dysbiotic and stable state will be the source of new discoveries in the field of disease etiology.

**Microbiota as stratification tool**

How to leverage these technological progresses in order to improve patient diagnosis and health care management? We propose first to explain how microbiota is becoming a novel stratification tool. High throughput metagenome sequencing campaigns published over the last 6 years cover a range of chronic disorders, from metabolic to autoimmune diseases.

**Metagenomic analysis**

Tools were different between studies and went from 16S ribosomal ribonucleic acid (16S rRNA) sequencing to whole microbiome sequencing associated with global gene count. Metagenome-wide association studies were built from varying protocols and populations. Surprisingly, the diseases that show a significant segregation between patients and healthy individuals on the basis of microbiome diversity are very broad and are summarized in Table 1 [18—49].

The first example of a possible exploitation of the patient stratification by metagenome analysis is the monitoring of the disease or health status. Obviously, colonization by pathobionts (i.e. any potentially disease-causing organism which, under normal circumstances, lives as a symbiont) could be envisioned but it does not require metagenome wide sequencing. Interestingly, a modification of the microbiota ecosystem enlightened in patient cohorts suffering from the pathologies gathered in Table 1 could become a way to identify the nature of the disease and to predict the disease onset. An example consists in the prediction of necrotizing enterocolitis in newborns where dysbiosis precedes disease and thus allows to rapidly pretreat with probiotics the children at risk [46].

Metagenome wide association studies (MWAS) were also used for checking resilience after dysbiosis induced by drugs, such as antibiotics or anticancer chemotherapies [50—52]. Through longitudinal metagenome analyses, disease severity and progression could be monitored. This was the case for instance in obesity, COPD, pancreatitis, chronic inflammatory arthritis and non alcoholic fatty liver disease (NAFLD) [53—57]. In this last disease, the presence of selected bacterial DNA, identified through gut MWAS, was retrieved in patient blood samples and is a way to get a simple and less invasive disease biomarker to replace liver biopsies [58]. Other non-invasive ultrasound observations are nonetheless emerging.

Follow-up of people at risk in a longitudinal mode led to the identification of bacterial strains presence/absence and of microbiota diversity modifications as predictors of chronic disease onset or aggravation, as illustrated in Type 1 diabetes, systemic lupus erythematosus (SLE), Crohn’s disease, cancers or food allergies [59—64]. Here also, monitoring may lead to preventive care measures, adaptation of clinical management and discovery of novel therapies by microbiota modifiers.

A last set of studies consists in exploiting microbiome profiles in order to stratify responders and non-responders to therapies. Examples can be found in the literature in the context of energy-restricted diet in obesity, statins, metformin or digoxin [22,65—67]. Here, specific components of gut microbiota are playing a fundamental role.
in drug metabolism, showing that they have to be taken into account in pharmacokinetics studies. Other examples of essential prediction of treatment outcome come from the recognition of the impact of donor microbiota diversity and relative risk of premature death after hematopoietic stem cell transplantation [68], or between gut microbiome and prediction of chemotherapy-related bloodstream infection risk [69]. Finally, this stratification tool was elegantly exploited with the metagenome-profiling studies of patients that respond to the anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and anti-programmed death 1 (PD-1) immune checkpoint inhibitors [70,71] and to cycloheximide [72]. This is being translated into the clinical practice in order to give efficient but toxic treatments only to patients that have a strong responder profile, and opens perspective of promoting responder profiles by adding live biotherapeutics (see below).

Thus, we have briefly reviewed how the MWAS tool is becoming an even more precise mean for patient stratification through enabling the detection of non-invasive and cheap biomarkers as well as offering new disease management and monitoring approaches.

A range of complexity is being added with the emerging meta-transcriptomic, metaproteomic and metabolomic studies, which complement the meta-analysis of microbiota and will likely also promote generation of mechanistic hypothesis and new potential stratification biomarkers [73].

**Recommendations**

**For basic and clinical research**

Promote the sharing of databases and standard operating procedures in order to create a public–private pre-competitive space able to favor rapid progresses in microbiome analysis and create opportunities for collaborations. Publications are poorly comparable because sample collection, DNA extraction quality, depth of sequencing and bioanalysis tools differ greatly between laboratories. Owing to this lack of standardization, it is even likely that many reports made to date may not be reproducible. Fortunately, standard operating procedures (SOPs) are being proposed that will be instrumental for the future. This is the case for SOPs produced by the International Human Microbiome Standards consortium funded by the European Commission [74]. One way to further mitigate these limitations will be through standardizations of reference materials. For instance, minimal standardized set of strains or DNA will be determined and distributed in order to permit comparability of two MWAS studies or methods performed by independent investigators. It can be envisioned that different mixes will be tailored to different applications according to population/pathology groups. Research to design these standards, their production and their distribution could be financed through public–private partnerships and international research consortia. The construction of dedicated arrays could also be proposed to investigate microbiota diversity.

**For health authorities**

The most relevant studies published to date consist in those presenting longitudinal follow-up of donors. These tools allow determining how microbiome profile may vary with the robustness conferred by each patient being its own internal reference. We should, through these long-lasting studies, be able to give a consensual definition of a dysbiotic microbiome as for instance the percent of reduction of microbial richness over time, the absence or reduced abundance of selected symbionts, or specific metabolites. Financing of these studies should be supported by international consortia or by national initiatives.

The outcomes will be generic, predictive signatures, in a limited set of high priority pathologies for which the microbiota profiling should have an impact on treatment outcomes. These can be selected amongst autism, type 2 diabetes, multiple sclerosis, inflammatory bowel disease (IBD), nonalcoholic steato hepatitis (NASH), hematopoietic stem cells (HSC) transplant, Guillain-Barré, neurodegenerescence, resilience test in antibio- or chemotherapies...

This should result in the validation of the registration of microbiota-derived biomarkers and companion diagnostics by *France Génomique* and other registration authorities, once reliability, specificity and sensitivity are validated through clinical trials. With this goal in mind, we recommend to enlarge the collections by inciting the sponsors to store and analyze sets of fecal sample before and after treatment in more clinical studies, especially in first lines, knowing that this requires to open information and to obtain enlarged patient’s informed consents covering the ‘use of data generated from the analysis of collected samples to the objectives of design of diagnostic, prognostic and monitoring tools of generic value in non-transmissible chronic disorders’.

We should also anticipate the tools for integrating these very complex datasets in clinical practice. Finally, these recommendations should result in the incorporation of microbiota as tool for diagnosis, prognosis and stratification in the nomenclature to ease adoption by testing laboratories. The anticipated bottlenecks in this context may encompass:

- development of confidential data management and confrontation with freely available omics data for general population (shared national database);
- development of algorithms appropriate for automated analysis from raw omics data;
- positioning of technical teams at the hospital to secure the link between subcontracted omics data generation, analysis and generation of appropriately formatted results, and delivery of results to the clinicians in electronic format;
- training of clinicians to the interpretation of patient metagenomic profiles.

**Microbiota as source of novel drugs, adjuvants and targets**

Once a clear relationship has been established between a pathological phenotype and a dysbiotic state, one should translate this into new original therapeutic strategies. What could we learn from these modifications of microbiome profiles and corresponding host microbe crosstalk? How can we
leverage how food-microbe-host interact as novel therapeutic solutions?

One tool, which has been recently set up to decipher host—microbes interactions, is called functional metagenomics [75]. Bacterial DNA is extracted from a microbial population, cleared from human donor sequences, and fairly large-size inserts are cloned into phagemids in order to be expressed by transformed Gram negative and Gram positive bacterial strains. Clones are distributed into screening plates, grown in adapted conditions and lysed. Supernatants can then be screened for a specific activity in phenotypic tests. These metagenomic libraries can be obtained from intestinal contents of healthy and patient donors [76].

Such a platform has been set up for the first time in the frame of the EU-funded consortium (MetaHit) and expanded in the Metagenopolis Unit of INRA (Agence nationale pour la recherche — programme des investissements d’avenir [National Agency for Research and Investment of the Future or ANR-PIA funding]) [77]. Symbiont-cell crosstalk mechanisms are then determined by identification of the gene(s) involved in the activation of the studied phenotype by insertional mutagenesis followed by the deconvolution of its mechanism of action. Several promising screenings were already implemented such as models of gut epithelial layer integrity, inflammation, immune cell maturation and immune-modulation, cell differentiation and proliferation, metabolism regulations and hormone secretion. These gave rise to the identification of original mechanisms of action and a range of publications and patents [76,78—88].

Obviously, this approach cannot take into account all the complexity of the intestinal milieu and it could miss some regulations due to pH, pO2, microbial cooperation and the complexity of the intestinal milieu and it could miss discovery processes [91—95].

A huge meta-analysis adapted to the microbiota complex issues of relevance for in vitro culture systems [89]. Nevertheless, it will allow exploiting 99% of human supraorganism genome for target and drug discovery by addressing the huge diversity of microbiome so far concealed from functional exploration due to non-culturability. In addition, unbiased metabolomics approaches can complement the above and give a fairly direct access to potentially beneficial or conversely toxic substances. More recently, differences in composition and function of gut microbial communities were recently identified to contribute to interindividual variation in cytokine responses to microbial stimulations in healthy humans as part of the human functional genomics project (HFGP) [90].

Moreover, these functional metagenomics studies were preceded by shotgun sequencing of gut and skin microbiome, and around 10 million of bacterial genes were sequenced. A huge meta-analysis adapted to the microbiota complexity, led to unknown strain sequence assembly. Bioanalysis of the sequences allows to identify new enzymes and to model their 3D structures. Once their physiological role determined, these can become a new validated source of druggable targets and will be managed through classical drug discovery processes [91—95].

Recommendations

Understanding how microbes and human cells interact for metabolite and molecular target discovery will be greatly favored by the quality of the tools. Here also, standardized protocols for microbial DNA extraction and cloning will give rise to a good representativeness of aerobes and anaerobes strain diversity. SOPs will be mandatory to obtain workable libraries, which could be shared through public/private consortia. Priority should be given to the most relevant clinical indications for which the solidity and reliability of patient stratification and transplantation experiments in germ-free animal models have shown that dysbiosis is causal in the disease. Another requirement lies in the availability of microbiome libraries, generated from pools of donors, for instance, which will mitigate various sources of inter-subject variations (e.g. genetics, food and lifestyle).

We would also like to recommend learning from what occurred around the sequencing and patenting of the human genome and, instead of blocking access to tools, setting up a way to give each player free access to shared libraries and databases. This framework will for sure accelerate the delivery of valuable knowledge based on high-quality starting material and means. Towards this goal, we propose to build these assets in the frame of a strategic Governing Group innovative medicines initiative (IMI) project, or of a European Institute of Innovation and Technology (IET)/health funding for product TRL>3 or through a joint action for biobanking financed by health and research ministries of the European countries.

Microbiota as target for functional modulation

Functional nutrition for preventive/curative outcomes

The offer along the concept of health preservation and even disease prevention via the use of functional foods supported the developments of first generation probiotics and prebiotics. The probiotics will be discussed in the section below together with other product lines containing live microbes. As for prebiotics, the initial concept called for a single defined molecular structure designed to promote a limited set of “beneficial” microbes in a rather specific manner. Accumulating observations are suggesting the need for a new paradigm in prebiotic/fiber-based modulation of the microbiota and thereby host—microbe symbiosis. Among key target parameters, we can cite overall ecological robustness and more precisely microbiota richness and resilience.

Low richness could be a consequence of major changes in nutritional habits over the past 2—3 generations. Indeed, what we called the nutritional transition (lowering fiber intake at the expense of simple sugars and animal proteins and fat) is bound to have a great impact on the microbiota. More than half of the energy supply to the intestinal microbiota is provided by exogenous dietary fibers while the other half comes from endogenous secretions, mainly mucins. For the greatest part of the history of the genus Homo, up to
60% of daily energy is derived from plant material (W. Wahly, personal communication). It is only through nutritional transition, initially in western countries and now worldwide, that we have brought this supply down, often below 10 g of fiber per day. Although not demonstrative of a causal role, this is consistent with the observation that the intestinal microbiota of rural dwelling, remote populations still harbor a more diverse community (we can cite here work on Burkina Faso versus Italy, Malawi and Venezuela versus USA, Yanomami Indians, Hadza versus EU [96—99]) (Fig. 2).

As illustrated in preclinical work in human microbiota associated animals [100], nutritional changes may induce durable losses of microbial commensals that would explain such observations although numerous confounding factors cannot be excluded.

Descriptive work also connected fiber intake within dietary habits and baseline intestinal microbiota diversity and enterotypes [101,102]. Enterotypes are preferred ecological arrangements of the human intestinal microbiota, the three described being distinguished by the driver genera, Bacteroides, Prevotella or Ruminococcus, respectively [103]. Intestinal microbiota diversity and enterotypes are connected such that the Bacteroides enterotype is a marker of low gene/species richness while the other two are associated with high gene count. Integrating dietary intake and baseline species richness data suggested the possibility to predict gene/species richness by a dietary fiber index based on habitual food intake of fruits and vegetable food items [104].

Conversely, a few studies already hint at a direct potential of dietary fibers to promote higher microbiota richness. In an intervention in obese individuals [23], a low calorie diet with high and diverse fiber content increase richness of the metagenome at the gene level by 25% in subjects with an initially low diversity. In an intervention trial with crossover design switching between 10 g and 40 g/day fiber [104], individuals of the Bacteroides enterotype at inclusion, typically low gene count, tended to switch enterotype more frequently than individuals of the Prevotella enterotype.

The mode of action of fibers can be conceptualized. Fairly specialized fiber degraders will occupy the tip of the microbial food chain (Fig. 3). Their activity will feed the rest of the microbiota with partially degraded simpler sugars all the way to the bottom of the food chain occupied by other specialists such as users of fermentation gases H₂ and CO₂. If fiber degraders can be seen as specialized, it is essentially due to the acknowledged requirement for a large and unique genetic equipment to perform such activities. Indeed, even to degrade a simple polymer such as cellulose, more than 20 genes and enzymes are commonly required. This suggests that actors in this ecological niche will have acquired their ability through evolution and gained a true ecological hedge. It has indeed been documented by metagenomic data [105]. This also suggests that removing any fiber structure from the diet may promote the loss of the corresponding specialized microbes, either to subdominant fractions of the intestinal community or definitely, as suggested by a recent study in human microbiota associated mice [100]. The above gain in richness with dietary intervention suggests the possibility to regain from subdominant populations active groups kept dormant for some length of time due to low fiber dietary habits. It also strongly supports the potential for a change in paradigm from the former prebiotic concept of single molecular structures to a global approach based on complex mixtures of fiber structures.

It is possible that the ecology, composition and overall functionalities of the microbiota drive its responsiveness to dietary modulators. It was recently shown that the response to a probiotic mix may be modulated by the initial microbiota configuration, the latter driving the permissivity to fermented food lactic acid bacteria [106,107]. Similarly, work by Chung et al. indicates that depending on the presence/absence of specific fiber degraders, the microbiota may be totally irresponsive to specific fibers [108]. This will have to be documented further if we want to determine the degree of personalization made possible by nutrition-based strategies of modulation of the ecology of the human intestinal microbiota. In a recent work, Elinav and Segal did
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Figure 3. Microbial trophic chain acting in the digestive tract illustrating the interaction of various functional groups towards the production of final products comprising short chain fatty acids (acetate, propionate, butyrate) and fermentation gases (H₂, CO₂, CH₄, H₂S).

implement a global approach interconnecting food intake, blood-glucose measurement and microbiome profile to come up with personalized nutritional recommendation [109].

In the context of colorectal cancer (CRC), epidemiological and scientific studies indicate that the risk of CRC is increased by processed and unprocessed meat consumption but suppressed by fibers, and that food composition affects colonic health and cancer risk via its effects on colonic microbial metabolism. Butyrate has a remarkable array of colonic health-promoting and antineoplastic properties: it is the preferred energy source for colonocytes, it maintains mucosal integrity and it suppresses inflammation and carcinogenesis through effects on immunity, gene expression and epigenetic modulation. Protein residues and fat-stimulated bile acids are also metabolized by the microbiota to inflammatory and/or carcinogenic metabolites, which increase the risk of neoplastic progression. These microbial metabolite effects can be modified by diet to achieve the objective of preventing colorectal cancer in Western societies [110].

Diet has been hypothesized to influence intestinal inflammation by alteration of the gastrointestinal microbiome leading to gastrointestinal permeability and immune sensitivity. Unfortunately, robust data that support diet changes to alter the course of IBD are limited. Thus, evidence-based dietary guidelines are lacking [111]. While there are differences in healthy versus IBD intestinal microbiome, there is not yet any evidence that changing the microbiome of an individual with IBD with diet is the mechanism through which the disease can be successfully treated. However, since differences in habitual diet intake that favor a Western diet (high in protein, high in saturated fat, and low in fiber and plant foods) lead to a higher risk of IBD, an alternative healthier diet approach can be envisioned [112].

Modification of microbiota profiles

We proposed above that low gene count can be viewed as a health stratifier, and thereby a target for microbiome modulation. It has also been documented as a relevant signature of responders/non-responders to treatments or a biomarker of severity and potentially indicator of the rate of aggravation in chronic conditions.

In IBD, it would be a signature of the relapse rate of acute phases, such that low gene count would be associated with more frequent relapses (more than once per year) or in other words, shorter quiescent phases (Guarner et al., personal communication). In liver cirrhosis, gene count was shown to be inversely linked to the severity of the disease based on model for end-stage liver disease (MELD) and Child-Turcotte-Pugh (CTP) scores [113]. These observations suggest a potential for monitoring over time with a potential to help the clinician in its clinical management and decision process. Appropriate diagnostic tools are yet awaited that will give a rapid, sensitive and specific indication of microbiome richness at a very reasonable cost (a few euros for instance).

The global parameter of gene count was shown to be associated to specific bacterial signatures, 4 metagenomic species being sufficient to offer a very discriminant model. This also suggested that beyond diagnostic, specific symbionts could express bioactivities with relevance for the preservation of gut homeostasis. We will address this point in the following chapter. One of the messages from this is that modulation of newly identified bioactive symbionts could be a specific target of innovative functional nutrition.

Dysbiosis was initially documented as a strict "microbiome" feature. As indicated in the section above, it has been described in numerous immune-mediated
conditions. After more than 20 years of descriptive work, recurrent features in dysbiosis are now structuring a vision that also opens the way for innovation. It most accurately stresses the importance of the fine interactions in microbes—host symbiosis. On the microbiome side, dysbiosis is typically characterized by low richness, loss of specific symbionts among those that are most conserved in the healthy gut microbiota — very often dominant Firmicutes — and proliferation of pathobionts — noticeably enterobacteria. On the host side in parallel, recurrent features of dysbiosis comprise alteration of intestinal epithelial permeability — hyperpermeability or leaky gut being the classical expression —, low grade to overt inflammation and oxidative stress.

Although this has not been truly designed as of today in humans, the strategic design of a holistic, combinatorial approach targeting the key features of altered host—microbes symbiosis would seem highly relevant. This would mean altogether promoting microbial richness and addressing host symptoms such as leaky gut, inflammation and oxidative signals. It could call for the next generation symbiotic, promoting symbiosis by synergistic effects of dietary bioactive ingredients and ingredients or microbial activities targeting altered features of intestinal homeostasis. Some of the available options based on dietary bioactives are discussed below.

**Managing abiotic stressors**

Abiotic stressors would comprise a variety of endogenously produced or environmentally acquired molecules challenging intestinal homeostasis.

**Managing oxidative stress**

Managing oxidative stress is one example. It will derive from inflammation and will lead to the release of radical oxygen species (ROS) or NO for example that are aggressors for human cells. Some bacterial enzymes are known to play a role in ROS scavenging. Although less well documented, they may also be expressed by molecules produced by human tissues, with a potential role of modulator for microbial signals as illustrated above. So far, a few lactic acid bacteria with catalase and/or superoxide dismutase activities have been documented. More information is available concerning the antioxidant potential of polyphenols and other plant-borne phenolics. Although recognized, the potential for microbial bioactivation and triggering of highest functionalities (e.g. rhamnosidase activity) is still under-documented. The whole domain of oxidative stress management is hence still very poorly explored to date and this clearly points at potential for innovation.

**Managing antibiotic resistance**

Managing antibiotic resistance is yet another example that has become a major clinical need to tackle the critical expansion of multidrug resistant (MDR) bacterial load in the hospital environment. Innovative solutions are awaited in this context and the French startup ecosystem is giving concrete hints of this potential. Da Volterra for example [114] is developing a solution designed to trap antibiotic residues with bioactives released in the intestine. This is geared to prevent exposure of the normal microbiota to antibiotics away from their expected site of action. Eligo Bioscience is yet another startup in the field [115], fine-tuning applications of the CrispR-Cas9 technology of gene silencing to design what would be the first directed ecological knockout approach ever designed. MaaT Pharma has set the first European good medical practices (GMP) platform, in support of its ongoing clinical trials, to perform an autologous fecal microbiota procedure following antibiotics courses to reduce MDR bacteria carriage. Clearly, other innovative solutions will be awaited to address what is becoming a very serious public health threat. Metagenomic profiling will prove ideally suited towards a finer recognition of gene dynamics and plasticity in the intestinal environment that in turn should help for the design of novel antibiotics.

**Recommendations**

**Change paradigm in functional nutrition: ecological management of microbiota**

Next to strategies aiming at promoting specific "beneficial" commensals, efforts should be made to:
- document the link between richness, ecological robustness and health status (risk of onset or aggravation of chronic conditions; response to diet or therapy);
- apply new tools including functional genomics, metagenomics, metabolomics and modeling to document means of shifting microbiota composition;
- assess possibility and benefits of modulating parameters of intestinal ecology.

**Need tools to monitor parameters of intestinal ecology with health relevance**

Ecology of the microbiota appears more and more as a solid indicator of health and resistance to a variety of environmental stressors, as well as in some instances a predictor of response/non-response or aggravation of chronic conditions. Translation of its potential as health indicator for the clinics will require the design and validation of tools indicative of features that should also be regarded as target for modulation via food bioactives or specific molecules. Devices and kits should allow easy and low-cost implementation of sensitive and specific, well validated tools, ideally with associated cumulative-learning means based on shared massive data. Specific features should comprise:
- richness (via signature species identified/patented or via more global surrogate biomarkers);
- robustness = resistance and resilience (requires longitudinal studies).

**Need to define minimal proof of concept (PoC) required for translational development**

The need for an industrialized product is quite clear but on a regulatory standpoint, there is a requirement for a delineation of the approach and minimal proof-of-concept demonstration needed for such tools and devices to be made available for the clinicians with likely re-imbursement when leading to proven benefits.
Personnalized treatments; where do we start and how far do we go?

In which indications? The working group speculated that initial efforts could be put on non-curable and/or most severe conditions (T1D, oncology...), as well as instances for which the medico-economic equation is in favor of some actions (immuno-psychiatry with severe psychiatric disorders showing an as yet uncontrolled exponential rise in incidence). 

The appropriate surrogate markers for fine monitoring may nonetheless still be lacking (see preceding point above).

Applications in the general population; where do we start and how far do we go?

Using microbiome knowledge for the design of preventive strategies should be geared to increase “healthy-life-expectancy”. It is delivering strategic information that could be leveraged for specific population groups; for example via quantity food supply to institutionalized elderly or school children.

Microbiota as source of live biotherapeutic products

At first sight, this chapter would seem to refer to well-established applications that correspond to the concept of probiotic for which the internationally endorsed definition is “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”. As we will illustrate, there is a very broad perspective for innovations in this domain that derive both from emerging knowledge on new generation probiotics but also from empirical applications in the clinic going all the way to fecal microbiota transplants.

Probiotics of first generation

On the whole, probiotics represent a fast-growing market for manufacturers and suppliers. The global market for probiotic ingredients, supplements and foods rose from €10.2 million in 2003 to €12.6 billion in 2008. The French market for probiotics used as industrial food ingredient represents 21% of the Western European market. In this context, Bioprox remains the only exclusively French company of lactic ferment and probiotic strains supplier and must cope with the considerable competition of large multi-national groups. A number of probiotic strains have been introduced in the market in dietary and pharmaceutical forms. Notably, lactobacilli and bifidobacteria constitute the main group of probiotics commercialized for human consumption as traditional food products. When this probiotic market is extended to include purely pharmaceutical applications, the potential market will be increased tremendously and could be doubled in the longer term. This means a considerable extension of clinical trials but also of employment in R&D. Functional food legislation is evolving worldwide by taking into consideration both applications of probiotics in food and in medicine.

It has been well established over a number of years of research that the effects of probiotics are strain-dependent. In the context of nutritional applications, much has been done concerning lactic acid bacteria and bifidobacteria that caused little concerns owing to their long history of safe use. The number of strains “on the market” has remained fairly limited in Europe and some probiotics with bioactivities and benefits were in turn well documented. Nonetheless, with the transfer of competencies of national food safety agencies to the European level with European Food Safety Agency (EFSA, set up in 2002), all initially accepted claims were withdrawn and applications had to be re-submitted. Since then, no claim was positively administered through a complete validation process except for the generic claim of yogurt cultures helping in the management of lactose intolerance [116].

The US Food and Drug Administration (FDA) enforces the same level of consistent quality in manufacturing that they would for any other medical therapy. For example, a probiotic used as a drug should not only fulfill the general FDA conditions stipulated above, but also be in agreement with existing national regulations and guidelines on good clinical practices. In contrast, the “Council Directive 2000/13/EC on labeling, presentation and advertising of foodstuffs” prohibits to link foods to any property related to prevention, treatment or curing of human disease. The International Life Sciences Institute (ILSI) [117] also defined some general principles and requirements. This document has been the basis for the current EFSA regulation. In conclusion, current challenges in the probiotics field require improving fundamental knowledge, including criteria of selection and modes-of-action, through private—public partnership in order to provide efficient and safe probiotic strains.

Lack of strain identification, lack of repeated human studies in healthy subjects corresponding to the target population and lack of overall supporting evidence were the major limitations put forward. The drawback of the present situation is that in the absence of an operating validation process, consumers are left on their own to perform their own trial-and-error based individual testing.

In spite of this, numerous double-blind randomized control trials have been performed and compiled in over 60 meta-analysis as of 2015. To give a few examples, effects were documented and supported by meta-analysis in antibiotics-associated diarrhea [118], gastroenteritis [119,120], irritable bowel syndrome (IBS) and gut comfort [121], lactose digestion [122], IBD (UC and pouchitis) [123], Clostridium difficile diarrhea (single RCTs) [124].

As far as drug status is concerned, there are at present only very few products (Ultralevure®, Bacilor®, Enterogermina®). This situation could stem from the lack of standard guidelines and absence of a regulatory pathway to marketing authorization. By extension, although a few clinical trials were implemented, there is also no status for genetically modified organism (GMO) lactic acid bacteria. In Europe, mechanisms are hence lagging behind and there is a real need for dedicated guidelines.

Single strain and mixed strains live biotherapeutic products

Among the reasons for the renewed and rather intense interest in the microbiota has been the recognition of
dysbiosis in numerous chronic conditions that have been shown to follow a fairly dramatic trend of increasing incidence since the 1950s. Initial descriptions of dysbiosis stressed the identification of often under-represented species of symbionts, challenging the Koch’s principle of a single strain of pathogen associated with the disease and able to induce the disease when administered appropriately. In some instances, alteration of the microbiota could be suspected as the causal link, especially when transfer of a patients’ microbiota to germ-free animals was able to promote a closely related disease pattern in appropriate models. Following this path, some species of commensals consistently under-represented in disease were highlighted and eventually well documented to exert relevant bioactivities in vitro on human cells and in vivo in animal models. This is the case for the following species Table 2.

For the above species, cultured strains are available that allowed to document their bioactivities. Occasionally, new strains were isolated that confirmed the rather widespread potential within the species.

Interestingly, even though the regulatory framework is not fully outlined, some are being offered as single strain live biotherapeutic products under the status of *aliments diététiques destinés à des fins médicales spéciales* (dietetic foods for special medical purposes or ADDFMS). For some, the mode of action or bioactive molecules has been identified. This is the case for candidate small molecules and a protein for *Faecalibacterium prausnitzii* [125,126] and for polysaccharide (PSA) for *Bacteroides fragilis*. Finally, strains of the above species are being mass-cultured and processed to be provided in capsules or sachets allowing oral administration for the implementation of clinical trials to provide a proof on concept of their bioactivity in man. This means that within the coming 4 to 5 years, this new generation of probiotics will emerge and thereby offer tools to address and mitigate some of the parameters of human gut homeostasis mentioned above (for example, gut permeability or low grade inflammation).

It is obvious that none of these strains will benefit from an acknowledged “long history of safe use” as food or drug, except to say that they have been in the dominant human gut microbiota for probably most of human evolution.

Next to these species that became candidates as they consistently emerged as differentially represented in patients compared to healthy individuals, some mixed strains emerged from the simplification of complex communities used in microbiota transfer. The process is ancient as it was used to identify mixed strains exerting anti-pathogen barrier activity in the 1960s, but it has recently led to high potential observations. One such observation is the promotion of a T-regulatory immune orientation by mixed spores of the whole microbiota (patent on the work of Kenya) [127]. The bioactivity initially observed with mice spore formers in mice was thereafter documented for human spore formers in mice and is being promoted as a solution by Vedanta Biosciences [128].

These observations being still very recent, the regulatory status remains non-defined. It is most likely that it will be considered as a drug, as some agencies in Europe and the FDA in USA consider intestinal contents for fecal microbiota transplantation as a drug for the context of recurrent *Clostridium difficile* infection.

Yet the regulatory context is not structured at all to allow validation of medical claims:

- standards to assess efficacy and safety are not completely defined;
- the question of dosage is difficult: no possible pharmacokinetics; and dosages in preclinical studies are not correlated with dosages used in humans;
- the question of safety also lingers: how to deal with antibiotic resistance, virulence factors, but also induced immune response (adverse event?)?

### Table 2. Following species.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Initial association</th>
<th>Other conditions</th>
<th>In vivo models</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Faecalibacterium prausnitzii</em></td>
<td>Under-represented in IBD</td>
<td>Same in IBS, extreme obesity, diabetes, cancer</td>
<td>Protective in induced inflammation</td>
</tr>
<tr>
<td><em>Akermansia muciniphila</em></td>
<td>Under-represented in weight gain</td>
<td>Leaky gut and insulin resistance</td>
<td></td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>In animal models with inflammation</td>
<td>Altered behavior animal models</td>
<td>Animal models of autism/anxiety</td>
</tr>
<tr>
<td><em>Blautia hydrogenotrophica</em></td>
<td>Under-represented in bloating IBS</td>
<td></td>
<td>Corrects IBS symptoms</td>
</tr>
<tr>
<td><em>Roseburia intestinalis</em></td>
<td>Under-represented in inflammation</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Christensenella minuta</em></td>
<td>Under-represented in metabolic syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Eubacterium hallii</em></td>
<td>Over-represented in insulin sensitivity</td>
<td></td>
<td>Associated with improved insulin sensitivity in fecal transplantation</td>
</tr>
</tbody>
</table>

IBD: inflammatory bowel disease; IBS: irritable bowel syndrome.
• with mixed strains, authorities may strongly expect a rational in the mode of selection of the different strains and a demonstration of their synergistic effect and safety.

Microbiota transfer

A long history in diarrhea and *Clostridium difficile*.

From Ge Hong in 4th century China to the first formal publication on fecal microbiota transfer [129], it seems that the empirical application of gastrointestinal ecological management gained some recognition among practitioners themselves. It progressively turned into medical practice under the term of FMT for fecal microbiota transfer. It is essentially a very basic, proficient and crude attempt to displace a pathogen by the replacement of a diseased gastrointestinal ecology by a healthy one. It has apparently been used for decades in the context of recurrent *C. difficile* infections still known to cause 14,000 deaths per year in the USA. The clinical trial published in 2013 [130] reported a far better cure rate of recurrent *C. difficile* infection with fecal microbiota transfer than with the last resource antibiotic vancomycin (93% versus 30%).

This observation of efficacy promoted recognition by regulatory bodies of this approach as a valid therapy. FDA, in the USA, is requesting the use of investigational new drug (IND) in fecal transfer clinical trial out of *C. difficile* whereas the use of FMT is accepted (under certain conditions) for patients not responding to standard *C. difficile* therapies. In France, the *Agence nationale de santé et de sécurité du medicament* (French health agency and drug safety or ANSM) also considers the FMT as a medicinal product. In the absence of a clearly established risk–benefit ratio, this approach should be reserved for rare, or serious situations where conventional treatment fails and in the absence of available therapeutic alternative. Germany and UK also assigned the medicinal product status to FMT whereas Italy and Spain assign the transplantation product status. Efficacy showed by initial studies and clarification of the regulatory framework, in turn led to the development of companies harnessing the promise of FMT. We also noticed that less formalized implementations are ongoing, including at the extreme the "do-it-yourself" advertised on the Internet.

Another development that was promoted by the initial observations in *C. difficile* infections is the potential of application of FMT in chronic diseases, hence current attempts to apply the concept beyond the strict clinical indication of resistant *C. difficile* infection. These include indications such as IBS, IBD, metabolic syndrome and autism. Corresponding ongoing clinical trial will be informative on the relevance of expanding the concept. They may also document the durability of the actual microbiota modulation since little is known as yet on the ability to alter the microbiota in the long run, or even on the potential compatibility of ecological replacement depending on the baseline donor versus recipient microbiota. Finally, safety is bound to remain a critical aspect in allogenic FMT where short-term risk of infection but also long-term risk of host–microbes symbiosis modulation should be considered.

The last application we should mention is autologous FMT. In this approach, the patient receives its own microbiota to restore its gut ecology after a stress (hospital/community) dramatically altering host–microbiota symbiosis. Programmed interventions such as chemotherapy, long-term antibiotic treatments or even certain surgical interventions are examples of situations calling for the preservation of healthy microbiota and its re-administration for the restoration of symbiosis. In this context where the donor and recipient are the same person, safety concerns are not as critical as for allogenic FMT. Autologous microbiota transfer is the core business of the French startup company MaaT Pharma.

Numerous aspects of the clinical practice itself are still not standardized. Among these, administration by upper route (nasogastric or nasoduodenal tube) or lower route (by enema or via a colonoscope) is still unsettled. Similarly, homogenization of stool in a house blender, using oxalic saline is still routine practice when one would suspect it will severely bias survival and composition of the fecal microbiota dominated by strictly anaerobic bacteria. The most logical expectation is that of industrialized and standardized practices that will ensure safe and reproducible processes, keeping in mind the importance to build further knowledge on the fine impact and to document modes of action.

On a regulatory standpoint, it is fairly evident that nothing is really settled. So far, competent authorities for Tissues and Cells at the European level and the European Directorate for Quality of Medicines and Health Care (EDQM) and the European Center for Diseases Prevention and Control (ECDC) did not retain the application of the directive 2004/23/EC on tissues and cells for FMT, such that member States are free to decide on the most suitable framework.

Recommendations

For basic and clinical research

Time is ripe to recommend a significant paradigm shift. There is indeed a fairly robust rationale behind the use of defined or undefined mixes of live biotherapeutic products that stems from the potential to combine complementary/synergistic benefits (occupy space, modulate leaky gut, inflammation and Ox-stress). Nevertheless, there is a need for robust substantiation in controlled trials including live biotherapeutic products (LBPs) and FMT in indications of severe chronic conditions, beyond *Clostridium difficile* infections, and therapeutic options inducing dysbiosis such as long multiple antibiotic regimen, chemotherapy...

Finally, exploratory work with the best available molecular tools needs to assess mode of action (durability; resilience; impact of baseline recipient microbiota; compatibility...).

Beside these crucial points: tools to select and sort specific bacterial groups or isolates of interest from complex microbiota samples are also needed to build microbiological collections and select additional (may be more relevant and less empirically selected) biotherapeutic strains [131].

For health authorities

Industrialized/standardized processes

Industrialized processes should be favored for the development of mixed/undefined microbial preparations. This may...
be a way to avoid ‘’do-it-yourself’’ FMTs; to ensure buildup of knowledge on safety, dose, etc.; to help draw or follow a regulatory path.

Regulatory aspects
A broad reflection on the standards of evaluation and assessment must be carried out at the European level. A repository of samples for retrospective evaluations could be proposed. Safety and efficacy of the products should be assessed in humans rather than preclinical models which happen to give very little information of relevance to humans.

On a broader perspective, for the regulatory context to evolve for ANSM-European Medicines Agency (EMA) concerning live biotherapeutic products and fecal microbiota transfer, the recommendation would be to join forces and share momentum. Indeed, there are generic aspects to both LBPs and FMT, including the status of drug for therapeutics containing live microbes.

Conclusion
Modification of our environment (delivery mode, food, treatments, pollution, sedentary lifestyle, stress...) could be considered, under the spotlights of microbial diversity analysis, as the cause of most of the chronic non-transmittable diseases that are the major unmet needs of modern health. In the last century, the cause of these diseases was investigated in scrutinizing the patient genetic, and here we open the field of research to a modifiable genome that is the human microbiome. For sure, this research field is still in its infancy and we are expecting that microbiome analyses will give rise to novel treatments. It will also promote another way to consider the human being, as a man—microbes supraorganism that should be cured in its entirety. Towards this aim, we would recommend that the use of these new investigational tools would be sponsored and encouraged by health authorities as the necessary investment to broaden the therapeutic arsenal. From this review, it is obvious and highly scientifically documented that variations in the intestinal microbiota composition may end up in dysbiosis with severe health consequences that can be acute or chronic as well as short- or long-lasting. It is also apparent that many avenues are explored currently in France and in EU for the development of means to prevent or counterbalance the causes or consequences of such dysbiosis. By contrast, the lack of clear consensus on what is a normal/abnormal microbiota prevent the ease for designing and financing development plans for these means. Synergistic work of various EU agencies including IMI, EMA, International Conference on Harmonization (ICH), EFSA and possibly others, should be strongly recommended to push ahead progress in that domain, given their potential for health as well as economic impacts.

Disclosure of interest
The authors declare that they have no competing interest.

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Human gut microbiome as source of innovation for health


