The International Cancer Microbiome Consortium Consensus Statement on the Role of the Human Microbiome in Carcinogenesis

Supplement

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Expert ratings of consensus statements:

Question One: How does the concept of "dysbiosis" relate to carcinogenesis?

1. With respect to carcinogenesis, "dysbiosis" should be considered a persistent departure of the host microbiome from the healthy, physiological state, towards a cancer promoting and/or sustaining phenotype.

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	0	Disagree	1
Evidence from animal studies	5	Neutral	0
Weak evidence from human studies	9	Agree	10
Strong evidence from human studies	2	Strongly agree	5

1. At the present time, there is no accepted quantitative definition of a "normal" microbiome.

Evidentiary Support		**Expert Agreement**	
No evidence	3	Strongly disagree	0
Evidence from in vitro studies	0	Disagree	0
Evidence from animal studies	1	Neutral	1
Weak evidence from human studies	5	Agree	8
Strong evidence from human studies	7	Strongly agree	7

Question Two: What are the broad molecular mechanisms by which the human microbiome may be involved in the aetiopathogenesis of cancer?

1. The mechanisms by which the microbiome may initiate and/or drive carcinogenesis can be classified into:
    a. Genomic integration

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	1
Evidence from in vitro studies	2	Disagree	0
Evidence from animal studies	3	Neutral	0
Weak evidence from human studies	2	Agree	8
Strong evidence from human studies	9	Strongly agree	7

    b. Genotoxicity

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	3	Disagree	1
Evidence from animal studies	6	Neutral	1
Weak evidence from human studies	4	Agree	9
Strong evidence from human studies	3	Strongly agree	5

    c. Inflammation

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	1

Evidence from in vitro studies	0	Disagree	0
Evidence from animal studies	5	Neutral	0
Weak evidence from human studies	4	Agree	8
Strong evidence from human studies	7	Strongly agree	7

d. Immunity

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	1	Disagree	0
Evidence from animal studies	6	Neutral	2
Weak evidence from human studies	6	Agree	9
Strong evidence from human studies	3	Strongly agree	5

e. Metabolism

Evidentiary Support		**Expert Agreement**	
No evidence	1	Strongly disagree	0
Evidence from in vitro studies	2	Disagree	1
Evidence from animal studies	3	Neutral	0
Weak evidence from human studies	7	Agree	13
Strong evidence from human studies	3	Strongly agree	2

Question Three: What are the conceptual frameworks that best describe the promotion of carcinogenesis by the human microbiome?

1. With respect to the pathogenesis of colorectal cancer, “the driver-passenger” model accounts for key observations from mechanistic studies and investigations of the on- vs. off-tumour microbiome.

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	1
Evidence from in vitro studies	0	Disagree	0
Evidence from animal studies	7	Neutral	2
Weak evidence from human studies	9	Agree	8
Strong evidence from human studies	0	Strongly agree	5

2. More broadly, the role of the microbiome in the aetiopathogenesis can be conceptualised as one apex of a tripartite, multi-directional interactome alongside the environment and an epi-/genetically vulnerable host.

Evidentiary Support		**Expert Agreement**	
No evidence	1	Strongly disagree	0
Evidence from in vitro studies	1	Disagree	0
Evidence from animal studies	5	Neutral	0
Weak evidence from human studies	7	Agree	7
Strong evidence from human studies	2	Strongly agree	9

Question Four: Is the relationship between the human microbiome and the aetiopathogenesis of cancer causative or associative?

1. At the single-organism level, the role of microorganisms as aetiological agents in carcinogenesis is well-established.

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	0	Disagree	0
Evidence from animal studies	4	Neutral	1
Weak evidence from human studies	2	Agree	7
Strong evidence from human studies	10	Strongly agree	8

2. There are plausible mechanisms by which the human microbiome may cause cancer.

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	1	Disagree	0
Evidence from animal studies	8	Neutral	1
Weak evidence from human studies	2	Agree	5
Strong evidence from human studies	5	Strongly agree	10

3. There is a causal relationship between the human microbiome and the aetiopathogenesis of some cancers.

Evidentiary Support		**Expert Agreement**	
No evidence	0	Strongly disagree	0
Evidence from in vitro studies	0	Disagree	0
Evidence from animal studies	3	Neutral	0
Weak evidence from human studies	8	Agree	10
Strong evidence from human studies	5	Strongly agree	6

Question Five: What are the key directions for future research to develop our understanding of the role of the microbiome in carcinogenesis?

1. Key areas for further development with respect to the investigation of the microbiome and carcinogenesis are:
    a. Large, international cohort studies

Expert Agreement	
Strongly disagree	2
Disagree	0
Neutral	0
Agree	4
Strongly agree	10

    b. Prospective longitudinal sampling

Expert Agreement	

Strongly disagree	1
Disagree	0
Neutral	1
Agree	0
Strongly agree	14

c. More focus on interventional, rather than purely observational, studies.

Expert Agreement	
Strongly disagree	1
Disagree	1
Neutral	2
Agree	6
Strongly agree	6

d. Integration of microbiome analysis with other oncological research projects.

Expert Agreement	
Strongly disagree	1
Disagree	0
Neutral	0
Agree	3
Strongly agree	12

e. Standardisation and transparency in reporting microbiome research

Expert Agreement	
Strongly disagree	1
Disagree	0
Neutral	2
Agree	3
Strongly agree	10

Selected quotes from the roundtable discussion:

How does the concept of "dysbiosis" relate to carcinogenesis?

Professor Hans Verstraelen (HV)

"I am always a little bit sceptical when I hear talk about pathobionts in this context [the microbiome] as… it gives me the feeling that we're going back to monocausal thinking [from] infectious contexts that we are trying to surpass by thinking in terms of community."

"In a community that has been called dysbiotic… there may be some community members that are increasing in abundance and have intrinsic pathogenicity but [this is] not an isolated phenomenon; they are in a community and it is the community that interacts with the niche."

Dr James Kinross (JMK)

"It's also a time-dependent issue: it's dynamic and niche specific."

Professor Christian Jobin (CJ)

"The microbiome is always with respect to the organ of interest."

HV

"There are a number of… definitions of dysbiosis but somehow they all describe… a departure of the community from what is expected to be healthy. Obviously the question emerges: what do we call healthy?"

"Most microbiome researchers tend to define [the] "healthy microbiome" as the microbiome that is present in a given individual in the absence of symptoms. I have major difficulties with [this]."

"If you screen for instance for BRCA and you identify someone as high risk [for breast cancer] do you call [them] a "healthy" person in the absence of symtpoms? Do we call [them] someone who is at risk? Do we call it disease? [This] needs to be very clear before you can define what is the healthy microbiome"

"You can't talk about dysbiosis… if you don't know what a healthy microbiome is."

Professor Rex Gaskins (RG)

"Another complication is the inter-individual variation. You can't group everybody together and think there's one solution."

Dr Claire Merrifield (CAM)

"The early microbiome is so key in developing the immune system.. which is tolerant to your microbiome. So your microbiome is always going to be unique. I don't know that we can [define] one healthy microbiome."

Professor Daniel Rosenberg (DR)

"What's dysbiosis to one person might be perfection to someone else."

Professor Julian Marchesi (JRM)

"At the moment we can't define a healthy microbiome. We can define a continuum, on which people sit but unless we can follow those individuals over time and capture their [associated] metadata we can't say that microbiome for you as an individual is a risk for a, b and c."

Professor David Cunningham

"What we need is longitudinal information so we can reach a view on what [is] a "normal" or "optimal" microbiome."

HV

"I always make the analogy with cardiovascular medicine where a lot of the definitions of what we consider normal come from the Framingham study."

JMK

"It's what the microbiome is doing rather than what is there that is important. We are making statements [about dysbiosis] on the basis of ecology; who is there. Actually what's more important is what are they doing when you stress them?"

Professor Jeremy Burton (JB)

"We shouldn't be looking at just the 16S composition because there's a lot of… biochemical redundancy."

Dr Alasdair Scott

"Can we define "normal" not in terms of… ecology… but in functional terms? A healthy microbiome would be commensal, immune tolerant, diverse, resistant to change, tumour suppressive. There might be lots of ecologies which would give the [same] result. Any departure from that you could define as dysbiosis."

CJ

"A consortium of microorganisms who's activity leads to… certain levels of butyrate, indoles… You don't corner yourself into specific sets of taxa."

JRM

"Maybe that's the consensus that can come out of this – do we need to start defining functions that we think would constitute a healthy microbiome? This is not just the gut. It's the skin, nasopharyngeal, vaginal… What cross-cutting themes can we identify that are important across the microbiomes and what are niche-specific?"

JMK

"In terms of cancer, dysbiosis is quite a divisive term because it probably doesn't describe what we need for explaining cancer risk. It's function that is the critical component rather than structure. What we can seek to do as a consortium is define what those critical functions might be for preventing cancer initiation. Of course, each organ system will have its own set of criteria."

Is the relationship between the host microbiota and the aetiopathogenesis of cancer causative or associative?

JA

"Stanley Falkow redrafted [Koch's postulates] in the molecular context. A bacteria is causative to a disease, not just by the presence of the pathogen but by the phenotype it expresses in vivo in a specific spatial context."

Dr Stephen O'Keefe (SJOK)

"H. pylori is part of the normal microbiota; it's not a pathogen. If you go anywhere in the developing world everybody's got H. pylori. They don't get duodenal ulcers and they don't have any increased risk of gastric cancer. Whereas in the West they do. Therefore there is a difference in the other

environmental factors which are very important. I think that the microbiome in general in patients with H. pylori should be examined in much more detail."

JRM

"Is it strain specific? Some strains [of H. pylori] are more virulent and would fall into the class of being a carcinogen but there are some that aren't as virulent that would be a pathobiont and given the opportunity may start causing inflammation."

JMK

"I don't think there is debate about [the role of H. pylori in gastric cancer]."

Professor Julian Teare

"It's important to try and understand the magnitude of any particular effect. Clearly your family history carries a risk. What about your diet? What is the risk of a differing microbiome? You can't change your genes but you can change your diet. You can change your microbiome."

JMK

"There is causation. [However], we need more [longitudinal] clinical data before a clinical audience [will] buy into this on a large scale."

"What is lacking from the evidence that has been presented is a really large-scale ambitious clinical study that.. recruits patients… and follows them from childhood all the way through longitudinally. We need something [like this] in cancer if we are ever going to get to the bottom of [causation]."

"I understand why we have these animal models and I understand their value and their function but I just worry… how translatable they really are to the complexity of a human."

CJ

"The bug itself is not enough… we need a host susceptibility in some way [to cause cancer]."

"With a cautious "yes" I think we could probably make some claim of causation as far as colorectal cancer [is concerned]."

"We are very biased towards bacteria… the microbiome is more than bacteria."

Assuming causation, what are the broad molecular mechanisms by which the microbiota may be involved in the aetiopathogenesis of cancer?

SJOK

"[We hypothesise that] diet affects cancer risk by its effect on the microbiota-produced metabolites which may be.. [healthy] for the mucosa, such as butyrate or toxic such as secondary bile acids or hydrogen sulphide."

"There really is substantial evidence for the role of short chain fatty acids in maintaining mucosal health and actually having anti-carcinogenic effects."

"There really is very good evidence that you can prevent most cancers by changing your diet. Now, whether that's induced by changes in the microbiota and metabolites… well, that's conjecture."

JRM

"I could play Devil's Advocate and say a lot of those things [biochemical processes associated with the microbiota] will be happening in the small intestine as well. So why aren't we seeing small intestinal cancers as much as we're seeing colorectal cancer? You've got butyrate in the small intestine, you've got bile acids in the small intestine, we've got all the nitrosamines… so why don't we see that [cancer] there?"

JRM

"But that's one thing I thought we were, in some aspects, trying to move away from. This [idea of] "an organism causing cancer, a pathogen causing cancer." Because that doesn't help [when] you look at people getting colorectal cancer; they don't have a pathogen in their large intestines."

JRM

" I don't think you have to have adherence of an organism to the mucosa to cause a pathology and damage. You can do it [via] organisms making metabolites that'll diffuse through [to the mucosa]. They don't have to be attached to the surface."

"I think you can… find a potential hypothesis where microbiomes in the gut could influence any cancer [outside of the gastrointestinal tract]."

RG

"In our own work… microbes are closely associated with the mucosa but they're not adherent. They are making a mutagen. Adhesion is not a requirement."

Dr John Alverdy (JA)

"What is the broad mechanism [by which microorganisms cause cancer]? Is it subverting the microbiome? Is it the vulnerable host who cannot counter-adapt to the pathogen that's driving the oncogenic process?"

JRM

"We can talk about the class one carcinogens, like HPV, Helicobacter pylori… a pathogen causing a known cancer. There are molecular mechanisms behind each of those. They have effector molecules… virulence factors. They interfere with some aspect of cellular biochemistry and immunology and then you get the cancer developing. Then you have… the metabolite-driven and the carcinogen-driven [cancers]. So, Desulfovibrio piger – would you call it a pathogen? No, but it produces a potent genotoxin. E.coli producing colibactin – is that a pathogen? [These organisms] are causing collateral damage."

SJOK

"We have a host of [intrinsic bacteria] which can get out of track if your host defence mechanisms are broken down."

JRM

"It is the organism-host interactome that's important here."

What are the conceptual frameworks that best describe the promotion of carcinogenesis by the host microbiota?

JRM

"These organisms [tumour passenger bacteria] do have a dynamic role to play on the carcinoma. They're not just passengers… some are active passengers. For example… Fusobacteria… are interacting with the carcinoma."

"Whether or not these [models of microbiome induced carcinogenesis] are applicable to other mucosal tumours [e.g. lungs, vagina], I don't know. It may depend on which cancer you are looking at, which model is generalizable…"

Professor Jun Yu

"If it [a putative driver species] is abundant in the early stage… pre-cancerous adenoma.. and the cancer tissue perhaps it's a driver. If it's only [abundant] in cancer then maybe it's a consequence [passenger]. What about the depletion of bacteria in cancer patients? The depletion of "good" bacteria that may have a protective effect."

JRM

"Driver-Passenger seems to have superseded it [Alpha Bug hypothesis]… [I've gone] to two DDW [Digestive Diseases Week] meetings back-to-back and not seen much mention of the Alpha bug, but seeing mention of this model [Driver-Passenger]."

"The Alpha Bug model… is a more classical microbiological model – it is an organism that causes a disease."

RG

"I don't think the two models are mutually exclusive."

JRM

"No, I agree with you. You may not get passengers, for example, in bone cancer but you might have a driver pushing it forward. There's not a lot of opportunity for an organism to colonise the tumour within the bone."

DR

"The idea that 20 years of exposure to one of these metabolic products [e.g. secondary bile acids] is causing colon cancer isn't easy for me to accept. [You said] bile acids are potent carcinogens – we're all exposed to secondary bile acids for many many decades of our lives and not many of us get colon cancer."

SJOK

"There's a threshold effect."

JRM

"It's a susceptible host, not being able to recover the mutation…"

RG

"In the context of a genotoxin, you're constantly repairing and it only takes one hit. So… over time… I would suggest that's why the colon cancer onset is at a late age. You constantly repair and then, at some point, with a susceptible genetic background… Different alleles are going to affect susceptibility."

DR

"What you're saying is that it's the bug-host-genome interaction."

JA

"It's a… bidirectional… conversation. And it's iterative… The final outcome is highly unpredictable. When you add sulfates to a cell line and you see DNA damage you've got a causative inference. But when you're talking about a human, eating a Western diet, travelling all over the world, taking a Z-pack [Azithromycin] every three or four months, with a sleep disorder, you're talking about a host that's highly vulnerable to a pathogen that's highly opportunistic. We should use the term "a vulnerable host" because we do know that lifestyle affects cancer. We ought to make it "interactome" – lifestyle, vulnerable host and pathogen/microbiome. This interaction can lead to the acquisition of a carcinogenic [microbiome], which, if there is a failure of [host] resistance… can [lead to the development of] a cancer."

CJ and CAM

"It doesn't have to be "acquisition", which would imply infection… it could be adoption."

SJOK

"So an example of the host defence side, is that there are about 18 genetic loci that have been identified as being associated with colon cancer risk. This is in studies of 5-10,000 individuals. What's very interesting is that about half of these factors; their effect becomes more pronounced if you have a high-meat diet. You have to be genetically susceptible. Why doesn't everyone who eats a high-meat diet get colon cancer?"

JMK

"Maybe [these models] are not particularly relevant. Maybe, it's the [vulnerable host-interactome] model that's more generalisable… actually that's what's critical here."

JA

"Cancer is susceptible to changes in the microbiome… but changes in the microbiome are susceptible to diet, stress, host genotype… Trying to get the interactome deconstructed down to the… putative causative agent… that's why this is so hard."

What are the key future directions for research within this field?

JRM

"It is an old problem, this concept of standardisation in biology… sometimes you run the risk of just measuring the standardisation process itself and not real biology."

Dr David Hughes

"[Existing epidemiological studies] have not really looked specifically at the microbiome – this is something we can tap into."

SJOK

"The problem with [microbiome research] is that too many people are doing observational studies."

Professor Robert Brown

"It might be useful to learn from some of the many mistakes that have been made in the cancer biomarker field. Probably 85%... of biomarker work is irreproducible. There are now a whole load of guidelines… about how to do good prognostic, predictive studies."