

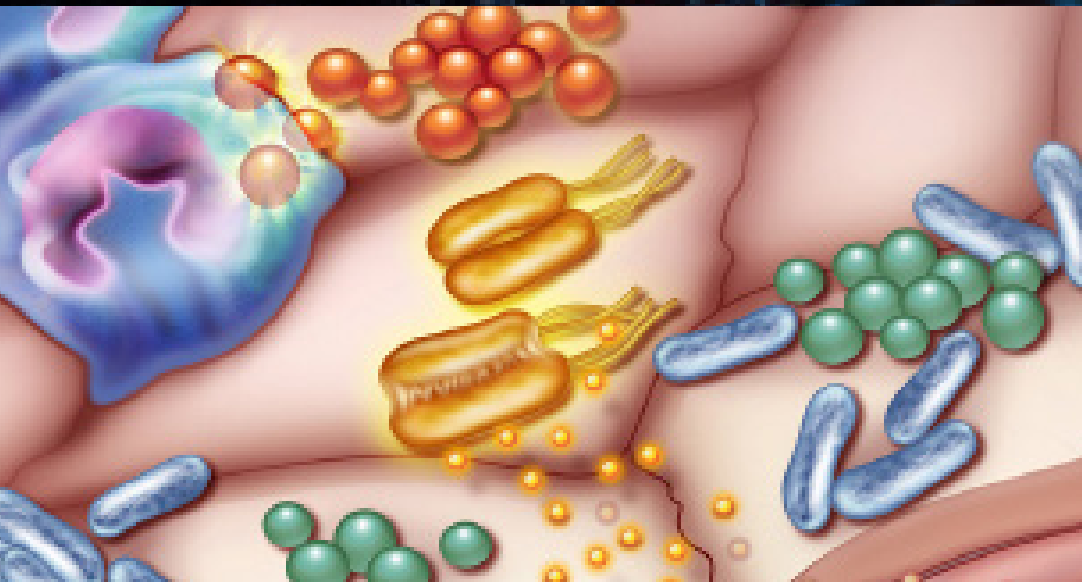
TURNING CANCER DISCOVERIES INTO TREATMENTS

# FRONTIERS

SPRING 2016

## Ecosystem Restoration

Can a black-raspberry drink repair oral microbiomes damaged by smoking and prevent cancers of the mouth?



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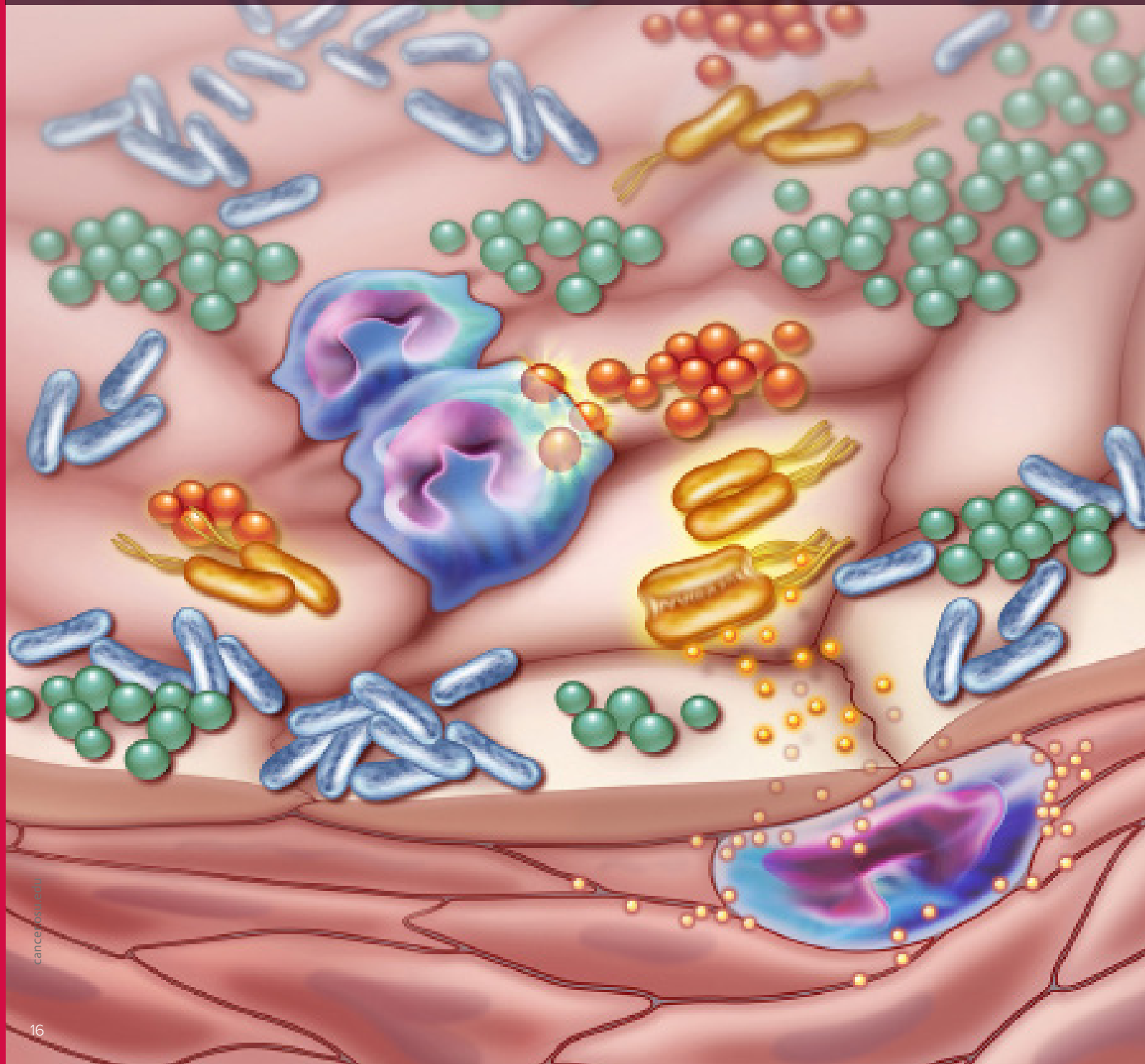


THE OHIO STATE UNIVERSITY  
COMPREHENSIVE CANCER CENTER



A Comprehensive Cancer  
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*The oral microbiome of a smoker. Healthy bacteria (green and blue) and pathogenic bacteria (red and orange) reside on the gum epithelium. Three neutrophils (blue) on the surface work to clear the pathogens. A neutrophil within the epithelial (lower right) releases cytokines to draw more immune cells to the site. The resulting chronic inflammation facilitates cancer development in the epithelial cells. (Note: cells and bacteria not drawn to scale.)*



# Ecosystem Restoration

*Currently, four OSUCCC – James researchers are collaborating to learn whether a food-based approach using a novel black raspberry drink might help prevent oral cancer, with a focus on how the microbiome may play a key role in this relationship.*

BY DARRELL E. WARD

Eukaryotic cells and prokaryotic cells have long enjoyed positive, intimate associations—including the most intimate of relationships.

Eukaryotic cells are the fundamental building blocks of the human body, and they prominently feature a nucleus that contains most of their DNA. Prokaryote cells are typically called bacteria. They lack a nucleus, are far smaller than most human cells and are popularly regarded as disease-causing and pathogenic.

Some 1.4 billion years ago, the two cell types shared the ultimate intimate experience: An ancestral eukaryote is believed to have engulfed an ancestral prokaryote. It was the start of something great. The nucleated cell gave the bacteria a stable, cozy place to live, and the bacteria became the energy-producing organelle called

mitochondria that have powered eukaryotic-cell activity ever since.


Intimate partnerships between bacteria and the human body continue today, with rapidly emerging research defining the composition and function of various bacterial communities, called microbiomes, that inhabit the mouth, colon, skin and other areas of the body. In fact, the National Institutes of Health's recently established Human Microbiome Project (HMP) estimates that a healthy human may have nearly as many microbial cells as human cells.

These microbial populations interact with the host, including the immune system, to maintain human health, but when the microbial population is deranged or microbes pass through host mucosal barriers, it can contribute



to disease risk—leading the NIH HMP to suggest that the human genome should be broadened to include the genes of its microbes. (Growing awareness of the body's microbial communities has given rise to the emerging field of microbiomics.)

There is great interest in understanding the role of the microbiome in cancer. Researchers at The Ohio State University Comprehensive Cancer Center –

Underlining and  indicate more information online at <http://cancer.osu.edu/Frontiers>.

*“All these disciplines are located on one campus, with the investigators integrated through cancer center activities. It illustrates what a cancer center should be doing: bringing diverse talents together to focus on a preventable malignancy like oral cancer.”*

**STEVEN CLINTON,  
MD, PHD,**

*The John B. and Jane T. McCoy Chair in Cancer Research and leader of the OSUCCC – James Molecular Carcinogenesis and Chemoprevention Program (MCCP)*



Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James) are carrying out studies investigating whether smoking-related changes in the oral microbiome raise the risk of disease.

Currently, four OSUCCC – James researchers are collaborating to learn whether a food-based approach using a novel black raspberry drink might help prevent oral cancer, with a focus on how the microbiome may play a key role in this relationship.

The study, titled “Interactive Omics: Black Raspberry Metabolites and the Oral Microbiome in Smokers,” is supported by a five-year, \$3.1 million grant (CA188250) from the National Cancer Institute.

Co-principal investigators on the study are **Purnima Kumar, DDS, PhD**, associate professor of Periodontology at Ohio State’s College of Dentistry; **Steven Clinton, MD, PhD**, The John B. and Jane T. McCoy Chair in Cancer Research and leader of the OSUCCC – James Molecular Carcinogenesis and Chemoprevention Program (MCCP); **Steven Schwartz, PhD**, professor of Food Science and Technology in Ohio State’s College of Food, Agricultural and Environmental Sciences; and **Christopher Weghorst, PhD**, professor of Environmental Health Sciences in the College of Public Health.

Oral cancer occurs in more than 260,000 people worldwide and kills

127,000 people yearly. Treatment for the disease is rigorous and often disfiguring and can reduce a person’s ability to chew, swallow and speak. Smoking increases the risk of oral cancer 6 to 27 times. Furthermore, smoking rates are rising in many developing nations, which lack the expertise and resources to effectively treat this cancer.

“We urgently need new approaches to prevent this disease, particularly in high-risk populations such as past and current smokers,” Clinton says.

Earlier work by the team showed that black raspberries can reduce hallmark molecular biomarkers in the oral mucosa associated with increased cancer risk. The bioactive phytochemicals from black raspberries also influence bacterial adhesion and biofilm formation, which may in turn impact the host gene expression of mucosal cells.

For the new study, the team developed and tested a novel black raspberry nectar drink that concentrates and preserves black raspberry phytochemicals, an effort that began with a collaboration with Dale Stokes and family, who manage a large black raspberry farm in Ohio. The novel project will determine whether the drink can restore the altered microbiome typical of smokers to a healthier state and provide a viable cancer preventive intervention.

“This research highlights the advantages of a land grant university,” Clinton adds. “It



involves Ohio State's College of Food, Agricultural and Environmental Sciences, the College of Dentistry, the College of Public Health and the OSUCCC – James.

“All these disciplines are located on one campus, with the investigators integrated through cancer center activities. It illustrates what a cancer center should be doing: bringing diverse talents together to focus on a preventable malignancy like oral cancer.”

### THE ORAL MICROBIOME

Kumar describes the oral microbiome as an ecosystem composed of some 2 billion organisms and having several distinct niches. For example, tooth surfaces above the gum line, pockets between teeth and gums, the tongue and tonsils each have a specific microbiome with characteristic species. “We’ve shown in previous studies that people of different ethnicities, of different ages or who have diabetes or are obese harbor distinct types of bacterial populations,” says Kumar, who has studied the oral microbiome for nine years.

Fifty to 60 species of bacteria live in the healthy oral cavity at any one time, but more than 700 species of oral bacteria have been identified across populations, she notes.

“We didn’t even suspect that many of these species were present in the oral environment until new DNA-based technologies revealed them. Now we are learning who they are, what genes they carry and

whether they are pathogenic, highly virulent or just gentle bystanders, or even promote health,” she says.

As in terrestrial ecosystems, environmental change can affect the oral ecosystem. “Altered microbial communities underlie the etiology of several oral diseases, especially in smokers,” Kumar says.

“Bacteria that live in a tooth pocket can also be impacted by smoking and cause bone resorption and gum disease, while related bacteria found on the mucous membrane are associated with oral cancer,” she notes.

A study led by Kumar suggested that, in addition to the direct effects of carcinogens on the mucosa, smoking makes the oral cavity less suitable for healthy microbes and encourages growth of pathogenic species. These species induce an inflammatory response that favors cancer development.

A 14-year-old boy has an established, stable, endemic microbiome, she explains. If he begins smoking, it changes the oral environment from oxygen rich to oxygen poor. The pH falls, levels of iron and other metals go up, and chemicals, metals and nicotine enter the bloodstream and circulate through the body.

Oxygen-loving aerobic species die off, and pathogenic organisms that thrive in an oxygen-poor, iron-rich, reducing environment overgrow the community.

“These include bacteria usually found in lung infections such as *Pseudomonas*, *Haemophilus*,

*“We’ve shown in previous studies that people of different ethnicities, of different ages or who have diabetes or are obese harbor distinct types of bacterial populations.”*

### PURNIMA KUMAR, DDS, PHD,

associate professor of Periodontology at Ohio State's College of Dentistry



*“Investigating all these interactions is really exciting and will provide fundamental findings on how foods, tobacco and the microbiome interact to impact cancer risk...”*

**CHRISTOPHER WEGHORST, PHD,**

*professor of Environmental Health Sciences in the College of Public Health*



*Acinetobacter,”* Kumar says. “We think that when a smoker coughs heavily, these bacteria may colonize the oral cavity.”

Many of the bacteria found in the microbiome of smokers have potent virulence factors and promote an inflammatory response in the host tissues. These changes may be identified by a specific series of changes in gene expression within the host oral mucosa, long before the development of cancer.

Once smoking alters the microbiome and an inflammatory process begins, the continued exposure to tobacco smoke promotes a vicious, self-perpetuating cycle that ultimately changes the oral mucosa and promotes the development of a cancer.

### **BLACK RASPBERRY EFFECTS**

The OSUCCC – James has a long history of research on the effects of the role of black raspberry phytochemicals in cancers of the aerodigestive tract. These fundamental studies, led by Weghorst, Clinton, Schwartz and others led to the newly funded project on black raspberry phytochemicals and oral cancer at the OSUCCC – James.

The studies included developing various black raspberry food products, an effort led by Yael Vodovotz, PhD, a member of the OSUCCC – James MCCP and

professor of Food Science and Technology. These food products, which range from confections to the nectar, have been tested in several clinical trials, suggesting that black raspberry foods provide for high patient compliance and demonstrate that the pattern of phytochemicals might be effective in cancer prevention.

The MCCP, along with funding from Pelotonia—an annual bicycling fund-raiser for cancer research at Ohio State—was instrumental in this intersection of food-product development research, preclinical anticancer laboratory testing and fundamental research on the oral microbiome.

An example of a supportive berry study was led by MCCP member Thomas Knobloch, PhD, a collaborator on the new study, and on a study recently published in the journal *Cancer Prevention Research*. It provided strong evidence for a gene-expression signature that is altered by black-raspberry phytochemicals.

The study involved 38 patients with oral squamous cell carcinoma. The patients took slow-release black-raspberry lozenges, made from freeze-dried black raspberry powder, four times a day from their diagnosis through surgery to remove the cancer (mean duration 14 days).

The researchers learned that the berry treatment significantly reduced the expression of genes

that promote inflammation and inhibit apoptosis. “Our findings also showed that critical anticancer compounds in black raspberries were present in the tumor tissue,” Weghorst says. This work was critically supported by highly sensitive analytical chemistry provided by Schwartz, director of the OSUCCC – James Nutrient and Phytochemical Shared Resource. The current study, however, focuses on smokers and nonsmokers at high risk for oral cancer. It is designed to learn whether consumption of the black raspberry nectar, which is high in bioactive compounds, can inhibit or reverse damage to the oral microbiome and mucosa caused by tobacco smoke.

Within that, the researchers want to understand the mechanisms by which black raspberries act. To investigate this, the Clinton, Kumar, Schwartz and Weghorst laboratories are teasing out the interactions between oral mucosal cells, the microbiome and the berries.

Key questions include whether the bacteria in smokers and non-smokers metabolize and further activate the berry phytochemicals or inactivate them, which would alter the preventive effect. “We’re using metabolomics to answer those questions,” Schwartz says.

On the flip side, the researchers want to learn whether the berries influence the microbiome population. Do they perhaps restore the smoker’s microbiome

to a healthier state or reduce the impact of tobacco on the microbial population?

“Investigating all these interactions is really exciting and will provide fundamental findings on how foods, tobacco and the

microbiome interact to impact cancer risk,” Weghorst says.

The researchers believe their findings will reveal whether oral bacteria influence the formation of black raspberry bioactive substances and will provide a



**CLINICAL TRIAL**

To explore questions related to the oral microbiome, Clinton, Kumar and the team have initiated a clinical trial involving 120 men and 120 women divided into four groups, with 30 males and 30 females in each. Two groups are smokers and two groups are nonsmokers.

One group of smokers and one group of never-smokers consume eight ounces of black-raspberry nectar daily for 12 weeks; the remaining smokers and never-smokers consume a similarly packaged placebo:

- 60 Never smokers + nectar**
- 60 Smokers + nectar**
- 60 Never smokers + placebo**
- 60 Smokers + placebo**



Yael Vodovotz, PhD, a member of the OSUCCC – James Molecular Carcinogenesis and Chemoprevention Program and professor of Food Science and Technology, developed the nectar. Initial testing demonstrated excellent compliance and feasibility, so it was chosen as the optimal agent for the large study.

Vodovotz used state-of-the-art food science technology available at Ohio State to develop the beverage. Each 8 oz. serving contains 10 grams of freeze-dried black-raspberry powder rich in bioactive black-raspberry phytochemicals. She and her lab also developed the placebo beverage using raspberry flavoring and color.

Before the berry treatment begins and at the end of week 1 and week 12, the researchers collect oral-cavity samples from each participant for the “omics” analyses, which include:

- Genomic and transcriptomic sequencing studies by Kumar’s lab of biofilm scrapings to learn how the oral microbiome responds structurally and functionally to the nectar treatment;
- Metabolomic studies of saliva samples by Schwartz’s lab for physiological and food-derived metabolites to learn the impact of smoking on berry metabolism;
- Transcriptomic studies by Weghorst’s lab before and after berry treatment to evaluate mucosal gene expression in response to nectar and tobacco exposure.

*“Key questions include whether the bacteria in smokers and non-smokers metabolize and further activate the berry phytochemicals or inactivate them, which would alter the preventive effect. We’re using metabolomics to answer those questions...”*

**STEVEN  
SCHWARTZ, PHD,**

*professor of Food Science  
and Technology in  
Ohio State’s College of  
Food, Agricultural and  
Environmental Sciences*



panel of bacterial markers for black raspberry exposure and validated timelines for berry treatment during a future phase III clinical trial in patients at high risk for oral cancer.

In the end, the researchers hope to find that carefully designed food products, such as those with black raspberries, offer a practical strategy that can prevent the initiation and progression of oral cancer in conjunction with smoking-cessation strategies.

“We’re looking for biomarkers and potential mechanisms that can be tested in a long-term phase III clinical trial,” Weghorst says.

“Our study will also help accomplish our long-term goal of developing food-based strategies for disease prevention, point-of-care diagnostics and biological metrics for successful treatment outcomes in high-risk populations,” Clinton says.

“Once the mechanisms of carcinogenesis are worked out, it might lead to valuable biomarkers that can define risk for each individual and perhaps identify new prevention or therapeutic targets,” Kumar says. “That’s the ultimate goal of all this research, of course: stopping cancer before it becomes a clinical entity.

“The oral microbiome is the canary in the coal mine,” she adds. “A smoker who is healthy might have no disease, but his microbiome tells us he’s at risk for disease.

“Keeping your oral microbiome healthy and stable is another reason

to avoid smoking, and for people who do smoke to quit. Otherwise you develop a hyperinflammatory state that can quickly lead to disease. So it helps to think about the oral cavity and our other microbiomes as ecosystems, because it encourages you to keep them well,” she says.

Efforts like this study by Clinton, Kumar, Schwartz and Weghorst to understand the role of the microbiome in health and disease are just the first wave of discovery.

“Next, we have to learn how we can alter these microbial ecosystems, not just in the oral cavity but in other tissues as well, in order to treat or prevent disease,” Clinton says.

“This is a whole new world of possibilities regarding how diet and nutrition impact cancer, and it opened up in just the last few years due to the exciting advances in defining the structure and function of various microbiomes.” ■