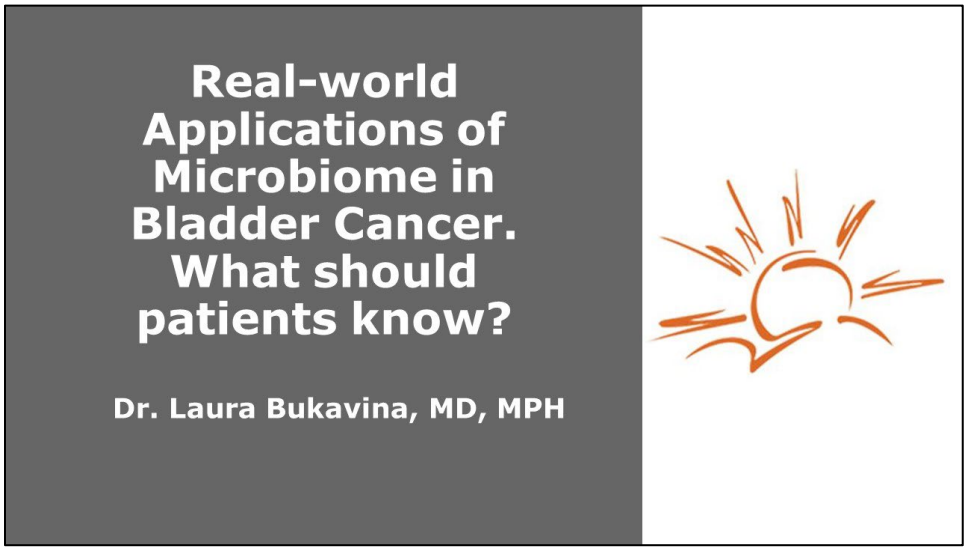
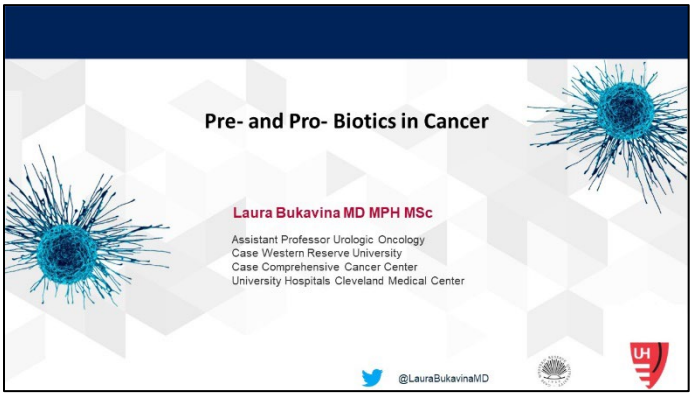


Stephanie Chisolm:

Welcome to Real World Applications of the Microbiome in Bladder Cancer. What Should Patients Know? Scientists have been looking at the relationship between the microbiome, probiotics and bladder cancer. That relationship is very complex and it's not well understood. Today's program is really intended to shed some light. It's not going to give you all the answers. It might give you some good questions to bring back to your healthcare provider, that really look at the potential impact of your gut bacteria on the tumor development and your treatment responses.

We're delighted to have urologist and scientist, Dr. Laura Bukavina from Case Western Reserve University Hospitals Cleveland Medical Center. In 2022, Dr. Bukavina received one of BCAN's Young Investigator Awards to support her research, and today she's going to explain that cutting-edge research that she did on the microbiome and it was showcased in BCAN's 2023 Bladder Cancer Think Tank.

Dr. Laura Bukavina:



Hi everyone. My name is Laura Bukavina. As Stephanie has said, I am a urologic oncologist. I do a lot of work in bladder cancer research and I'm at Case Western University Hospitals. So today's focus really is to talk a little bit about the research that we have been doing in bladder cancer, but also, I wanted to just give an overall presentation of what is microbiome. What is probiotics, prebiotics? Because I think having that level of understanding really

allows you to be able to transition to microbiome and ask the appropriate questions. So we have to start at the bottom and work our way up to what microbiome is.

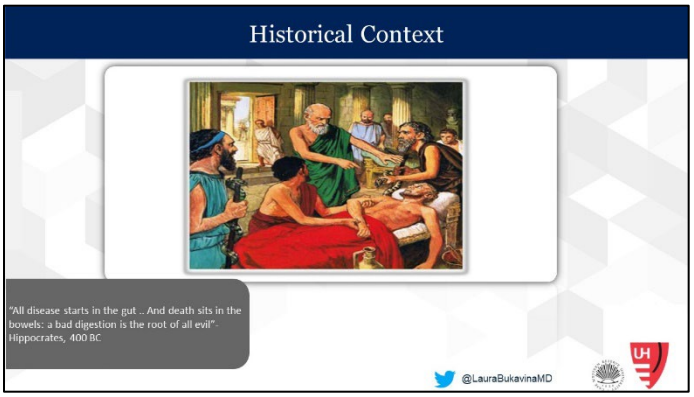
So as I said, we're going to talk about the pre- and the pro- and the syn- and the post-biotics, which are a lot of confusing language, and I think everyone who looks at this is really confused. I know even a couple of years ago when I was looking into it, this was a very confusing topic overall.

Dr. Laura Bukavina:

So there are no disclosures that are relevant to this presentation. As Stephanie has said, a lot of the research that I have presented is supported by BCAN Young Investigator.

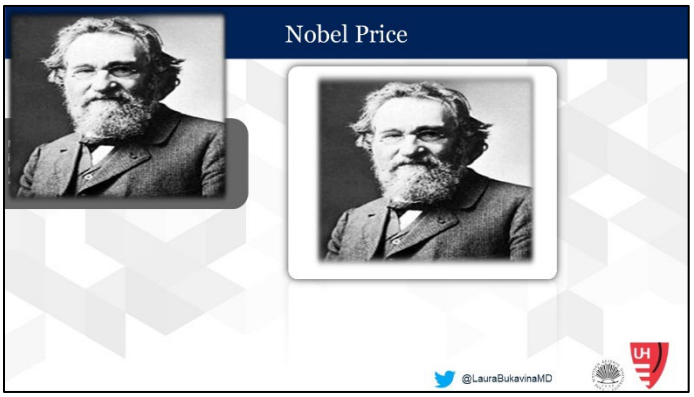
Dr. Laura Bukavina:

So in terms of historical context, the concept of probiotics and prebiotics really started a long time ago. As Hippocrates was a huge proponent of probiotics, he said on multiple occasions that, "All disease starts in the gut." That's one of his quotes, and, "Death sits in the bowels and bad digestion is the root of all evil." So clearly he was a fan of microbiome and probiotics even before the term was coined.



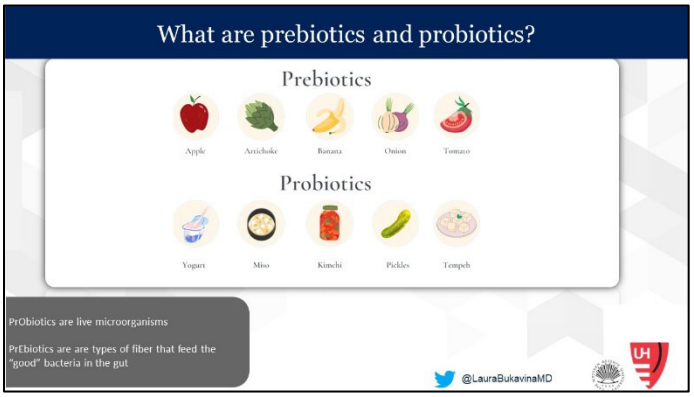
Dr. Laura Bukavina:

And really not until about late 1800s, 1900 was the father of modern immunology, Ellie Metchnikoff coined that term of probiotics. He is considered to be the father of immunology and he's the one that started looking at the relationship between the gut and how your immune system develops.



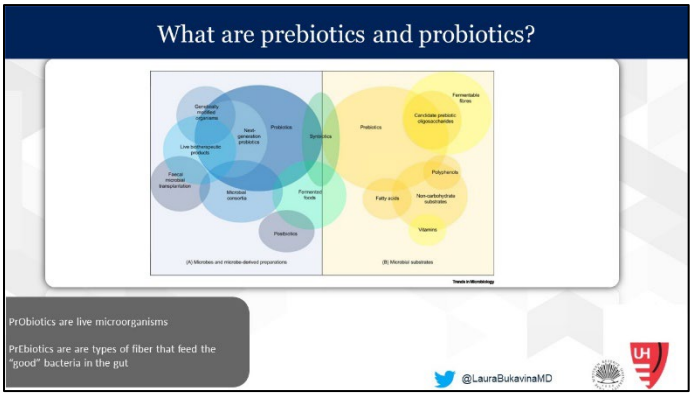
Dr. Laura Bukavina:

So what are prebiotics and probiotics? The simplest way to explain it is prebiotics are not alive. Those are the potential things that bacteria might digest, that help certain types of bacteria grow. Now, probiotics really do mean that it is a live organism. Typically, they're considered to be the good type of organisms within your gut that have a host bug positive relationship.



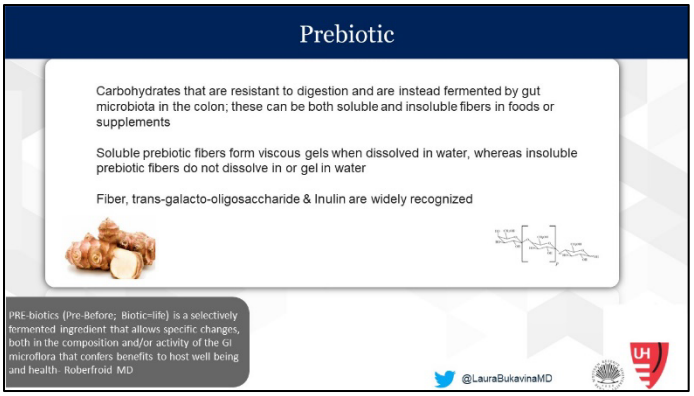
Dr. Laura Bukavina:

Everything in-between is highlighted here. So the synbiotics are the combination of the bacteria as well as the food for the bacteria that sometimes you see manufactured and marketed. And then the postbiotics, which are in that purple right here are the byproducts of bacteria. For example, vitamin K, which is produced by bacteria, is one of the postbiotics. So this is just a wording game. A lot of the research has been focused on the probiotics, which means live bacteria. But the Nalo research really recognizes that in order for us to really move forward with microbiome, we have to almost focus on the pre and the probiotics together.

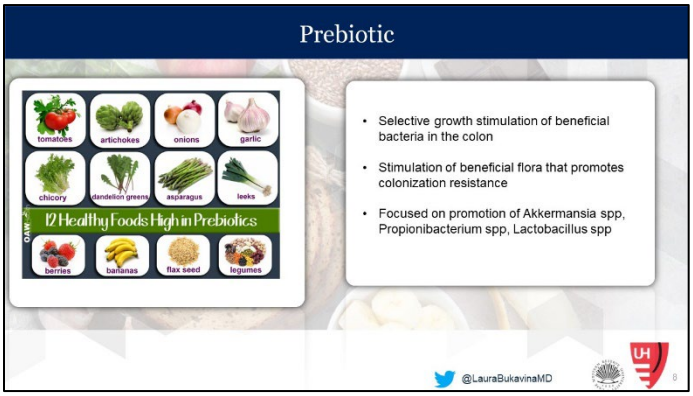


Dr. Laura Bukavina:

So prebiotics, short version is anything that is resistant to digestion. So I think the most commonly way we think about it is fiber. Some fiber is digested, some fiber is not digested. However, the things that are not digested by us, by our own enzyme will be tolerated and digested by bacteria. The most commonly are what called inulin and some of the carbohydrates. Now those are used as food by the bacteria which are further broken down into other metabolites that can affect your immune system.

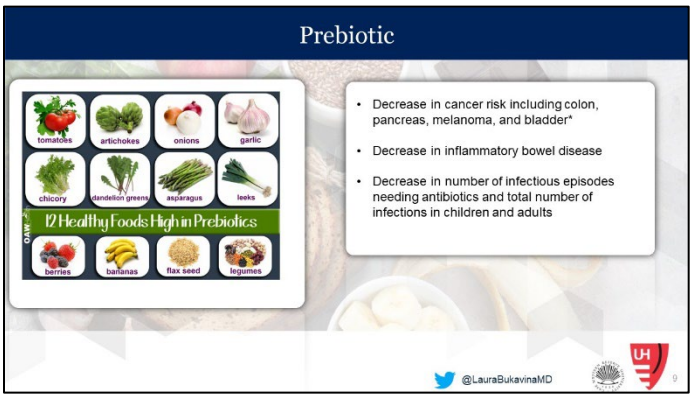


Dr. Laura Bukavina:



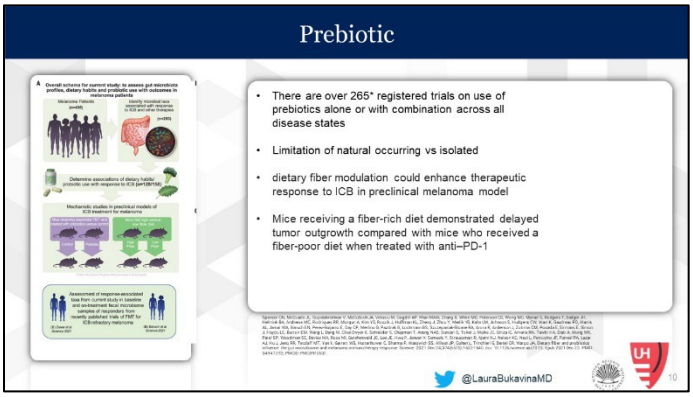
Prebiotics are really in every vegetable possible. The most common vegetables that have a high concentration of prebiotics are your berries, your bananas, your green vegetables, your artichokes and onions. Now, the stimulation of the flora is thought to be accomplished by these prebiotics really being beneficial at increasing the growth of the good bacteria, which are your Lactobacillus. I'm pretty sure everyone has heard of Lactobacillus species being good. Your Akkermansia, which has been shown in research in humans and in mice to be beneficial to our cancer patients. And really, it selects specific bacteria because those bacteria are able to digest these prebiotics much more efficiently.

Dr. Laura Bukavina:



Research has shown that these prebiotics, which is a healthy diet, a combination of high fiber diet and not digestible carbohydrates, not only decrease the risk of multiple cancers, bladder, melanoma, colon, pancreas, I think, but also other diseases including inflammatory bowel disease and Parkinson's disease. And then if you look at overall infection complication, children who have a high prebiotic diet have less need for antibiotics and have decreased number of total infections in their lifetime. So if you look at many of these studies in history that looked at having a good diet, balanced diet, high in vegetable content, high fiber diet decreases the risk of colon and many of these diseases, this really pinpoints to the fact that many of these are because of the prebiotic potential to increase the growth of these good bacteria.

Dr. Laura Bukavina:



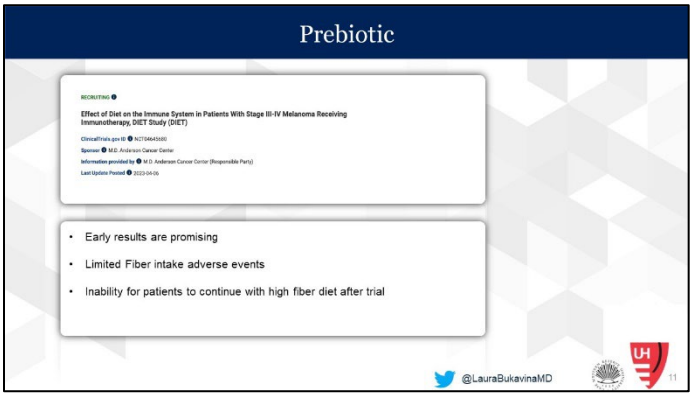
Now, I wanted to dig a little bit deeper into what actually is happening within the science on the prebiotic research base. So right now as we look, there's over 265 trials that are looking at prebiotics, probiotics, or combination of two within multiple disease states. Many of them are in cancer. The limitation of a prebiotic is that you can only eat so many leafy green vegetables and so many vegetables overall to be able to reach that five-gram recommendation of a prebiotic.

Now, dietary fiber modification, meaning that if patients or mice are given fiber in a clinical trial have been shown to correlate to response not only with development of cancer but also because it increases your response to many, many immunotherapy types. And this is one of the study that I highlighted here in melanoma. And melanoma really has been the poster child for a lot of these studies because it's such a high morbidity disease, but also some patients have an amazing response to immunotherapy.

Now, what Dr. Vargo's team looked at is that they noticed that mice who received a fiber rich diet in combination with probiotics, so in addition to giving supplements of healthy microbiome, really had almost an exaggerated response to immunotherapy, in compared to those mice who had a fiber poor diet. And what they're doing now is they're actually translating these findings to humans, to patients who are receiving immunotherapy within the clinical trial.

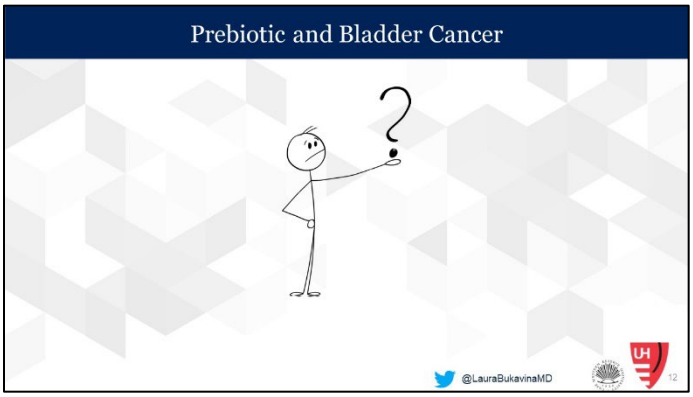
So I wanted to highlight this because I wanted to make sure that patients and overall public understands that microbiome modulation or supplementation with prebiotics and probiotics is never a single therapy. It is never meant to be a therapy taken alone. It is a therapy meant to be taken in combination with what we consider to be standard of care because it's never going to be curative. However, it increases the efficacy of current therapy and it decreases the number of side effects associated with that therapy.

Dr. Laura Bukavina:



So this is a trial that I was talking about in melanoma and prebiotic, and really, they're currently recruiting many patients and some of the early results they presented that I was able to see had incredible response to immunotherapy while patients were taking fiber supplementation. However, the limitation is that as soon as the patient are off the trial, the fiber intake drops dramatically and back to baseline. So it's a catch 22. We know that this helps. There is a high fiber intake to better immune response. However, it's very difficult for patients, really for anyone to have such level of dedication and to continue that high fiber intake throughout their daily life.

Dr. Laura Bukavina:



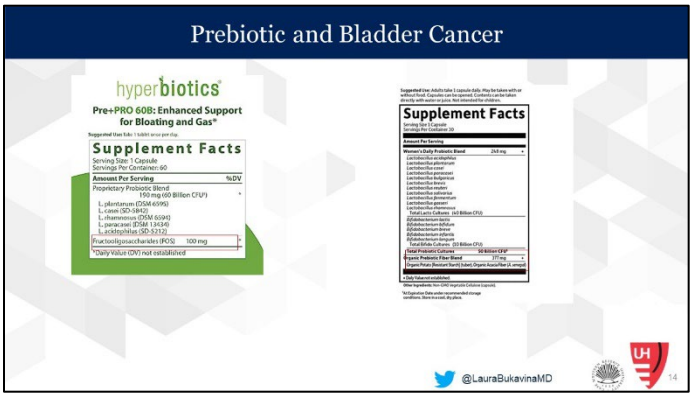
So what do we know about prebiotics and bladder cancer? We really don't. So prebiotics research and bladder cancer is non-existent. There is some research that I'll share with you that we have done on probiotics. However, prebiotics has not been explored and really should be the target of more research within bladder cancer in combination with probiotics.

Dr. Laura Bukavina:

As I said, prebiotics dose recommended by the Gastrointestinal Society is five grams. So just to highlight how varying amounts of this probiotic is actually listed within the supplements that we currently get, this is an example of a very popular pre and probiotic supplement on the market. And you can see here that's highlighted by an arrow, the recommendation is five grams versus the actual content within that one capsule is 362 milligrams. That's less than one-tenth of the recommended dose. So for one to be able to even obtain the necessary prebiotic fiber that's recommended, you have to take 10 of these capsules, which you understand that's highly unrealistic.

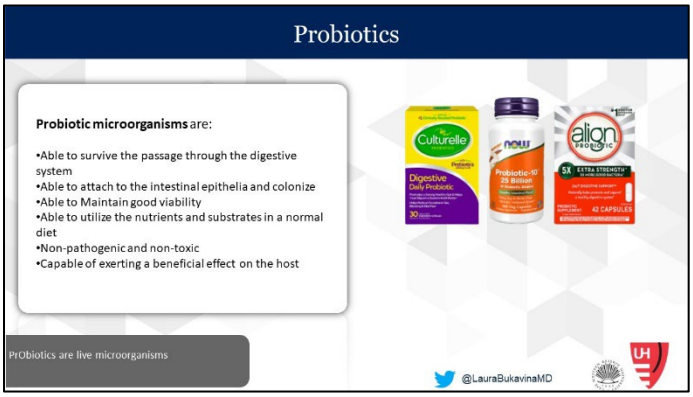
Dr. Laura Bukavina:

This is other examples. This one's even worse. It's a 100 milligrams and you can see this one, the prebiotic fiber is 377 milligrams. So for anyone going to the store and considering buying any probiotic or prebiotic supplementation, I wanted to highlight a couple of the things that everyone should look out for because a lot of this is unregulated and really heavily marketed towards people without really any justification for what the guidelines recommend. So one of the things you should definitely look at when you look at probiotic and prebiotic pills is the amount of prebiotic fiber that's within these capsules.



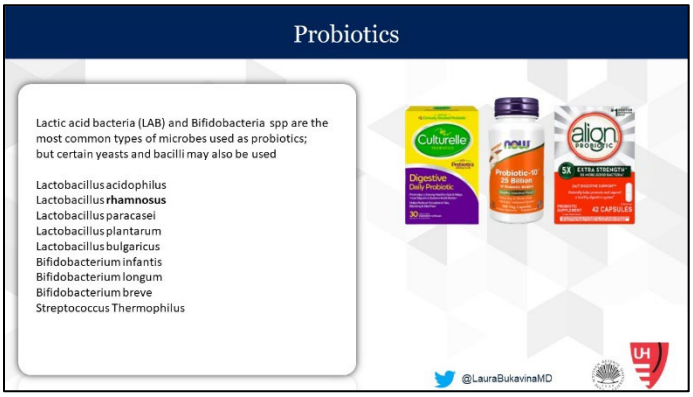
Dr. Laura Bukavina:

So I want to move on to probiotics and talk a little bit about probiotics because that's where a lot of the research is. So probiotics are live, these are live bacteria. Typically, they're the good type, the good bacteria such as Lactobacillus, they typically do not cause disease. They don't cause sepsis, they don't cause urinary tract infections. These are bacteria that are living in tune with your body and stimulate your immune response. And they're almost always capable of exerting some sort of beneficial effect on the host.



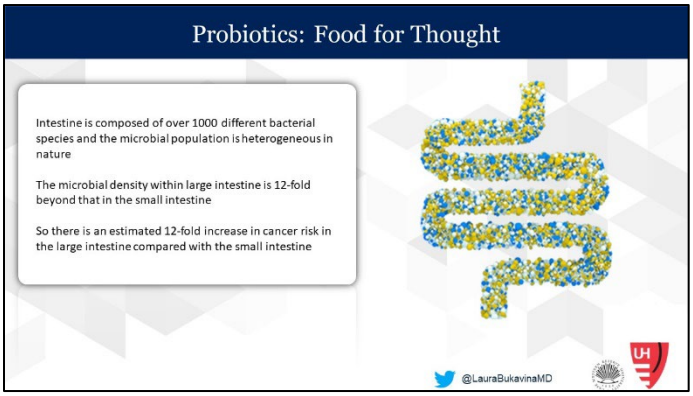
Dr. Laura Bukavina:

These are some of the commonly listed probiotics. Lactobacillus species really dominates this field as well as Bifidobacterium, which has been known to be prevalent in children who are breastfed and have multiple immunomodulatory, meaning stimulating the immune response, but also gut protective effects.



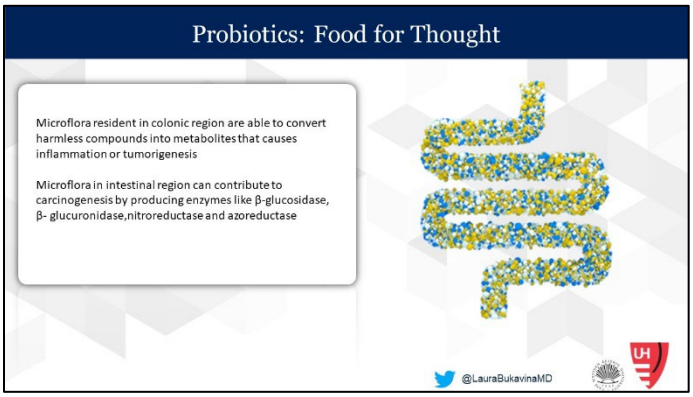
Dr. Laura Bukavina:

And I wanted to throw this out there, and this is really just an association, but I thought this was an interesting finding when I heard is that we hear of colon cancer fairly often in terms of the incidents and the prevalence. However, if you look at the diversity of bacteria from small bowel to large bowel, which is your colon, the microbial density within the large intestine is about 12 times higher than that of the small intestine. However, if you look at the cancer prevalence, the cancer prevalence within small intestine is almost 12 to 15 times decrease compared to colon cancer. And that really is thought to be secondary to multiple bacteria that is within the colon in addition to other factors. And also the metabolites that are produced by this high burden of bacteria.



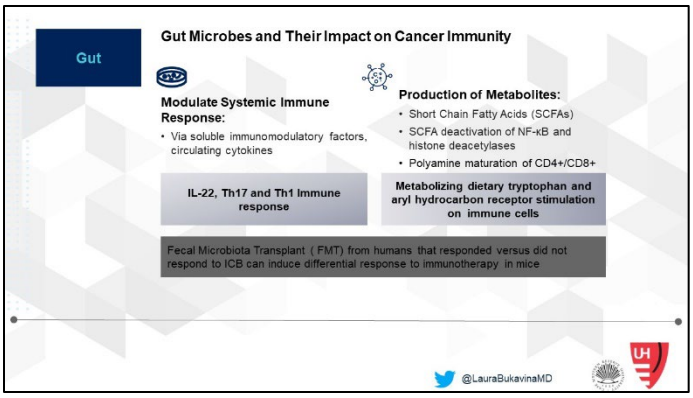
Dr. Laura Bukavina:

And how do the bacteria really cause cancer? In addition to the immune stimulation, the bacteria do. They also have the ability to produce multiple byproducts. So those prebiotics, they're not digested, can sometimes be digested by the bad bacteria, by these enzymes that it can later produce these toxic metabolites that cause then immune dysfunction. It can cause genetic mutations and it can cause cancer down the line.



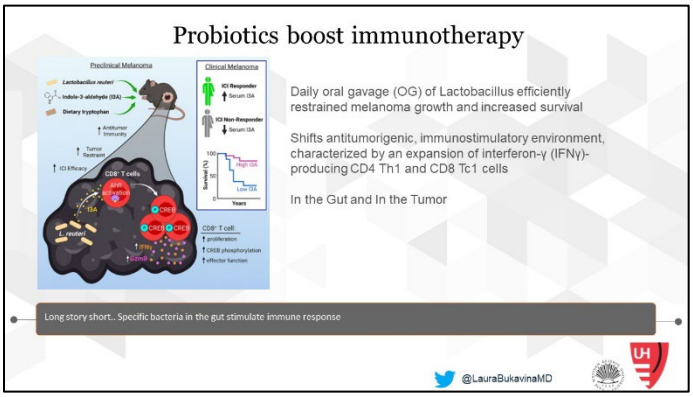
Dr. Laura Bukavina:

This is some of the things that I talked about within bladder cancer, additional ways that your gut microbes can impact cancer immunity. Not only can they... As I talked about enzymes, can they really produce those metabolites, but also these microbes can also produce short-chain fatty acids. So short-chain fatty acids, depending on the fatty acid can actually be protective against cancer development.



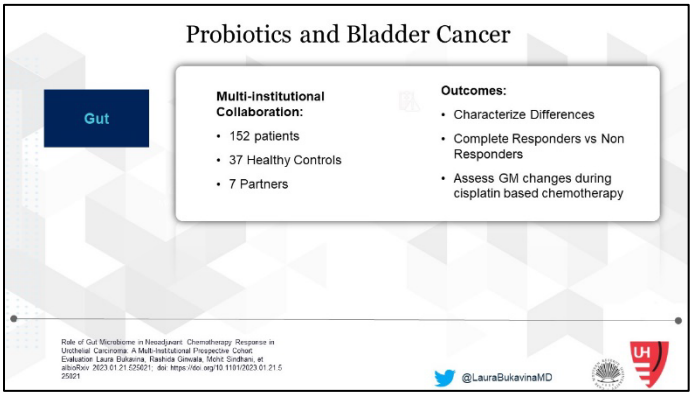
Dr. Laura Bukavina:

So I wanted to dive into a little bit into the research again within melanoma and immunotherapy. So another story is combination of prebiotics and probiotics. So when we look at just supplementing someone with, for example, Lactobacillus, without supplying the necessary digestive nutrients for that bacteria, which are your prebiotics, the effect on immunotherapy response is much lower. However, if you have a mouse with cancer and you give them prebiotics and probiotics, so combination of Lactobacillus, in addition, you can see here Indole, which is the most common prebiotic, this really shifts your immune response to cancer. In this study, you can see that the mice who had melanoma when they were supplied with both Lactobacillus, which is the probiotic and the prebiotic Indole, in combination, their survival almost doubled over their duration because of this immune response.



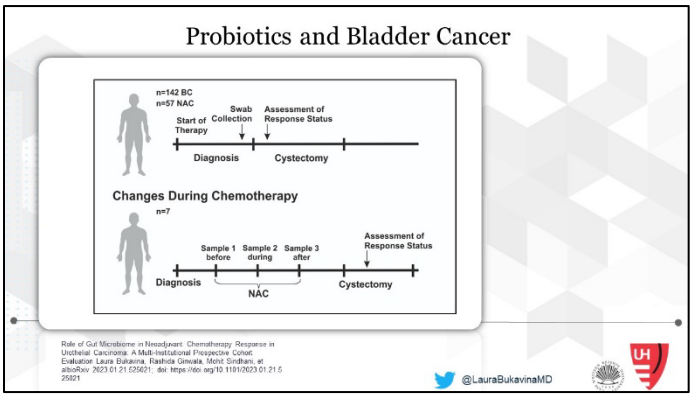
Dr. Laura Bukavina:

So we did something similar in bladder cancer by first looking at the differences in microbiome. So we wanted to see if there's specific bacteria that provide a signature for patients who respond to chemotherapy. A lot of you know that if you have muscle invasive bladder cancer, your treatment really starts with chemotherapy if you're able to receive chemotherapy followed by surgery. So we wanted to see in addition to just the tumor differences, are there differences in the gut microbiome in our patients who respond to neoadjuvant chemotherapy better than others.



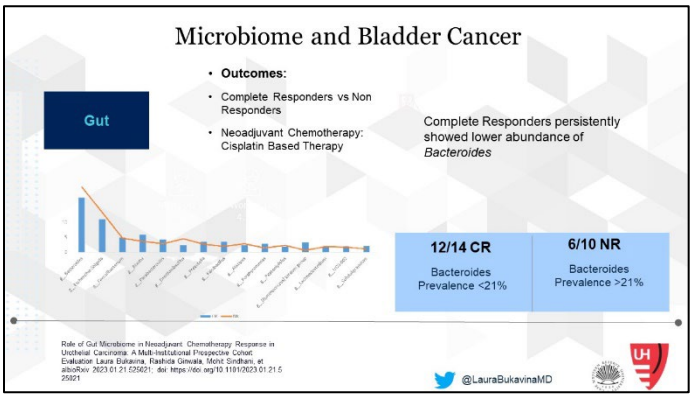
Dr. Laura Bukavina:

And what we did is we collected several stool samples and urine samples throughout the study to see if there are differences.



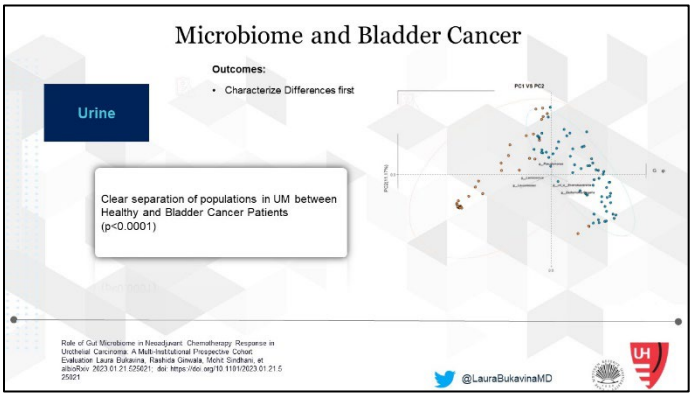
Dr. Laura Bukavina:

And we did find a difference in one bacteria that was very interesting, which is Bacteroides. Again, going back to melanoma. This previously has been shown in melanoma as well where patients with a high Bacteroides count were less likely to respond to neoadjuvant chemotherapy. In fact, the response was so strong that we found that patients who did not respond continued to have increased number of that Bacteroides throughout their therapy, while patients who are going to respond to chemotherapy actually had a significant decrease in that bacteria. So that was very interesting to us and that's something that we continue to explore right now with other studies.



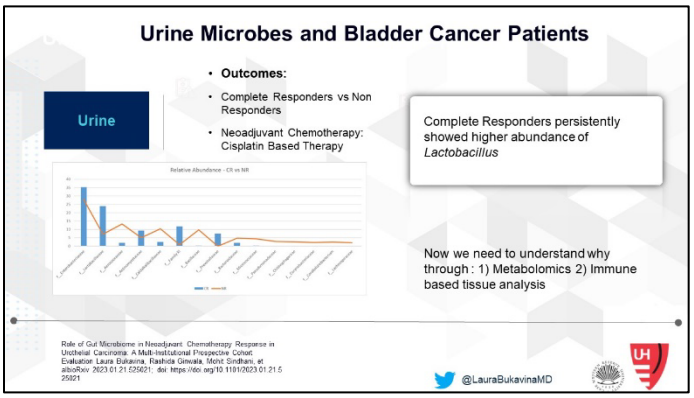
Dr. Laura Bukavina:

As I mentioned, we also collected urine as bladder. We have the ability to really collect the urine from these patients and we know that there's a clear difference in the composition of the bacteria in patients with bladder cancer and without bladder cancer.



Dr. Laura Bukavina:

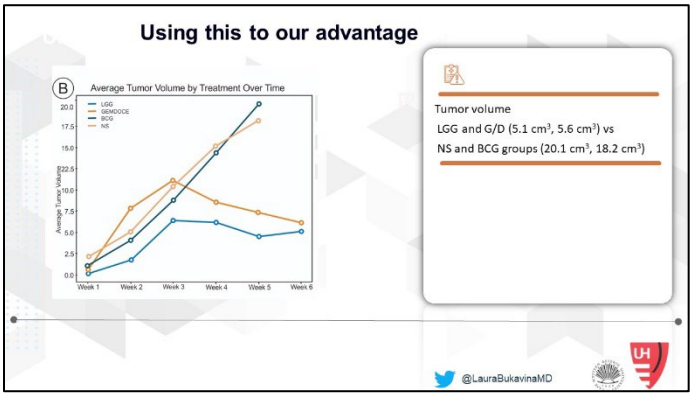
And we continue to look at response status. Again, patients who received neoadjuvant chemotherapy and had a response, had a much, much higher Lactobacillus in their urine. And this is males and females. I think we typically think of Lactobacillus to be associated with females, with vaginal flora. But really, this was within urine and actually about 60% to 70% of our patients were male.



And so Lactobacillus continued to be present in urine in many, many of our responders.

Dr. Laura Bukavina:

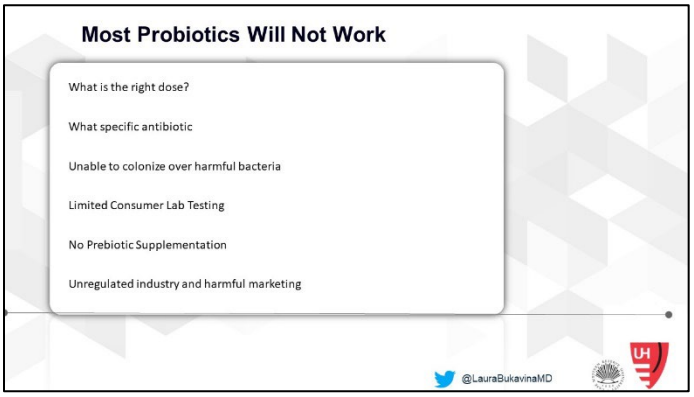
So based on this finding, we actually applied this to our mice and what we found is that supplementing mice with Lactobacillus specific species called Lactobacillus Rhamnosus, which is the LGG. Intravesically, so instilling this Lactobacillus during treatment, we were able to obtain a 50% complete response in these mice.



So just to interpret that, if you compare that to chemotherapy, just giving someone intravascular installation of LGG, meaning we put into the bladder the LGG and compared it to BCG and compared it to chemotherapy, gemcitabine docetaxel, the two best responses we've seen was actually with LGG and with chemotherapy and they were almost equivalent. BCG on the other hand did not work so well.

Dr. Laura Bukavina:

However, if you are considering going to the store and buying probiotics off the shelf, I do want to warn you that there's a lot of difficulties in finding the right probiotic and really understanding that there's a lot of nuances behind it. We really don't know what the right dose of probiotic is. We know that there is a certain amount called colony forming units that has been used previously, that we have used in our studies, which is 10 to the six.

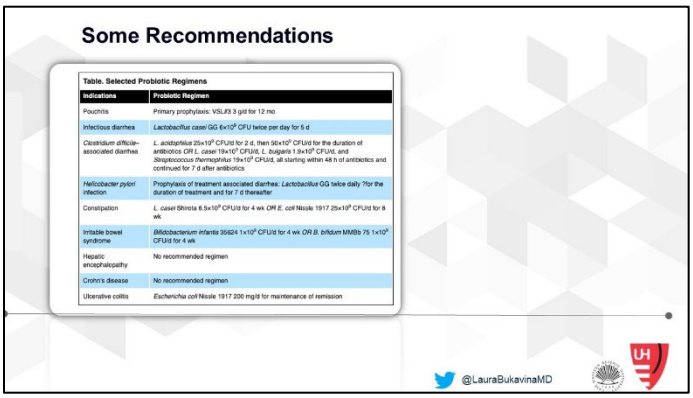


What specific antibiotics affect what microbiome? And if you are an antibiotic at the same time as your probiotic, does that affect it? If you take probiotics right now and you are already colonized with many, many harmful bacteria, it really is very difficult to change your microbiome because there's no room for the good bacteria really to sort of colonize. So does that mean that we have to clear, sort of reset our gut before we even start to think about taking probiotics? Which is in mice studies, which is what exactly happened. You have to be able to completely clear your existing microbiome, almost like a colonoscopy prep, before you start taking probiotics.

A lot of these companies are really targeted to marketing and less so towards actual consumer testing. So their reported number on the bottle might actually be different than what is actually in the pill. A lot of them don't have the prebiotic supplementation. So taking probiotics without really providing the food necessary for these bacteria really has limited response overall. And a lot of this is just harmful marketing over promising to many of the patients because they are just aimed for their financial incentives.

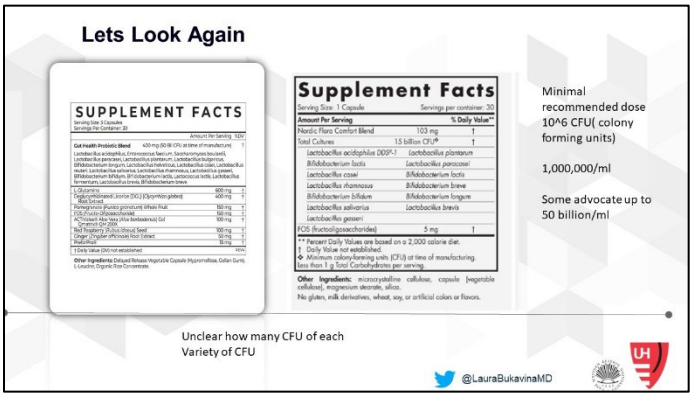
Dr. Laura Bukavina:

So there are some recommendations currently out there from many of the societies in terms of what has been very rigorously tested and is currently recommended for treatment. As you can see, a lot of these recommendations are again focused on Lactobacillus. And again, this is that Lactobacillus GG, the LGG that I talked about, in addition to the Bifidobacterium. But again, many of these recommendations are within the infectious space. None of the recommendations currently exist for any of our cancer patients.



Dr. Laura Bukavina:

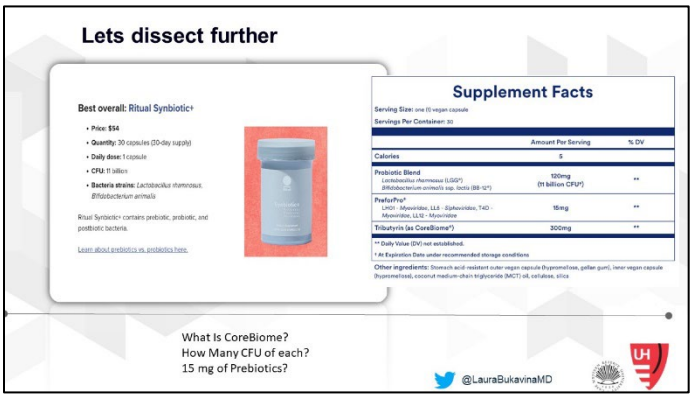
So just as we did with prebiotics, I wanted to take some of the most commonly used probiotics out there and look at the composition. As I said, CFU, which stands for Colony Forming Units, is typically how we look at the content of bacteria per milliliter. So if you think of BCG as bacteria, the typical amount of BCG per milliliter is 10 to the six, and that's the minimal amount that has been shown to be effective. Now, some of these probiotics have 50 billion CFUs and some of them have 50 billion and some of them have 10 to the two CFUs. So it's very important to look at the CFU content in the back.



In addition, as you notice, a lot of these probiotics actually lump all of these cultures together and give you a lump sum. So if you see in the gut health probiotic blend, there's a 400 milligram probiotic blend with 50 billion CFUs. However, it's a combined CFU, so you're not really getting the information on each individual bacteria. And I've looked through about 15 of these and I really couldn't find one that actually

gives you specific number per each bacteria. And a lot of this has to do with marketing, a lot of it has to do with just not understanding the process. But if you find one that specifically lists CFU per each bacteria, would love to see it.

Dr. Laura Bukavina:



Now, this is what someone called the best probiotic out there and it's called synbiotic. So I wanted to dissect this synbiotic. It's what they consider to be the pre, pro and the post-biotic supplementation. I do like that they only list two probiotic blends, which is your Lactobacillus Rhamnosus. Again, the LGG that our research focused on, in addition to the Bifidobacterium, which is great. They also listed it as compounded. So you see that they only have 11 billion CFUs. But again, this is combined. Again, let's go look at the prebiotics. Remember we said five grams is a recommended dose. Here they have 15 milligrams is all you're getting. And then the post-biotic, which we didn't talk about much, but this Tributyrin, the post-biotic has never really been shown to really affect much of the outcomes within cancer outcomes. However, they also listed it here.

So as I finish up this talk, I really wanted to make sure that many of you understand that there are a lot of nuances to these supplements and when you consider buying any pre or probiotic supplement next, it's really important to turn the bottle and question what is in the back, what is marketing and what is real and how much of this is really going to help me?

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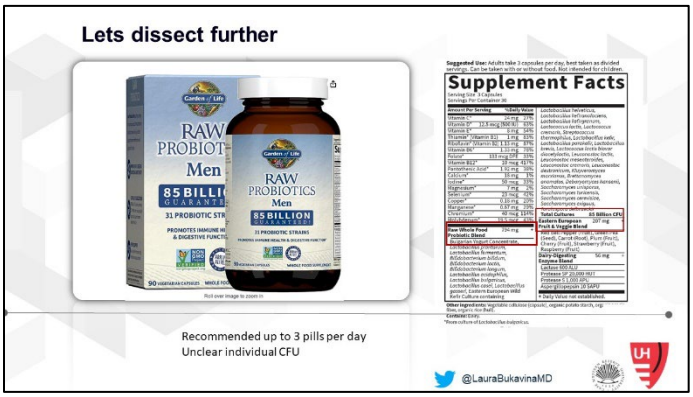

I looked at another one just to highlight, and this is number one seller on Amazon currently. I'm not sure why it's just for men. I couldn't find anything that's men specific except Selenium in this. However, again, if you look at this probiotic blend and the prebiotic. So again, they're lumping everything together and this one gives you 85 million CFUs, but there's 85 different bacteria in this. In addition, if you look at their prebiotic, which they list as a fruit and veggie blend, which it doesn't necessarily mean it's a prebiotic. There's only 207 milligrams within this supplement.

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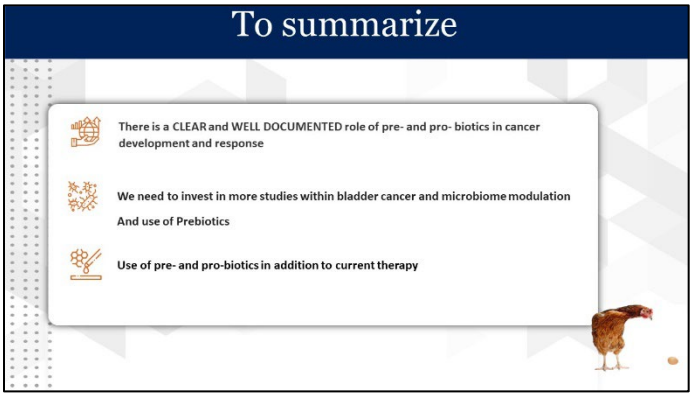

So to finish off, there is a clear and well-documented role of pre and probiotics in cancer development response. It's a well-studied phenomenon in melanoma and we're starting to understand it in bladder cancer. And I really look forward to exploring LGG more in bladder cancer patients, seeing how we can use this to our advantage. The use of pre- and probiotics is always in addition to current therapy. This is not meant to be a standalone therapy. However, it is meant to augment and increase the efficacy of the current therapy and decrease the side effects. And I think overall as society, we need to focus on more studies and really more trials within microbiome modulations and the use of prebiotics within the clinical trial setting. So we're able to do vigorous signs and really be able to provide the results for our patients. And with that, I am happy to take some of these questions.