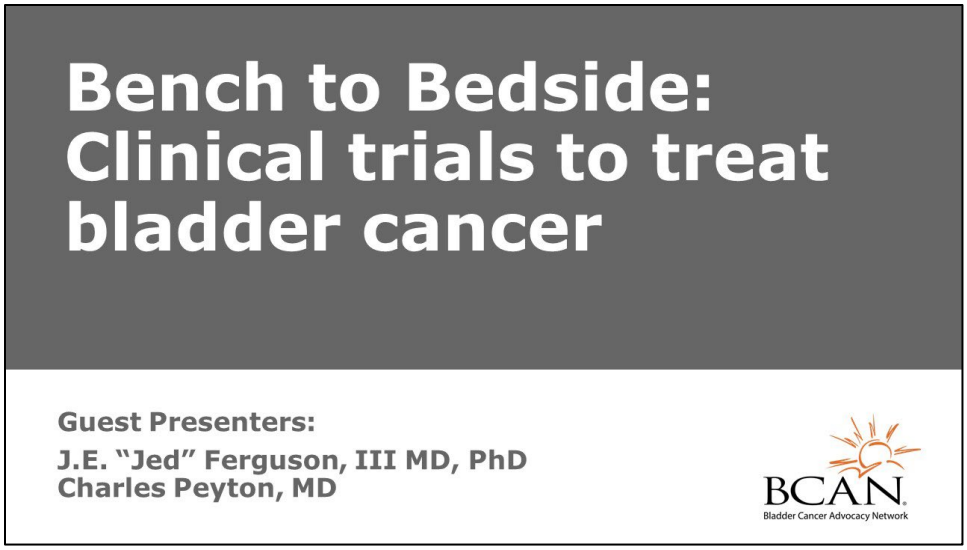
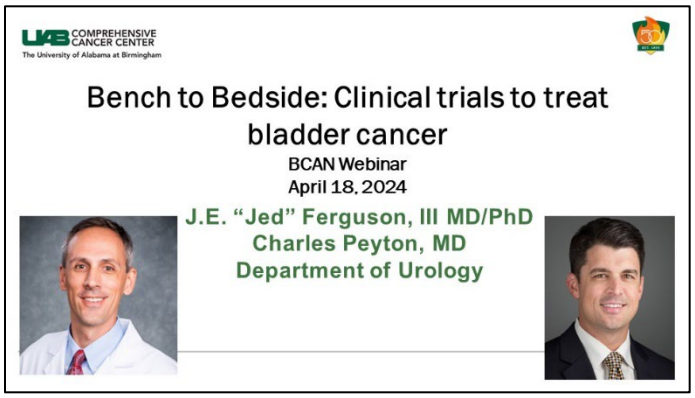


Patricia Rios:

Welcome to the Bladder Cancer Advocacy Network Patient Insight Webinar series. I am Patricia Rios, Senior Education and Advocacy Manager and your host for today's webinar. I'd like to begin by thanking the sponsors of our Patient Insight Webinar series, Merck and UroGen.

Now, today's topic is Bench to Bedside: Clinical Trials to Treat Bladder Cancer. The phrase bench to bedside describes the process of taking research results from the laboratory into the clinic so that it can directly benefit patients. It's a short phrase for what is often a very lengthy and complex process. To help us understand this process and the role of patients as it relates to bladder cancer, we have two very special guest presenters, Dr. James Ferguson and Dr. Charles Peyton. Dr. Ferguson is a urologic oncologist and assistant professor at the University of Alabama at Birmingham, also known as UAB. He's also a staff physician and scientist at the Birmingham Veteran Affairs Medical Center. Dr. Ferguson's clinical area of focus includes cancers of the bladder, prostate, kidney, testes, and penis with a strong emphasis on urothelial carcinoma. He's currently investigating the biological ramifications of ARID1A mutations on the biology and therapeutic vulnerabilities in bladder cancer, and has received a Veteran Affairs Merit Award for this project. You will hear more about this mutation during his talk.



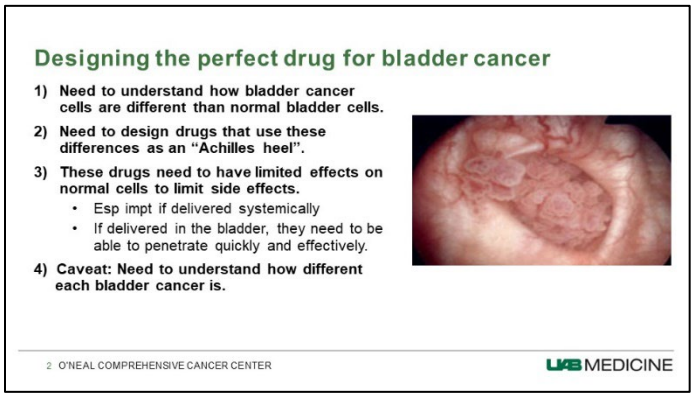
Dr. Peyton is an assistant professor also in the Department of Urology at the University of Alabama at Birmingham. Dr. Peyton serves as co-chair of the Genitourinary Oncology Clinical Trial working group and is the principal investigator on several clinical trials at the University of Alabama at Birmingham. He serves as a member of the NCCN

Guidelines for Bladder and Penile Cancer and is an AUA core curriculum author. His research interests include quality of life, therapeutic, and outcomes research related to urologic cancer.

I will now direct your attention to Dr. Ferguson and Dr. Peyton so they can begin their presentation.

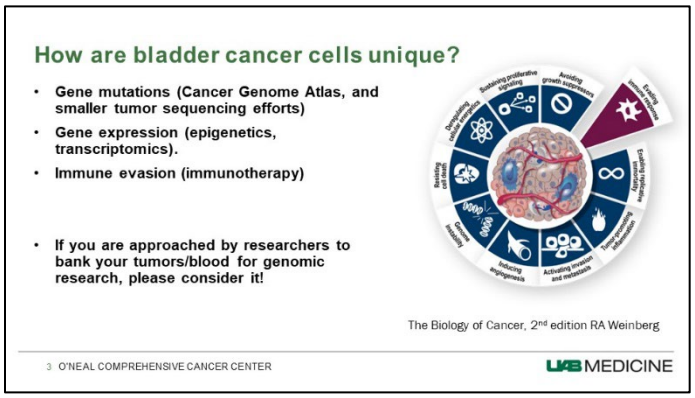
Dr. James Ferguson:

Thanks so much, Patricia. I really appreciate it and thanks everybody for joining us. We're splitting up the talk kind of in two parts. I'm a physician scientist, as Patricia talked about, and I'm going to share a little bit about how we get from the bench to almost the bedside. And then Chas is going to take over and talk about clinical trials and bladder cancer and what they give us and how we get there. So if you could advance the slide for me.



Bladder cancer, just like a lot of things, we spend a lot of time thinking about what's the perfect drug for bladder cancer and how do we design the perfect drug? We want a drug to be absolutely lethal for the cancer cells, but totally benign to all of their normal cells. So that increases your therapeutic ratio so you have cure without side effects. That's the perfect drug.

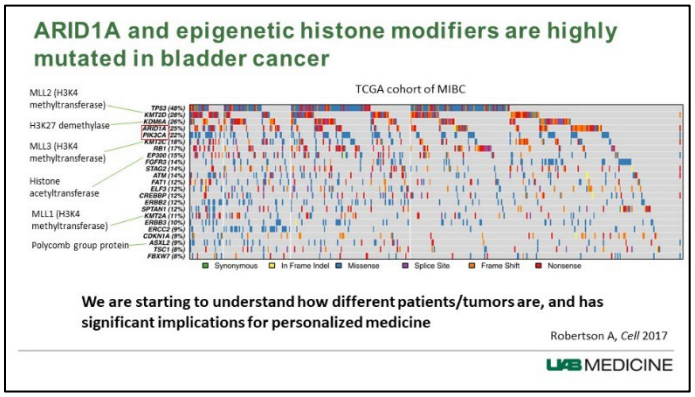
In order to get there, number one, we need to understand how bladder cancer cells are different than normal cells and different than normal bladder cells. And we need to design drugs that use these differences as an Achilles heel. These drugs need to have limited effects on normal cells in order to limit the side effects. This is especially important in systemically delivered cells, but even if we deliver it in the bladder, they need to be able to penetrate quickly and effectively through some of the natural defense mechanisms that the bladder has in order to get down to the root of the cancer and kill it. A huge caveat to this is that as we're learning more about bladder cancer and other cancers, we're realizing that maybe not every cancer is the same. Maybe there are subtypes of cancer that can be treated similarly. Maybe every single cancer is unique and every single patient is unique and we need to focus on what makes them unique rather than what brings them together in order to truly design effective treatments in a personalized medicine and a strategy. Next slide please.



Dr. James Ferguson:

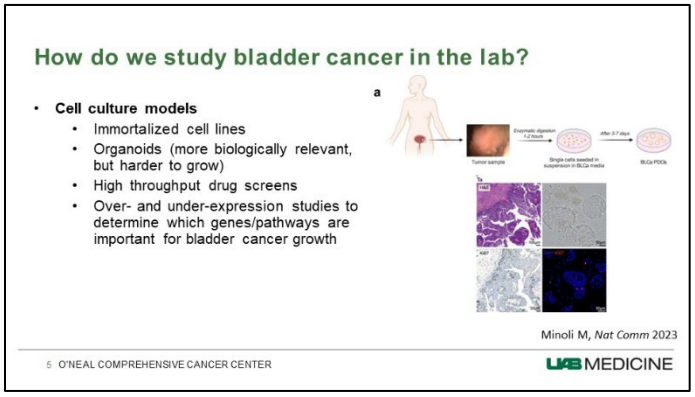
So just a really quick theoretical view on how bladder cancers are unique. Well, they have unique mutations. We know this from the Cancer Genome Atlas trial in smaller tumor sequencing efforts that bladder cancer cells accumulate specific mutations that are in a fairly reproducible pattern, but different tumors can have

different mutations that are driving their growth. They can have different gene expression than normal cells either through epigenetics or transcriptomics, which I'll talk about a little bit later. They develop ways to evade immune surveillance, which makes immunotherapy that y'all may have heard about very effective. All of this to say that we need bladder cancer samples in order to do good basic science. So on the fourth bullet point, if you are approached by researchers to bank your tumors or blood or urine for genomic research and other research, please consider it highly because it really helps us to have those banked specimens to do really meaningful research on human bladder cancer samples. Next slide please.



Dr. James Ferguson:

This is a screenshot of the cancer Genome Atlas, also called TCGA. And basically what they did was took muscle-invasive bladder cancer samples from about 400 patients and sequenced them to find out what mutations were accumulating in each patient's tumor. So on the Y-axis... Can you all see my pointer? Probably not, huh? Okay. You can see Patricia's pointer. So as you go across each column, there is one patient, and every single color in one of those columns is denoting a mutation in whatever gene is listed on the left. Then those genes are ranked in terms of the percentage of cancers that have mutations in them. So TP53 is the most commonly mutated tumor suppressor in bladder cancer at 48%. And then as you go down the row, you see some other genes that are important, including ARID1A, which is number four and is present in mutated form in 25% of patients. So this starts to give us an idea about what genes are really in bladder cancer, try to understand patterns, and see if we can develop drugs that can target specific mutations in genes. Next slide, please.



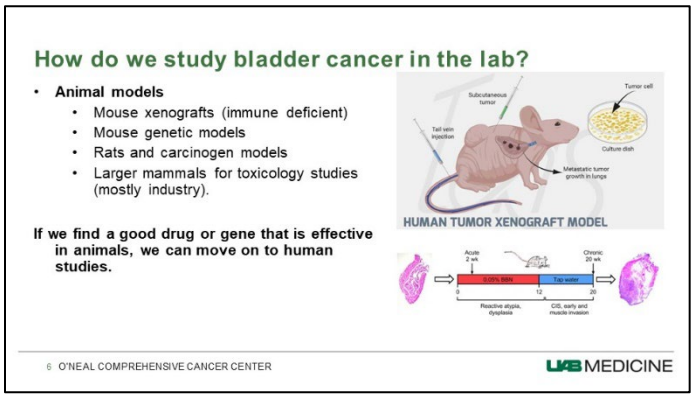
Dr. James Ferguson:

So just really briefly about the broad strategies of how we study bladder cancer in the lab. The tried and true model is cell culture models. We can take cells from bladder cancer and grow them in the lab on plastic until they become basically immortalized and we can passage them kind of indefinitely. They're easy to work with. They're somewhat hard to kill. The caveats are that you lose a little bit of the biological context when you grow cancer cells on plastic. There's organoids, which if you look to the right on subgroup A or figure A, kind of denoting how we grow organoids in the labs. You take a tumor, you seed it on a matrigel, which is kind of a jelly-like substance, and these tumors will grow in kind of three dimensions.

They lack blood flow, but they have some of the stroma and some of the connective tissue and even some of the inflammatory cells that bladder cancer has. They are short-lived so they're difficult to

passage and they lose some of their biological likeness to bladder cancer in vivo with each passage. But that's one way to get a little bit closer to the biology of bladder cancer in the lab. These two models allow high-throughput drug screens to sprinkle different medications on these cultures to see what tumors are killed by certain medications.

Then the exciting thing about organoids is that you can imagine a situation where you harvest an organoid from a patient, grow it in the lab, test different medications, and then find the medications that work and put it back in the patient. The trick is doing that quickly enough so that you're not allowing tumor progression in the meantime. And then finally, the kind of tried and true approach is over and under expression studies to determine which genes and pathways are important for bladder cancer growth. I'll talk a little bit about that later. Next slide please.



Dr. James Ferguson:

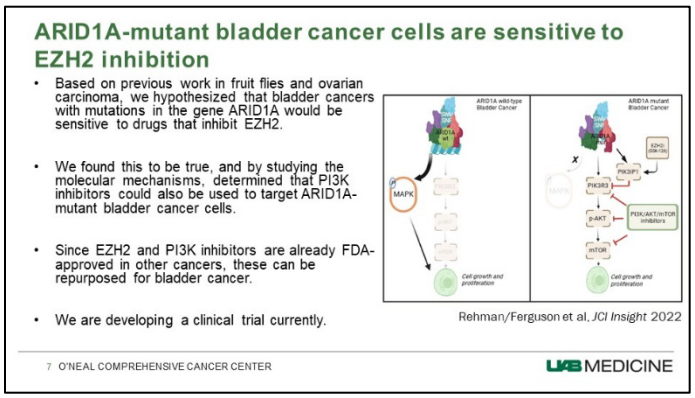
The next step up from cell culture models are animal models. Probably the biggest workhorse in cancer research is the mouse xenograft. And the way that works is you take a mouse and generate a mouse that doesn't have an immune system so that you can instill human cancer cells in a way that won't be cleared by the immune system of the mouse. That allows the tumor to grow, and then it allows us to treat the mice with different medications or different genetic changes to see how they affect both bladder cancer tumor growth and also metastasis and spread. So on the right you can see a mouse there with a needle going into its subcutaneous space and planting some cells and resulting in a subcutaneous tumor on the flank. That's one approach. Another approach is we can inject it directly into the bladder to form orthotopic tumors in the bladder.

And then finally, we can inject cells in the vein of the tail and look for how well and efficiently they form tumors in the lungs as kind of a metastatic model. So that's probably the most commonly used tool in the lab for studying bladder cancer. Secondly, on the left, there's mouse genetic models which are not quite as prevalent in bladder cancer as other cancers just because of the genetic drivers of bladder cancer are not quite as robust as other cancers. Third, there's rats and mice carcinogen models. So this is where we take carcinogens that we have purified initially from cigarette smoke, put it in the drinking water of mice or rats, and they form bladder cancers within months. On the very far right, you can see a schematic there where it's the cross-section of a normal mouse bladder.

Then we give them in the red 0.05% BBN. Please don't ask me to pronounce the name of that chemical. It's very long and complicated, but we call it BBN for short. So we treat them for 12 weeks and then switch them to tap water and then at 20 weeks, we cut out their bladder and you can see that a muscle invasive tumor on the bottom right in the mouse's bladder. I think that's important for

patients to hear that we can take carcinogens and cigarette smoke and give them in relatively higher doses to mice and give them bladder cancer within a matter of months.

Then fourth and finally, larger mammals for toxicology studies. Once we have a medication that we think is effective for bladder cancer, we can give it to animals. If it's never been used in man, we want to make sure that there's no major toxicology issues before we give it in clinical trials. That's mostly in the sphere of industry where that's done currently. To wrap up, if we find a good drug or gene that is effective in animals, we can move on to human studies. Next slide please.



Dr. James Ferguson:

This is just one example of some work that I've been doing with my group in the lab, just to give you an example of how we determine and find new targets for therapies and then hopefully get them into humans as quickly as possible. We had noticed that from some previous studies, both in fruit flies and ovarian carcinoma, that fruit flies with mutations in ARID1A and EZH2 didn't survive. They died. And a group of ovarian cancer researchers had found that ovarian cancer cells with ARID1A mutation can be killed with pharmacologic inhibitors to EZH2. And we hypothesized that bladder cancers would behave the same way. And bottom line is we found this to be true and we studied the molecular mechanisms and determined that bladder cancers with an ARID1A mutation underwent a switch in their signaling pathways that led to cell growth and proliferation.

So initially, ARID1A wild type cells proliferate through the MAP kinase signaling cascade. But then on the far right as you see with ARID1A mutation, they switch up MAP kinase signaling and then activate PI3 kinase signaling through the MTOR cascade. And then when you hit them with EZH2 inhibitors, it upregulates an endogenous inhibitor protein of PI3 kinase that turns this pathway off and results in cell death. And on top of that, you can hit it with PI3 kinase inhibitors that are a bit more specific. So EZH2 inhibitors and PI3 kinase inhibitors are already developed and tested and FDA approved in humans for other indications. So we know that they're safe and we think that they're effective, so we're currently working on opening a clinical trial for patients with stage IV bladder cancer with ARID1A mutations to be randomized to EZH2 inhibition. So next slide.

So that's all that I was going to talk about and that gets us to clinical trials and Chas can take over.