Question 1: Do clinical trials cover the cost of genetic sequencing, or is this a burden that the patient needs to pick up? How do they get this done, if they're interested in having a treatment that's specific to their tumor?

Dr. Iyer: Maybe I can start to try to answer that. It depends on the clinical trial. The majority of clinical trials do not support the sequencing that occurs, and so oftentimes that needs to be done at institutions that might provide that sequencing, free of cost in some cases. Other times, actually, the tumor tissue can be sent to a for-profit center, so a commercial center like Foundation Medicine, for example, where the sequencing will take place. The burden of cost for that, at least some of that, will be or may be covered by insurance, but it will also need to be shouldered by the patient, as well.

The goal, though, is that if we can identify, for example, a mutation for which we can actually come up with a drug that works for that mutation, and that leads to FDA approval of that drug in bladder cancer, then likely insurance companies will be more open to covering the costs of the genetic sequencing.
required to screen patients. Other than for that, it depends on the trial, but I would say the majority of trials right now don’t shoulder the burden of paying for genetic sequencing.

**Question 2:** This question ties back to what Dr. Lerner talked about in terms of exceptional responders. Do all patients need to have their tumor sequenced? What do you think? Is this something that everybody should be looking at doing, just in case they have a recurrence down the road, if say they have their bladder removed, and should they know what it is so that they would know if they could respond to one of these different types of therapy?

**Dr. Lerner:** That’s a good question. I think the short answer to that is no, perhaps with some exceptions, in part because of what Gopa was saying about the potential cost, and because we don’t yet have the validated drug-target relationship. For instance, I perhaps may have painted a fairly bleak picture about chemotherapy, but we’re still going to go through the things that we know or we think we know work in a particular patient. If a patient has adequate renal function and is otherwise able to get the standard chemotherapy, we’re going to start there. We have these new immunotherapy drugs that are available for treatment, but I think that most of us, when we’re thinking about how to properly care for and prepare for the various contingencies in a patient with an advanced form of bladder cancer, I think many people are encouraging and discussing getting the tumor sequenced so that we have that information as a backup in the future.

I know that, and Gopa can certainly expand on this, that many institutions are doing this as a matter of routine, getting the tumor sequenced so they have that information. It obviously also serves a very significant research purpose, but Gopa, how are you counseling your patients with respect to getting their tumors sequenced? How would you advise them?

**Dr. Iyer:** I would underscore exactly what you said, that there are already a number of standard of care treatments available for bladder cancer patients, thankfully, in the form of chemotherapy and now immunotherapy, that we would typically recommend doing first before moving to targeted therapy. I would say that given especially the recent FDA approval of immunotherapy, that probably the targeted therapy studies in bladder cancer should probably move to the next step for those folks for whom immunotherapy doesn’t work.

Just as Seth said, we do like to ... At least at our institution, we do encouraging sequencing to be done, mainly because it takes some time for sequencing results to come back. At least for us, it takes anywhere between four to six weeks for the results to return to us, and then to analyze that to be able to counsel patients appropriately about clinical trials that are available, that’s a time-consuming and difficult ... can be a challenging process. If we already have that information because we sequenced a tumor early on in the patient’s course of treatment, then that takes away that extra time. Oftentimes, when patients have progressed on these standard of care treatments, time becomes much more important for us, because we’d like to start them on a treatment as quickly as possible and not wait. In that setting, I think I typically counsel my patients to try to do sequencing as quickly as possible and as early as possible in their disease course.

I do think, though, that what Seth said is exactly correct. That’s not possible in every institution that doesn’t offer sequencing on a routine basis, and for many patients, the cost does become a limiting
factor for them. There's an additional level of complexity here, in that the alterations that we identify within a patient's tumor when they have muscle-invasive bladder cancer may not all be the same as the ones that might be present within a metastatic site. We know that there are actually differences in the genetic profile of tumors from the same patient, whether it's taken from one location in the bladder, or from outside the bladder at a metastatic site. That can also make eligibility onto targeted therapies a bit more challenging.

**Question 3:** To what extent is their genetic heterogeneity within a primary tumor and its daughter metastases, and how is this represented in The Cancer Genome Atlas, if at all. Are biopsies of mets analyzed in addition to the primary tumor, or do they just analyze the primary tumor?

**Dr. Lerner:** That's a very insightful and appropriate question. It unfortunately was not part of The Cancer Genome Atlas Project. We use a term called bulk tumor analysis, so whatever we had from a given patient's tumor, it was all analyzed in bulk fashion. In other words, there was no separating out or dissecting out what appeared to be maybe differences or different amounts of tumor in a particular part of the specimen. As a result, there's really no opportunity to interrogate tumor heterogeneity per se, and there were really very few cases where we had tissue from metastatic sites. That's one of the limitations of The Cancer Genome Atlas Project.

There are many other projects going on in individual laboratories where they have really done a very detailed and thorough analysis of heterogeneity, both within the tumor in the bladder, and analyzing the metastatic sites. It's showing what's been shown in many other tumors. There's a lot known, for instance, about kidney cancer, about the genomic differences between the tumor and the metastatic site, and often between different metastatic sites. For instance, a lymph node metastasis may look very different genomically from a lung metastasis from the same patient.

Oftentimes, though, the genomic events that are so-called driving the cancer behavior can be identified in the primary tumor in the bladder, but at the same time, there may be different genetic events driving the metastasis. I think it's one of the great questions that still needs to be worked out from biology and how that information will be used to select the best treatment for the best patient. I know that this is also an interest of Gopa's, so he may have something to add to that.

**Dr. Iyer:** No, I think that covered it all, actually. Yeah, I agree. I think it's a big challenge. Probably one of the ways to tackle it is just to be able to sequence a lot more tumors, to really be able to identify the landscape of genetic alterations in the metastatic setting, and to try to delineate what the drivers are in metastases as compared to the primary tumors, just as Seth had said.

**Question 4:** If a patient has both the P13K and the MAPK mutation, how do you select a drug for this patient? How do you pick the most important mutation in these patients' sequenced data?

**Dr. Iyer:** That's a really wonderful question. I would say the short answer to that really is that we really don't know yet how to choose one versus the other, how to prioritize one versus the other. Sometimes, some of the information that we get from the tumor sequencing is not just the list of mutations and the list of genes that have those mutations, but also at least some sense of maybe what percentage of the tumor cells within a given tumor may have that mutation, as well.
That data is not really something that we can use in a standard fashion, because when we do a biopsy, for example, of a patient's tumor, oftentimes we get both tumor as well as normal tissue that mixes in with that. That affects the numbers that we get in terms of the overall frequency or percentage of tumor cells that carry a specific mutation, but something to consider is that if you have, for example, a PI3-kinase mutation and you have an FGFR3 mutation, but one is perhaps present in a much higher frequency than the other, then you may want to consider that one first in terms of a clinical trial. We don't do that on a very routine basis.

The other is we oftentimes basically choose, and oftentimes that's based on logistics, in terms of what clinical trial is available in the area. What do the clinical trials involve in terms of the screening process to get onto them? Do they have slots available for patients? Actually, those more practical I think issues come into play quite a bit in making that decision, and I think honestly, we just need to learn a little bit more about the biology of these signaling pathways in bladder cancer to be able to delineate whether one is perhaps more of a driver than the other.

**Question 5:** Now I have a really interesting question, and I think it speaks to whether or not patients should automatically have their tumor sequenced when their tumor is removed. Here's one from a patient. "I had bladder cancer that had spread to the pelvic lymph nodes and was resected three years ago. A tissue sample was submitted to a private sequencing company called FoundationOne. No actionable mutations were found, but CRKL was found to be equivocally amplified, and FGFR1 was also found to be amplified." Do these findings, only the amplification category, give this individual any hope if their cancer returns? Are there now new things that maybe would be applicable?

**Dr. Iyer:** I can take an attempt at that one. I think it's a very good question.

**Stephanie:** I know there's no guaranteed answer to that, but I think this is speaking to should we really consider having our tumors sequenced just in case. Is this what we're getting to at this point?

**Dr. Iyer:** Right, yeah. No, that's a very good point. I think that just to speak specifically to that question, a lot of these clinical trials are not just looking at mutations within genes, but also looking at amplifications where perhaps there are more than one copy of the gene and so more of a specific protein is being expressed, and perhaps causing a signaling pathway to be activated because of that. There are other mechanisms, not just mutation, that might result in activation of a signaling pathway, and so there are trials that are going to be out there and that are already out there that are testing drugs for patients who have amplifications as well in certain genes, not just mutations.

One reason, perhaps, to do sequencing is that even if no actionable mutations may be identified, that's right now in time. As we start to continue to develop newer drugs, and start to understand the biology of these tumors better, there may be options present in the future that are just not available right now for some of those mutations. In other words, a mutation that's considered in that other category, where we don't know the biology too well and we don't have a drug, might very well go up to one of the level two or level one categories, where now there may be an FDA-approved indication for that drug, over time. So, having that information, having that knowledge might be useful in the future even though it's not useful right now.
**Question 6:** Do either of you know if there are any trials that are targeting non-muscle-invasive bladder cancer in terms of doing any sequencing of those tumors, or is it strictly for the metastatic?

**Dr. Lerner:** I think I can take that one. I think the quintessential target would be FGFR3, which is altered in about 70% of tumors. There's actually been a trial that Noah Hahn led that didn't show efficacy, but there was a lot of very interesting work done in terms of the biology and target identification.

One of the challenges in targeting FGFR3 for non-muscle-invasive bladder cancer is most of the drugs are delivered systemically, so intravenously, and there's some toxicities associated with that that may not be in the range of what we might accept for treatment of what's commonly the low-grade tumors, which most frequently have this alteration. So, a lot of work still needs to be done, and we're very intent on doing trials that target FGFR3 in non-muscle-invasive bladder cancer.

What's got the field very excited now is there is a disease state that we call BCG-unresponsive. In a patient with a high-grade cancer that's not muscle-invasive, the standard of care is to use a vaccine called BCG. That's give into the bladder through a catheter weekly for six weeks, and then we give maintenance treatments. If a patient has not responded to that treatment or has recurred with another high-grade cancer after that treatment, the current standard of care for many of these patients would be bladder removal.

There's been, really starting back in 2012, through collaborations with the U.S. Food and Drug Administration, we now have what's called a registration pathway for drug development in this space. There's an intense amount of activity with some of these new immunotherapy drugs, because we know that this type of bladder cancer responds well to immunotherapy. That's what BCG is, as a vaccine. There's a lot of work being done with those drugs, and I think that there's still a lot of interest in the so-called targeted therapies, FGFR3 just being one example. I would say to the audience, stay tuned, because there's very exciting work being done for developing new drugs in non-muscle-invasive disease, too.