SPEAKER 1: Today, we have the pleasure of hearing from Dr. Charles A Powell, chief of the Division of Pulmonary Critical Care and Sleep Medicine and CEO of the Mount Sinai National Jewish Health Respiratory Institute. Dr. Powell completed his medical degree at the University of Chicago Pritzker School of Medicine, his residency at Columbia Presbyterian Medical Center, and his fellowship training in Boston University School of Medicine.

Dr. Powell's clinical and research interests are in lung cancer and mesothelioma. His research focuses on understanding the genetic and susceptibility factors for these diseases and the molecular events that are important in the early stages of lung cancer development and progression. He's an elected member of the Fleischner Society and has served as the chair of Thoracic Oncology section of the American Thoracic Society. He has published widely and been the recipient of numerous awards. Please join me in welcoming Dr. Powell.

[APPLAUSE]

CHARLES POWELL: Morning. So today, my goal is to take you on a journey, a journey looking into lung cancer in-depth. And I first became interested in lung cancer when I was a fellow in training, mainly because it was the disease for which we were nihilistic in terms of hope for positive outcomes at the time, but also hopeful that advances in science and understanding the genomic complexity of cancer and understanding the potential for screening for cancer might eventually pay dividends in improving outcomes for lung cancer. And so I'll take you on that journey. We'll cover the clinical issues in lung cancer, and then we'll spend a bit of time trying to unravel some the mechanisms that drive the clinical phenomena that we are very interested in.

So for disclosures, I'm a consultant for these three companies. I'm not going to talk about anything they do or produce. Now, to introduce to problem-- the problem is lung cancer. So lung cancer is a common cancer. But it's not the most common cancer in terms of incidence. There are going to be about 230,000 cases of lung cancer in the United States this year. There are more cases of prostate cancer in men and more cases of breast cancer in women.

But when we look at mortality, lung cancer is clearly the number one cause of cancer mortality in the United States and for that matter, in the world. Last year, about 200,000 people will die from lung cancer. So why are there so many people dying from lung cancer, even if it's not the most common cancer in terms of incidence?

We can get some insights if we look at the trend of five-year survival for four common cancers in the United States over the last 35 years. And so if we look at that blue line, that's prostate cancer five-year survival. That's increased to about 98% statistically. And then the magenta line is breast cancer, which now has about an 85% to 90% five-year survival. And colon cancer, as well, as increased to about 60% five-year survival.

So you can contrast those three lines on the top with that flattish yellow line on the bottom. And that flattish yellow line on the bottom is the trend in lung cancer survival over the last 35 years, which has increased from 12% to 19%, but still lags far behind. So then, the question becomes, what may account for this discrepancy that we see between the five-year survival for lung cancer on the bottom and the survival for these three other common epithelial malignancies on the top? And so I would say there are at least two major reasons that can explain this discrepancy. And we'll take them one at a time.

The first thing is, lung cancers typically are detected late. Over half of lung cancers are detected at stage four metastatic disease. We have a long history of approved screening programs for colon cancer, prostate cancer, and breast cancer. And until recently, we did not have an approved screening program for lung cancer. That changed. That changed after the publication of the National Lung Screening Trial that was performed in 2011 and earlier, where 50,000 high-risk individuals were randomized to receive low-dose CT scans and sequence for over three years or a chest X-ray. A chest X-ray really is placebo. We already had known that chest X-ray is not an effective screening modality.

And the study was conclusive, in that those who were screened with low-dose CT scan had a reduction of lung cancer mortality of 20%. And not only that, there was an overall reduction of mortality from any cause of 7%. So this really was a home run study to demonstrate the efficacy of screening for lung cancer.

And so now we've embarked on implementation of lung cancer screening in the United States. We are guided by our payers, who have determined that lung cancer screening is approved and paid for, for certain high-risk individuals who meet certain criteria. And just, in general-- this is probably familiar to some, if not all of you-- the high-risk individuals are those between the ages of 55 and 77 who are asymptomatic.

Of course, that's what you need in order to be qualified for a screening exam. They need to be current or former smokers, meaning they have 30-pack year history, and they're either current smokers or they quit within 15 years. And they need to receive a prescription from a provider for the low-dose screening that stipulates the patient received counseling about the benefits and the harms of screening, in the form of a shared decision-making visit.

The stipulation requires that there be at least one printed material used for the shared decision-making visit. And here's an example of one. This is available from the American Thoracic Society. And so what it shows is what happens if we screen 1,000 people, on the left, and if we don't screen 1,000 people, on the right. And so from the data from NLST, it would indicate, that if we didn't screen these 1,000 people, 21 would die of lung cancer. But if we did, 18 would die from lung cancer. That means three lives would be saved from screening.

But at the same time, any screening procedure is associated with risks and harms. And some of the harms involve the diagnostic procedures that are done, the follow-up findings on a CT scan, some of the risks involved, some of the complications from the treatment, such as surgery. And so it's estimated that perhaps three individuals in the screen group would have major complications from screening.

So this is the type of information that is to be presented to patients in a similar type of form, and then a decision is made to move forward with screening. And then a referral will be made to an approved screening program. And this is all overseen by the American College of Radiology or other bodies.

Well, right now, it's moving ahead. But uptake is low. About 5% of eligible individuals are getting screened for lung cancer across the country. So that either indicates two things. One is there are barriers to implementation of lung cancer screening that we need to address. Or two is it's just going to take time to achieve the high rates implementation that we see with other screening procedures.

And when we think back about mammography many years ago, it took a while for the rate of uptake to increase. So it's probably a combination thereof, but we all think that is important for us to enhance the implementation of lung cancer screening. And there are a lot of ways to do that.

It's not only the purview of primary care physicians. This is a pro/con debate that was published in*Chest* not too long ago, where the question was, should only primary care providers be those to recommend patients for and guide them towards lung cancer screening? That was the opinion of a primary care provider at a academic medical center in Boston. And then there was a counterpoint that it doesn't need to be restricted to primary care providers. Anybody with access to high-risk individuals and sufficient knowledge and education about the benefits and harms of screening could also provide that type of access.

And to further push the implementation forward even more, there are now nice guidance documents that are available to all. This is a really good one from a combined effort of the American Thoracic Society and the American Lung Association, that goes through all the elements that are important for an effective screening program and all the elements that you should look for when you're referring a patient to a screening program. And so I refer you to this as you wish to learn more about CT screening and how you can get your patients in, and what you should be looking for, and what your patients should expect when they enter into that.

So that's take-home message number one, that it is possible to prevent deaths from lung cancer in high-risk individuals, using lung cancer screening, without it being said that the most important intervention to prevent lung cancer in high-risk individuals who are currently smoking is to encourage them and help them with smoking cessation, which is part of every single lung cancer screening program. OK, so that's one reason to explain this discrepancy. But there's another one that may be as important or may be even more important. And that has to do with the understanding about the biology of these cancers.

So the histology of the three cancers on the top-- colon, breast, and prostate-- they're all adenocarcinomas. But the histology of lung cancer is mixed. Lung cancer histology for non-small cell lung cancer is comprised of adenocarcinoma, squamous carcinoma, and neuroendocrine tumors. And until about 10, 12 years ago, we treated all non-small cell lung cancers the same in the early stage of lung cancer, in the advanced stage of lung cancer. And we now appreciate that the mechanisms that drive these cancers is different in each histology and that there are opportunities to leverage those differences to target therapies to those tumors that are most likely to respond to specific treatments.

So now, when we think about lung cancer, it's no longer sufficient just to label lung cancer as non-small cell. We need to know the histological subtype and again, adenocarcinoma, which is the most common subtype in the United States and in Europe, versus squamous, versus neuroendocrine. There are very nice studies in advanced lung cancer that show that different chemotherapy regimens, specifically those that contain pemetrexed partnered with a platinum agent, are much more effective in non-squamous tumors than they are in squamous tumors. And therefore, there are different regimens that are used in squamous tumors. That is now the standard of care for treating undifferentiated non-small cell lung cancers, adeno, versus squamous.

But now we also know that there are specific drivers of lung cancer that can be targeted and halt the cancer and are associated with wonderful response rates and better outcomes than we ever saw before with traditional conventional chemotherapy. And so here is a table that outlines the key drivers that have been discovered in lung cancer. And every time I give this talk, I have to draw more red circles. And that's good. And I think the hope is, someday, every number here will have a red circle on it. But the field is moving fast.

So when it first started off, the red circles only were on mutations in EGFR and translocations and out. Those are the first two targetable driver alterations noted in lung cancer. And please also keep in mind that all the alterations that are key drivers that are actionable are an adenocarcinoma to date. And so EGFR mutations are not so common in the Caucasian population, 10% to 20% prevalence, but they're much more common in the Asian population, up to 45% to 50%, enriched in females, enriched in never smokers.

And now more recently, there are drugs that target other drivers, such as BRAF mutations and translocations in ROS1 and in NTRK1 as well. These have all entered into the therapeutic armamentarium for patients with advanced lung cancer and are associated with better outcomes than we're able to see before in patients who were treated with conventional chemotherapy, for example. So I just want to give you an example of how this information and these discoveries about drivers have been translated into practice for advanced lung cancer.

So we'll start with the first one, and this is EGFR. So the traditional response rate to conventional chemotherapy for advanced lung adenocarcinoma was about 30%. Then an important discovery was made that showed that there were subsets of lung adenocarcinoma that harbored a mutation in EGFR that had response rates of up to 70%. And so the mutations in EGFR cluster in a couple of different exons. They cluster in terms of deletions in exon 19 and point mutations in exon 21.

But there are also important mutations at a lower frequency that can occur in other exons. The mutations and alterations in exon 19 and 21 confer exquisite sensitivity to treatment with tyrosine kinase inhibitors targeted to EGFR. The initial tyrosine kinase inhibitors used in the first generation where gefitnib in Asia and erlotinib in the United States.

And to show you how these drugs can be applied, I like to show the data from this trial. This is 10 years ago, but it's really quite informative. This is the iPass Trial. It was done in Asia. And what was done was patients who had demographic characteristics suggestive for enrichment of the EGFR mutation were randomized to receive either conventional platinum-based chemotherapy or an EGFR tyrosine kinase inhibitor to fit them in this trial. EGFR status was not measured before entry into this trial.

So the overall data are shown on the top left. And it showed, that overall, patients who were treated with gefitnib had better progression-free survival-- longer progression-free survival, I should say-- than those treated with conventional chemotherapy. So then, one might say, OK, so here's a population. They're enriched for having a high prevalence of EGFR mutations. Why don't we just treat everybody in this population with the tyrosine kinase inhibitor, gefitnib?

Well, the data suggests that's not going to be optimal. So some of the patients in the trial, in a post-hoc fashion, had EGFR mutation data analyzed. And so the top right graph shows the data on the patients whose tumors had an EGFR mutation. And it showed that those treated with gefitnib had a markedly better progression-free survival than those treated with conventional chemotherapy.

But the real important part is in the panel on the bottom left, that shows, in patients whose tumors were wild-type for EGFR, their progression-free survival was worse, if they were treated with gefitnib than with conventional chemotherapy. So this really established the paradigm for care that exists to this date, that targeted therapies are beneficial in patients whose tumors harbor the target. So therefore, for patients with advanced lung cancer, molecular testing is required to determine the presence of the driver. And if the driver is present, then the appropriate target therapy can and will be prescribed for those patients.

Now, the outcomes are wonderful and much better than we had before. But there are limitations and roadblocks towards achieving the types of survivals that we see in other cancers. And so one of the points to keep in mind is every endpoint I showed you thus far with regards to the targeted therapy is progression-free survival, not overall survival. And so we have to think about why overall survival endpoint is not achieved.

And there are a couple of different explanations. One is that trials may have been confounded by crossover bias. That's possible. And the second is that what has been observed is that acquired resistance to these therapies is really inevitable. Almost all patients who are treated with these targeted inhibitors will develop acquired resistance.

So now the field has evolved to try and understand the mechanisms of these pathways of acquired resistance. One of the most common mechanisms of resistance to EGFR mutation is a acquired, or de novo mutation, in exon 20, and that's a T790 mutation, which is targetable by a third generation EGFR tyrosine kinase inhibitor, osimertinib. And there are other pathways that can also bypass EGFR TKI therapy, and there are drugs that attack that, as well.

So now the landscape really has moved from when we were relying just upon first generation tyrosine kinase inhibitors to gefitnib or erlotinib for EGFR or crizotinib for ALK permutations, to now even consider using third-line agents that were designed to bypass mechanisms of resistance, that also show improved efficacy in the front line. So now osimertinib is considered to be a viable frontline agent for patients with EGFR mutations. And alectinib and/or brigatinib are considered to be frontline agents for patients who have ALK alterations.

So the field has really evolved. It's really moved forward. But again, the key point to keep in mind, especially for those of us on the front line, who will be diagnosing patients with lung cancer, is the importance of making sure that the patients are diagnosed fully. And that means, that for advanced lung cancer patients, the samples are acquired and evaluated for the presence of these driver alterations.

Now, the field continues to move forward. And so much of the enthusiasm, which is warranted, comes from the developments and understanding the impact of altering the immune environment in the context of cancers such as lung cancer. And so many of the drugs that target this pathway are involved in the checkpoint mechanisms, that we all have endogenously, and that have constituent functions in our body to try and prevent autoimmunity whenever it's relevant. But tumors are really smart, and they leverage this checkpoint mechanism to try and have the immune system evade detection of the tumors.

And so the way this works is that T cells have a receptor that, when engaged, will decrease the function of the T cell response. That receptor is called PD-1. When that receptor is engaged by a ligand, amongst the ligands is a ligand called PD-L1, then the T cell response will be decreased. Tumors have expression of PD-L1. And so therefore, it will diminish the immune response to the presence of a foreign antigen, which can be a tumor.

So now, to try and take advantage of that system and to inhibit that system, drugs have been specifically developed to inhibit the receptor, PD-1, and the ligand PD-L1. And the first generation of drugs targeted the PD-1 receptor. And that was nivolumab and pembrolizumab. And now new generation drugs have been developed to attack the ligand, and those are atezolizumab and durvalumab.

Originally, these drugs were developed for and tested in the second-line setting, for patients who had advanced lung cancer, who had progressed traditional therapy, and then recurred. And then the studies were done to show a benefit in those individuals who were treated with the immune inhibitors. But now it's moved to the frontline setting.

And so here is a recent paper that was published in*New England Journal* last year, that shows patients with advanced non-squamous lung cancer, who were treated with either pembrolizumab, which was the PD-1 antagonist that I showed you, versus conventional chemotherapy. And what this showed was, in the front setting, the overall survival was significantly improved in those treated with pembrolizumab.

So please note I said overall survival. I didn't say progression-free survival. So the overall survival endpoint has been met by all the trials, using immunotherapy, in the context of lung cancer.

And so now the standard of care for the treatment of patients with advanced lung cancer has changed for both squamous lung cancer and non-squamous lung cancer, such that incorporation of these PD-1 antagonists is used routinely in the upfront treatment for patients with advanced lung cancer and for patients whose tumors have high level of expression of the ligand PD-L1, as measured by immunohistochemistry in any routine lab. The patients of have high levels expression, as manifested by 50% or greater, then single agent pembrolizumab can be used for those individuals. And for those patients who have some expression, but not over 50%, then pembrolizumab with chemotherapy is used. So the outcomes in terms of overall survival are better now for patients with advanced lung cancer than they were before.

So again, the other point of emphasis to keep in mind is that it's important to know the PD-L1 status for your patients with advanced lung cancer. That will guide the treatment. So we need to know how to select patients for targeted therapy in the context of lung cancer. All patients with advanced lung cancer should have PD-L1 testing done. And I showed you how that information is used to guide treatment.

Patients with advanced lung cancer should have molecular testing done. We are fortunate here it's done as a reflex test. Anytime the patient has advanced lung cancer, then reflex testing is performed on a large panel of genes, using next generation sequencing, to evaluate for the presence of driver mutations.

But the burden really remains upon us to make sure, when we do a biopsy, we acquire sufficient samples, so that there's enough DNA to be evaluated for molecular testing and enough cells that can be evaluated by immunohistochemistry. And that doesn't fall on all of you. That really falls mostly on the rows of the pulmonary folks who are here, who would be doing a lot of the diagnostic testing, and then the interventional radiologists, who may or may not be here.

And so the other key point to keep in mind is I told you about targeted therapies, and I told you about immunotherapy and that they both seemed to provide advantages. And then one might say, well, why don't we just combine them? Well, the data seemed to show that combining them doesn't really have efficacy. In fact, patients who have targeted alterations, meaning mutations in EGFR or translocations and ALK or mutations in BRAF, they do better if they're treated with a targeted therapy than if they are treated with immunotherapy.

The other thing to keep in mind is sometimes, there's not enough tumor that's accessible for performing molecular testing. But now there are at least two tests that have been developed such that-- they're called liquid biopsy assays. So tumor cells that are shed into the blood can be analyzed by methodology that's really quite sensitive and very, very specific to detect the presence of driver mutations and can be used to guide therapy for patients with advanced lung cancer. And those tests are available here, of course, as well.

So that's the landscape of advanced lung cancer. And I think there are some important take-home messages for us. One is, again, the importance of screening for lung cancer. And the second is the importance of completing the diagnostic evaluation, such that we can make the appropriate histological diagnosis and acquire specimens for molecular testing that would be able to help drive treatment for lung cancer.

But I'm a pulmonologist, and most of the cancers that we detect are in the early stage. And the research that I've been doing in the lab has focused on understanding the mechanisms that are important in driving the progression of early stage lung cancer. And all along, the hope has been that the advances that have been made in advanced lung cancer would be able to be translated to improve outcomes in patients who have early stage lung cancer, as well.

And so let me tell you this story. So this story really has to do with understanding the heterogeneity of early stage lung cancer. And I show you examples of two CT scans that show typical types of nodules that we can detect in the context of an incidental finding or in the context of a screening study. And on the left-hand side is a CT scan, where in the left lower lobe, we see a ground glass nodule. And sometimes, people say, what do you mean by a ground glass nodule? And really, the reference means you can see right through it. You can see the architecture of the lung through this little, hazy opacity, just very-- it's transparent, to some degree.

And then, on the right-hand side, we have a sub-solid nodule, or part solid nodule. So there's a little ground-glass component around the outside. And the inside, you can see there's a solid component. You can't see through that. That represents deposition of desmoplastic stroma and an immune reaction. So these represent the spectrum of early stage lung cancer-- ground-glass part solid and then, fully solid, as well.

And early on, when I began my research into lung cancer, when I was assistant professor at Columbia, I partnered up with a wonderful scientist and pathologist, Alain Borczuk, who was now a Cornell. And what we did is we examined a large number of resected early stage lung cancers. And we evaluated the pathology of these lesions. And what we were able to do was to classify these tumors, based upon their histology, into tumors that were purely solid, therefore purely invasive cancers, versus tumors that were purely non-solid, which would be classified today as adenocarcinoma in-situ. And then tumors that had a small degree of invasion-- those are called microinvasive cancers-- five millimeters of invasion is the cutoff, and those just in between.

And what we saw was a marked difference in the five-year survival after resection for these types of cancers. So for patients who had the non-invasive cancers, or AIS, pure ground glass, their five-year survival was 100%. And for patients who had minimally invasive cancer, their five-year survival was 95% and 98%. Whereas those that had more invasion had lower survival rates, similar to what we see with many patients with early stage lung cancer.

So really, there are two takeaways here. One is there is a subset of lung cancer with markedly different survival than that flat yellow line that I showed you twice already. We're talking about a type of lung cancer that has survival rates of 95% to 100%. The second take-home was our observation is generalizable. So around the same time, similar work was being performed around the world.

And I'm showing you two studies from Japan, below ours, and another study from the United States. And it all showed the same thing. Clinical outcomes for patients who had non-invasive cancers after resection hovered between 95% and 100%. So really, the story becomes that there is a pathway of potential progression of early stage lung cancer that we can clinically observe multiple times, where a tumor can progress from a non-invasive ground-glass opacity, that correlates with pathology. It looks like this.

This is the pathology of adenocarcinoma in-situ, where there are tumor cells. This is chock full of tumor cells on the left. But the tumor cells nicely line the alveolar membrane-- they don't invade-- versus tumors that are represented radiographically by part solid, where there is a component of desmoplastic invasion, that you can see quite clearly over here, surrounded by the non-invasive pathology in the rim. And so this progression not only can be seen under the microscope, but it can also be seen clinically.

And our question is, what drives that progression? So to try and get some insights into this process, we, amongst others, started off-- and this is a while ago now-- by doing some relatively straightforward studies to look at the transcriptomics of lung adenocarcinoma. And at this time, the platform of choice was a microarray platform, the Affymetrix platform. And what did is we looked at a small number of lung cancers, 35, that represented the pathological spectrum of invasive and non-invasive cancers.

And when we looked at the gene expression profiles, and we clustered the samples together, solely on the basis of the similarity of the gene expression, without knowing the pathology, we saw that there were three groups-- one group of tumors that had genomics associated with a pure invasive pattern pathologically, one group that had most of the non-invasive cancers, and one group that had the tumors that had both the component of non-invasive and invasive cancer, the part solid lesions, if you will. So this was what we did at our lab in New York, and it was generalizable, in that multiple groups around the world were investigating the same question and found the exact same phenomena. Whether this study was done in Japan, on the top, done in Michigan, over here, or done through a United States consortium, on the bottom, same pattern emerged, that lung adenocarcinomas would cluster into three different groups, based upon their genomics. And those groups were associated with the pathology differential between a non-invasive cancer, versus a solid cancer with the lepidic type of mixed cancer, represented by the [INAUDIBLE] lesions in between.

So this really provided biological plausibility to support the clinical observation that different pathological types of lung cancer were associated different biology and therefore, would be important to take into account as we made a diagnosis and recommended treatment for early stage cancer. And these concepts are manifest and written up in a really nice document that was published in the *Journal of Thoracic Oncology,* about nine years ago, that reclassified pathologically and histologically lung adenocarcinoma, change the terminology to terms that we use today, and have helped to drive the field forward.

There have been many applications of these advances in our understanding of early stage lung cancer. I'll just give you two. One is that the tumor node metastasis staging scheme, that most of you have now committed to memory for many different cancers, the new eighth edition takes into account the invasive size, when we measure the size of lung cancer, as opposed to taking into account the entire size of the lesion. For practical purposes, what that means is, if you have a ground-glass nodule that has a solid component, the T stage is determined by the measurement of the solid component, not by the overall component. Because that is what is associated most closely with clinical outcomes. That's one manifestation.

And a second manifestation is our approach for how we evaluate nodules that are detected in patients who either have an incidentally detected nodule, which is much more common than patients who have nodules detected during the screening. So here is the Fleischner Society Guidelines, published in 2017, that provide guidance on how to evaluate and manage patients with incidentally detected pulmonary nodules. And the guidance is different, depending upon whether we're talking about a solid nodule or talking about a nonsolid nodule. The reality is the prevalence of cancer is much higher in a nonsolid nodule that is stable over time, but the properties-- biologically and clinically, those tumors are much more indolent. So there are differences in the recommended follow-up algorithms and approaches for diagnosis for the nonsolid lesions, compared to the solid lesions.

But really, our focus in the lab is trying to understand the specific mechanisms that drive the progression of lung cancer, from that nonsolid, non-invasive tumor, to the more invasive solid tumor, that potentially is capable of metastasis to the lymph nodes and systemically. And so really, what we're focusing on are the drivers of the process that will transition to tumor from over here-- this is a adenocarcinoma in-situ microinvasive tumor, the minimally invasive or non-invasive cancers, to the invasive cancer. And so our work previously had shown that alterations in the TGF beta cell signaling pathway were important in driving lung cancer progression.

And so we discovered, that using human tumors, the same tumors that we used to derive those dendrograms that I showed you a few slides ago-- and when we honed in on specific genes and pathways, we identified that loss of the Type II TGF beta receptor was a key driver in promoting lung cancer invasiveness. So that was discovered in human tumors. It was validated using human cells.

And then we took the step of generating an animal model of lung adenocarcinoma, where we took a lung adenocarcinoma model, and we knocked out that Type II TGF beta receptor gene. And we showed that mice that had knockout of this gene receptor had tumors that were invasive and metastatic, compared to those that had wild-type TGF BR-2 expression. And then, subsequently, we've done studies to identify the downstream signaling pathways that are activated when the TGF beta cell signaling system is disrupted. And we've now published that mediators that include lysyl oxidase and CCL5, which is RANTES, are important in driving this process. And they represent potential therapeutic and diagnostic opportunities in these cancers.

So this is all work that was done when I was at Columbia. And subsequently, we've now driven this work forward in our lab here at Mount Sinai. And so what we focused on was in collecting larger sample sets of tumors that are clinically annotated and applying some of the new and improved technological advances to understanding the genomic basis of cancer and understanding that there is no one particular alteration that's going to be important in every circumstance, and there are multiple and different potential mechanisms that can drive the progression of cancer. They can include copy number alterations, deletions and amplifications. And this is work that is centered by efforts done at Cornell with Alain Borczuk, that I'm going to go into detail on.

There are key mutations that can occur in lung adenocarcinomas. And I'll show you a little bit of data from a paper that is ready to be available online from the Blue Journal. And that's work that's done in collaboration with Pierre Massion's group at Vanderbilt. And then I'll show you the work that we're doing, predominantly focused here, looking at transcriptomics and network analysis.

So first, this is the paper that will soon be available in the Blue Journal. This is looking at the mutational landscape of lung adenocarcinoma. So now we're taking 101 lung adenocarcinomas. 50 represent the spectrum of AIS and minimally invasive cancers, and then 50 represent the more invasive cancers. And we're examining them, using next generation sequencing, using a targeted cancer panel of 347 genes.

So what did we find? Well, first off, amongst the mutations, most of the mutations were missense mutations, about 83%. And as the tumors progressed from non-invasive AIS or to minimally invasive cancers, then eventually, the adenocarcinoma, the frequency of mutations increased, which you can see over here. And so then we characterized different mutations, that you can see in the rows over here, with the specific types of tumors. The invasive tumors are more purple, and then the non-invasive tumors are pink and such.

And you can see that EGFR mutations are quite common, and they're enriched in this population of relatively non-invasive tumors. KRAS mutations are more enriched in invasive cancers. And there are other important drivers, that you can see over here on the right.

Now, many of these drivers are associated with pathways that are important in lung cancer. And so we looked at the relevance of these pathways. And what we saw was that there was an enrichment of activation of some key pathways that are important in lung cancer. And focusing really on the bottom, there's P53, and there's AKT, and the MAP kinase pathway. These were all preferentially activated in the invasive adenocarcinomas. And activation of these pathways was associated with outcomes in these patients.

So we've learned a lot about the distribution of mutations. We actually dug in a bit and discussed the clonal evolution of these mutations in these tumors. And I think that's going to provide nice insights as we think about the progression of these alterations from an epithelium that is normal, to an epithelium that is preneoplastic to one that is characterized by early lung cancer, that is non-invasive, to one that is invasive. So there's a lot of information that will come from that particular study.

This is what I want to focus on for the last part of my talk. So here's our lung cancer group, at least most of our lung cancer group. And so the work I'm going to focus on is looking at the transcriptomics of early stage lung cancer. And most of the work that I'm going to show you has been done predominantly by Abby, over here, with wonderful help and partnership with our colleagues in genomics and Sema4. And that's Seungyeul Yoo over here, and Jun Zhu, who is partially obscured by David, over here. But there's Jun.

And so this is what we do. So again, tight collaboration with Alain Borczuk, at Columbia, and now at Cornell, who provided us with the samples that were enriched for the presence of a tumor. And we're focusing on the tumor here. We had 53 lung adenocarcinomas. And then, we were able to identify genomic signatures that distinguished the invasive, versus the non-invasive cancers. And then we went on to show that these signatures were generalizable at step 1.

So here we go. So here is the unsupervised clustering dendrogram where the samples get sorted on the similarity of the gene expression. And we then use these data to identify signatures of invasiveness. So we have 21 tumors over here that are in the invasive category and 32 in the non-invasive category and different gene expression in the rows, in blue versus red, associated with these different categories of invasive and non-invasive tumors. So this helps us to derive an invasive signature.

So then, one might assume that tumors that are enriched for a signature that is characterized by invasiveness might have clinical behavior patterns associated with metastasis and death. So that's a testable hypothesis. And one of the ways to test that hypothesis is use external data sets that are derived from adenocarcinoma tumors that have annotated outcome information. So that's what we did.

And on the right-hand side, I show you the results when we overlay our signatures of invasiveness onto seven published data sets of lung adenocarcinoma. And these are acquired from data sets that were produced in the United States. This is the Cancer Genome Atlas data set, which is probably the largest. This is another large US consortium database. Then we have two from Asia and two from Europe.

And I think the key point I want to bring home is that we have seven data sets here. And in every single data set, our signature invasiveness is associated with differential survival in all these different tumor cases from around the world. So this is robust external validation about the clinical importance of this signature. That was step one.

So then what's step two? So step two is we want to understand, what is driving the process that leads to the invasiveness signature? And then, what, if anything, might be targetable to alter the progression of these tumors moving from a non-invasive to an invasive cancer? So to take advantage of the wonderful expertise we had here, Jun and Seungyeul, who have developed methodology to do network analysis of genomic data sets, were able to create a network map of key drivers that are important in cancers, regardless of organ system, and to overlay our gene expression data set on that network analysis.

And so that's what you see on the bottom, that spider web, if you will, where the red are the genes that are upregulated in our invasiveness signature, and the green are the genes that are downregulated. We see a lot of red. And you also see these circles that are helpful. And so the are really-- the key drivers are transcription factors, such as TPX2, and then [INAUDIBLE] 5. And this is also known as surviving.

These are not targeted, but some of their downstream mediators are. And so two of the downstream mediators are circled over here. This is a aurora kinase B, and this is aurora kinase A. So this data sets up a testable hypothesis. The testable hypothesis is that inhibiting the function of aurora kinase A and B can potentially retard invasiveness and result in prevention of progression of lung adenocarcinoma. That's the next step.

So now we test this in-vitro. So we set up systems, and Abby did this work. And we were able to knock out aurora kinase A and B, using a variety of different mechanisms. First, we use genetic mechanisms and the CRISPR methodology. And then we used small molecule inhibitors. Some were non-specific. They would take out aurora kinase A and B at the same time. And then, also, Abby's done studies where she specifically knocked out individual aurora kinases A and B. And then, we looked at the important properties of cells in-vitro to determine the impact on the mechanisms of invasiveness in these cells.

On the left-hand side is a Western blot. So here we're looking at protein expression in tumor cells, two different tumor cells-- one different set of tumors on the top, another set of tumors on the bottom. And what we're looking for here specifically is activation of aurora kinase A and B, which is shown by the presence of this band of phosphorylation that is present in activated aurora kinase A and B. And when you use the drugs, the inhibitors, pan or kinase inhibitors knock out expression of the phosphorylated state of these proteins. So we're inactivating aurora kinase A and B.

So then, we can use these drugs in our in-vitro systems. And we measure three things in our in-vitro systems. We measure the ability of cells to traverse across the artificially-made wound in a cell culture dish. That's the wound-healing assay. We measure how quickly they can move through a membrane in a well of cells. And we measure how quickly they can move their way through the basement membrane, which replicates the process that we think is important in-vivo.

And so all three systems showed the same thing, that when we knocked down expression of aurora kinase A and B with these inhibitors, we were able to abrogate invasiveness, migration, and wound-healing. And Abby and others in the group have gone on to do additional studies to characterize the mechanisms of how these inhibitors may impact these properties, and have gone on to use a variety of ways to validate these findings. But I don't have time to show you that now.

But what I do want to just do is bring it home. And what's the path forward here? So really, when we think about lung cancer, and pretty much, the way we think about any clinical disease, the key to success is to integrate all the important information that we can acquire for a specific case. And the sources of information, of course, include the clinical information, imaging information, and molecular and biological information.

So well, if you apply that to lung cancer, we can focus really on the imaging information and the molecular information. And we believe the path forward is to combine the two, to use radiomic or similar approaches to characterize the status of the ground-glass opacity. Is it part solid? Is it fully solid? And if it's part solid, how is it changing? There are ways to precisely measure that and record it over time, and there is work that is published by groups, such as Claudia Henschke and Dave Yankelevitz, who run our screening program, that show the radiomic analysis of these part solid and solid lesions correlates really well with what we can see under the microscope pathologically.

So we want to correlate those data with the molecular data. And I showed you some of the ways that we're identifying the key drivers that are important in moving a noninvasive cancer to an invasive cancer. Some of the mechanisms include activation of aurora kinase A and B. Some of them include specific mutational signatures and the pathways they activate, the overall transcriptional signatures, the loss the TGFBR2 and then the downstream mediators that are elaborated when TGFBR2 is lost, not to even mention yet the immune environment and how that is impacted by many of the changes that I showed you, over here, and can similarly be targeted in parallel or separately for driving forward the treatment of lung cancer. That is the path forward for how lung cancer at early stages can be addressed, with the hope of having similar successes that we're seeing in the advanced stage of lung cancer.

So now just a few concluding points to reiterate some of the take-home messages-- so the key about understanding lung cancer and making advances in lung cancers is to appreciate it's a heterogeneous disease. And the heterogeneity is in the biology, and the biology drives the clinical outcomes and offers therapeutic opportunities for intervention that can be successful. Lung cancer screening is effective. It should be done more than we're doing it right now. And we in the pulmonary group and the rest are very happy to help facilitate that for anybody who has any questions. And you can certainly take advantage of the lung cancer screening program that we have here, which is truly a pioneering very effective program.

We need to perform molecular testing anytime we're making a diagnosis for advanced lung cancer. And early stage lung adenocarcinoma drivers, that include aurora kinase and other specific transcriptional and mutational signatures, they can be leveraged now and in the future to precisely diagnose and treat these cancers and improve outcomes for patients who have lung cancer, now and in the future. So thanks very much for your attention.

[APPLAUSE]

SPEAKER 2: We have time for a few questions.

AUDIENCE: So if you have a patient-- the original biopsy shows how you should treat the patient. The patient responds, but then, later on, develops a new tumor. If you look at the gene signatures, was there a small population that has now grown out, or was there a new mutation that occurred?

CHARLES POWELL: So that's a really key question. So what happens as a tumor progresses from one that is responsive to one that is resistant? And to answer that question, I would refer us to a beautiful paper, really a case report, that was published in the *New England Journal,* by the Mass General, group led by Alice Shaw. And what she talked about, in that case, was a particular patient who had an ALK driver mutation in a lung tumor. And that patient was treated with crizotinib and developed resistance.

They repeated the biopsy. And then, they were able to show the mechanisms that were causing resistance and a suggested utility of using another drug, called lorlatinib. And then the patient eventually acquired resistance to lorlatinib. And then another biopsy was done to show that the patient now was sensitive to crizotinib.

But the really key analysis, to answer your question directly, is they did [INAUDIBLE] analysis on all the mutations that were present. And at the very beginning, most, if not all, the cells had a specific alteration and out that was sensitive. But there was a real small population, maybe 1%, of cells that were resistant.

And over time, as those patients were treated with crizotinib, the population of cells sensitive to crizotinib, of course, reduced. And then the population of cells resistant increased. So they were present all along, in that case. And they were selected for-- upon treatment with crizotinib. So that is a likely process that is going on, in not just that patient, but in other patients, as well.

AUDIENCE: So I'm curious. When you're looking at some of the molecular pathways that are involved, very common, it was mTOR on the AKT pathways. Is anybody looking at [INAUDIBLE] normally?

CHARLES POWELL: Yes, so Pierre Massion's group is doing some nice work, looking at AKT and looking at mTOR and in some in-vitro models. It looks like it may be effective, as well-- just now to move it more into the preclinical stage and then see if it has any clinical applicability. [INAUDIBLE].

AUDIENCE: Just because we use it in kidney transplant patients for [INAUDIBLE] melanoma. We've had good success with melanoma. So it's not a big leap for us to do. We just switch immunosuppression solutions. It's really [INAUDIBLE] is not enough of the cases, but they respond very nicely and they're AKT driven.

AUDIENCE: Your genomic signature has to do with the tumor itself and also its microenvironment. Could you discuss a little bit about the field effects of cancer and how we might be able to do some kind of screening for cancer, just sampling the environment around these things?

CHARLES POWELL: Yes. So I think it's important to think about tumors as an organ, if you will. And so the center of the organ are the tumor epithelial cells. But they live in a neighborhood, and the neighborhood is the stroma, that's comprised of immune cells and endothelial cells and fibroblasts. And they all contribute together to allow a tumor to grow and to proliferate and metastasize.

Until recently, every effort was focused just on the tumor. But now we see the benefit of also taking into account the immune environment and then impacting that, as well. We don't have any strategy that I know of that will be able to be used in screening to characterize the microenvironment and say whether or not the microenvironment makes an individual at higher or lower risk for cancer. Over time, we'll learn more.

Over time, we'll learn how maybe the microbiome influences the microenvironment and may change the susceptibility of individuals. We have to keep in mind that 10% of smokers develop lung cancer, and 90% don't, even though they're exposed to the same carcinogens at the same level. So there are differences in individual susceptibility to develop disease, even upon the same exposure. And some of the approaches that you suggest may allow us to understand that a bit better.

AUDIENCE: So I'm referring to the next generation sequencing.

CHARLES POWELL: Yeah.

AUDIENCE: You showed a slide that had non-invasive and invasive. And most of the non-invasive mutations were around the EGFR. But there are also some invasions on the other genes, which are also present for mutations in other genes which are present in a the highly invasive cancers. Does that mean that the non-invasive has already some cells with all these lousy bad mutations. How do you explain that? Do you understand [INAUDIBLE]?

CHARLES POWELL: Yeah, I do. I understand. The question is a really good one. And so what I'll say is that there are some mutations that we see in advanced lung cancer-- and I'll use KRAS as the example. I mean, that's the one that is a prototypical mutation in very advanced lung cancer. That same mutation is apparent in preneoplastic lung tissue. There's a lesion called atypical alveolar hyperplasia. And those lesions are enriched for the population of KRAS mutations.

But the behavior of KRAS is much different in the context of a cell that's in that AAH environment, compared to the behavior of the KRAS mutation in the context of a invasive tumor. And when I think about that, I think about pancreatic cancer, which is a similar issue, where KRAS mutations are associated in the tumors themselves, with more of an indolent behavior, as opposed to aggressive behavior. So the context is driving the behavior of the tumor in the presence of that mutation. And the work really is now on trying to understand how the context manipulates the response. So yeah, you're right. There's overlap in terms of the mutations across the pathology. But we don't know what influences behavior that's driven by those mutations in most contexts.

AUDIENCE: Thank you.

SPEAKER 2: Thank you very much. Thanks.

[APPLAUSE]