

Dr. Janne: We can start moving on in, can you show that. Can you put up that slide that was just up? As you get coming in, just to highlight that there continues to be interest in this area this is a slide that Dr. Scher meant to show in his presentation, but there is an upcoming conference focusing on a very similar topic a Gordon Conference in Mt. Holyoke at Massachusetts. I hope there is AC at the dorms where people are staying.

Okay, so in this session we will focus on liquid biopsies in lung cancer drug development and clinical use. We will have two presentations and then a panel discussion and I'll invite the panelists to come up afterwards. Now lung cancer works out as a really an ideal disease in which liquid biopsies may be used. Lung cancer is of course a disease where genotype directed therapy is the standard of care and we have multiple targeted therapies approved for specific genomic subsets of the disease such as; EGFR inhibitors, ALK inhibitors and RAS inhibitors. Lung cancer patients of course ones where biopsies, traditional tumor biopsies can be challenging, either the tumor is in a location that's difficult to biopsy such as next to the aorta or next to some other structure or the patient's comorbidities prevent you from doing a biopsy. Somebody who was bad COPD for example where the risks of biopsies are as significant, and hence having alternative technologies that are able to not only diagnose the specific genotypic subset of the disease but ultimately be used in monitoring etcetera.

Of course we have agents that are approved when patients develop resistance to targeted therapies and understanding the specific mechanism or resistance, again which can be determined from a non invasive method may help guide the particular therapy that the patient should receive at that point. As Gideon mentioned earlier we now have just a reason approval and we will hear more about this later on today of the first cfDNA assay for EGFR mutation, EGFR activating mutation detection recently approved and available for clinical use.

There is a lot of interest in this particular disease four liquid biopsies, again our first two presenters will talk about sort of the clinical use and where things are headed. Then I will go on to the panel discussion where we have representation from not only the first two speakers but also members from different companies that are making targeted therapies in the lung cancer space, and also have interest in and liquid biopsies in that as well. Without further ado I will introduce our first speaker Geoff Oxnard from the Dana-Farber Cancer Institute who will speak on plasma genotyping for treatment selection of advanced non-small cell lung cancer, Geoff.

Geoff: Thanks Pasi and thank you for inviting me to speak today. I want to talk about plasma genotyping in treatment selection for advanced lung cancer. I'm a clinical investigator, I don't work in the lab I work with labs. I'm going to try to bring this really focused on patients and how we can use this to help patients using three patient cases; a patient with newly diagnosed lung cancer, a patient with a quite resistant EGFR-TKI and a patient with suspect reoccurrence of lung cancer. All of these are patients of mine who over the past months I've used liquid biopsies try to help guide their care.

In the first is a of forty nine year old never smoked who presents with cough and headache, CAT scan seen here shows a long mass and multiple lung nodules. I know to you forty nine and never smoke it doesn't seem like lung cancer, but this is the kind of

patient population we are enriched for at academic centers. This is a unique patient who sure looks like they have lung cancer but doesn't seem like your average type of lung cancer. This is a patient where cancer genetics has a potential to make a big difference in finding a targeted therapy for them. Brain MRIs done shows an eight millimeter mass in the cerebellum, we can't rule out leptomeningeal disease based on the scan which might be causing the headache. There is a super [inaudible 01:42:49] lymph node and a biopsy of this is done by the surgeon who is often the first person to see a patient with potential lung cancer and that shows non-small cell lung cancer.

The patient comes to see me the oncologist four days after seeing the surgeon, which is sort of a pretty quick timeline but appropriate for a motivated patient who is sick with symptoms. At that point the pathologist is still looking at the slides trying to make sure the diagnosis hasn't finally signed it out yet, could be lung cancer but it's non smoker maybe it's something else. The story is being sorted out and the patient comes to me and says, "Well, I feel sick I want treatment what do you got for me?" I've got to get around to making a decision for him.

I think it's worth recognizing the multiple steps involved both in tumor genomics and biopsies versus liquid biopsies right. For a patient if I'm seeing and I think need a biopsy for genetic testing, that patient I order the biopsy that gets scheduled and performed by some procedure a surgeon or initial radiologist. It then goes to the first pathologist who reviews it, confirms the diagnosis, makes sure it's adequate and signs it out and then that eventually goes to the molecular lab who then preps the DNA performs the assay. When you read about the turnaround times that is recommended for genotyping, that's eight business days for this step, but the fact is the clinical course is quite a little longer for our patients and there are many places where this gets delayed and it takes more time.

For a liquid biopsy, I see the patient and I draw the blood and it goes to the molecular lab. We skip out a bunch of the places where the whole process can go wrong, so for a patient like this with lung cancer who is sick and getting sicker and needs treatment now, a liquid biopsy has an urgency that can quickly get them a result perhaps.

We recently published a validation our dPCR assay we've developed it at Dana- Farber, and the median turnaround time of our assay was three days from blood draw to result as opposed to twenty seven days from ordering a biopsy to result. Three weeks of delay can be a big time period for a patient with progressive advanced lung cancer. With this patient I order plasma genotyping while we are waiting for the tumor results to complete, I see him on a Monday on Wednesday I have a result back in my hand. 38% AF of L858R this patient has an EGFR activating mutation detected, and I can based on this result done in the clinical lab in LDT not an approved test, I order off-label or erlotinib to help get him treatment and get him better quickly.

Can I rely in the result like this, this is a validation we did over a hundred and eighty patients recently published in [inaudible 01:45:23], where we looked for these key EGFR and KRAS mutations are using ddPCR and the sensitivity for the known tumor genotype and patients with a known genotype we could detect in 64%-82% of patients depending upon the assay being used. The key point is as you have more disease, all these are

metastatic lung cancer but as you have more disease your sensitivity goes up. If you have stage four lung only disease your sensitivity was in the range of 50% or 60%, because a lot of these patients just aren't shedding DNA into their blood for you to detect. If you start getting; brain, bone, liver metastasis, if you start getting sick with a high volume disease your chance of shed goes up and chance of a liquid biopsy being successful goes out.

These are most successful in patients with liver mets and bone mets based on some analysis, but in patients with more limited disease in the lung, lymph nodes only the chance of finding that circulating DNA goes down. Sensitivity is variable depending upon the patient's disease state. How is the specificity though, for the key driver mutations in EGFR and in KRAS there were no false positives seen with this assay, so you can trust a positive result and that's really how we developed this; 100% specificity but sensitivity that's below 100%. Trust a positive and act upon it, but if you have a negative you may need to fall back on the tumor result because you may have missed it. I will point out one caveat which is 63% specificity for the T790M resistance mutation and this we think is because T790M resistance is complex. It is heterogeneous.

A single tumor biopsy might not capture the entirety of their resistance, but a liquid biopsy may detect circulating resistance mutations not present in a single site of biopsy disease, and this is a problem I'll talk about more a little bit later. When this patient I start erlotinib based on the positive EGFR result, the patient symptoms rapidly improve headache resolves, cough gets better, he responds like this and he is doing great months later. A liquid biopsy can quickly get me to a result that can help impact patients care. This is a setting where the FDA recently approved the liquid biopsy for detecting these key EGFR driver mutations in plasma which we will hear about more later.

To conclude there are practical logistical challenges when we do widespread tumor genotyping right, we love tumor genotyping but the fact is it's not happening for everyone everywhere. If you want to make it happen for everyone everywhere, perhaps liquid biopsies can supplement this as a way of getting patients to a result. It is really DNA testing that impacts lung cancer care and that's why it's circulating DNA testing that is the tool that's making an impact in clinical care today. Plasma genotyping can allow rapid initiation of targeted therapy, a high positive [inaudible 01:48:00] value means trust a positive result, but sensitivity really in most of these assays maxes out around 80% you may miss something all right, so a negative result cannot be trusted. As we communicate this to Doctors and to providers, we have to understand that a negative liquid biopsy result can't be trusted you need to fall back on tumor.

If we trust negatives we will miss a lot of patients who could benefit from targeted therapy, but you aren't seeing that in their circulating DNA. This is a trial we are launching now Dana-Farber, where we are enrolling and across Dana-Farber Harvard Cancer Center. We are enrolling patients with advanced lung cancer and rich for having EGFR mutation but they don't have tumor genotyping yet. We perform rapid plasma genotyping over the course of days, start the monitor erlotinib based upon this blood test and follow their outcome. Looking to show that the outcomes are just as good when you treat based on a blood test as if you waited for the tumor biopsy. This by the way uses our LDT test that we have developed for use for our Harvard patients.

All right, case number two, now let's get to resistance. A seventy three year old female EGFR mutant lung cancer she has been on erlotinib for two years; she had a great response, she is tolerating it well, she feels terrific, she sees me every three months, she is living the dream, where comes a lung cancer patient okay. Has a normal life and she comes in and her scan shows that her mass is getting bigger, her lymph nodes are larger, she is asymptomatic but she has what appears to be resistance. At this point is it standard of care to get a biopsy, yes.

Osimertinib ACD9291 was just approved now in multiple countries for T790M positive resistance, you need to have the right kind of resistance to benefit from this drug. If you have T790M on your resistance biopsy 62% response rate, PFS nine point seven months median you do great. If you do not have T790M, you have a 26% response rate only a median three month PFS you don't do that well chemotherapy is right for you. For the first time there was a drug approved requiring a new biopsy years into a patient's care at a decision point to decide if we go a tolerable active drug or standard chemotherapy. The first drug approved based on a resistance biopsy this change is oncology care, suddenly we are doing more multiple biopsies for our patients and it's part of routine care, a great place for a liquid biopsy.

This patient in particular has a growing paramediastinal mass right next to the aorta, getting a biopsy this will require going through the pleural twice, this is a high risk biopsy; the patient feels well, she has a lot of comorbidities, heart disease, diabetes. She doesn't really want this biopsy I don't blame her, so we say can we get a liquid biopsy. We sent genotyping of cell-free plasma DNA, I won't mention which lab we use here and they report 'detected for her known L858R mutation.' Now I will say this isn't a positive result, this means we saw something, we are not sure if it's positive or not, we'll tell you it's detected you make up your mind. I speak to this because it is hard to know what a positive and a negative is with these assays, there are low level results you are seeing, you are not sure if it's in the noise range or not. Defining what a positive is rigorously for your assay is really important, and it's a place where really a lot of these assays are struggling.

This patient does not have T790M in this liquid biopsy, can this be trusted? We study this using the first line oral trial, the phase one oral trial of osimertinib is a collaboration with AstraZeneca. We looked a beaming I'd from seismic agnostics and other digital PCR assay, and we found just like in the prior data I showed you sensitivity is in the range of 70% or 80% for these various EGFR mutations. Sensitivity for the resistance mutation goes up to 80% from 70% if you have the sensitizing mutation, if you don't have the driver mutation don't look for the resistance mutation you are not going to find it. Specificity again is very high for the driver mutations, but again a 30% false positive rate for T790M resistance is heterogeneous and there is discordance between the blood result and the tumor result sometimes.

When we look at the outcomes based on the blood result instead of on the tumor result, what do we see? If you are plasma positive or T790M 63% response rate, median nine point seven month PFS the same grate outcomes, if you are positive in the blood test versus positive in the tumor test this is great right. The negative result is a little

more complex, they do better than expected. If you are T790M negative 46% response rate higher than the 26% and an eight point two month median PFS higher than the three point four median PFS. The negatives do well because the negatives are a mixture of true negatives and false negatives as I just told you. A positive result seems actionable, but a negative result can't be trusted. If you then do tumor genotyping on the plasma negatives, you see that the plasma negative tumor positives do great and the plasma negative tumor negatives do poorly.

Using this data we advocate for a paradigm that's slightly different, instead of the current FDA proof T790M test leads to positive osimertinib negative chemo, a paradigm where you first do a screening plasma test looking for the T790M. If positive skip the biopsy start the drug, if negative proceed to the biopsy and use that to pick your treatment. Now this does not yet have an FDA approved T790M blood test to go with it and that is something that we need, and how much do we need it? I have patients who have died waiting for their biopsy results, wanting to get on these droughts. The result was positive but they couldn't get the result because of the delays, so I think there is real urgency to help patients get to this place.

This particular patient the T790M was not detected, we can't trust it, I want to proceed to biopsy I just told a biopsy was not feasible what do I do. She says, "I feel fine let's just wait," we give it a couple of months this is standard in lung cancer when there are [inaudible 01:53:50] progression. We give it a couple of months and see if overtime they develop another site to biopsy or they develop more shed, we repeat scan in two months. Except six weeks later she is hospitalized with a PE, with more progression of her cancer, she is sick, she is even sicker now, she is too ill for a biopsy, she needs anticoagulation, we get another liquid biopsy and we find that L858R has gone up to now 2% and the T790M is reported as negative asterisk.

Here is a small print, in contrast to other low positive results this one seems really high sixteen of two thousand and seven and forty seven droplets, this is really kind of high than we are used to seeing but is too low for a positive so this is negative. What do I do? I start off-label osimertinib right okay this is what I had to do for this patient, but in the end what I really want is an assay that's clear about what's positive and what's not and can help me help my patients. This particular patient got sicker and died of a PE and was not able to benefit, so I don't have a happy ending for you but I think the need is clear. We need to know what is a positive, what counts and we need an FDA approved assay that we all trust in.

To conclude case number two there was a clear clinical need for plasma genotyping to aid in the management of drug resistance, not just lung cancer resistance but drug resistance is happening in breast cancer and in prostate cancer and in other spaces. What we are setting, what we are establishing in lung cancer will be emulated across oncology and yet we still don't have an assay. There was an unclear reference standard, a single biopsy does not represent resistance, we need a better sense of what a reference standard should be and maybe that is in fact treatment outcome and many of the available assays have not been optimized because of these complexities to meet our clinical need.

This is a trial that will be launched from BI the Alexis six trial where patients who are plasma T790M positive without a biopsy will start a mere selective EGFRt semi inhibitor or mutnib, sixty patients treated looking to show great high response rates when treating based on a liquid biopsy we don't need the biopsy. This is the kind of trial we need where we just go with the blood test, skip the tumor and show the outcomes are just as good.

Case number three a seventy four year old never smoker with a prior history of resected lung cancer comes in with spots in his bones. He had stage two lung cancer three years ago, he got adjuvant chemotherapy and then serial CAT scans. As CAT scans now show new sclerotic lesions in his ribs, bone scan shows uptake in sorry ribs not rubs, spine pelvis. This is his bone scan, what does it look like, does it look like lung cancer? Look like prostate cancer, what does it look like? It looks like cancer in his bones, he needs a biopsy right? You know what a bone biopsy can be tricky let's do a liquid biopsy I say. I check it he is positive for the EGFR L858R mutation, 3.5% AF does this confirm that this patient now has recurrent metastatic lung cancer which will be terminal, incurable would you want a biopsy?

I asked my docs we all said, "The poor man wants to know what he has give him a biopsy." That it's hard to give a patient a diagnosis of incurable cancer, to really make a treatment plan for advanced disease without that tissue diagnosis. We are very dependent upon the tissue and even though this is really, really consistent with recurrent lung cancer and if I couldn't get a biopsy probably I just start a EGFR inhibitor.

He deserved a biopsy to confirm the diagnosis, there was really no clear data available to say that plasma genomics can make a diagnosis really all the data is in patients who have a diagnosis and use the plasma genotyping to supplement it to guide treatment. We do know however that tumor NGS can make the diagnosis, we have cases at our center where tumor NGS has changed the diagnosis from what looked neuroendocrine carcinoma to Ewing sarcoma. What looked like a esophageal cancer to out positive lung cancer, what looked like poorly differentiated non-small cell to mesothelioma.

Tumor genotyping can do this because we understand tumor genomics so well today, but still that is a patient who has a diagnosis of cancer and the genomics is helping you to better clarify what the diagnosis is. Otherwise there are some mutations which really offer no insight, if this patient had a callus mutation in his plasma we would say there are ten different cancers that can give a callus mutation you still need to biopsy. It's not clear that the plasma can make the diagnosis yet, though I certainly think I hope we'll get there someday as we gain more trust. This patient proceeded with a biopsy of his right hip lesion, positive or adenocarcinoma, tumor genotyping was done on this which showed an L858R EGFR mutation exactly what was predicted based on his blood test he started on a erlotinib and is doing well today a year later.

Conclusions number three, it is unclear whether a biopsy for cancer diagnosis or staging can really be replaced by a plasma genotyping at least today, but we might get there as we have more trust and maybe when combined with other tools like CTC analysis like other liquid biopsy tools. This could come together to really definitely make a diagnosis without needing the tissue, but the tissue is the issue and we trust the pathologist to

make that determination for us and that stands today. I will acknowledge my collaborators which are varied and my funding and we'll take questions later, thank you for your time.

Dr. Janne: I think we'll take, if there are questions for Dr. Oxnard from the audience, there is also for those of you who are standing in the back there are seats up in the front if you would like to come and sit down. When you ask a question if you could please introduce yourself and where you are from.

Dave: Hi Dave Eberhard genomic health in University of North Carolina, one aspect is in stage four progressed patients ...

Speaker 1: I think that we might want to be careful about thinking that we know what mutations are diagnostic when we're basing those on what's been found in primary untreated patients. What I also wanted to think about is in our experience with circulating tumor DNA in this patient population, particularly those who have progressed with non-small cell lung cancer after EGFR TKI therapy, we've been finding that we often see loss of the primary sensitivity mutations. I'm wondering, in your experience, is this something that you've been seeing? In addition to acquisition of something like T790M being a basis for resistance, do you also see loss of sensitivity mutations as a basis for resistance?

Geoff: It's a great question. It's a great question. The first thing I'll say is I don't think we should be doing discovery based on plasma yet, because the technology's still evolving. There are a lot of resistance biopsies that have been done. There are hundreds and hundreds of resistance biopsies that have been done after EGFR therapy, and I have never seen loss of the driver mutation. It's not lost. It is a truncal event. If you don't see it in the plasma, it is merely because your shed has decreased. The tumor is no longer shedding, and therefore, there's no tumor DNA to be detected. Rather, I use the driver mutation as the adequacy step. Is there driver in there? If there's driver, look deeper. In fact, you probably need at least 1% or higher of the driver to even look deeper for the subclonal resistance mutation, which is in fact inherently less than half or 10% of the total [leels 00:01:35].

The short answer is no, we don't see loss of that, and we need to be careful. We need to use the basic principles we know from tumor and use those to inform our plasma results.

I will also say that EGFR mutations are truncal and do not change over the course of a patient's metastatic disease. As they progress more and more and more, it doesn't go away, and it doesn't change to another genotype. That may be different in some other cancers, but the fact that we know that truth based on tumor, we can use that to interpret and develop and validate our plasma assays.

Speaker 3: Geoff, could I just, if you don't mind, question your approach? Now, I've read your article online where I study the PFS's of the two groups, plasma-positive/tissue-negative, plasma-negative/tissue-negative. I do not believe there is any difference in PFS between those two groups.

Geoff: Which two groups? I'm sorry, clarify which groups you mean?

Speaker 3: Plasma-positive/tissue-negative, plasma-negative/tissue-negative. Of course, it's inferior, as you have presented before, to tissue-positive/plasma-positive, which means there is a concern that really the clone is so small or irrelevant or, as I said, the drug's active, and ... Sorry, I'm caught. Robocall. I think it was Trump. I really think that this is a very dangerous area. I think this drug's active, more active when it's a T790 mutation, and less active when it isn't, and it's really still active against the majority of the tumor. If I was to treat this patient, I'd just give them the drug anyway. I wouldn't even bother doing the test because I don't think it distinguishes a patient that's going to do better.

Geoff: Have you treated these patients?

Speaker 3: What?

Geoff: Have you treated these patients?

Speaker 3: I know a number of people who have.

Geoff: Okay. I will say that-

Speaker 3: It's a better drug, and it's less toxicity, and of course-

Geoff: But there are definitely patients who just progress and die on this drug. We know that.

Speaker 3: Oh yeah, sure, sure.

Geoff: One of the problems is the drug is a T790M inhibitor. All these drugs are T790M inhibitors. Almost every trial and every data set everywhere is in patients who are T790M-positive. There's no hundred of patients who are T790M-negative who are showing up saying, "Please give me that drug that's not going to work for me." There was one that trial.

Speaker 3: Now, there's a report from Wash. U. at the ESMO Lung Cancer conference with 60 or 80 patients, treatment-naïve, and their PFS was longer than-

Geoff: Yeah. That's treatment-naïve, though. That's not resistant.

Speaker 3: Yes, but I'm just saying, didn't-

Geoff: That's very different. T-

Speaker 3: Bottom line is, the PFS in those two groups is no different.

Geoff: I think maybe we could do it.

Speaker 3: I do commend you on your front line test. I think your point about specificity-

Geoff: We should discuss later. I disagree with you.

Speaker 3: Well, I disagree with you.

Geoff: Okay, great.

Speaker 3: This is very important-

Geoff: Good, report that one.

Speaker 3: Because chemotherapy could be better in these patients. That's the question. Chemotherapy's selective in these patients.

Dr. Janne: All right. Maybe you guys can take the discussion during lunchtime. We're going to need to move on for the interests of time, so I wanted to introduce Dr. Lecia Seaquist, she's an associate professor of medicine at Harvard Medical School and at Mass General Hospital who's going to talk about looking toward the future, liquid biopsies for treating, monitoring, risk stratification, and early detection. Lecia?

Lecia: Thank you, [Pasi 00:05:12], and thanks to the organizers for inviting me. When the three of us were planning out this session and thinking about what we wanted to cover, we wanted to give a broad landscape of the different types of applications that liquid biopsies might have in lung cancer. I think Geoff got the straightforward situation where we have lots of data accumulated about drugs that are active in patients with specific tumor mutations and what we're looking at is can we use a less-invasive technology to substitute for that tumor biopsy.

What I'm going to talk about is certainly more circumspect and more theoretical, less data at this point in time, using liquid biopsies for some of these other applications that might happen in clinical lung cancer. Here's my conflicts of interest. Basically what I'm going to try and talk about in the next 20 minutes is where we are today with respect to being able to use liquid biopsies to meaningfully monitor response to treatment. This is for patients with advanced disease, what about risk stratifying patients of any stage of disease, and then can we incorporate liquid biopsies into early cancer detection. As I said, there's less data here and we're talking more about using our imagination and trying to envision what the future might hold. This is one of my patients who took this picture in New York at the John Lennon memorial, and I just love the picture.

When thinking about advanced lung cancer patients, traditionally we start a new systemic therapy and we wait eight or so weeks to determine the tumor response via a CT scan. Sometimes if patients have tumor-related symptoms, we have a sense earlier as to whether or not they're responding, but many times patients don't know, and we don't know, and it's an anxiety-creating situation. Could liquid biopsies be used to give us an earlier clue to response, and could they warn us about emerging resistance?

Well, I think the area where we have the most information about this so far is the specific area that Dr. Oxnard covered with EGFR mutations and looking at CT DNA serially to try and understand some of this. We do know there are many published

examples of individual patients or small series of patients who are following CT DNA levels, looking at EGFR, looking at the resistance mechanism T790M, can correlate with clinical outcomes and how patients perform.

These are a couple of patients that were published by my colleague at Mass General, Dr. [Piatroska 00:07:48], looking at a different T790M inhibitor, in this case rocletinib, and how patients did over time, monitoring both the tumor response, traditionally a radiographic tumor response, which is in blue, and then also following serial plasma levels of CT DNA. Here, the T790M, the resistance mutation is a solid line, and the founder mutation, the activating mutation, is the dotted line. This particular patient had an initial response, but when they started to progress, you see a divergence between their activating mutation, which is rising, and the T790M mutation stayed low. In fact, that correlated clinically with what we saw on a repeat biopsy, which was done here at the green arrow, and that patient had lost their T790M mutation. We could have predicted that from the plasma.

You can also see that the plasma started to go up before the CAT scan started to go up, and this is what gives us hope that you could maybe predict emerging resistance based on this type of serial monitoring. Here is just another case example where the patient had a response by plasma and radiographs, and when they started to progress, both the activating and the T790M resistance mutation were going up, and indeed, that correlated with what was seen on a tumor biopsy, the gold standard biopsy at that time.

Yes, serial CT DNA can correlate with clinical outcomes in individual patients. There's been some data published about larger groups of patients as well. This is a study out of the Italian group with Marchetti looking at the Cobas EGFR PCR test, and these are first line treated EGFR patients, and so basically looking at when you first start a TKI and you have baseline detectable CT DNA, how quickly does that signal drop out if you do serial measurement over the initial course of their treatment? They showed very nicely that among 14 patients who seemed to have a rapid decline in their CT DNA level that ultimately they had a very nice tumor shrinkage on a median of 59%. For this group, in contrast, there were six patients who had a slower response in the plasma or even a failure to respond in the plasma. When you looked radiographically, these patients only had an 18% tumor shrinkage radiographically. Again, we do see that this correlates with clinical outcomes.

Now, this is a randomized trial, the Fast Act II trial, in which many patients, some EGFR mutation positive, some not, were randomized between chemotherapy that was intercolated with an EGFR TKI versus placebo. It's a complicated study, but what I'm showing you here is just a subset of patients who did have an EGFR mutation and also had a detectable level of mutation in their baseline blood sample. Here, you can see the baseline distribution of EGFR quantity in the plasma for the group that was randomized to chemo plus placebo, and then here's the group that was randomized to chemo plus erlotinib, the EGFR inhibitor. You can see that over the course of three cycles of treatment, the chemo plus placebo arm has some decrease on average in the level of CT DNA and the chemo plus erlotinib group has a much greater decrease. The overall trial, by the way, ended up being positive for this arm of the trial, and there's a appreciation of a greater decline.

Then, when we focus on the group of patients treated with erlotinib who had some, after three cycles, when they looked at those who still had residual detectable positive plasma tests versus those who had become undetectable, this actually was a method of risk stratifying. The difference between the patients here and the patients here bore out in the trial with both improved progression through survival and improved overall survival for those who had cleared their plasma. You can get the sense that there could be utility here, but I think one of the key issues is what do we do with this information? What we don't have is clear evidence that early therapy switching based on this potential for emerging resistance gives us improved outcomes.

If you can imagine with this cartoon, that maybe there are three potential treatments that a lung cancer patient may receive over time, and traditionally, now, we use clinical assessments, so radiographic progression, to tell us when to switch from, say, the red therapy to the green to the yellow. If we had some kind of marker, a liquid biopsy assessment that gave us an early clue that the treatment wasn't working, that doesn't necessarily get you a longer outcome. It just helps you cycle through these therapies quicker. We really have to be careful about what we're using this test for.

Now, for those of you who are lung cancer specialists, you may wonder about all the randomized trials showing maintenance therapy has a benefit. That's really a slightly different scenario. Maintenance therapy was compared in randomized trials against a break from therapy, which used to be our standard. We're talking about some therapy versus no therapy, and the focus was really on those who weren't progressing rather than those that were progressing. I don't think that all the data that we've accumulated supporting maintenance therapy really fits into this issue of early switching for non-response.

The other point I'll make is that in EGFR disease specifically, what we do have clinical data for, although not extremely robust clinical data, is we have data for treatment beyond progression. When patients do progress on the EGFR TKIs, more often than not, they progress indolently, slowly, and we do have a lot of data that clinical benefit can be achieved by staying on the treatment even in the face of radiographic progression. We have a lot of room here that we still need to validate the clinical usefulness of these tests.

I did learn about this breast cancer study in the process of getting ready for this talk. This is exactly the type of question that we need to ask in this scenario in lung cancer. This is the SWOG 0500 study, which was based on the Cell Search CTC assay, which, as you may know, there's a cut-off for five CTCs as being a positive versus a negative test. There were a number of treatment-naïve metastatic breast cancer patients that were assayed, and I'll refer in the next slide or in a couple of slides that were negative at baseline. For this purpose, we're going to focus on those that were positive at baseline. Two hundred eighty eight patients had greater than five CTCs at baseline, and they were started on first line chemotherapy, any standard treatment at the discretion of their docs, and the CTCs were assayed again after one cycle. Basically, 123 of these patients were still positive.

Many of the patients converted to negative after just one cycle of chemo, but for those who remained positive, they were then randomized to continue with the same chemotherapy, or, based on the notion that maybe this was a sign they weren't going to do very well with this chemo, ultimately, they were switched to another chemotherapy at their doc's discretion. The primary outcome here was overall survival.

What did they find? Essentially, looking at the two randomized arms, there was no difference in overall survival or progression through survival by making this switch. Switching therapy early for failure to respond did not necessarily impact survival or outcome of the treatment. This failure to respond was, however, prognostic, as you might imagine. Arm A was the group of patients that I didn't put in the diagram that had no baseline elevated CTCs. They ended up having the best prognosis overall. Arm B was the patients who started with positive CTCs but turned negative after one cycle. Their prognosis was in the medium range. Then, the patients with the poorest prognosis were those who failed to clear their CTCs. However, switching therapy, as we know, didn't impact their prognosis.

CTC assessment of a failure to respond was prognostic in this large study, but intervention did not salvage patients, and I think that's the real message here. Risk stratification is not necessarily helpful for a patient unless you can act in a positive way to somehow modify that risk.

Is there a situation where monitoring makes sense today for clinical lung cancer patient care? There's really no evidence basis for doing this, but the wide availability of the tests, they're multiplying like rabbits, the type of tests that are available for us in the clinic, especially for EGFR detection, makes it very appealing for both patients and doctors to measure these levels. We should be cautious about using such monitoring to drive our treatment decisions until we really have data that suggests that this can improve outcome.

Now, switching to a totally different topic: Early stage lung cancer. For those of you who are not familiar with screening for lung cancer, I'll just give a brief background that lung cancer is the largest cause of cancer-related mortality in the world and in the US, more patients die from lung cancer every year than from breast and prostate and colon combined. Four hundred and thirty three Americans every day die from lung cancer. However, early stage lung cancer can be curable. Five-year survival can be up to 70% for stage 1A here in the blue line. However, most patients present with advanced disease because there are no pain receptors in the lung. Patients do not feel a tumor growing there until it spreads somewhere else and causes symptoms. This is a big problem.

There were many negative trials for lung cancer screening in the '80s and '90s, but now, four years ago, five years ago, the NLST, the National Lung Screening Trial, was published. This was a huge effort: 50,000 patients between 55 and 74 years of age. They were all importantly heavy smokers, either active or former recently quit within 15 years. The trial compared annual low-dose CT scans over a three-year period to annual chest x-rays, and the bottom line is that there was a 20% reduction in lung cancer specific mortality and a 7% reduction in all cause mortality, leading to low-dose CT scans now being recommended by the US Preventative Services Task Force for patients that fit

this eligibility criteria.

However, there are many issues that affect the implementation of such screening. Twenty-four percent of the participants in the NLST had a nodule detected; however, 96% of these turned out not to be cancer, upon further workup. These CAT scans detect a lot of stuff, and most of the stuff is not cancer. There are estimates of 8.6 million Americans that would qualify by that smoking criteria, and if you conservatively estimate that a low-dose CT scan is \$200, then this would cost \$1.72 billion per year for the first scan only, not including the subsequent workup of all these false positive nodules that are found. Cost has been a huge concern and a topic of policymakers. Other than costs, there's also concerns about overdiagnosis, medical complications of biopsies, radiation exposure from repeated CAT scans. There's also a large portion of advocates that are saying we're not even screening enough people. What about patients who have less than the required smoking history, because we know that lung cancer in never-smokers is on the rise?

All of this makes it a setting ripe for a great interest in some sort of companion diagnosis that could improve specificity, and liquid biopsies certainly would fit into this situation quite well.

What do we know about different research that's been done? I'll show you a couple of small studies, but again, there's no real definitive study that has been done in this space. This was a very interesting study from a French group looking at circulating tumor cells in early detection of lung cancer, and they used the ISIT filtration technique, which is a size-based filtration technique to look for CTCs. They studied 168 patients with COPD who were negative for significant lung nodules at baseline, and these patients also got annual screening CT scans. Five patients in their population had a baseline positive CTCs detected, and I found it amazing all five of these patients actually went on to develop lung cancer over the next couple of years, while none of the other patients without baseline-detectable CTCs developed lung cancer, with a median follow-up on those patients of [60 00:21:40] months. All five of the detected cases turned out to be Stage 1A, the earliest possible stage of lung cancer, and they all underwent surgical resection. I don't know if the results would turn out this clean and tidy in a larger patient population, but certainly promising early results from one group.

Now, we heard earlier about microRNAs and their promise. There were several studies, actually, that looked at microRNAs in early detection of lung cancer. Hennessey examined plasma for a variety of different microRNAs and basically came up with a signature that was used in a training set of 50 patients and then validated in a second set of 130 patients. The trouble with this paper is that they had patients who did not have lung cancer, but the lung cancer patients that they used had a variety of different stages. Although most of them were earlier stage, there were also patients with metastatic lung cancer. That part is a little bit unclear, but they came up with sensitivity of 100% and specificity of 84% in this recent paper.

Then, there's a group from Italy that's been doing a lot of work with microRNAs in conjunction with their various CT screening trials. They had two different randomized lung cancer screening trials, and amassed a cohort of over 800 patients that had blood

samples to look at microDNA. They came up with a complicated ... I know it's hard to read here, but a complicated signature using four different combinations of microRNAs, and then they had this stratified high, intermediate, and low-risk, what they called a signature classifier. It's a bit complicated, but ultimately what they showed ... I think this is what we're striving for in the field, is that when they did the CT scan screening alone in these patients, they had a false positive rate of 19%. This is similar to what was found in the lung cancer screening trial, the NLST, remember, that there was about 25% of patients had an abnormality, but 96% of those abnormalities were not cancer. Similar false positive rate. Combined with their liquid biopsy signature classifier, their false positive rate fell to 4%. This is exactly the type of thing that we would be looking for liquid biopsies to achieve, and this group is doing more prospective work with their signature.

I think we're just on the cusp of really what will be able to be achieved in lung cancer screening and using liquid biopsies as an adjunctive type of test. We are, as this conference is evidence, we're experiencing a technology explosion with all sorts of different platforms for liquid biopsies right now, and we happen to be right in the same time period where we're broadly implementing lung cancer screening clinically in this country. The situation is just right to look at these different liquid biopsies and how they can potentially help us.

I'll just tell you in the last couple of minutes what we're doing at Mass General right now. We've been doing a lot of work with the iChip for CTCs. This is a technology developed by Daniel Haber and Mehmet Toner, and the third version of the chip, called the iChip, is really quite elegant, where you take blood from a patient and mix it with magnetic beads with antibodies against white blood cells. Then the sample goes through a size-based separation microfluidic exchanger, where the RBCs, platelets, and other blood components are funneled out and what you're left with is the larger nucleated cells. Then, there's a microfluidics inertial focusing section of the chip, which basically gets all the cells to line up in a single file where they can then go through a magnetic deflector and the white blood cells that are tagged will be drawn off. What you're left with is non-biased selection of CTCs. All of this now is on this neat little plastic thing that looks like a DVD. We're looking at that for its potential utility in early-stage lung cancer diagnosis.

[Sosha 00:26:02] Petroschka and Mike Lanuti at MGH are also collaborating with Gardens and the Lunar study looking at resectable nodules and using a CT DNA technology to assess pre-op, inter-op, and post-op levels of CT DNA to see how that can help us. There are many, many hundreds of efforts going on around the world looking at liquid biopsies and early stage, so as I said, I think we'll have much more data next time this conference is held.

In summary, most of our data to date is about disease monitoring in advanced patients, particularly using CT DNA platforms to detect specific mutations like EGFR. Though appealing, disease monitoring and risk stratification based on these types of tests are tricky without evidence of what you do with positive results, and that early switching can improve outcomes. There are many platforms, as we heard about this morning and will continue to hear about that could be used in other applications. I think a real big

area in lung cancer is in conjunction with screening to improve the specificity of the low-dose CT scans. Many studies are ongoing in this arena.

Thank you for your attention, and I'll take any questions.

Dr. Janne: Okay, if there's a quick question for Dr. Seaquist, and in the meantime, maybe our panelists could come up to the podium as Lecia's answering the questions.

Lynn: Hi. My name is Lynn [Saborra 00:27:41], I'm from the NCI and the Early Detection Research Network. I see that you have a decrease in your false ... Or, the study had a decrease in the false positive rate for low-density CT scans when it was compared to the liquid biopsy. How does that compare to a bronchoscopy? Is it better than bronchoscopy? In other words, if you compared the low-density CT and bronchoscopy to reduce the false positive rate, is the liquid biopsy better than that? Or, has anybody done that?

Lecia: Yeah. I think if I understand your question, there is a large field of research looking at different bronchoscopy-derived markers and how that could potentially help us improve our ability to detect cancer, whether it's looking at genomic signatures from the shed cells or a variety of other markers. There's volatile exhaled gas tests as well. There's lots of different platforms that could potentially improve the specificity of CT scans. Since today's seminar was about liquid biopsies, I really only focused on the liquid biopsies. I'm not aware of any particular group that has compared bronchoscopy-derived methods to liquid biopsy-derived methods, but I think you're right. There's many different ways to skin this.

Lynn: Well, I was just asking because obviously a patient would rather take a blood test than a bronchoscopy.

Lecia: Absolutely!

Lynn: So would the clinician, I'm sure.

Lecia: So would the payer!

Dr. Janne: Howard?

Howard: I think the SWOG trial raises several issues. The first relates to the trial design, and also, I think, reflects that we're so anxious to do things with a liquid biopsy. We're jumping in with both feet in the absence of data. The fundamental flaw in that trial, much as I hate to say it, is they never show that the regimen that they were switching to actually had any degree of efficacy. You don't have to be a rocket scientist to figure out if you get two things that don't work, they're going to equally not work.

The second thing is, we're seeing the same pattern of emerging resistance, as I showed earlier, with the rising PSA trial. When we treat a patient with hormones, and in fact, we're giving maintenance therapy, because we don't stop them. The idea of switching is one question, but one can also think of add-on. In particular, if you start to look at the

[heterogeneity 00:30:15] of the cell populations, and if you look at some of the early work I've seen in circulating tumor DNA and CTCs, you will eliminate subsets of cells. Then, if you can characterize either what's resistant or what de novo or what has acquired resistance, you can do more educated either add-ons or changes. The trial designs are going to be very, very different than the all-or-none margin of straight line. I think more planning has to go into these as we do them going forward.

Lecia: I agree. Very nice comment.

Dr. Janne: Thank you. We'll go on to our panel discussion. Two of our panelists have a few quick slides. David, do you want to start? Maybe you guys can give the slides and then we'll do questions after both of you have given the slides to the entire panel. You can come up here.

David: Okay, yeah. Thank you very, very much for inviting us to talk about some of the things that I think are quite exciting that we're doing at GenenTech. I wanted to take this opportunity to announce the start of a trial which we call the Blood Fast Assay Screening Trial. It's basically an umbrella study where we're using a next-gen sequencing platform to identify patients for each of the intervention arms.

The study has been in the works for quite a while. The idea is that the initial study will include intervention arms for EGFR, ALK, and RET. We're looking at multiple different options for the EGFR component here. The basic idea is that we would screen patients using the foundation medicine effect assay platform. First line non-squamous or squamous non-small cell lung cancer, patients with PS0 to 2. We're collecting quite a bit of plasma in the study at multiple different time points to look at some of the questions that Lecia brought up. The intent also is to look at, in future cohorts, to look at primarily rare mutations, of which many of you know, there are many small segments in lung cancer. It's very difficult to run trials in those populations. We're also looking at combinations: for example, [ALK/MEC 00:32:41], [BRefMec 00:32:44], and antiPDL-1.

Some of the elements of this that I think are on the cusp or on the edge of drug development are really looking at end points in areas where, for example, you have very high response rates. How do we develop drugs or combinations of drugs that are comparing to a standard of care where you're seeing 80%, 90% response rates? Obviously, patients need to do better, but perhaps in corporation of duration of response or time in response may be considered approvable end points in the future.

Again, I think the other element of this that we're interested in talking about ... We are interested in working with other parties, including industry, academia, and government to incorporate new arms into the study based on essentially anything druggable. Where there's an active drug, we would be interested in potentially talking to whoever has those to incorporate into the study, either as to identify patients for further activity or for potentially approval in the future.

Thank you.

Ken: Okay. Thanks for the organizers for inviting me. Just a few quick slides. We talked a lot

about using hot-spotting assays to detect yes or no mutations and specific key genes for patient selection, but maybe we can talk a bit going beyond that about using actually next-gen sequencing to detect not only multiple genes, but some real specific details on specific genes and specific mutations that might help guide patient treatment. This is in the context, again, EGFR mutant lung cancer, and again, in the context of third-generation inhibitors, all of which now that have progressed to clinical development have been shown. We see T790M after first generation TKIs. We're starting to see the 797S mediated acquired resistance and response to third-generation TKIs. Again, this has been seen now for all third-generation TKIs that are sufficiently advanced in clinical development.

This particular mutation, 797, is very, very much predictable. This is the site where EGFR, where the compounds, the covalent compounds, bind onto the EGFR protein, and it is imminently predictable. Certainly we're seeing that in clinical studies now. This snapshot is from three different papers, actually, that reveals some of the complexity of the 797 mutation that is revealed by next-gen sequencing of plasma DNA. The reason why this is important, because again, we think that understanding the complexity of these mutations is key to potentially future patient care. These next-gen sequencing reads, as you can see, because the T790M mutation and the 797S mutation are so close, the sequencing, next-gen sequencing reads cross both sites. You can see in both of these cases ... In several of these cases, we see that this mutation, the 797 mutation that's seen here, can be quite complex. You can see multiple nucleotides can result in this particular change also, in this particular case and in this particular paper, from multiple different inhibitors.

What you also see, of course, is that in every case presented on this slide, you see T790M and the 797S resistance mutation occurring on the same allele, or in cysts. This is very important, potentially for the following reason. Geoff Engleman's group has presented in vitro work that suggests that first-generation inhibitors, which are not these irreversible binders like third-generations are, can actually have activity against the C797S mutation. They showed this in in vitro work, but showed very clearly that this is only true, actually, these first generation inhibitors like [Aresso 00:36:45] and [Garciva 00:36:46] and [Ephatnim 00:36:47] only work in the context where T790M is not there, of course, or when the 797S and the T790M mutation are on different alleles.

These triple mutants that occur with the two mutations on the same allele actually are insensitive to any current TKI combo, whereas when they're on different alleles, you might be able to use first-generation inhibitors to treat subsequent resistance to third-generation inhibitors. What's important to note is that while I've just showed you that many of these cases, these occur on the same allele, which would make them insensitive to any context of EGFR TKI as presently. Sorry.

There is a particular case, one case example that's been published in the literature to date, and all the end numbers need to increase here. We do see one particular case that's been published where we see the T790M allele, T790M mutation and the 797S on different alleles. Based on the in vitro work that's been done, you might suggest that such a patient could now be sensitive and could respond back to a first-generation inhibitor. I present this particular example not because it's definitive, certainly, the

numbers need to increase, but the allelic context of these particular mutations revealed only by next-gen sequencing and not necessarily by these yes or no hot-spotting assays, may actually prove to be critical going forward to identify appropriate therapies for patients once a fourth-generation inhibitor or other inhibitors become available.

I just referred to a recent paper published by [Pasi's 00:38:19] group showing that such mutations, at least the theory of such inhibitors, has already been put forward, and so understanding the allelic context of the mutations, specific mutations using that next-gen sequencing, may in fact turn out to be important. Thank you.

Dr. Janne: Okay. Thank you, Ken. Okay, we'll open the questions for any of our panel members, either from the audience or from the internet. Maybe I'll start and I'll ask Victoria a question. Geoff showed a trial design where you're thinking about using a next-generation EGFR inhibitor in a trial selecting patients purely on a plasma-based T790M status. We've heard different aspects about different assays today. How do you and how does your company ... How do you go about choosing which assay do you pick? What kinds of things are you looking for, characteristics? What are important pieces there when you decide that you're going to work with a diagnostic company and pick an assay for a clinical trial development?

Victoria: I guess when we design a clinical trial, we always think about the pragmatism of the solution that we're taking, so it has to be something that has already shown that it works. Maybe if this is the pivotal trial with some regulator interruptions later on, you would want to be on the safer side and go with a partner which has already got some interest in this area as well, and you might go as a companion diagnostic partner as well. You don't want maybe to go with a smaller company who don't even know how they're going to register their test. They'd rather go take a safer bet.

Also, something more reproducible that can be extended to other countries, because the trial that Dr. Oxnard showed is actually going to take place in the US. In the first place, however, if you can't recruit the patients for various reasons of competitive situation, you might want to branch out to other countries. Then, again, your partner needs to be prepared to branch out as well. I guess all these considerations. Plus, of course, cost, and plus, the multiple choices or multiple decisions that your investigators later might need to take.

The test that we are choosing for this one is actually [Roshkobas 00:40:31]. It's a yes/no EGFR T790M positive or not. We thought about NGS, but with the panel that NGS can generate, you might only confuse your investigators, potentially. As I said, it goes back to pragmatism.

Dr. Janne: Great. Important practical considerations. Question?

Peter: Peter Everhard, genomic health, University of North Carolina. Clearly, the detection of emerging resistance mutations linked to alternative therapeutic decisions is a huge advantage with circulating tumor DNA analysis. In general, for disease monitoring or early progression of treatment detection, what's the difference between circulating tumor DNA, where we are now, and PSA or CA-125, and we're going down the route to

clinical utility and establishing the evidence for that. Is this the same old thing over again with something new, or what are the differences here? I'd just love to hear your thoughts.

Geoff: One potential, if I may, is that it's more disease-specific, potentially, right? When you find the known [KRS 00:41:40] mutation emerging back, it's not because the patient had a bout of colitis or whatever. It's not the non-specificity of some CR markers, so it is disease-specific. When you see your KRS levels go down with your emerging phase one drug, we have some data on how we've used this on dose finding on phase one trials, there's a compelling sense that you have an on-target effect of your key drive mutation. I think when you're tracking drive mutations go up and down, you can feel that that is really biologically relevant to that patient's case.

I don't know if that's necessarily cost effective, right? Your CEA costs, I don't know, a couple bucks? It gets reimbursed for 30 bucks, maybe, right? Most of these assays are a hundred times as expensive as that. You need to be sensible that no one's ever going to be able to show CEA really changes care. You need to be a lot better and as cheap. It is a dilemma, I hear you, but the potential is that it's more biologically specific about that patient's cancer.

Dr. Janne: Anyone else on the panel want to comment on that? David?

David: Yeah, just to add to that. Yeah, I think it's key that for many of these things, it's actually the target of the drug. It's not just disease-specific, it's actually what you're trying to hit.

Victoria: Maybe I would like to add here as well. When we're doing the clinical trials now, there is always this gold standard of tissue biopsy in between anyway, so you do a plasma test, but you still have to validate through tissue. The vision would be for the convenience of the patients, physicians, payers, everybody, is to skip the tissue biopsy step and do PSA equals prostate cancer almost always. If not, then there could be some additional workup, but it doesn't have to be applied to all the patients. That would be the vision, ideally, to develop it this way.

Dr. Janne: Okay. Question?

Speaker 1: Victoria, in this trial of 80 patients with plasma positiveness of which you'll never know how many are tissue positive, but the majority still will be tissue-positive, so the result will be weighted by tissue-positive tests. What's your end point? What are you trying to achieve with this trial?

Victoria: We're looking at the objective response rate. What we are trying to demonstrate is that there is this utility of the test. We're doing pivotal development in parallel with the use of tissue biopsy and validating the test, etc. This is more of a clinical, practical solution for physicians to inform that actually if you have a blood test only that could give you almost similar if not the same result-

Speaker 1: But it will be the fact that their test is about 70 ... What's your test specificity, Geoff?

Victoria: I think-

Speaker 1: It's going to be way-

Geoff: Depending on what your reference standard is, if the reference standard is accurate. Some of the tests have 80% sensitivity and 70% specificity.

Speaker 1: No, no, no, excuse me, [inaudible 00:44:25].

Geoff: Yeah.

Speaker 1: The frontline tests are fantastic.

Geoff: Yeah.

Speaker 1: They've got fantastic specificity.

Geoff: Seventy percent specificity, depending upon the data.

Speaker 1: Yeah, which means 70% will be tissue anyway, so unless you have hundreds of patients, we've got a good number of plasma-positive, tissue-negative, I don't see what conclusions you can come to. Have you got something more sophisticated to try and determine whether this clone is significant, like some allelic ratio measurement to call [inaudible 00:45:00] positive?

Geoff: There are a lot of assays that are investigators studying that question. I think that's a very interesting question. I think the problem is, for assay development, the first step is getting an assay that can actually say positive or negative in some rigorous way. There's one test that managed to pass regulatory approval based on drivers and not [inaudible 00:45:17], but then it's a whole separate clinical development of you to say, "I now can quantify accurately. Quantify accurately against what? What's your reference standard for quantifying CF DNA?" Now you want to create a ratio. What's your reference standard for calculating that ratio? It gets very hard to be validated.

Speaker 1: Well, you know, in this particular clinical space, Geoff, it would be to the founder mutation.

Geoff: I agree with the calculation, it's just the validation that's challenging. I agree with that, those are all great questions that we're studying, but it is a long road to get there.

Speaker 1: Yeah. That's what I said, I'd just like to point out again that truly, in your paper, I think it clearly shows that chemotherapy could be superior in this subgroup of plasma-positive/tissue-negative, and we have to be very careful to try and sell this drug with a plasmar assay when it's clearly an active drug and will be in the first space eventually.

Geoff: There were 18 patients in that cohort, so that, I agree, it's hypothesis-generating and it needs more study.

Dr. Janne: Yeah. Howard?

Howard: First of all, I want to commend Geoff for being practical and realistic and living in the real world. As a clinician, you're trying to get information. If you have nothing and you get something, even if it's not perfect, it's better than zero. This is not a competition between assays. It's a question of in what patient context can you get the information that you need in real time to make a decision. If you can get it with a mutation-based cell-free DNA in three days, that's outstanding.

We have the same issue out waiting for the biopsy results. One of the major problems we have is trying to get outside tissue in. We haven't really discussed the logistics of acquiring specimens. They don't just show up in the laboratory. We have a dedicated group of eight people. When a patient's going for a biopsy, they go to the interventional radiology suite, they hold that patient's hand, they watch the specimen, and they transport it directly to a GU pathologist who handles it. That costs money. If you want to get a specimen from an outside laboratory, even if it's a young man, high-grade tumor, you have to start dealing with the pathology department. They want the informed consent, and they want a check right behind it. It's not a priority for them.

One of the biggest backlogs we have is just getting the tissue in-house to actually analyze. It's wonderful to try to do these studies, but if you start adding up all the costs, it's not just the data at the end to do your analysis. It's all the front-end planning. I think more care has to go into that aspect of it, and really make sure that your assay is going to be with you for the whole ride.

I look at this as some ways, it's like getting married. It's very easy to see 4000 new ... I get, once a week, "What's new in circulating tumor cells?" There's a lot. Twenty assays a week, and every one looks better than the next. When you commit to that path, it's like you've formulated your drug and you've got to be ready to go all the way, in steps, and say, "We've done the phase one, it's worth doing in phase two," and then getting a real, large-scale, appropriately-powered trial to answer the question. It has to be a collaboration with the sponsor.

Your point was excellent. The worst thing that can happen is when your sponsor runs out of money, or you can't do it even when you have strong phase two data. This requires long range planning. It's not just wake up one day and say, "let's try this."

Geoff: You describe complexities at [inaudible 00:48:45]. Imagine trying to take genomics out into the world. Everybody's doing it, right? No! No! People aren't doing it. Patients aren't getting targeted therapy just because they're like, "Who do I get to do the stupid biopsy? Who am I supposed to call? The radiologist two hours away?" The patient's not going to show up for that. We're trying to make this happen for everyone, and maybe liquid biopsies, if done well, can help us get there.

Howard: Right. But also, it requires very close collaboration between the clinicians and the pathologists and the radiologists and the nuclear medicine person and the interventionists. We actually do this in a conference to pick the site.

Geoff: I know my favorite pathologist. I mean, I've got her on speed dial.

Howard: Right. I'm lucky I have more than one.

Geoff: The local community doc doesn't talk to the pathologist, do they? I don't know. Maybe.

Howard: No, but seriously. It takes a while for the interventional radi- ... We used to sit in clinic and say, "Okay, here's a bone scan. Stick a needle in a spot." Half of those are burnt out lesions that are essentially all bone. Then we say, look at PET scans, and you still can miss certain areas even when you see the needle right in it, so one of our dedicated radiologists actually developed a classification scheme of the changes on CT scan, and we know which part of the tumor will actually give us a yield. It's not random, send the person to an interventional radiologist who may be excellent, but they haven't had the same training to pick out that site. It really takes a lot of focus, collaborative effort, to do this right, and even then, we're still only 50% positive in bone.

Dr. Janne: Excellent points. Just like we have team science, we need to have team medicine, and I think as we are doing more and more genotyping, be it from tumor, CF DNA, I agree with Dr. [Schirr 00:50:19] that the collaborations are critical.

Speaker 12: [Prahad Shah 00:50:22], FDA. I heard a lot about tissue biopsy. Can you explain to me what exactly you mean by that in the sense ... are you talking about resection specimen? Are you talking about core needle biopsy? Are you talking about cell block from cytology specimen? If you're talking about core needle, how many cores? I mean, I'm a pathologist. I know how many cores I need, given what is happening in not-small cell. I need at least six. But in your institution, when you're talking about core, how many cores? Does it has any background on the test?

Lecia: These are great questions with no specific answer, and I think from a practical point of view, which tends to be where we keep going back to, in treating patients who are going on trials and who are going on experimental drugs, what we get is often dictated by the confines of the trial. I think in lung cancer and in the EGFR space a few years ago, all of the trials were requiring core biopsies because of this notion that potentially they are better. I think at least I can speak to my own experiences that core biopsies often are not better in lung cancer, and often FNAs and cell blocks actually have more genomic material that you can extract.

I think on a practical level, there's not a big difference between these different types of biopsies. All you need is that somatic DNA. Whether you get it from a core or whether you get it from a thoracentesis cell block, it doesn't really matter. Tissue is tissue, in my opinion.

Geoff: We have cytologists and surgical pathologists, which are the two branches of pathology, now competing to find out who can be more useful, right? The cytologists were feeling left out, so they've developed this rapid trick where they can get you the DNA before the surgical pathologists even sign out the specimen. Right? It's like, how can we? Because in the end, the patient's got the tissue, right? If I get plural fluid, I'm using the plural fluid. If all I can get is ... I've done an FNA of a skin metastasis, and sent the DNA

from the fluid straight to the molecular lab, who took it and looked at it. We'll just do whatever I can do to get a result for the patient.

Unfortunately, I know from a regulatory standpoint it's a lot more concrete than that. In the real world, anything we can get our hands on, understanding that there are adequacy requirements and you have to be cautious of false negatives, right? Bone biopsies can be falsely negative because the DNA gets degraded, etc. you need to be careful with that stuff. For the patient who's got progressive cancer, you've just got to go for it and do your best.

David: Just to add a comment there, I think along those lines, I think historically, certainly in lung cancer trials at Genentech, we've been pretty particular about the types of samples that we allow. I think recently and certainly in a number of the pivotal studies that we have ongoing, we've increased the flexibility to include cytology samples, FNAs, to gain experience and to see how well they actually correlate with blood or other things that we're doing.

Victoria: [crosstalk 00:53:34], from the clinical development trial protocol, typically we request about ten slides per patient, just because there are some eventualities. The derma tissue might not be there on one slide, but will be on another. Also, you might need to retest because the test result in the first round will be undeterminate. That's why ten is a magic number, but we don't always get that.

Dr. Janne: David, you showed a diagram where the schema for that study ... Tell us a little bit about it. Are you using CF DNA analysis only in those patients, or are those patients also getting a tumor biopsy at some patient, or it's optional, depending on the patient?

David: For that study, the enrollment will be based on the plasma result. Tissue would be optional.

Dr. Janne: Not required.

David: Not required. I think we're going after a patient segment which I think has been left out of what's currently happening in terms of the drugs are approved for tumor with a positive mutation. We're trying to go beyond that.

Dr. Janne: Again, as you think about the diagnostic aspect, what kind of turn-around time do you anticipate in that trial? What do you think is acceptable from a clinical standpoint?

David: We're working with our friends in Cambridge at Foundation Medicine on that, and obviously, we would like that turn-around time to be less than a week. I think in reality, it's going to be between ten and 14 days, which is within the period of routine screening.

Dr. Janne: This trial will be US, or around the world?

David: It'll be a global study.

Dr. Janne: Do considerations, and maybe all of you guys can comment on this, and Victoria mentioned that a little bit ... Are considerations different in those designs around the world, or acceptability, do you think, of CF DNA-based studies, same, different?

David: I think from the perspective of actually enrolling patients based on a plasma result, there seems to be ... I'm not going to say unanimous. It's not unanimous, but certainly, there are a number of ... A large majority of lung docs are okay with doing, provided there is some history of an actual lung cancer diagnosis, primarily for the reasons ... There are mutations that are across different tumor types, so you definitely want to know that it is lung cancer. Yeah. There are different regulatory requirements in the EU versus the US, but this is a trial that we're trying to use as a platform to have those discussions.

For rare alterations like RET or BRAF, it's just not possible to run a 500-patient randomized study. That's, I think, where the question around what is an improvable endpoint for even the initial standard of care in a particular targeted segment, but also, then, how do we follow that up when you have an 80% response rate? You can't use an overall response rate anymore. What are the endpoints that we could use? Maybe duration of response or something like that.

Dr. Janner: Question?

Speaker 13: The new guideline has come up. It's still in the common period from the [inaudible 00:56:46]. They are testing EGFR on everything: small cell, squamous cell, non-small cell. What's your opinion about that?

Lecia: Well, I think there's two things. I mean, we do see sometimes rare patients who have squamous histology or even the small-cell histology that has an EGFR mutation. Certainly it's rare, but there can be mistakes in the histology, I think, more commonly that you can have a misread of the histology, or you could have a mixed tumor where you only happen to be sampling one portion. Certainly in small biopsies, that can be an issue, as you well know. I can say clinically, in my institution, we've been testing all non-small cell lung cancers for EGFR mutations for eight or nine years, and we certainly do find some in [non-adno 00:57:49] cases.

Geoff: It should be worth saying that there are ... That tumor isn't always truth, even for drivers, and that in one validation where the tumor gene typing is done at an academic center who never gets it wrong, we can find 100% positive [inaudible 00:58:05] value. Out there in the world, first local assays can miss variance, routinely can just test a poor specimen. It appeared EGFR-negative, but that's because the DNA was inadequate or the assay was inadequate, and then it's positive in plasma. "Is this a false positive? What is truth?"

Then, there's the problem of local assays being falsely positive. I'm definitely seeing lots of false positive [ALK fish 00:58:31]. As you take an assay that, when done in a central lab with the right people was very reliable, you start getting it everywhere, and everyone's finding these borderline results that they're calling positive. Truth gets really messy. That's, in some ways, I guess, great if you're developing a liquid biopsy. You can

say, "Ha-ha! We never have false positives. Just blame it on the tumor being wrong."

I'm not sure that's true, but I do think if you're developing a validation strategy, you need to be very cautious about who's doing your truth and who is your reference standard. Because if it's out there in the world, if you take an assay, a liquid biopsy, and develop it in places where you can't trust the tumor genotyping, suddenly you have 50% sensitivity and 50% specificity, and no one knows what's right, and your assay looks terrible. These problems about how reliable tumor is and in what circumstances are very real if you're trying to develop the competitor and do it in a way that's reliable.

Lecia: I think the other issue is that we're moving away in lung cancer from this one-off testing of different mutations. We've been talking a lot about EGFR today, but there are ten other mutations that are important to test for in lung cancer. Some of them are more common in squamous or are relatively equivalent. Met-skipping mutations are found in both adenocarcinoma and squamous. Rather than trying to make a complex algorithm of who you test for what, testing everybody for everything becomes, in some ways, more cost-effective and efficient.

Geoff: That being said, a 1% false positive rate is fine in a population that's really enriched for a variant, but a big problem if you're looking for 1% prevalence [ross 01:00:06] rearrangements, especially in smokers and small cell. You do need to be cautious, because a little bit of false positive can go a long way in the wrong direction if you test all the wrong people, perhaps.

Speaker 13: Hearing you correct that for validation or a section specimen of that tied tumor is going to be the truth?

Geoff: No, that's not what I said. I just think you need to be cautious and intelligent. We did a successful validation where we just made sure that we had our pathologists on board doing the tumor genotyping and it worked out with variable specimens, but if you allowed it to be any pathologist anywhere with any assay, you just have to consider that there are going to be a lot of variables and you won't be able to reach 100% positive [inaudible 01:00:45] value.

Speaker 14: Geoff, I had a question. You showed that slide with the report. It read like a radiology report, like "cannot rule out clinical correlations suggested." I just wanted to, from a real world ... You're obviously very dialed in to molecular data, so you can interpret these tests, but if you're out in the community, how challenging is it if you're getting all these different tests from all these different vendors? Does there need to be some sort of discussion on how best to caveat it and read out some of these analyses?

Geoff: A lot of the assays that are commercially available have done no clinical validation, have only done technical validation on constructed specimens, and have never validated against any clinical truth. Most of the results you can get from clinical tests probably shouldn't be given to doctors, and that's my feedback to the FDA. I think, but, in the end, it's happening. What I have to do is, I get an 80-year-old sarcomatoid carcinoma with a "ross rearrangement," comes to me, and they progressed on crizotinib, so they're coming for a ross resistance trial. I was like, "Oh my gosh, guys. This isn't a ross

arranged patient, it's just an error." That's not the way most docs think. They just take it as truth. We have to recognize that the results you're sending out ... Don't be the next [Theranostics 01:02:16]. The results you're giving out, docs believe them. The docs, I don't think we can give the docs skepticism. Otherwise, it just gets really, really messy. They're going to trust the results, and it's dangerous, and we need to keep doing what we're doing to work on FDA-approved assays and making them used.

Speaker 14: Okay.

Howard: I will say that many institutions, ours as well, we have these molecular tumor boards where we look at NGS data from tumors but increasingly from plasma because we need to help docs interpret that, not just that there may be an EGFR mutation, but what do you do with the litany of other alterations, and do you choose a therapy for a patient based on these alterations? I think there is a large educational component as we start to interpret NGS data, be it from tumor or from CF DNA in the clinic.

Dr. Janne: Ken, did you want to make a comment?

Ken: Yeah, just another comment. Ever since osimertinib's been approved in the US, on a weekly basis I get these comments from colleagues of mine that are in the field. I get these panicked calls saying the rate of T790M mutations is much lower than what is out there in the literature of 50% or 60% after first sign TKI. When you dig into this, it's because these doctors from around the country now are getting tests from all kinds of different commercial sources, and not only the analytical maybe hasn't been done properly, but the interpretation of the results.

In a lot of these cases, the commercial suppliers are just supplying the data back without any sort of interpretation, in the sense that it turns out that a lot of these patients weren't actually ... doctors are just asking for this test before first line treatment, so the T790 is close to nothing. Or, they get a result where there's no CT DNA in the sample at all, and they're reporting that as negative and not interpreting that, when in fact, that patient is a non-shedder and that sort of interpretation needs to be coupled into the results. It is an issue, not only from an analytical step, but interpretation of even a valid analytically correct result needs to be interpreted properly.

Speaker 1: Could I just follow up on what you've said? That you know, I've seen several results of a T790-positive without the founding mutation being there in a heavy smoker, so it's clearly false positives, and that's not uncommon. You probably can comment on [crosstalk 01:04:37] Geoff.

Geoff: Yeah, and they're giving those patients osimertinib. Makes no sense.

Speaker 1: Then the company says, "Oh, they're on an EGF TKI. That's why you can't see the founding."

Speaker 15: [Lada Luthra 01:04:46] from MD Anderson Cancer Center. Very interesting debate, and I think everyone of you touched one key point in this implementation of liquid biopsy in patient management. One question I have is, when do you, even if it's analytically

validated through two different platforms and everything, if I say that my sensitivity of the assay is 0.1, would you consider that as a positive and change your treatment? When do you change your treatment?

Geoff: Lecia mentioned this really nicely. She was like, "Don't just be sending the tests, letting the tests guide your care," right? I only send it when there is evidence of clinical resistance. If the patient has progression on scan or progression of symptoms, if I have suspicion, start looking. If you do it before there's suspicion, yes, then you start getting distracted by the potential for false positives. Yeah, if I trust the assay and they've done a rigorous validation and it's really positive, then preferably with an FDA-approved assay, then that should count as my positive and I can shift. I don't think the level ...

What I was just telling you, that case I shed was 16 droplets, but was below the 1% threshold. Everyone sets their threshold different ways. I just want to believe they've done it well.

Speaker 15: I understand that, but also if you have two, three different platforms which you come from. Another question is, when you don't have a biopsy available, like you were saying, and you depend on liquid biopsy, in that scenario, what would you do? I know it's not that easy to answer.

Geoff: I think the message to the world should be take the results you're getting with a grain of salt.

Speaker 15: Correlate with them.

Geoff: Look at numbers, perhaps. That's why I think quantitative assays are potentially very valuable, because they can potentially have a gray zone where you can say, "I'm not sure," and then a truth zone. Everyone's setting ... Gray zones aren't done well in the community, either, because docs don't know how to interpret them. I don't have an easy answer.

David: Maybe I could just add quickly to that answer. I think while these are very interesting and cool technologies, I think the key point is that decisionmaking needs to be driven by clinical outcomes, not so much whether you can detect something in the blood, right? We have to run, ideally, fairly large studies to actually figure out which sequence is better, or do you switch early. I think in some cases, it should be defined by overall survival, not continuing therapy with the same targeted therapy or slightly different versions of this.

Geoff: Right.

David: While that would be hard, I think in order to really use these things appropriately, it would have to be defined clinically.

Speaker 15: Yeah, I think the clinical validation is ... Yeah.

Victoria: I just wanted to add in this context and the previous question, you know with the

abundance of the PDL-1 positivity and different tests, there is now an ongoing effort, which is called Blueprint, where the pharma companies who own this PDL-1 inhibitors and also the test companies and also FDA, they're trying to define what is positive, what is not positive to help the clinicians out there to use the drugs. Maybe with the amount of platforms that are being developed for EGFR ALK, etc., something similar should happen and somebody needs to be in the lead to unite the people to define the thresholds and acceptability of different tests for patient decisionmaking.

Geoff: Right. You could have some constructed plasma specimens that you then send out to the various labs in a blinded bakeoff and say, "Send me results pass or fail," and they may get it, they don't. One of the problems is the plasma is so scant, right? It's not like a research where you have tumors sitting in the fridge and you can just cut out slides and send them around. Any given real in vivo plasma specimen is extremely tiny and you can get a couple tubes for a given patient. You can't send it around to 20 labs.

Doing such a bakeoff in such scant specimens is hard. It would probably need constructed specimens, but yeah, get these companies to say, "Here's my result, am I right or wrong," and then start giving out grades.

Speaker 15: Yeah. I think the main take-home lesson is we have to do large-scale studies combining companies and academic institutions. I think that's a major point.

Dr. Janne: We do have a session this afternoon on liquid biopsy test development, and I think also some of these point should be brought up there as well as to what should be our common standards or principles around a particular test, what kind of validation, analytical, clinical, clinical utility would we like to see because of the wide spectrum of different assays that are out there. I think we can continue that discussion.

Let me ask Ken a question. Ken, you made a case why NGS may be useful above and beyond a Singleton assay. Most of the things, of course, what we've been talking about are mutations. NGS can also, of course, detect copy number alterations, and these two may have clinical meaningfulness in terms of resistance or targeted therapies. Do you think we're there yet in CF DNA copy number analysis, or not?

Ken: No, I don't. That's the short answer.

Dr. Janne: Okay, that was quick.

Ken: I think that determining amplifications, the BGFR, MET, whatever else, from an NGS approach is a tricky business, and even those groups that have had many years experience in doing this have disparate results, actually. There's been some evidence recently ... Some presentations and publications have been published recently that are quite different for certain resistance amplifications than has been previously published. I think we're not there yet, but I think it's an extremely important piece, of course. MET amplifications, EGSR amplifications, are very important obviously resistance mechanisms to first-generation, but clearly to third-generation inhibitors as well. The short answer to me is not yet.

Speaker 16: Chris Raymond, Resolution Bioscience. What do you need to see to be convinced? What sort of data gets you there, where you do believe copy number variation assays out of blood?

Ken: Yeah. I think that ... We talk about gold standards and what is truth. I think for amplification events, you need to compare it with whatever the gold standard is, whether it's [fish profiline 01:11:05] ... I can see that tissue to CT DNA and establish those threshold cutoffs. A lot of the thresholds that have been established for papers that aren't in the literature are from cell line spike in experiments where you determine a particular threshold based on cell line. MET amplification cell line's an example. That's just a different beast than if you're using clinical samples.

My ask is actually ... It's a very difficult thing to do, to get enough of fish-positive MET-amplified patient samples to be able to do with paired plasma samples to be able to do that correlation, but to me, that would be the first step to get there on case by case example.

Geoff: It's particularly hard because we don't know what positives should be on tumor, either.

Ken: Exactly.

Geoff: There's no reference standard in tumor, and there's false positives. It's a very hard space, and we haven't yet made up our mind on tumor what really counts and what doesn't to then get there in plasma, or you could call it as an opportunity, where you could show you're even better than tumor, but you need the clinical outcomes. When there's no truth, it gets really harder to develop an assay that wins.

Howard: [inaudible 01:12:02] except for that it's clinically relevant, right? The point is you can actually do something with the information. What do we need to do to prove it to you?

Geoff: It can be clinically relevant if it's accurate, so treat a bunch of patients, and certainly I'll respond.

D. Janne: I think the themes that we're hearing from our sessions this morning and certainly from the panel is that clinical validation, showing clinical utility, showing that the finding leads to therapy and that treatment improves or alters the patient's condition, I think are critical, and again, something that we should strive towards. Many of the trials that you've heard about this morning certainly are trying to address those, and I think that is one way that we move this field forward and show that non-invasive diagnostics and liquid biopsies are useful.

Any other last questions?

Speaker 17: I think there's two areas here. Of course, the FDA has just released comments on next-generation analytical validity. We at Multi-X have got standards and we would encourage people that the tissue concordants they use fit those analytical validity standards. They can use an orthogonal method on tissue, and then similarly waive release standards on cell-free DNA. I think we just have to have common standards.

Once you've done that, then you can question whether you do the clinical utility answers questions. You should, at least, follow standards or reply to the FDA's release.

Dr. Janne: Okay, and more on that in our afternoon session on liquid biopsy test development. Now, for lunch. Thank you all for attending.