



Dr. Nathan Pennell
The Emerging Role of Molecular Markers in Managing
Non-Small Cell Lung Cancer

February, 2010

Dr. West:

Hello everyone. My name is Dr. Jack West. I'm a medical oncologist and the President and CEO of GRACE, the Global Resource for Advancing Cancer Education. Today we are starting a new format fore into webinars which I hope will be a very effective and efficient way to reach a community of interested people all over the Country or even the world through electronic media.

It's very fitting that the first person who will be delivering this content is Dr. Nate Pennell, who is a longtime, now approaching a year with GRACE, faculty member. He is medical oncologist and lung cancer expert at the Cleveland Clinic Foundation.

So with that I'll turn it over to Dr. Pennell to cover the topic of today's program which is the evolving role of molecular markers in managing non-small cell lung cancer. Thanks for coming out and doing this with us.

Dr. Pennell:

Thank you Dr. West for inviting me to give the inaugural GRACE webinar. I want to welcome everyone in the GRACE community for coming and thank you for participating in this. I hope this will be as interactive as it can be with questions at the end. I hope this will be an educational experience for everyone.

The topic today is the evolving role of molecular markers in managing non-small cell lung cancer. Today, we're going to be talking first about what I like to think of as the many faces of what we call non-small cell lung cancer. Although this has been lumped together as a single diagnosis, in fact, we're beginning to understand that this is more of a grab-bag of individual diseases that have all been lumped in this one category. That's becoming important as we're starting to piece these apart.

Second, I'm going to define a little bit of what I think of as a molecular marker and what that means. We'll talk about the current status of molecular testing in lung cancer practice today. Then I would like to talk about a few promising molecular markers that should be coming up in the near future. This is in no way meant to represent a comprehensive list, but a few that I thought were most promising.

For the last century and into the early 2000s, when a patient walked into my office, all I really needed to know in terms of how to treat them was what type of lung cancer they fell into in these two categories: small cell lung cancer or non-small cell lung cancer. It is not at all unusual, certainly ten years ago, to have gotten a pathology report that said non-small cell lung cancer and no further identification beyond that.

However, what I want to point out is that its been known for probably most of the last century that non-small cell lung cancer is in fact a heterogenous disease and the pathologists who look at these samples under the microscope have been able to identify specific recurring subtypes and have defined these very well. We think of them in terms of squamous cell carcinoma,

adenocarcinoma, and large cell carcinoma; but in terms of the various subtypes there are probably dozens of different types.

From the standpoint of treatment options, I'm happy to say that it has changed somewhat despite non-small cell lung cancer not otherwise specified being the primary diagnosis in 2000; in 2010 I can say that we have some molecularly-defined and functional clinical definitions of various types of non-small cell lung cancer. For example, about 10% of North Americans with non-small cell lung cancer have mutations in the epidermal growth factor receptor (EGFR), about 4% have translocations of the ALK gene which is anaplastic lymphoma kinase; about 20% have mutations in the KRAS gene which is another well described oncogene. From a clinical treatment standpoint, some patients are eligible for treatment with various drugs such as bevacizumab which is also known as Avastin.

The question is why is this important for us to be able to define these different subtypes?

The goal of all medicine and especially oncology is to move towards personalized medicine which is matching the specific type of tumor with the perfect treatment. If you could figure out what the underlying cause is and actually turn that off; much like finding the right key to open a lock. The question is how do you identify the right key? For the purposes of today's lecture one answer is called a molecular marker that defines that tumor.

So what is a molecular marker? There is no Webster's definition of molecular marker, but this is how I think of it: it's a molecular characteristic and by molecular I mean either something involving proteins, DNA or RNA of a cancer cell; or of the patient who has the cancer that carries either a prognostic or predictive value.

A prognostic marker is something that indicates a better or worse outcome irrespective of the treatment. Good example of that would be tumor stage. So you would imagine that a patient with stage I cancer is generally going to have a better prognosis than stage IV cancer no matter what you do.

On the other hand, a predictive marker is something that the presence or absence of would predict how the patient will do with a specific treatment. It predicts whether they will benefit or not from this treatment. The best example for lung cancer and I'll talk quite a bit more about this today is the EGFR mutation and treatment with Tarceva or Iressa.

What are the current molecular markers in lung cancer practice today? I only want to focus on two: epidermal growth factor receptor and in particular the mutations in this gene; and second we're going to talk a bit about KRAS mutations because this is something patients are coming to see me with second opinions and they've had this checked. This is something that is commercially available right now.

The epidermal growth factor receptor (EGFR) is something that has been known for a long time to be important in cancer, and in particular lung cancer. Its something called a receptor tyrosine kinase. Essentially it has two main parts: one on the outside of the cell that binds to the ligand or the growth factor such as epidermal growth factor receptor. Once bound, the inner part here which is where the tyrosine kinase sits is activated in that much like the ignition key in a car, it turns on the machinery of the cell. When this is overactive as in a cancer cell it can promote all sorts of bad things such as angiogenesis, proliferation, protection from appropriate death and increased metastasis and invasion.

Recognizing the importance of EGFR, there have been a number of drugs that have been designed to inhibit this. The ones that we are going to talk mostly about today are Tarceva which is currently the one of these available in North America; and Iressa which is available in much of the rest of the world.

This is something that has been discussed extensively on the GRACE website, so I won't belabor it. I do want to use this to illustrate one of the earlier trials. This is the BR-21 trial that led to the approval of Tarceva in second- and third-line treatment of non-small cell lung cancer. Patients who had failed one or two previous chemotherapy regimens were randomized either to Tarceva or a placebo, and this did show a two-month improvement in median overall survival (this is the top line here showing a better survival). Now this in and of itself was interesting, but not incredibly interesting.

What was very interesting was what we've seen in specific clinical subgroups of these patients. For example, patients who had never smoked had a dramatically better survival when they were treated with Erlotinib than patients who had never smoked who were not treated with Erlotinib. In current and ex-smokers there was only a very modest improvement in survival. So something was clearly standing out here.

When they looked not just at this but at every trial that treated patients with EGFR TKIs, these clinical characteristics stood out. Patient who had never smoked, patients with adenocarcinoma, women, age and ethnicity, all had a much greater chance of having a dramatic response to EGFR TKIs. The question was what is the molecular marker or markers that might predict who would benefit from an EGFR TKI? In other words, when a patient walks in the door, how can I tell to whom I should be giving the TKI to take them from this to this?

There have been a number of candidate markers for EGFR TKIs, certainly the simplest and least expensive is to use clinical criteria alone. If you only want to treat non-smoking Asian women with adenocarcinoma, is that a sufficient criterion to pick the ones who will benefit the most. We'll talk about checking for EGFR mutations and what was thought to be a quite promising biomarker which is EGFR team copy number assessed by a test called Fluorescence In Situ Hybridization or FISH so patients with high numbers of gene copies are usually defined as called FISH positive, the proteomic pattern. This is not a tumor marker, but in fact is a marker in the serum of patients with lung cancer that can be detected in the blood.

Then KRAS mutations which are something that is certainly perceived in much of the lung cancer world as a negative predictor of response or a predictor of people who would not benefit from TKIs.

The first paper that published the discovery of mutations in the epidermal growth factor receptor gene that was responsible for these patients who had these dramatic responses to TKIs; in this case gefitinib which is known as Iressa. Since then, we've come a long way. We've studied this extensively. We now know that mutations enhance the activation of EGFR leading to cancer cell addiction to the signal from the mutated receptor. In other words, the cancer cells now are relying on the activation of EGFR to stay alive. It makes sense then that if you inhibit the EGFR with Tarceva or Iressa that they would then be much more likely to die.

In fact, we now know in prospective trials the patients with EGFR mutations have objective response rates that are 70% or greater in median survival that is approaching two years when treated with TKIs; which is probably double of what you would expect from an average non-small cell lung cancer trial.

The question still comes up, however: are these truly predictive of benefit in terms of survival from TKIs; or are these EGFR mutations only prognostic? Is this a different disease and patients simply have a better prognosis and do better no matter what you do?

That brings me to truly a landmark study in this particular field called the IPASS study, again, probably all of you are familiar with this from the website. This was a trial done in Asia in patients with adenocarcinoma who were never or light smokers. They were randomized to either Iressa or chemotherapy with carboplatin and paclitaxel. Two important questions were answered by this trial. First, is it important to check for EGFR mutation or can you simply use these clinical predictors in order to choose the patients who should be treated with EGFR TKI? Second, the patients with EGFR mutation, which is better: the TKI or standard chemotherapy?

This is the funny looking progression-free survival curve that everyone was puzzled over at first because it's got this weird hourglass shape. Numerically there was no difference in progression-free survival, although the overall curves favored gefitinib. The explanation for the funny looking curve was that it was a superimposition of two different groups: those with EGFR mutations and those without.

As you can see in the EGFR mutation positive group, those who were treated with Iressa had a vastly superior progression-free survival than those treated with chemotherapy. On the other hand, those without EGFR mutations, so-called EGFR wild type patients, did much better with chemotherapy than with Iressa

This is the overall survival curve that did not show any difference. Keep in mind that patients who progressed on chemotherapy were able to get Iressa in second-line making it almost impossible to ever really show a survival difference in this type of a trial.

The conclusion from what we know about EGFR mutations now, I think this is no longer controversial. EGFR mutations are a predictive marker for both response and certainly improved progression-free survival with EGFR TKIs in the first-line treatment. They probably also have a significant impact on overall survival. That is going to be difficult to ever prove.

Clinical characteristics are clearly not sufficient to predict benefit from TKIs in the absence of mutation. The point I want to make is that mutation testing for patients certainly at least who you have a high clinical suspicion of mutation should now be a standard practice. I am working with my own institution to make sure that this is done on a standard basis. This is happening more and more because people recognize the importance of this test.

Now, testing for EGFR mutations is another matter. The tried and true way to do this is, is something called DNA sequencing where you simply find the code of the DNA throughout the entire tyrosine kinase domain of the gene. This is currently patented by a company called Genzyme in Massachusetts. It requires a substantial amount of tissue. Many patients who have simply a needle biopsy or even a cytology specimen from a bronchoscopy do not have enough tissue to adequately run this test.

In addition, the turnaround time is about 3-4 weeks. In addition to many patients not having enough tissue, many of them are not able to wait 3-4 weeks before starting treatment; that's problematic.

Another way to test for EGFR mutations which I personally believe is going to become much more common and may, in fact, become the standard way of testing for mutations is called

allele specific PCR-based assays. There's a technology called Scorpion ARMS (Amplification Refractory Mutation System), it doesn't sequence the entire gene. Instead it looks only for the most commonly known mutations in the EGFR gene. It requires substantially less tissue. It's much more sensitive. It has a turnaround time of less than a week. This also now is commercially available.

Aside from mutations, another potential molecular marker that has been extensively investigated is increased EGFR gene copy number by Fluorescence In Situ Hybridization (FISH). If you focus on the upper right here, you can see this is normal cells, each cell has two chromosomes and these red dots are the EGFR gene; there are two copies in every normal cell.

On the other hand, there are cancer cells that have multiple chromosomes and have multiple copies of the EGFR gene; or in this case sometimes they duplicate the EGFR gene. All of these red dots are dozens of copies of the EGFR. The hypothesis was that this increases the reliance of these cells on EGFR and therefore might predict benefit from an EGFR TKI.

Going back to the BR-21 trial, this is early data from the Canadian group looking at those with EGFR amplification treated with erlotinib had a significant survival improvement compared to those treated with placebo. This magnitude was substantially larger than what was seen in the overall group.

This was argued back and forth quite a bit in the scientific community. However, I think that the nail in the coffin of FISH was finally hammered home by the IPASS trial and the molecular analysis of these tumors that was presented in 2009. What we now know because they were able to analyze copy number and mutations in the same patients which had never been adequately done in the past, most patients with EGFR mutation positive tumors also are FISH positive so some of the past studies may have been contaminated by mutations and they never knew that. More importantly what they showed was in patients who did not have mutations, FISH positivity by itself did not predict an improved outcome on the IPASS trial. In conclusion for now, EGFR gene copy number by FISH should not be used routinely to guide treatment for EGFR TKIs.

Something else that I think is very interesting is using serum proteomic signatures. This is not a test on tumor tissue, but instead is a serum test. All you need to do is draw a patient's blood. They will then use something called MALDI (matrix-assisted laser desorption/ionization mass spectrometry) to analyze the pattern of the proteins in the serum and give you a distinct signature. The hypothesis is that you can find a distinct pattern of proteins in patients who will respond or who will get a benefit from TKIs compared to those who will not.

This was led by Dr. David Carbone's group at Vanderbilt University, when they did the multitest on a large number of patients who had been treated with EGFR TKIs and then asked the computer to come up with an algorithm that will pick the people who did well from the people who did poorly; they were successful in doing that. As you can see, patients with a good signature have a substantially better survival than those with a poor signature.

Importantly, when they tested it on patients who had not received TKIs, but who had lung cancer, there was no difference in survival between the two signatures. So, this was clearly related to treatment with a TKI.

It's important to remember that this is all retrospective. These were all patients who had already been treated with TKIs and they had serum left over afterwards. Before this can be appropriately used in the clinic it has to be proven and validated in a prospective fashion.

They are doing that now. It's something called the PROSE Study, which is a European trial of patients who failed first-line chemotherapy. All of them will have this proteomic profile done and then are randomized to either erlotinib or chemo with an endpoint of hopefully being able to show that patients with the good profile will do better than those with the bad. At that point it may be useful.

Finally in terms of EGFR TKI, this is still a relatively controversial topic. KRAS mutations are a common finding in adenocarcinoma. This is much more typically associated with heavy smokers as opposed to EGFR mutations which are more common in people who have not smoked. This is fairly well described.

The presence of a KRAS mutation is a prognostic factor. This is a meta-analysis of a bunch of trials looking at KRAS mutations that in the end when they've combined them all indicate that probably there is a worse prognosis just in general with patients who have KRAS mutations.

The question here, though, is, can the KRAS mutation predict for a poor response or lack of benefit from EGFR TKIs? Why would we think that? There is some evidence that suggests that this could be a poor prognostic factor or predictive of poor outcome.

First of all, EGFR mutations and KRAS mutations tend to be mutually exclusive. I'll explain on the next slide why that is. An immediate use is that if you detect the KRAS mutation, the patient is unlikely to have an EGFR mutation. In this particular paper, published in 2009, looking at 11 different trials of patients treated with Iressa or Tarceva, they showed that of the patients who have KRAS mutations, essentially none of them had objective responses to Tarceva or Iressa.

This was used as an argument to say that perhaps that KRAS means you shouldn't even try using those. But, in fact, what I would point out is that patients who do not have EGFR mutations, so EGFR wild-type patients, even in the absence of the KRAS mutation typically had response rates in low single digits. So I'm really not convinced that this is all that different from those who would be KRAS wild-type, but simply don't have an EGFR mutation.

If you think about how RAS works it makes sense that they are mutually exclusive. It is what we call downstream of EGFR. If this is the ignition key, you can say that KRAS has hot-wired the machinery of the car. Once this is turned on it doesn't really matter what happens up here in the EGFR. It's essentially taken out of the loop.

This argument will probably continue to go on for quite some time. There was some evidence presented in 2009 that made at least a reasonable argument against KRAS being a significant negative predictor for using EGFR TKIs and that was the molecular analysis from the SATURN Trial.

SATURN as everyone remembers from some of Dr. West's posts on GRACE is the randomized trial of patients once they've completed first-line chemotherapy and were randomized to either Tarceva or placebo in the maintenance setting. What this trial showed overall was a progression-free survival and an overall survival benefit in the patients who were on maintenance Tarceva.

They then presented the molecular analysis. So patients who were KRAS wild-type had a curve that looked very much like the overall population with a statistically significant improvement in progression-free survival.

In those who had KRAS mutation positive tumors where maybe people expected these lines to be superimposable, there did appear to be a benefit in the Tarceva-treated group. Now, this was not statistically significant because there were fewer than 100 patients total that had KRAS mutations that they could test. However, the hazard ratio for the benefit was very similar to what was seen in every other subgroup analyzed with about a 23% improvement in progression-free survival. It did appear that it was a poor prognostic factor, and both groups did worse than they did in the wild-type group. I think this is at least not proof that KRAS is a negative predictor of benefit.

For now, I would say there's not enough evidence that KRAS mutation should be a contraindication for the use of EGFR TKIs in the second- and third-line. Certainly, I would never recommend using one in first-line treatment if there was a KRAS mutation simply because IPASS showed that in the absence of an EGFR mutation, patients should receive chemotherapy.

I would go as far to say that in standard clinical practice today there really is no need to look at KRAS mutation status outside of a clinical trial. Although if you do have that information, it can provide indirect evidence that the patient does not have an EGFR mutation.

In conclusion for molecular markers that we're using today in the clinic, EGFR mutation testing should be done routinely on patients with a high probability of having mutation so the non-smokers in particular. Ideally this test will be available to everyone once it becomes more readily available and less expensive. Anyone who has a positive mutation should be treated with EGFR TKIs. For now, EGFR gene copy number by FISH, KRAS mutation testing should not be used to guide treatment decisions in 2010.

How about markers we can expect to see over the next decade and potentially significantly sooner than that? I'm going to focus on three: the first is the ALK translocation so the anaplastic lymphoma kinase, which has been quite played up in the news and a lot of discussions on GRACE's website, too. Many of you are familiar with this I'm sure.

Second I'm going to talk about two potential predictive markers for response to traditional cytotoxic chemotherapy, including the excision repair, cross-complementation group I or ERCC1 and ribonucleoside-diphosphate reductase, or RRM1.

First, the ALK translocation, I won't belabor this because all of you have probably heard quite a bit. This is brand new, it's been discovered in about 4% of patients with non-small cell lung cancer. The most common partner with this rearrangement is a gene called EML4. This is present in up to 20% of non-smokers, although in non-smokers who are EGFR wild-type it maybe one in three or perhaps slightly higher. There do not appear to be any overlap with EGFR mutations as opposed to EGFR mutations which are more common in women; these appear to be more common in younger men with adenocarcinoma. Importantly in pre-clinical models in the culture dish or in a mouse when you inhibit ALK in these tumors they die.

This is an easy rearrangement to look for. All you need to do is an ALK-specific FISH test very similar to EGFR gene copy number. Most hospitals have been testing for this in a very similar rearrangement with ALK in lymphoma for quite some time. You can also detect it with

immunohistochemistry so this is something that any pathology department should be able to test for.

Importantly, there are drugs in development which target this rearrangement. The one that is farthest along is the Pfizer drug 02341066 which has now been tested and presented in a Phase I trial, 32 patients were presented in late 2009. The first 29 of them, 59% responded to this drug and 83% had stabilization of their disease. This is very early. However, based on these very promising early results, the company has already stated a Phase III registration trial with the hope that they can accelerate getting these drugs out and available to these patients as soon as possible. It's fairly impressive how quickly this is moving. So I would anticipate that if this confirms that this is beneficial in these patients, then these drugs might be available within three to five years; probably not sooner than that outside of a trial.

This is a little bit different. Instead of talking about a targeted therapy, what I want to talk about are predictors for benefit from traditional chemotherapy drugs. The first is ERCC1, certainly the best described of these markers. It's a critical protein in the DNA repair pathway. In particular, the excision repair pathway is responsible for repairing damage caused by a platinum chemotherapy—cisplatin, carboplatin. As all of you in the audience know, these are the backbone drugs for treatment of non-small cell lung cancer.

It's been shown that low levels of ERCC1 expression are associated with a poor prognosis. However, low levels of expression also seem to make the cancer more sensitive to damage with platinum chemotherapy. It makes sense if you think about it. If the cancer cell can't fix the damage, the DNA is going to die. Whereas if they have high levels of these repair enzymes then they can fix the damage from the chemo before they're killed.

The second one we'll talk about is RRM1, which is an important protein in producing the deoxy ribonucleotides or the building blocks of DNA. RRM1 is an important target of the chemotherapy drug gemcitabine or Gemzar which many of you will recognize as one of our most important drugs for treating non-small cell lung cancer. It's been shown that low levels of RRM1 may increase cancer sensitivity to Gemzar.

The first study that showed an important benefit to checking these markers, in my opinion, was the investigators of the IALT trial, the International Adjuvant Lung Trial, which was the first randomized trial to show a benefit from adjuvant chemotherapy. Patients with early stage disease were randomized to chemotherapy or observation; and it did show a significant benefit. However, the investigators went a step farther, took the tumors that were resected and stained them for ERCC1 and then compared the survival between those who had high levels of expression versus those who had low levels of expression to see if there was a difference.

For this (I don't know how well this projects on peoples' screens), I want everyone to focus on the red circles for the next few slides. The upper left-hand corner, this is the overall survival curve for the IALT trial. There was a very slight separation of the curves between the chemo and placebo groups. There was a statistically significant benefit but it was quite modest—4% improvement in overall 5-year survival.

Now, in patients who had ERCC1 negative tumors by a simple immunohistochemical stain, there was a much more impressive improvement in survival in the chemotherapy group compared to the control group. The median survival was 56 months in the chemotherapy group versus only 42 months in the control group indicating a significant benefit from chemotherapy in patients with low ERCC1 as opposed to those with high levels of ERCC1. Again, the ones you

would predict with platinum chemotherapy would be less effective, in fact, there was no benefit from the chemotherapy detected in the subgroup. Survival in fact was numerically inferior at 50 months compared to 55 months in the control group providing very strong but still retrospective data supporting the use of ERCC1 in patients who would be treated with platinum chemotherapy.

Just like any retrospective study, this needs to be validated in a prospective fashion. And it always leaves us with the question, "Well what do you do with the patients who have high ERCC1? Are you really not going to treat them based on this?" It's difficult to say. I think was a smart design. This is an ongoing prospective trial by the Southwest Oncology Group SWOG 0720 using only the patients in whom it's already a little bit controversial about using adjuvant chemotherapy—stages IA and IB. They'll have RRM1 and ERCC1 levels determined. Those who have high levels who you would predict would not benefit from chemo, will not get chemo and that actually be considered the standard arm because chemo has not been shown to be beneficial in this group anyway.

In those, however, who have low levels of these repair enzymes who might be expected to have a higher benefit from chemo, they will receive adjuvant cisplatin and gemcitabine. I think this will be a very interesting study when this is presented. That's early stage disease.

What do we know about these enzymes in metastatic disease? There have been a great many retrospective studies looking at those treated with platinum and gemcitabine chemotherapy; this is just four of those illustrating that some have shown significant improvement in overall survival with low levels of ERCC1 compared to high levels; low levels of RRM1 compared to high levels. However, I will point out that this is slightly inconsistent. There are number of trials that did not show statistically significant benefits. Certainly enough of them were positive that it was worthwhile to move this into a prospective trial.

This was published in 2007, the Spanish Lung Group led by Dr. Rosell randomized patients with previously untreated non-small cell lung cancer to either a control arm of standard chemotherapy for everyone—cisplatin and Taxotere—or they assessed their ERCC1 and mRNA levels. Those who had low ERCC1 mRNA, so this is actually a technically more difficult test to do than the staining from the study I told you about last, these are the ones who would be expected to benefit from platinum; they received cisplatin and Taxotere. Those who had a high level of ERCC1, those who may be resistant to platinum received a non-platinum doublet of Taxotere and gemcitabine. This has been shown in Phase III trials to be equivalent to two platinum doublets.

(I apologize if this is slightly blurry. If you'll just focus on the red circles.) Those in the non-genotypically driven arm had a response rate of 39%. Those in the ones where they determined the treatment by ERCC1 levels had a statistically significant better response rate of 51%. However, the overall survival was exactly the same in the two groups: 9.8 months; 9.9 months. It's a little bit puzzling and I think this reflects, even retrospective data which has been inconsistent, clearly there does appear to be a higher response rate with low levels of ERCC1, but the survival benefits have been hard to find. I find this particular study a little bit disappointing.

There has been another prospective trial, in this case a Phase II trial, done by the folks at the Moffitt Cancer Center in Tampa called the MADeIT trial. Patients had ERCC1 and RRM1 levels determined again by mRNA, a more technically difficult test than immunohistochemistry. In this case, 85 patients were assayed and 53 were enrolled on the trial.

This is the algorithm. Those who had high levels of ERCC1 did not receive platinum; those with low levels did. Based on RRM1 expression, those with high levels did not receive gemcitabine, in this case carboplatin and Taxotere; those with low levels did receive Gemzar; so carboplatin gemcitabine in the ERCC1 low group; and then the non-platinum doublets with or without gemcitabine in the ERCC1 high groups. This is a little bit confusing but hopefully people are following along with that.

These are not lines of randomized trial. This is an overall survival and this is a progression-free survival line, but the response rate was 44% with a median overall survival of 13 months. While these numbers are certainly promising, I will say that it is not at all unusual for a Phase II trial to show response rates in median overall survivals in this range. So it's certainly not a slam-dunk. It is promising enough to move onto a Phase III trial which is the MADeIT II trial and that is ongoing.

So in conclusion, ERCC1 and RRM1 maybe predictive of benefit from adjuvant treatment in early stage non-small cell lung cancer and we do have at least one trial ongoing right now to verify this. If I had to guess, I would say this is probably where this will play out first and move into standard treatment and that could be in a relatively short period of time.

These markers are less clearly predictive of benefit in the metastatic setting and the testing is somewhat more complicated given the general lack of large amounts of tissue as you would normally have in a surgical specimen from an early stage patient.

There actually is emerging evidence right now that both a primary tumor and a metastasis can have different expression of predictive markers. In particular ERCC1, the expression can be quite different between a primary tumor and a metastasis. This differential expression may complicate things and make it more difficult to see any benefit depending on where you biopsied the tissue.

In conclusion tonight, molecular markers clearly have the potential to guide treatments by aiming us at the underlying cause of the malignancy. However, we need to identify more important targets for non-small cell lung cancer and develop effective treatments against those targets before molecular markers can be applied to a large number of people.

In 2010, I think EGFR mutation testing is the only validated molecular marker that guides treatment of non-small cell lung cancer; although I would not be surprised in five and certainly ten years to see a number of others including EML4-ALK and ERCC1 moving into routine practice.

I hope when I give this webinar in a hollow dome in 2020, instead of showing this early graph which shows us defining perhaps a third of non-small cell lung cancer, it'll look more like this, where you'll be spinning the wheel of multiple molecular characteristics, lining them all up and finding your specific treatment for your specifically molecularly defined cancer. Thank you very much.