I’d like to set the stage for where we are regarding EGFR tumor mutations and talk a little bit about resistance, because that really is the next frontier in thinking about EGFR biology.
So what do we know about EGFR tyrosine kinase inhibitors? From 2004 to 2006, it became pretty clear to us that erlotinib prolonged survival when given as second- or third-line therapy compared to placebo. And the data emerged then that suggested that FISH may be a good way for predicting who benefits in that setting. We knew that erlotinib and gefitinib can cause dramatic responses in a subset of patients and that mutations best predict this group. And we also learned that combination with chemotherapy back in 2004 to 2006 didn’t likely provide a great benefit.
Now, it was the identification of the epidermal growth factor receptor mutations that ushered in an era of molecular medicine in lung cancer.
There are generally two important groups of mutations. These mutations occur in the tyrosine kinase domain. They tend to be either exon 19 deletions or exon 21 point mutations. There are some mutations we find in exon 18, and there are some resistance mutations that we find in exon 20. But that gives you the range of where the mutations are. And over the past, from 2004 to 2006, we’ve actually been able to understand what most of these are, which has simplified testing and evaluation.
Now when one prospectively treats a patient who has a mutation, you can see a dramatic response. This is a patient of my colleague Dr Bruce Johnson, who was treated on Lecia Sequist’s study, where she was treated with gefitinib up front, identified by a mutation and found to have a very nice response to primary tumor.
Lecia’s study screened 98 patients: 35 percent had EGFR mutations, with a 55 percent response rate, 9.2-month PFS and 17.5-month OS.
And if you see the waterfall plot, the vast majority of patients had very nice shrinkage in their tumors.

So what we learned from not only Lecia’s study but studies which were done in Korea, Japan and in Spain is that when you prospectively treat mutation-positive patients, you see responses in 70 percent of patients and about 30 percent of mutation patients don’t respond to treatment with EGFR TKI. I would argue that’s one of the most important groups to look at and try to figure out why that inherent resistance emerges.
Resistance

- There are 2 robustly described TKI-resistance mechanisms: T790M in EGFR and MET amplification
- 1 patient with both T790M and L858R had a best response of SD and remained on treatment for 55 days

But we also know that resistance is important as an acquired phenomenon, because unlike CML, where a patient who’s in a molecular CR early on with CML can have a very prolonged, almost normal life span from treatment with a drug like Gleevec®, we know that when you treat EGFR mutations with TKIs, resistance emerges relatively quickly.

This is a patient that I took care of at Mass General before I went to Yale, a patient who had an EGFR mutation and, you can see, had slowly progressive enlargement in the size of these tumors. And this patient turned out to have a T790M mutation, a secondary mutation.
Here’s a second patient, treated on Lecia’s trial, a patient who was treated in Fairfax, Virginia, who presented with an exon 19 deletion, did not respond at all to Iressa®, died rapidly and in retrospect, turned out to have MET amplification. And MET amplification has been found to be an important mechanism of resistance, initially described by Dr Jeffrey Engelman at the Mass General, who at the time was at the Beth Israel Cancer Center.
An interesting question is, why do tumors become resistant? EGFR is phosphorylated and dimerizes — often to ErbB3, among other partners that are preferred binding partners for EGFR. And it sets off a signaling cascade within the cell that causes the cell to proliferate.
What you can see from this cartoon is that when you use gefitinib or erlotinib, you inhibit the phosphorylation of the tyrosine kinase domain and inhibit dimerization and downstream signaling. Since EGFR-mutated cells are addicted to this oncogenic pathway, the cell stops proliferating.
Now, what are some ways that the cell can become resistant? T790M is a secondary mutation which occurs in the active tyrosine kinase domain, and that essentially prevents gefitinib or erlotinib from being able to inhibit the enzyme and, therefore, normal signaling occurs. And this is not terribly different from the story in chronic myelogenous leukemia with secondary resistance mutations to Gleevec.
MET amplification is another mechanism of resistance as shown nicely in the work of Dr Jeff Engelman. I want to give credit to Dr Carlos Arteaga, Jeff Engelman and Dr Pasi Janne for the cartoon conceptualizations, which are a terrific way to take a look at resistance.
With this second mechanism of resistance, MET amplification, the cell, even with gefitinib or erlotinib on board, finds an alternative way to signal through AKT and PI3 kinase, allowing the cell to grow and proliferate.
And this led Jeff and Pasi to ponder whether if you could inactivate PI3 kinase in some fashion, would that be enough to overcome resistance to TKIs?
And it’s caused many to look at ways of combining multiple drugs to block many of these resistance mechanisms to try to restore activity to this area.
There's also a potential additional mechanism of resistance, which is signaling through IGFR or insulin-like growth factor receptor activation. This might be an alternative way of becoming resistant to EGFR tyrosine kinase activity.
One question I get asked a lot from a clinical standpoint is, what is the role of RAS mutations? And this is some data that Paul Bunn and Fred Hirsch generated from the SWOG, where they looked at EGFR-activating mutations, K-ras and FISH. And what you see is there’s no overlap between K-ras mutations and EGFR-activating mutations. In my practice, I’ve seen two patients that I can recall who have had both K-ras and EGFR-activating mutations. It is remarkably unusual to see them together.
Some have argued that K-ras mutations are a negative prognostic factor with regard to treatment with tyrosine kinase inhibitors. This is data from Frances Shepherd, from the BR21 study. You can see that while they’re small numbers, the hazard ratio actually favored placebo in patients with K-ras mutations when compared to treatment with erlotinib.
However, BR21 may not be the whole story. There is some interesting data that Dave Jackman published last year, which looks at patients who had both EGFR and K-ras mutations and found that when you pull out the EGFR mutants, there didn’t appear to be a big difference between K-ras mutants and K-ras wild types in terms of survival following EGFR tyrosine kinase inhibitors.
And so it becomes a very interesting question. I tend not to think of K-ras mutation as a resistance mechanism but rather as a completely unrelated oncogenic driver. Because they’re so distinct from EGFR mutations, I tend to think of them as being the rationale for a different mechanism of proliferative advantage for the cells.
Where are we now in terms of resistance mechanisms? T790M accounts for up to 50 percent of resistance, and some have argued it is as high as 80 percent of resistance. c-MET amplification and IGF signaling account for perhaps 25 percent of resistance, and still others have argued that the nonkinase role of EGFR is important. There is still a large group that would still go under the category of “unknown.”

How do we overcome this resistance? There are dual kinase inhibitors that inhibit T790M, possibly irreversible inhibitors having an advantage here. c-MET and IGF-1 are both targets worthy of study.
I would argue that EGFR mutation testing in 2009 is ready for prime time. The test can be done reliably on 10 unstained slides from a core biopsy. Almost always you can get an EGFR mutation test from a larger resection specimen. And as the technology gets better, we’re getting to the point where doing this on a fine-needle aspirate is certainly possible.

The two- to three-week testing interval remains a challenge, but we now know that the actual time to do the test can be brought down to just a day. And most of our time is spent gathering the specimens and processing them for testing itself.

Rebiopsy of patients, I think, is also a very important trend that we need to look at. Vince Miller presented some terrific data at this IASLC meeting and at ASCO, which looked at the Memorial experience of rebiopsying patients who became resistant so we can figure out why those patients have abnormalities.

And then, finally, I think the generation of analysis of circulating tumor cells will provide yet another way to look for evidence of resistance.
This is some data that Lecia Sequist and Dan Haber provided from Massachusetts General Hospital, where they have devised a new circulating tumor cell chip, which traps cancer cells and allows their quantification and genetic profiling. And you can see in this figure that the cancer cells get trapped against a small micro-post. You can then wash off the cells, analyze them for mutations, and you can actually find the presence of EGFR mutations in the cancer cells.
We now know that many, many patients with advanced cancer have circulating tumor cells when you look carefully enough.
And the ability to assay circulating tumor cells for molecular abnormalities will be important.
So to conclude, I think mutated EGFR provides a model for the paradigm of oncogene addiction. Tyrosine kinase inhibitors, whether they’re done in Boston or Beijing, shrink a tumor in 70 percent of patients with EGFR mutations and should be considered as a care standard for patients who harbor these activating mutations. Never and light smokers with adenocarcinoma should be tested as early in the course of therapy as possible, but I think it’s very important to know that mutations do still occur in smokers. And using a rationale for how to have a reasonable testing strategy for smokers is also important. Rebiopsy of responders at the time of progression can definitely help guide future therapy.
This is a brief overview on pharmacogenomics of angiogenesis — a simple but clear illustration of how pharmacogenomics can be used to approach angiogenesis biomarker discovery. Today we study cancer and lump patients together based on the site of disease, whether it be breast cancer or lung cancer.
Our goal in using pharmacogenomics is to be able to identify subgroups of patients who will or will not benefit from a given drug or find a subgroup susceptible to the associated toxicity. Each color represents a different subgroup. The green group (everyone wants to fall in this subgroup) represents the group that benefits but without the toxicity and then there is the purple group that will obtain no benefit and no toxicity. Many of our recent studies have also suggested that the subgroup enriched to experience both better efficacy and more toxicity (blue group) is quite common. It is for this subgroup that we must adequately understand this benefit to toxicity ratio so we can jointly decide with the patient whether proceeding is worth it.
This is a great place to start when thinking about biomarkers, in that these are the fundamental hallmarks that define cancer. And many are really specific to a given tumor cell, including the ability of a cell to evade apoptosis, having cell sufficiency and growth signaling, insensitivity to antigrowth signaling, the ability of a cancer cell to invade and metastasize, and of course, limitless replication potential.

But the hallmark of sustained angiogenesis is really quite different. Although the tumor elicits this response, it is the host that has to comply. And so this allows for a higher yield of host-imprinted genetic variability in terms of thinking about biomarkers.
Angiogenesis is important for tumor proliferation, including a variety of cytokines, growth factors and receptors that are fundamental to this process. One of the most central is vascular endothelial growth factor, or VEGF, which we know to be secreted, in part, by the tumor. This then is bound to a variety of VEGF receptors. One of the critical receptors is VEGF receptor 2, which helps recruit bone marrow-derived endothelial precursor cells to help form the vasculature to the tumor itself.
Evidence that angiogenesis is important in tumor pathogenesis is as follows: Increased microvessel density, which is the histologic surrogate for angiogenesis, has been found to be associated with advanced stage and poor outcomes in a variety of cancers. Data also exist to support high expression of proangiogenic factors and can be correlated with poor clinical outcome in a variety of malignancies. Most importantly, if you block tumor angiogenesis, it disrupts growth in a variety of tumor types, which is the strongest indication that tumor angiogenesis is important in tumor pathogenesis.
There are several ways to think about targeting angiogenesis. One can disrupt vascular endothelial growth factor itself. Probably the most mature drug in this class is bevacizumab, which is a humanized monoclonal antibody against VEGF, or by use of a ligand sequestrant, such as VEGF Trap. The VEGF receptors have also become targets. Monoclonal antibodies target the extracellular membrane portion, and a variety of intracellular tyrosine kinase inhibitors such as sunitinib and sorafenib, both of which are FDA-approved in several malignancies, target the intracellular portion.
Unfortunately, although these are truly targeted therapies — whether they target VEGF or the VEGF receptors — to date we really don’t have a good idea of what subpopulations to target these agents to. Data are now emerging looking for biomarkers to indicate which patients would be best suited for each drug treatment. This table demonstrates some data to date looking at VEGF single nucleotide polymorphisms.

The VEGF minus 2578 allele was associated with improved overall survival in the E2100 trial, a Phase III trial implementing bevacizumab in breast cancer. The minus 1498C allele was associated with more hypertension in E2100. The minus 1154A allele, again, was associated with improved overall survival in E2100. And the minus 634G allele was associated with more hypertension in E2100, improved overall survival in E4599, a Phase III lung cancer trial, again implementing bevacizumab, and was associated with more hypertension in a meta-analysis implementing axitinib in renal cell cancer. It’s also important to note that all of these alleles are in tight linkage disequilibrium.
When thinking about a pharmacogenetic approach to angiogenesis biomarker discovery, there are four essential ingredients. The first, genetic variability, must have the ability to impact the biological system that is being tested.
Plenty of epidemiologic data suggest that genetic variability impacts angiogenesis. This includes variable risk and prognosis in a variety of conditions where we know angiogenesis is an important player such as, in a variety of malignancies, retinopathy, nephropathy, pre-eclampsia, recurrent pregnancy loss, and vasculopathy. The majority of these studies look at VEGF, HIF1, and nitric oxide synthase; however, there are newer data looking at VEGF receptors in other critical genes.

One major limitation to these studies is conflicting outcomes. Also, many of these studies only examine a single gene or a single SNP. Finally, clinical variables, which we know to be very important in terms of altered risk, are often completely ignored in these studies.
Data suggests that variability may be associated with the site of metastases. One data set shows the VEGF minus 1498CC genotype was more common in visceral metastases, as opposed to bony metastases. A provocative paper by Menendez, published in the *PNAS* in 2006, demonstrated that a VEGF receptor-1 promoter polymorphism was associated with differential induction by p53. As a result, for this first time, we have been able to intimately tie the cancer pathway with the angiogenesis pathway together.

And finally, a small study showed that variability and complement factor H may actually affect treatment outcome in macular degeneration for patients receiving intravitreal bevacizumab.
This is not level one evidence, but when reviewing the body of data as a whole, it strongly suggests that variability is biologically important. I'm going to use breast cancer angiogenesis as a model, briefly.
Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

Essential Ingredients:
- Genetic variability must have potential for biologic impact
- Genetic variability must exist in drug disposition or destination
  - Metabolizing enzymes/transporters/targets
- Drug evaluated must be heterogeneous in outcome
  - Mix of success and toxicity
- Variability must be frequent
  - Generalizability of results

Source: Walgren et al. JCO 2005; 23:7342-7349

A second fundamental property is that genetic variability must exist in the drug’s disposition or destination, and this can include variability in the metabolizing enzymes for the drug, drug transporters or ultimately the drug target.
Angiogenesis is a great place to look at genetic variability, as there is robust variability within VEGF itself. The majority of variability in VEGF is within the promoter and regulatory regions. Here you can see five polymorphisms. These are five SNPs that tag for the most common haplotypes and actually account for about three quarters of the variability seen in a mixed population of Caucasians and African-Americans. These polymorphisms do appear to affect the functionality of and expression of VEGF. Likewise, within VEGF receptor 2, there is a promoter polymorphism, which appears to affect the function, and two common nonsynonymous SNPs, as well.
Pharmacogenetic Approach to Angiogenesis Biomarker Discovery

Essential Ingredients:

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A third important quality for a biomarker is that the drug evaluated must be heterogeneous in outcome, obviously, so you need to see a mix of success and toxicity to have a good biomarker.
A good example of heterogeneous outcome is with the use of bevacizumab in breast cancer. E2100, a Phase III trial, randomized 722 patients to either paclitaxel or paclitaxel with bevacizumab as first-line therapy for metastatic breast cancer.
As you know from the parent trial, there was a significant prolongation in progression-free survival from about six months to 12 months with the addition of bevacizumab, and this was statistically significant with a \( p \)-value of less than 0.001. Likewise, there was an improvement in objective response rate from about 25 percent to 49.2 percent when evaluating measurable disease. And, again, this was statistically significant.
Bevacizumab also increased Grade III/IV toxicities. The toxicities listed in this table were statistically significantly different. And I think these can really be broken down into three major categories, the first of which includes infection, fatigue and neuropathy. And I think this category could be explained, at least in part, due to an increased duration of taxane exposure in the experimental arm.

The second category includes CNS ischemia, headache and proteinuria. And this category represents serious bevacizumab-induced toxicities — fortunately, they were quite rare.

The final category is hypertension. This is a serious toxicity, which is frequent and clearly bevacizumab-induced. And because of its frequency, this becomes a very nice toxicity to study from a biomarker standpoint.
These are the Kaplan-Meier curves for the overall survival from E2100 in our pharmacogenomics substudy. This was a retrospective evaluation of E2100 where we had DNA available for 363 patients, divided approximately equally between the experimental arm and the control arm. Now, if you look at the Kaplan-Meier curve on the left, what you can see in purple is the paclitaxel median overall survival, which is 25.2 months, compared to the orange line, which is paclitaxel with the bevacizumab, which was improved only to 26.7 months, and these were not statistically different. However, if one looks at the experimental arm broken down by genotype, one can see a dramatic improvement in median overall survival to 37 months.

Equally important, if you were to break down the paclitaxel-alone arm by genotype, there is no difference. Therefore, this serves as a predictive marker, not a prognostic marker.

On the right Kaplan-Meier curve, one can see the VEGF minus 1154AA genotype has almost a two-year incremental benefit over the paclitaxel arm alone.
Genetic variability of VEGF also predicted clinically significant hypertension in E2100. The VEGF minus 634CC genotype had essentially no Grade III/IV hypertension. And this was compared to about 20 percent for the alternate genotypes. Those with the VEGF minus 1498TT genotype had less than 10 percent Grade III/IV hypertension, and this was compared to about 20 to 25 percent for the alternate genotypes.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Percent Grade 3/4 Hypertension (no./%) by Genotype</th>
<th>p-value</th>
<th>p-value</th>
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<tbody>
<tr>
<td>VEGF-634</td>
<td>CC=6% (n=27, 15.3%) vs. GC=22% (n=62, 46.3%) vs. GG=19% (n=98, 38.4%)</td>
<td>0.013</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>CC vs. GC+GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF-1498</td>
<td>TT=6% (n=20, 33.9%) vs. CT=22% (n=62, 46.3%) vs. CC=22% (n=35, 19.8%)</td>
<td>0.056</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>TT vs. CC+CT</td>
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</table>
We also compared this toxicity of hypertension with overall survival, retrospectively, in E2100. And what we found is that, indeed, those patients who had experienced Grade III/IV hypertension had a significant prolongation of median overall survival — 25 months for those who did not experience hypertension compared to about 38.7 months for those who did. And this was statistically significant.
The association of hypertension with survival is a theme seen across several trials now. As mentioned, E2100 was associated with an improved overall survival. E4599, which was the Phase III lung cancer trial implementing bevacizumab, also was associated with improved overall survival and progression-free survival. In this study, hypertension was defined as any grade hypertension and that greater than 150/100. The NCI Canada’s BR-24 study, a Phase II trial implementing the anti-VEGF drug cediranib was associated with improved response rate and progression-free survival. They defined hypertension as new hypertension or any worsening grade of hypertension. Finally, a meta-analysis of several axitinib trials demonstrated an association with improved overall survival. They defined hypertension in that study as diastolic blood pressure greater than 90.
We'll now look at the E4599 lung cancer trial. This trial randomized 878 patients with advanced non-small cell lung cancer to either the standard carboplatin/paclitaxel or the experimental arm of carboplatin/paclitaxel/bevacizumab. They also performed a pharmacogenetic substudy of 133 patients, again split fairly evenly between the control and experimental arm, which evaluated several candidate polymorphisms, including those from VEGF, interleukin-8 and ICAM-1.
E4599 Results

- Median OS:
  - PC arm=10.3 months (95% CI: 8.2-15.6)
  - BPC arm=13.0 months (95% CI: 10.2-16.6)
- Median PFS:
  - PC arm=4.6 months (95% CI: 3.6-5.6)
  - BPC arm=6.5 months (95% CI: 5.4-8.3)
- Treatment by genotype interactions tested for in a multivariable model:
  - Gender
  - PS (0 or 1)
  - Stage (IIIB/IV vs. recurrent)
  - Adrenal mets, liver mets, and bone mets

In the parent trial, there was a significant improvement in survival with the addition of bevacizumab from 10 to 13 months, and also an improvement in progression-free survival from 4.6 to 6.5 months. They looked at treatment by genotype interactions tested for in a multivariate model, analyzing for gender, performance status, stage and also, site of metastases.
They evaluated progression-free survival, classifying patients by their SNPs. These are a combination of polymorphisms, including the VEGF 634, the interleukin-8, as well as ICAM-1. Then they created a subgroup of patients that were categorized as a “good SNP cluster” and a “bad SNP cluster.” As you can see from the Kaplan-Meier curve, those patients that had the “good SNP profile” and received paclitaxel and bevacizumab had a long median progression-free survival of 9.2 months, as demonstrated in the blue line.

However, the experimental arm of paclitaxel/bevacizumab in the unselected, or “bad SNP arm,” had a progression-free survival of only 5.4 months. What’s also interesting is if you look in the control arm, paclitaxel alone, there was no major difference in terms of median progression-free survival, whether they had the good or bad SNP profile. And when looking at treatment by marker group interaction, this was statistically significant, with a p-value of 0.003.
Patients were classified by SNP category and evaluated based on overall survival. The bottom table shows that in the patients that received chemotherapy alone, those with the “bad SNP profile” had a median survival of 8.5 months compared to 10.2 months for those with the “good SNP profile.” Patients that received chemotherapy with bevacizumab and had the “bad SNP profile” had a median survival of 10.7 months and extended to 16.8 months for the “good SNP profile.”
Demonstrated in Kaplan-Meier form, it is visually easy to see the differences here. Again, for the paclitaxel/bevacizumab with “good SNP profile” (blue line), you see a huge separation from the green line, where the median survival drops from 16.8 to 10.7 months, whereas in the paclitaxel-alone arm, you see a fair overlap of the curves. Again, when looking at treatment by marker group interaction, this was statistically significant.
More recently, the pairing of drugs and genes has hit the lay press. This is from last year’s *New York Times*, where you can see all three major categories of variability being demonstrated to associate well with the drug of choice, the first of which is mutational variability, where HER2 gene amplification has been used successfully as a marker for the drug trastuzumab. Mutations in K-ras have successfully predicted benefit for those receiving cetuximab. Likewise, EGFR mutations have been associated with outcome for the drugs gefitinib and erlotinib. Several metabolizing enzyme polymorphisms have also been able to predict outcome, including CYP2D6 with tamoxifen and UGT1A1 for irinotecan. Most recently, variability in VEGF has become recognized as a potential biomarker for bevacizumab.
Our hope now is to prospectively validate some of the prior findings from these VEGF polymorphisms in E5103. E5103 is an adjuvant trial for lymph node-positive and high-risk lymph node-negative breast cancer. All patients have HER2-negative disease. And the randomization goal is for about 5,000 patients to be randomized to 1) a control arm of AC followed by paclitaxel, 2) an experimental arm of AC followed by paclitaxel with concurrent bevacizumab, and 3) a second experimental arm of AC followed by paclitaxel with concurrent bevacizumab followed by a maintenance phase of bevacizumab.

Here we plan to either validate or refute the VEGF polymorphisms that we looked at from E2100 and to perform a more comprehensive analysis by genome-wide association. We have also implemented a quality-of-life assessment to more thoroughly understand the risk to benefit ratio.
Conclusions

- Pharmacogenetics (biomarkers)
  - Improves therapeutic index
  - Leads to drug discovery
  - Benefits patients

In conclusion, using pharmacogenetics as biomarkers allows for improvement of the therapeutic index of existing drugs, has the potential to lead to new drug discovery, and clearly can be beneficial to our patients.
Angiogenesis is a hallmark of malignancy. We know that its inhibition is effective in multiple tumor types, but there’s real therapeutic heterogeneity. Because of this, biomarkers are clearly needed to best select drugs for a given patient.

Some of the early work suggests that germline genetic variability might be important; however, validation of these findings and further understanding of molecular biology is essential before these can be used in clinical practice.
The identification of EGFR TK domain mutations in lung adenocarcinomas and their profound sensitivity to treatment with small molecule EGFR TKIs has revolutionized how we think about non-small cell lung cancer.

Individuals without EGFR or K-ras mutations are now tested for ALK FISH looking for rearrangement of the ALK gene. Dr Kwak and Dr Shaw recently presented data on the promising efficacy of a kinase inhibitor for this fusion protein in patients with non-small cell lung cancer. ALK FISH testing in lung cancer could have important clinical and research implications.
ALK fusions result from translocations analogous to BCR-ABL in CML. Why they happen and how particular partners are identified is the subject of a lot of research at a very basic level from multiple labs. In lung cancer the chimeric protein involves the anaplastic lymphoma kinase or ALK fused most commonly but not exclusively, to the echinoderm microtubule-associated protein-like 4 or EML4. This was first described two years ago, which is encouraging in that it took us five years to get the EGFR story to prime time but in just two years, we’ve gotten enough momentum in the field to have an agent in trial that’s working.
Although there are multiple partners for the ALK protein, nearly all the fusion proteins seem to include the ALK kinase domain, so they should be susceptible to kinase inhibition.

Activation enhances proliferation/survival through some of the players you’ve seen before, things like PI3 kinase, JAK-STAT, and K-ras and ERK.

The frequency of this fusion in the initial papers was reported to be about four or five percent in adenocarcinoma, which would require screening of a large number of patients to find one mutation, making timely completion of a clinical trial difficult.
PI3-kinase mediates, in part, growth signals from the ALK protein. Therefore, if a patient had a PI3-kinase mutation or some other dysregulation in that axis, they might be insensitive to effective inhibition of the ALK kinase.
Clinical Activity Observed in a Phase 1 Dose-Escalation Trial of an Oral MET and ALK Inhibitor PF-02341066


These are results of the trial that Dr Eunice Kwak presented at ASCO and which were updated at this IASLC meeting by Dr Shaw, of the Pfizer drug, 02341066 — I should point out that this drug is an inhibitor both of MET and ALK, which is not necessarily characteristic of all agents in that class.
This trial began with accrual of patients with different types of malignancies, and then, based on early response in a patient with an inflammatory myofibroblastic tumor, it was expanded to include a cohort of lung cancer patients with this ALK fusion. Like patients whose tumors have EGFR mutations, patients with ALK fusions also seem to have unique clinical characteristics.

These patients tend to be former or never-smokers and to have adenocarcinoma. Notably, many of these patients were heavily pretreated.
Unlike EGFR mutations, early data suggests these ALK fusion mutations may not be prognostic. Like EGFR mutations, these appear essentially to be exclusive from K-ras mutations. There have been one or two cases reported where individuals also have an EGFR mutation.

One of the ways we think we can enrich for detecting this abnormality is to test the subgroup of patients who are EGFR and K-ras nonmutated and never-smokers. The incidence there — as you saw at this meeting and as previously reported by Dr Shaw’s group — can be as high as 25 percent in that population.
This is the waterfall plot presented at ASCO and then subsequently updated with about 10 more patients at this meeting, showing a curve very reminiscent of what we saw with EGFR tyrosine kinase inhibitors in patients with EGFR tyrosine kinase domain mutations. Very impressive responses, although it is noted that a number of these responses are early on, so we don’t have data yet on their durability.
The overall response rate at the time of the ASCO presentation was 53 percent, and four patients had progression at first evaluation. Again, it will be important to try to characterize what coexistent genetic events prevent those individuals from responding.
These scans of a patient in the study were shown at the ASCO presentation by Dr Kwak.
These are some of the pretreatment characteristics of individuals on this study and their duration on the ALK inhibitor on the far right.
This is a druggable target. Phase II and Phase III trials of the agent, 02341066, in patients with ALK rearrangement are imminent. Comparison of multiple platforms for screening and diagnosis are ongoing.
Conclusions

- Non-small cell lung cancer is no longer one disease treated empirically with one or two chemotherapy regimens.
- NSCLC, and in particular adenocarcinoma, must be subtyped both pathologically and genotypically.
- EGFR mutations served as a “watershed event” for this fundamental paradigm change, but other “druggable” kinase mutations will continue to be identified.

I would like to conclude by emphasizing again that lung adenocarcinoma is now not one disease. It’s probably moved on to four or five diseases, and it may well be like lymphoma or perhaps broader than that in the coming decades. I believe that non-small cell lung cancer, and particularly the adenocarcinomas, must be subtyped both pathologically and genotypically. EGFR mutations are not the end of the story — they’re the beginning of the story. They’re a watershed event and have led to a fundamental paradigm change. I think other druggable kinase mutations will continue to be identified, but, again, that, in turn, is not the final solution to our problems, as an identifiable kinase mutation is probably present in only about half of advanced lung adenocarcinomas.