Refining Prognosis of Early Stage Lung Cancer by Molecular Features (Part 3): Evidence to Support a New, Biology-Based Staging System with Dr. Johannes Kratz, Surgeon, Massachusetts General Hospital

Dr. Kratz:

When we started off our project we had a hypothesis that we could develop a practical prognostic gene signature that would allow, for one, improved staging, and two, identification of patients that were probably most likely to benefit from adjuvant therapy. Hence our specific aims were to develop this practical molecular tool to identify high-risk early-stage patients and we also wanted to subject this molecular tool again to large-scale blinded clinical validation.

So we started our study in a particular fashion. One, we had to first develop the assay technology to measure gene expression reliably in a medium other than snap frozen research-grade tissues, which we’ll talk about in a second. But we had this basic overall idea that we could take patients from UCSF, which is our home institution, and measure a limited amount of genes. One of our strong tenets early on was that looking at a small select number of genes will give you about as much information as looking at hundreds or thousands of genes because we found out through positive studies, that it was really just a few key players that were driving the majority of the information that was given. So these smaller gene signatures were almost basically as good as these incredibly difficult and unwieldy gene signatures involving tens of thousands of genes.

We wanted to develop a very practical assay that would give us a prognostic algorithm, which could then be independently validated again in the blinded clinical fashion in two large cohorts. We were lucky to partner with two cohorts that were of an international nature. The first was Kaiser Permanente in Northern California. This also had the advantage of being a community-based setting, which was lung tumors which had been resected in the community, stored and archived in the community and then sent to us for analysis in a non-research institution fashion.

We were also lucky to partner with the China Clinical Trials Consortium, which is a big Consortium in China that allowed us to have access to a large international population just to validate our assay.

Again as mentioned, the first challenge was to develop an assay that could be used in a practical fashion. And this we decided had to be through the use of formalin-fixed paraffin-embedded tissues, or FPET, that are commonly used in clinical practice. Whenever any patient gets the tumor specimen resected in surgery, you can send it to the pathology lab and store it as this particular medium.

We knew that all the data was based on frozen samples thus far and we knew this was going to be challenging because these processes involve formalin fixation and paraffin embedding that degraded RNA every time and we knew that the amplification technique and the way to measure RNA expression were non-trivial. They were exceedingly difficult microarrays and we knew that quantitative PCR was better at accurately assessing mRNA expression levels in FPET. So that’s what we chose.
We had this challenge, we had these blocks, had to get somehow information out of these blocks to develop patient prognosis. Of course, the black box is in the middle and we had to develop techniques and the ability to transform that idea of paraffin blocks into a practical and prognostic assay for a patient with lung cancer.

We talked about RNA from frozen tissues. These are the snap grade frozen tissues that were used in all the previous studies. These are tissues that are very high grade so the RNA that you get for them when you run them is intact.

This is in contrast to the RNA that you get from paraffin tissues, which is fragmented and cross-linked, and unfortunately fragmentation and cross-linking makes it very degraded, as shown in this gel, side-by-side and it’s very difficult to extract information. So again we had to develop novel techniques, more than that we had to define them specifically for lung cancer tissues.

I won’t go through all the details of how that was done but there were three key steps. The three key steps that we found through various trial and error and all our experimentation were:

1) Using gene specific reverse transcription.
2) Pre-amplifying our cDNA prior to PCR.
3) I think this is very key: using custom designed TaqMan assays.

So these were basically molecular techniques that were developed specifically for the purposes of this test and specifically for the purpose of working with this tissue. We could not buy these off the shelf, they’re not commercially available, you can’t just put this test together. It needs to be very carefully thought of what you’re targeting and how you’re going to target it, in order to measure gene expression reliably and its fragmented RNA.

This is an overview of the patients included in our study cohort. There were close to 1800 patients, by far the largest validation and clinical design of any prognostic test in lung cancer and probably all the cancers. There were over 300 patients that we used in UCSF training cohorts, approximately 400 in the Kaiser cohorts and almost 1000 in the Chinese validation cohorts.

Some clinical differences of note, the age in the Chinese cohorts were somewhat younger than their counterparts and there were fewer females and fewer smokers. However, the medial survival and the overall survival was similar between all three groups as well as the histologic breakdown.

Of note in the Kaiser cohort, these were mainly Stage 1 patients. The Chinese validation cohort and UCSF cohorts included later stage disease as well.

We had picked basically 11 genes in the pilot study that we had published in 2008. This pilot study started with over 200 possible candidate genes and we whittled it down to between 60 and 80 potential genes, which we then ran quantitative PCR on, and we really identified 11 key genes we thought were key for prognosis in non-squamous non small-cell lung cancer.

So these 11 genes were the ones that we then went on to use in the future test cohorts on these 350 some patients that we then tested using these paraffin blocks that were available to us.

The algorithm was developed on the tissues of training cohorts. Ultimately after the extraction of all RNA we ended up with 337 patients with usable samples. These 337 patients, the gene expression of these 11 genes that we identified were measured in all these patients and we developed a prognostic algorithm.
It really related to the patient’s risk of mortality within five years to their gene expression profile. What this really means in layman’s terms is how aggressive is that tumor and how likely are you to harbor micro-metastatic disease at the time of surgical resection. The gene profiles are a proxy for tumor aggressiveness and really aggressive tumors meant that you had a really high risk of passing away unfortunately from the disease within five years. So you see as the risk increases in the UCSF training cohorts the probably of mortality at five years also increases.

Once we had developed the technology and measured all these genes and developed the gene expression product out of the training cohort we were then at the point or the moment of truth to finally and independently validate this prognostic gene signature on our validation cohorts. And we were pleased to see that our prognostic gene signature validated quite nicely in both of our two large international cohorts.

What’s striking actually is despite the differences in the stage background between the two patient populations, but also if you think about it, two completely independent international cohorts with different genetic backgrounds, these patients basically acted the same when they were identified by this prognostic gene tool to either low, intermediate or high risk category. If you were a high risk category patient with a tumor resected at Kaiser Permanente, University of California or in China, your outcome will be about the same. And that was true even regardless of stage because remember Kaiser cohort patients were all Stage 1 patients.

This really spoke to the power of the tumor biology and understanding tumor biology to determine patient prognosis. When we looked at these patients independently by stage in China, we may have thought that maybe this test was just a proxy for stage, all you’re doing through this molecular profiling instead of profiling tumor aggressiveness you’re profiling tumor stage and why can’t you just do stage.

Well, if you break down the China cohort by stage, you still saw good risk stratification by prognosticating each one of those stages. Even at Stage 3, you have low, intermediate and high risk patients reliably identified by the assay.

One other way of assessing whether a test is useful or not is through multi-variable progression analysis. This is a way of assessing with other common clinical variables how good is the assay or how good is your product’s model in identifying high risk patients over and above what are commonly thought of as a typical clinical risk factors.

Again it is striking to see both in the Kaiser and the Chinese cohort the high risk category as identified by the genomic and prognostic assays was the most powerful predictor of patient outcome despite inclusion of all these other commonly associated risk factors in patients with lung cancer.

Lastly, there’s one other way that is commonly used in the clinic to see whether this prognostic assay or prognostic feature adds in useful information and that’s the area under the current analysis. The physical description is probably not totally relevant for this audience and I won’t bore you with that but suffice it to say that by this criteria that’s commonly accepted by clinicians the genetic assay did outperform the conventional measures used to identify high risk Stage 1 patients by the NCCN and we’ll come to that. We’ll talk a little bit more about that in just one minute.

So the results of this were published in the Lancet in January 2012, and we’re pretty pleased by the response. But one of the things that we want to push forward is, what is the utility of this
prognostic assay? That just goes back a little bit to what I was talking about earlier, the interplay between these predictive and prognostic biomarkers and how they’re useful in a clinical setting.

Well it turns out both are extremely important and I hope to give you some examples now of how prognostic assay can be immediately clinically relevant to clinicians and the patient.

One of the features that we noticed recently is that, as I’m sure a lot of you know, there’s been new CT screening guidelines that have just recently been published in the JAMA. If you have a smoking history and you’re between 55 and 74 years old and you meet certain other criteria, you’re supposed to now go in and get a CT scan to see whether you have a lung tumor.

We know that because of CT scanning there will be a lot more of these small tumors that were not going to be picked up otherwise. That will lead to the resection of a lot of small, basically tiny tumors that were found through these new lung cancer CT screening guidelines.

And so we thought to ourselves, well a) is that a problem and b) if it’s a problem, what are we going to do about it. Not that the CT screening is a problem but that you have a small tumor that’s resected, now we’re going to suddenly have more millions of more of these small tiny tumors resected and are they dangerous and if they’re dangerous is there anything with our current methods that we can do about them.

These tumors are actually very dangerous. If you look at tumors that are less than 2 cm that we know have not spread anywhere, the published reports for T1a disease- which is what we’re talking about, tumors that are less than 2 cm – you see that up to a quarter of these patients die within five years.

In our particular validation cohort we had 259 of these patients in the Chinese and Kaiser cohorts and over 30% of these patients were dead within five years. So these tiny little tumors actually do matter and they do impact mortality quite significantly.

The second question is, okay the prognosis is not good if you have these little tiny tumors. Remember we’re going to pick up potentially hundreds and thousands or more of these tiny little tumors that are going to be resected in the next years. What are we going to do about them if they’re so deadly? The current guidelines do nothing about them because there’s nothing we can do about them. There isn’t any proven benefit giving these patients additional therapy, there’s never been any proven benefit giving these patients radiation or chemotherapy. So the current standard of care by both ASCO and the NCCN is just observation.

We can’t be satisfied with this result so this is one of the things we did. Let’s go back and assess our validation cohort and say well for our assay is there anything that our assay can offer these patients who will now go on to be detected and resected at a very early stage of their disease, they may go to their oncologist, their oncologist may say well you have 25% chance of death within five years, but we’re just going to watch you closely.

Now potentially we could offer them prognostic information of genetic tests that helps them verify what their risk stratification is and may clarify in the minds of those physicians whether anything else should be offered to these patients.

So we went back and we looked at our validation cohorts. We had 272 tumors that were T1a disease, less than 2 cm that were also node negative and had no metastases, so T1aN0M0 tumors. We were able to show that our assay reliably identified patients with almost 50% risk of
mortality at five years and the multivariate analysis which I have described was again a very, very strong predictor of risk when you were a high risk patient with this disease.

This other common statistic that we talk about is the area under the operating receiver characteristic curve analysis. Again, this is a great benefit over the conventional criteria that were used by the NCCN to identify high risk patients. These results were just published two weeks ago in JAMA: the Journal of the American Medical Association.

That tells you that for patients who will now be detected with very tiny tumors, the assay that was developed or any genomic prognostic assay clinically validated on a large scale may potentially be useful for these patients because now we can just identify those patients quite accurately even if they have tiny little tumors.

The second utility that we can think about and is already being used in clinics today is identifying high risk patients. So, if you look at the current guidelines, these are the most recent guidelines I believe, that were published by the NCCN on how to treat non small-cell lung cancer.

If you start with the left, you start with surgical resection. If you track this all the way to the right and you follow stage IB disease, after it’s been all surgically resected, the current recommendation from the NCCN is clinical. You can either observe these patients or you should or you can consider chemotherapy in “high risk patients”.

Now, if you then go on and see what the NCCN defines as high risk patients, they define poorly differentiated tumors, vascular invasion, wedge resection, tumors greater than 4 cm, visceral pleural involvement, etc.

With the exception of probably tumors that are greater than 4 cm, none of these clinical factors that have been identified by the NCCN really have a lot of strong clinical trials validating their prognostic significance. Yet the recommendation is that adjuvant chemotherapy should be considered for these patients. If that’s the case, if we’re making a clinical decision based on what makes intuitive sense to the NCCN identifying patients with “high-risk” tumors because they know that these tumors are so dangerous to the patient, why not use the clinically validated prognostic assay that can tell you that these patients in a clinically validated, rigorous way have really been identified as being high-risk patients.

The last topic we’ll talk about is a new staging paradigm. So, you can use this information that’s from a prognostic assay to look at tumor biology, help stage your patients. I mean there’s been a big call for this recently with the lung cancer staging guidelines, the new guidelines that were adopted in 2009, per that adoption there was a footnote at the bottom that said we couldn’t use molecular information but boy would we like to.

And here is an example of how that can be used. So you take patients that are low-risk, you bump them down a conventional stage; you take patients that are high risk, you bump them up a conventional stage. If you apply this to the UCSF training cohort of early stage I to IIa patients, you’ll see that doing that helps you immensely with risk stratification and helps you identify patients who are at high risk of dying from the disease and patients who will actually probably be okay after resection of their disease.

If you apply reclassification statistics, which is the new way to look at and compare these prediction models, you’ll find that with the addition of tumor biology, 70% of patients get reclassified, there’s an increase in the accuracy of their classification in over 20% of all these
patients, and the ability to distinguish between who’s going to live and who’s going to die improves by almost 25%.

So, in the future I think you’ll probably open a text book and see something like this. Instead of a TNM staging system you’ll see a TNMB staging system in which B stands for Biology. And whoever gets this test, you can do this now today with the assay that we developed, you can risk modifying, you can assign patient-stages based on what their TNMB profile is, B takes into account what the molecular biology of that tumor actually is.

And we do have a randomized control trial planned for the future. This is will help us identify patients that are high-risk and assess the utility of adjuvant chemotherapy in that setting. I won’t go through all the details in the interest of time but for those who are curious we do have a trial underway.

So in conclusion, I hope I gave you a little overview of the prognostic and predictive molecular biomarkers that exist today. They are here to stay, they are already being used in the clinic, and their future is very promising for the evaluation and treatment of non small-cell lung cancer.

We described a little bit the practical molecular aspect that was developed by our laboratory and then I went over the different ways that I thought were maybe clinically useful and needed development to this audience, and how prognostic assays can help us identify high risk small tumors, identify high risk stage 1 tumors for which the NCCN says to consider chemotherapy and they may ultimately evolve our cancer staging paradigms.

So thank you again, Dr. West, for inviting me to present today. Thank you to everybody who made this work possible.