**BRN Symposium, Advancing Cancer Through Biospecimen Science**
Transcript for March 13, 2008, 8:30 a.m. – 12 noon

**Welcome**
Anna D. Barker, Ph.D.

….That began my journey. I have gone through three phases, the 'gee, why doesn't everybody fix it' phase; the 'I don't know what you are thinking, why would you think about fixing it because it isn't broken phase;' and now the 'why haven't you fixed it' phase. We are getting into that phase.
>> In the truth is that journey started in 1999. I think it's 2008. This has been a long journey in terms of defining the issues, bringing the scientific community with us to the point where we find ourselves today when we all realized that this is not a simple problem. It is a complex problem involving virtually everyone who participates in cancer research and I would argue this doesn't relate just to cancer, it's other diseases. But it's been made so important the day we started talking about personalized medicine. I can't turn on my computer in the morning without getting an invitation to a biomarker meeting or personalized medicine meeting. In this town you can find three to five a week. That's great, but changes in the genome have translated to -- you heard from Helen interesting programs to sequence the cancer genes, also controversial. But as the data starts to come out of that, the major thing is bio specimen. The specimen we have, over 350 million this country, the quality of those, in so far as high throughput scientific endeavor is coming in that about 30% of those specimens qualify. Means it's very expensive, going slower than it should, and we have a lot to learn if we are going to deliver on personalized medicine. Also a learning curve in that everyone means well, believes that in their freezer is a treasure trove of high quality samples they want to own and not particularly share. When they find out only 30% qualify for something they want to do they are really willing to share then. Getting a lot more sharing now.
>> One area, to look at glioblastoma. Ovarian and lung following that. As we move into the possibilities of applying the sequence of the human genome to disease our questions got a lot tougher. To do high throughput science we are going to have to fix the bio specimen problem. That means my journey led me someplace where I met someone, and it's usually about people, who helped change the landscape as much as anyone this room, more than anyone I know. In a follow-up meeting or two I actually heard about someone named Dr. Carolyn Compton, lived in the wilds of Canada. I sucked her into coming into the U.S., joining one of these meetings. She's not a shy person, as you know. We were having lunch, and she said to me this is all very good, I am excited, this sounds great, but how you actually know Dr. Barker that when you are taking a specimen out of patient, into a sophisticated study, [indiscernible] study, say. How do you know you are not measuring the effect of the anesthesia on that tissue -- I said gee, I don't know the answer to that question, but in fact I did. We didn't know. For all the other variables we didn't know. So we quickly found out that in our endeavor defer and well meaning endeavor to collect bio specimen over many years, well intentioned though we are, we paid very little attention to questions that matter.

>> If you are getting a grant at the National Cancer Institute to do this work, I would put virtually at zero, can you imagine coming to a study section, how to bring bio specimens out of patients? Probably wouldn't be well regarded.
>> I promised money -- Oprah in the afternoons --
>> Some day we will deliver on some of those issues.
>> We have at NCI, over the last -- Carolyn arrived, there are a lot of people, standing on the shoulders of a lot of people, worked with us on the issue, even before we started in 1999, the national dialogue. Cancer was very contributory, publishing the national blueprint, national bio specimen, kind of a beginning wake-up call. With Carolyn's help, answering some of these questions, we are here to talk about how to do that.
>> We have money to do that. The good news is, the community is awakened regs we are in the why haven't you fixed it phase. We are all here n to figure out how to do that. How to ask, answer, be involved in terms of research and how to ensure we don't create new problems that might ought shine some of the old problems.
>> I really am glad you are here, glad you have an interest in the area. We need first-class scientists working in the area. We need the very high quality pathology world to be in this area, especially molecular. We need surgeons on board. We need the whole community working this problem from end to end. We need an end-to-end solution. We have a lot of you here and I am gratified by that. I think this will start a new era in this question.
>> There is -- I talk a lot, when I talk to groups about where we are in cancer research, we are in a unique position. We are at a crossroads where we can actually probably make maybe near exponential progress if we are right about these things. If the pro-- turns out to be the source of biomarkers we think it is, but if we can't measure from lab to lab, achieve -- if you can't depend on data you won't move toward personalized medicine. I was at a meeting, nano technologies, for example, able to weigh a cell, able to move down into -- ranges, well below for sensitivity to detect these changes. We will be able to measure very very very minutial quantities of what we want to measure. Even signals we want to measure, but you have to know what you are measuring.
>> So with that, I think we have a way forward now, and the way forward is, actually, I think real team science, based on very very solid biostatistical assumptions and the kind of work this group can produce.
>> David R has actually agreed to go, actually before Dr. Compton this morning. Many of you who know David know this is appropriate, a sign from God that this was it is way it should have been organized anyway. Many of you know David. It's a great pleasure to have him here, professor of medicine, epidemiology at UNC-Chapel Hill. During the clinical epidemiology -- research established the field of methods for evaluating. Held faculty position at Case Western Reserve University and Yale. Current -- clinical -- molecular -- collaborates with NCI groups, clinical -- one very close to my heart, seeing, I think enormous progress in terms of what it's going to take to do proteiomics and what biomarkers will become one of these days. David, looking forward to your talk and appreciate you joining us.
>> Applause.

**The Role of Biospecimens in Biomarker Research: Challenges and Opportunities**
David F. Ransohoff, M.D.

>> Thank you, it's nice to be here. We will discuss today the promise and limitation of cancer biomarkers. The disconnect between claims, products, and fundamental problems in study design, the most important topic. We have to address the problems of chance and bias, how to address them. There's many topics we could talk about. We could talk about this for hours. We are going to focus on one, the local of specimen in understanding and addressing the problem and avoiding disconnect.
>> We will start with the disconnect. This group stops at 2008, I talk with people at the FDA a couple of months ago to learn what's new, the curve doesn't look a lot different now. Here's what Walter Blackstock said in 2006. This is strong. I do not understand the surge of activity in the surge for biomarkers. The available evidence suggests that proteiomics, despite almost a billion dollar investment has so far failed to deliver any new biomarkers or commensurate returns.
>> These are strong words, but I hope he's wrong, we need to address it. This is a story in science, 2004, but much applies in 2008, thousands of papers reported results, obtained using -- multiple genes simultaneously, but are the results reproducible. The point is there seems to be a disconnect between claims and products in multiple --
>> There's a lot more claim than product we are doing things inefficiently. We haven't reached the exponential growth stage Dr. Barker mentioned we would like to reach.

This requires understanding financial problems in study design. In considering the problems we wrote it gutted by even more fundamental principles of science that we all know. This is going to have some work to do. Principals explained in this wonderful article where he says -- he makes remarks on science, pseudoscience and learning how not to fool yourself. What might be wrong with my results? Details you throw out must be given if you know them. If you know anything at all that is possibly wrong to explain it. This principle asked what might be wrong. This is what John said is the reason why some fields advanced faster than others. He asked this in his brilliant article Strong Science. He was looking at the field of molecular biology of the gene. Why does that field advance so rapidly? What he said was, this was the reason. On any given morning -- can you imagine walking through the lapse and looking at their plight or? It is either just invite letter of brimmer. On the next line will be two or three explanations, if the list of what he did wrong. Underneath is a series of suggested experiments are controls that can reduce the number of possibilities. He was saying that progress is based on considering alternative explanations and avoiding over interpretation. He said when scientists in the field return and ask what might be brought, that is when the field advances. We have to ask ourselves, are we doing that enough and our field? Now, you might say this fundamental principle is nothing new. He would be right. This principle is going to have specific work for us when we start considering specimens. We will start with chance. That definition, this is one kind of problem. In a multi variable predictive models we are fitting what seventeens being expressed to some kind of outcome. If we are fitting a lot of pieces to some kind of diagnosis. This occurs when a large number [ indiscernible ]. This may fit random variation within the original data that doesn't represent true relationships that hold for independent data. In this article he showed how this worked in situations where they've made up gene expression data on thousands of patients and then randomly assigned cancer and did my verbal analysis to see if it could fit, to define cancer versus normal. And found that many methods produced a perfect fit. It is by

chance. The basic problem is if he lets the computer run long enough it is fitting in at dimensional plane to your data and it can't find a fit. It is idiosyncratic. It just fits that they did that you drive them off on. The consequence is that the results won't be reproducible because they don't describe reality, and that method to demonstrate that the overfishing didn't occur, it is to assess reproduce ability and an independent group. If it was totally independent of the Mont -- of a group that you direct them are from. This is used to drive a pattern or a rhythm. You can derive anything you want.

You are generating a hypothesis. This is where overfishing can occur. You can't do cross validation, but this is all a hypothesis generations deaf. Then you must tested on a totally independent group, independent of the one that you train. You can't go back and recombine these things and do it again. To demonstrates that overfitting didn't occur. This validation group has to be totally and completely independent. This doesn't happen a lot. So we aren't going to talk about chance because we don't have enough time, but the status as this is a rookie mistake. And I know that because we've made this mistake in my field. This is what I'm seeing now in the field of diagnosis and prognosis and response. It is fairly easily avoid it. It is hard to get the specimens, but conceptually it is easy to address.

The situation is improving because others are becoming aware. The bigger problem that we're going to spend time on has big implications for specimens. Carolyn said, what about the method of anesthesia? This is the kind of crazy detail that can totally blind side you and mess up what you're doing. We have to think about bias. It is the biggest problem. It is neat. It's fun. We just all have to be aware of it. Bias is the main threat to the validity or reliability of clinical research. Bias is the systematic difference between compared groups so that the comparison gives a systematically distorted answer. It is wrong and misleading. For example, this is an article. They deserve credit for posting their raw data on the web and for being transparent so that others could look at this. There is nothing new that I will say that they haven't heard or discussed proteomics. This is per diem expense to identify ovarian cancer. Subjects were persons with and without apparent in cancer. This pattern was applied to an independent blended validation. And this is a schematic of what that looks like where you take your specimen here and your ion source. He might sign a laser on it and get these things to disassociate. They flowed down the tube at different rates based on mass and charge. They are picked up by a detector, and you end up with a pattern that essentially ends up being multiple variables that you plug into a multi variable analysis. And the results were that the discriminatory pattern incorrectly identified all 50 ovarian cancer cases for sensitivity of 100 percent and specificity of 95%.

It almost led to a commercial blood test until the FDA stepped in. So there was a lot of interest, but this bias explains some of the result as proposed. Was bias introduced by the wrong order of the specimens? If the cancers were run on different days spent in because of the data published on this, Keith was able to look at this and figure out the days that they were run. And if the mass spec machine drifts over time than it not biologics signal associated with cancer can get hard wired into results and both the training and

validation groups, signal is present. There is a difference between cancerous is not. It is just not do to balance your cancer.

For this kind of work he has been termed a forensic statistician as reported in Nature genetics. We know it is correct. Somebody who after a study is published tries to understand what was actually done. It took him at one time, a year even with that data on the Web to figure this out. There is a lesson here, if we need forensic statisticians to figure out what happened in a study it means our journals are failing. The scientific process some more is failing. This is the kind of detail that must be described investors and results, and we, as altars ideally -- this is an ideal. We should actually discussed this problem.

If anything might be upon you must explain it. We should all be trying to do this. Authors, readers, editors. A typical report has varied little discussion of what might be wrong. What is published in papers is often so incomplete that if I want to learn the details of the study methods I go to a Gordon conference and take the time for a walk in the woods. It is like taking history from a patient. How did you start? Where did you get the samples? Where were these blood drawn? What happened to them? Where were these blood drawn? If this is the way we need to learn about methods and do science there is something fundamentally wrong with the process. This is what are in site is supposed to be about.

There are many sources of a biased in a study of diagnostic test prognosis or response to therapy after specimens are received in your lab. That is to the right of this red line. When they are received in your lap cancer might be on a onetime patrol. The discipline that deals with problems here might be called laboratory science and includes quality control issues. Here is an example of a difference time -- kind of bias. Differential x a protease. And other birds is peptide patterns and not proteins. It is very sensitive and specific. This is where the signal of discrimination is. If this is right we have to pay a lot of attention to this outside the body.

If the compared groups are different the cancers are 67 years old. The controls were 35 years old and 58 percent were women. Now, this difference doesn't necessarily explain discrimination. It really doesn't. We talk now. But it is high in the differential diagnosis. This may look in retrospect a like an obvious and dramatic problem if that received virtually no attention from the editors in the original article. What might be wrong? This problem is arguably so important that it should have received extensive discussion and perhaps it precludes publication of such a strong conclusion. Now, this problem may look obvious in retrospect, but when I sent this article and supplemental material to the 30 junior faculty and fellows and the development program that I direct -- I sent them quizzes like this all the time. I said, can you find the pious? Only one person got it. I said, Margaret, how long did it take you? She said four hours. Even though it might look obvious to most people it doesn't leap out at you. This person had training in epidemiology and it was still hard for her to figure out. In contrast when I sent this to the National Cancer Institute he responded with an aborted struggle within minutes. The point is this can be very hard to figure out. We're dealing with different disciplines. We

are dealing with at different -- disciplines. Things can be confusing. It is hard to find people with enough expertise.

So this is an example of specimens received in your lap. We're going to talk about that in just a second. That there are differences as Dr. Barker mentioned. On this side of the Red Line to the left it is a clinical study design or clinical epidemiology. That discipline is different. So there is a culture clash and communication problem with different disciplines. Today we are going to scratch only the surface of the problem to be on the left side there are many really difficult Tobiases. This is what I and others have taught courses about -- like your long courses. There are celebrated examples that we all have to learn. His forms the canon of the clinical research literature. Here is the problem to be an even one can be fatal to your study. One group was collected under anesthesia and the other was and, if that hardware's your signal to a fast track.

That may make things hopeless or at least very confusing. Duane even one bias can be fatal in a way that you can't fix on the right side with any amount of laboratory analysis or buyout informatics as you might do. And rescission of the specimen, collection methods, it doesn't follow. It describes what she does and that your reader to side of if the problems were there. If the fatal virus is there because of design you might be stuck with it. There are very rare instances where you can do something. You might have misled the specimens. That is a new phrase. I'm a victim of the specimens during its think about it. When you have them in the lab your stock. That means you need to know what happened before hand. It has implications for this group. Bias is the main threats to policy and clinical research. The goal is to avoid bias. We understand where it comes from. When we design the study had is when you randomize. We aren't sure what might be all the sources, but we want to equalize everything. We want to avoid it. Avoided if you can carry as this is most important. That is why it is in bold. Then if you actually did what he designed --

We routinely ask people if he broke the blind? We blinded it, but that might have broken it. We have to find out if the design might have worked. In reporting you are transparent said that editors and readers can judge. You think about it and consider all of these things. At the end of the day it is designed to read it is the determinant. B, C, and D are about conduct and reporting of the design. If it as fatal flaws -- and I'm talking about design of the right of the line. You can't erase that by doing interpretation. How do we address problems?

Specimens are arguably the determining factor. Everybody wants specimens. Without the right specimens you can't even evaluate -- Discover or validate the marker that he might have. If a possible blood tests were created to mark it couldn't be evaluated because there are no blood specimens banks anywhere that I know of. Meaning from early cancer -- because that is what you want to detect. And collected before a biopsy which would very possibly introduce some signal into the blood. You don't have the right kinds of specimens. It is hard to collect fees. This situation for colon cancer would love to have the right kinds of blood specimens. They are very hard to get. With the right specimen's -- recommends clinically relevant. It is related to some three is.

We can describe that and write that up in the discussion section of an article. Whenever you collect specimens you want to think, what with an article look like if I discovered a test and burn it up? And you have to have some clinically relevant specimens and it has to have no fatal virus. You could do some amazing and unexpected things. In discovery, development, and validation. Look at this thing at the bottom and black. The person who controls the specimens controls the field. That is the opportunity. It is tough, but specimens are absolutely critical to both Discovery and validation. Let's look at things that might be done.

Some of the spectrum, but we can begin to think about it. Discovery and validation -- discovery means pattern or analyze, whatever it is you want as a marker. And validation -- maybe even FDA validation. It uses the same group of specimens treatise's this side, you do discovery. Patterns are an alliance. Heat test or validate that on the right. It all came from the same big group on the left. If this were is our right specimen, clinically relevant and snow blindness like a repository, it might be possible to do to fight and there may be others. Tutu FDA the validation from the same group. Not specimens, but from the same group. We don't have time to discuss how this works in detail. You can read more about it here. It may be difficult to get the right group. It isn't impossible, and it's something we need to think about aggressively and imaginatively. You could also do discovery and validation of multiple test simultaneously in a kind of bake off for everyone gets the same ingredients and then tries to find discrimination. Here is how to work. For colon cancer working with the early detection research network, I have just completed a small -- using this kind of approach. But others are starting to use this approach as well. Suppose you collected 10 mm of serum before the -- this is a big end. This would be extreme. Suppose that labs can do discovery on 50 my religious.

If this is a really big is the position. A lot of labs need more than that. With technologies like multiple reaction lot during this kind of thing might be possible. It is just a thought experiment. You could make 200 sets for validation with lots of controls. My wife is a chef. You give the chefs all the same ingredients involving 200 labs. And you ask, can you guys bake the batter cake? You might be able to use bank specimens. There is very important repository representative blood. There is no bias because there were collected and stored before the diagnosis. These bloods could be ideal.

We have formally fixed paraffin embedded specimens that can be used to assess markers are prognosis. And this is the source of specimens that genetic health used and [ indiscernible ] to validate or to assess the set of markers that date to arrive in some other area that resulted in there 2004 New England Journal of Medicine study. This is the study using already medicines be back on to a clinical trial that made the case for this might be clinically useful. This is how the test would behave in some relevant group of human beings. All right. These approaches are not easily [ indiscernible ] and don't apply in every case. They aren't impossible, and they are at least worth aggressively exploring. These kinds of approaches can't even be thought about in drug development. Market development is different from drug development. In drug development we use phases, but may be renewed to rethink that. Quacks and Drug research we have to do things

incrementally and a prospective member. We have to give somebody a track and see what happens.

We have to be cautious because people have side effects. It we have to study dosing and then efficacy. And markers is different. We can do cross sectional studies. We don't have to administer them. We don't have to worry about side effects. And because of those differences between drugs and markers e may have these approaches, one, two, and three, that are different. And an opportunity with the right kind of specimens for faster -- I don't know about exponential, but faster and more reliable because we have the right specimens. Discovery and validation. It may not work for many things, but for the field is at least work of thinking about. That way we can try to export these kinds of opportunities.

All right. So these slides have shown how if you have the right specimens, clinically relevant and not by as you might use them. Let's discuss a different topic about specimens with very practical implications for the specimen collection that is the concern of this group. This is the main point. By that time specimens are collected the study was designed and down to read you may plan to do things and the laptop but you have already done a study. They are just specimens. There was a study that preceded that.

By that time specimens are collected you have done the whole clinical study to the left of the bread line. Now, a lot of what this group is concerned about is, hat happens at the Red Line when specimens are collected? And what happens to the right? This is important. But you have to realize that you could be a victim of specimens if Bias is hard wired and. If it is fatal and serious you may be stacked. Don't get blindsided by what is on the left side. He meets and ask, do you know where your specimens have been? Do you know where your specimens have been? And will you be able to describe those details and the masses and considered the impact and the discussion of when you buy out the article? Because that is what you are going to have to do.

There is a big implication that if we are going to have to describe and discussed that we need to design its rights in the first place. It is not to specimens. We have to think about this as if when we collect specimens that there is one specific research question we are answering that we are to have to describe in the methods, results, and discussion. We have to think about it as a study. It might be for multiple purposes, but it is a study. Guidelines are for reporting. They tell us what to report and information. These are the guidelines for reporting status of diagnostic accuracy. Markers are for reporting recommendations. Miami, my great experiment. All of this is about reporting and information.

Is telling you about design. And guidelines apply to both sides. It isn't black and white. The cream applies to the right and the blue applies to the left. There is a lot of reporting that we might do. This is one example of the kind of relevant detail you much reports to the left of the red line and a study about Siegel DNA markers. For colon cancer screening we don't need to always be so detailed, but we can't totally ignore what happens on the left. This is based on what we know about biology, the major focus of this group. Steps

must be taken to avoid bias including things like uniform handling. On the left side there is a parallel set of concerns, generally less familiar to this group, I would expect.

We are down to the last few slides. Avoiding bias is for some later phase of research. If that is what you're thinking, think again. Bias is particularly important in the early research you might like to do. My friend, the inventor of a medical diagnostic designs that is now a billion dollar business, knows how to do development. He says the worst thing you can do a is a week where you have early study because you set off in the wrong direction and it takes so long to figure out what goes wrong. In 2008 we might turn conventional wisdom on its head. That phrase of my colleague and early detection research network and use high-quality strongly unbiased specimens as early as possible and development or discovered. Other topics we can't talk about. No time. The role of journal is the systems of correcting. I don't think it is nearly as efficient as it could be. What is the roles of incentives? Are they working and they're right or wrong direction? This is the last slide.

There is promise and by markers for cancer because we know so much biology. We have wonderful tools. We can measure anything we want about the biology, but there are some limitations. There is very large disconnect between products that are related. Deaf threats to the legacy. Specimens have a critical role and provide a huge opportunity. The person who controls the specimens controls the field. The left side is done. And to improve productivity and efficiency of the entire process of discovery and development. I look forward to the work of this group which has that opportunity. Thank you very much. I believe we have time -- I believe ee have like 10-15 minutes for questions. You tell us when to stop. Questions and comments please.

Thank you for your lecture. I thought all your points were very good. I have two comments. Forensic epidemiologist should talk to the scientists of the study there criticizing because in that case the criticisms were based -- and those were returning studies to look at variability. Notwithstanding potential source of bias in any study, at least it would be good if we -- a forensic epidemiologists actually talks to the investigator to get the right data to analyze. That is very important because it causes the individuals and our field to be shy about posting their data on the Web. We need to have that. A second issue is one way to avoid bias or to validate your bio markers. Measure it with another independent method. If you have a mass spec the best thing to do would be to identify that protein and then measure it with an amino acid and another independent method. You didn't touch on that for a time, but but we would like to do in the bio marker feel this may be discover and prove you can measure it with a totally independent platform so that you know the bias isn't due to how your individual system works. At least for that for the piece we identified now and chemical to five clinical chemistry we use a different method to identify in sequence. So that was a way to validate a study that may have been questioned in terms of bias. So those are another source of we don't know what the proteins are. So we identify and sequence proteins that we can measure by routine immunoassay.

Thanks. I appreciate your comments. I think you are right about communication. I'm worried like you are curious. You guys get you produce points for posting information on the Web. That is one of the problems we have in science we need to manage. Regarding how Keith interpreted the initial study and whether you were trying to shepherd the disability, I think what he was concerned about. And I know this because I've lost a close when Keith read his articles and you read the response. Tiber to editorial and got pretty familiar as you know. We got pretty familiar with the details. What he was concerned about was that the People's take-home message from the original article was not just about sources of variability, but here is a test that we think really can discriminate ovarian cancer versus not. And that was the message that she was concerned about. And some of this relates to what was the intent and what was the conclusion? With regards to the issue of showing that bias didn't happen, that is a very important principle. The specific example was -- and the much larger picture the kind of thing that needs to be reproduced is Canada's permit varying cancer versus not in another group and also where there isn't a possible by desk. Sometimes the same biased it's repeated. Buyers can be repeated in in multiple studies. Just reproducing it somewhere else may help, but -- and it is very complicated. We just have to keep all of this on our radar screen. I appreciate your effort. Yes, ma'am?

Thank you so much for your comments this morning. So many of them are just absolutely right on the mark. Particularly when you talked about the disconnect and the disconnects in your field as it relates to science. What I thought was interesting is the example you gave about pancreatic cancer. Even if we have the right tests today the samples don't exist. And that is an interesting -- that is an interesting example of the disconnect that exists in the world of science because the reality is there actually are some good sets of specimens, but yet everybody does not know where everything is. So there is a major disconnect with even knowing what is there. So if you had the bake-Off, who has what? So there is a consortium. I appreciate your bringing light to that fact because it is a lot of this other fundamental logistical issues that are going to be as much as a barrier to accomplishing the science as it is the science itself.

I think you're raising a couple of important points. One is there is awful lot of the specimens as around that we don't know. I am skeptical. I think this is worth aggressively pursuing. I'm concerned many of them won't be useful. I think aggressively cataloging and looking is extremely important. My career has been based in part on when I have a question I look around the world. I have some fascinating stories.

You have to have an idea about what if I found it? What I really want to maquis have to go into it with an idea about what you are looking for. Just having a big and with lots of different groups isn't going to solve the problem. Coming back to the point is that doing this -- you know, every PRG says we want specimens. It is very unsexy. It is very unglamorous, and it is the kind of thing that is a common thread. This is hard to do and there isn't a lot of incentive. I don't know whether some of the strange and wonderful things I described, how many can be done, but that is the kind of thing we need to think about to motivate us, actually finding or developing those specimens such as a real challenge. We simply need to take it on.

Just as we have individuals with experience in statistics on editorial boards and so on, what is your view of having more individuals with experience in pathology, molecular pathology and so on on editorial boards because I can tell you most journals of Anna. Those are the kinds of things that many investigators in this room are getting hit with.

This is, what sort of expertise we need in journals. I think this is a really big problem. It is more than molecular pathologists. As a clinical epidemiologist interested an observational epidemiology we don't randomize people. A lot of that expertise and my shield can apply here at the molecular markers. It is just like a blood glucose. The guts of the study is a crazy Apsaras saw [ indiscernible ] that gets done. There is a lot of different expertise. I see in manuscripts that are received from good journals or that it published, it is like there is just a gigantic gap. I periodically talk with journal editors. It is much bigger than just one field. This represents a serious problem and transitional research. If we have the release sophisticated labs stuff that intimidates people like me until I took a sabbatical and learned about molecular methods. I was terrified about this stuff. --

To figure out how to do my sabbatical and went to Oliver Smithees and I said, here is what I want to learn. I said do I have to get lost in the details? I figured out how to solve the problem, and it isn't that hard to learn about molecular methods. I could not do them, but they are easy to understand. It is not hard to cure me out to understand what the methods work, but until I did that I was intended. If that was the problem with having a regular epidemiologist during their review. I was intended.

People in the lab down appreciate or think that anything that happens to the left of the line is important yes, sir? Is a big problem and we have to work on it over the long run. Yes, sir?

I appreciate your comments. I am all for increases in position, however there is a limit to what can occur. He gave the example of the [ indiscernible ]. Well, I am not sure because I wasn't part of the study, but I estimate is standard fixed steady. The materials were from a variety of different hospitals was probably use different buffer formulations. Is this was probably set around for different periods of time before they got fixed. The processes were different. My point is that the reason why they are so successful is because they chose a panel of an alliance that are robust enough to actually accounts were still be present despite all these variations. My one concern is that we send us to much on the research specimens, the ability to transmit to actual clinical practice must be hard.

It is a problem. They did have markers that were robust enough to reach with regard to the source of specimens this is from an NCI clinical trial, and I don't know the details of how different filters is interesting, but here is how you look at that. There could be differences from different centers, but if the study is like it was his date collected the specimens before anybody knew what the outcome was going to be because they had to follow people overtime. There is no bias. It is nice. Noises were you have differences between the centers, and that may reduce discrimination if it's there. It isn't a systematic

Association of the annoyance with one group versus the other. In other words, if one soldier had all cancers and the other half are controls and they had different methods of standardization. That is impossible. The point is you don't have to have total centralization. That could have had some nice job but they still found a signal. It is systematic differences during cancers and controls. Things can be a menace that way.

Cris Carter from George Russians and university. You have not mentioned that samples --

Pardon?

Matched samples. To what extent have you had cancer from a mastectomy and adjacent normal sense for proteomic analysis. What are some of your biases? Some of the mass spec --

We need to talk about specific details. You might be able to do it. The problem in general is using this is -- in general. It depends upon the specific question. You have to think about what could be wrong. When you are trying to [ indiscernible ] you might not know all the things to match on. It might not be able to. Whether it works that way depends upon the idiosyncratic things and the question you are answering, but for example we can use these if we are trying to understand therapeutic efficacy. That is why we use transition to make everything equal.

I'm talking about matched from the same individual.

Depending upon the question that could work. It depends a lot upon the details of the question to be if you have to ask the things that could be wrong. In general you have to be cautious about matching samples on things to the left of the line is we don't know all the things to match for. If that is widely used transition. We have time for one more question.

At least for tissue best specimens we really have no standards of normal. Normal, some people with cancer are obviously not really normal. Obtaining truly normal tissue from molecular studies especially when they are related to the desperation of exposure and racial demographics and menstrual cycle difficulties and changes in women and so forth, all of those things are really a very high part that I think it could to beat a lot to getting more specific by markers with a good positive predictive of values.

Good. Thank you. I think I'm turning it back to you, Helen.

**Introduction of Carolyn C. Compton, M.D., Ph.D.**
Helen M. Moore, Ph.D.

Thanks, David. I think we -- do we need to take a few minutes to load up? One minute to load up the next presentation.

If you can sit down we can get started. Sorry, we had technical difficulties. It looks like this is going to be an awesomely conversationalist group which is a good sign. First of all, thank you. Thank you for setting us off on a good path. I will add one thing. I know he told you to think about where your specimen has been, but I really am concerned about thinking about where they're going to go. And I think as we talk about this convergence with the advanced technologies that are really asking the questions we want asked, which we are attempting to do here increasingly is interrogate the complexity of cancer. And that is going to involve, I think, enormous changes in technology.

We are seeing this in genomics, as you know. If we are just getting ready to shift to new economic platforms which will happen in proteomics. David is working with us to standardize some of these issues. Our timing on this actually is perfect. At which it happened five years ago. We have some ketchup to do, but we can. I mentioned before that Doctor [ indiscernible ] had been [ indiscernible ]. Many as you know -- I hope that she doesn't need a lot of introduction. But Carolyn is a wonderful person. Nearly everybody in this field has to be hard, but I have a lot more admiration for people who work from their heart. She understands the issues we are dealing with. 1500 people will die from cancer today. She is close to those issues as a colon cancer pathologist. She trained well for the position she is holding. She trained at Harvard.

At Harvard she moved on to become professor of pathology and director of the gastrointestinal pathology. Her interests are quite broad. In terms of transitional science especially, most of you know her as an expert in colon cancer pathology and pancreatic cancer is another interest of hers along with one of hers as well as mood healing. She is absolutely untiring in her dedication to the professional groups that are attempting to move these fields forward, especially the College of American Pathologists. The study of chemical oncology. Before coming to NCI she was professor and chair of pathology and pathologist in chief. She came in knowing full well the magnitude of the issues, the difficulties of the problems we had to face. And she has done that with enormous energy. She works from the head and the heart. I am convinced that if we are going to solve this problem Carolyn is going to help us get there. As I said before this is a position we have had for a few years now, and we are well on our way to doing something about it by putting some resources behind this finally and engaging the broader community to actually begin to put some real substance onto the field.

It is our pleasure to have you up here with your presentation.

**The Importance of High-Quality Biospecimens to the Research Enterprise**
Carolyn C. Compton, M.D., Ph.D.

Thank you, boss. I'd really appreciate David going first because in point of fact I've left my presentation and my car, and I don't know whether that came from the head or at the heart, but they ran back and got it, and here it is. I appreciate your indulgence. I meant this to be an introductory talk. To set the stage to make certain that everyone in this room understands what we are here. We are not actually here because of bias specimens. We are here because cancer is our number one health problem. Cancer is the number one

killer today. One American dies every minute of cancer. Over 500,000 people will die of cancer this year. A million and a half people will hear the words, you have cancer. The current rate of incidence of cancer in this country, one out of three females and one out of two males will develop cancer in their lifetime. Look around the ground.

We are all potential cancer patients. We spend almost $200 billion a year on health care costs for cancer on. And when we consider the yearly budgets which is respectable, $4.8 billion declining unfortunately per year invested in transitional and basic research. It is a proverbial drop in the bucket when you consider that $16 billion a year spent on cigarette companies for advertising. So we have a substantial flow we are fighting, and we are now developing powerful tools with which to fight it. This is a new era of molecular technology which promises to transform this field. There have been many articles in of the late press and in the professional press about this to alert us to the fact that we now have the tools we need to evolve to an era of molecular oncology.

Advances in molecular technology and research will move us from where we are now, where we are diagnosing disease after it has established sometimes, where we are making more for the diagnoses and classifying to rest in its typically. In the same way that we have for the past 100 years we are using generic is our critic regimens with unpredictable adverse events. This is created and patients sometimes making them worse rather than better. We are evolving to a new way to look at cancer. By its molecular biology and the most fine and precise sense to use that knowledge for early detection and prevention, molecular characterization of tumors to which we can design targeted their peace and we can actually predict adverse events and therefore avoid them based upon our knowledge of the underlying molecular biology of the patient, AM or herself. So we are moving toward a whole do understanding of the biology of human beings as well as the biology of their disease.

This is our view of this process. It starts with the patient. And a collection of it by a specimen from the patient's which is essential for molecular medicine and translation of research leading to molecular medicine because, in fact, that is where the molecules are. And from that patients specimen processing and packing of the specimen, use and molecular analysis, it comes back to the patients in the form of transitional research that will in fact lead to new therapies and more than new therapies, it will change the demographics. It will change the outcomes for this disease. So in fact in a world of personalized Madison the buyer a specimen becomes the center of the universe. It defines the molecular character of the host, the disease susceptibility, the treatment efficacy, a whole new field has grown at around this approach of predicting reaction to therapies based upon the underlying molecular biology of the patient.

Molecular characterization of the disease while -- and I and looking forward to this. This is a whole new way to characterize heterogeneity which has been the bane of our existence. And hold new feels that didn't exist when I was training have grown up around our powerful technological ability to look at whole class is up by molecules all at once. When I was training and had my first lap this was about looking at my favorite molecule or my favorite molecules one at a time. Now we can get them all together. Not only that

we can look at how they interact with one another in a metabolite mix approach. If so fill in your favorite class of molecules. You are looking at a field which allows you, through technology suit SS whole class is of molecules at one time and in fact this leads us to something that I have found Carolyn dropping.

I am certainly not an expert in the field of human knowledge, but there are experts who just study the evolution of human knowledge. One of the most impressive statements that I have read it was an the law of decelerating returns. We won't experience of 100 years of progress in this century. It will be more like 20,000 years of progress at today's rate. Dr. Parker of the attitude this acceleration of accumulation of human knowledge. And this is mostly due to technology. So the technology development has accelerated our difference between produced data.

And we can turn data into human knowledge. So in fact this will become the era of biology because of our ability to probe biologic systems in a way that we, we never before have the ability to do. Service has been estimated that scientific knowledge to five all scientific knowledge up to this moment in time will double in the next three years. And biological knowledge will double and the next five. And that some of all of human knowledge is just 1 percent of what it will be in the year 2015. So this is pretty impressive. That is, the ability said use technology to our advantage, to advance our knowledge of biology of human beings and the biology of their disease, but with this power comes powerful risk. This is what we're here talking about the topic today. Where have your by a bio specimens been?

Where has that object and that houses all of this important data which we can now probe. But what is the quality of the specimen? This is the risk that we now take because we now have the technological capacity to produce low-quality debt from low-quality and alights from low-quality biospecimens with unprecedented efficiency. So now we can get the wrong answers with unprecedented speed. And in fact this is partially Burberry are today. So we are in this room at the right moment in time to avert this disaster. Unraveling the mass of metrics of misleading data may in fact compromise progress in unprecedented ways. The faster you go the behinder you get. And this may be in fact where we are today with our technology applied to this paradigm. This is something that's was a logo, a phrase invented by the computer industry.

But in fact it is all too applicable to every field of science. In fact at the end of the day it doesn't matter how smart your investigator is, how wealthy -- how wealthy or Cancer Institute is and your investment in cancer research. This paradigm still applies. If you don't start with the right stuff you are crunchy and output data that represents trees. That is what we are after. We are after the production of data for its own sake. We are after Protection of data that will benefit cancer patients. So we have taken steps towards -- the very first baby steps towards taking out the garbage. And in fact, that is why I came to the National Cancer institute. It is a big job. There is a thought of garbage.

And in fact it has been piling up in the back of the Institute for a long time. I only now need forklifts to remove it, but in fact it is daunting task. That is why this issue of bias

specimen quality has incurred in this dark shadow at the edge of our science. It has been too daunting have fought on how to solve this problem. To assume that it isn't your problem but happens and somehow get ahead in the sand approach is the one we ended up taking. So we are looking at this issue square and the eyes. We want to standardize the quality that will drive the development. Human beings aren't standardized and the specimens are even less standardized to read this is the raw material that is driving the personalized Medicine carried is an on standardized product on which we must confer the standardization lest we end up with non standardized unusable data. We need to remove the barriers to cancer research represented by this limited availability of high-quality platform appropriation in by assessments.

If this is the single biggest bottleneck to progress the onus is on us to remove it and to do it immediately. It is a moral imperative and we need to do this to lay the foundation for tomorrow's standards of care. Because what we discover that is true is based on the technological platforms and the standards of the analysts that go into those today, if it represents truth and if it benefits patients will become the standard of care for tomorrow. So that is in fact what I feel we are doing.

We are taking this approach and a stepwise fashion. We first had to assess the state of the science as it exists right now and [ indiscernible ] specimens. We did this with your help, people in this room. We have developed a state of the science guidance. This is now NCI policy and was accepted as such by the two boards that govern activities. We are using this as a platform to go forward to improve the state of the science using this symposium as a start point. We want to create -- in fact, the NCI best practices are not evidence based in total. They only represent the evidence such as it exists at this moment which is a very scanty. So we want to legitimize the science of by specimens, their study as biological entities and use that data to improve the standard operating procedures for processing, accessing, and storing by assessments for the research enterprise where want to use these standards to harmonize by a banking practices across the NCI. And we are doing that in all of the venues that UC.

We are doing this and institute wide practices. We are partnering with accreditation bodies to ensure that these standards are implemented because as I have learned -- it took me a long time to learn the following friends. We are the NCI. We are not a regulatory agency. We don't regulate anything. We can't make anybody do anything. We can entice them to do things, things went attacks, but in fact that people with the sticks, the people that check on compliance are really strategic partners with whom we need to ally ourselves. And we are. And those would be people like a professional body like the College of American Pathologists. To the point brought up here in the last discussion session, yes, we are using specimens in clinical research based upon -- borrowed from the clinical enterprise. But we also need to be sensitive to the fact that the clinical enterprise may need to be changed. Enterprise itself may need to be upgraded.

To serve patients better remain need in the facts to partner with the professional bodies that have the ability to do that. You are helping us here today and we are facilitating the

creation of a scientific evidence by bio specimen activity, procurement, processing, civilization that will enable us to move forward and find the truth faster and more efficiently. So we are building better bio specimens. That is what we are all about developing and implementing data driven for as much data at that exists. Processes that answer the integrity used in cancer research and medicine. This is our vision, and you will see this little diagram in other talks from my teammates. You think about this when you talk about where you're by specimens began Carias they started out as being part of the anatomy to read and and separating themselves from the patient's anatomy. These are non physiologic. And in fact the most powerful is it was stress is known to human beings is encountering the surgeon.

This is like getting hit with a Mack truck, you don't feel that because the anesthesiologist has already given you a drug that is so powerful in terms of changing our physiology that you are now unconscious and in some said. So clearly this has some kind of physiologic impact on every tissue in your body. And then the surgeon removes the cancer, the tissue by the vast horizon and, cutting off it's parts supply inflow and outflow. Now it has no blood supply and it has no oxygen. And then is removed from the body and it changes temperature. It does from body temperature which in a biologic cents  is absolutely critical to maintain within a very narrow range. In fact if everyone in this room were reduced to a room temperature rebut all be deaf. So this is a stress of the first quarter. And then it comes out and sets on a bench.

It is cut into different Alcotts what's cool and desiccate at different rates of speed. It is put into different stabilizers which penetrate at different rates of speed. So in fact the cells that encountered this on the outside react to that in a different way than the cells on the inside. So all of the things that we are doing to is up by a specimen, the question being, where has it been? It has been plenty of places Terry its normal human beings never go. And we have absolutely no concept. While this is going on the specimen is a living entity. Until we suspended by fixing it or freezing it, it is living.

So in fact it is capable of and is in fact reacting to all of these stresses. So its changes the molecular composition of each by a specimen in ways that we don't yet understand but that are critical to understand if we are going to separate the artifacts from the biology of the disease. So all of these pre acquisitions variables that happened in the operating room and the Post acquisition variables that happen in the pathology sweet, a short list of which are shown here need to be understood.

And it is also the vision -- and this makes perfect sense because not all of your organs are the same. They are in fact designed to react differently. So in fact, all of the difference desman types shown down here -- the fact that this is a quite abbreviated list because all normal tissue -- each type of normal tissue should react in a different way. Your brain doesn't react in the same way as your liver or your bone to these different stresses. So in fact we need to understand how those variables that were on that previous list effect each one of the specimen types in terms of the bio molecule classes which we can now so precisely detect and measure in the different platforms that measure them.

There is a lot of knowledge that we must gain in order to fill and this matrix that is specimen tech dependent by molecule tech dependent. An analysis platform attack dependents. So in fact we need to fill and this ice cube tray. This has become an icon for our office, the ice cube tray. This is what we intend to understand, how each of those variables on that's previous list actually affect your ability to detect these molecules and these different platforms in these different issues. Now, this is very complex. But it isn't infinitely complex. It is absolutely critical that we understand this if we are to go forward. And this is the visual that I would like to leave you with because in fact I think this is where the scientific world has made its big mistake.

We think of the patients with cancer needing tickets insight into the patient's disease as taking the specimen which we regard as many. We equate that specimen precisely with the biology of hat pace is deceased. We never take into account the fact that despite a specimen has reacted to this panel plan of powerful physiologic stresses that have in fact turned it into something else. It is no longer in many me. It is a unique biological entity that has endured physiologic tresses that note human being -- in fact it physiologic stresses that the buyer specimen interest are incompatible with human life.

They are extreme and extraordinary and they will change the molecular composition of those living and reacting cells. S o w e need to understand how those affected by specimens and understand their unique biology. In fact the reason we are in this round is because this by a specimen as the object of research in and of itself -- it has never been regarded as a valid form of investigation. It has never been fumble. It has never been publishable. Yet it is absolutely critical that we understand this biology if we are to change this paradigm of the taking of the molecular composition of the bio specimen through the transitional and personalized medicine process in order to benefit the cancer patient. We have to understand what the differences, or we will never be able to get to this vision of being able to treat the patients disease with specific, exquisite, targeted approaches which is the hope and the possibility of the future. I thank you all for being here this was meant to be an introductory talk.

We are here to discuss and legitimize this new method scientific investigation, to talk about what it is critical. There is going to be a lot more discussion on this. This has already created major problems for the brightest of our investigators across the country. Why is crucial to start now and what is at stake and to the stakeholders are. They are to scientists. They are the patients, and that includes all of us, as I pointed out. All of us potential cancer patients. We are here to discuss how to begin, how to move forward, and how to integrate the science into the fabric of medical practice because that is, in fact, what we will change the world with. And it is time for change. There is this famous quote, if you always do what you have always done you always get what you have always gotten. And the rate of progress in cancer research is not going to change until we address this fundamental problem. What we have an our banks and what we are doing as we sit here is no longer good enough to fit the needs.

In order to serve patients we must enable chains that will change the world of medicine. And removing the single most significant barrier to the development of personalized

Madison. I'm grateful that you are all here. I'm grateful that you are all willing to talk about this and to participate, not only in defining the problem, but into designing the solution. I open the floor to questions. Thank you very much. Questions? Comments?

University of Virginia. I agree with everything that he said, but I just wanted to point out that before we throw out his histo morphology --

I would never throw it out.

It has been my direct observation and personal experience that the majority of garbage actually comes from a lack of quality control. Those are still very important things that must be considered.

No question about it. And one of the things that I actually pointed out that the recent meeting that Doctor Parker refer to, we were talking about cutting edge ideas and biology has seen the three that eyes of the chemists, physicists, and other scientists who have never applied their methods of thinking to biologic problems. But one of there reasons why histology has endured as the current standard of care for cancer patients is because it is so robust. It is a technology that is over 100 years old, and it has changed very little. And I would defy anyone in this audience to give me another example of a currently used standard of care of medicine technology that is 100 years old. In radiology they are barely 100 days old. The technology perhaps so rapidly. It costs only $0.25 to produce as slide. And they both turned pathologist can derive more clinically useful information from that slide and deliver it true interpretation, their medical knowledge in a way that is -- in a way that is par for the importance. In fact, in terms of cost effectiveness I would defy anyone to show me a better example.

The stethoscope.

The stethoscope. Good. That is good because we are using phenotype. We are looking -- we are looking at there shapes and sizes and patterns.

[ speaker unclear ]

Never. Under the microscope, and we are able to -- we are able to classify the disease and predict what it will do based upon is phenotype. But now we are able to look beneath the phenotype. What makes this a look that way? And what makes it grow and those patterns? When we are able to look in a subcellular, sub molecular, sub anatomic level then you are raising the bar for at least stopping the action at some common points where all of these technologies will give you the right answer.

A phenotype is so hard. Pathologist's know this. You can take the specimen that leave it sitting out. You can drop it on the floor. If we can put it back and still make a diagnosis. But in fact this is not good enough. Not that we will ever -- I think this is the tree on which the Bosnian to behind. If we will never give it up. In fact, people understand it better.

Carolyn, President of American Culture Collection. I want to talk about the business of bio specimen banking. I will give you some sobering data related to that. If you to a process diagram of bio material management's you can distill it down to six letters. AAPPDD, acquisition, authentication, production, preservation, development, and distribution. To read those are -- the nibbling parts of that system. 90 percent of the costs is in double A. Acquisition and authentication. The reduce the cost of an acquisition of a tissue culture line to 15000-$12,000. Hopefully voted it down to nine. 90percent of the cost is all centered on the acquisition of quality specimens.

The second sobering fact is recent data. 30percent of all by materials is invalid. That is conservative. We've really have a serious problem. You talk about the basis of the problem. How are we going to deal with the financial logistic issues related to the is cost analyses and getting support for what you want to do?

I'm so glad you brought that up. Because this is one of the major initiatives that is now ongoing. We are very interested and the economics of by a banking. And it is very difficult to five in fact, we first had to address this issue because our best practices stakeout right that we do not approve debt we don't support profit making. But we do support -- in fact, encourage cost recovery. That presupposes that you know how much it costs you to acquire and store a quality specimens. In fact my getting that information is very difficult.

The way it is time to find these come from real people. You get them from real people because they enter the medical enterprise. So they come from an institutional culture, working economic setting where the business model is quite different from place to place, from state to state. And it is subsidized and different ways by different groups within the hospital or institution. So it is quite difficult, in fact, to cost account when it comes to the collection of the biospecimens. But we are working quite hard on trying to [ indiscernible ] as this to give guidance to the community when they're trying to figure out how much it costs. And how much lesser charge investigators to come and use their collections in order to sustain themselves.

They themselves need a business model to sustain themselves over the long run. They need to know these things. We are also working with medical economists because the foot side of that question is, what will it cost you if you don't do this? And the investment in clinical research -- I mean if this is what curls the toes of the board of NCI. Suppose we are investing $5 billion a year in research. We cannot afford to do this -- not only can we not afford to do it because it is waste of money and it compounds the field because 30 percent of the data is wrong. Now we have to go back and revise the text books.

But we aren't making progress against cancer. And that is why the cancer institute excess. So the cost of not doing this are also extremely important to compute. I hear here, it isn't insignificant and it is an inexpensive, but PRI and fact it is worth the investment. Otherwise you are wasting your money. As per David's point, it doesn't gain as anything to be producing data that is biased, incorrect, and [ indiscernible ].

The largest source of problems is the undocumented sharing of materials between investments.

[Audio and video feed have frozen.] goal that we have, and not only -- everyone in this room I think is here because you are the choir. So we are talking about, this is a chance for us to talk about this together, but we don't need to convert it. What we need to do is to reach out and educate those people who aren't singing in the choir gets, and I can tell you. Everyone in this room is not delegated because we can't do this alone. You have to have help us. The awareness of this issue. Educating and training is what this is going to take. It is absolutely correct that this has been regarded as beneath contempt. People won't even treat it with the seriousness -- and yet it underpins everything. So it makes no sense, and we really need to change people's thinking.

Pending hearts and minds is what this is about.

A comment on that. I think we all want to change hearts and minds. Over some perio d on time we will do that. If there are emerging some critical policy issues here that really go beyond changing hearts and minds. I am not so sure when it comes to this issue we are going to have choices. You all remember HIPAA with no good feeling. We don't want to have the same issue occur here rehab superimposed on us a set of standards that may or may not be in the best interest of science. But we are working hard to do is to get these evidence based Best practices developed. It is a real time process but to also make it is important to our current process as to taking appropriate care of animals -- I would argue that taking care of samples from humans is every bit as important or ensuring that your clinical trials infrastructure meets requirements. If so I think over the next several months you are going to hear more about this. I am hopeful that this will become part of our granting process in terms of award.

And of course I'm going to hear back from everybody with an unfunded mandates, but I can tell you we are spending tens of millions of dollars collecting samples. Should not cost us that much more to do is references to it that way we are doing it.

Come up to a microphone because we are telecasting this.

Be are having trouble getting funded, and in CI actually started a new study section which is harder to do that and amendments to the constitution. That is called the cancer bio marker study section. I was on it for four years. There are some other people in this room who were on it. I got routed off because you can only be on it for so long. It is bunch of people like us who care about what we do. In fact many of the things David went through were critical to the study section. So as people who said, it isn't going to be easy. These people understand what we do. I have to say that pre analytical issues and banking were occasionally but rarely discussed, but we are starting to be on the radar screen as we move that is especially true for things like proteomics of plasma and all other regions. So this is happening, but even better guidelines will be quite valuable for that committee which is buying to beat the critical committee.

This is going to be the convergence of a lot of different efforts. It will come through the grant process. It is going to come through the College of American pathologists who are not in discussion with us about starting a fire repository acquisition program. It is time to come through the American College of surgeons who on a hopeful that have asked me to give a keynote at the American oncology Group annual meeting in June because in fact that surgeons themselves think that -- they only focus on the patients during the operation. The specimen, once it comes out is beneath their notice. It goes into a bucket, and they sit in that pocket for hours before somebody perhaps it out and takes it to pathology.

The point is that there has to be a change in the third vision and a recognition that their responsibility to the specimen is just as great as the responsibility to the patient on the table. In fact, their ability to take care of the patients properly after the operation is going to be determined by the integrity of the specimen if it's in fact house is the molecular data that they need in order to administer therapy. So this is going to take a massive mine shaft, and as Dr. Barker points out a few sticks as well as carrots. Jim?

As you are kidding organized I encourage you to broaden your thinking in terms of the band with. Right now there is very near a needle through which this passes, and that is the policy diagnosis. Fans moreover the specimen is harvested at the end of the disease which is when it is taken out of the patient. If it is necessary to take, whether it is molecular or spectral data and maps into the patient population before a diagnosis is made. Because at some point clinical decisions can be made without a diagnosis and somebody is going to to have to be brave enough to act on the molecular signature and optical signature.

So this by repository effort is really at the tail end of a patient population management turf, and I would encourage you to think about broadening out through which he passed information.

And we think about this, but that can only be brought on the basis of this has to be validated. Court has to be validated, no question. Even pathologists have to be thinking about running back. If we talk on the patient.

Fair enough. One more question.

One quick question about the big carrots and sticks. I am a molecular biologist and I worked very closely with pathologists and surgeons and by a specimen collection. One of the things that I have been seeing that for breast specimens at least they're really may be in molecular and point where the specimen is set on for a Micro rates steady. It's a question of liability if it isn't properly care for.

Now, there is a stick.

Absolutely. That's got everybody's attention, and this is beginning to be a conversation in the background for prostate as well. So one of the things you might consider is introducing a building code for collection of molecular biology quality material so that you aren't collecting just were the standard H and E3 you have another level of care, of fiduciary responsibility that you can then build four. I did the surgeon in the operating room where the clinician at the outpatient center. If it is biopsy or wherever it might be, that, I think, gives you -- you can document the fact that this was done properly. If there is some sort of objective and point. There is a mechanism to get reimbursed, and the liability issue is covered.

Point about taken. If to that point when it was quickly on one of my size, but the initiatives, and that we are integrated and is called the Interagency on policy task force which will bring together the --

This will bring together the FDA and CMS on these issues in making determinations and paying people to do something so labor-intensive but require the expertise. And what we're talking about in a more global sense, my parting words to you is a study of how to change professional behavior. Coming from the world of medicine, where professionals can be conservative. Because the stakes are high. Is life and death. So, in fact, the way that you change behavior, it is limited to it is possible through three mechanisms number one is that you can pay people to do its and people will do what they are paid for and number two is that you can link it for accreditation or licensure. In order to practice they must do it in a certain way. And the Number three, this is the hope of a dash of this type of approach, if you show them the data, people don't want to be bad scientists or positions and to show them the data that shows them that they must do with this way in order to get the right answer, they will also change their behavior.

With that, I think you for the lively discussion of this reception for David and I of this session and I have the pleasure of announcing a coffee break.

Ten minutes.

[ applause ]

**Lessons Learned: Effects of Biospecimen Quality on Genomic and Proteomic Research**
Session Chair: Carolyn C. Compton, M.D., Ph.D.

Hi. Would you please take your seats, and we will get started again.

If you would please state -- take your seats. We will get started again.

Were going to get started again.

Just so people know, we do have a program at lunchtime today, informal or you can go sign up and talk about a subject of interest for you. The sign up for that is just down the hallway in the next ballroom.

It is my great pleasure and honor to introduce our next speaker, Patrick Brown. He is a Howard Hughes investigator and professor of biochemistry at Stanford. His doctoral thesis investigated the basic molecular mechanisms of DNA so he is trained as a pediatrician and then went on to do a postdoctoral fellowship at UCSF with Michael Bishop and Harold [ Indiscernible ]. But his claim to fame I am sure well known to all of you in this audience because he invented the expression, microarray. And we are honored to have him here today to discuss his viewpoint and share his wisdom on this topic and to expand our horizons. Thank you, very much, for coming.

**Pitfalls and Gaps in Research Using Human Biospecimens**
Patrick O. Brown, M.D., Ph.D.

Thank you. I appreciate having been invited here. I should say that I was typing fast this morning during the Carolyn's -- it during their presentation because I want to make my presentation complementary to the ground that they covered. I hope you will indulge me because it was a little bit on the fly and I hope I -- I will be deliberately provocative and some people might call that obnoxious, actually, but I feel like it is part of what I see my role as is to poke some discussion and maybe expand the scope of the discussion. I want to ask your indulgence for that.

I want to focus not so much on the issue of specimen handling, I think that was addressed very well by Carolyn and David. Instead, I want to focus much more on what kinds of specimens we need to collect. And this sort of outlines the things that I want to talk about, which I think are huge deficiencies and our fundamental knowledge that are really at the heart of what we are -- the purpose of the cold by a specimen collection business -- the purpose of the cold by a specimen collection business.

Our knowledge of a human variation, noctis molecular variation -- which are enormously deficient in but just basic things like variation and anatomical variations in the normal not overtly diseased population is a huge gap in our knowledge. And I feel like I will get to this point, but I feel like this is absolutely at the heart of Disease detection and diagnosis. Implicitly, he was talking about something that is a deviation from the scope of normal healthy variation. It implies that you actually know what that scope is and we haven't done nearly enough to do that.

So that is a key goal. The second thing is with respect to early detection of cancer which is one of our important goals here. There is this weird paradox which is that we are, or we don't in general know what we are trying to detect. You know, if we clinically detect a cancer, we don't observe its. And we don't know what it looked like before hand. That may sound like a philosophical point but it is actually fundamental and, in fact, I'm going to give you an illustration of about how wrong we are about the assumptions we make

about the preclinical natural History and when we are trying to detect cancer to try to make a difference.

Another point that I think was very well covered by both David and Carolyn is comprehensively describing the source and handling of the descried -- of the samples. There are numerous examples and I will illustrate one, of slobby standards that make it impossible to trash -- trust the results.

This is something that I come across all the time when you are talking about precious biospecimens that have potential immediate utility but they are also precious enough that you want to observe them for future uses. And the problems that you and colder -- the problems that you encounter of getting the used out of them and observing them for the long time. It sounds like a silly technical thing but it is an important thing to address and I think there are technical ways of addressing it.

Okay, so -- again, I am when to say that I am not went to talk about sample handling and processing because my experience has been, and just about anyone who has done characterization of any biospecimens is the variation and the results that you get, with molecular profiling or what ever, when you try to track the sources of extended variation, it is not taxable on what you are trying to study the majority is not due to experimental factors and handily factors is due to biology and things that happen before the sample is collected. It is the biology. It is a much larger parameter space and it is harder to control.

And I want to give an illustrated example to be provocative and obnoxious and to motivate the discussion that leads into some of the topics. This was a paper that was published less than a month ago or something like that. It got a lot of press and it was very heavily hyped by the Yale press office. It was a paper that purported to describe a multi Mercker, a sixth marker early detection test for ovarian cancer, were in the conclusion they say it has a sensitivity of 95.2% and the specificity of 99.4%. There are a million problems with this paper. I want to focus on one that is most relevant and what I want to cover.

So let's look at some of the details. The sample collection, good for them because they made a concerted effort to use a very standardized way of collecting and handling the blood. Okay? But this gets to the point of the real problems of this paper -- there are many problems, where the problems and I want to focus on take place. And that is, it is biology. So if you look at the two groups that are being compared there is an unfair and cancer groups where the samples were collected previous to diagnosis at the clinic. These are people that were coming to the chronological oncology clinic and they knew there was a problem. They're going for the regular exam.

These are the six markers and again I don't want to take it to you all, but I did want to point out these few things -- these are familiar to many of you. And why is this, why do I want to focus on these? Well, because this is a case where all of the careful sample handling and the world does not save you from the fact that there is a built-in bias to these samples, a confounding factor that has to do with biology that is not taking into

account and it is just the tip of the iceberg of things that we don't adequately appreciate and recognize that can just totally trash a study like this.

So this is a previously published study that looked at one of these markers and I encourage you to look at it, it was published in -- several months ago looking at prolactin. And they weren't interested in looking at this marker for ovarian cancer motivated in part by a study from the same group.

It found -- prolactin, not too surprisingly, it has been studied -- it is a wonderful Mercker of impending surgery. And incredibly, look at the paper but is just beautiful for that, it is useless to receive the correct for pending surgery it is useless as a marker for ovarian cancer.

Another marker that they looked at was leptin. It has been steady quite a lot actually and it is incredibly sensitive to all sorts of things. It is insensitive to weren't you last eight -- when you last ate, and stress and other variations. This was published about a year ago and it looks at the relationship between the average amount of sleep you get every night and your leptin levels.

This is another paper looking at the relationship of Ceram serum leptin levels -- And the point is that there is all sorts of very large-scale variations in this biomarker. There are a million -- I don't even know what I put this in, but there is nothing particularly unusual at that but there has been a lot of studies looking at circadian variation. A very large fraction of genes show a significant of circadian variation, this is not 18, but cortisol. It.

The point is that if you have samples at different clinics, one in the morning and one in the afternoon, if you could have a diagnostic marker for time of day that you think is that it knows in what ever that clinic specializes in. Any way I won't be that into the ground and there is all sorts of factors, psychological stress and so forth that have huge effects on blood chemistry and physiology.

One that is familiar to me is where someone was claiming to have an early detection test for breast cancer based on gene expression and blood and if you look at the jeans identified as markers they tell you what your relative neutral bill account is -- you give a shot of epinephrine to someone there counts double inches -- bit doubles.

And the major assault type that is due to physiological stress that can be confounded that things you're trying to detect. I am just reading this into the ground because I just I want to make the point that if we don't have a detailed map and a profound understanding of the normal factors and Variations, physiological variations, stress, drugs, diet, you name it, and all these other molecular patterns we will be going on wild goose chases all the time. And they will be throwing millions of dollars of investors' money down the toilet Filene it just a misleading data. This is just to illustrate another point that I think is, again, recognized but not nearly systematically studied enough and that is from a steady my lab did many years ago looking at for feel blood samples from people were recollected samples. One of the interesting things that we found is that if you cluster based on all the

pattern of gene expression from these blood samples, what you find is that they cluster almost perfectly by individual despite the fact that the samples are collected over periods of weeks.

This is again quite well recognized for a large fraction of the genes in the human genome. There are many variations in their expression patterns. Again, tremendous potential for come pounding if you are looking at diseases where there are ethnic variations in the current and stuff like that. Or just different genetic basis or samples are collected from different geographical areas or what ever.

And this is just the same thing looking at the protein levels in the blood and the point is the same, there is it a lot of stable and from variations that if you are not care -- careful it could be mistaken for something more interesting.

So okay. This is all of this stuff but the very fact that there is this prominently featured paper that was published in a extensively peer reviewed journal that neglected these factors and came up with the bogus results said that it is still worth seeing. So for any molecular marker things that we need to take into account are the following things listed here.

This is grossly underestimated work if people don't even have their definitive diagnosis get are coming for a work of of something bad. There are a great many things. So I can wind about this all the time -- I think we need to do with it and I think we need to -- this needs to be a very high priority for anyone who is in this whole biospecimen

The thing is that we have a database or a knowledge base of molecular, physiological, anatomical variations in the human population were if there was a systematic and quantitative and rigorously documented and we can look at how these relate to any of the parameters that can be influencing them, environment, history, and so on he would have a much better interpreted framework for any study that we're doing where we are looking to all of those other variations for the specific signal of a disease or what ever.

So again, we need to think of the variations in terms of differential diagnosis and if we think it is cancer as opposed to the other sorts of things, we agreed to be wasting a lot of time -- we are going to be wasting a lot of time.

The point is that for any cancer -- oh, what am I doing wrong? Oh, sorry. Okay.

Okay. The point is that I think this might have to do, I am sorry to be politically incorrect, it has to do partly with the whole disease orientation of the NIH, that anything is not a disease falls between the cracks but yet for every disease is with reference to that that you're trying to make the diagnosis. There is not nearly enough information in mapping the range of normal and yet its is foundational. Just look in the literature and the database and so forth what a tiny fraction is voted to trying to look at any kind of normal variation or any one individual systematic look in detail at the variation and molecular characters of cells and tissues and so forth.

It is the masonry deficient -- it is amazingly deficient. They should be a huge priority to look at this database. I feel like why, how can that not be already a high priority?

So I think that this needs to be a large-scale effort and it needs to be a very large population scale effort to look at variation because we are looking at many cases trying to detect a very low prevalent disease state. Where if you are looking at a population, 99.99 times out of 100 the thing you are trying to detect and diagnosis isn't there. Which means you need to know the theory, freer variants of the normal range -- very rare variance of the normal range.

Is a big task, but I feel like the fact that it is thick and hard does not mean that we should just blow it off.

So let's see, okay. There is a lot of practical issues. 1, I think I would like to see a serious effort this is a tough problem to rejuvenates the whole autopsy routine. There is -- academic medical centers in the United States are incredibly rare these days. At Stanford they do one a week or something like that, I think. And when they are done they tend to be rather ad hoc, or small series. We should do a very large-scale, tens of thousands of meticulous systematic -- systematically documented autopsies characterizing all these things, and addressing things like not only does the normal variation but in real quantitative terms.

Also something that is fundamental coming up with this cancer early detection think, weeping and we know -- we pretend we know how prevalent a cold neoplasms are and the current population.

I remember when I was in medical school way back in the day it was interesting news that most men by the time they are 60, you will find something in their prostates that you would call prostate cancer but yet very few of them with no and they will die of other things. There was a similar surgery about kidney cancer -- if you just observe them many of them just go away or never grow. We don't really know how prevalent occult neoplasms are in the population and if we do early detection tests, there is the enormous risk of formal diagnosis in detecting a bunch of things were if you just didn't know about them they would not be a problem. We need to know how common that is. So it is a tough problem. Normal people usually aren't dead so it is difficult to get a lot of autopsies of normal people. It.

Get I think we can think sensibly about this. I don't think there has been enough of a systematic effort of doing this is a transplant donors are a good potential source of someone who at least until very, very recently was just walking the streets apparently normal and healthy. I think medical examiners are another potential source of doing these kinds of autopsies of people who die suddenly of trauma or what ever.

Anyway I don't have a solution to this, it is not easy but it should be on someone's agenda.

Another issue that I want to touch on is there a tremendous gap in our knowledge, and we make a lot of assumptions of the preclinical natural history of cancer of looks like. We think we know what we need to detect and what we are trying to detect. Maybe we don't think that that we convince ourselves that we do. But yet it is critical because we know what we are looking for if we are trying to figure out how to detect it.

Okay. It is a difficult thing to do because, of course, you are not going to detected early cancer and watched to see what happens in general. Yet I think that is clear and we keep coming across this that the prevalent assumptions that underlie this whole early detection process are -- This is an example from an analysis that I have been doing in collaboration with a scientist at the Canary foundation, just trying to build a model for the pre -- preclinical natural history of ovarian cancer.

This is basically taking advantage of the fact that in high risk populations often women will have a prophylactic surgery where their ovaries and troops are removed despite no evidence that they are harboring early cancer and low and behold when do section them, you find tiny cancers, typically. Most of the time they are so microscopic -- 90 percent of the time they are too tiny to detect, only on microscopic exam.

The point is that gives us a window on cancers that are in people that don't know who have them and you can use that as a base for modeling and natural history.

This is just a curve, the x axis is the diameter and the y axis is the parameters that have gone to stage three or four. And the medium size -- median size is .9 centimeters in diameter. It is more than a thousand times smaller in volume than the median size of clinically detected early stage tumors. So that the tumors that you have to detect to have 50% sensitivity in terms of detecting the cancer is that you need to detect when you need to detect them to save lives are 1,000 times smaller than the tumors that people typically use and declare victory for early detection when they are doing their validation studies, which is complete early stage tumors. That is just to make a point we think we know and we pretend we know and we don't know what the preclinical natural history of one of the cancers is trying to detect and that needs to be a priority. It is a top problem but not impossible.

This is just kind of random but it is something that I have encountered a lot. Is that people are reluctant to use their precious samples for characterization or any kind of studies because they are so precious and you don't know when you are going to come up with something better than what you're thinking about doing now.

One of the problems that you come across is that you can't -- many of the markers are trying to detect if you freeze and thaw multiple times it degrades the ability to be measured. So you have to -- have some ideas. It occurred to me that I don't think it would be hard to come up with better ways of doing this. One of the things I'm glad to try to pilot in my lap, just to illustrate this point, is collecting serum samples in long, skinny

plastic tubes, so if I want a micrometer, I could slice of a peace and resealed it, so you can do it at different sizes without having to thaw them out.

So this is a recurring problem and it causes people to hang on to specimens and feel like they never are actually going to -- There will never be a steady good enough to warrant actually using my precious samples for it. So we should think in advance how we can make these things are perturb the divisible and renewable and stuff like that. I'm not the first person who raised this issue, I know.

Okay, I think that is all I want to say. So I will take questions. I hope I have this to enough people off that there will be some people come to get up and challenge me.

Yeah?

I am Susan from Indiana University and, first of all, I would like to thank you for a very provocative talk. We have taken you up on your challenge and I think we have the first normal bank to my knowledge in the world's and is funded by Susan Coleman is best to shoot from normal volunteers -- breast tissue, and we have the medical history and everything you could use for the risk factors . It is only difficult to get it started and we have 100 women who want to give tissue and we have 72 people who are going to get on a bust -- were going to get on a bus from have been -- the only bottleneck we have is to have enough surgeons to get it done. So stop by poster five tonight to get it started.

I think it might be harder if you were studying pancreatic cancer [ laughter ]

Or prostate [ laughter ]

I feel like we take a defeatist attitude that it is never clear to happen, if you say that it is a heart problem and you can deal with it, I think there are ways to make progress, so I love that, that is great.

Jim -- you have make me happy and I think you highlight one of the key take comes from this morning. We don't not use a normal reference range for our test we use reference lane, we don't stay normal range. So there is no normal, we are on Newtons. You have to be very careful in your design, and your study to pick your controls precisely to the task that you are going to get because there is no normal. I think that has been very well how late did and I appreciate, Dr. Brown, what you have done because even if patients come in and give their breast to issue, some have been smokers and pollution that they have been in their own communities so you have to be very careful on where you are calling for control and for normal.

David from NCI, a plug from the Health and examination Survey which does not have to issues but has body composition survey and serum and urine samples in five to 7,000 people per year and that you best dating back a decade -- in the United States dating back a decade. It has detected leptin and the bursts serum measures so I think there is a little more material out there available for the comparison with pathological samples in the

analysis of these sort of molecular issues that you are discussing. It is worth exploring that more, perhaps.

I think there are other opportunities like that. PLCO is another example and the Women's Health Initiative, where there are large collections of samples from normal people with varying degrees of other data collected. But I think a very useful thing for the people organizing -- First of all, there is the issue that some of these things are expensive, just funding and adding value to these kinds of things -- and the other thing is that there is tremendous added fell you if you can integrate the data and do with it in a systematic, quantitative format and put it in a place for everyone can have access to its at the highest level of resolution, and there is privacy issues and stuff like that -- where every parameter can be tracked to the individual sample level so that you can look at systematic relationships between different variables.

There is a lot of latent potential and some of these population based studies, but I think it really requires some of the Kent effort to attack it and get the full value -- it requires significant effort to attack it.

[ Indiscernible ] I trust your response more than anybody to this column in question. In physics there are a number of things that you can measure which can be too much affected by the observation event so much that they can't be used and you have to step back and use secondary phenomena. Based on what you have seen with their own hands, do think that are an April filing is in or out of that category and can you trust that -- RNA profiling, and is it stable enough to get meaningful data or is it too much affected by observation?

There is no categorical answer to that but I would say that my experience doing a lot of profiling and all sorts of things in terms of RNA expression patterns, it is that the dominant source of confounding, extrinsic variation is biology. The experimental factor is handling the way that you do everything down stream of that, not to trivialize it you have to be careful and, of course, if you just let it rot or something like that you will have problems. I have been quite astonished sometimes at the robustness, if you know which things to pay attention to, the robustness of the profiles that you get at an RNA level.

An example of that, many years ago we were having a study of collaborators in Norway that were sending us frozen samples of breast tumors and we were profiling the RNA and looking at systematically -- and we had a mishap where one of these samples sat in customs for a week and it arrived, the dry ice had vaporized it was room temperature and stuff like that, we figured, why not? We don't want to throw away. And so we profiled the RNA patterns in the sample, I feel like if we waited another week week it would have been smelling bad, but it had not been handled in a careful way.

We have another sample, this was a before and after chemotherapy trial, and we have another sample from the same patient in the same tuber and low and behold the carefully handled sample in the very badly handled samples -- they matched up almost perfectly. You could tell out of 100 samples once one came from the same patient despite the fact

that one of them was sitting around at room temperature and the other one was properly handled.

There were differences, there were sets of genes that have a characteristic response that have a -- that said in a department for six hours for what ever -- but most of the things that are to issue specific patterns are stable and robust enough that I think they tolerate that kind of thing.

I don't want to over generalize this, but I think -- and obviously is really important to be meticulous and careful of the handling and documenting the handling of samples. But the main point is that in that case, despite the corbeil potential for artifacts, biology dominated the variation in that expression pattern.

Down from Rubicon genomic. Is there a way to take a small ally, and profile of the DNA? [ Indiscernible ] sequences and then mind that data in many different samples -- I'm sorry, in many different studies as opposed to taking a fixed sample and placing it up into smaller and smaller pieces?

Yeah. I don't think those are mutually exclusive things to do. Obviously with nucleic acids in this kind of a special case, with a caveat about biases and anything that amplifies them and stuff like that, at least to have the potential for a reasonably permissible way of amplifying RNA signature and the total genomic DNA or what ever. The principal the almost arbitrarily so that is a specific case. And absolutely, I feel this is done quite commonly, because a lot of times this is a situation that you run across all the time for you have an adequate amount of sample to do all the things that you want to do and then people will pre amplify the DNA and RNA and you can recover a lot of the label information that way. Still it does not solve the more general problem but this is definitely something to do. It is a way you can make something much more divisible if it is the and A for RNA and send it off for profiling -- if it is DNA or RNA.

I would like to make a plug for, because we have been trying to do some of the things that the suggested on normal and I think it would be great if NCI came to the sense that that is a priority and put a big shoulder to it because it is a very difficult example. We did a transplant donor study to collect breast tissue and it is critical to get the -- is critical to get normal tissue from young people with a spectrum of risk factors acting on their normal tissues. It took us two years to collect 138 quadrants and frozen fixed samples and part of the reason was that we were unlucky with transplants. I think the two issue is really needed to do this, and this is very difficult, one, a very big effort to get a lot of multi disciplinary people together including people like recruiters and people underground working with patients and two to get a really good public support behind this transplant donation. It rises and falls, getting people next of kin to cooperate. There are damages and we have tried a variety of things but the great thing about the transplant donors is that you get the call systemic potential the of people. You can get a normal biopsy of breast and appreciate that study that is ongoing. And what comes out, I don't know what normal is because I am a pathologist in used lead a can be fibrous tissue.

A ticket and there and to give a big specimen and it is put in the context of the full physiology and it is from and for people, you get the tissues when the risk factors are acting because they often act in very young people and we see decanters 20 years later.

I think if and see I really wanted to -- I think this is really a big high bar effort even though conceptually it seems obvious.

Cannot, thank you very much it makes a point that it is a high bar but it is doable. And staff NIH put its way -- if that NIH put its weight behind it and get the public engage in terms of backing of the variation in normal people and stuff like that, you could have -- there would be a substantial group of people that would be willing to have a donor sticker on their driver's license or what ever for Medical Research or who knows what.

High. I am [ Indiscernible ] in Cambridge. Given the very ability that you see in the normal or controlled issue is it imperative for the conditions when they are collecting tumor tissue to also collect normal or controlled tissue? I mean -- Because how are you going to fish out the real sin a tour of the cancer or something giving the capabilities.

I think that is a good question, one thing that might lead spent some time looking at a question related to that. And I would say that to a first approximation, the differences between a cancer tissue in one person and another -- and another person and between a normal person counterpart or what ever are much greater then the differences, despite the fact that those are important.

It becomes much more of an issue when you are in a situation where there is a noise problem like Orrin -- early detection of tumors. There is a very small signal and the variation in the background is huge by comparison. I think in terms of a lot of cancer classifications and diagnosis, I think it is important to consider, but it is probably not tremendous factor. It is still great to have normal tissue for a bond of things if you are doing genetic or mutation profiling or something like that. So I say, when in doubt you should always corrected because it will always be useful for something.

Tom Wheeler from Baylor University, a comment about the -- organ tissue donors. One of the things that we've noticed is that for a long time we considered the tissue normal but, in fact, they are put on my support for a couple of days in order to be declared brain dead. And they get steroids and a lot of other factors and a notice that there is a marked increase of eight the Towson's in the cells. So to consider that normally would not be inappropriate. We have normal tissue in patients that have bladder cancer for example and tap their prostate removed. But they are not a normal population. So what is normal? How do we define normal? I can think of a scientific experiment based several hundred years ago popularized in the movie young Frankenstein in the nine -- in the 1970's and Gene Wilder asked for a normal brain and his assistant went and got the brain and transplanted it. When the monster was acting erratically he said did you get the normal brain? And he said, yes, it was written on the jar, AB Normal. So the search that what is normal is the paradox here. And I think the Coleman Foundation should be congratulated because this is the closest that we are going to be getting to that.

I am wondering if I am overstating my time here, I don't know what the schedule is.

I wanted to mention a new partnership that more Roswell Park is entering. The unit is the clearing house for transplanting issue so when a person dies and indicates they would like to be a tissue donor that goes through units and they actually do the consent. We partnered with them to take advantage of their infrastructure to be able to collect tissues from anyone that has indicated they want to be a donor. In partnership with Russell we are moving forward to identify our patients that we have the whole medical history of that would be a donor to collect illegal phenotype.  to collect phenotype. So it does raise another whole other level of complexity on how long postmortem is the tissue and other issues with that but people may want to think about partnering with people with in their local community just to actual -- actually address these issues.

Thank you, that was a great discussion. I just want to say one thing myself about acquiring normal tissue. If it is done organ by organ it is a certain approach, and your dedicated to a certain type of disease but we really need total body tissues. And that is the problem that we need to be able to compare the site specific differences in biology. We are even looking into this ourselves as to why can't we partner with, for instance, the living will types of initiatives that are taken on by a state-by-state basis when you sign up for your driver's license. Can you also donate more body if you are in a car accident to research? Right now only people think about donating the organs for medical use and transferring to another human being but they don't think about how valuable those tissues would be in research. That would be another awareness approach that we could think about taking.

With that I would like to introduce a longtime friend -- longtime friend and colleague that I am proud to introduce, Lance Liotta, he is currently the co-director for the center of applied protein mix and a molecular medicine. He was formerly the chief of the laboratory of pathology and chief of the section of to Merck invasion and the fastest since at the NCI and the director at the NIH.

He was just leaving as I was coming, but we still are closely related and work together. So, Lance Liotta has devoted his career to be steady -- to the study and he invented a series of micro deception techniques and protium mix methods that are used in Labs across the country and he has 80 patens to shout across the world. So it is with great pleasure and pride that I introduce Lance Liotta.

**Critical Pre-Analytical Variables in the Real World of Tissue Procurement**
Lance Liotta, M.D. Ph.D.

Thank you, very much. I am grateful to represent the path elegy Committee seat and to present some new data that what is happening with the mini meet that Pearland talk to you about -- that, Maryland talked about and it is struggling to preserve -- survive and the collection Pan.

We know that the tissue that is removed from the patient is alive and it stays alive until it finally is fixed in the inner core of that tissue by formaldehyde in the usual situation.

So how do you know how long to leave a piece of tissue on the bench before is no good for your molecular analysis? It depends on what you are measuring. Is there a test that you can provide on a piece of tissue and say that this is acceptable or not been acceptable? Would it be possible to take the tissue immediately out of the patient and preserve all of your markers' instantly that would allow the graphologist to to a section and allow the patient -- tissue to be preserved at room temperature, and that is what I will be discussing, and I will touch on the same for blood.

We were very fortunate to be recruited from NCI and FDA to George Mason University with a wonderful support by Virginia for a compliant proteomics Laboratory for analyzing tissue and blood. And the individual who has conducted the work that I will be talking out is Virginia and I am proud to represent her data.

The problem with the George Mason University is they don't have a medical school and we have a big interest in analyzing the tissue samples covey could get from various clinical studies sets. So what are V-22 about putting the tissue with no medical school? We are very fortunate that MCI had previously established a relationship with an entire country of Italy -- all of the different cancer centers are participating in a tissue and blood consortium and we brought over to George Mason University when we left.

We were also very grateful to established a relationship with a vocal Community Hospital -- a local community hospital that has 15,000 specimens per year. We thought this would be a great opportunity to collect tissue. When we went into the operating room of the Community Hospital we saw how tremendously difficult it would be to prepare to shoot reliably and reproduce it. Even though it came out of a patient and it was frozen immediately when we checked on it was unknown how long it stays on the bench before it was put into the freezer.

So we thought that in order to do any molecular analysis we thought that it is very important to understand what is happening immediately after the tissue is move from the patient and what fluctuations are occurring in the molecules during the life and death struggle of that piece of tissue.

We are potentially studying this biomarker -- and all of the molecular targets of the future are in signal pathways and if we could just measure whether an individual signal path in a patient's piece of tumor tissue is inactive that would help us to understand the appropriate inhibitor to get that patient or what combinations we should give that patient.

So I will be talking about tissue biomarkers and proteins.

How do we measure the fluctuating States of these signaling pathways particularly when they can change very rapidly depending on what is happening in that environment or inside the cell.

With regard to kinase pathways, so we can imagine that from minute to minute the state of a protein would be changing and that makes a very tough biomarkers to measure although it provides a fantastic information about what is happening in that cell.

It is not only one bus for a nation, it is many sites on what it is talking to downstream and what are interconnected. So a very important new class of biomarker. You need to study its quantity and big differences could make a deal in terms of the -- in terms of this and most of these are very low in terms of the piece of tissue so you can't even see it.

Fortunately it there a large number of Adam bodies have been developed that recognize this residues of the quantitative leap measured these proteins into samples.

So how long should we let a piece of tissue set out and how will it change with regard to these important markers, could we identify biomarkers in that changing tissue that would allow us to predict whether that tissue is adequate or not for the analysis?

This is the major question about what is happening to the tissue after is removed from the patient there is a three large unknown time. Where we think we know how long tissue sat in the operating room and how long it sat on the path elegist table but most of the time is not recorded adequately, how long it took from the patient to your fixative. And once it is in your fixative there are other kinds of changes that can be cussed as well.

So we have to take into consideration in the real world tissue is collected, processed, and how that can change it. And we have to integrate any new molecular profiling tests seamlessly into the standard of care because no pathology laboratory will accept it. They can do in an artificial research environment, but that does not want to help us when we want to take this out to routine use for doctors and patients in a community hospital.

What happens? It is taken out in need to be subjected to a frozen section and it needs to be cut, and after the embedding, the histology needs to be preserved. We can't is take a tissue out and rented up and analyze it because the pathologist used to look at its and eight diagnosis has to be rendered.

It has to support all of these issues for routine standard of care. Tissue preservation methodologies are from a research perceptive comment it could be very deficient. Formal and fixation although it is robust, when you do this for hundreds of years it has raised low penetration of tissue PRI is a very big problem for any kind of molecular analysis.

And while freezing is ideal, it is never possible in a routine hospital or clinic or out in the field for you might be conducting a clinical trial.

Here is an illustration of formulation -- formalin penetration, the tissue is not CrossLand for many hours. So there is going to be a pretty between the outside and the issue, the tissue is still alive -- so it depends on how large the piece is and what the composition of the fixative is.

So we want to understand in a real world community hospital housing you can get a piece of tissue from a surgeon's cutting knife and how you can get it into a preserve a Ted and what happens immediately following the collection of the tissue regarding the signal path with proteins that are present in the tissue that we can analyze.

Our objectives were to indicate to shoe collection -- tissue collection, determine how long it takes to acquire tissue, analyze effects, discovered fluctuations, and compare the profile of different issues. Is each tissue individual and the way that it responds to the environment? We want to develop quantitative data for determining national data driven basis for collection for the cause and markers to guide presentation chemistry and preservation methods, and prefers -- parameters for preservation technology.

Those were our goals, so we went in and work with the pathologist and the surgeons so that we could collect tissue that was not required for diagnosis, cut it up into uniform pieces in measure everything and know all the time -- and then let these different pieces of the same tissue sit on the bench for various lengths of time and we would analyze the tissue to see what was the effect on the time course of these very important signaling proteins that we were interested in.

Here is an example of some of the clinical trials that we conducted with uterus, colon, lung and so forth. We've recorded as much information as we could think of as to what is calling on with that tissue and its temperature, specimen size, and we've looked at the elapsed time that we could get the tissue. And our conclusion that the average time was about 18 minutes and the earliest time was four minutes and the long this time was 40 minutes and mold was ten minutes.

We looked at 50 individual proteins that were important and this just, in color, lists all of the different classes of pathways in the proteins that we looked at.

We used very rigorous antibody allocation methods, that we practice vigorously every day and are glad to qualify are antibodies to make sure that they are as specific as they could be and that might include competition with phosopeptides.

Than they looked at proteomics and to integrate into cellulose and it is preserved indefinitely. Then we come back with antibodies specific for the proteins of interest and we have both -- built in reference standards and positive and negative controls and we print everything in a curved so we stay in the right dynamic range.

So this essay has been proven to be reproducible and for a set of 100, it was independently validated in a blind fashion. It is as good as the individual and the bodies,

and it can be very sensitive because it only requires one and a -- antibody -- here are some examples.

So here are some -- the most interesting finding immediately for individual pieces of tissue is that these signal pathway proteins instead of the cain of weight and dying went up. Most of the problem was the -- in increase with time and then gradually declined. That was because the tissue was alive and that pathways are reacting to the situation and you can see examples of here of individual protein planes that are fluctuating and going up with time compared to the baseline.

Some declined away at everything eventually goes away but very reactive in the beginning. Because the tissues are living and reacting to the trauma.

Here you can see upward fluctuations followed by declines. So we want to see if this was -- how this very from tissue to tissue and was this reproducible so we looked at the time course for fluctuations and signals and pathways for this defect type of tissue and everything labeled in a yellow here is going up significantly. More than 20%, two standard deviations. Many markers were going up and not down in the first 90 minutes after procurement.

Then we look at the position analysis for individual studies and these are all P Values for the markers that were elevated.

Here is another example wormy looked at pathways that were augmented or hyperactive -- another example where we looked at the pathways -- early or late in the process all with statistically significant variability.

We then could take some of the data and qualify certain markers that went up and down over time by a Nova analysis to say that these might be circuits to say whether a tissue is surviving or if it has perished or not.

We need to have a subset of markers, what do we see if the preservation chemistry is working? We have to have a subset of markers to identify and this is how we have to come up with some examples.

So why were they going up instead of going down? Because the tissues are alive and they are active in how could we prove that? You might think that the way to preserve the protein is to give an inhibitor like to give another and have the chair, that is not going to work because the fossil destine him richer will shut the downward decline and allowed the pathways to add more phosphor relation onto the things of interest. So the inhibitor is not went to work. And it does not work with tissue.

What happens is that while there are fluctuations this is worse the fluctuations are even greater -- compare to the pro cream -- proteins are not involved.

And if we use and other inhibitor to block that upward fluctuations we could see more of a stabilization of the phosphor nation.

So this demonstrates they have -- they can be --

As a tissue dies and depending on what type of organ is it might be different for cancer tissue as opposed to non cancer tissue it goes through a series of stages of pathway activation that we can imagine relates to what is happening in the life and death struggle where you have hypoxia, metabolic exoduses and decline in glucose and inclement Tory task -- and eventually you have cell death at the end.

So in conclusion, from this new data, at least with regard to the protein, the optimal time is 20 minutes and we can get to she with and 20 -- which in four to 40 minutes and the biggest issue is false allegation.

And that the phosphatase inhibitors alone are not adequate and A a commendation would stabilize the tissue.

We want to apply this knowledge to a real world clinical situation. So we tested this hypothesis. Are the signal pathways the same or different than the primary tumor that it came from? Because the metastasis is in a different micro environment.

So we were able to measure 75 patients in the operating room where the tissue was collected randomly from the primary tumor or the metastasis at the same time in the same operating setting, with in 20 minutes the two she went into -- 80 and we pleaser Micro dissected the tissue.

I would presume that both of these tissues were subjected to the fax of anesthesia. So we then analyzed the samples that we had, and then we found a season of and points that were statistically significantly different between the matched tumors and metastasis, some going up and some going down, the of the image of looking at signal pathways is that we can look at signal pathways and see if they are interconnected and if this goes up something else would go up and this is the advantage because you can functionally verify that it is a pathway changing, not just a random event of a signal pathway.

We also attempted to independently validate the results with 17 other independently collected specimens from the same operating area. And we used Western plotting to make sure that we were measuring by the reverse based protein a rate essays that was found -- Of course using a lot more tissue for the western one.

We were able to identify a series of interconnected pathways that were augmented in the metastasis compared did to the primary tumors in the colon cancer patients.

We presented this data who is now going to be supporting a clinical trial with a Fairfax to study what the signal path for a profile is and to use this to set up by patients for the

inhabitants of their be because one of the important pathways that was altered was that pathway.

You can't collect frozen tissue routinely and putting in the freezer likely did in the Italy study. We need a preservation technology that preserves the morphology and the proteins right out of the patient. So that we don't have that unallowable variable of how long is that on the bench for what happened to its between removal from the patient and when you got it.

There has been a lot of pork in our field and many of it is supported -- there has been a lot of work in our field test to look at thermal activation, microwave assisted rapid Foreman exaction -- formalin fixation, and a lot of exciting technologies.

What we would like to use in the real world of the pathologist is something that would not be a machine or a box that sits in the operating room, we want to have a chemistry that we could sunburnt the tissue and immediately at the time of procurements does that we could submerge the tissue in.

For room temperature preservation, and four frozen section diagnosis, and that most civilized the proteins and also must maintain the morphology so it can be shipped at room temperature.

We have made an attempt to achieve these goals and here is something we are working on.

Our idea of preservative that we are working with takes the vantage of the knowledge that we have gained and it is a precipitating fixative, so that we can still do a frozen section. We have reversed a bow lifted soluble and water soluble crosscuting for chemistry.

And then we have these inhibitors to keep the system steady wild the enhancers' take the fixative into the tissue.

And if we look at some of the end points that we identified early on and others we can see that this fixative chemistry at least the reiteration of the experiment shown here did a pretty good job of suppressing the upper elevations in the pathway proteins that we were worried about.

That was measured before and that a later time than in the fixative for markers that we knew would change from endlessly -- changed tremendously.

So we are taking this board in actual clinical trials and using this fits with it chemistry in a breast cancer trial supported by [ Indiscernible ] and Frank Holmes and that trial, the tissue is collected in 12 different centers and put in a biopsy Bank and submerged in the chemistry that I just mentioned and sent to our laboratory where we are analyzing the phosphoproteins and we can look at the time it took to shipping and variation from one

clinic to the next. It is a very good study. We are also using this for a multiple myeloma project in another funded project.

So we're trying to take this chemistry that we are developing all the way to a potential room temperature preservative at least for the markers that we are interested in and it also preserves our NA as well.

In the many attempts in the field -- so we don't have to worry about those on noble time delays -- unknowable.

I want to take a plug for poster Number 13 will be take the same possibly foresee am by no markers -- the same philosophy for serum biomarkers.

There can be all sorts of epidemiology issues with Ceram but biomarkers are in low concentration and they are obscured and they are rapidly degraded by enzymes and some biomarkers that we know of our labile and only last a few minutes in the blood.

We are working on a technology that allows the investigator at the exact time that the blood is procure to have the biomarker proteins of interest captured and sequestered and concentrated and protected from degradation.

So she will present her poster and this was and the Journal.

So these can conduct collector -- the is can do the following.

Bueno and did completely excludes albumin in high abundance proteins.

We have heard about other particles that sequester proteins such as done by our lab and in cooperation with [ Indiscernible ] a surface capture of workers. This is a coarse salt, so we would only allowed the protein that passes through a barrier to get inside the particle that it is captured by 8 feet and said the particle like a lobster trap.

So concentrate all of the protein that is in the solution of interest and it ends up inside the particle and with the high surface area it occurs in less than ten minutes as documented and the data shown in the poster and the published paper.

So this is what these look like and to conclude, we think that this is another direction for immediately preserving blood so that we don't have stability problems. We could envision putting the particles into so that the blood immediately enters the tube and the buyer markers are preserved -- and the biomarkers are present so you can harvest them later.

Thank you very much.

[ applause ]

That was so exciting in light of the fact of how we are claimed to do the research on the specimens is so critical. First I do need to acknowledge to anyone who owns a prostate gland or works on prostate cancer that even though we work on breasts we appreciate the prostate gland and its lack of accessibility. The thing is, can you extrapolate these kind of exciting understandings of what we have to do on how we will look at the whole body biology and what happens with a cadaver for a trauma victim that inhibits the use of those practices that you are working on, or what would have to be done to preserve that for a whole body?

I think the question is focusing mainly on autopsy for whole body procurement there has been some examples such as the hot Pickens' group for warm body autopsies were the tissue is prepared after the patient is deceased but before the body in store for an evening of time in a freezer, or in a refrigerator.

We can see that there are many changes that take place in the tissue depending on the temperature and how they file that tissue is to the requirement for oxygen and brain tissue changes minute by minute after you take a piece of brain tissue out there are case sensitive as you might expect to the oxygen concentration and glucose concentration.

Other tissues may respond more slowly to the situation. We think an ideal -- this is an ideal situation Tuesday was everything right out of the patient with different kinds of chemistry -- this is an ideal situation, you can treat it with a microwave or you can defeature everything and that will stabilize the proteins, but then that compromises the morphology and the histology.

Maybe will -- we will see a future where we take a piece of tissue and process it in various ways to preserve the different and alights of its ship -- and our observations is that the tissue is alive, it is going to be reacting and changing. If we want to know what those important changes are and if they have anything to do with the disease that we are setting we need to do it at the minute that it comes out of the patient or even at the time of surgery.

It is very hard to do this type of research in the clinical hospital. But I think if we can integrate our methodology into the routine clinical practice, the surgeons seem to be very excited to participate. It makes it more problematic as we are measuring markers to understand that the tissue is alive and stays alive for a very long time. We to think that it comes out of the patient that it is dead, and that is wrong. There is a lot of data coming out about changes and fluctuations in tissue. We want to believe that nothing is changing and that is perfect and that piece of tissue was removed from the patient but that is not the case.

I think this is very interesting work and very important to understand the edition -- the difference between mini me and life of its own, and this is very important for us to understand. What I'm trying to understand for work that Carolyn and Helen's group is trying to do in the future, are there practical implications for what you can tell them about collecting tissue? I am trying to think of a way to make this easier because we can't wait

for all of the state of the artwork to be done. I am thinking there are two principals, one is, can you provide advice about what is good enough? Something like over 20 minutes old then throw it out or something like that. That is one kind of thing. I think minimal criteria will help Helen and Carolyn.

The other thing is whatever the method of this specimen collection is is to try to avoid bias differences, for example in the Italian study, but if the normal tissue versus the cancerous tissue, for if one was done at three minutes and the other it was done at ten minutes, can somebody who was collecting a sample -- it was the cancer -- and then it got frozen, that is the kind of thing to try to minimize in this as a mine collection. minimize in a specimen collection and that is the systematic difference between cancer and lung cancer plops.

And about noise, and the end of the day that is when to secure a difference that there are both important problems and we can't accept -- expect perfection. And what my concept is that we could use good specimens to discover if there is any signal that at all, and then if that is the case to go back and once we have an interest in marker or interesting disease than me can understand all of the details that can mess it up. I know you are not suggesting this but we can't get paralyzed by waiting -- you are not suggesting this at all -- we can get paralyzed by waiting for all this to to be done and have the perfect specimen. Perfect is the enemy of good, and what is perfect enough for us right now to build on?

Thank you for that good question, with regard to the Italian study, the metastasis was sampled at the same time randomly taking the metastasis or the primary tumor in the same patients at the same time. These aren't liver metastasis and primary tumor in the same patient that were done.

It depends on what you are measuring and in by a specimen banks today if we look at those individually -- and the biospecimen banks today, some of them may be useless but some may be useful for some other kind of markhor. So the guidelines relate to what you are measuring and to qualify an individual specimen there are certain chemistries if you want to look at Los causation -- that localization that can be very robust and survive the cycles or delays in preservation for if you are pressuring some other marker you might want to have been frozen within 20 minutes.

Ideally he need to get things frozen or stabilized in York LeClair ethanol fixative as quick as possible -- in your molecular ethanol fixative and we need to integrate that and to be clinical practice. I know they are trying to use the ethanol practice right in the operating room right when it comes out of the patient.

Just a comment in that regard, David, and that is filling in the ice cube tray, it is just as important for clinical purpose to know what isn't important and what doesn't skew the data as what does because it gives us the knowledge to be confident that we can ease up on the Richter at least four things that don't matter and focus on what does and how they do affect things and design things -- this is the quintessential example of designing an SOP based on data. Because you have the data what happens that shows you what

happens for the thing that you are studying. But it also, something that he kind of glanced over that I find it very important is that when you see something that changes on a break other bases with some kind of thing -- that changes on a rate of the basis that you can use as a quality indicator and it always does this, then you can go into your freezers and pull up your specimens and assess the quality of those individual specimens for that particular piece on that qualifier.

And we need those kind of data driven qualifiers' because we have millions of specimens that we don't want to discard, but we have to know what they are good for and what it agreed to give us.

Ideally you would have a series of tests that you do on a piece of tissue from a biospecimen bank, and maybe it is no good for phosphoproteins or maybe it is good for DNA analysis and we would go through that with knowledge to come up with a protocol that is data driven as Carolyn mentioned and individual for the class of marker that we are interested in.

Of course, our preservation methodology itself might perturb the system so we have to be careful.

One more question.

I would like to compliment you and your colleagues for working with a community hospital and to -- expanding the diversity. I was curious whether you need to devote additional resources and the Community Hospital to the informed consent process.

Yes, of course of the stations, even though it is left over tissue, it is informed consent ahead of time, the protocol specific for tissue collection. And we have a dedicated person who participate in the tissue collection and an own scientists from our side and in the hospital, the cancer center has hired a person whom participate and absolutely requires. The surgeons, pathologists, don't want to be bothered with this. You have to have your person there and they didn't need to develop a very good report to -- they need to develop a good reports of the are not considered intruders. And then it can be workable, depending on the surgeon.

Karen is a experts that advises our office on this. And we have to say this is focused purely on the physical aspects of biobanking it, and there is a whole lot of a fax on this, even in speaking in these terms once it comes out of you, you don't even own it. So there are all kinds of -- by our legal system is defined as some of their complete entity that is no longer equated with you. But over which you have control but it is part of your anatomy. So we do have guidance related to those because, in fact, you can have the perfect Putnam grade biospecimen and never use it for research if these issues are not addressed.

With that said, we are going to break for lunch now.
-- We will see you back here at 1:30.