Overcoming the Unknown:
New Approaches to the Diagnosis and Treatment of Carcinomas of Unknown Primary.
London 15th October 2009

Improving understanding and evolving therapies for Unknown Primary Cancer patients

Dr F. Anthony Greco

I recently saw a patient with CUP. He was a very intelligent gentleman, who was on the internet all the time wanting to find every little detail about everything. He asked me how many patients have I seen with CUP. I thought for a while and was not sure about it, so I asked him to give me a little time to think about the question. I went back and looked at the calendars for the past 10 years. I have been doing this for the past 35 years and I figured out that the average number of CUP patients that I saw per week – not just from my regional hospital of course – was about 4. I did the maths over the past 35 years and I told the patient this figure and he said 'Well now tell me, are you any different from Napoleon's mules?' I said 'What do you mean?' and he said 'Well Napoleon's mules went from Paris to Marseilles a hundred times during his campaigns and they did not know the way on the hundredth time any better than they did on the first time'. I sort of feel like that in many ways as this is a very difficult, frustrating area where we can argue about the definition of whether it is a proved CUP. Which is a little ridiculous in my opinion because there is no such thing as a true CUP – if we could do post-mortem exams on patients before they died there would virtually no CUP (I will talk about that a little more in a minute). I know what people mean when they say 'true CUP' but the fact is that the definition is not agreed upon as was discussed this morning. We need a definition however, otherwise we are lost.

If you look at carcinoma of Unknown Primary in the tumour registries in the United States, or you go into the Netherlands and Australia and other parts of Europe you will find that it is not rare. I have heard the term rare, or not common cancer, in fact this is a common cancer. In the United States about 3-5% of all advanced cancers are actually classified in this category. I think that it is even more common than this for reasons that we could talk about in detail, in that a lot of these patients are given diagnoses because of other issues:

1. The patient feels uncomfortable so the doctor is going to go ahead and give them a diagnosis when they have a tumour in their liver and their CA99 is elevated, even though their pancreas is totally normal. Often that patient would be called pancreas. A patient who has a medial style mass will frequently be called lung cancer, even though there is no primary found in the lung. Those patients get coded as pancreas or lung and therefore the true incidence of CUP is probably higher than the tumour registry data suggest.

2. In some practices in the United States CUP is rarely seen. Friends of mine are in those practices and the reason that CUP is rarely seen is that they define every case, whether you have clinical information or not. You simply call it something other than CUP. That is done for various reasons, including getting drugs paid for for these patients, which is very difficult with a diagnosis of Unknown Primary Cancer.

The important aspects that I am going to talk about today are:

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1. Recognition of groups of patients that we can list. It takes 30 seconds to write them out, but it has taken 30 years to determine these subsets of patients, who are clearly different. They used to be classified as CUP, and many still are, but clearly have a more favourable prognosis and are treated in specific fashions. About 20% of all CUP patients fall into one of the favourable subsets.

   We can recognise these subsets now but it has been an evolving recognition as technology has improved. Thirty years ago patients with germinal tumours were almost never recognised unless they had a testicular mass. Now that subset of patients is usually recognised and is highly treatable.

2. The second area I want to talk about, which is more controversial, is that in the last decade empiric 'one size fits all' treatment in my opinion has actually shifted the survival curve to the right for CUP patients. I am not an advocate of 'one size fits all' but this is 2009 and in 1995 and 1997 we did not have nearly the technology we have today so 'one size fits all' was at least something. I want to show you old data compared with new data on that and I think that we have made some progress but it is not the way we are going in the future.

3. The third important area is new diagnostic technology. You have already heard a lot about this and I am not going to give a lot of new information but I am going to talk about a couple of studies, some of which you have already heard about, and put my own perspective.

Patients can present to physicians in a myriad of fashions. I could stand up here for an hour and tell you all the ways they can present, but they basically present with what looks like metastatic disease. They are evaluated. Yes we do still do histories and reviews of systems and physical examination – very important – including stools for ??? blood, which is sometimes left out of the examination, and pelvic and rectal exams. When you have a patient who looks as though they have metastatic disease on initial evaluation most of the time you are going to find the primary and I can estimate, based on data, how often but it is the majority of the time. You are going to find the primary - they are going to have a lung lesion or they are going to have a bowel lesion, or a stomach cancer etc. There are patients, after an 'adequate evaluation and biopsy' where we do not know where the primary is. Over the last 35 years specialised pathology has improved remarkably, in my opinion, and the data on this has become more comprehensive in the last 5 years, although some of it was available before that. We also have our clinical observations. Clinical observation is not dead, there are groups of patients that you can recognise by clinical features that have important therapeutic implications, particularly when you combine it with the specialised pathology.

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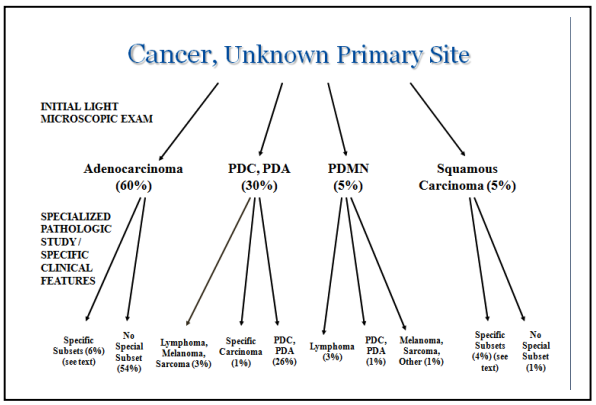


One problem with this diagram is that the arrows are all going one way but this is not the case. For example: you see a patient, you do an evaluation and eventually a biopsy. The patient has a work up (you need a minimal standard work up), and then we have specialised pathology on the initial diagnosis. Sometimes a specialised pathological test should then be communicated with the physician, who then does another clinical test that wasn't done initially. For example: a patient has a CD20+ve CBX +ve tumour. Some would say that every one of those patients needs a colonoscopy but let's say for some reason that that patient did not have one or the patient had one that was 'normal'. I think that in that setting you need to do another one, or you need to repeat the initial one. A patient with a TTF1 +ve tumour – you need to look at the lung very carefully and you might even consider a bronchoscopy in that situation. The error can go back up to the evaluation. Once in a while a patient will have a clinical finding that will tell the pathologist which direction to go with the staining technology, so the errors work both ways.

Recently I have been wondering in women who are CK7+ve if we should do MRI's on their breast even though all the other breast markers are negative. Obviously if another of the breast markers is positive we need to do an MRI on both breasts. These are examples where a clinician and pathologist need to work together in CUP patients.

As time has gone by the specialised pathology has got more sophisticated, to the point where now we are down to the molecular level. Immunohistochemical markers and stains and molecular diagnoses are related. Proteins get translated from DNA through RNA, or in some instances micro RNA also reflects tissue constituent function. I like the analogy that hepatic cells have certain functions, breast ductile cells have certain functions. Some of those functions are retained in cancers and if we can identify them by a stain, or by molecular histology, that may give us an answer when we otherwise don't know where these cancers are coming from.

For all practical purposed Unknown Primary Cancer patients represent carcinomas. We can now sort out the other lineages fairly well, although four or five times a year I see patients where we have trouble even with the lineages. Is it sarcoma, is it melanoma, is it carcinoma? Most are adenocarcinomas that are easy to identify under the microscope, although it is not so easy to know where they came from. Some are poorly differentiated carcinoma (a minority) with no adenocarcinoma differentiation. In a small minority, although an important

group touched on earlier, are squamous cancers which are usually in the head/neck region or the inguinal area, but can actually present anywhere.

20% of all the patients who present with Unknown Primary Cancer have a subset which is different. If treated appropriately they have a substantially better prognosis than the group as a whole, so obviously we need to identify these patients. These patients weren't identified in one year – it has taken forty years of research to sort this out – when you look at it from today's world some of it seems ridiculously obvious, but it was not as obvious 30 or 35 years ago.

There are a number of favourable prognostic subsets of patients with Unknown Primary which have been recognised during the last thirty years:

1. The most dramatic example is the Extragonadal Germ Cell cancer syndrome. This is usually a young man who has disease in the lymph nodes and often the retro perineum, often with pulmonary nodularity, with some times other lymph node involvement and who has a rapid clinical course and, as I mentioned, thirty years ago this would have been just another Unknown Primary Cancer. There was no typical germinal histology on diagnosis and we did not have immunohistochemical stains then, we were working on a HCG and alphapheta protein stains and even they are not specific to germinal tumours. We have better stains and markers now. We have carriertypic information from cytogenetics that can diagnose these patients by Fish testing as well and we have molecular profiling, so in this clinical picture we probably would not miss this diagnosis. This is highly treatable but rare.

2. Another group of patients that are not considered by many are those who have lymph node involvement. We recognised years ago, and MD Anderson also recognised this, that patients with lymph node involvement alone have a better prognosis than those that have liver and bone as well. This is, in my view, a favourable group of patients where we now have more data that their prognosis, even treated with empiric therapies, is much better than those with liver and bone and multiple other metastases.

3. I have mentioned squamous cancers of the head/neck and inguinal area, which is where PET scanning can be quite useful in pointing out a primary site that you could not find in any other way, but if you find it, then the patient is out of the Unknown Primary group. The patients I am talking about are ones who don't have a primary found. We have to presume that when they are in the head and neck that they have an occult lesion in the head and neck area. These patients have a relatively good prognosis, even with local therapy, but seem to have a better prognosis with combined modality therapy just like head and neck squamous cancer. This can also occur in the inguinal area and there are a couple of highly treatable occult lesions, particularly from the anal canal, but also the uterus, cervix and vulva areas which can present with inguinal squamous cancers and you do not want to miss the possibility. You look for those primaries, but if you don't find them in my view the patient needs aggressive local therapy in some form of systemic treatment.

4. Studying women with high isolaria axillary adenopathy wasn't so easy years ago, but now we know that many of these women have occult breast cancer. How do we know this? Because many of them had mastectomies in the past and those that had them (before the era of MRI) had normal mammograms. Those that had pathology looked at carefully, and there are large groups of these published, usually had primaries from 2mm to 1.5cm. One of the reasons that post-mortem examination show very few breast cancers is that when you have a patients where you cannot find any liaisons in their breast by physical examination and they have a normal mammogram, so you have to make hundreds of sections of both breasts to find a 2-3mm lesion. That simply was not done in many post-mortem examinations as they had no idea that there was a breast cancer, so of course you are not going to find a breast cancer in post-mortem. I think that this is a more logical explanation of why Unknown Primary at post-mortem is rarely found in the breast and yet we know from molecular studies that breast is more common than that.

5. Women with peritoneal carcinomatosis – this is now so well recognised that these women, when they have cirus histology, are included in ovarian cancer trials. They are not considered an Unknown Primary any more. I think it is reasonable, but keep in mind that there are patients with peritoneal carcinomatosis who have primaries in the pancreas and colorectal or gastric, or even breast or elsewhere that aren't this entity and you can confuse the two. This entity can now be recognised by molecular profiling and other testing, including immunohistochemical special markers, which can help to identify this in most patients.

6. Men who have an elevated PSA and an Unknown Primary Cancer obviously have occult primary cancer in their prostrate. Sometimes a blind biopsy of the prostrate will find it, sometimes it won't. These are treatable like prostate cancer.

7. Neuroendocrine carcinoma of unknown primary sites is a very interesting and important subset. There are two major varieties, well differentiated carcinoid like tumours which have an intholm biology. It is probably not so much treatment that makes them do better, it is the nature of the disease. The other is the poorly differentiated neuroendocrine carcinoma which can be small or large cell. These patients do better if they are treated with platinum based treatment. They do substantially better than the group as a whole. When a patient has a large cell carcinoma it is very important to do neuroendocrine stains or you have completely missed this diagnosis. It is easy when the patient has a small cell histology, but when it is large cell there is really no other way of knowing it is a neuroendocrine type tumour.

8. Lastly it is becoming clear that a patient who has a single site metastasis with does a lot better than the group as a whole, who has multiple metastasis. It does not matter where it is – they can have a single brain met, a skin liaison, one group of lymph nodes, a small mesentery involvement, adrenal gland etc. One site and that is all you

> can find. That group of patients, even with empiric therapy and with aggressive local therapy, surgery and/or radiation does much better than the group as a whole with a lot of metastases.

That is an important group, one of the subsets which we can recognise. 80% of the patients with CUP do not fall within one of these favourable subsets. Maybe we will recognise more. There seems to be a profile of colon cancer CUP's if that group in fact does as well as colon cancer then we have found another subset that we can add to the list. In my opinion unless a patient has an anatomically defined primary we are making a mistake by defining that patient, or resolving their case. I am going to get argument on this, and that is fine. I feel the same way about molecular diagnosis – unless a patient has an anatomically defined primary site I am not going to call them colon cancer based on immunohistochemistry. Yes, they may well have a colon cancer. I am not going to call them lung cancer based on immunohistochemistry although yes, they may have lung cancer. I am going to call them CUP lung cancer profile, CUP colorectal profile etc. Until we can sort all this out with prognosis and patient outcomes we can be making a major mistake by resolving these cases when we do not have the known primary anatomically defined. Again I will get some argument here, but that is fine.

I now have a number of slides, a lot of which we have talked about already. The workup of these patients and the stepwise evaluation by immunohistochemistry is important. You need a stepwise approach and communication with clinicians back and forth, in order to make the ideal workup.

I want to talk a bit about post-mortem data. I agree that this information from 'Cancer Treatment Rev. 2009 May 35 (3) 221-227' is very difficult to interpret as this data came over 60 years. Can you imagine seeing a patient 50 years ago, before we even had CT scanning and saying 'We don't know where your primary is from' and then you do post-mortem and you find the 2cm pancreatic mass. That was an Unknown Primary. Today a CAT scan would immediately find that mass, so some of the patients in this series were like my example, others, like breast cancer, which seemed very unusual, are also like my example. You have to do multiple sections through the breast, hundreds, in order to find a 2, 3 or 4mm liaison unless you are lucky. The more 'modern' autopsy series reported 20 to 25 years ago did find a little more breast cancer but by and large these common cancers are what is represented in CUP. That is important. CUP patients have a primary, we just cannot find it during life. This brings us back to how much workup a patient should have.

We recognised a long time ago that certain types of histology were more common with certain patients. This was years ago that we defined this and at that time there were a lot of those subsets of patients that were still being included in CUP as a general sense. The younger patients tended to have poorly differentiated carcinomas and more rapid growth of their tumours, and involvement of the lymph nodes more often. Whereas the elderly patients tended to have multiple metastatic sites, well differentiated adenocarcinoma and they did poorly on any treatment given, particularly before 1997.

As we learn more and take the treatable subsets and treat them appropriately then we have 80% of the patients remaining with CUP that generally have a poor prognosis. Over the past thirty years within the definition of Extragonadal Germ Cell Syndrome in young men there are a number of cancers that are found:

- Germ cell neoplasms
- Neuroendocrine carcinomas of unknown primary site
- Undifferentiated sarcomas
- Lymphomas
- Thymic carcinomas
- Midline carcinoma in children and young adults
- Primary peritoneal carcinoma
- Most never identified.

These are not all germ cell tumours by any means. Not surprisingly as technology improves we have subsequently learned that in the syndrome that we defined that there are many other cancers.

For poorly differentiated tumours we would use platinum based treatments like that used for small cell lung cancer. In such a group of patients the survival curve is substantially better than patients in the unfavourable group (80% of all Unknown Primary Cancer patients) where we have nearly a quarter of the patients with poorly differentiated neuroendocrine carcinoma of Unknown Primary site still alive at 3 years.

The Empiric Chemotherapy regime of 'one size therapy fits all' before 1992 was an exercise in futility basically. Most of the regimens were used in GI cancer or breast cancer. Platinums were used and by and large the response rates were low. There was no complete response data and in fact most of the studies did not even report patients surviving for one year. I presume that a few did. None of the patients survived two years in these early studies. I can show you data for 15,000 such patients which suffer from small trials, lack of control groups and offer no real information that you could improve the survival in CUP.

In the late 1990's a bunch of cytotoxic treatments became available that were broad spectrum: the taxanes, gemcitabine and irinotecan drugs which we knew could treat a lot of known cancers, so investigators began to plod along doing mainly Phase II trials to look at those new drugs and I want to show you a little different perspective on this. These trials in the last decade have excluded patients, for a large part, with favourable prognostic features because you can at least control that part. They also excluded neuroendocrine tumours as part of the favourable groups.

These trials virtually all gave Phase II data but we wanted to look at this differently. Instead of just concentrating on median survival we wanted to see what the one, two, three year and beyond survival were in the trials. Since there was no two year survival ever reported from any trial we could find we had to assume that very few, if any, patients lived after two years before. If you look at natural history data of CUP, which is not a good way of doing it but is all we have, the median survival is about four months. The one year survival is around 10%, which is pretty miserable over all.

Our group began to do Phase II trials in sequential fashion looking at these empiric, broad spectrum, combinations. Not something that I am advising any longer – I agree that 'one size fits all' is delegated to the history of medical oncology and CUP in my opinion, although there is still going to be some more trials like this. This study was a decade ago and we used broad spectrum antimicrobial therapies, different Phase II trials all relatively small in size, but accumulating a total of 451 patients, 350 of which were followed up long term. 80% were poor prognostic patients. When you look at the 396 cases and concentrate on median survival it is only 9 months, but if you look at the longer survival – one, two, three to ten years – you definitely see patients still living even in the unfavourable groups. After three years about 12% of these patients survived, so was there something here? Even some patients with progression free survival from these empiric regimens survived, so I believe that there is something here.

You then say 'Let us look at other trials around the world'. The problem is that we all have the same limitations and small numbers. I can show you some other Phase II trials, using more broad spectrum antineoplastic agents, all published since 2000, looking again at one, two and three year survival as benchmarks rather than median survival. The bottom line is that for 928 patients in the last decade, all Phase II, median survival is 9 months and one year survival is 34%, two years 13% and 3 years 12%.

Why do I not like median survival as a gauge? Median survival can be very close to the same when patients all die within 18 months or whether 30% of the patients are still living at two or three years. To miss that would be a major miss, so in my opinion there has been a shift of the curve to the right with these empiric broad spectrum regimens and I have used them for good performance status patients with CUP when we don't have any other information. I am not going to talk about other Phase II trials with other drugs which are also empiric 'one size fits all'. This slide shows the result of work reported in the Journal of Clinical Oncology 2007; 25; 1747 which does show some promising control of disease but is again a small trial. We used the same therapy with chemotherapy with one of the empiric combinations (this will be published soon) and results looked reasonable but again, without the two or three year survivals I am not going to say much about median survivals.

We then decided, and this is years ago, to look at a Phase III trial – Randomized Phase III comparison of Paclitaxel/Carboplatin/Etoposide Versus Gemcitabine/Irinotecan, both followed by Gefitinib in patients with CUP – which this is the largest Phase III trial in the worlds literature in Unknown Primary Cancer, with one hundred patients per arm. The reason we did this was because we wanted to find something that was less toxic and we wanted to show that survival was no worse. I won't go into great detail but this was a Taxol Carboplatin etopiside regimen which we knew had certain toxicities that we did not like. This was an easier regimen, a day one and day eight regimen that we paletted and it was much better tolerated. What we showed was that the survivals were very similar and they are similar to what I showed you for these more modern broad spectrum drugs. There are no differences in these treatments; median survivals are low but again there are patients surviving out to two and three years with curves similar to those I showed you before. These results

are unprecedented in the literature that I can find before 1997. Take it for what it is worth. The toxicity was substantially less with the gemcite being an irinotecan regimen. This will be published soon.

I don't like empiric 'one size fits all' treatment in 2010 because we can do better. We can define these cancers better and in my opinion we will then treat the patients better as you have heard already today.

You have heard several times today that Unknown Primary Cancer is really a compilation of many primaries. I am convinced that there is probably some other biology here. There is the different biology of why a cancer remains 2-4mm or 6mm and spreads all over a patient and you can't find it – that is different. There is a different biology when pancreatic cancer is a call (later proven) and spreads to the bone much more frequently than the liver, or prostate cancer, which is a call (later proven) spreads to the lymph nodes and does not spread frequently to bones. There is a different biology going on here but we do not understand it yet. We have not found any genetic signatures to prove that it is different however, than known primary cancers.

In this slide you can see the survival of the known primary cancers, based on large Phase III trials. The worst one is pancreas and, believe it or not, the survival has actually been improved recently in pancreas and you can accept or not accept that. In non small cell lung cancer survivals are creeping up. In colorectal survival has gone up substantially from a median of about six months up to twenty two months. There I would accept medians! Obviously looking at the one, two and three year survival, Harpreet mentioned that 30% die within a year, but there is also 30% more who live more than three years, so you can consider that angle too. I think that Unknown Primary is a compilation of what we are seeing here of the actual primaries given empiric treatment. This is not the best approach.

Therapy for virtually all the cancers is not the best we can give when we use 'one size fits all'. Let me give you an example as an American: Taxol carboplatinum is a common regimen used in the United States for Unknown Primary Cancer. The only disease I can say that that represents state of the art treatment for is ovarian cancer and that is probably going to change before long. We can treat lung cancer better know as we treat it with Bevacizumab plus those two drugs. We now know that certain subtypes of lung cancer are treated better with other drugs. Taxol carboplatinum is not the ideal treatment for breast cancer, is useless in colorectal cancer, not good for renal or hepatic cancer and it is not ideal for bladder cancer. I could go on and on. I think that Dr Bridgewater's slides showed that area, that there are several cancers to the right of that area and CUP was right there with seven months.

We need to identify these cancers and if we can 'identify a profile of CUP patients'. Keep that definition in mind – a profile of CUP patients not a resolved CUP – I don't like a 'resolved' CUP unless you have an anatomical primary. If we have groups of these patients that we can identify by immunohistochemistry, molecular profiling or a combination of both, and don't forget the clinical features which are very important. Those combination of factors, if we can find profiles, and prove in those CUP profiles that patients can do better either by

appropriate randomised control trials or by other forms of perspective trials then I think we have made an advance for these patients. Until then I am not sure that we have made an advance.

There are three commercially available molecular profiling techniques which you have already heard about – bioTheranostics, Rosetta Genomics and Pathwork Diagnostics. We are involved in research with bioTheranostics and will soon be starting research with Pathworks. I think that the microRNA technology is very interesting. I am not sure that a comparison of these technologies is going to help us very much. They are all accurate in identifying known cancers but they are not 100% accurate. When I have a patient with a lung cancer who has liver metastases and a big inter-bronchial lesion I am 100% sure what that cancer is, yet the molecular profile is only 85% sure. That is not very good. When you don't know where the primary is I will take 85% any day of the year! I can't see that any of these tests is a whole lot better than any of the other – they are all very accurate in defining known primary cancers.

There have been a couple of studies (Dr Bridgewater showed his earlier) looking at indirect validation of molecular profiling in Unknown Primary Cancer. Two of these were published in the same journal last year and one of Dr Oien's associates did an editorial on this which put it in perspective.

- The first study Gauri Varadhachary and our group were involved in (Molecular Profiling of carcinoma of unknown primary and correlation with clinical evaluation. Journal Clinical Oncology 26: 4442,2008).
    - The Veridex assay had some limitation and only identified six tumour types but they were common tumours.
    - We had a large group of Unknown Primary Cancer patients, the majority of who we looked at retrospectively.
    - The group at MD Anderson had several that were prospectively evaluated, so we combined the series. They all had CUP – what I mean by that is that they all fitted the definition of CUP when they went on the study. If you define CUP differently some of these patients might not be on the study.
    - The assay was successful in 104 patients. This showed that you could do this on formalin fixed tissue, small biopsy specimens. Fine needle aspirations were excluded.
    - The primary site of origin was assigned in only 61%, perhaps because only 6 tumour types could be identified, but there could be other reasons.
    - The important point here is that, at least from a clinical and pathological standpoint (again we did not have exhaustive immunohistochemistry here although we had some immunohistochemistry) it looked as though most of the common cancers were in this and most of the patients had clinical and pathological features that were consistent with the molecular diagnosis.
    - Another important aspect of this was that a colon cancer profile was recognised and in the retrospective group they all received 'one size fits all' Taxane platinum type treatment. They did very poorly, with only a response

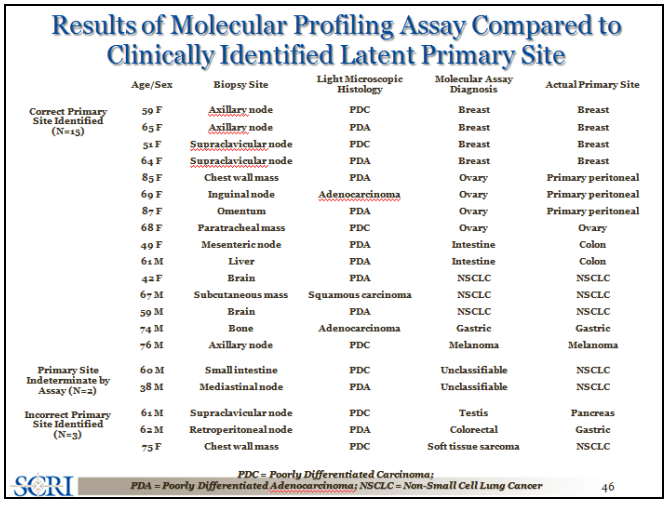
or two in a group of 12 or 13. In a similar number of patients, when they had, colorectal site specific type treatment like FOLFOX they did much better and a majority actually responded to therapy. This is a little small sample size for us to be convinced that this CUP colorectal subset will do as well as a metastatic colorectal but it is a hint that they will.

- This is indirect validation. What do I mean by 'indirect'? None of these patients have an anatomically defined primary site so we cannot be sure what the primary was. All the studies suffer from this, except for one, which has its own set of problems – this is the one I will discuss in a minute.

- The other study done here (Gene expression profiling to identify the Histogenetic origin of metastatic adenocarcinoma of unknown primary. Journal Clinical Oncology 26: 4442,2008) was by Dr Dolphine's group I believe, and from Amsterdam Dr Horleen and others. This was a very good study.
  - There were 38 patients with Unknown Primary Cancer and they had a very detailed immunohistochemical panels around these patients.
  - They thought that they had classified 16 of these cancers. Again we could argue about this and I accept what they mean here – it had a profile consistent with that primary. But without an anatomical I am not going to accept it as a primary yet.
  - Interestingly enough of these 16 patients the molecular profile said the same thing in 15. That is very encouraging to me as they validate one another. The molecular profile validates the immunohistochemistry but more importantly, the immunohistochemistry validates the molecular profile.
  - In the other patients 22 could not be classified by immunohistochemistry but they had 3 different possibilities.
  - They correlated the findings in these patients with the clinical pathologic variables. In 14 of the 22 patients they felt that they had a potential site which could have been clinically relevant and they thought that this was adding to the already known way of evaluating these patients. Again an indirect validation.
  - How do we do a direct validation? I only know of two ways:
    - You do post mortem and find a primary. You have the original diagnostic biopsy and you know what the primary is, so you do a molecular profile on the initial diagnostic primary and see how accurate it is.
    - Find patients with latent primaries. You do not know how much trouble it is to find latent primaries in patients with Unknown Primary Cancer. First I have a group of doctors telling me ' If you find them they are not CUPs' and I tell them that if I do post mortem I am going to find 75% of them, so where do you draw the line? Obviously you can find the primary in some of these patients two months later, six months later, eight months later, sometimes years later. We had enough denominators here – a large group of CUP patients – that we could look back through our clinical trial database and find patients in

who we indeed found the primary later during their life. This is called latent primary. We drew the line at 2 months. You could make an argument that that is too soon and they had a defined primary. I am sorry, if they fit the definition of unknown primary you can define it and they are an unknown primary. If two months later you decide that you are going to do a colonoscopy again and you may find their primary. You found the primary but they are not excluded from unknown primary – they were an unknown primary at diagnosis. It is just a matter of what testing you want to do. If you do post mortems you are going to find most of the primaries, so I don't think that argument is a good one.

- We found 38 patients and over 500 who had latent primaries; most of these patients were treated on clinical trials but not all of them. We had to exclude 10 of the patients because we excluded fine needle aspirations, so that left 28. In 8 of those they didn't have adequate DNA for the testing. We used the bioTheranostics RT-PCR, so that left us with 20 patients with latent primaries. But we knew every single one of these patients' primaries so then we took the initial diagnostic biopsies (all CUP patients to begin with) and we subjected that to molecular profiling.
- The results have not been published yet but are in the process.
  - This slide shows the 20 patients and the biopsy sites, light microscopic histology, molecular assay diagnoses and actual primary sites found. This is direct validation – direct, not indirect.



Results of Molecular Profiling Assay Compared to Clinically Identified Latent Primary Site

	Age/Sex	Biopsy Site	Light Microscopic Histology	Molecular Assay Diagnosis	Actual Primary Site
Correct Primary Site Identified (N=15)	59 F	Axillary node	PDC	Breast	Breast
	65 F	Axillary node	PDA	Breast	Breast
	51 F	Supraclavicular node	PDC	Breast	Breast
	64 F	Supraclavicular node	PDA	Breast	Breast
	85 F	Chest wall mass	PDA	Ovary	Primary peritoneal
	69 F	Inguinal node	Adenocarcinoma	Ovary	Primary peritoneal
	87 F	Omentum	PDA	Ovary	Primary peritoneal
	68 F	Paratracheal mass	PDC	Ovary	Ovary
	49 F	Mesenteric node	PDA	Intestine	Colon
	61 M	Liver	PDA	Intestine	Colon
	42 F	Brain	PDA	NSCLC	NSCLC
	67 M	Subcutaneous mass	Squamous carcinoma	NSCLC	NSCLC
	59 M	Brain	PDA	NSCLC	NSCLC
	74 M	Bone	Adenocarcinoma	Gastric	Gastric
	76 M	Axillary node	PDC	Melanoma	Melanoma
Primary Site Indeterminate by Assay (N=2)	60 M	Small intestine	PDC	Unclassifiable	NSCLC
	38 M	Mediastinal node	PDA	Unclassifiable	NSCLC
Incorrect Primary Site Identified (N=3)	61 M	Supraclavicular node	PDC	Testis	Pancreas
	62 M	Retroperitoneal node	PDA	Colorectal	Gastric
	75 F	Chest wall mass	PDC	Soft tissue sarcoma	NSCLC

PDC = Poorly Differentiated Carcinoma;
PDA = Poorly Differentiated Adenocarcinoma; NSCLC = Non-Small Cell Lung Cancer

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  - It was accurate in 15 of these patients. In 2 it was unclassifiable, in other words there was more than one site that was listed and could not have a statistical significance.
  - Errors were seen in 3 - Incorrect predictions.
  - Again 75% accurate
  - We also studied the patients in more detail; the sites of metastases, initial histology, what we treated the patients with and what their

outcome was (not very important in this small study, but we collected it anyway).

- This slide shows the patients, the biopsy site and initial immunohistochemical staining done on the patient. Keep in mind that these were done over nine years. Seven years ago we did not have many of these stains and this was not a prospective, this was all retrospective, so we are lucky to even have this.

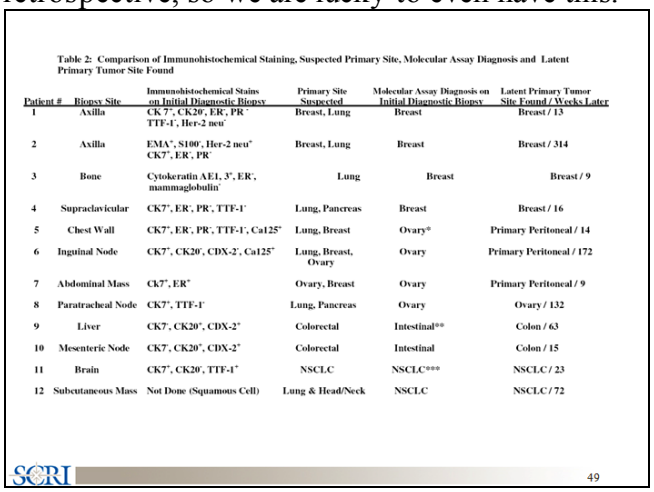

Table 2: Comparison of Immunohistochemical Staining, Suspected Primary Site, Molecular Assay Diagnosis and Latent Primary Tumor Site Found

Patient #	Biopsy Site	Immunohistochemical Stains on Initial Diagnostic Biopsy	Primary Site Suspected	Molecular Assay Diagnosis on Initial Diagnostic Biopsy	Latent Primary Tumor Site Found / Weeks Later
1	Axilla	CK 7+, CK20-, ER+, PR+ TTF-1+, Her-2 neu+	Breast, Lung	Breast	Breast / 13
2	Axilla	EMA+, S100+, Her-2 neu+ CK7+, ER+, PR+	Breast, Lung	Breast	Breast / 314
3	Bone	Cytokeratin AE1, 3+, ER+, mammaglobulin+	Lung	Breast	Breast / 9
4	Supraclavicular	CK7+, ER+, PR+, TTF-1+	Lung, Pancreas	Breast	Breast / 16
5	Chest Wall	CK7+, ER+, PR+, TTF-1+, Ca125+	Lung, Breast	Ovary*	Primary Peritoneal / 14
6	Inguinal Node	CK7+, CK20+, CDX-2+, Ca125+	Lung, Breast, Ovary	Ovary	Primary Peritoneal / 172
7	Abdominal Mass	Ck7+, ER+	Ovary, Breast	Ovary	Primary Peritoneal / 9
8	Paratracheal Node	CK7+, TTF-1+	Lung, Pancreas	Ovary	Ovary / 132
9	Liver	CK7-, CK20+, CDX-2+	Colorectal	Intestinal**	Colon / 63
10	Mesenteric Node	CK7-, CK20+, CDX-2+	Colorectal	Intestinal	Colon / 15
11	Brain	CK7-, CK20-, TTF-1+	NSCLC	NSCLC***	NSCLC / 23
12	Subcutaneous Mass	Not Done (Squamous Cell)	Lung & Head/Neck	NSCLC	NSCLC / 72

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- These slides (one example above) show you the primary site that was suspected, based on clinical and immunohistochemical and pathologic findings, not one exclusive of the other. You can see that in most cases we could not give a single diagnosis. We had several, however, where we did. For instance in one example we thought that it was a lung based on clinical features and other issues, and another we thought was colorectal based on the staining pattern and liver metastases, while in another we thought it was colorectal based on mesenteric node as well as liver metastases and the immunohistochemistry. So we suspected those. In another we suspected non small cell lung cancer based on CK7 positivity, CK20 negativity, TTF1 positivity.

  You might look at those three that I have just pointed out to you. The molecular profiling diagnosis also said the same thing in those three examples. Again molecular profile validates immunohistochemistry. Immunohistochemistry validates molecular profile. Plus the actual primary was correct in each case. In fact in six of the patients who had a single site predicted by immunohistochemistry five of them would have been predicted correctly. Only one, which we thought was lung, and we made special effort with this one because it was a woman with bone mets, and I understand that, of doing some breast studies, both were negative. Again keep in mind retrospective immunohistochemistry.

17	Mediastium	CK7+, TTF-1+, PLAP+, CK20+, chromogranin+	Lung	Indeterminate	NSCLC / 19

Here are the results of another lung diagnosis and in this case we had pretty good information clinically, and staining wise, that this was lung. The Initial Diagnostic Biopsy was indeterminate.

15	Axilla	cytokeratin AE1,3', HMB45' S100', CK7'	Unknown	Melanoma	Melanoma / 56

In one case (above) of a male patient with other metastases we simply did not and it turned out that that one was a melanoma. All the melanoma stains here were negative.

We have here five out of twenty patients predicted by immunohistochemistry accurately. Five out of twenty – not bad but keep in mind that we did not use all the panels we could have used in 2009 retrospective. Fifteen out of twenty predicted accurately by molecular profiling.

This is what it is. We do not have a lot more data than this now but to me this is very interesting and important. Based on my own experience of lots of other patients molecular profiling and immunohistochemistry are complimentary and when you combine them with the clinical features you are going to be able to make an intelligent estimate of how to treat your patient and not treat with 'one size fits all' any more. We have to study that to prove the outcomes and that is what we are doing.

I am not going to tell you all the studies that we are involve in as it is too time consuming. I like Dr Bridgewaters 'design – he showed electro profile treatment or empiric treatment – random design. That is the ultimate study. We cannot do that in my clinic. I cannot have a molecular profile of colorectal and give them Taxol Carboplatin it would not work. Patients are too sophisticated and doctors are too sophisticated. I have to realise my limitations but maybe it can be done somewhere else. What we are doing is looking at prospective trials treating the patients according to the molecular profiling, immunohistochemical and clinical diagnosis. Again, briefly, we are taking all patients with unknown primary and we are excluding the treatable subsets. We are doing immunohistochemistry, we are doing RT-PCR bioTheranostics test and then we are treating patients according to what it says. Basically we are treating:

- Breast, we are treating with Taxane and bevacizumab. A combination of the standard in the United States.
- Ovarian we are treating with Paclitaxel, Carboplatin and bevacizumab. Bevacizumab is not standard, but you know who is helping to sponsor the study.
- Colorectal we have a number of regimens that we can pick from:
    - FOLFOX and becacizumab
    - FOLFIRI and becacizumab
    - 5-FU/LV weekly X 6 then 2 weeks off plus becacizumab
- Non small cell cancer lung cancer can be treated with

  - Paclitaxel, Carboplatin and bevacizumab
  - Docetaxel, Carboplatin and bevacizumab
  - Gemcitabine, Carboplatin and bevacizumab
- Pancreas we treated with Gemcitabine and erlotinib
- Renal cell we treated with Sunitinib or bevacizumab plus or minus Interferon
- Prostrate we treated with Androgen deprivation therapy.

This study is half done. We have substantial numbers of subsets of CUP colorectal profiles, CUP lung profiles and CUP breast profiles. Now I like good randomised control data but if I have twenty patients treated with a colorectal regimen, twenty solid patients who have a colorectal profile and they have a median survival of two years, I am sorry, but I am going to accept that and that patient no longer is in the same realm as the rest of the CUP patients and are shifted over into the favourable subset group. For other patients, where it is marginal, we may need randomised data as Dr Bridgwater showed. Some of those are only two or three months different.

I am going to stop here but I am very excited about this new era that we are in. I did not even mention that we have to crawl before we walk and we are crawling now to try to determine what cancers these patients actually have. Walking and running is actually looking at molecular characteristics of a patient's cancer regardless of where it is from and treating the patient according to that rather than the actual primary site. We are going to get there.