Overcoming the Unknown:
New Approaches to the Diagnosis and Treatment of Carcinomas of Unknown Primary.
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The CUP-One Trial
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John Bridgewater's talk has really given quite a lot of overlap with my talk but I am going to try and think of the situation from a different way , thinking of the opposite angle. Over a period we have started to tease apart how to do a clinical trial which would be statistically valid in this disease area and it has become very clear that there is more to it in terms of what we might get out of it. This has been the longest trial in incubation that I have ever been involved with. This is because it is a rare cancer and also because it has been very difficult for peer review bodies to understand exactly how you could get a good study out of this work. In fact this delay might have worked in our favour because things have developed very rapidly over the last four years and ,since we think all the paperwork is now complete and we are ready to roll, it might be that this is an appropriate time to adapt to the rapid changes you have heard about today in terms of the molecular technologies.

Starting as a sceptic I am certainly more convinced that the diagnostic aspect of the trial is getting close to something that might be clinically usable in some way although we do not know which way. Therefore it gives the opportunity to move it one step forward.

The question is 'Is it possible that CUP is actually just another way of saying that we want to treat metastatic disease, irrespective of site?', 'Do we really want to care about the site?' There is a point, that has been raised on many occasions, that, although we question the cost of the investigations of a CUP microarray or equivalent as a few thousand pounds, we should not underestimate the cost it takes for patients to go through the factory line we have just discussed - both in time and resource. If you are in the private sector in the UK just getting a CT scan these days costs £1,500 plus reporting. Should one be doing colonoscopies and colonographies and CT and PETS, which are about £2,000, whether you actually need them or not? There may be some utility in actually understanding that there might be a fast track to knowing what you actually need to do.

In terms of the biology the studies like those that have been presented today, studies that are prospective with validation, and all the work that you have heard about so far today are really going to power us forward.

The question is how to define the cancer. This was one of the first problems in thinking about the trial. The other way of thinking about it is that you sit at your MDT and you have patients coming in every week with metastases and so you could start off by saying that there is your diagnostic challenge straight away. You will have an unknown primary at presentation in almost every MDT you go to because it is very common that they will say 'OK it is a suspected colon cancer. Let's do a colonoscopy'. The cancer is unknown at that point as it has not been defined as being certain. When you find the primary you treat it and you classify the rest as CUP. Many of them, however, remain as Uncertain Primary Origin and we are forced, for all the reasons we have heard today, to actually classify it both for patient reasons, drug access reasons, individual reasons and suspicion based on a radiologist or a pathologist saying 'I am confident it is x, y or z'.

Does any of this matter? What matters to patients is actually outcomes and it is not really the diagnosis in this case as you have heard. The outcomes are, importantly, palliative care and quality of life.

How certain are we? How can we define certainty? Everyone has presented some fantastic data showing that they have got certainty in their diagnosis. I do not actually know what the gold standard is that you are comparing it to because you are all saying that you are certain that it has arisen from that primary. I do not know how you are defining that because there is no way of actually knowing in the current publications what the quality assurance is. Are you treating a CUP as a CUP , a true CUP, or you are just suspecting that it is a CUP or do you think it is really an

unknown and you think 'Well I will treat this as a pancreas cancer because there is a spot in the tail of the pancreas and I do not whether that is a CUP or a pancreatic cancer'. Real experience constantly leaves me uncomfortable.

This slide shows the details of several real patients that I have seen in the last few weeks.

- The first one had an almost identical case to what we heard this morning in the Case History. This would be treated by everyone seeing it as a colon cancer and again we have talked about colonoscopies and in this case they were negative twice. What do you treat the patient as because it looks like a Barn Door colorectal cancer? I do not know what that patient has. I will come to show you why it might be that it doesn't matter.
- The next results are for an immunohistochemistry G3 neuroendocrine tumour. IHC questioned that it was of biliary origin because some of the markers were coming out as biliary markers. There are lots of bone mets and it fitted a poorly differentiated neuroendocrine tumour. The CA 19.9 was normal, the CA 125 was grossly elevated. Would you treat this as a poorly differentiated neuroendocrine tumour or as an advanced biliary cancer? A colandula carcinoma? I do not know the answer to that. I opted for colandula carcinoma and the patient had a complete response. He could have had a complete response if I had treated him the other way, I do not know. Sure enough, within five or six months of stopping treatment, the patient relapsed.
- Liver lesions only. What about cases like this one which I saw recently where I was told that there was a poorly differentiated PatA cellular cancer. The AFP was 30, the CEA was greater than 40. There is no cirrhosis. Do I apply for Sorafenib? We cannot find Sorafenib in the UK so do I apply for it and spend hours trying to get an application in?

I have uncomfortable zones with all these patients.

Is there a value in hunting the primary in a highly metastatic carcinoma? The assumption is that the patient management and prognosis are linked to the tumour site of origin. Let us challenge that a little bit. John Bridgewater presented some data that he made up, I made up this also from the databases, which is quite similar. This slide shows that there is population ranking of the top 10 cancers

There are fairly similar survivals in terms of untreated patients and with treatment we have improved it marginally in some patients. These form a huge disease burden. The argument is always that if you have a CUP that falls into a category and you treat them with a platin agent they are going to do just as well whether you know the diagnosis or not. The agreement is that that there might be some subsets which have a good prognosis and the only one that John Bridgewater rightly excluded was hormone refractory prostate cancer. If you look at Stage 4 breast cancer, Stage 4 colon cancer, prostate hormone refractory you get the top three cancers again and you know that there are specific treatments. Now it is very difficult to know what the survival of the untreated patients are because we are now dealing with clinical subsets of subsets. In this modern era we do not know how long these patients would actually survive with best supportive care because we don't do those trials any more. Certainly they look on the face of it as though they survive a lot longer.

The recent CRYSTAL Trial basically looked at the addition of Cetuximab and Folffiri as first line therapy to a colorectal cancer population who were advanced and with poor prognosis. The results appear to be a landmark because on an intention to treat (these aren't split into KRAS, WAI-type yet) we have made a small incremental gain in survival, with the addition of Cetuximab. I would say 'Why not look at it a different way?' and look at the patients who don't do so well. What the results actually show is that 30% of the population are not surviving beyond a year. So I don't know that if that patient presented as a CUP whether I would actually do him any benefit at all giving him any standard colorectal cancer regimen. I think that this has not been discussed. It is the populations that are not doing so well in the groups that we are saying are doing really well. That is probably the overlap subset with CUP. You have to put forward the hypothesis that you may not get your answer by picking out your good subsets because of the heterogeneity of the cancer.

If you look at that the 30% and you say 'What does it represent in terms of a significant population burden?' it is absolutely huge because you are looking at a third of one of the top three cancers in the country. Are these subsets not really conventional response colorectal cancer? I don't know the answer to that but it is very important to consider that there are a lot of colorectal cancer patients who do as bad or worse than CUP patients. With a Cancer of Unknown Primary on average the median survival is about 8 months and there is no obvious best regimen. You must remember that the outcomes are similar to most advanced common tumours.

Why don't we do more post mortems? I had a look at the original papers and they are so poorly quality assured that I cannot even understand them. There are selection biases all over the place as to why patients did or did not have post mortems.

At post mortem in a highly metastatic disease you are looking at the last six weeks of a patients live when the tumour population is doubling probably at the rate of knots and there is tumour everywhere. I don't know how in post mortem studies you can validate gold standards. John showed a graph about the differences between anti mortem studies in the molecular profiling era and the post mortem studies and there was one obvious difference. That was that the breast cancer population seemed to suddenly expand in the modern molecular profiling era. Why might this be?

There is a lot of molecular heterogeneity; which has major clinical implications, which has got nothing to do with the site of origin. In John's example earlier you have a triple -ve breast cancer, a gastric cancer and an overlap in terms of both their molecular phenotypes, which signify response parameters, and in this case herceptin treatment, where suddenly these patients are doing significantly better when they have Her2 expression. The one question you have to ask yourself is: 'Are we just picking up some overlap that the technologies are picking up?' This is not a breast cancer so how is it on post mortem studies and on clinical studies you miss a primary cancer? It is difficult to fathom that one. The reason is probably that they are not breast cancers but actually your test is picking up, as has been known for many many years, that the inside shoe over expression of ERand PR is very common in GI tumours. If you bias your molecular assays, whatever they are, MRI expression RNAs, it is likely that you are going to get biases in the system. As you are probably aware there have been liver and pancreas studies based on immunohistochemistry of ER and PR using drugs that affect those receptors which have all turned out to be a bust. So there are a large number of GI cancers which actually over express ER and PR at the RNA level. I do not know if you want to call that a breast cancer, if it is highly metastatic, or whether you want to call it a gastric cancer.

This raises a lot of issues about what you are actually saying. Is the source of origin based on the biases that we have.

There is also the Phase II study promise and the Phase III failure phenomenon. I am not as experienced as some of my fellow speakers, but we have all had patients who were in Phase II which we said did fantastically well with the drug of interest and then the Phase II trial failed. We said that this is not possible as we saw the tumours shrink when we did the Phase II. It is not possible that that trial failed. All that is saying is that there is molecular and clinical heterogeneity . There must have been a subset of patients in that Phase II who responded to that drug because they had a particular molecular phenotype. It is actually, therefore, not the tissue of origin that counts, because our clinical studies by and large have seen more -ve Phase III's than +ve Phase III's. It is because we are not selecting the right subsets for treatment outcomes, whatever that parameter might be.

These were the thoughts we had when we were trying to develop this study and asking how we could capture, overcome and understand some of these issues. What we are saying is that clinical heterogeneity and molecular heterogeneity are probably equivalent and maybe we can use CUP as a model for all metastatic disease to test the hypothesis. This has been said for hundreds of years.

The question 'If I am sceptical about new technologies and saying 'Find your primary but it won't make a difference to the patient'' is probably too extreme a view. I don't think that in the future they are going to have a big impact on patient outcomes. They might have other impacts, however, which are extremely important. We have heard some of these today:

- Certainty – it is nicer for patients to know and the clinicians to be confirmed with an independent test that maybe they are right, whatever the biases are.
- Molecular profiling will gradually move towards directing us towards treatment, so we are learning all the time in terms of research. That is very important, and all of us who have done oncology and used drugs know that you are always at the bottom of the pyramid when you start and you move upwards with double drugs and triple drugs and this is the same sort of analogy in that sense.
- We are going to diagnose more favourable subsets. I am not quite clear that we are going to do it in the way that we have discussed today, but then we do not know what the outcomes are going to be until those studies are published.
- We do know that in some cancers, such as breast and colorectal but lymphoma we are already using genetic taxonomy to subset the patients. These diagnostic ID tests get more and more sophisticated and if we do the right studies on the back of them will allow us to do that in more detail.
- One of the things that has become more obvious in the last few years, and we talked about this in terms of the pathway, is that maybe we are actually positioning the concepts of all these tests in the wrong place. Maybe what we should be doing is positioning them at the outset of a cancer diagnosis biopsy. That is what we did in this study because it gives you clues to speed up the process and there may be a big health economic benefit if you can prove it in randomised trials.

These were the thought processes we had to try and incorporate into a study, hence its long incubation because of the confusing aspects of treatment, diagnosis etc. We got a whole group of people together , some by coincidence and some by fortune, which was essentially:

- the group at The Hammersmith
- the group at Glasgow – the trials unit as well as Karin, who had been publishing immunochemistry in this area
- and also The Peter McCallum Cancer Institute in Australia who contacted us to say that they had their own in-house profiling technology and would we be able to test it?

We decided, because a lot of CUP trials have actually failed because of poor recruitment, to think about this in a way that we would get some translational output and clinical output because irrespective of what I am saying today about the sceptical nature of the way people are classifying CUP it is actually quite difficult to get large numbers of studies in clinical trials. We first said 'Let's do a translational side of the study, which will be primarily diagnostic to start with'. We decided to use three technologies and compare them:

- One would be an immunohistochemistry, which Karin leads and has published extensively
- One would be the Bowtell Melbourne group, which have published two papers on RT-PCR-based, which is very similar to some of the technologies you have heard about today.
- We had a discussion with cupPrint, which is now Biotheranostics. Even encouraging them to be involved with us was very difficult but I am pleased to say that the superseded test, which is the Biotheranostics test is going to be incorporated into our study.

We are going to try to test these technologies.

The second part of the challenge is how to do this in a blinded way. Also from the patients point of view we had to have some treatment options for them. This was quite an interesting problem. If you ask people in the UK what they treat their CUPs with two regimens came up consistently: one was ECX, which is a epirubicin, cisplatin and capecitabine, and one was gemcitabine and cisplatin. Basically this is because they cover a lot of tumour types and there is some published date on those regimens and both contain a platin, which seem to be the common theme. There was no real evidence that was very strong one way or the other.

We wanted to do a head to head on the two regimens but were turned down by The Grants Committee, who said that it was an unpure study, so we had blocks all the way. In the end we decided to do just epirubicin, cisplatin and cpecitabine +/- a new biological.

The next hurdle was then to try and interest a pharmaceutical company to do CUP research when there is no commercial return and you will find it is very difficult. We approached about nine companies. Some said yes verbally but when it came to the final 'put your money where your mouth is' they said no because they saw the costs of running the study. We actually decided that we could run a single armed Phase II with the epirubicin and cisplatin until a drug partner came along. Thankfully through the AstroZeneca /NCRI collaboration we have now got Vandetanib, which is a dual inhibitor of EGFR and VEGFR.

This is a 114 patient study. There is some logic in the way that we chose Zactima, which is Vandetanib, and that was based on the fact that there is data that the VEGFR pathway and the EGFR pathway will probably play some roll in some of these tumours. Tony Greco also published a paper on the use of bevacizumab and erlotinib, which actually showed some of the longer survivals his group did. So we used a combination of those two drugs without chemotherapy and there was some logic and rational behind using these drug. There was also data that the EGFR pathway was over expressed.

The design of the study.
This is quite complicated because of all the issues raised.

- The patient comes first to an MDT and presents with metastases. At that point they are unconfirmed, or uncertain, because you do not know whether a primary has been found.
- We undertake a minimum clinical investigation as per our protocol guidance. Patients who are in treatable subsets, or of non-carcinoma pathology are excluded at this point. The clinical investigations are fairly loose on purpose in order to reflect real time practice but conclusively also means that if you want to participate, at some point you are going to have to call this a CUP or a non-CUP by the decision of the MDT. This is, therefore, a pragmatic study reflecting what happens in real life weekly for all our MDTs.
- At some point you call it as a known primary, because you feel that your clinical investigations have given the known primary, or as a CUP. There are now tissue biopsy samples for both data sets .
- The known primaries are then split into three, four including the base pathology unit who has to do some diagnostic work. They are sent blindly with only age and sex on the samples to Karin, who distributes them to the other two groups to do their molecular profiling validation.
- At this point all three centres are not aware of what the local decision was as to whether it was a primary or a CUP.
- The patients can benefit from going into a clinical trial which has two parts:
    - Treatment part
    - Translational part where we want to learn from the data, with multiplatform analysis for different things depending on how much funding we can get, which is looking more promising all the time.

This complex design took a long time to finalise and to convince peer review committees that you could statistically validate a hypothesis.

We did the statistics and basically for the clinical part of the trial it is always much easier as it is a simple randomised Phase II with the Vandetanib, or not, with the EXC . 114 patients are needed to show whether the Vandetanib is giving a signal of benefit, or at least, non inferiority. The clinical part of the trial is, therefore, easy to structure as long as you have 114 patients in the trial.

The results of the three outputs will be difficult as no one has ever done this before. How you power those is very difficult. Essentially what we are asking the pathologists to do is to call their top primary – say where this has come from. Obviously as time goes on they will have a mix of patients which are known and unknown primaries and we can make some expected correlations between the known primaries as your validation said, of how accurate they were. We can then adapt that to the unknown primaries if one of the tests shows a very high concordance with the real clinical conclusions that the clinicians reached.

We are hoping, with this study, to begin to think about how CUP genetics might actually tell us about not just diagnosis, which I think we are going to clinch in some form. We are going to validate a lot of these methods and my guess is that there is going to be a lot of developments which validate these methods. It is just a question of how cheap one is and how easy it is for the clinician to do it. How does that compare to the pathology? As time moves on it is likely that they will be embedded in some way or other and it is just a question of how good are they going to be compared to the other technologies.

We are beginning to unravel in this study the whole concept of metastases because we have got blood, genes and are hopefully going to be able to get a higher level of microarrays. This will actually give us prognostic and predictive information across the two subsets. We will also be following the known patients for survival and we have some limited data capture for what treatment they got as well. What this study is really to do is to organise things. Probably the real endpoint of this study is that, hopefully, we will be able to organise CUP research, at least in the UK.

The current status of the trial is that it has taken a long time, we have, we think, got the final paperwork signed recently and we are looking at the initiation meeting, starting by teleconference very shortly. We have had a lot of interest from the study abroad but at the moment we are sticking to the UK because there are more drug funding issues.