Overcoming the Unknown:
New Approaches to the Diagnosis and Treatment of Carcinomas of Unknown Primary.
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CUP research: diagnosis and treatment

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It is a great pleasure to be able to speak about this, one of my research interests, in oncology.

What I have tried to concentrate on in my work is the true CUP, so other sites excluded clinically, PSA's, the exclusion of the sub-sets ,which I am sure that you have been through already, and I understand that PET scans have been deemed unnecessary and in further investigation and that is certainly currently our standard practice. These are the sub-sets about which I am sure you have spoken already

- squame cell carcinoma in neck nodes,
- carcinomatosis in the female,
- the adenocarcinoma in the lower neck nodes,
- PSA's,
- the axillary lymph node in the female and
- the midline adenocarcinoma in the male or germ cell tumour, which is now very specifically defined.

These groups are here, of course, because there are data to demonstrate that their survival is just as good if you treat them as head/neck cancer, ovarian cancer and so on. It is very important to exclude those sub-sets and there is something to underline that fact later on.

We have heard a lot about uncertainty and this surrounds the doctor as much as the patient. The uncertainty is as much around the diagnosis as around the nature of the data that is out there. There are, in my view, several aspects to this:

- One is that CUP is not a rare cancer, it is an uncommon cancer. But currently there is no centralised treatment process and Harpreet Wasan and I both treat other rare cancers for which the management, the treatment, the research, the whole picture has been completely revolutionised by the development of the central MDTs . Central Multidisciplinary meeting in which, in all cases, are focused and there is a responsible, enthusiastic physician, who leads the team from a medical point of view. There is a specialist nurse, who then guides the patient through that, often precarious cancer journey, and that has absolutely revolutionised the treatment of other uncommon and rare cancers and if the NICE process, which is currently going through at the moment, comes out with anything at all, surely this is that a CUP specific MDM is a no brainer here. It is absolutely essential to move this subject on.
- There are often poor performance data.
- The studies are invariably retrospective, or for the most part retrospective, and include specific sub-sets which have very good prognosis and so the small publications are very difficult to believe.
- There are lots of other biases, for example published this year in the EJC somebody has gone through all the Phase 2 studies – 29 Phase 2 studies, with almost 1400 patients in it – looking at response rate as an indication of what is the best treatment to give patients with CUP. They went through the whole lot and came out with some hazard ratios, which is a measure of benefit, and yes, cisplatin and doxorubicin did seem to have some kind of improved benefit, curiously, runertike and carboplatin did not but, equally important, in terms of relating to this positive hazard ratio was the general impact factors, so the publication bias is a significant factor. If you have a good response rate you are going to go into a high impact journal, more people will read it and more people will consider this to be a standard treatment. That is probably not the way to go about it.
- Single centre studies have a higher impact rate, central radiological review of the response rate brings that response rate right down and, as I said, earlier, the inclusion criteria, if they include one of those sub-sets then it completely impacts on that response rate.
- Randomised data would, in fact, overcome most and many people would say, all of these issues. So I would argue, at the risk of upsetting some of my colleagues who are about to

speak and those who have already produced data in this area, that the single arm Phase 2 study can provide little evidence, I would argue no evidence, about the best way to treat our patients with CUP. I would argue that it is not useful.

This slide takes us through the CUP thought process and at various points I will throw in some of our data, both clinical and laboratory, and studies I think we should be doing to really underline and nail the issue.

The first proposition, hypothesis, assumption – it somewhere in between all those – is that CUP are hydrogenous. It is not one single strange adenocarcinoma of the left ear lobe which everybody has, these are common cancers that are missed. Are they common cancers that are missed? The data on this slide shows the autopsy found primaries in CUP patients and then the DNA assigned primaries. In fact n=500 actually is not accurate as the number includes a number of known primaries which were analysed using some of the RNA technology and if you look at the studies that are analysed the true number is probably closer to 130 and 90 out of a 130 people were identified. So these numbers down the side of the graph (starting with 11.5 at the top) are probably inaccurate. Much more reliable are the autopsy found primaries. So it looks as though these studies show there is a range of tumour types. Curiously breast is very under represented which I find needs explanation and questions the validity of the whole series. You get a feel that these CUPs are in fact probably a range of common cancers. You could argue that that simply reflects the false negative rates of standard investigations. In other words you have 7% bowel cancers in there because there is a miss rate of something like 7% for colonoscopies. That's probably an underestimate, the colonoscopy failure rate in some of the larger series is around 20% and this has been improved recently with the CT colonograms and classically you now go on to perform your CT colonogram if you fail your colonoscopy. But nevertheless there is a failure rate amongst those and if you take that as a percentage of the total number of colorectal cancers in the UK every year I think that would theoretically contribute to the CUP population.

Similarly there are problems with breast cancer screening in mammography and the mammography, for instance, is much less likely to be positive if you have lumpy breasts, lobular breast cancer histology and small tumours. That has been known for some time, so is there a miss rate in the standard investigations that account for the instance of CUP in the population? I would argue yes.
We have some data to support this from a feasibility series we did around 2007. We took paraffin sections from 23, what I would consider to be true CUP patients. The analysis was done through Agendia which I think no longer exists, we then went through them case by case with pathologists, molecular biologists and a bio mathematician. I would completely accept that there is GI bias in the case selection because I am primarily a GI physician and these are the patients that came to me. It is a little bit old and diagnostic techniques may have improved a little since then. I think it is reasonable to say from the data that, again, it supports the premise that CUP are missed common cancers. From the MRNA analysis four tumours were thought to be ovarian, two breast, eight large and small bowel (The Agendia test at that time did not distinguish between large and small bowel, which is one of the flaws of that original data set). It came from the original pathological data set from which it was drawn large and small bowel are not distinguished. One each of cholangio, endometrial, small cell lung cancer, mesothelioma , pancreas, urinary bladder and stomach. Very roughly speaking a reflection of the common cancer distribution which supports the hypothesis that CUP in fact missed common cancers.

This slide shows some case reports, just to say that it is not necessarily the case:

- One of those patients was a sixty year old man with a peritoneal disease. The peritoneal biopsy was done via a laparotomy adenoma glands were found in the peritoneal fat. The Agendia diagnosis came out as mesothelioma, which is of course not true. It simply reflects the fact that this was mesophyllial tissue that was taken at the time of surgery, so with that patient it clearly got it completely wrong.

- A forty three year old man with an axillary lymph node who had lumps excised over six years from his left axcilla and nobody had a clue about what the situation was. The MRNA suggested breast cancer and because he is an Essex man he completely refuses to accept the diagnosis and hasn't had any treatment for this in any way and refuses to see the breast surgeon. The other aspect about this which I do not know if you have addressed or discussed but at this point, when you find out that the patient is likely to have breast cancer and indeed it becomes blindingly obvious it is a male breast cancer you send the patient back to the breast cancer colleagues – or do you? You don't see the patient again, you should really send them to your breast cancer colleagues.

  So your role as an oncologist is diagnostic, not therapeutic. Unusual for us, but perhaps something we should think about.

- Finally, a current case, a sixty three year old woman with a left ?????? lymph node excised was adenocarcinoma. Nothing found anywhere else and we resorted to PET scan I am sad to say. Ultimately the MRNA suggested endometrial cancer and we went through the pelvic investigation. It was completely normal. Six years later she represents with a node in the other groin, which was exactly the same thing and had a hot ?????????? on the PET scan. At the time we did the RNA the Agendia test couldn't really distinguish between endometrial and ovary. Again that is something wrong with the original pathological data set. So this could easily be a gynaecological malignancy and she is having an operation any time now.

You have to, very much, take the molecular diagnosis in the context of the clinical picture. Because, after all , although we say that CUPs are missed common cancers they are clinically and, clearly, biologically different. There is something different about them and we need to take that into account when managing these patients.

What about the 'one size fits all' chemotherapy rule? I would propose that 'one size fits all' chemotherapy is a ludicrous concept and I will try and back that up by showing you this slide of randomised Phase 3 trials of chemotherapy and CUP. Four have been published, two are quite old and some data which is yet to be published and will be discussed later today. If you take out the very old studies which I think you should, and look at the survival results I would suggest that there is no obvious winner that you can pick out of any of these regimes combining up to seven drugs. I would argue that this suggests that 'one size fits all' is, with great respect, nonsense.

There is another way of looking at it. If you look at the efficacy of standard chemotherapy drugs, of which three are discussed at random on the slide, and their efficacy in certain tumour types you can see that there is very disparate activity of these drugs in these cancer types and really if you are using a 'one size fits all' you are only going to hit your appropriately responding cancer in a certain number of cases.

Are CUPs molecularly similar to their known primaries? I would argue yes. Consider the data from the same series that we sent to Agendia relating to breast cohort, colorectal, endometrium, kidney clear cell, kidney adenocarcinoma, lung, ovary, prostate, thyroid and bladder (which did not work out tremendously well). Again it roughly agreed with what the Agendia data said and visually makes sense. With 24 micro RNAs you can actually get to your cancer. You can get to colon and breast cancer in 5 or 6, which is very good. This currently rocks in at 34,050$ for doing this test. You put your sample in an envelope with your cheque and you get a result. That is perhaps part of the discussion.

The other thing that there is relatively little about at the moment is what is known as the cancer methylomes, when DNA is methylated and the pattern of this methylation reflects the type of cancer that it is. These datra were in the middle of generating these data but it really is the same as, and in many ways the principles are identical to, the MRA and the micro RNA analysis and you can generate a very simple metholation cascade that describes your cancer and this what we

are doing currently. We have a series of 86 true CUP patients, we have the immunohistochemistry, the micro RNAs are being done at the UCL Chemistry Institute, the methylation assessment is being done by
Dr Fernandez in Barcelona and we should be able to directly compare these three ways of looking at unknown primaries and come out with a number of foster primaries. It will be very interesting to see whether this gets anywhere and perhaps help us to choose the easiest, the most effective and the cheapest molecular diagnostic tool. After all the Rosetta roxin is 3400$, and most of the other MRA technology, as I understand it, comes in at £2000 and this is expensive. How successful is it? One of the huge advantages that metholation has is that you can do it on DNA so there is no difficulty with the quality of the MRA extraction which has been a hurdle until a few years back. This is what we have running at the moment.

Identification is feasible, foster primaries can be found. Is it worth it? This slide represents the survival for the common solid tumours, advanced incurable common solid tumours and the numbers represent mortality per year in the UK. The further you get to the bottom of the list the more lethal the cancer is and the more people will die from that cancer that year. The median survivals are taken from the latest data. The four good ones are kidney, ovary, breast and bowel and they come out very well. CUP, I would propose, rocks in at around 7 to 8 months. Any diagnostic process that you undergo, any molecular test, will have to really put people onto the right hand side of the line. If you make a diagnosis of pancreatic cancer it will probably not make much difference but if you make a diagnosis of kidney cancer it will make a huge difference, ovary cancer: a huge difference, breast and bowel; a big difference. One of the criticisms of this approach is 'Why don't you just exhaustively look at your patient for those four cancers?'. I would argue that that is not a particularly useful way of looking at it, after all kidney has only got up to 30 months in the last few years, up until two or three years ago kidney cancer had a terrible survival rate, and similarly bowel cancer has only had a good survival rate in the past four or five years. Up until four or five years ago the survival would be similar to that of CUP. The principle is there; you have to move your patient from a not particularly effective 'one size fits all' therapy to a foster therapy which then puts them into one of the specific categories which gives them more life. The thought process is slightly artificial but I think useful to go through.

The other way in which you demonstrate, or prove whether it is worthwhile, is probably in a randomised study. The idea is very straightforward: you have half your patients treated as your molecular tests suggest and half your patients given a 'one size fits all' approach. 'Blood out of a stone' does not describe the difficulties I had extracting the total number of patients from the statisticians for this because you have to go through a rather complex analysis of exactly how much benefit you are going to get and the likely incidence of getting somebody with breast or bowel cancer out of your CUP population. Three hundred patients was the number that I managed to get from them.

Just to touch on the role for biologicals Can the choice of the biological treatment be determined by the molecular profiles? Forget an anatomical primary, look at the biological nature of the tumours. So for instance, if your patient is KRAS Wild-type offer the patient eGFR inhibition whether you think it is a bowel cancer ,or a breast cancer, or whatever. The big problem, of course, is that the KRAS pathway is probably the most well described, and even that we do not understand tremendously well. I am sure that this would be one way of approaching it.

To sum up: Are CUP hydrogenous? :

- I think they probably are. I would like to think that they are missed common cancers.
- I do not believe 'one size fits all' chemotherapy is effective.
- They are molecularly similar. Similar being the operative word. They are biologically different but molecularly similar to known primaries and can be identified.
- Foster primaries can be found and we need to determine whether our primary specific chemotherapy will be more effective.

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