Ulster Medical Society

Thursday 20 October 2011 at 7 p.m.
'*Personalised Medicine and Modern Pathology*'
Professor Manuel Salto-Tellez
Queen's University of Belfast

Professor Cupples:

Good evening, once again and you're very, very welcome. I've had a couple of people have joined us on the way through, and that's great, and we may have some more to come still, but I think this is probably more comfortable than the crushed quarters next door.

I'm standing in tonight for Professor Johnson, who sends his apologies. He's had to go to the Medical Research Council, so we couldn't argue, by asking him to stay here, I think, in terms of competition from the Ulster Medial Society and the MRC. My name's Margaret Cupples, for those of you who don't know me. I was the President of the Society last year.

So, without further ado, can I extend my welcome personally to Professor Manuel Salto-Tellez, and we are delighted to have him at Queen's. He brings a truly international perspective to the University, to Belfast, and to the Society this evening. He is the new Professor of Molecular Pathology, but he's also the Visiting Professor, Department of Pathology & Cancer Science Institute at the National University of Singapore, and I had to look to see the different categories he has been in already, because I know that his homeland is Spain, he's also worked in Germany and the Netherlands, and he has specialised in pathology at the University of Edinburgh, sub-specialised in gastrointestinal pathology in London at St Mark's Hospital, and in molecular pathology in the United States.

He's worked in Singapore for over ten years. He has published many papers, books, and he is going to tell us something more about a perspective of pathology which we're not perhaps very familiar with, and that is the personalisation, personalised medicine and modern pathology. Professor Salto-Tellez, thank you for speaking to us, and I can say he's happy to take questions during the talk, as well as at the end.

Professor Salto-Tellez:

Thank you very much. I'd really like to thank the President for the kind invitation to be here, and the past President for the very, very kind introduction. As I say, we are a small group, so if there is any burning question that comes to mind, please don't hesitate to stop me and we can go through it.

I understood that this is usually a very varied audience, from all parts of science and medicine, so this is the aim today, to give a presentation probably as general as possible, so that we can also have a good discussion, and when talking about molecular pathology, about personalised medicine, about modern pathology in general, I always think that it's a good idea to have a little bit of a historical perspective, to understand exactly where we are coming from and what we've been doing in the past, and in the very past, it was essentially the pathology of dead people, so essentially we were looking at bodies, we were looking at organs, we were looking at how those organs looked, and that gave us a clue, together with the symptoms of the patient, of what was happening in the bodies of those that were diseased. This is probably the very early days of physiopathology, and it's starting in Greece, as far as we know, continuing in probably one of the prettiest cities in the world, if I may say so, in the south of Spain, and taking us into the Renaissance, where really there was a clear push to understand how the results of an anatomic dissection was telling us, together or coupled with the information that we have on the patients, on how essentially disease has happened, and from this point onwards, you can see one of the big things among pathologists, that this is still now the one that has done more autopsies is the better pathologist; Morgagni, apparently, 600, if you go a century or two later, Rokitanski 20,000, so it's difficult to beat that one.

Really, what we are doing here is really looking at the microscopy of organs, and trying to learn medicine from that, and then something has started to happen, with Virchow and with other people—we started looking at the microscope, and we realised that the changes that were happening in tissues and that were happening in cells were probably at least as important as the ones that we saw with the naked eye, to understand diseases, and here is probably the first description that I've seen of cells related to a benign or a malignant potential, and probably this is the first description of an infiltrated breast cancer into the skeletal muscle around it.

If anyone, if any pathologists in the audience wants to blame why we do frozen sections these days, one of the things that we hate most, you can blame this gentlemen, [/ Heim?] who was the one that pioneered it. And so at this point it's very important, because we are moving from an anatomical dimension of pathology to what somebody called at some point, the anatomical and clinical dimension of pathology, which is essentially transforming the pathology of the dead into the pathology of the living. This was an extraordinary world revolution, probably in the 1940s and the 1950s, the fact that several people, they started to bring a new dimension to pathology. At this point, the pathology report was essential, and this was becoming an essential piece to decide on therapeutic intervention on diagnosis and on prognosis, so much so that at that time, it was clear that no surgeon, no physician, no oncologist, that were known at the time, would be able, or should, make a decision on treatment or prognosis unless there was a full pathological report, and that essentially brought pathology to the centre of the life in hospitals, are the moments in which clinical autopsies were shown to all the residents in the hospitals, the moment in which there was a pathological discussion of the disease. That became a remarkable moment in the life of a hospital.

So, from centuries, we move from microscopy, we move to histology, and sometime in the 1970s we started bringing immunohistochemistry and immunocytochemistry to the armamentarium of pathologists. But see how [?] in disease. The DNA was discovered in 1953. Since then, there's been a significant amount of work telling us about the nature of diseases and biomarkers that are associated with those diseases.

Let me take you to 1992. This is the time in which I started my training as a histopathologist in the University of Edinburgh. At that point, which is probably 40 years or almost 40 years after the double helix was characterised, the amount of cases that had molecular analysis were probably 0.05. We used to do B and T cell rearrangements. Occasionally we would take a partial or a full [mole?] for ploidy analysis. That was really everything that we did, and this was after 40 years of scientists providing remarkable information about the nature of diseases, about the molecular biology of diseases. It is now 2001, and at this point we can say that probably at most, 10% of the samples that come to the pathology laboratories are analysed from the point of view of molecular diagnosis.

Why is it so? Why is that taking almost 60 years to get all that information into our diagnostic armamentarium? Well, maybe this is another way of looking at it, so this is a bottle, and this is a bottle that is full of basic science discoveries, and this is a glass. This is a glass that is supposed to be full with the diagnostic and the clinical applications of those discoveries, and this here is a bottleneck, so for years we were saying that "Yes, we knew something about the molecular basis of disease, but it wasn't enough to bring it in to the diagnostic applications." Sometimes we were saying that we really didn't have the technology robust enough to analyse all that in a meaningful way. Sometimes we are beginning to think that what we have is a lack of robust research design, and probably design clinical trials, to make sure that those biomarkers really can be used in diagnostics. Whatever the reason, there's been a gap, and if we want to understand what is happening in the area of molecular pathology, of personalised medicine and modern medicine nowadays, from a practical point of view, I suggest that instead of looking at the bottle, we can look at the glass. That will give us an idea of where we are, and how useful this new version of pathology can be, and this is essentially what I would like to do in the first part of today, so I would like to give a definition of what personalised medicine, molecular pathology, molecular diagnostics is, I would like to take you through some of the technology very briefly, of what we are using nowadays for molecular diagnostics, maybe conceptualise some of the uses of that information as well, and take you through some of the validations of how we do a test these days, and how we are helping the patient that way.

So let me start with some definitions. So if you apply molecular biology techniques, and you apply the knowledge of molecular mechanisms for three main purposes, either to diagnose, to prognosticate, or to treat patients, then you are doing molecular diagnostics. If you are not doing any of these, you are probably doing something very interesting, but it's in the area of research, it's not in the area of diagnostics, and that, as you will see through the talk, is a different work. So if you ask out there, molecular diagnostics means "Let's see if there is a mutation, if there is an inherited pattern of disease", but we know nowadays that the bulk of molecular diagnostics probably comes from infectious diseases, and even more than even from genetics, it could come from oncology and solid tumours together, and these two areas are really the areas that mean the application of molecular diagnostics to what we know essentially in terms of histopathology and cytopathology.

So, we could define diagnostic molecular histopathology and cytopathology as the application of molecular diagnosis, to the samples that normally come through our routine histopathology or cytopathology department. What will these be?—well essentially, anything that has to do with formalin fixed paraffin-embedded tissue, anything that has to do with fine needle aspiration, with pleural effusions, with exfoliative cytology, the cell blocks that are generated by this, those will be the natural materials that we normally use for what we would call diagnostic molecular histopathology and cytopathology.

What are the technologies?—this is just very briefly. So we can usually analyse the molecular basis of diseases at three different levels, and the degree of complexity of the technologies that we are using is also there, so from the point of view of DNA, you can do conventional PCRs, you can do single sequencing, you can do para-sequencing, you can do the HPLC, and now we are beginning to bring next or third-generation sequencing into our molecular diagnostics. From the point of view of RNA, and there are some extraordinary experts in the audience, you can take anything from a routine RTPCR to the highest group of gene expression profiles that we are using now in inputting. And we get, looking at structural chromosomal abnormalities, gene copy numbers, again anything from simple FISH to other forms of in-situ hybridization that are not necessarily fluorescent, but our ACGH would be some of the options that you would have in mind. Obviously the bread and butter of molecular diagnostic laboratories are on the top of these columns, but more and more often, and this is something that we are really aiming to do here in Belfast, both in Queen's and in the Trust, is to be able to incorporate these high throughput technologies into routine molecular diagnostics as well.

So to me, the best way to explain to you how this test can be useful for the patient is really to take you through a menu of these tests in a laboratory that does these kind of tests, and this is the menu that we had in my lab in Singapore, and the one that, with changes, we would like to adopt here in Belfast. We can divide this test normally in three groups: one which I call tests with predominantly a diagnostic value. We do these molecular test wide, because the pathologist looks at the tissue, looks at it under the

microscope, and cannot make up his or her mind, and it's important, because depending on what label we give to that material, the patient has a different diagnosis, and probably also a different treatment, and there are different ways of looking at these. Translocations of sarcomas, translocations of sub-lymphomas, have been the most relevant ones, and sorry for the misalignment. Essentially what is happening here is that, if you have a sarcoma, and you don't know what is the precise sub-type, but you are able to detect the translocation, which is the product of the fusion of two specific genes, then you will be able to give a diagnosis, because this signature is pathognomonic. The same would happen with lymphomas. Lymphomas, there are two main types of the studies, the chromatin studies that have been with us for a while, and then all these translocations, that again, are becoming essential to decide which sub-type of lymphoma the patient may have, and therefore what is the prognosis and what is the therapy that we need to implement.

Just to give you an example of this, I brought some examples to just indicate how this helps individual patients. This is a 63-year-old lady with the imaging features of [?], which is how our imaging people recognise this kind of image with bilateral [?], worsening dyspnoea and two main lesions in the thorax, a very clearly hypo-dense defined lesion there. This measured almost 10 centimetres in maximum dimension, and here you can see another one, a smaller one in the anterior mediastinal area, measuring four centimetres in maximum dimension. So the doctors went in, took an FNA, a fine needle aspiration, and this is what we saw. I'm making that point to bring today cases in which molecular diagnostic has helped in materials that are particularly small. We are talking about FNAs, we are talking about small biopsies.

The aficionados in the audience may recognise that this a tumour with a biphasic pattern, one area that was definitely cellular. You can see a vascular pattern coming out of it, and then another one that is much more loosely arranged and probably with fatty tissue in it, and if you look deeper into that, you can start recognising things that pathologists will recognise as part of a liposarcoma, these cells with these small nuclei and these compartmentalised empty spaces, these other ones that are coming as a multinucleated area, multinucleated cells, with a significant degree of pleomorphism. In other areas of this material, as you can see, the lesion was much more fatty looking and even so, you could see these rosette-like cells with several nuclei, and in other parts of the biopsy, of the cytology, once we spin down the material and we've created a cell block, you can see some of the features that led to a provisional diagnosis of liposarcoma.

We are not used to give a definite diagnosis of something like sarcoma or lymphoma, on FNA material only, and yet one of the interesting things that molecular diagnostics is providing is that because some of the markers that we are looking at here are pathognomonic of a disease, you can do so, and that is what we did in this case, so we knew that myxoliposarcoma had a specific translocation. We looked for that translocation and we found it here, as you can see, in duplicates in this material. So that was the diagnosis, and based on that diagnosis, surgeons went in, dissected the specimen, and really when we look at the huge material, the information that we got was very similar to what we got in the very little material that I mentioned earlier on, taken just by the needle. So we saw essentially a cellular and yet adipose-looking lesion, with the same vascular pattern that I described earlier on, with a significant degree of pleomorphic areas, and the diagnosis was myxoliposarcoma, and this is how the patient evolved.

One of the interesting things here as well in this case, is that the pathologists will recognise that it is extremely difficult, when you are resecting and re-dissecting the samples, to know if the lesion is present in the margins or not, so we use molecular diagnostics in this case for that as well. So to ensure the surgeon that the lesion was fully excised or not, because morphology wasn't good enough, we did molecular diagnostics for that purpose.

Another interesting use of diagnostic molecular, pathologistically speaking, comes with [hydo?]pathology, another area of difficulty where we try to do fine needle aspiration inside of nodules of uncertain significance, and we know that using a panel of different genes looking for mutations, primarily BRAF, we can differentiate those that were originally indeterminate from those that have a high chance of being malignant from those that have a lower chance from being malignant, and this work that is from Pittsburgh has now been done in Newcastle, and the results are equally encouraging.

So keep BRAF in mind, because BRAF mutations seem to become very useful right now for different reasons in modern medicine. In therapeutics, they are telling us what is the likelihood of patients, particularly with malignant melanoma, but with other cancers as well, to respond to certain personalised medicine; in diagnostics, because of thyroid cancer; in colon cancer, because it's related to prognostics and therapeutics as well, and we learned a couple of months ago that also in hairy cell leukaemia, are likely to indicate a possible intervention.

So these are tests with a diagnostic value. We do them because the pathologist doesn't know what he's looking at. There are others that we do because they have a genetic value, and the classic test is microsatellite study, so remember the adenocarcinoma sequence.

This is something that we've been telling our students for many years now, the first time in which we were able to link a succession of morphological changes under the microscope with a succession of molecular changes at the DNA level. Well, it's probably true that this is false, and in fact we are moving into other models which obviously are becoming more complex, in which we are looking at hypermethylation, and we are looking also at specific mutations,

followed by models in which we are bringing together the microsatellite instability status of the colon cancer with the methylation status of the colon cancer, it's difficult.

One of the things that has puzzled me by the way, and I put this slide while I was doing this this morning, when I knew that Richard was going to be in the audience, is that if you look at breast cancer, there is always a link between single biomarkers, ER, PR, CRV2 and the gene expression signatures that we are beginning to identify. Even in gastric cancer, this is the work that we did in Singapore published just two months ago, we saw that there was a relation between what we understand as diffused gastric cancer, intestinal gastric cancer and gene expression parameters. I seem to see an association between what we know about the single biomarker or a small biomarker nature of colon cancer, and what the gene expressions are telling us, and I'm just wondering if this would be an area in which bringing them together, we will know more about the nature of colon cancer.

From a practical point of view, microsatellite instability is a very useful test, and is so because the diagnosis of hereditary non-polyposis colorectal cancer, this is the commonest known cause of colon cancer, and essentially you have three ways of looking this problem: either by the microsatellite instability test I am going to show you in a moment; immunohistochemistry with a mismatch repair proteins, or mutation analysis of the mismatch repair genes.

Just to illustrate this in a way that hopefully everyone will understand. These are two patients from the study that we published a few years ago. This is a very young colorectal cancer patient, 31 years old, but it was microsatellite stable. There was maintenance of the expression of the mismatched repair proteins, and there was no mutation in the mismatched repair genes.

Here you have another 31-year-old, but this time, as you can see, with a strong family history of colon cancer, and this patient was unstable. The patient lost expression of one of the mismatched repair proteins, because there was some mutation of one of the mismatched repair genes, so you can look up this material, you can look at your own patients, and you can start deciding what is the most cost-effective way of using this test, one after the other, to make a final diagnosis. There is a case to start doing this in patients under a certain age, and we really hope that very soon we'll be able to do these here with our own patients.

Now, what is really changing the paradigm of everything that we understand in pathology and in molecular diagnostics is these kind of tests. The tests that we don't do because the pathologist doesn't know what he's looking at, or because we think they may be inherited, we are doing them because it would be able to tell us which patients are more likely to respond to which drugs, and this is a true change. This is a list that we collected two years ago, and the list has been growing, of antibodies that are small molecular inhibitors, so these are new therapies that are targeting specific key genes and specific pathways that are helping oncologists to treat old enemies with new drugs. This is a change of paradigm, because essentially what this is saying is that, at the end of the day, the decision of which patient gets which drug is not so much based on just the wish of the clinician, but it's based on the result on a biomarker, and if you think that this list is already long, think about this—last year it was calculated that there are somewhat in the order of 850 reputed clinical trials with some of these new drugs.

So even if you are very pessimistic, and you think that only 1 or 2% of these trials are going to be successful, this list is probably going to double in the next year or two, and this revolution in oncology is also deeply transforming the way we practise pathology, because we know that there are now at least 6 cancers, [?] tumours, breast cancer, gastric cancer and colon cancer, malignant melanoma, where there are single biomarkers, the result of which is going to tell us which patient is more likely to respond to which drug. What my labs have been trying to do in the last few years is to show that we can do this confidently, that we have a body of published evidence showing that we can do this well, and also that we can do this in the best, which I think is best, which is considering that modern pathology cannot do this in isolation. It has to be a synergy between morphology, immunohistochemistry and molecular diagnostics.

Very briefly, let me take you through some of these tests. We do seek mutations in gastrointestinal stromal tumours, and we do them because that will tell us which patient is more likely to have the first response to [?], and also because in cases of diagnostic difficulty, if you find a mutation, you will be able to diagnose something as a gastrointestinal stromal tumour, instead of some other parts of the differential diagnosis. This is one of our, of the work that we did. Again, I bring you this particular study to show you that, with the small material, with the small cytology, we are able to detect confidentially the mutation, the same mutation that we would be detecting if we analyse the fullest specimen, the full surgical specimen.

Now, this is changing the way we do pathology, and it's changing the value of pathology, and I'll give you some examples again throughout the next few minutes. Pathologists are very good at diagnosing [?], but we are very bad at prognosticating [?]. What do we do? We count the number of mitosis, we see what is the size of the tumour, we see where the tumour is located, we look out for differentiation markers, and we give an idea of how the tumour may behave.

Now, I would argue that this classification of this cancer, and remember pathologists are par excellence the classifiers of diseases, that this classification should probably be super-imposed by a molecular classification, because based on the mutation profile, will give an information that is probably more meaningful for the patient, which is how these patients are going to respond to certain [treatments?].

We do KRAS mutations in some cancers, particularly in colon cancers, and this is the way that we explain why we do it in our reports. Patients with wild-type KRAS labelled negative are more likely to have disease control with this drug. Patients with any mutation may not benefit from this treatment. So this is one of the studies in which we were involved, and these were groups from Australia, from Singapore, and I think, yes, the [NDHS?] from the UK joined us, and it was essentially a way of looking at the same material with several different technical approaches to understand what was the best way of looking at these mutations. And again allow me to bring you this paper showing that, on the small cytology samples, we can confidently detect KRAS status, so if you see a patient again, or you see a patient for the first time, and all you have is the material from an FNA from the liver, you can confidently indicate there what is the KRAS status.

Now, these are some of the models, or some of the numbers that began to tell me that molecular diagnostics is not only absolutely necessary for our patients, but may also be a good business proposition. From that moment in which we started doing KRAS testing, we moved from 200 cases a year to, as you can see, more than 500 within the first half of 2010, and in fact, when we look at the source of the cases that we were analysing in my previous lab, you realise that there may be as many industry-sponsored or clinical trial-related, as many of the others together. In fact, our own hospital probably contributed 14% of this, and this is the model that I would really like to bring in the new lab that we are building here, and I will tell you in a second. The fact that if you do molecular diagnostics well, there is no reason why others will bring their tests to us.

Now, I don't know if there was ever a histological classification of metastatic colorectal cancer. What I can tell you is that the oncologists know one very well, and it's the presence or the absence of KRAF mutations particularly, probably not [BRAF?] any more, and the response of [?]. We are doing a lot of each year, of our mutations these days, and this is one of the tests that is going to come online now, in our laboratory here very soon, and we do this because again morphology has taken us as far as we could get. From a morphology point of view, for many years we were differentiating between a small cell, and not a small cell, because those were the treatment options that we had. Now, we are increasingly seeing that it is important to indicate where adenocarcinoma is, and I'll mention this in a second, so we are doing EGFR mutation analysis, again to predict the response to a drug, or to two drugs probably. The EGFR gene is large, but the section of the gene that has the genetic information that is telling us how the patients are going to respond is relatively small, so with four PCR reactions, we really can't cover the four exons that are telling us, as you can see, which mutations tell us that the patient is going to be sensitive to the drug, and which mutations are going to tell us that the patient and the tumour are going to be resistant to the drug. This starts giving us very interesting information about many things that are related to molecular diagnostics. For instance, the clinical materials—we are living with what somebody has called the small sample revolution. The needles are getting smaller, the material that comes out of the needle is less, and yet the information that we are requested to provide is more.

How do we do this? Well, life probably provides a very good model for this purpose, because as you know, these tumours can be, are difficult to access. The first time that I was convinced that you could really do molecular diagnostic in small samples was in this study that we did together with other people from Australia and Japan. We analysed more than 100 samples, including this one, a 66-year-old female, a non-smoker, with a routinely recognised tumour of 4.5 centimetres. They put an FNA, the amount of material that was obtained was somewhere of 1 or 1.5 millimetres maximum dimension. The DNA was extracted, the mutation was identified. When we could go back to the surgical material two or years three back for the resection specimen of this patient, the mutation was confirmed, so we started making sure that our tests were validated for the small samples as well as big samples, and these are some of the results that we just reported a couple of months ago in an annual scientific meeting. You can really get material for analysis from lung cancer from four main sources: those that come from excision during surgery, core biopsies from peripheral lesions, the small bronchoscopic biopsies from central lesions, and cytology from FNAs and other places. When you ask yourself, as we did, what is the sample that gives you more often a good result?—in other words, the less number of unsatisfactory results?—we were very surprised to see that it was the cytology material. Always the cytology materials can be better fixed, and therefore the DNA preservation is much better than any of this, and believe me, remember this comes from a laboratory that was doing molecular testing for 30 or 40 hospitals in 20 countries throughout Asia, and as you can imagine, the quality of the DNA was very variable. So much so that if you look at the two main hospitals for which we were doing EGFR testing, the hospital where our histopathologists or our oncologists trusted, that you could do reliable EGFR testing on cytology samples, the vast majority of the EGFR testing was done on cytology cases, so in other words, we don't choose which is the best tissue to do molecular diagnostics. We make our techniques so that the first specimen that we get from the patient is good enough to do molecular testing, and in fact some of the original descriptions of how things like EGFR testing are done should probably change because of this evidence that our group and other groups are putting forward.

Now, what this means is that, if you are a physician, and you are involved in the whole process of treating these patients, your approach to tissues should probably change significantly. Just let me give you an example.

A patient with lung cancer, we sampled the tumour. The first thing that we wonder is, can we make a diagnosis? If we can, we do so; if we cannot, we're going to have to go back and get more samples. The next question—can we do molecular testing to decide on the therapeutic intervention? Well, if we do, we do so, if not, we'll have to go back and get more samples, and this is putting extra pressure to three groups of doctors. First of all, those that are at the front line of any material for diagnosis, because they have to remember that there are more requirements to that material; definitely for pathologists, because we have to make very good use of that material. Maybe we shouldn't be asking for 10 or 15 immunohistochemistries, if four are enough, because we have to keep material for DNA extraction; but also the oncologists, because there will be occasions in which, even if you do this by the book, you are not going to have enough material, and therefore re-biopsying patients may have to become an issue in many situations.

The other thing that I think is very important about analysing EGFR is, who asks for the molecular diagnostic test? I've been working with AstraZeneca, and other companies, on this matter. So imagine, a pathologist gets a diagnosis of non-small cell lung cancer, the report is issued and goes to the physician. The physician produces a report, it's referred to the oncologist. The oncologist meets with the patient, says "Wow, you have lung cancer, we need EGFR testing." EGFR testing is done, the patient meets with the oncologist again, and the decision is made. These, in our own practice, took an average of 26 days. If you empower the pathologist to decide on EGFR testing at this point, that report is already going to have EGFR information, which means that that patient can be directly sent to the oncologist, and this process has a turnaround time which is less than four days, and we are beginning to work with the industry which is going to pay for some of these tests, and also with the people that are making these kind of decisions in the hospitals, to make sure that we streamline this process in the best possible way.

Now, the way we classify adenocarcinomas of the lung is changing dramatically. This is the WHO classification of lung adenocarcinoma: it's long, it's cumbersome, and I still haven't seen an oncologist making a specific treatment decision based on the sub-type of this kind, maybe bronchial or alveolar, maybe. Now, it's clear that we are beginning to understand the molecular basis of adenocarcinoma of the lung, and not only we know the molecular basis, we know it based on value markers that are potentially treatable, that are potentially druggable, and this obviously has a significant advantage which I will discuss with you in a second.

So maybe what we should start doing is bringing into the hospital models that will allow us to combine conventional pathology and modern pathology for the interest of the patient. For instance, if I look at this list, there are already three tests that are really defining adenocarcinoma: EGFR, KRAS and EML4-ALK because of the ALK inhibitors that we know these days. Maybe one way of doing this would be, if the patient has a small cell carcinoma, there will be other non-personalised medicine treatments, but if it is a non-small cell, we'll do EGFR. If it is mutant, we treat accordingly. If it is a wild type, we do KRAS; if it is mutant, there are some options. If it is wild type, we do EML4-ALK, and based on the result, we do [?]. A sort of cost-effective way and logical way of starting integrating testing in our routine work.

Now, molecular diagnostics is not kicking pathology out, provisional pathology, but it's changing its value. Remember the days, as I said earlier on, in which our only problem was the small cell carcinoma versus non-small cell carcinoma? Well, these days are gone, and they are gone because where pathologists plays that case in this continuum between adenocarcinoma and the squamous cell carcinoma, can change the fate of the patient, because we know that if we are at this level, the patient is likely to get a GFR mutation analysis, and therefore being considered for anti-EGFR treatment. If it is on this end of the spectrum, it will not. If it is somewhere here, it depends very much on what is the policy in the hospital. So who is going to be tested, and therefore who is going to be treated? And also, what are the chances of getting the mutation for quality control and quality assurance purposes, are very important. So the value of traditional pathologies are still there, it's very important, but it's changing.

HER2 testing has been probably the first and only example of personalised medicine done in years, and I just want to take one minute to tell you about the complexity of what is coming. So at the moment we are doing HER2 testing to decide on the treatment options for two possible drugs. We are seeing new drugs that are going to help in improving the blockade of HER2 testing, and we are seeing drugs for other inhibitors that are not necessarily HER2, but are related to HER2, and what this means is that in the future, what we are going to have is many different treatment options, and many different biomarkers associated with those treatment options. We have new pertuzumabs, we have new PI3 kinase [entor?] inhibitors, some of which work with the specific cases where there are specific mutations, we are getting interested in growth factor inhibitors. Again, the amplification of this gene is key to decide which patients are going to respond to that drug, and obviously we have the example of truncated P95 protein and [hepatamine?] as a second line of treatment in breast cancer. So the complexity is going to be tremendous, and I think we are going to see HER2 in other places as well, and see how things change, see the difference in the way we do things.

Biomarker analysis prior to therapeutic treatment, I think is here to stay. There is a new type of pathology that is already here to stay. It's here to stay not only because of what we already know, but because anyone that is doing a good phase zero and phase one trial, are going to incorporate analysis of those materials to try to find which biomarkers are

going to predict which patients are going to respond to this drug. This is now in many ways the standard practice. We are going to see more complex technology being involved in molecular diagnostics, and we are changing the effects on [?] cancer, and this is probably one of the most extraordinary changes that we are seeing.

In 1999, the then director of the National Cancer Institute challenged the scientific community. He said, "Do molecular analysis, and apply molecular diagnostic technologies, to make the classification of tumours more informative. This should change from a classification that is purely morphological, to a classification that is molecular." How did the pathology community feel about this?—extremely bad. [Juan Jose?], one of our main leaders, was trying to write papers to justify that morphology was still important. Other pathology friends were just announcing the end of pathology [resection?].

Now, where are we 13 years after the challenge? Well, every year there are more samples coming to pathology departments, and not only that, we're beginning to learn that new molecular diagnostic approaches need to be based on very solid morphological interpretations. In other words, phenotype and genotype are not mutually exclusive, they are complementary, but they are changing. They are changing in chest, they are changing in the breast, they are changing in the colon, and as I said earlier on, they're changing in adenocarcinoma of the lung. Look at the base classification of adenocarcinoma of the lung—not very objective, not very reproducible, believe me, with very little clinical relevance, very cheap, because pathologist's time, as we know, is very affordable. Now, how is this? It's certainly much more objective, because we are looking at an analysis of a test. It's more reproducible, although note, I'm not putting as reproducible, because as usual we always have issues with technology. The clinical relevance is beginning to be much higher. It's definitely more expensive, but it will not be more expensive in the future.

So allow me to take you back to history. We were at a moment in which, in the mid-50s or 60s, in which pathology was essentially the core of our hospitals, and then as you can see, something has started to happen. We started to put a molecular dimension to pathology. Essentially it meant that not only we had to look at this end of the spectrum, we had to look at the whole end of the spectrum, and because of that, we started to create levels of molecular complexity between the traditional pathology description and the clinical decision made. We are seeing these with single biomarkers, we are seeing these with the coming of multiple biomarker analysis, we are seeing these with pharmacogenomics, but what I would like to argue is that the best people to do this kind of tests at the end of the day are those that understand the molecular, the clinical and the pathological knowledge of the disease. In other words, it should be pathology departments, the ones that are adopting these tests, and together with the traditional pathology, offering it to oncologists, to physicians and to patients. Certainly pathology should be doing these single biomarkers, as I've mentioned earlier on, but also the multiple biomarkers that I mentioned earlier on as well.

This is another way of putting it. This was an opinion paper in the American Journal of Surgical Pathology, with some of my colleagues in the Massachusetts General, probably an evening where we shouldn't have written anything, but you know, it happened that way. Now, to maintain our central role in pathology, we need to embrace molecular diagnostics. If we don't do that, certain things are going to happen. Others are going to do it for us, that's very clear. We are going to compromise our strategic position as a crossroads between science and diagnostics, and what is very important is this, we are going to lose revenue, because molecular medicine and molecular diagnostics is the fastest-growing area in medicine at this point, and that's why our recommendations, and I'm sorry for the emphatic style, but this was at night with probably a couple too many whiskies that we shouldn't have had, is that it's time for surgical pathology to embrace molecular pathology, that this should happen where it really matters which is when you are signing out the patients, and that we should really start training our people in that kind of activity.

If you want to see it in a different way, what are pathologists at the end of the day? We are integrators of information. We look at the morphology that we see, we look at the immunohistochemistry and the electron microscopy, we look at the clinical information and the imaging, etcetera, and we make a diagnosis. It is essential that we integrate the molecular information as well, because only then, we are going to continue maintaining the central role that 60 years ago, a pathologist managed to have in the hospital. So my conclusion is, a stronger molecular pathology operation will become major pathologies and cytopathologies.

Now, allow me two minutes of your time. How do we do this in Belfast? How are we going to bring that to our own situation? Well, our challenge is to bring together our hospitals, the Cancer Centre, and the local industry that obviously is having a significant role in the way we are doing molecular diagnostics as well. Now, what we are doing is that we are creating a comprehensive operation that is taking this pipeline through basic science into diagnostics, and as you can see in this pipeline, there is a section that is definitely research, there is a section that is definitely diagnostics, and then there is a section that is going inbetween. This is a section that we know these days is research, but to make it fully meaningful, it must happen in laboratories that have the rigour of molecular diagnostic operations.

So how do we start doing this?—well, by integration. The laboratory that I'm going to present to you now is an integrated effort between CCRCB Queen's and the Trust, an integration between what you could call traditional pathology and modern pathology. This is the layout of the lab. I'm told that it will be ready at

the end of March of next year, although the work has already started. As you can see, we are organising things so that we have a large area for molecular tissue diagnosis, we have a pre-PCR room, a post-PCR room. The bio-imaging group of Belfast, that is extremely important, is coming to this laboratory, because they are beginning to understand that it's good that they are where the tissues are being generated, and the biobank, which is an extraordinary good development for Belfast, is also going to be incorporated into this. We have a significant amount of technology, including next-generation sequencing, and probably this is the most important thing, this is a luxury—this is the people that are involved in this.

We have six pathologists, of at least national reputation, many of them, that are spending time through CCRCB and CR UK here. We have the leader of the bioimaging group with us, and the leader of the biobank, and scientists that have been working in tissues for a long time. We have very good secretarial support. We have three clinical scientists that are coming directly from the Trust, and are bringing a significant amount of expertise. We have two extraordinary post-ops that are going to allow us to look at the molecular biology and at the bioinformatics and data analysis of all this work. I'm very pleased to tell you that two of our brightest pathologists have decided to do PhDs with us, so they are going to be in this laboratory at least four or five years, and we have a very strong support of technical staff, including, thanks to the help of Friends of the Cancer Centre, a research nurse that is going to allow us to bring the clinical information that we need for our studies.

The goal is to have molecular pathology translational research, molecular pathology diagnostics, supported by a very strong biobank, and by a very strong bio-imaging component, molecular pathology research in this way, so we have a lot of techniques and technologies, we have a biobank, we have bio-imaging, always analysing cases that come from our hospitals, and therefore the clinical information is known to support our research, the research of our researchers, and the activity of [?]. And the way we are doing this is integrating things like, tissue [?], very robust protocols for extraction of DNA, RNA, etcetera.

This is some of the work that is already going on. These are the main sets of main cancers that we are aiming to get ready within a year. Some of these are already ongoing. For instance, in the prostatic, this is some of the work that we've already done in prostate cancer, analysis of biomarkers in an animal model, in breast and in ovary as well, in head and neck, together with [Dennis?] group, and in other groups as well in which we are essentially bringing the tissue and the clinical dimension to the molecular biology that they are generating, and very important, the groups that are looking at what the future of molecular diagnostics is going to be, is the integration of high throughput data and high throughput analysis into the therapeutic decision-making of the patients.

The biobank and the bio-imaging are very important, the biobank because it's providing not only the quality of the tissues, but also the ethical component of it. Things have been organised by Jackie James in such a way that not only we have high-quality materials, but we also have a very robust ethical framework in very little time, which is very important, and obviously the bio-imaging group that we have here in Belfast is of international reputation.

When we are doing molecular diagnostics, we are setting up these tests, and other tests. In fact, this is the many of tests that we are aiming for, and in an adult, you have the ones that will be ready within a month. Here are some of the results. This is some of the validations, looking for instance of a mutation in KRAS, or a mutation of EGFR, and as I say, integration being the main component of what we are doing. Now, what this means is that we should really integrate ourselves. We have two pathology departments in Belfast that are independently holding some of the best pathology talent nationwide. They are ten minutes apart from each other. They are in buildings, one of which is more, Victorian times. The other one is beside this nice building, in a place that is full of asbestos. It doesn't make sense. Someone in this audience told us a while ago that consolidation of these services is the best way forward, and when I read the report of Dame Allen, I could look at it word by word and agree to it. We need to bring these operations together, because if we do that, we are going to have something that is going to be hardly beatable in the rest of the UK. We are going to have a laboratory with a volume of 60,000 biopsies, 12,000 cytologies, more than 20 senior pathologists and a similar number of trainees; modern protocols, as you have seen, that we are trying to develop, a dedicated state-of-the-art molecular diagnostic operation, as I mentioned earlier on, and privileged links with academia and with the industry. If we do this, we are going to develop a pathology operation that is going to be second-to-none in the UK context, and this basically is because of the people that we have. I cannot over-emphasise how important it is that those that make the final decision on this kind of activity realise that bringing these two departments together can bring not only the treatment of our patients, but also the academic dimension of our medicine significantly forward.

So, allow me to tell you that this is happening because of the tradition that we have in pathology in Belfast. When I started, I'll tell you a little story and I promise I'll finish. When you come to a new place, you want to know what has happened before, and obviously there is a history of pathology in Belfast, so I arrived in what is called the Institute of Pathology in that Victorian building, and I said, well, what is here that can tell me how is pathology in Belfast? So the first thing that I saw was this picture, and this picture is full of people that 28 years later, because I think this was somewhere in 1984, but it's still here, can see a very [cheeky Steven McQuade?], an extremely young Peter Hannon, as you can see, Dame Allen somewhere there, and many other people that 28 years later are still here, and are still being the backbone of the pathology that we are doing these days,

and trying to find clues of what was the style of academic pathology in Belfast, there was something that I saw in a filing cabinet, so this, maybe Dame Allen can … it seems that it's been very much used, and it seems that it has been used by more than one chair until now, and I was thinking, I was wondering if this was the style of academic leadership in pathology here in the past, but what I can tell you is something—if we do things well, we are going to build up a very solid tradition of pathology, with very solid research and very solid molecular diagnostics, and this is going to be a very bright future. Thank you for your time, thank you for your patience, and I'm very happy to take any questions.

Professor Cupples:

Thank you very much indeed, for bringing modern pathology into life in here. Tricky questions—anyone like to start the ball rolling? Yes, Patrick?

Dr Patrick Bell:

I'm always struck, when I hear somebody talk about molecular techniques and genetic techniques, about the lack of discussion of, do you ever get results wrong? I was struck that you talked about unsatisfactory samples. I suppose as somebody who's used to more working with physiological parameters and numbers, and has ranges and so forth, but in your sort of work, do you ever make mistakes, and do you quantify those and quality control them?

Professor Salto-Tellez:

Yes, so if you look at the criteria to accredit a laboratory for molecular diagnostics, and I'm now thinking about the criteria of the College of American Pathologists, two-thirds of that document, and it's a document of more than 100 pages, is aimed so that you don't make mistakes, so that you control the work that you do, that you have a very strong quality control and quality assurance mechanism that will allow you to detect any possible wrongdoing that your laboratory is doing

I don't think that molecular diagnostics is necessarily better than other areas of medicine, but because it's beginning to be very strongly regulated, it's certainly not worse, and one of the reasons of why molecular testing becomes more expensive than it should is because of the redundancy that it will bring to the system, the amount of samples that we repeat, the amount of controls that go in each sample, the number of people that are analysing the same case. It's a must, and in fact that's probably why, in one of my slides, when you saw the layout of the laboratory, I had three acronyms in the slide. They're essentially the accreditation bodies that we would like to bring into our laboratory to convince you and to convince others that quality is paramount. Otherwise, it would be very difficult to clear a hybrid laboratory, that we are aiming to do.

Professor Cupples:

Roy?

Professor Roy Spence:

First of all, congratulations on your vision. I think it's fantastic. Can I ask you a kind of conceptual question? Who owns the tissue? Let me explain what I mean—there was a case in the States some years back, where a cell line came from a patient who had their spleen removed, and they found a very rare lymphoma and the cell line came from that in due course, and was commercialised, and it became a very major case in the States, who owned that spleen, so when you now have an installation that starts going from the diagnostics right through to commercialisation, and supposing this year or next year, in two years' time, you get a discovery from [?] for example or a lymph node biopsy, say the same thing, the cell line appears that can be commercialised, or something a bit more sophisticated, or in due course, through Richard here, a drug appears down the line, do you know what I mean? I'm asking the question almost rhetorically, but who owns that discovery, from my spleen?

Professor Salto-Tellez:

Different countries have different views, but there are some things that I think are common. The tissue is owned by the patient, so the patient at any point can go to a pathology department and say, I want to retrieve my tissue from here, and they can do that.

Now, what we are trying to put in place are avenues in which the patients are happy to, if you want, donate those tissues to those that are going to make use of them, or to allow the use of that tissue anyway, until they say that they don't want to, and that's why they sign forms, they sign consent forms, and they allow for that work to happen, so that point is very clear. The patient owns the tissue until the patient decides that he or she doesn't own the tissue any more.

The interesting question is, then afterwards, who does? Is it the hospital, is it the researcher, is it the government? It's very difficult to know. What is very clear to me is that, in many cases, rather than ownership, who owns the tissue, what we should have very clear is who is the custodian of that tissue, because that is really what makes a substantial difference, to make sure that if that tissue is used for the future for any reason, is with the interest of mankind in mind, that it's fully anonymised, that it's fully confidential, that no-one is going to send results back to that individual person for that purpose, to me the main question here is not so much ownership but custody, and good use of the material, but again, if you want to translate that into a legal concept, it's a minefield.

Professor Roy Spence:

It is but supposing, I did a splenectomy last Thursday, it was a very complicated [?] spleen apparently, supposing that spleen was used for research, and in due course, you get a cell line from it, in due course, I'll pass it over here to Richard, and he becomes a millionaire in five years' time?

Professor Salto-Tellez:

Well that, Richard shouldn't have used that cell line unless there is a paper from the patient indicating that he or she is very happy to donate that material and the rights of that material afterwards, for science, and then what we have to make sure is that, at the end of the day, if there is a huge profit from that, the people that get most of the profit is not Richard, but it's the institution for which Richard is working, and all this means legal agreements, but if we have them in place, there is no reason why what we perceive now as fear or danger, is something that could be profitable for society eventually.

Professor Cupples:

One more question, I think.

Dr James Douglas:

I think Roy's point is very apt. I think (?? 1:11:23) excellent talk, (?? 1:11:27) will benefit from advancing that forward, but we do not really know what (?? 1:11:32) and in some cases in the States, people have brought cases relating to (?? 1:11:37) and I think have succeeded.

We have our own very complicated and difficult Human Tissue Act, and it's still not perfect, because at the bottom of the whole problem is, and it's slightly incorrect what you said, nobody owns their own tissue, according to the law, nobody owns tissue but you have the right to take it away and bury it, if you so desire, and that means that there's still a big hiatus in the law, which I think as yet, and in fact, paradoxically, the more molecular biology and other science is developed that values cell line tissues, the more likely it is to come into a difficult head, and it can't be resolved, but I think although it's nice to think that advances of this sort will actually bring benefits to institutions, I think Roy's point is correct—we cannot rule out that [?] of the day that individuals [?].

Professor Salto-Tellez:

No, you cannot rule out, but then, allow me for a second, what is the alternative?

Dr James Douglas:

The alternative is, we don't do anything.

Professor Salto-Tellez: (?)

The alternative is that we have an institution that is teaching the students, and there is no advancing; in other words, an institution that is taking of the patients, of the present, because we are teaching them very well, but not the patients of the future, because we are not doing research.

Dr James Douglas:

All I'm saying is, it will become a difficult issue in the future. I agree with you, I'm not advocating that the patients get that money, that profit. I'm just telling you that it's an unresolved issue.

Professor Salto-Tellez:

Absolutely, I fully agree.

Professor Cupples:

There's one last burning question.

Professor David Hadden:

David Hadden, I'm very ancient and retired, so long ago that I go back in pathology to the days of your predecessor, who also came round the world through Edinburgh, and that's John Henry Biggart, whose name you will have heard of, and he taught us ethics, and the people in the back row that are worrying about ethics, we were taught ethics by the professor of pathology, and we kept these blood specimens, and we looked at them and we did our research on them, and we discussed them with the patients, and we didn't regard it as an ethical problem. I accept that there have been ethical problems, but we learnt our ethics from the pathologist, my generation, and we were very grateful to that, but one special question for you: you've talked all the evening about cancer. Have you plans ahead for what you might call the other parts of medicine, such as the autoimmune diseases, the diabetes, the endocrinology, all those vascular disorders which we're much concerned about? Will they respond to your genetic knife?

Professor Salto-Tellez:

Let me put it in the best possible and politically correct way. Life is complex, and the Belfast Health Trust is complex, and there are already groups in Belfast that are doing very good work in molecular diagnostics in other areas. The genetics department is an excellent department. The haematology department is doing top molecular diagnostics in some tests. Infectious diseases, Peter Coyle, is a leader in molecular biology. Now, what I would like to see is a situation in which we all synergise from each other. When you think about it, what we are applying is similar techniques to different problems, how good it would be for the whole of this community if we had a single institute of molecular medicine or molecular diagnostics for the whole of Belfast, or perhaps the whole of Northern Ireland. That is what I would like to see. I have no doubts that the rest of the molecular diagnostic community is doing a good job, but I think we could organise ourselves better to be much more meaningful.

Professor Cupples:

Thank you very much indeed. You bring excitement and colour, I think, to the world of pathology. When I was a student, you actually knew a little bit about the tradition of pathology teaching in Queen's. Professor Allen, as she was then, I think, brought the excitement to pathology in terms of the lunchtime post-mortems. We thought if we listened to her, that we would know everything that there was to know about pathology, but I think tonight is actually revealed the fact that the more you know, the more you know you don't know, so thank you again.

There is a cup of tea outside, a cup of coffee for those who would like it. Please take time to chat. Manuel will take some more questions, if you would like to ask him them. Can I remind the members and visitors please, that there is a book just sitting on the table over there. We'll be delighted to have your name put in for the history, that you were here tonight, and to invite you all again to the next meeting of the Society, which is 24 November. It's in Londonderry, so it's a bit of travel, but the Chief Medical Officers of both the north and south of Ireland will be there, to discuss the way forward, and better collaboration.

Thank you for this evening.