Ulster Medical Society

The 2020 Belfast City Hospital Lecture

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Professor Ann Mullally
The Physician-Scientist: Rewards and Challenges. A Personal Perspective

Harvard University

Professor McMullin:

Good evening everyone, to this meeting of the Ulster Medical Society. This is actually the 2020 Belfast City Hospital lecture. Several of us found out that there's supposed to be an annual Belfast City Hospital lecture, which they pay for, and I've managed, with a little collusion with Patrick Morrison, we've had a 2019 one, and we've had a 2020 one as well, so this is the 2020 Belfast City Hospital lecture. However, for our 2020 Belfast City Hospital lecture I am delighted to introduce Professor Ann Mullally, who's flown in from Boston today. Ann is originally from Mullingar, she qualified from UCD in 1999, did medicine for a couple of years in Ireland, and then went out to America, where she has trained as a haemato-oncologist, and then is working as a translational scientist. She came on my horizon quite a few years ago now, at various MPN [myeloproliferative neoplasms] meetings, where this person appeared with wonderful mouse models and research into CALR, and she works as a physician-scientist in Harvard. She clinically works in the Dana-Farber Cancer Institute, Boston, but has her labs in Brigham and Women's Hospital—I hope that's the right way round—and she has a big interest in myeloproliferative diseases, and myeloid malignancy, and has an extensive publication record and grant income.

She's going to give a talk to us tonight. I have to say, if it had been left to me, I probably was going for, "Of Mice and Men", or something along those lines, but she actually said, which I think will be to everybody's benefit, to widen it out a bit, and she's going to tell us about the physician-scientist rewards and challenges, a personal perspective, with a bit of "Mice and Men", I'm sure as we go through. Thank you, Ann.

Professor Mullally:

Okay, thank you so much to Mary Frances for inviting me. It's a real honour to be here, thank you for coming out. I'm going to tell you about some of the work that I do in my lab, and some of the reasons I really enjoy being a physician-scientist, and then some of the challenges I think that the field is facing, and just talking over dinner, it seems like it's not an American problem only. I strongly believe that physician-scientists can really make tremendous contributions in our understanding of human disease, and in advancing the care of patients, but it can be challenging, so just to tell you a little bit about my own career so far.

I'm going to really, hopefully this, it's how to pitch this. I hope I haven't made it too scientific and too haematological, but please feel free to interrupt if you want, or ask questions at the end. I'm very informal, so there's no problem with that.

I want to tell you just two stories from the lab. I work on blood cancers and myeloproliferative diseases like Mary Frances, We have a really good understanding of the genes that cause these diseases, but we have substantial deficiencies in the treatments that we have for our patients, and so my goal is to try to understand the biology of these diseases with the hope of advancing the treatment, and so I'm going to tell you one story of a patient that we're studying, a series of patients, but I'll highlight one in particular, and then this focused around the gene JAK2, which we'll talk about in a second, as one of the causative genes for these diseases; a second story around the gene called calreticulin, which my lab has worked on a lot, and then finally I'll just highlight some of the things that have been in the medical literature in recent years around the physician-scientist, and really sort of, the paucity of numbers of physician-scientists there are in the United States, and sort of almost as if physician-scientists is an endangered species, so I'd be happy and interested to hear your thoughts on that at the end.

My lab studies myeloproliferative neoplasms, so these are diseases that arise in the haematopoietic stem cell compartment, and we've been fortunate in the haematological field that we've been able to study tissue for a really long time, as it's been always easy to get bone marrow and blood, and I think this has really helped us in understanding the biological basis of these diseases. There's been an enormous amount of work done around normal haematopoiesis and normal haematopoietic stem cells, and we have a really good understanding of how our body maintains blood production our entire life. Essentially we have these so-called long-term stem cells that reside within our bone marrow, and these are rare and quiescent, but they're capable of self-renewal, and essentially that's what allows us to make blood all our lives, and also they can make these different lineage commitments, so they can become broadly speaking two different types of cell, one lymphoid cells, or secondly, myeloid cells. And the myeloproliferative diseases occur because of mutations, somatic acquired mutations, so when that stem cell replicates, it acquires mutations

and we know that with every decade of life, we acquire more mutations, and that's a process of ageing, and most of the time the mutation has no consequence to the cell, but sometimes it does, and gives a growth advantage to the cell, and that's what happens with myeloproliferative diseases. So these mutations arise in these stem cells, and then they change the behaviour of that stem cell in two major ways: one is, it gives a growth advantage to the stem cell, so now that mutated stem cell grows a normal haematopoietic stem cell, and it also skews the lineage bias of the cells towards myeloid cells, and hence the name myeloproliferation. So these are diseases that often times can be just picked up incidentally. Somebody goes to their primary care doctor, they have a CBC drawn, and they're noted to have an elevated blood count, and then it gets worked up and we found out they have myeloproliferative disease. Or they can present with some of the consequences of having high blood counts, often times strokes, heart attacks can be their first presentation. Then, these very interestingly, a very fascinating aspect of these diseases, that we have a poor understanding on, is that these myeloid cells proliferating over decades can actually damage the bone marrow micro-environment, and cause this disease called myelofibrosis, which is where we have scarring or fibrosis of the bone marrow, and actually bone marrow failure and low blood count. So this is sort of like the fundamental basis of these diseases, and as I said before, we have a good understanding of what are the genes that cause these diseases, and so the diseases, the haematologists in the audience will recognise the names, polycythaemia vera, that's a high red cell count, essential thrombocythaemia, a high platelet count, or myelofibrosis, this fibrotic bone marrow. So these are all terms, descriptive terms for how these diseases were originally described, and really it wasn't until the '50s that somebody postulated that they probably have a common genetic basis, and then in 2005, the first gene that was at the root of the cause of these diseases was discovered, and that was the JAK2 gene, which is the most common gene that's mutated. Remember, these are acquired mutations, so it's not an inherited mutation, it's something that's acquired within the bone marrow stem cell.

The second most common mutation is in this gene CALR, or calreticulin, that was just discovered in 2013, and then the third mutation that causes these diseases is in the receptor for thrombopoietin, which is the growth factor for platelet production, and that's the MPL mutation. And as you can see, these mutations occur in a mutually exclusive manner, typically, so if you have one you don't have the other, and we think that's because they all converge on a common pathway, so once you activate the pathway by one of these mutations, that's sufficient, so there's no pressure for the cell to mutate another gene in that pathway. The pathway that they activate is what's called a JAK-STAT signalling pathway, so this is a signalling pathway that mediates signals between cell surface growth factor receptors and the nucleus, and basically these mutations activate that pathway, such that it's constitutively active, so normally our body has a lot of feedback mechanisms. When our red cell count is high enough, we shut down red cell production, and there's a feedback between the amount of red cells and oxygen sensing and erythropoietin, and it's very tightly regulated, but in the situation of these diseases, you can see that the pathway is turned on all the time And so this is just illustrating that normally you have a ligand, so for example, this would be erythropoietin binding to the receptor that would activate the pathway, but in the case of these three diseases, you have pathway activation in the absence of the ligand, so the pathway is constitutively turned on, and as a consequence of that, you make more blood cells than you need, and that's myeloproliferative disease.

So, as Mary Frances alluded to, we've generated a lot of mice recapitulating these mutations, to allow us to study in more detail the consequence of that mutation on the stem cell. So one mouse that we made several years ago, when I started out originally in Gary Gilliland's lab at the Brigham, is we made a mouse that just had this JAK2 mutation, and as you can see, the mutation is at a very specific location. It's this V617F residue, a valine to phenylalanine substitution at position 617, and we created a genetic mouse that just has this mutation, so it's a single base mutation that causes this amino acid change. Actually when you do that, the mice develop a disease that's actually identical to the disease that human patients develop. So they develop a lethal myeloproliferative disease, so this is just a survival curve here, so here are the mice with the mutation median, survival is around 150 days, and then these are the wild type, the litter mate control mice. Basically they developed very elevated red blood cell counts, as patients do, so elevated haematocrit, and they develop very large spleens, so this is the normal mouse spleen and this is the spleen from the mouse that has the mutation, and then this is the normal spleen with the normal splenic architecture, but if you look at the spleen from the mice that have the mutation, it's full of what we call extra-medullary haematopoiesis, so blood production outside of the bone marrow, so it's full of erythropoiesis, granulopoiesis and megakaryopoiesis. So, by just introducing this single mutation into a mouse, you can engender all the features of the disease you see in people.

That's important, because that tells us that that mutation is really important in causing the disease, and therefore if we had good ways to target it, we

would have a high chance of curing the disease, since we know the causative mutation. So since that time, we've moved on in human MPN to do a lot of gene sequencing, so now, if you're a patient who comes to Dana-Farber with any kind of blood problem, we run a next-generation sequencing panel, and that panel comprises 100 genes, and they're genes that have been demonstrated to be mutated in any kind of blood disease or blood cancer, and so there are some mutations that are gainer function or point mutations, and we just sequence that particular gene, or that particular region for the gainer function mutations, and some are loss-of-function mutations, so they might be anywhere in the coding exomes of that gene, and for those, we sequence the entire coding region of the genes. So it's what we call a targeted next-generation sequencing panel, so it tells you what mutations are there, and then it tells you what percentage of the sequences have the mutation, and so that kind of gives you a sense of, the genetic make-up of this patient's blood cancer. This data, we started doing this at Dana-Farber in 2014, and this data we extracted, I think, about four or five years later, looking at 750 samples, all of patients who had myeloproliferative neoplasms, the disease my lab studies, so we've excluded anyone who had lymphoma or myeloma, AML or anything else. We just really focused on MPN, and this was like 750 samples from about 700 patients, and this is just the spectrum of mutation. Just to orient you here, each column is an individual patient, and each row is a gene, and these three causative genes are up here, and as you can see, they're mutually exclusive, so if you have JAK2, you don't have CALR and you don't have MPL. Dana-Farber's a tertiary referral centre, so we tend to see patients with more advanced disease, patients who've been in the community for a while, and then something is not working, and they get referred, but even in that group of patients, you can see in approximately one-third of cases, they have a JAK2, a CALR or an MPL mutation, as their sole mutation, again really getting to the point that these are the causative mutations, and if we had good ways to target them, we would have a big impact on this disease.

As patients progress, they acquire more mutations, so their myeloproliferative disease undergoes genetic or clonal evolution, so you have a cell that has one of these mutations, and then it acquires an additional mutation in that cell, and therefore becomes more genomically complex, and potentially more resistant to treatment, particularly any treatments that are going to target these mutations. So you can see, there's a lot of genes that are co-mutated, and if you go here, these are patients who have just a sole mutation, but we have some patients up here who have eight individual separate mutations, and these are patients with more advanced disease who typically have had the disease for a longer period of time, and have undergone genetic and clonal evolution.

So we're really starting now to try, our goal is to build this information over long periods of time, so this is 700 people over four years or so, but if we continue to collect this information over 10 years, 20 years, we think this will be very powerful in helping us to understand why do some patients stay very stable over time, and not actually progress or change in their myeloproliferative disease, whereas some other patients progress more quickly. They go to myelofibrosis much more quickly, or sometimes they go in transforming to acute leukaemia, and we think a lot of this has got to do with the genetic complexity of their disease, and what concomitant mutations they have, and we are hoping that over time we'll be able to get better predictive models to help us really understand who are the high-risk patients much earlier in the course of their disease, and try to intervene more aggressively, before they sort of progress and it's more challenging to treat them.

So I'll switch gears a little bit, and just tell you about a patient who I saw in my clinic, and one of the big rewards, I think possibly the greatest reward of being a physician-scientist, is that you can actually study your patients, and you can study their cells, and you can sort of say, why does this happen to this person, and how can I understand it?

So this is a patient, he's a 55-year-old man who is a runner, and he's very athletic, and he logs everything, his weight, how fast he runs, all of this type of thing, and he goes to his primary care doctor every year, and he always has a CBC drawn and he looks at it very closely. And he noticed that his red blood cell count every year was going up a little bit, and it was still within the normal range, and nobody really made very much of it, and then it started to creep up a little bit more, so that it was outside of the normal range, but he was feeling pretty good and was actually running great, really fast times, and then he noticed that after he ran, he would get itching or pruritus, on his torso, which he never got before. And he thought that was unusual, so he went on Google, and he put in "high red cell count and itching", and lo and behold, the disease polycythaemia vera came up, so he went to his primary care doctor, and he said, I think I have polycythaemia vera, can you check me for the JAK2 mutation?—it's the type of patients in Boston maybe, I don't know if they're like this in Belfast, but anyway, so lo and behold, he had the JAK2 mutation. I think one of the reasons the patient was so very focused on his health was that he, despite the fact that he was an athlete, a vegan, a really healthy person, had actually severe coronary artery calcification on his coronary CT, and he had been monitored closely by the cardiologists, because his father had died at 45 from an acute MI, with no real reason for that, not a smoker,

not a diabetic, not any particular reason, and so this person, since his father died, had been very focused on his health and very focused on trying to do everything you possibly can to stay healthy, but despite this had this severe coronary artery calcification on his coronary CT.

So anyway, we sequenced his peripheral blood, and we found that he did indeed have a JAK2 mutation, and then he had a TET2 mutation, which is one of these other concomitant mutations that can occur, and he has the disease, polycythaemia vera. It's not clear whether his heart disease is independent or related to that, we'll talk about that in a little bit of time. There's really fascinating literature now emerging around this JAK2 mutation, and how it can actually be detected in healthy people, and may contribute to coronary artery disease development. But what we've been doing in this patient is, he participated in a trial that we're doing currently, where we're doing what's called single-cell RNA sequencing, so what we're doing is, we're taking bone marrow from individuals, including him, and then we're simultaneously sequencing each individual cell for the entire transcriptome, at the same time as we sequence for the mutation, the TET2 and the JAK2 mutation. And then what we're doing, I'll just try to give you an example of this, so this is basically single-cell, so this is sort of an illustrative example of, in the same cell, we're getting the transcriptome sequence, but also we're getting the mutational status, and so therefore, within the same individual, we can discriminate between the cells that have the mutation and the cells that don't. And that, I think, hopefully will be very powerful, because that will allow us to identify unique things about the mutated cell that the normal non-mutated cell does not have, within the same person, and that might give us a better understanding of unique things that we could target to treat this disease.

So this is his single-cell RNA sequencing, so what you're looking at here is just a plot of all of the cells in his bone marrow, and they're colour-coded to represent the different sub-type of cell that they are, and they cluster based on their similarity. So how it works is, it's a bioinformatics algorithm that takes that transcriptional sequence of that cell, and then puts it next to the cell that has the closest transcriptional sequence, and then you form clusters like this, so for example, the stem cells are up here, these are the erythroid cells, these are the megakaryocytic cells, these are myeloid cells, these are lymphoid cells, dendritic cells, and these are monocytes down here. And then what we do is, we're able to take that single-cell DNA genotyping, so we're genotyping each individual cell for the mutation, and then we're going to say, okay, where are the cells that don't have the mutation, and where are the cells that do? So these are the unmutated cells, and you can say that these are interspersed everywhere, but there's a lot of them in the stem cell compartment, and less maybe in the erythroid compartment, and remember he presented with a high red cell count, and then if you go on and you superimpose where are the JAK2 mutated cells, you can see they're very much enriched in the megakaryocyte and erythroid cells, as you might expect, but what the big surprise was, we found this population of cells here which are monocytes, that are almost all mutated cells, and this, we would never have been able to tell this by looking at his peripheral blood count, because his monocyte count is normal, and we don't think of monocytes as like a part of the real disease, a cell that's indicative of the disease.

So here what I'm showing you is, we've looked at the ratio of the mutated cells to the normal cells within each compartment, and you can see it's very high in the erythroid and the megakaryocytic cells, as you would expect, but really very, very high down in these monocyte cells, and this is a completely novel finding, and we've found this now, and we've sequenced about ten patients using this technology, and we found it in about five or six of the ten, and we've been able to identify specific inflammatory programmes that are activated in these monocytes, and we are doing a lot of studies to try to understand what is the role of these cells in the disease progression, and in contributing to some of the phenotypes, potentially even the cardiac phenotypes that I alluded to.

So I think this is really exciting, because this is the first time we've been able to discriminate the mutated cells and the wild type cells in the same person, and we're getting very high resolution information, and hopefully identifying mutated-specific consequences and therefore better targets. Because right now in the clinic, we have JAK2 inhibitors, and they don't work so well in terms of eradicating disease, and the big problem with them is they also inhibit the wild type form of JAK2, so we're trying to get to better, more specific, more targeted therapies, and we're hopeful that techniques like this, such as single-cell RNA sequencing and single-cell genotyping, will be informative in that regard.

So just to get back to the coronary artery disease, just to give you some perspective, this guy, 55 years old, with this family history, the standard of care treatment for him today, in Boston and in Belfast, is for him to get therapeutic phlebotomy to lower his red blood cell count, to go on aspirin, and that's pretty much it. He could go on potentially some sort of medicine to bring down his blood cell count, but we have no way of eradicating or eliminating those cells that cause the mutation, and therefore essentially we manage this disease in this person for their life, and that seems obviously pretty unsatisfactory, given his family history of coronary disease, and just

given that therapeutic phlebotomy is more than 100 years old, and we're still doing it, and we need to do better, so that's sort of part of the motivation of trying to study this patient and others like him in more detail.

What has been very, very intriguing is some recent studies that have come out, and I don't know if the haematologists, or people in general, have followed this literature, but what has become increasingly apparent now is that, as we age, our tissues, our bone marrow, but also other tissues, acquire mutations over time. And patients don't have the full disease, but they have mutations that cause these diseases detectable in their blood, and this originally was published in 2014, and there's been a slew of papers since then, so I just want to highlight one study. So this was a study that was done, it was a collaborative study at the Broad Institute, and there were other groups who did it as well, but essentially what they did was, there were big gene sequencing studies, whole exome sequencing studies going on, to try to understand what is the genetic basis of diabetes, and what are the genes that cause diabetes, and what these authors did was, they actually exploited the fact that the DNA that was sequenced to determine the genetic basis of diabetes was taken from blood, and so then they went back and looked at those whole exome sequences, and looked for mutations that they know have been associated with the development of blood cancers, 160 altogether, to see if they were detectable in the peripheral blood of normal individuals. So remember, these are people who do not have myeloproliferative disease, they do not have leukaemia, they do not have any blood cancer. They're entering a study because they have diabetes. There was a control group to try to understand what are the germline heritable genes that cause diabetes, and they focused on the blood, because that's where the DNA was extracted.

Lo and behold, they found actually a relatively high frequency, approximately 10% of people over 70, have these mutations detectable in their peripheral blood, and they don't have an overt blood disorder, they just have detectable mutations, and this is what they call Clonal Haematopoiesis of Indeterminate Potential, or CHIP, and interestingly, the fifth most common gene amongst the genes that are detected was this JAK2 gene that causes myeloproliferative disease, and so now we know there's this entity, what we call JAK2 clonal haematopoiesis. So you have the mutation, you haven't gone on to develop an overt myeloproliferative disease, but just the fact of having the mutation without the disease seems to have consequences. There've been some follow-up studies that have been done around this. So the first thing that has been identified is that the presence of these clonal mutations, and JAK2 in particular, in your peripheral blood, is associated with an increased risk of cardiovascular disease, and as you can see here, this is the hazard ratio here for the JAK2 mutation is 12. It was around two or three for these other mutations, these epigenetic mutations. And there's been some research studies that have tried to understand why this is, and so one idea is that, if you have mutations that are in your blood cells, in particular in your monocytes or in your macrophages, that these cells don't respond normally to cholesterol, or they're more pro-inflammatory. And so when you have an incident event like a plaque rupture in your heart, and you have these macrophages or monocytes that respond, they respond in a different way than if you didn't have a mutation, and they're more pro-inflammatory, and this may be the reason why they're associated with increased risk of cardiovascular disease, which is really kind of a paradigm shift in terms of thinking of cardiovascular disease as a disease of inflammation, rather than a disease of platelets and vessel obstruction. So there's been this study and then there's been a subsequent study that's also shown that just having this mutation, this JAK2 V617F mutation, in your peripheral blood, not having an overt blood disease, is also associated with an increased risk of venous thromboembolic disease.

So this is a really fascinating area of research now, so what is the clinical relevance of this? Why do some people go on to develop overt myeloproliferative disease, some people don't? What should we do about this?—if you have risk factors for coronary disease, and you end up having a JAK2 clone, should we do something about that, what should we do?—so a lot of ongoing interesting work in this area around clonal haematopoiesis.

We've also done, I'll skip this in the interests of time, but we've also done some studies in the mouse around this. We'll go on, in the interests of time, to the second story.

So the second main gene is calreticulin. It's the one in red, and this is a really fascinating story, and again speaks to the power of genomics and genetics. I know you had Dr Jyoti Nangalia come and talk, so Jyoti was involved, and led the work actually, that identified this mutation as a causative mutation in myeloproliferative disease, and that study was done because they took 150 people who had these diseases, and they just sequenced the exomes, so they sequenced all the coding genes to say, what are the genes that might cause this disease, and rather than sort of hypothesising that it must be something to do with the JAK-STAT pathway, they just sequenced the exome. And they identified calreticulin, as you can see here, there's a big chunk, the second most common causative mutation, and that was very unexpected, because calreticulin is not a signalling gene. It has nothing to do with cell surface receptors, it has noth-

ing to do with the JAK-STAT pathway, it's an endoplasmic reticulum protein, and this is just showing some of the domains of the protein here. So normally what it does is, it's involved in protein controlling and folding in the endoplasmic reticulum, so when your cell is making nascent proteins, they have to fold normally, and they go through what's called a calreticulin/calnexin cycle, and they bind calreticulin and then are released after normal protein folding occurs, and it also has an important role in calcium homeostasis, so if you knock this out, if you knock calreticulin out in the germline of a mouse, they die from cardiac defects. They don't make a normal heart, they don't have enough calcium so they can't pump, there's not normal muscular development within the heart.

So really unexpected, that a gene like this, sort of like housekeeping, a resident chaperone gene, how could mutations in this gene cause a myeloproliferative disease?—and so we became very interested in this, and a very fascinating part of this is that the mutations actually all cluster in the last part of the gene, so in exon 9, and there occurs these insertion/deletion mutations in exon 9, and these cause a frameshift mutation, and basically they change the C-terminus of the protein quite substantially. So normally you have this KDEL sequence which is important to retrieve calreticulin back to the ER, but these mutations, you get rid of that and you have this novel C-terminus, and that's actually shown here. So you can see here's the sequence of the normal protein, and then here's the mutated protein, so you have 40 amino acids of mutant-specific sequence at the end of the protein, and that's already been used by pathologists diagnostically, so they've developed antibodies against this C-terminus, and that can be used for diagnostic purposes, so this is immunohistochemistry on the bone marrow, and here you can see, here's a JAK2 or MPL mutated cases, you don't see any positive staining. Here's this so-called triple negative, where you don't have any of the mutations, no staining, and then these are different types of the calreticulin mutation, and you can see there's very strong staining, so this can be used for diagnostic purposes, these antibodies.

So we were very fascinated to understand how can mutations in calreticulin cause myeloproliferative disease, and we did some studies around this. We did the typical thing, we over-expressed it in a mouse, and just like with the JAK2 gene, if you just introduce that mutation alone, you get a high platelet count and high megakaryocytes, which is what the patients get, and then if you look here, here's the [empty vector?], here's the wild-type calreticulin, here's the mutated form of calreticulin, you see all these megakaryocytes. So clustering and all of the features we would see in a patient, you can engender those in a mouse. So again this speaks to the power of this, as a causative gene and a causative reason for someone to develop myeloproliferative disease.

We went on and did a lot of studies, I won't go through these in much detail, but essentially what we hypothesised is that, because the phenotype of the disease in people and in mice is very megakaryocyte-rich, we figured that something about megakaryocytes and megakaryocyte signalling must be important, and we figured that thrombopoietin receptor MPL must be very important, and through a series of experiments that, in the interests of time, I won't go too into detail, but we went on to show that the thrombopoietin receptor, MPL, is absolutely required for mutant calreticulin to function as an oncogene. And this is just a cellular transformation assay, so we over-expressed these oncogenes in cell lines that are cytokine-dependent, and when we withdraw the cytokine here, the cells die, but if you do the same experiment now where you're co-expressing MPL, the cells survive, but if you do it with the epo receptor, they don't survive, so this demonstrated that MPL, the thrombopoietin receptor, was absolutely required for mutant calreticulin-driven MPN, and then we went on to show that mutant calreticulin actually binds MPL, and that's shown here in these [coning?] and precipitation assays, and by doing so, it activates the JAK-STAT signalling pathway, as shown here, so MPL becomes phosphorylated in JAK2, and the STAT downstream of MPL are activated.

So this is a little cartoon, so there's been a lot of work, these mutations were discovered in 2013, and over five years, ourselves and other groups have really worked out the mechanism by which these mutations cause this disease, and essentially what they do is, this mutation changes the C-terminus of the protein, and then the protein binds the extracellular domain of MPL, and it activates the JAK-STAT signalling pathway in a completely ligand-independent manner, so again resulting in this constitutive activation of the pathway, and that's shown here.

So normally, MPL would bind, calreticulin be released, but because of this mutated C-terminus, it holds onto it, and forms this complex that traffics to the cell surface and activates the signalling pathway. We think that's really important, because now that we understand the mechanism, maybe we can come up with better ways to treat these diseases. So the current treatments we have are JAK2 inhibitors, which block the signalling downstream, and they attenuate the disease, but they really don't cure the disease, so we're working on ways to develop a mutant-specific antibody that would be not just diagnostic, like I showed you in the pathology slides, but would actually also be therapeutic, and could block the signalling, or if there were ways that we could interfere with the binding interaction between mutant calreticulin and MPL inside the cell.

There's also a lot of work around immunological targeting of calreticulin, so as you probably know, any of you who work in oncology or any disease really right now, immunology is huge, and immunotherapy is really huge; and so because of that mutant-specific C-terminus, there's a lot of sequence that is entirely specific to the tumour cell, which the normal cell does not have, and so developing immunological ways to target that is an area of ongoing research, either by making a vaccine that would augment autologous immune responses, or by activating the T-cells using ENTPD1, or engineering T-cell receptor, if you knew which epitopes were processed and presented. So there's a whole focus around immunological targeting of calreticulin, and I'm hopeful that these will yield results, and because these are causative mutations, I'm hopeful that those will be very impactful in terms of altering the natural history of these diseases, which is the major deficiency of our current therapies.

So that ends the sort of science part. I'll just now allude to a few recent things that have been published around the physician-scientist in the United States. This was a New England Journal perspective from a couple of years ago, around this idea of the endangered physician-scientist, and the authors made the point that really, the numbers are really going down, so about 40 years ago, about 4.5% of physicians were engaged in research, in scientific research, and now it's of the order of 1.5%, so it's really actually a very small group. This is just data from the NIH, looking at who has NIH funds, so this would be sort of the equivalent of an R01 grant from the NIH, and this is over time, going from like 1996 all the way up to 2012, and you can see, so MDs, straight MDs, or MD PhDs, it stayed pretty steady, and is actually decreasing now, but it's only of the order of around 8,000, and over the same time frame, straight PhDs is gradually increasing. This has caused a lot of cause for concern, and here are some of the ideas that the authors of this perspective had, and I think maybe many of them are relevant here also. I know you have like the clinician scientist model here. I think this is a big one in the US, the time spent in training before you become independent, so for an MD PhD, they do four years of undergrad, then they do four years of medical school and four years of PhD training, so that's 12 years to the point where you start internship, and given the cost of medical school in the United States, and medical school debt, this is a big barrier, I think, to people sort of going down this academic track, so there's a lot of programmes around repaying loans and NIH debt, etcetera.

Another big one in the United States is sort of how healthcare is financed, and all of the challenges around reimbursement, both from academic centres, and then insurance companies. We've recently introduced the electronic medical record, EPIC, and a big part of what we do is document and bill, and that imposes a lot of time and energy, time that maybe is taken away from doing research things. There's protected time for research … I'm just putting these up, maybe they'll be more of a topic for discussion at the end.

The major other part is the stagnation of the NIH budget, so this is shown here, so this is the NIH budget from 1959 to 2016. I think it started out at around $200 million. I mean, it's increased almost 200-fold since then, but the recent years, this is up to 2016, there's been stagnation or not increased funding within the NIH, so that makes funding more competitive, so I have an R01 grant, will renew it this year and next year, and yet the funding rates are around 20% for NIH funding, so there's a lot of good science that's not getting funded due to constraints from federal funding.

Then another aspect, I guess, another challenge I guess is the issue of gender, and in the United States, and I think this is true maybe the world over, we've seen big shifts in sort of the gender representations, so if you look at people entering medical school or graduating medical school, it's approximately 50/50, or more women than men. For a long time now, over more than 10 years in the US, there's been equal numbers of medical school graduates, but if you look then at senior academic percentages, so percentages who are deans, department chairs, full-time professors, it's still very much a minority. This was an opinion piece, this is the title of the opinion piece by Dr Gwen Nichols. She's the Chief Medical Officer of the Leukaemia Lymphoma Society, which funds a lot of our research and a lot of research in the United States around this problem, and there's a big drop off around the assistant/associate professor level, where people go to that level, but then drop off to the more senior ranks, and obviously there's a lot of issues and challenges around that.

So those are just some of the kind of bigger picture challenges that the field is facing, but hopefully we're up to the challenges, and we'll continue to have great physician-scientists into the future.

I'll just end there. I'll just acknowledge, this is my lab, so there's seven people in my lab currently, five post-doctorate fellows and two research technicians, so these are people who have done undergrad, and then want to go to graduate school or medical school. They come and spend one or two years in the lab. I have a mix of straight PhDs, MD PhDs, and straight MDs, so a mix of all those three. We've also had some people who have graduated at the lab, and gone out to start their own labs, so this is Shannon Elf, who's a former post-doc with me, has her own lab at UChicago, and Dr Edwin Chen has his own lab at the University of Leeds, UK, now, and then some people have gone to graduate school, and then some other

people who went back to their home institutions. Most of my funding comes from the NIH, the Leukaemia Lymphoma Society also was a big funder, and then, in the United States, we're fortunate—we have a lot of foundations that fund research, so the MPN Research Foundation, Gabriel's Angels Foundation, this is internal funding, and the Chan Zuckerberg Initiative all contribute to the funding in my lab, and help us to do some of the studies that I showed you.

So I'll end there, and I'll be happy to take any questions or discuss.

Professor McMullin:

So thank you very much, that was a wonderful mixture, so any questions? So maybe I'll get you to start off a little bit, we talked about the way some patients do well, and we know these patients who go for many, many years and nothing changes, and when you're as old as I am, you've had someone in the clinic, it may be genetic, but do we have any idea what the differences are?

Professor Mullally:

I think that one thing that we're starting to realise is that there's enormous heterogeneity in the patients. Obviously you have the genetic make-up of the person, so most people who get these diseases are in their sixties and seventies, but about 20% of people are under 40, so if you're under 40, you have a longer time to live with the disease, and probably there's some reason, genetic predisposition that you've got the disease earlier, and then you have a longer time to live and have genetic evolution. Then I think genetic complexity, acquired mutations, is definitely a big factor, and then I think the other factor, and this is hopefully what the single cell will get you, is the cell of origin that acquires the mutation. Which cell gets the original mutation? Is it a very primitive stem cell versus a more differentiated stem cell, that can change the phenotype of the disease that you get?—so I think those are some of the factors, but I think we need big, multi-centre, longitudinal studies. There's probably environmental factors as well. There's a lot of study now around clonal haematopoiesis, and why does that not expand in some people and do in others, and probably environmental factors like smoking or radiation exposure. These things have been shown to influence that, the immune system, all these factors, so I think lots of things hopefully to keep us in funding for years to come.

Professor McMullin:

And if we give Peter the microphone, what about the cardiovascular, are the cardiologists going to have to screen everybody for their JAK2 mutation? I have a few patients come to my clinic who have been found to have a JAK2 mutation with normal blood count. It's very difficult to know what to do with them.

Professor Mullally:

Speaking with the cardiologists, what they tell us is that you consider it like another risk factor, like cholesterol or hypertension or whatever. Consider it in the context of the whole patient, and what are their other risk factors. It's not clear what you can do with anything to modify it, so if they have other risk factors, I tend to put them on low dose aspirin, but I've had young people who have turned out to have a JAK2 mutation detectable in their peripheral blood, don't have any cardiovascular disease, unclear what we should do with them.

Professor McMullin:

And do your cardiologists screen everybody for JAK2?—because our lab would have kittens if that happened!

Professor Mullally:

No, we don't. It's very interesting, now that we're starting to pay attention, so for example, before I came here on Wednesday, I had a clinic, and I saw a woman who was 47 years old, and she'd just got diagnosed with new polycythaemia vera, like our patient that I told you about, and in 2017 she'd had a TIA, like two real TIAs, MRI-documented, no cause found, and two years previously she'd had a DVT, again a real DVT, no cause found. So I think maybe we should start it as a screening in patients who have DVT or TIA, or some vascular event at a young age, without real reason. I think that to me seems as reasonable as the screening for Factor V Leiden or one of those things, so she had no apparent cause, and then comes in three years later with polycythaemia vera. I didn't have time to go into it, but we've done these very cool tracing studies, where we can work out when the mutation first occurred using whole genome sequencing, and in most cases it's like more than ten years, or 20 years, if you trace back through the lineages, so probably, from the point the patient presents, it's many, many years, decades probably, from the point of when that initial mutation occurred, so they could have the small clones for many years contributing to some of these outcomes.

Professor Peter Maxwell:

Thanks very much, Dr Mullally—a lovely lecture, thank you. I was very interested by the panel … 750 patients who had other genes, full genome sequencing approach. Many of the genes that seem to come up beyond the ones which were originally known, like JAK2, are genes which might be epigenetic modifiers, TET and [?] and going back to the question Mary Frances asked you, what do you think is the link between the environmental triggers potentially, some of those other mutations are common, but the epige-

netics [in the middle?] pushing things along?

Professor Mullally:

These are somatic mutations, and so we know that, as patients, so if you look at patients with myeloproliferative neoplasms, if you look at patients who have a more advanced phase of the disease versus earlier patients, they're highly enriched for epigenetic mutations, so they undergo clonal evolution with epigenetic mutations as they get older, but we also know that clonal haematopoiesis, about two-thirds of clonal haematopoiesis is accounted for by three genes: TET2, DNMT3A and ASXL1, so it's very possible that those patients have a clone, like a CHIP clone, and then at some point they acquire a mutation, like a JAK2 mutation, or a CALR mutation, that gives them an MPN disease phenotype. Or it could happen, the converse—they could have the JAK2 mutation first, and then they undergo clonal evolution. So all of the myeloid malignancies, epigenetic mutations, somatic [epi?] mutations, are a very big player, and very challenging, because we have generally a loss of function mutations. We have really nothing to target them, but definitely a big contributor.

Then in terms of the environment, so there is some work, particularly around the DNMT3A mutation, that in an inflammatory environment those cells are selected for, this is mostly in mouse models. So if you take normal stem cells and you compete them with DNMT3A mutated stem cells in a mouse, and then you expose them to some inflammatory stimulus, the DNMT3A mutations will do better, they will competitively out-compete the normal cells. And so I think this may be the intersection between the immune system, ageing, and the clone mutations within your bone marrow, and why they're very age-associated, so clonal haematopoiesis is very age-associated. Under 40, you find almost nobody with clone mutations, and then at 70, it's 10% or more. I think it's probably the age-associated acquisition of mutations, and then the intersection of that and the immune system altering as you age, and then whatever other environmental exposures occur, that allow these clones to grow out and advance, but a big challenge, like therapeutically, because of being loss of function and really no good ways to target them.

Professor Peter Maxwell:

Thanks very much. There is one other question, and that is, you actually showed us lots of reasons why you shouldn't really want to become a clinician scientist? There was lots of negatives, it's too hard, it's too long. Why don't you tell us why it's such great fun to be a clinician scientist?

Professor Mullally:

I think it's the research. It's very, very cool, to see a patient in your clinic, and think about, why did this happen to them, and try to understand it. I work at the same institution as Bill Kaelin, who along with Peter Ratcliffe and Gregg Semenza, just won the Nobel Prize, but if you talk to them about, and I've seen Peter Ratcliffe give talks about this, the original question was, why did this happen to this patient?—and so this kind of curiosity-driven science, which is actually not to be negative, is hard to get funded now, is kind of the fundamental basis of why we do research. I think those are the positives.

I also think there's a lot of other positives. It's an incredibly flexible schedule, you don't have to be in clinic. You travel a lot, if you want, maybe not in the middle of a coronavirus epidemic, pandemic. I think for me also, mentoring junior colleagues is really amazing. I am a product of exceptional mentorship. I had amazing mentors at the Brigham, Ben Ebert, [?], and so helping people early in their career and seeing them grow and evolve is very, very gratifying, I think. It's just really cool to find out something new. For me, the thing, when our lab worked out how calreticulin mutations cause MPN, it was a source of enormous pride, and the publications are there, and they stand hopefully the test of time, so I think those are some of the reasons.

Professor Pascal McKeown:

Thank you very much, I really enjoyed your presentation. We've heard a little bit about the potential advantages of having a clinical academic career. You've also talked about the plateau effect, so what's actually happening in the States at the moment? Are there active programmes to try and restore this kind of traditional pathway, [?] kind of model for how you actually make this achievable and interesting?

Professor Mullally:

I think there's a lot of work around, so for example, the American Society of Clinical Investigation has a taskforce, and people are involved in doing this. I think some of the things that are being done, so they have these fast-track programmes now, so to try to shorten the duration of your training. So for example, if you're going to be somebody who runs an academic research lab, do you need to spend five years taking care of patients if ultimately at the end of it all, you're going to run a research lab?—so there's some of these sort of short-tracking type things. There's a lot of stuff around NIH loan repayments, but I also think there's a big struggle. I really do think it is, I mean, it's a real problem. And then I think the other part that's been addressed with is the NIH funding, so for example, funding early stage investigators, so the traditional word in the US is what's called a K award, so kind of, if funding goes down, they really try to protect those awards, that they don't suffer, and then they've introduced these K to R transition awards, so

that, when you get the K, you're already on the way to the R award, so you don't have to keep going back. Those have been some of the initiatives, and we even have initiatives, right, for example, I have an R01 and I'm going for my first renewal of the R01, so there are, that has a little bit higher level funding than if I was just going back for my R01 like a third or fourth time, so those are some of the types of things that are being addressed, I think.

But I don't know, I will say, at my institution, the biggest source where people leave is to the pharmaceutical industry, so, like, a lot of my colleagues, physician-scientists, take leadership positions in the pharmaceutical industry, so that's another source, particularly in oncology, with just the rapid development of new drugs and immunotherapy and all these type of things.

Professor McMullin:

Does anybody else want to comment? We have a number of young people in tonight, and even young female people in as academics?

Audience member:

I was just interested actually, I suppose it goes back to the patient, your first case, of someone who looked like they were very much in control of their own health, how they deal with all of this extra data, things that we now generate, and that we don't necessarily know how to process ourselves, how does that impact on the patient?

Professor Mullally:

I will say, America's probably, we share all the information. In fact, the patients can actually read, for better or worse, they can read all their notes. They get all their information, like so when my patient, a lot of times, I come into clinic and they have the app on their phone, that looks, and they already know their CBC before I walk in the room, so I think that's actually a good thing. You don't have to filter, what do you tell them, what do you not tell them, because they already know everything. I think people process it differently, so he's very much like a numbers person, so he's very obsessed, so he went on therapeutic phlebotomy. His MCV went down, and his RDW went up, and he obsessed about all of those type of things, but I think in terms of information-giving, the patients have all the information now. There's really no barrier to that, so that's how it is, and we deal with that. I'm not sure if that, still in love with that, but maybe how you write your notes might alter. His situation, he's a very interesting patient. I don't know if, it's very possible he has some genetic inheritable reason to have premature coronary artery disease, and that's why his father died. I don't think his father had myeloproliferative disease, as best we can tell. It's possible that he's had, like he had JAK2 CHIP for a long time, that contributed to his cardiovascular disease, and so it's actually, if you think about him as a polycythaemia vera patient, he's under 60, he's never had a vascular event, so he should get aspirin and therapeutic phlebotomy, but that seems insufficient actually, so he ended up getting very iron-deficient, and that ended up affecting his running ability, and so, then his white count went up, so I ended up putting him on hydroxyurea, because he's not that far off 60, but even that seems very insufficient, and we've studied, like we've actually done a ton of studies on his neutrophils as well, and they're very inflammatory, so we're in that interface between, what is our data to support doing, based on clinical research, versus what does my biological mind want to do to stop him from having a heart attack? Then he flat out says, stop me from having a heart attack.

Professor McMullin:

If Adam Mead or other people were sitting here, they'd be saying, why are you getting him interferon to reduce, on the basis of reducing the clone more than anything, and possibly the inflammation, if that's something?

Professor Mullally:

Yeah, that's a good consideration. He has a TET2 mutation, which is, they have been demonstrably more resistant. He also has some liver function abnormality.

Professor McMullin:

Any of the girls want to comment on clinical academics?—because I think that's quite important? That's the really hard group to get.

Audience member:

Thank you for a lovely talk. I suppose I, you alluded to some of the pressures in your time, particularly in America with delaying and all of that. I'm conscious of … considering a career as a clinical academic. What's your top tips for trying to balance that?

Professor Mullally:

I will say, one advantage of the American system, or the academic clinician system, is that, so like, if I have an R01 grant, the terms of the grant are that my time is protected, that I have 80% of time to devote to research. My institution has to respect that, which they do, they very much do, but I think if you're in a conundrum where you're not able to protect your time to do research, I think sometimes it comes down to like, what is the non-negotiable part of it, so if you have funding, I think that's, for me, has always been a way to protect your research time, because it's sort of non-negotiable. You can't get pulled in to do more clinical work if you're funded. My salary gets paid from those grants, so there's really no reason that I

can get pulled into the clinic, but you have to have a big infrastructure that's there around, that the clinicians have to be able to do, you need clinicians to take care of the patients, so there needs to be an infrastructure. So I guess that all goes to say, the environment, I think, is really important, like to be at a place that really supports academic research, because you need that at an institutional level, at your division level, and you need to have people who really believe in the physician-scientist model, and are going to protect you, so that you can do research. I think that's really really important.

Then, I don't know, I think I struggle with it. I try to prioritise the things that, the things that matter the most, which is funding and publications, and then obviously when I'm in the clinic, I'm in the clinic 100%, and taking care of the patients is incredibly important. I think those would be kind of some of the principles that I try to use.

Audience member:

I'm an obs and gynae trainee, so how does it work for people who are in craft-based specialities? Obviously you speak about your clinic, but I would have to be spending my time between labour wards, and ante-natal clinics and post-natal follow-ups and things like that.

Professor Mullally:

I think that's very hard, like so, for example, that's part of, I don't think I could be an acute leukaemia doctor, for example, and do this, because 20% clinic doesn't happen in acute leukaemia, because the patients get admitted, they're in the emergency room, you get called all the time. I actually think you have to be thoughtful about what clinical work you do, and whether that's compatible with being away from the clinic for long periods of time. I mean, in myeloproliferative disease, our patients tend to be stable in the main, so most of my patients come back every three months, six months, a year. I mean, sometimes they call, but not that often, for example, but if I had somebody who had acute leukaemia, and was getting induction chemotherapy, that would be entirely different, and I think that would be a lot more challenging in terms of doing research.

Professor McMullin:

But you do cover the wards from time to time?

Professor Mullally:

I do, but that's not that much.

Audience member:

And how do your colleagues, how they are in those rules? How do they manage it?

Professor Mullally:

There's many different tracks. We have the research track, I'm on the kind of lab/science track, then you have people who are on the clinical research track, so those are people who are in the clinic and doing clinical trials, then we have some people who are on what's called the education track, so they're involved in teaching, and those type of things. There're different tracks that you go on, and you get promoted on those different tracks, so that's how it works. Honestly, I think it's like, 80/20, it's really hard to make enough time, because research just takes an enormous amount of effort, just to get the thing funded. To bring a project from start to publication is an enormous amount of effort in every way, in terms of the funding, in terms of the people who work on the project, in terms of the data, in terms of putting it into a manuscript, in terms of submitting it, so you really do need dedicated time, and that's why I think you need like support from your supervisors and the division and the institution to really enable you to do that. That's like, being honest about it, that's what I think.

Audience member:

This is a question about the advice, about lifestyle, for example sports, with MPN?

Professor Mullally:

So in general, lifestyle, things that we recommend for them, I don't recommend anything particularly, other that they don't smoke, they absolutely can't smoke obviously, and that we control all other risk factors for cardiovascular disease, like blood pressure, cholesterol, that sort of stuff, a generally healthy diet, regular exercise, and then for some patients who I think are at high risk for thrombosis from long-haul flights, we do sometimes give them prophylactic, like a DOAC, like rivaroxaban 10 mg before each flight, some of those type of things I do do for some patients who have either had thrombosis, or are at high risk for thrombosis, I do that in some specific cases.

Audience member:

Just another question about triple negative patients; are they really negative, or is something that maybe becomes in the future … a condition?

Professor Mullally:

So there's been some studies, there are a minority that are definitely less than 10% of patients. A subset of them have mutations in JAK2 and MPL, but at different sites so not at the 617F or W515L, at other sites, so if you sequence it, the whole of JAK2 or the whole of MPL, you can find other sites of JAK2 and MPL. A small number have link mutations, SH2B3 mutations, and then some of them, I mean based, there was this paper from Grenfell, that again Dr

Nangalia was involved in, some of them probably don't have clonal haematopoiesis, young women with high platelet counts, I think in those situations, there was a subset, a small subset who they didn't identify any clonal mutation in, who may have a non-clonal reactive thrombocytosis, but I think those are definitely the minority, and if you sequence JAK2, CALR and MPL, you're going to capture more than 90% of them, so in our [MPN?] sequencing panel, we get all three, but you can also do it sequentially. I know at some institutions, you do JAK2, and then if that's negative, you go through in order of frequency or more likely. I mean, JAK2 is the most common, and if they have … I mean, polycythaemia vera is a disease of JAK2. You either have a V617F or an EX12 mutation, so you really only need to sequence JAK2 probably in that situation. So there may be a small number of genes left to discover, but I think it's very small, and I think it's probably, the JAK-STAT pathway is really the major pathogenesis.

Professor McMullin:

Thank you very much.