Ulster Medical Society

The 2020 Sir Thomas & Lady Edith Dixon Lecture

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Held Online

Too Much and Too Little—How Does an Extra Chromosome Cause Leukaemia in Children with Down's Syndrome?

Professor Irene Roberts
Oxford University

References to unpublished work have been removed. We hope that the full lecture may be presented once the work in question has been published.

Professor Mary Frances McMullin:

Welcome everyone, to this year's Ulster Medical Society, and our first-ever online lecture. Just to give you a little bit of background, this time last year in March, we were due to have Professor Irene Roberts' talk on 19th March. We had had Ann Mullally on 5th March, and we were looking forward to the last lecture in the year, and unfortunately as we all know with Covid, it had to be cancelled at short notice, along with the dinner, and everything else. We got to the summer time and we decided that I should stay on as a president for two years, and just leave things for a few months, the idea being then when the next president, Dr Seamus McAleer, takes over, he would be able to have a full lecture programme, because that obviously wasn't going to happen this year And the idea originally was that we hoped things would improve, and by the time we got to about this time of the year, we would be having lectures and have a couple of things towards this time of the year.

Unfortunately, of course, things haven't improved that much, at least not to the extent that we're all socialising, but what we are all getting better and better at is running our lives online. So we thought to keep this society going, it would be great if we could have a couple of online talks this year to finish off what was really the end of my presidency last year, and I'm delighted that Professor Irene Roberts agreed to give the talk that she would have given last year. And we've then got, and I'll come back to this, an extra lecture in two weeks on Egyptology, just to whet your appetite for that.

So tonight we are having the Sir Thomas and Lady Edith Dixon lecture of 2020 which is being held in April 2021. Sir Thomas and Lady Dixon, and you'll hear that we've all been looking this up as we were talking beforehand, but Sir Thomas Dixon was known to us all, the pair of them, as sort of philanthropists and did lots of things in Belfast. I'm told that Sir Thomas was a wonderful man, a good businessman, a good administrator, a farmer and a judge of cattle and horse flesh. So that was what he did, but the two of them did a lot of things, and eventually, when he died, Lady Dixon wanted to finance a medal and a lecture. But from what I'm reading, Sir Ian Fraser, he apparently himself, and I'm sure this is true, insisted that it was called the Sir Thomas and Lady Edith Dixon lecture, because he had a big hand in it.

We're arguing about when the first lecture was actually given, and this is of interest for us, particularly as haematologists, but what I found out was, the first lecture was given by Sir Lionel Whitby, an expert in as, Sir Ian Fraser said, blood diseases. But actually as haematologists, we would know, and it was a name we all learnt as we were going through, that he actually organised blood transfusions during the Second World War, but he died the year I was born, and that's a long, long time ago.

The lectures continued, and as I said, there may be some other discussion as to what was the first one, but that's what Sir Ian Fraser seemed to think was the first one. It was originally in the Royal, but eventually it, as described here, has a loose attachment to the Ulster Medical Society, because it was felt that Queen's had plenty of lectures and didn't need any more, and certainly has been that for many years since, so that is what we are having tonight, supported by the Sir Thomas and Lady Edith Dixon bequest with a medal to celebrate the lecture at the end.

So as the lecturer tonight, it is my great pleasure to introduce my very good friend, Professor Irene Roberts, so Irene, I was trying to think when was the first time we met, and I think it was probably about 1986, when we were both up for some British Society for Haematology research competition, which I certainly didn't win, but I think you did. We worked together when I went to the Royal Post-Graduate Medical School in Hammersmith as part of my rotation in 1989, and at that stage, in my recollection, Irene had just been appointed to set up paediatric haematology transplant, care of sickle cell disease, and anything else to do with children and haematology, more or less single-handed with no resource, was my memory of it, and I used to work there. We had really very interesting times and then I also worked in a lab, and we had great fun torturing little cells and trying to make them grow. However, the bit I remember particularly about Irene, and nobody knew anything very much about neonatal haematology, and in the Hammersmith it was really natal—they were very, very small, and she looked at every baby's blood film every day for a year, to become an expert on how to interpret these blood films. But Irene has gone on from then to build up an astounding career in paediatric haematology, both from a research and a clinical

side, and in 2013, she moved from being professor of haematology in Imperial to Oxford, where she's the professor of paediatric haematology. She has a lot of research, astounding publications, and also is an outstanding paediatric haematologist.

She's particularly interested in the impact of Trisomy 21 on haematopoiesis, and on the stem cell, in the context of leukaemia in early childhood, and it is on that, she's going to talk to us tonight. We've actually changed the title a little bit from last year, but it's, "Too Much and Too Little—How Does an Extra Chromosome Cause Leukaemia in Children with Down's Syndrome?"—and just before I start, I'll ask you if you can put off your camera if you wish during. I think we'll probably mute everyone, and then at the end, we'll get everybody back on again, and can have questions and get people to put their hands up. Sorry, I should have said that earlier, so it is my delight for you to give your lecture tonight, thank you.

Professor Irene Roberts:

Thank you very much, Mary Frances, for your really warm and generous introduction, and you're quite right—we did have to set up paediatric haematology from scratch, but luckily in those days, nobody asked me for a business plan, so I was able to get on and do it without being challenged too much about how much it was costing. I don't think that would happen now.

Anyway, back to this evening and this lecture, which I'm genuinely very honoured to be asked to give, having read about Sir Thomas and Lady Edith Dixon as a result of the invitation, and my only disappointment is that I've not got the chance after all to come to Belfast, because I'm embarrassed to say that I've never been, and I was genuinely really very excited about having the opportunity to come for the first time last March, but that's just something for the future.

What I want to do this evening is to tell you what I think is an intriguing story about a particular type of leukaemia that, as Mary Frances said, affects young children with Down's Syndrome, and this link with Trisomy 21, and in fact the link between leukaemia and Down's Syndrome is really extraordinary, and I think it's best illustrated by the results of this population study from Denmark, from Henrik Hasle, which shows you several things. It shows you the standard incidence ratio, which is on the right-hand side of the table, and the first thing to point out is that, compared to children without Down's Syndrome, the increased risk of acute myeloid leukaemia is over 150-fold greater than in children without Down's Syndrome, but it's not just the myeloid leukaemias that are increased, it's also the lymphoblastic leukaemias which are increased, suggesting that there's something potentially different about the stem cells of these children, that you should get two such different lineages of leukaemia involved. Secondly, you'll notice, because I've highlighted it in blue, that this increased risk is particularly so in the young children under the age of four, and then finally, intriguingly, the risk of other cancers of solid tumours is actually half what you'd expect, 0.45, rather than 1, and this suggests that there's something very particular about haematopoietic cells which makes them susceptible to leukaemic transformation. So I want just to tell you, in the next couple of slides, a little bit of background information about the leukaemia, and then about the pre-leukaemic stage which many children with Down's Syndrome will go through before developing leukaemia.

So first of all, the leukaemia. I'm not going to talk about the lymphoid leukaemia at all this evening. In children with Down's Syndrome, the AML, acute myeloid leukaemia, is known specifically as myeloid leukaemia of Down's Syndrome, or MLDS, and we know now that this originates in foetal life, and it presents before the age of four years, so there's this very defined time window, and the cases of leukaemia in the children with Down's Syndrome are preceded by a neonatal leukaemia which is unique to Down's Syndrome, and it's known as transient abnormal myelopoiesis, or TAM. Some of you might know it as transient myeloproliferative disorder, both of these are the same condition, but I use TAM because it's easier to pronounce.

So just to say something about TAM, because for those of you who aren't paediatricians or neonatologists or haematologists, you might not be very familiar with this strangely named condition. This is a clonal haematological disorder, and it's characterised by increased circulating blast cells in a baby with Down's Syndrome. And this shows you on the right-hand side a typical blood film, where I've put a couple of arrows at the blast cells that we commonly see in this disorder, TAM, and it's unique to babies with Trisomy 21, either with Down's Syndrome, or even with mosaic Down's Syndrome, where the extra chromosome is only in the blood cells. And this condition usually presents in the first few days of life, it might even present during foetal life, and it never presents after the age of three months. So this is telling us that TAM is a foetal disorder which is strongly linked to Trisomy 21, and we've been working on each stage of this leukaemia development for about 15 years now altogether.

So if I summarise just some of the work that's led up to us developing the model which I'm showing you on this slide now, we know that it requires the presence of Trisomy 21 in foetal haematopoietic cells, and we know also, thanks to work from a large number of labs, which is summarised at the bottom with these references here, that transient abnormal

myelopoiesis, the pre-leukaemic stage in the middle, is due to acquired mutations, they're not inherited, in the erythroid megakaryocyte transcription factor, GATA-1, and these are already present at birth.

Although many cases spontaneously resolve, we know that for leukaemia to develop, it's not enough to have a GATA-1 mutation, you have to have additional mutations in other genes which then occur in cells which persist for more than two to three months of life—the mutant GATA-1 cells persist, and then they acquire additional mutations.

So we've had a series of questions, we've been studying each stage of this process, and I'm going to go through them in three separate sections of the talk, starting off with the first question, but actually going to spend most of my time on the second question. So our first question is the obvious one, and the hardest one to answer, and as you'll see, we haven't really got there yet, but we are slowly homing in on the kind of answer that we're looking for, and that is the simple question: how does Trisomy 21 affect foetal and neonatal blood cells?

So to do this, we knew that we had to study the cells before the GATA-1 mutation had been acquired and we've done this in both neonatal cells and foetal cells. And all haematologists, as Mary Frances will know, are fond of showing this typical diagram which probably makes non-haematologists fall fast asleep, but I have to use it to make a few simple points so that I can then explain what Trisomy 21 does. This shows you, in summary, haemopoiesis in one diagram, starting off with the HSC, the haematopoietic stem cell, which we know undergoes a series of decisions, in order to decide whether it's going to be a myeloid progenitor going through the multi-potent myeloid progenitor stage, MPP. Here the myeloid progenitors are up at the top, and these are the cells that give rise to red cells and to megakaryocytes, via a megakaryocyte erythroid progenitor, or to granulocytes and monocytes via a granulocyte macrophage progenitor. On the other hand, if at an early stage they decide that they're going to adopt a lymphoid fate, they do that via these progenitors down here, which I'm not going to say anything more about today.

Now, we can study what Trisomy 21 does to this pattern of normal haematopoiesis in several different ways, and we've largely focused on flow cytometry to characterise the numbers and frequency of these cells, their non-functional assays, to show how Trisomy 21 interferes with function, and then on transcriptomic studies and more recently epigenetic studies, which I'll say nothing about today.

So first of all, using flow cytometry, what can this tell us? I just want to walk you through this slide slowly. What this shows you is, the proportion of each of these different cell types in trisomic foetal samples, compared to the disomic, normal samples, and if we take first of all the first box, which is the most primitive haemopoietic cell type, haematopoietic stem cells and multiple progenitors, and we compare the frequency in trisomic samples in red with disomic samples in blue, what you can see is that the numbers of HSC, haematopoietic stem cells, up here, are increased. More dramatic even than that is what happens in the myeloid progenitor compartment, where you can see particularly here, there's an increase in the frequency of megakaryocyte erythroid progenitors, which is interesting because this is the cell which gives rise to platelets, megakaryocytes and red cells, and interestingly the GATA-1 transcription factor is also very important for making these cells, so this is already a clue about why there's an interaction between Trisomy 21 and GATA-1.

And then the third box over here shows you the frequency of immune-type progenitors, granulocyte monocyte ones, GMP, and the LMPP, and lymphoid progenitors, which are reduced. The bottom line from all of this is that Trisomy 21 causes multiple changes, both in foetal haematopoietic stem cells and in progenitors of all lineages.

What then about the effects of Trisomy 21 on the function of these cells? Well, I just want to show you one piece of data about the function, because it nicely makes the point about why it might be important in leukaemia, and what this shows you, is if you grow these megakaryocyte erythroid progenitors in culture dishes in the lab, the trisomic cells grow much more quickly than the disomic normal samples, showing that Trisomy 21 megakaryocyte erythroid progenitors are highly proliferative. Of course, this begs the question immediately of, what is driving these changes?—and if we look at what might be driving it based on the genes that are on chromosome 21, this just shows you a simplified cartoon with some of the 240-odd genes on the chromosome marked, and the ones that are marked are favourite sort of genes, because these are largely ones which are either implicated in leukaemia specifically, for example like RUNX1 down here, or we know that they're very important for haemopoiesis, such as BARK1, or they're involved in the inflammatory pathways, such as the interferon receptors shown over here, so one of the first things we did was to look at gene expression in trisomic cells versus disomic cells, and I'm only going to show you one piece of data from this before moving onto the middle section, and this is micro-array data. We've also looked at this using RNA sequencing data, and we've looked at it at a single cell level and in bulk cell level, but the results are actually very similar, and the conclusions are very similar, which are that Trisomy 21, as you might expect with an extra chromosome, does cause there to be a large number of differentially expressed genes. As you can see in this work, we found 833 differentially

expressed genes, if we took the haemopoietic progenitor, stem progenitor cells from trisomic compared to disomic samples. But what was surprising was that only 34 out of these 800-odd genes were actually on chromosome 21, and 799 of them were not on chromosome 21, and indeed they tended to reflect the same pattern of abnormalities that we'd already seen in flow cytometry, with increased expression of erythroid genes and also reduced expression of B lymphoid genes. And the bottom line of all of this is that Trisomy 21 doesn't just cause alterations in expression of chromosome 21 genes, it causes much more profound genome-wide perturbation of gene expression. 21 25 58

So if I summarise this part of the talk, and try to answer the question, how does Trisomy 21 affect foetal and neonatal haemopoietic cells, from this, from what I've shown you, but also from a lot of work which I haven't shown you because of time, we can say that Trisomy 21 causes an expanded proliferating haematopoietic stem cell and myeloid progenitor pool. This progenitor pool has very strong megakaryocyte erythroid bias. This is before there's any mutation in GATA-1 at all, and this is driven by very complex genome-wide changes in gene expression, and what we know now is that this is most likely controlled by epigenetic mechanisms which allows the survival of the trisomic cells, probably as an adaptation of these cells to being able to survive with the presence of an extra copy of chromosome 21. So I want to move on now to the second question, or the second aspect of this leukaemic transformation, and think about transient abnormal myelopoiesis now, and to ask some specific questions for this section, which are, how common are GATA-1 mutations in neonates with Down's Syndrome?—and what effect do these mutations have on their blood cells?. And before I go onto tell you about a study that we established some years ago to document this, I need to tell you a little bit more about the GATA-1 gene. So as I've mentioned already, the condition, transient abnormal myelopoiesis, is caused by acquired mutations in the GATA-1 gene. Now, these mutations, and there's some shown in this cartoon here, GATA-1 gene, are largely clustered in exon 2, with a few occurring at the very beginning of exon 3. As I mentioned already, it's very notable, given the abnormalities that Trisomy 21 itself causes, that GATA-1 is a transcription factor that's absolutely necessary for the normal production of red cells, platelets and megakaryocytes, and it's a gene which is on the X chromosome, the relevance of which I'll explain in a moment.

Now, when you get, in Down's Syndrome, these mutations in transient abnormal myelopoiesis, these mutations, as I mentioned, they cluster here, and the effect of this is to cause translation of a short GATA-1 protein, so here's the full-length protein here in blue, and when you have mutations in exon 2, or the beginning of exon 3, what you end up doing is using this start site here, and you get the production of a short protein, GATA-1S. Now, the most amazing thing to me is that mutations like this are not leukaemogenic in the absence of Trisomy 21, and we know this because there are patients who have inherited mutations in this gene, and they do not develop leukaemia, or at least they very, very rarely do, so there's a very strong association between exactly this type of mutation and leukaemia, and as I mentioned already, these mutations are already present at birth, and so because of this, we knew that we wanted to study the impact of GATA-1 mutations on neonatal cells. We would actually have to set up a study to follow babies with Down's Syndrome, and so that's what we did, and I'm going to spend a little bit of time telling you about this study, and about the results that we found. So we established it with the following aims. We wanted to determine the frequency of these mutations in babies with Down's Syndrome, and we wanted to relate this to their clinical features and the haematological features, and to define exactly what kind of mutations occurred in these babies, and then armed with this information, we then wanted to follow the babies up to determine the natural history of GATA-1 mutations in Down's Syndrome, and to work out the true risk of subsequent leukaemia in that setting, so I'll show you, in the next slide, the study design which seems beautifully simple, but turned out to be incredibly complicated to actually deliver. I'll describe it first.

It was a prospective multi-centre study with the aims I've just described, and we picked 18 centres in England and in Scotland, two in Scotland, 16 in England, and they were all chosen for their links to the Hammersmith neonatal unit, which as Mary Frances already told you, was where I was very interested in studying the blood pictures of all the babies in the unit on a daily basis. And so we picked consultants to be part of this study who I knew would be pleased to work with us, and would deliver, and they certainly did. All we wanted them to do was to collect a full blood count, arrange a blood film, and then send us the leftover full blood count bottle and the film, and we would do GATA-1 mutational analysis on the leftover full blood count, but we needed to have this collected in the first week of life, and then after we'd enrolled the baby, we would take serial samples and follow up and follow them until they were aged four years, because that was the time window for the development of the leukaemia. And our first problem was that this condition, TAM, was not very carefully defined by the WHO at that time, and so we had to come up, it was very woolly, we had to come up with our own definition of TAM, and we decided it would be any neonate with Down's Syndrome who had a GATA-1 mutation, and peripheral blood blasts of more

than 10%. So with that in mind, what I want to show you first of all was, how we answered the question about the impact of Trisomy 21 on the babies' blood cells, so we could only do this in babies who didn't have a GATA-1 mutation, and this shows you the platelet count, and then some pictures on the right-hand side, the platelet count of babies without Down's Syndrome compared to babies with Down's Syndrome, and these were gestation-matched, and they were healthy babies, and through this, as you can see, with the non-Down's Syndrome babies in the open symbols, and the Down's Syndrome babies in the closed dark ones, that thrombocytopenia is much more common in the babies with Down's Syndrome, and the platelet count has shifted down, and in addition to that, under the microscope, we could see babies who had no GATA-1 mutations, but they still had megakaryoblasts in their peripheral blood, and they had great big chunks of megakaryocyte cytoplasm, and some of them even had circulating megakaryocytes. In other words, just having Trisomy 21 was enough to perturb neonatal megakaryopoiesis and platelet production, even in the absence of GATA-1 mutations.

What then about the red cells?—well, these were abnormal as well, perhaps you were not too surprised by this, and the first thing is just simply the haemoglobin, where you can that the Down's Syndrome babies in the dark symbols had a higher haemoglobin than the babies without Down's Syndrome in the open symbols. They also had larger red cells, they were macrocytic, and they had increased numbers of circulating erythroblasts, and under the microscope, there was a very high frequency of abnormalities of erythroid morphology. You can see this odd erythroblast here, we'd see target cells, binucleate cells, and then even basophilic stippling in some of the erythroblasts, so what this told us was that Trisomy 21 itself increased red cell production and caused dyserythropoiesis in almost all the babies with Down's Syndrome.

And then finally, considering what Trisomy 21 did to the white cells, it was a similar sort of pattern. We saw an increase in white cell count in the babies with Down's Syndrome, an increase in the neutrophils, and an increase in blast cells, so this was babies, as I mentioned already, where we had not detected a GATA-1 mutation, and very frequently the morphology of these white cells was rather odd. So in some, Trisomy 21 not only perturbs foetal liver haematopoiesis, but it also caused tri-lineage perturbation of neonatal haematopoiesis.

What then about the frequency of GATA-1 mutations? Well, after we'd collected samples from about 200 babies, we went on to analyse all those where we had enough DNA to perform direct sequencing, sinosequencing, so we had 186 samples from the first 200 babies initially, and what we found was that 9% of them, and all of those in fact with blasts over 10% at this point in the study, had GATA-1 mutations, and so we designated these as TAM. But the fact that we were able to see blast cells in such a high proportion of babies without GATA-1 mutations made us wonder if we might be missing GATA-1 mutations, and we decided to adopt, after the first few years, a more sensitive way of looking for GATA-1 mutations based on next-generation sequencing, and we were really amazed to discover, when we did that, that we could actually detect GATA-1 mutations in 29% of the babies, and most of them, or rather the majority of them, had no clinical signs of TAM and no increase in blast cells.

References to unpublished work have been removed.

We have been trying to develop simpler ways of assessing the GATA-1 clone other than relying on next-generation sequencing, because this is quite time-consuming and quite expensive, so recently we published a quicker way of doing this, which is to actually look at the protein, the GATA-1S protein, that's produced as a result of having a mutation in the end terminal of the gene, and you can measure this using flow cytometry. And so you can actually count the cells which are expressing the short GATA-1 protein, and that way also get an estimate of the size of the mutant GATA-1 clone. And that's what's summarised over here on the right-hand side of this slide, where we've got the percentage of GATA-1S cells measured by flow cytometry on the y axis, and then the VAF measured by next-generation sequencing on the x axis, and you can see that there's a pretty good correlation, and that this also seems to be reasonable, if you look at the bottom panel, even when there's a small mutant GATA-1 clone. And again you can sort of see the same thing if I show you this plot on the left, divided into TAM and silent TAM, you can see that even these samples would likely be detected by using this flow cytometric method.

Now actually, when I was plotting this graph, I then noticed something else which turned out to be interesting, which is these samples here, where there was a clear discrepancy between the VAF, which was much higher than you'd expect for the percentage of blasts, and we realised that four of these samples were discordant samples, so the VAF was not done on the same day that the blood film was done, even though that's what we thought we were measuring. And it just illustrates that it's very important, when you're looking at the blood film, actually to get a blood

film within the first few days of the first week of life, because the problem here was that the blood film, something had gone wrong with the early sample, and we'd been sent a late blood film. But there's also another interesting thing, because one of these samples, you'll see, is a star, and this is the only baby which had a partial Trisomy in our series. All the other ones had full Trisomy, and this poor baby, who had a partial Trisomy, only part of chromosome 21 was extra, was the only silent TAM that transformed to acute myeloid leukaemia, and also subsequently they got acute lymphoblastic leukaemia. So this kind of case would be very informative if they were not quite so rare, of being able to tell us which genes and which parts of chromosome 21 are important in the leukaemic process, but it may well be that this baby is missing the parts of chromosome 21 that drives the proliferation that we see caused by Trisomy 21.

In the last few slides, I just want to carry on trying to answer the questions that we posed about predictive factors, and what this shows you again is the VAF, but this time, instead of correlating it with blast cells, it's correlated with the clinical features. And we came up with a clinical score, which was made up with one point each for the main clinical features of TAM, which is, for those of you who look after these patients will know, are hepatomegaly, splenomegaly, effusions, skin rash and jaundice, and when you do that, you can see that actually there's a pretty good correlation between the VAF and the clinical score as well. So we did hope that might mean that you'd be able to identify babies at birth who were more likely to transform based on their clinical features, but this did not prove to be true.

Here we have, with a clinical score, those babies who developed MLDS, and as you'll see, these clinical features are just not good enough to predict whether a baby's going to develop MLDS or not, so it's certainly not inevitable that a baby that's got all of these features, or even lots of them, is at any greater risk than any other baby, of transforming to MLDS. What then about the type of mutation?—well, I've said already that they're all in the end terminal of the gene, which they are.

This shows you all the mutations for the babies that we've studied, and it makes several points, first of all that the mutations occur right across the whole of exon 2. If you look at the type of them, they're a mixture of mis-sense and nonsense mutations. Some are splicing at each end of the exon, and the most common are frame-shift mutations. When you look at how they divide between TAM and silent TAM, it doesn't look as if the mutation type explains whether you get a large clone or a small clone, TAM or silent TAM. When you look at whether they predict for whether they get MLDS or not, with the yellow stars, there's maybe a suggestion that babies with a frame-shift mutation are more likely to transform, but in fact, because these mutations are more common, this isn't statistically significant, of course, and far more babies who have a frame-shift mutation, don't develop MLDS than do, and so actually, the type of mutation is also not a good predictor. We then looked at whether the number of mutant GATA-1 clones would predict for the risk of myeloid leukaemia of Down's Syndrome. I hadn't mentioned, I don't think, that a number of these babies had more than one mutant GATA-1 clone, which is really quite remarkable. Some of them even had eight clones. The majority, if they had more than one, was two, but about one-third of babies overall had more than one mutant GATA-1 clone. It was similar in TAM and silent TAM. The cases of MLDS are marked on with a yellow asterisk, and you can see that having more than one mutant GATA-1 clone does not increase the risk of transformation.

So the final thing we looked at then was the relationship between the size of the mutant GATA-1 clone and the percentage of blasts, and these two features do predict for transformation to myeloid leukaemia of Down's Syndrome, so shown here on the left is leukaemia-free survival, depending on whether your diagnosis is of silent TAM in blue, or TAM in red, and you can see that you're more likely to transform if you have TAM than silent TAM, and then over here is the risk of transforming if you have a variant allele frequency of more than 0.2 versus less than 0.2, and again you can see that's significant as well. So bringing all that together in the last summary slide, what I've tried to show you is that these extraordinary end-terminal truncating mutations in GATA-1 occur at very high frequency in newborns with Down's Syndrome, 28%, and they're frequently multiple. The mutations are acquired only in foetal cells, not postnatally, and they usually occur or expand after 20 weeks' gestation. Fortunately the mutant GATA-1 clones are usually small and clinically silent, and they resolve spontaneously, and confer a low risk of MLDS, but Down's Syndrome neonates who have large mutant GATA-1 clones do have a higher chance of then acquiring additional mutations, particularly for some reason in cohesion genes, and then of going on to develop myeloid leukaemia of Down's Syndrome. I have had some names on the slides as I've gone through, acknowledging their contribution, but this slide shows you just 20 or 30 out of, I think hundreds of people, who have contributed, particularly to the neonatal part of the study, and I've highlighted in red those people who I want to give most credit to, Natalina, Anindita and Gemma in my lab, Gillian in my old lab at Imperial, the people that worked particularly on the neonatal study recruiting babies, and helping to analyse the data, Neha, Amelie, Laure and Helen, and the people in the Vyas lab, who were

instrumental in helping to develop the initial GATA-1 mutation assays, as well as their collaborators and funders, and that's us in happier days, in a very famous Oxford landmark on our Christmas night out.

Thank you very much indeed.

Professor Mary Frances McMullin:

So thank you very much, Irene, that was a wonderful talk! So I'm sure you'll be happy now to take some questions? I think maybe the idea would be if you stopped screen sharing, that would show people on the screen, and you can ask questions, put them in the chat or put your hands up or just wave at us.

Before we start, I just want to say one thing before people leave, and please don't leave yet—what we would like you to do, there are 40 people on the call tonight, so that would be a really good Ulster Medical Society meeting if we were all in the lecture theatre. We would like everybody to email Kathy to tell us you were here tonight, because that will give us a record of the attendance. There's a very ancient old book that everybody signs their name in, we're not going to have it, so if you could email us, we'd know who's here, and we will then send you back out a CPD certificate. So please can I ask everybody who is on the call to just drop an email to Kathy at UMS, it's the email address that you get the notifications from.

So any questions?—Peter Watson.

Dr Peter Watson:

Professor Roberts, thank you very much indeed for your terrific lecture, very interesting. As a non-haematologist, for years and years I've been aware of the Philadelphia chromosome problem in leukaemia, and I wondered with the Down's Syndrome chromosome abnormality, are there any analogies, or is there any commonality in terms of the mechanism by which leukaemia occurs in these two conditions?—or are they completely different, and tell you something else about the mechanism of leukaemia?

Professor Irene Roberts:

It's a very interesting question. I think there possibly are two ways in which one can draw parallels with the Philadelphia chromosome. One is that characteristically they have abnormalities of tyrosine kinase signalling, and that's clearly something which is very important to have carefully regulated in haematopoietic cells. If tyrosine kinase signalling goes a bit crazy, then that makes them proliferate more and it also interferes with differentiation, and that's certainly something which happens as a secondary event in the full-blown leukaemia in Down's Syndrome, although it's not something which is probably relevant to the pre-leukaemia or the effect of Trisomy 21 itself. The other thing is that, actually interestingly, some of the abnormalities that we see in babies with Down's Syndrome are also seen in adults with chronic myeloid leukaemia, the typical myeloid type of Philadelphia chromosome-positive leukaemia, so I think there may well be some, as yet to be undiscovered, common threads between the conditions, so that was a very interesting question.

Dr Peter Watson:

Thank you.

Professor Mary Frances McMullin:

Anybody else with a question?

Dr George J Calvert:

It's Jonty Calvert. It's a long time since I've practised medicine, I'm retired a long time. Thank you, Professor Roberts, very much for your talk.

I just wonder, I had a clinical interest in Down's Syndrome, I wonder, is myeloid leukaemia less likely in a translocation form of Down's Syndrome, and less likely on the mosaic form of Down's Syndrome, for individuals?—in other words, if you have a mosaic disposition, are you much less likely to develop a myeloid leukaemic picture?

Professor Irene Roberts:

That's an interesting question as well, because one might imagine that you would be less likely, because the environment of the blood cells is not trisomic. The support cells within the bone marrow in the liver and so on are not trisomic, it's only the blood cells, but actually that's not the case. Individuals who have mosaic Down's Syndrome are just as likely to acquire a GATA-1 mutation as a baby with Down's Syndrome, and they're just as likely to transform to the myeloid leukaemia of Down's Syndrome, so it seems to be a very, very powerful interaction between this extra chromosome 21 and GATA-1 mutations, even without you having the other constitutional features of Down's Syndrome. And yes, you get it whether you have a Robertsonian translocation or a more typical extra copy of chromosome 21 without a translocation.

Dr George J Calvert:

Thank you very much indeed. Thank you.

Professor Mary Frances McMullin:

Any other questions?—Michael Trimble?

Dr Michael Trimble:

Yes, if you're inching towards a mechanism, is there any hope of a prevention or a cure?

Professor Irene Roberts:

That's also a very interesting question. We actually set out with the idea that we might be able to

prevent leukaemia by identifying babies with GATA-1 mutations, and then developing a simple treatment to accelerate the disappearance of the mutant clone. In fact, because there's such a low rate of transformation, our study showed that it was not going to be feasible without an enormous study to try to target the step, which was GATA-1 mutation to full-blown leukaemia, although there are people who have suggested that you should try to get rid of all the GATA-1 mutant cells in all babies with a mutation, to prevent all cases of leukaemia. We've not taken that stance, because you'd have to treat 95 babies where the mutation would go away on its own. Now, with respect to whether you could prevent the mutation in the first place, I think what we would need to be able to do, and we're beginning to do some work on this now is, you'd have to understand what caused the GATA-1 mutation in the first place. Is there something which is operating during pregnancy in some cases, which is causing a high frequency of GATA-1 mutations? We don't know the answer to that.

Professor Mary Frances McMullin:

Graeme Greenfield has his hand up—go ahead, Graeme.

Dr Graeme Greenfield:

Thank you very much, that was a really fascinating talk. I hope I picked this up right, but in the individuals that go on to develop the leukaemia, the clone, the GATA-1 clone, does it disappear in its entirety for a short period of time, or is it undetectable in that kind of interim stage before the reoccurrence? Professor Irene Roberts:

Well, it doesn't disappear completely in the sense that we know that it must be there, even if we can't detect it, and the reason that we know that it's there is because the mutation in the leukaemia in GATA-1 is exactly the same as the mutation at the TAM stage. I didn't say that actually, but that's why we know that it must be there, even if it's under the radar, and you might need to have a level of sensitivity of, down to one in a million cells or something like that. Where we've seen cases like that, we've used a very, very sensitive technique like digital drop PCR, and we can see that it is there, if you see what I mean, even though using ordinary next-generation sequencing, we can't detect it. So there's two ways of answering that question, one is directly, with a better method, or an even more sensitive method, but you can also infer it.

Dr Graeme Greenfield:

And I suppose, just as a follow up to that then, at the time of reappearance, is there any suggestion of a trigger or anything that might cause that, such as infections or inflammation or anything, that might cause a change in the environment as such?

Professor Irene Roberts:

That's a really interesting question, and it may be analogous, for example, to other kinds of childhood leukaemia, like lymphoblastic leukaemia, where that's been one of the mechanisms that had been postulated to cause development of leukaemia from a pre-existing clone that started in utero. It's not really known, to be honest, but what we did observe was that, if a baby, or if a child, gets an infection, then it causes an amplification of the clone that's otherwise maybe at a very, very low level. So the infection, probably because of various cytokines and things, does cause amplification of the clone—what we don't know is whether it causes the acquisition of the additional mutation, or it just amplifies what's already happened.

Dr Graeme Greenfield:

Thank you.

Professor Mary Frances McMullin:

Anybody else? Maybe I could just get in as an adult haematologist, I think the figure we were told was something, 20 times the incidence of AML in Down's, but the adults get this as well, and we see this, is the adult AML different?—because your average age of developing, 16 months, is it in some way related to the Trisomy 21?

Professor Irene Roberts:

That's another interesting question. It's not. The adult leukaemias don't have GATA-1 mutations, so it's, if you like, more bog standard AML, if there is such a thing. I don't think we've done enough work on understanding what the haematology is in adults with Down's Syndrome. It's been really rather neglected. They remain at a higher risk of leukaemia when they're older, not nearly as high as when they're young children, lymphoblastic and myeloid, but they also get other things, like myelodysplasia and aplastic anaemia, but I don't think any, there's only been one, as far as I know, really systematic study of the haematology of adults with Down's Syndrome. That was an Irish study, it was published in the Irish Medical Journal.

Professor Mary Frances McMullin:

You certainly see more adults with Down's Syndrome coming through than you'd expect.

Okay, has anybody anything else to say? Well, it just remains for me to thank Professor Roberts again and I'll post her the Sir Thomas and Lady Edith Dixon medal in due course. Thank you.