Text: Louise Hagbard, Ph.D. Photo: Ola Jacobsen

STEM CELL INNOVATORS

JO MOUNTFORD

"I think we still have big gaps between academic science, what people call translation, what really is translation, and then what's clinical."

PROF. JO MOUNTFORD, Ph.D.

THE HEAD OF CELLULAR THERAPEUTICS
AT THE SNBTS JACK COPLAND CENTRE IN EDINBURGH

ABOUT: **Prof. Jo Mountford is a specialist in translational processes and focuses on the development of stem cell-based technologies and regenerative medicine. She is an Honorary Professor at Heriot Watt University and also holds Honorary Associate Professorship a the University of Glasgow and is the Head of Cellular Therapeutics at the SNBTS Jack Copland Centre in Edinburgh. The SNBTS Cell Therapy Group is supporting the development of different cell-based technologies and cellular therapies with particular focus on the development, translation and manufacturing of clinical-grade cellular therapies according to Good Manufacturing Practice (GMP).**

Jo's academic interests are in the generation of mesodermal cell lineages for therapeutic use. This includes molecular and biochemical analyses and the overall aim is to fully dissect key signalling events, transcriptional networks and epigenetic changes that lead to effective differentiation to these lineages.

SNBTS JACK COPLAND CENTRE

In 2017, the Jack Copland Centre became the new national headquarters for The Scottish National Blood Transfusion Service (SNBTS). The Jack Copland Centre contains several core activities and services, and delivers a first-rate service in the processing, testing, supply, research and development of blood and human donor tissues and cells.

What has been the biggest thing during these last 20 years of pluripotent stem cell research, from your point of view?

"I'm sure you're going to hear this from everybody. It really was Yamanaka. That work was just astounding. It seemed like alchemy and I really wasn't sure that it was going to be true. The fact that they're already doing trials with these cells in Japan is amazing. So, yeah, for the iPSC particularly I think that was game changing because this whole ethical issue with ES cells was still big. At the same time there would often be somebody with a religious take on a situation – endless debate about this – and countries where it wasn't possible. So no, I think iPSC was as earth shattering as it seems, still."

"But then, the thing in the last 20 years that's probably surprised me the most was when bone marrow or cord blood stem cells seemed to become a panacea for all diseases. The idea that poorly defined 'stem cell' preparations from these tissues would directly rebuild another different tissue when placed at that site. There is no solid scientific basis for this, clinical trials in heart attack have

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↓ "The Jack Copland Centre is a fabulous place."

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shown it to be safe but there is little evidence of persistent efficacy, but these kinds of stem cell therapies are still touted by many unregulated clinics all around the world for a huge variety of disorders.”

Do you feel that the public opinion about stem cell research has changed during these two decades?

“I do think that public awareness around cell therapies is rising and that there is a better understanding around what we do and people can see the benefit of it. I teach, and I used to go into schools and do public education stuff, and you’d have kids saying, ”Oh, I don’t mind if you crush babies up to make stem cells.” ”Okay, well that’s not what we do.” I think the public are really willing to go into clinical trials and to do whatever they can to help and I would even say that they’re impatient to see something happen, frustrated by the lack of tried and tested therapies. I really get that with people with diseases where there’s no drug therapies. I see both sides of that. However, there have already been examples of bad practice in unregulated clinics harming patients. That’s why we’re doing what we do, making sure there are no safety risks. If that were to happen in a proper clinical trial then we’d be in the same situation as the early gene therapy trials in America when everything stopped. Now we’re finally back where we should’ve been 15 years ago. It’s that fear of what could happen to stem cell- or cell therapy if anything goes wrong, but the public just want treatments. I find that a really difficult line to walk.”

Tell us your story. How did you end up where you are today?

“When I was young, I don’t think I ever knew what I wanted to do. I fell through school. I hated physics with a passion but was made to do it. But I loved biology and chemistry, and then I applied for biochemistry and genetics, and I hadn’t really got any idea what it was. I got on a general biology course, and then in the third-year project of my degree, I was in a laboratory in Birmingham where

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they were working on hematopoietic differentiation. That's what hooked me. The people clicked, and the job clicked. It was just the right place, at the right time, doing the right thing. I was never a driven academic person until I hit that lab, and then it all made sense. So, I got into the stem cell field via experimental hematology. I did my Ph.D. on CD34-positive cells, just as CD34 was being discovered. Then I was doing myeloid differentiation where I spent many, many, many hours with an HPLC analyzing chemical changes during differentiation to different lineages, and then trying to look for therapeutics for leukemias based on those lineages. Then I wandered around a lot. I went to Strasbourg and did a year of molecular training. Then I went to Oxford for two years and started growing megakaryocytes and platelets before going back to Birmingham for a fellowship. From 2000 to 2002 I was in Memphis, Tennessee. In Britain, in the late 90s, there was the Alder Hey Hospital scandal, where a pathologist was retaining child tissues from autopsies without consent. That became known and understandably, getting human tissue samples became more difficult and I was not able to obtain cord blood to derived CD34s for a long time. So, I went to America because I could still work there, so I did a couple of years in America. During that time hES cells were discovered and became big. I couldn't work on those in America because I was working in a hospital that was charitably funded and the political situation at the time was not supportive. Then I realized I wanted to work on hES cells and had to come home to do that, so I came back to Glasgow in 2002 to work with Tessa Holyoake who was a big myeloid leukemia specialist."

"In Tessa's lab, they were working on hematopoietic differentiation, leukemic therapy, your standard hematological methods. I wanted to derive hematopoietic cells from pluripotent, I wanted to derive endothelial from pluripotent, and that whole hem-endo idea was coming at the time. But deriving blood from pluripotent cells was not the first thing people thought of, because why would you need it? This is what I always said as well, 'Well, why do you want a new source of red cells? We can get them from people.' But then it was that whole chemical decision points between endothelial and hematopoietic, and how do cells choose to be what they want to be. Because that had always been the massive debate in hematology, how do hematopoietic stem cells differentiate. Is it stochastic? Is it directed? Is it supported but not directed? So that same kind of thinking applied to pluripotent cells was what attracted me, I think, at that point. So, I set it all up myself from scratch, got technicians trained, trained myself, and got that up and running. The Scottish Government have a local development authority called the Scottish Enterprise. They funded a large program looking at pluripotent stem cell differentiation and potential use for tools and technologies in their ITIs division. It involved the company Cellartis who moved over here to do that work, and the University of Dundee Drug Screening Unit. They set up a stem cell lab in Glasgow, on on campus that needed additional stem cell support and I was brought in as a pluripotent SC consultant to that program and ended up moving into the lab full time. So, we got great space, great facilities and that was the big start for me really. Then once that program finished, I managed to fund those staff to stay. Then we started the red cell differentiation work and various other programs, and endothelial differentiation much more in depth. I've been pretty much pluripotent since."

Tell me a bit about your current role here at the SNBTS Jack Copland Centre.

"I'm very much stem cell and stem cell-derived therapies. That includes pluripotent and somatic stem cell derived products, derived and differentiated under GMP as advanced therapy medicinal products (ATMPs). So, my background is academic but I'm no longer really an academic. Here, we have positions at the universities, and we have research interests at the universities, but the primary function of SNBTS Jack Copland Centre and my new role is to make therapies, both stem cell-derived thera-

“I set it all up myself from scratch, got technicians trained, trained myself, and got that up and running.”

pies and non-stem cell-derived cellular therapies. We have manufacturing facilities which makes us one of the few institutions in Britain that can actually do everything from originating the idea, through developing the process and taking it into GMP. Then we have the clinical links to go into trial as well, so it's a fabulous place to be from that point of view. We've got lots of stuff going on. So, there's John Campbell who is more focused on immune cells, and me, and between us we've got about 35 scientists, and then there is the GMP production department, our Head of Production, Neil McGowan, is amazing. So the whole Tissues, Cells and Advanced Therapeutics (TCAT) department is about 90 people. It's the biggest opportunity. It's huge."

Is there anyone in particular that has been an inspiration for you?

"Tessa was huge to me, to us all, I think. We knew how special she was at the time, but I think now she's gone you appreciate it all the more. She just did stuff. She had a thought and she made it and she was a tremendous finisher, completer. She built the new building, she fundraised for the work in the new building while we were there, so she was a great inspiration."

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"Here, our boss Mark Turner is a clinician, and he's the guy that's actually pushing us to take these things to clinic, and pushing us to realize, rather than just talk about it. He's also a consummate politician. I mean, compared to me, I'm sometimes perhaps a little bit too outspoken, he is really skilled and does a great job for us."

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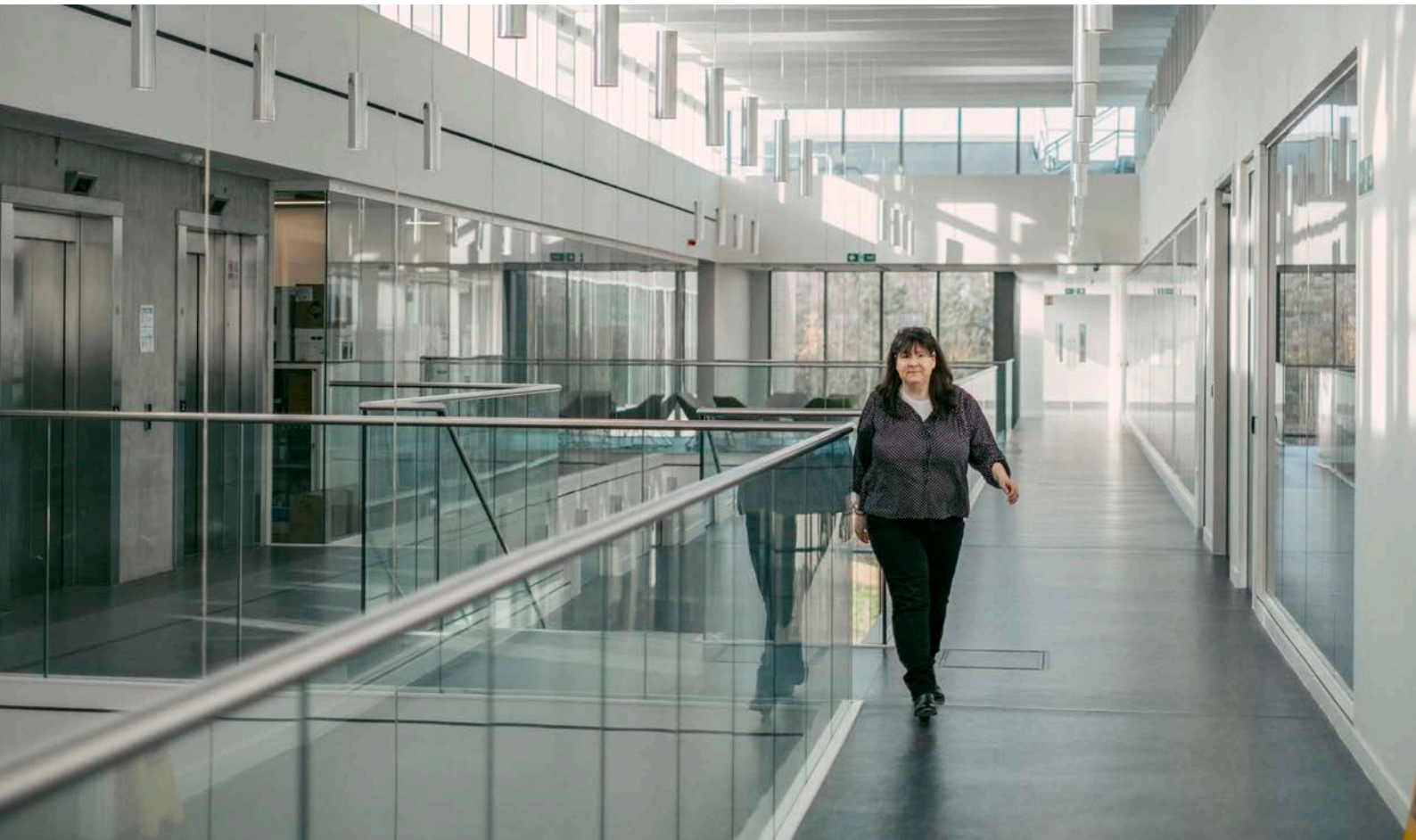

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What is your specific research focus?

"My primary focus at the moment is on pluripotent, derivation and differentiation, of derivation to clinical grade and to GMP. So, we've had to start from scratch and think about how we would start deriving new lines that would be suitable for clinical use. We're a good way down that process now, and we're hoping before the end of this year to be manufacturing GMP grade iPSC lines. At the same time, we're also banking cell lines. So, if other people have cell lines that have been derived at GMP but in small amounts, we're also expanding those up to master cell banks, the working cell banks. We're also collaborating with a number of academics to look at using derivatives of those, like in stem cells for therapeutics. I think we should really have more of an academic push than a commercial pull. Really pick out the good bits of science and make them real. However, I think we need a mindset for realizing therapies. Make it more about, 'Do you have a clinical development route?' I think we still have big gaps between academic science, what people call translation, what really is translation, and then what's clinical. We just are so far apart. I think there's huge amounts of valuable stuff being done in the universities, and if people don't consider translation from the beginning, they can go down a pathway where they can't come back from. Our boss, Mark Turner, the one thing he always says is, 'You start with the end in mind.' That is as simple as using a substrate and a medium that you can take all the way through the different grades. You can start with the research grade, and you move to a cleaner one and end up with a GMP one. You don't have to incur the cost until late, but the reagent should be the same. But the number of academics who still use Matrigel ... I hate Matrigel, and all the other off-the-shelf unknown ECM components. And it doesn't need to be a choice based on cost. People think more defined substrates are expensive, but if you're going to basically waste 10 years and then have to go back and start again, it's not expensive. And write your grants accordingly! I've rarely had a grant for stem cell work that has less than £30,000 a year consumable on it, but people are still asking for 15. You just don't. You just have to ask for what you actually need. The funders understand it."

What can we do narrow these gaps between academia and clinic?

"So, if I wanted anything to change, I think it would really be that we could truly all connect a bit more and break down those barriers and just smooth the way, because it's better for everybody. So, at the moment in Britain we have these things called ATTC, which is Advanced Therapies Treatment Centers. They're virtual centers, aiming at realizing and delivering cell therapies from all levels, including cold chain, starting material, everything. If the funding were there, I would like to make them concrete buildings where the sole focus is on the full process. They should have cleanrooms and trained staff who can then advise the developmental staff, who can then advise the basic research staff. Then, I think, you could really make a difference. I mean, it's kind of what we aspire to here on a small scale, but you know, manufacturing space is at a premium. In general, quality assurance, quality control support for these products is lacking in many places. We can't easily find trained staff to do these jobs. I think those kinds of centers would really help do that. And we haven't even started to talk about the viral processing. Vector production is a big black hole for cell therapists. So, we need all of that as more genetically manipulated cell therapies are developed. But I think those kinds of topic-specific centers could really help."

Do you think pluripotent stem cell treatments will reach the clinic? Will cell therapy be applied in the scale that we hope?

"I do believe that cell therapy will be an off-the-shelf treatment, for some diseases. The Parkinson's trials are really advanced and I like the way they've come together to plan trials with different cell sources. That's very sensible. Maybe that's the first big disease that will get treated. I think that's going to be really exciting to watch. I think we also have to do something for heart attack and heart

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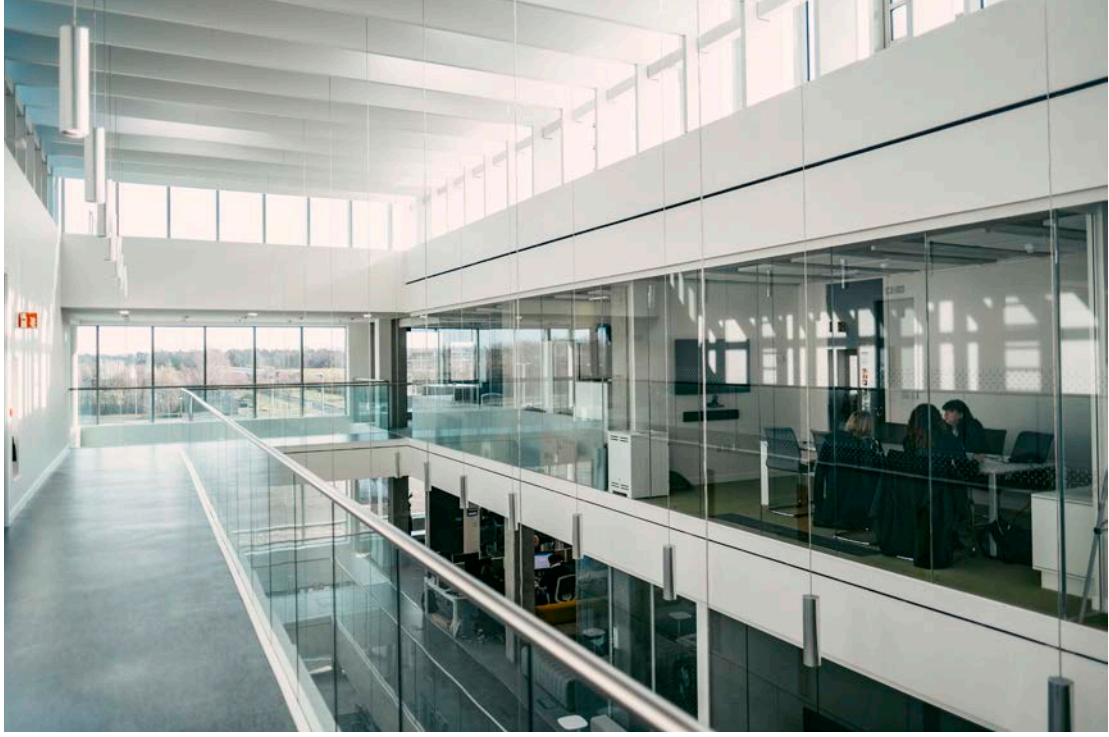
disease. If we can replace cardiomyocytes, you can improve quality of life. Diabetes is another example where I don't see any other options for that either. The difficulty, of course then, is that these are also very high-prevalence diseases. So, the demands could be huge, which brings in itself huge problems with scale up. The problem, of course, is funding. It's so tremendously expensive to grow these cells to any scale, let alone the kind of scale we're going to need for the big diseases."

"Where I think we can contribute and what the Global Alliance for induced Pluripotent Stem Cell Therapies (GAiT) is about, actually, is to serve as a central, international resource for those organizations that are developing therapies from clinical-grade induced pluripotent stem cells, and to support the expansion of this nascent field. So GAiT is trying to set up a network so that essentially you can take a donor line from anywhere in the world. If each country produces their own top 10 HLA haplotypes, the top 10 lines will give between 30% and 70% coverage depending on your population. If each country makes their top 10 and banks them to a similar quality and standard that they can be internationally exchanged, all to the benefit of patients globally. It's a really big aim. Trying to get anybody to agree to anybody else's standards is incredibly difficult, but this is something that Shinya Yamanaka, Ian Wilmut and Mark Turner set up. I think that the GAiT initiative is really important because we will not be able to get whole-scale coverage of large populations without that number of lines. Having any individual entity make that many lines is not really sensible. Whereas doing it on a franchise basis almost, you know, seems to make sense to me."

"But then we would also need agreed protocols for differentiation of tissues from those lines, and there'd have to be comparability between the lines. So, there's also some regulatory work there too because any product from any different cell line is a different product and would have to go through different trialing. So, to be able to get the regulators to appreciate that a bank would consist of different donors, different cells, but then the differentiation would make them equivalent, and that the comparability is in the final product is tough. But I think we're going to have to get there to make it work."

Apart from funding, what do you think will be the biggest obstacle for pluripotent stem cells to reach the clinic?

"My biggest concern at the moment is about the safety of these cell lines just from a long-term culture exposure and adaption point of view. I think there's real risks. I think a lot of the work that's been published to date on differentiation of these cells will almost certainly have been done on cells that are abnormal, but if people have only G-banded occasionally there's no way they're going to know. So, we use SNP arrays now for all of our karyotyping, just to show no change, and I use different density of SNPs depending on what depth we want to look at. Clinically, it's really tough to know what to do because if we look, we know, but we might not know what it means. So, you can't make any risk analysis on it, so why look? The standard at the moment is just G-banding. If we sequenced a clinical iPSC line and found, for example, a p53 point mutation, we would probably chose not to use that line, however there is no data demonstrating the effect of such changes *in vivo* so we cant fully define the risk. So, until we have really good models to be able to predict the effect of those changes, it is unclear what we do with the data from whole genome seq or similar analyses. However it is important that we start to accrue that information so that it can be correlated with *in vivo* effect over time. I think we're going to start getting into a bit of a confusion soon about that. In general, I think we need a new transformation and tumor formation assay. Some regu-



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lators are looking at alternatives or tests in addition to teratoma assays, but I don't know that we yet have a good replacement. Whether that is purely computer modeling and becoming predictive, you still need to feed data into those models. Where will that data come from?"

"So, this issue is a focus of the International Stem Cell Initiative, on genetics testing, and trying to correlate phenotype to genotype, genotype to phenotype, and trying to see what the risk is of these different changes. Not necessarily calling them mutations because they might just be natural variants. You know, the Japanese trial on RPE paused because a potentially oncogeneic mutation was found in the autologous iPSC from one patient. It was unclear if it was a pre-existing mutation present in the fibroblasts, but they still completely redesigned their trial based on that. I think we're going to hit that scenario more and more as we start to make more clinical-grade iPSC lines and everybody will be doing some kind of genome seq. So that information's going to really confound things, I think, unless we find a way to deal with it. So we have to start collecting that data from day one. It's the only way that we're going to start getting that corelative, if not causative, data – that will also require openness and international data sharing."

What is your view of working with hES vs. iPS cells? Do you prefer one over the other?

"Yeah, it's a really good question. At the moment I don't see gross differences in anything except maybe the safety profile. iPS have that additional derivation step, which is far more stressful than the derivation of ES cells. So, I think the chance for introducing culture adaption or spontaneous mutation during that derivation process is a risk that's additional to iPS that requires that additional safety eye. I think as long as you are then making fully-programmed robust iPS cells that are cleared all of the exogenous material and that can be shown to be genetically the same as the starting material, I think everything that we're doing with them is equivalent. If you have a really robust protocol, it works on any pluripotent line."

"I think, generally, people are developing protocols that are very much dependent on an operator, or very much dependent on a single lab. We can never make that GMP. That's a real issue especially when you work with academics. They come to us with their protocol that they've only worked on themselves. Even once you take out all the animal components and you move to GMP reagents, there's still green fingers needed. That can't be the case in a GMP environment. It's that robustness and reproducibility of the protocol that overcomes anything that's not just interindividual variation in the cell lines, that to me is the biggest thing. It's not an hESC or iPSC thing, it's the individual variation in the cell lines."

Is there a stem cell standard? I mean, how do you know whether or not you have a good stem cell line?
"It's really difficult. It's functional and phenotypic. So, we have, as part of GAiT, derived a set of criteria, which include the expression of surface markers, the expression of Oct4, cleared exogenous material, differentiating all the three lineages, spontaneous differentiation in embryoid bodies as well as three-lineage directed differentiation. And normal karyotype. If it's functionally pluripotent, and it has markers of stem cell identity, and it doesn't carry any exogenous material if it's an iPS cell, I think that's the best definition we can use for the moment."

The interaction you've had with regulatory agencies, what is your experience from that?
"We have a lot of workshop meetings from the different lettered organizations, and I do find it very useful. They have tended to be very pragmatic and very accepting of the fact that this is all new. So, I think for the moment they're actually being very supportive, and they get it, and they understand the difficulties and tend to be pretty open to discussion."

If you look back at you career so far, what are you most proud of?
"I am proud of the team I have built, a really tight team who get on really well. I've got Ph.D. students who've stayed with me. I've got staff that I picked up from other projects who've stayed with me. They've all moved across to here with me. That has been great."

How are you as a leader?
"That's a really good question ... My leader style is, I think, very much 'come with me'. A supportive and relatively low-impact leader. They're free to develop things if they come and talk to me about it and we do it together. 'No fear' I think is a really important one for me. I think a lot of scientific misconduct comes from fear. I've seen it in other places. I think yeah, if you mess up, you do something wrong, if you don't get the answer that you want, I would a million times rather that you came and said, 'This is not working. I've screwed this up' rather than just trying to work it through. It's that honesty and integrity that's key for me. They know that I will always back them up and that I will go to war for them if necessary."

So, what's the plan for the future?
"We have a number of adult stem cell therapies in clinic, but nothing pluripotent yet. So we need to take a pluripotent therapeutic to the clinic, clinical trials with iPSC-derived cells that we've made. To make the iPS cells all the way through from starting material, bank the cells, and then derive from them and get them into clinic has got to be our aim. Not sure though what the first one will be. We've got a nice ES cell-based endothelial product for critical limb ischemia. Some patients have non-healing wounds that are often infected and last resort treatment is to amputate the limb. So, the clinical trial will be into the late but not-too-late stage to try and reestablish vascularization. We've proven our ability to induce differentiation of hES cells to vascular endothelial cells in a highly efficient feeder-free, serum-free manner with potential for scale-up. We're looking at taking this to clinic with the ES at the moment, in a study led by Andy Baker at the University of Edinburgh, but the protocol could easily be switched over to iPS with the ability to reprogram somatic cells to iPS cells from patients with cardiovascular disease. So, we've done all the pre-clinical and we've got a grant application in for the first-in-human trial. If we get funding, that's five years to clinic. We may choose to change the cell line and redo the pivotal animal studies. It's a possibility."

"We've also got quite a big mesenchymal cell program so iPS cell-wise, one of the things we want to do is to derive mesenchymal cells from iPSC and use those instead of donor mesenchymal cells. For the iPS we're using adult peripheral blood, mononuclear cells, because it's non-invasive and we take them from whole blood. We're using BioLamina products to derive these new lines. We never have any problems switching from the culture system other people's cell lines come in on to Biolaminin, and for derivation we had it tested vitronectin versus Biolaminin with different media, and you win. We're a good way down that process now, and we're hoping before the end of this year to be manufacturing clinical grade iPSC lines. At the same time, we're also banking cell lines, adult cells and potentially pluripotent-derived as well. So, if other people have cell lines that have been derived at GMP but in small amounts, we're also expanding those up to master cell banks, the working cell banks. Long-term we're looking at genetically engineering those iPSC-derived mesenchymal cells to change their tropism, particularly to try and get them to be retained at the site of action. So that's on a bit of a longer horizon, but that's a really interesting project too." •


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