

Imperial College November 2011

Professor Schneider Interview – interviewer Alan Keys

A-Good morning Michael, I really appreciate you giving your time this morning. I'm fascinated to learn how heart treatments are going to progress into the future, and I can't wait to hear what you've got to tell us about the way that research is going. I would like to start at a mundane level by asking you why you do heart research.

B- Sure, for me the story begins with the immensity of the public health burden. 40% of people here in the U.K across Europe and in the U.S and in other industrial nations will die of cardiovascular disease. That's makes the public health burden immense, equal to all cancers combined. I sometimes say with a little bit of sarcasm that if you are not studying cardiovascular disease or cancer, the rest is orphan disorders and chronic annoyances by comparison in terms of the scope of death and disability. What some people think is that the problem of heart disease has been solved.

We know that there is a contribution from smoking, from high blood pressure, from blood lipids and the diet that people eat. It is sometimes thought that if we just take control over those known risk factors the problem of cardiovascular disease will go away. Part of the dilemma is that's far from the truth. We can lower the risk of having a heart attack by reducing those things but the single biggest contribution to cardiovascular risk is age. So even though we are reducing the number of heart attacks that one has in one's 40's, 50's and 60's, as the population ages into the 70's and 80's the actual rate of having heart attacks, the volume of heart attacks in the population is going to go up and not down.

In addition, because people these days can survive their first heart attack which they might not have 20 or 40 years ago, the end result of heart attacks is heart failure; the inability of the heart muscle to pump as effectively as needed to do the work required of the heart. Heart failure is ballooning in the elderly population and represents the biggest public health challenge in cardiovascular disease.

A-That's getting a bit close to home for me actually..., who funds your research?

B-My work is funded largely by the British Heart Foundation, it plays a unique role here in the U.K compared to similar charities in other countries. The BHF funds roughly 30 endowed professorships like mine, it funds four large centres of research excellence across the U.K, including one here at Imperial. It funds a variety of training grants, project grants, programme grants, instrumentation grants besides the BHF was a major contributor to constructing the new cardiovascular labs that we will have Hammersmith opening in about 6 months' time.

In addition, no one funder is enough to bear the cost of the work, we also have research funding from the Medical Research Council, I also have research funding from the European Research council and smaller grants from the E.U.

A-I see, thank you. I believe your research actually involves stem cells. Would you like to tell me what stem cells are in more details please?

B-The distinguishing feature of stem cells and the property that has made them attractive to scientists, and clinicians and has excited the imagination even of the lay public is two things. The first is their growth potential, stem cells are endlessly self-renewing so they can be grown in the laboratory again and again and again to essentially limitless numbers. The second feature of stem

cells is their ability to turn into many of the clinically relevant cell types in the body, including heart muscle, eyelet cells; that make insulin in the case of diabetes, neurons and glials for disorders like Parkinson's disease and spinal cord regeneration.

So, it's this ability to take a nondescript cell that hasn't turned into anything yet and direct it down one or more different paths to create human cells that have the properties that are needed to rebuild tissues, to heal tissues or even build three dimensional organs that excites the imagination of clinical investigators and the patients who will ultimately benefit from that kind of work.

A-And they occur naturally in the body?

B-They occur naturally in the body, those are frequently referred to as adult stem cells and a big part of the work in my own lab is about these unexpected adult stem cells that we discovered that are dormant in adult hearts. I say that they were unexpected because adult heart muscle in mammals has very little ability to rebuild itself after injury such as heart attacks by laying down new daughter cardiac muscle cells to replace the ones that have died. Recent studies have shown that there is some regenerative capacity but it's clearly not enough to meet the clinical burden of heart muscle cell death that occurs in disease.

So these dormant stem cells that we and others have discovered in adult hearts including human hearts are exciting for two reasons. One might be that we can take those cells out, we can grow them in the laboratory, we can expand their numbers into tens, hundreds, thousands of millions and then those self could be re-injected into the patient himself or herself either in a catheter or at surgery. When injected into injured hearts, these cells can turn into cardiac muscles better than many other kinds stem cells can. In addition, such cells would overcome the potential transplantation barrier, coming from a patient himself or herself they wouldn't be subject to immunological rejection, like a heart transplant or lung transplant would.

But there is another reason that the cells in adult heart muscle are particularly exciting, and that is the theoretical possibility that one might devise small molecules, drugs or hormone like protein that could target those cells as a magic bullet and activate those cells and promote self-repair using the stem cells that are in the heart already without the need for cell expansion in the lab or in a factory followed by cell grafting.

A-So if I understand you correctly, you are saying that you can manipulate the cells in the lab at the moment and that is what you are working on but the end result is actually to be able to manipulate the cells in the body, so that the body repairs itself. Is that right?

B-The holy grail would be the sugar cube or other construction that contain the signal, heal thy self.

A- How do you actually do this at the moment in the lab?

B-Here in my lab we take an approach that is driven in part by developmental biology, by answering the question what are the hormone like proteins or other instructive signals that cells see early in the embryo that guide heart muscle formation. Either by causing the cells to become more heart like or by causing those cells to proliferate in early life while the heart is being formed. There are a number of candidates that come to one's attention from those kinds of studies in model organisms ranging from flies, fish, chick embryos, mice, frogs and others and we learn from each of those in turn.

A second way that we do here in my lab is through so called Omic technologies, not testing one protein at a time or one gene at a time, but asking the question what are all of the proteins that are expressed in these cells. Measuring gene expression for 20,000 different genes on a so called gene chip the size of my thumb nail, and from those kind of analysis, and by comparing those patterns of gene expression with the patterns of gene expression in other stem cells, in adult hearts, in embryonic hearts and across a variety of 60 or 70 other tissues, we begin to pin point these cells molecular signatures and identify antenna like proteins called receptors that are on the outside of these cells that might sense particular growth signals and be used to activate these cells in the lab or in the patient himself or herself. And, indeed through that kind of approach we were able to predict a small number of candidates and test them using robotics and high through-put cell culture in the lab that allow us to test not 10 or 20 different conditions side by side but many hundreds of conditions side by side and in that way we were able to pin point 2 proteins out of many, dozens, that actually act on these cells and are able to drive their proliferation.

A-And from what you said at this stage you use Animals for research in the lab. How do you do that?

B-The reason that we use Animals in research depends on the question to be answered. At a late stage of a project, animal research is absolutely indispensable to prove that a potential product, whether it's a cell or a drug or a hormone like protein will be safe when given to patients in the context of dangerous diseases.

In some other instances and cell grafting to the heart is a good example; you need to know what is the right cell type, and is that cell type safe, you also have to figure out technical difficult matters like how to deliver it best to the heart. Is it best to give it down the coronary arteries, at surgery, in a sheet or with a scaffold of other factors to help those cells survive and function? Those are not the kinds of experiments that could be answered just from a soup of cells in a dish or even through mathematical models and computational biology. As much as we use both of those kinds of tools, there are certain pieces of the problem that have to be solved in large mammals that are close to the human in their anatomy and their physiology for the products that we take to patients to be as assured of safety as is humanely possible.

Now at earlier stages of the work, particularly for discovery biology we use animals for very different reasons from that. One of them is, that some animals; flies, zebra fish, and in my lab mice are capable of being manipulated genetically with immense precision and detail. In my lab, those experiments involving mice are of several different kinds. One uses a series of genetics tricks to make the cardiac stem cells turn florescent colours that tell us exactly how those cells arose in the heart during early life when the heart is just being formed. Do those heart muscle stem cells come from the blood vessels that grown into the heart muscle? Do those cells come from an immature precursor of the heart that remain undifferentiated even weeks and months later in the adult? Do they come from the lining of the heart, either from the outside or the inside? Both of which have stem cells of different kinds. We use genetically tagged Mice in ways that allows us to study the origins of our cells.

In other projects in my lab related to the genes that contribute to heart muscle cell death during a heart attack or other injury, we use Mice because in Mice we can delete a gene for a specific protein with almost surgical precision; just in heart muscle, just in the adult, allowing the rest of the animal to develop altogether normally, allowing the heart muscle to develop altogether normally until adulthood. To lead a gene at a predetermined time come back a week later, give the animal a heart

attack and see if removing that gene confers the predicted protection from injury that our other studies in cell culture would lead us to predict. That's one of the most conclusive tests now available and that would fuel interest in that target as for potential work along the lines of drug discovery to find a clinically applicable remedy that would suppress that gene or protein.

A-That's fascinating. Are there any examples of better treatment starting to emerge from this manipulation of stem cells?

B-I would say that one that is directly relevant to your question involves a molecule called Thymosin Beta 4. It's studied here in the U.K by Paul Riley (who is moving from UCL to Oxford) and in the States by Deepak Srivastava in the Gladstone Institute in San Francisco. This was a molecule that was known for many years to be a potential agent of use in the context of wound healing. What Deepak went on to do was to show that Thymosin Beta 4 is expressed at high levels in the embryonic heart of Mice while the heart is being formed. On the basis of that tested whether Thymosin Beta 4 might contribute to better wound healing after heart attacks, and it did. What Paul has shown this past year is that one of the ways in which Thymosin Beta 4 works is by activating the dormant stem cells in the outer lining of the heart, the epicardial stem cells, and directs them to turn into heart muscle after heart attack if Thymosin Beta 4 is given to Mice. So on the basis of the earlier studies by Deepak clinical trials are under way and on the basis of the more recent work by Paul I think the interest in Thymosin Beta 4 will increase further.

A-Is it possible for stem cells to be produced or manufactured artificially?

B- Well if you mean, is it's possible to start with something that ... The best example of what someone might think of a stem cell made artificially is what investigators in the jargon of the trade refer to as induced pluripotent stem cells or IPS cells. These start with normal cells from an adult, it could be skin fibre blasts from a skin biopsy, it could be cells taken from a blood sample, it could be cells taken from other sources. Using cocktail of stemness transcription factors which investigators learned about through many years of research on embryonic stem cells, putting the genes that are essential for embryonic stem cells to behave the way they do into a skin fibre blast, or into a blood cell, turns them into something that for most intents and purposes is embryonic stem cell like.

There are some subtle differences, there are open questions as to which kind of stem cell will be the better stem cell in different diseases or clinical situations. It remains true non-the-less, despite that limitation that embryonic stem cells and IPS cells, starting with the skin biopsy can be converted into beating heart muscle in the laboratory.

Starting with a patient's skin biopsy or blood sample becomes a way to make patient specific cardiac muscle from stem cells which in turn can be used either as a cell product that would overcome the transplantation barrier for grafting, or alternatively, has already been shown by many labs around the world to be useful models to study the exact mechanisms where a given mutation leads to a cardiac muscle disease. So from studies of human genetics we might know which gene is the cause but we don't know exactly the details of how that defect in protein leads to the disorder in the heart muscle. So having human heart muscle in the dish bearing a series of mutations for heart failure, for cardiac rhythm disorders is proving to be an immensely important tool in lots of centres.

A-So this is also offering the prospect of better treatment as well?

B-Yes

A-That's good. Tell me, what has been your notable learning in your own lab recently, the latest thing you are seeing in your research.

B-I'm going to have to think a bit before answering that. My spontaneous answer would be that I wouldn't want to go out for public consumption because it hasn't been published yet. While I'm happy to present it in abstract form at academic meetings it's not something that I would want to go out. That's a really good question. Let me take a stab at it.

One of the things that we have learned over the course of the past year is that..... That's not good 'either that would be too discouraging a message ...

One way to answer your question is to say that research is a little bit like rock climbing. You solve a problem and that tells you what the next problem is and you advance one problem at a time. One of the things that we are very excited about right now are studies using human IPS derived Cardiomyocytes to give us access to human heart muscle in a dish for pathway dissection and ultimately drug discovery.

Over the years my lab has found a number of enzymes called protein Kinases that are activated by cell stress in diseased human hearts, in mouse models of heart disease, in heart muscle cells grown in tissue culture and subjected to different death signals. In spite of all the evidence we have for several of those, the critical missing piece of information is that even though we know that it goes up in diseased human hearts we don't know what the function is in a human context. We can block the gene's expression or function in Rat cardiac muscle cells and see superb protection from death signals, but if we were going to take that forward to patients we would want to know that the gene has a function in a human cardiac muscle cell itself.

One of the projects that we are most excited about is taking human IPS cells derived Cardiomyocytes as a way to have human heart muscle in the dish, in the lab, subjected to different conditions that lead to the cell's death in ways that mimic heart attacks and then test whether our specific candidates indeed have an essential role in human heart muscle the way it seems to in the rodent cells. This would provide a strong impetus to focus on those genes and those enzymes for potential drugs that would inhibit their activity and confer protection to the cell. Beyond those 2 or 3 candidates that we are studying first, those kinds of experiments are amenable these days to higher and higher through-put using the kinds of robotics that we have here in the lab, ultimately knocking out more than 500 protein Kinases in the human heart muscle cells and testing their function in cell death and other aspects of heart disease.

My answer at timescales will surprise you.

A-Oh, will it? I would have thought a long timescale.

B- All of the work at my lab one way or another concern the problem of heart muscle cell number. Heart muscles die, the regenerative growth that occurs is not sufficient to replace them. If one were an Economist or working in the city of London , one could think about that from the supply side, lets make more, lets give them to the patient and part our work concerns that. The other aspect of the work is the demand side. What specific genes and proteins couple cell stress to cell death during a heart attack and how might we block or preserve jeopardised heart muscle cells. The experiment that we are doing is built around several stress activated enzymes called; MAP4 K 4, MAP3 K 7 and a few other Kinases besides that we found are activated in diseased human tissues, in mouse models

of cardiac injury, cardiac muscle cells subjected to stress. If we mimic the increase in activity in genetically engineered Mice or heart muscle cells, it sensitises those muscle cells to death or kills them outright. If we block the activity in cultured Rat cardiac muscle cells it protects them, making it sound like a useful route to preserve jeopardised heart muscle in patients having a heart attack.

At the moment the single most important thing we don't know is whether these proteins have an essential function in a human heart muscle cell. We use human stem cells to make human heart muscles and to provide access to human heart muscle to solve that problem.

A- As a patient, I hear about the business end of it in the NHS. How do you communicate the work you are doing in research to the medical doctors and then test it out in the community to see whether it actually works as you would expect it to do?

B-That is a terrific question. It's one part of the work that I think it's actually easier to do here in the UK than in most other countries. The research arm of the NHS, (National Institute for Health Research) has funded a series of biomedical centres and biomedical research units across the U.K and I'm in all of those. NHS Trust is affiliated with Imperial College including Brompton, Herefield and Hammersmith. We meet regularly with the clinical leads in those areas to assess needs and build a joined up strategy.

A-Two questions on timescales: From the research that is going on, what in the relatively short term do you see emerging as new treatment? Looking further ahead to maybe when my sons are of an age when heart treatment maybe an issue, where do you see the research taking us?

B-The answer to what cell therapy or what product is best for heart repair is really going to depend on which time horizon you are talking about. This sounds like science fiction or something that's over the horizon to many people, but the important thing to emphasise is that there are more than 1000 patients who have been treated by stem cells of different kinds for heart disease already.

Typically bone marrow cells that may not be as effective as others in terms of their ability to turn into new beating heart muscle cells. Bone marrow may benefit the heart in other ways including contributions to blood vessel formation and wound healing. For cells with proven ability to turn into heart muscle effectively, mainly the dormant stem cells from adult hearts; there are already 4 clinical trials going on world-wide 2 in the U.S, 2 in Japan chiefly for adults who had heart attacks and heart failure. One of the trials in Japan is to rebuild the heart in a serious form of congenital heart disease.

So when you ask about your son and your son's offspring in the future, I think the direction of travel will be from the current from trials which are simply injections of naked cells, to injection of cells that have more of a tissue engineering component; with gels, scaffolds, cells as sheet, cells as prosthesis, cells that ultimately have been engineered to have blood vessels along with them and get oxygen and nutrients. There are some investigators who are already seeking to build a three dimensional heart by creating a fibrous scaffold and seeding the fibrous scaffold with stem cells where upon they take up residence and ultimately start to beat after a couple of days. So, if you ask what does the future hold, I think the direction of travel will be from naked suspension of stems to stem cells that have been activated in the lab, to treatments that have increasing degrees of a three dimensional engineered component, and ultimately perhaps prosthetic hearts themselves created from engineered scaffolds and seeded with stem cells.

A-That is an incredibly optimistic note on which to end. It has been absolutely fascinating, the future is clearly well beyond the average persons' expectation and understanding. It has been wonderful to learn what you are doing and I do appreciate you just lifting the lid a little on all the exciting research that is going on. Thank you very much indeed Michael.

A-There has been some stalling in the development of drugs by the big pharmaceutical companies. Does this research offer a new horizon for those companies in the development of fascinating new drugs?

B-Almost anybody, either from industry or academia who talks about current prospects for drugs discovery uses the same introductory slide. The amount of money being spent in industry in drug discovery is increasing and the number of new compounds being taken forward into human trials is falling. One reason for that is that drug companies don't always know what the emerging targets are as potential points to intercept in ways to preserve, protect and defend the jeopardised cardiac muscles in the setting of a heart attack. Conversely, academic labs in the past have not had the right tool kit to produce the kind of evidence that a company like GSK, AstraZenca or any of the leaders would respond to. The ability now to have human heart muscle in the laboratory as an endlessly renewable resource, creating the human heart muscle from human embryonic stem cells, from human IPS cells becomes a way for academic labs and industry too, to have unprecedented access to human heart muscles whether it's for toxicology and safety screening in industry or for target or drug discovery in both settings?

A- Does that mean that the use of animals in toxicology is being reduced?

B-There was a Royal Society meeting on the subject of, is the mouse a useful organism for biomedical discovery and the answer in the case of Toxicology, part of it become a catch-22 situation. Everything is judged by the standard that every other thing is judged. I would be glad to affirm that conceivably the use of such model might reduce the need for animals in the kinds of Toxicology. Incidentally, the increasing use of human stem cell derivatives, whether is heart muscle, neurons or other clinical relevant cells may increasingly become fruitful as the test bed for toxicology screening and drug safety screening and hopefully would reduce some of the need for animals at least in that kind of research.