Ulster Medical Society

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The Robert Campbell Oration

Advanced Therapeutics for the Acute Respiratory Distress Syndrome

Professor Cecilia O'Kane

Professor Mary Frances McMullin:

Good evening everyone, and welcome to what is going to be the Robert Campbell oration tonight. Robert Campbell was born in 1866, in Templepatrick, County Antrim. He eventually trained as a surgeon, become a demonstrator in anatomy, and then got his LRCP and his FRCS in England. He came back in 1897, as honorary surgeon to the Royal Belfast Hospital for Sick Children, and he worked there for the rest of his days.

He joined the Ulster Medical Society in 1897, and he was Vice President, and then President, in 1916–17. A lot of information about his work is very interesting. He was basically a practical surgeon, a paediatric surgeon, and he eventually, one of the most interesting things I've found out was that he had large amounts of discussion in the outpatients' department of Queen's Street Children's Hospital, to be allowed to perform operations on children in an outpatient capacity. He also wrote a little and taught, but he was well thought of as a teacher.

He eventually died of Bright's disease before the age of 60, and it was quite interesting then, because a fund was established by his friends, and they wanted to have something in the fund to be used for something with recurring practical utility, and they ended up setting up the Robert Campbell memorial prize, which was to be given for distinguished work in any branch of medical service to the members of the profession in Ulster. It's quite interesting, as the descriptions go on, as to who this person should be. They're always medical men residing in Ulster.

So eventually the terms were to perpetuate the memory of the said Robert Campbell, and to advance the cause of medical and surgical science. So that is the Robert Campbell oration. I have asked, this year, Professor Cecilia O'Kane to give this oration, so Cecilia, whom I've known for many years, qualified in 1997. I think she won most of the prizes in her year, as well as an honours degree. She was a houseman in the Mater, of course, like many of us were; went on to train in medicine and respiratory medicine, and then undertook a PhD in the Hammersmith Hospital in London, before coming back here as a senior lecturer. Since that time, she's built up her research group, has extensive grant funding, well over 100 publications, and is now a full professor within the University, and it is my great pleasure therefore to ask her to give us a talk tonight on *Advanced Therapeutics for the Acute Respiratory Distress Syndrome*, trying to keep the theme of *Looking to the Future*, so Cecilia, thank you very much.

Professor Cecilia O'Kane:

Thank you very much, Mary Frances, and thank you for the honour of asking me to talk tonight. When I was looking through the Ulster Medical Society archives, I was a bit discomfited to see that this talk was once given by Alexander Fleming on penicillin, and my nine-year-old really summarized it fairly succinctly when he said, "Oh, no pressure mum, then!" He said, "What do you have in common with Alexander Fleming?" Well, I really wanted something inspirational, but the best I could come up with was, "I've prescribed penicillin!"

Again, looking through the Ulster Medical Society archives, one of the things I learned about Robert Campbell was that when he gave a lecture, he said he didn't like to do what was in the standard textbooks, and talk about that, but what he really liked to talk about were the difficult problems and the unanswered questions, and what I'm going to present tonight is probably not a neat body of work with all the questions answered, but really a body of work that's been trying to address some of the hard questions in ARDS, and a summary maybe of some of the problems that lie ahead and the solutions that we have to try and reach.

So the ARDS condition that we all come across in our critically unwell patients, it's not common at ward level, but very common when patients become unwell, and they end up in ICU, so about one in five patients who are on a mechanical ventilator will fulfil criteria for ARDS, and globally that accounts for about 7% of ICU admissions. Again, an under-recognised disease, because when you talk to the public about ARDS, most of them have never heard of it, unless they've had a patient in intensive care, but more people die of this than people with asthma, breast cancer and HIV in any one year, so it's a huge cause of mortality, and we have 4,000 to 5,000 deaths per year within the UK and Ireland from this condition.

It presents as gross pulmonary or respiratory failure with bilateral pulmonary infiltrates, and the definition of this, initially described in ICU in the late 1960s, as a clinical syndrome that was really characterized then by pathological follow-up of the patients who'd died, but clearly waiting for pathology to make the diagnosis is not good, and the diagnosis is, as a clinical syndrome, of bilateral infiltrates on your chest x-ray or CT scan, pulmonary oedema that is not entirely explained or completely explained by cardiac failure or fluid overload, and impaired oxygenation, and it's graded into three groups of mild, moderate or severe disease, according to the degree of oxygen impairment, and those gradings reflect the increased mortality as the oxygenation is worse. And it happens in response to a variety of insults, so it can either be direct injury to the lung, and the commonest reason for that would be pneumonia, but also very topical at this time of year, is a not infrequent

complication of flu in the critically unwell; aspiration of either gastric contents or other inhalants is a potential cause of it, and then the less common causes would be things like pulmonary contusion or re-expansion after lung surgery, or potentially after actually, re-expansion after pneumothorax or pleural fluid drainage.

Indirect lung injury accounts for a significant proportion of our patients with ARDS, so patients who develop systemic sepsis often have their condition complicated by the development of ARDS, and trauma is a non-infrequent cause of this condition also.

During ARDS, there are a number of different pathological processes at play, so the initial pathological definition or description of it really talked about the loss of the alveolar epithelium, and the replacement of the alveolar epithelium with these so-called hyaline membranes, and obviously if you've lost your alveolar epithelium, you lose both your surfactant production, so you get alveolar collapse, but you lose the ability to clear fluid from your lungs, and as the lungs fill with fluid, you develop respiratory failure for gas exchange.

As well as the alveolar injury, there is also a characteristic endothelial injury, and often that's the initial insult or the initial driving factor within the lung, a systemic or indirect lung injury, so the leak from the alveolar endothelium, and that then is complicated by the recruitment of a neutrophil population to the alveolar space, and further release of proteases and damaging cytokines or chemokines.

In order to recover from ARDS, you need to really grow your epithelium, and I'll talk about that in particular, because it will come back to the function of trying to repopulate the alveolar epithelium later on. So you need to clear fluid, you need to reduce the inflammatory infiltrate, and you need to recover your alveolar epithelium and lose the leak from your endothelium.

There are essentially three major processes, when I'm talking to the medical students, or to my colleagues in science about the pathological processes that are happening in our patients. It's essentially inflammation, epithelial denudation and endothelial disruption, and either causative infection, or often superimposed infection, because these patients end up on a ventilator, a piece of plastic communicating directly from their upper airway down into their lungs, which allows the passage of micro-organisms readily into the lungs.

Any treatment that we think about to try and help recovery from ARDS needs to try and address all those three mechanisms, and you can see why that could be quite complex, because anything that suppresses inflammation predisposes us potentially to infection, and when we stop inflammatory pathways, we often stop the pathways that initiate the repair process, so we're dealing with a number of different processes at one time.

Because respiratory medicine and intensive care really know how to celebrate, when it was the 50th year anniversary of the first description of ARDS in 2017, the Lancet respiratory medicine published a celebratory edition, and they devoted that edition of their journal to ARDS and to subsequent developments of it, and revisited the initial history of it. This is the front cover of it, and I like this slide because it really does reflect the poor advances we've actually made in it. When it was initially described, the doctors who had been looking after the patients that were ventilated, and who they described the ARDS in, they talked about using a mixture of ventilator strategies and various fluid regimes with limited success, and the treatments that we have for ARDS today are still essentially those two, so protective ventilation for the lung, and managing fluid carefully. In terms of therapeutic advances for ARDS, we've actually had very few, so no pharmacological therapy that changes the outcome from this disease.

In contrast, there are a myriad of studies of animals with models of ARDS, where we have successfully cured the animals and achieved great results. I think ARDS is one of those great diseases for actually really reflecting the limited value of animal models in some conditions. Again, when I'm talking to our students, I always refer to this paper, because I really like it, and it was a paper back in 2006, where they carried out a review from the previous about ten years of papers that had been published in really the very top scientific journals of the time, so Science, Nature, Cell, Nature Medicine and Nature Genetics and so on, and they looked for articles where a therapeutic had been tested in an animal model of disease, and where the article had been cited more than 500 times. Now remember, we're looking back at the pre-2006 era, so for a large part of that time there wouldn't have been electronic journal access, and so citations were from the old books, and citation at that stage for any one paper was actually a lot less than it might be now, so these were really incredibly highly cited papers, and they looked then for the studies that had shown success in animals, and then looked to see whether these studies had been replicated in a human trial. In only 37% of these really top therapeutic interventions in animal studies, were they able to see that some replication of the effect had been shown in a clinical trial. In 18%, the opposite findings had been found in clinical trial, and the remainder were either untested or unpublished, and so again just really citing that animals are important in medical research, but have limited value.

Why should it be that there is such a divergence? Again, I like to reflect on this, and so when we do, I don't do animal research, but when my colleagues do, we're using mice usually, who genetically are very similar, and the animals are generally the same age, usually the same gender in any one experiment; they're bred in a pathogen-free environment, and they get the same diet for all their life, so all their epigenetic insults and the nature versus nurture issues, those are all tightly controlled.

When you model disease in animals, the animals get an identical insult in terms of its nature, its size and the timing of it, and the animals usually don't

have any co-morbidity, and they're rarely on a whole big list of other medications like our patients. When you look at animal studies, and see the variability within an animal, in terms of response to treatments, I often wonder how we ever show a positive effect in a human study, because these are really so difficult to control in patients. Of course, apart from the logistic experimental set up and controls around it, animals are not human, and for the conditions like ARDS, there are a number of key molecules that are important, or that we know are important in regulating the immune responses or inflammatory responses in ARDS, and they're missing in mice. So for example, IR8 is a critical chemokine for regulating neutrophil influx to the lung in ARDS, and is prognostically important, was actually absent in the mice, and there are profound species variations in response to insults.

In a paper, in 2013, in PNAS, really explored this, and they looked at a number of different models, and I'm just going to give an example of lipopolysaccharide in this case, and LPS is a bacterial antigen that's seen in Gram-negative organisms, and is a common model to use for systemic sepsis, or for ARDS, for example. When they did, they injected healthy volunteers with a low dose LPS, and gave an equivalent dose of LPS to mice, per body weight, and when they looked at the genomic responses in the blood from mice and from humans, there was minimal correlation, and in fact what they said, among genes, changed significantly in humans, the murine orthologs are close to random in terms of matching their human counterparts. I think that makes us need to think very carefully about how we pre-clinically evaluate anything for ARDS.

So can we improve pre-clinical models?—and this has been a big focus of our research group for the last ten years, and there are a couple of models that I want to talk to you about tonight, and we're really very keen to try and model in humans before we go onto clinical trial testing, and you can do that with isolated cells, and we do do that, but isolated cells are quite limited in what they can actually, the information they can actually give, or the complexity of disease that they can actually model, and one of the models that we've set up here in the past is an LPS challenge model.

So you can use these to stimulate a low-grade inflammatory response. You can sample the plasma compartment, you can sample the lung compartment and you can sample the urine, to try and measure biochemical and biomarker responses to injury.

Usually in this model, how we set it up, is we inhale LPS, or we get a volunteer to inhale LPS in the morning, and six hours later we will sample the pulmonary compartment by performing bronchoscopy and lavage, and then 24 hours later, we sample plasma, and we've been able to show a very reproducible low-grade inflammation and injury response to that, and if it all does sound a bit unethical, the amount of glycopolysaccharide that we're asking them to inhale is equivalent to the amount of glycopolysaccharides that contaminates five standard cigarettes, so it's actually quite a low dose model.

This is some of our research group, and just as a disclaimer, I and the other PIs in the group have all been victims of this model as well, and volunteered for it, so I can reassure you that it's reasonably comfortable This is one of our post docs, and she's had her LPS inhalation. You can see she's pretty bright and happy, and [Kumar?], who is one of the cardiothoracic surgical fellows, is very happy because he didn't inhale LPS, and he's getting to do Meghan's bronchoscopy.

This model induces inflammatory cytokine production within the lung compartment, so we can see up-regulation of cytokines and chemokines that we know are pathogenic in ARDS, and prognostically important in ARDS, such as TNF alpha, IL1β, and IL8 among others. It also drives neutrophil recruitment to the alveolar space, and causes some protease activity to be up-regulated within the alveolar space, and you can also measure markers of both alveolar and epithelial and endothelial injury in it, and it is self-limiting. We occasionally get people with a mild pyrexia after, but that's probably actually more due to the bronchoscopy and lavage rather than the LPS itself.

The value of this model for me is that I think this is proof of concept that the intervention, or testing might actually work on a whole human being, and it also allows us to predict the effect size in terms of how much am I dampening an inflammatory response. We also use it sometimes to give us an idea of pharmacokinetics and pharmacodynamic, and data in this inflamed model, and the samples that we get from this are really incredibly valuable, in terms of trying to understand the mechanistic events underlying inflammatory processes in the lung. It is obviously a limited severity of an insult. You can't give live bacteria, and you can't make people particularly sick, and the response is really biological rather than physiological, because clearly we don't want to make people unwell with it, and there is limited sampling in terms of, we can get blood, urine and airway and alveolar space sampling. It's a bit difficult to go back with repeated sampling or repeated injury, although we have on a couple of occasions done a repeat bronchoscopy several weeks apart, but you can't really ask someone to have a bronchoscopy three or four times in one day.

To complement that, we have also set up another model, and this is what we call the *ex vivo* lung, and this technique was developed in Canada to try and optimize or improve lungs that have been offered for donation but weren't suitable for transplant, to try and wash out cytokines and inflammatory responses, and make these reconditioned lungs suitable for transplant. In this model, we have human lungs that have been offered by brain-dead donors on ICU, who have consented to organ donation, and whose family have agreed that if the organ isn't suitable for donation, then that it can be used in research. I think that's a really important thing to acknowledge, it's such a valuable set of samples and such an incredible, generous gift from the donors' families at this time,

and I think we always take this very seriously, that when we get human lungs, we do want to use them to the maximum effect, and really get good information back out of them, because they are so precious.

In the human lung model, we can ventilate the lungs, as I've just shown you, and we can perfuse it with blood, and we have set up a number of different models of injury with either bacterial antigens, bacteria or other mechanical stimuli, and we then intervene in some way, so try to have preventative or a therapeutic manoeuvre to see if we can reduce the inflammatory risks run here.

The first model, or the most common model that we have used here, is again to use a lipopolysaccharide injury, and we can ventilate the lung for about four hours, and after that we still have fairly intact fluid clearance within the lung, and a very intact pulmonary physiology. Obviously it is a time-limited experiment, because the lung won't survive for prolonged periods *ex vivo*.

When we injure the lung with LPS, we find, as we do, and we expect that we get neutrophil infiltrate and to the lung, we get histological evidence of ARDS. We see that the lung does not clear fluid from the alveolar space in response to LPS, and then we get the inflammatory infiltrate, or the inflammatory cytokine up-regulation similar to that, in the healthy volunteer LPS inhalation model, and also similar to the profile of cytokines that we see in the large compartment from patients with ARDS.

Its advantage over the human inhalation model with our healthy volunteers is that in this case we can give a more severe injury and induce pulmonary oedema, which is part of the pathophysiology of ARDS, and we're able to see changes in markers of permeability, which we won't see readily in the more mild limited inhalation model.

Because it is an *ex vivo* lung, we can use more aggressive stimuli, such as live bacterial infection, and the model does allow us to measure physiological parameters around oxygenation compliance and pulmonary artery pressures, so again this is a whole lung tissue environment, and then we're able to sample histologically, which clearly we can't do in our healthy volunteer model. Again, like the healthy volunteer model, this gives us proof of concept that the intervention that we're testing can have an effect on human tissue.

But it does have limitations, of course, and so it's an isolated, perfused lung, and importantly you don't have a bone marrow, you don't have lymph nodes and those sort of factors or an immune response, are not easily addressed in this model. There is no liver or kidney to process any drugs that you might have added. It's short-lived, and because the lungs are not suitable for transplant, by definition if they're not suitable for transplant, it's because they were impaired at baseline, so we're not looking at a very clean model at baseline, because clearly if the lungs were pristine, they would have been used for transplant.

But like our patients, and this is an advantage but a disadvantage, it's a noisy system, so the lungs are different ages, the patients may have smoked, they're mixed gender, potentially different races, and the patients who have donated these lungs may have had co-morbidity and have all been on a number of medications, so in some ways more real life model than many of our experimental models in the lab.

One of the other issues is that, depending on where the lung has come from, there is a variable cold-ischaemic time, and that does affect the viability of the tissue that we receive.

So how do we use these models?—and I'm going to talk about a couple of situations where we've used these for testing therapeutic interventions, and the first one is the story of KGF, or keratinocyte growth factor, and KGF is a growth factor that we make normally by our fibroblasts in an attempt to support epithelial growth. KGF is used as a licensed medication for the treatment of oral mucositis in patients who've had chemotherapy or radiotherapy, so it's already an existing medication, and in mucositis it obviously aids the recovery of the buccal mucosa and the GI mucosa after radiotherapy or chemo.

In the late 1990s and early 2000s, there was a huge amount of interest in KGF as a potential intervention for ARDS, and extensive and experimental work being done on this, in [?] and in isolated cell systems, and I'll just put up a few of the experiments that have been published. There are hundreds of papers in this field from this time. Things that have been shown were where mice were infected with bacteria and treated with KGF. Mice that were treated with KGF cleared the bacteria faster, and this was dependent on an increase in GM-CSF production by the mice that had been treated with KGF, and GM-CSF enhances the ability of macrophages to take up bacteria and clear them.

In a caecal ligation and puncture model (so essentially the caecum is tied off and punctured to allow intra-abdominal sepsis), then in this model, in this case it was followed by acid aspirations, I guess mimicking the situation of an older person in hospital who has bowel sepsis or a perforated bowel, who may develop a superimposed pneumonia, aspiration pneumonia, or hospital-acquired pneumonia, and in this model, and once they were treated with KGF, they had reduced neutrophilic inflammation in their lungs, and increased clearance of bacteria again, and this was mediated by a reduction in a number of different chemokines.

Finally, just as an example, in sterile models of lung injury, when the lungs were injured by bad ventilation and treated with KGF, KGF reduced the amount of pulmonary oedema in those injured lungs, and in these bottom photographs, you can see the proliferation of the type II alveolar epithelial cells, so KGF was inducing the growth of the type II cell to allow it to repopulate injured alveoli. So that all looks really incredibly interesting, but again all animal work. So we used it in our healthy volunteer model of ARDS, so we randomized healthy volunteers, to either a placebo or Kepivance—palifermin is the generic name of Kepivance, the trade name, and the healthy

volunteers undertook LPS inhalation after three days of KGF, and we looked at various different markers of inflammation in the lung and various different markers of alveolar epithelial function, and we found that, when we treated the healthy volunteers, pre-treated them with Palifermin or KGF, that they had higher levels of surfactant protein D, and that suggests that they had more well-functioning type II alveolar epithelial cells. When we took that fluid out and added it to alveolar epithelial cells in the lab, the alveolar epithelial cells grew faster as we would expect, so it was an environment that was promoting epithelial expansion. Like the animal models, the treatment with KGF was associated with higher concentrations of GM-CSF within the pulmonary compartment, and that was functional in that there was GM-CSF mediated an increased uptake of either dead epithelial cells or bacteria by macrophages, when they were stimulated with the lavage fluid from these patients, so essentially we were showing very similar data to the animals, that is promoting GM-CSF dependent good macrophage function, and supporting alveolar epithelial proliferation to allow repair of an injured alveolus.

Around the same time, some of our colleagues in the US were looking at KGF in the *ex vivo* lung model, and similarly found that KGF improved alveolar epithelial function in an LPS injured model there, and was associated with reduced inflammation. So great, we had this very compelling data that what we were seeing in the animal models was really very similar to what we were seeing in the human models, and we progressed to a clinical trial in patients with ARDS.

We treated 60 patients and 29 were allocated to KGF and 21 to placebo. Our primary outcome from this trial was to look at their degree of oxygenation in response to this treatment, and the actual marker of oxygenation, called oxygenation index, which is a measure of both the degree of impaired oxygenation, and the amount of ventilation that's applied, but the take-home message is that the lower your oxygenation index, the better.

So when we looked at our primary outcome, which was day seven oxygenation index, to our surprise, and I think great disappointment, we found that actually no improvement in oxygenation index, in the KGF treated group, in fact it was higher at day seven in the KGF group than the placebo group, and do you remember I said that the lower oxygenation index is better.

We looked at a number of other markers of relevance or clinical outcomes of importance to our ARDS patients in terms of degree or duration of ventilation and duration of ICU stay, and hospital and ICU mortality, and the signal was opposite to where it should have been, and we were underpowered for all of these outcomes, but there was certainly no evidence of benefit from KGF. So the animal models, the human models, very disappointing in terms of their predictive value for what would happen in our patients.

Aspirin is another drug that we've looked at, again very strong data that even low doses of aspirin are beneficial in animal models of ARDS, and when we have tested that in our human models in our healthy volunteer study, we pre-treated for seven days with aspirin, and then undertook a bronchoscopy and blood sampling, so we randomized patients who are healthy volunteers who fulfil the following criteria, so essentially nothing very exciting. If you've got a history of aspirin intolerance or peptic ulceration, or low platelet count, you weren't included, but they were generally very easily fulfilled by our healthy volunteers. We randomized 21 to aspirin and 14 to placebo, and one patient withdrew, and one patient was excluded because he subsequently developed an upper respiratory tract infection and we didn't want to do the lavage when he was unwell.

We similarly looked at aspirin in the EVLP model, where we instilled LPS and added a higher dose of aspirin to the EVLP model, and in both models we found that aspirin was associated with reduced neutrophilic influx to the lungs, with encouragingly very similar results across the board. In the lung, the *ex vivo* lung, we found that aspirin was associated with significantly reduced histological damage, and in both the human volunteer study and the EVLP study, we found that aspirin was associated with a reduction in cytokines in the alveolar space, so again very encouraging data that low-dose aspirin would be a potential intervention for ARDS.

So, we are probably the masters of naff acronyms in our group, so STAR is a reasonably contrived acronym for our clinical study in patients with ARDS. STAR has just been completed, and the analysis of that is just in its final phases. It's not published yet, so I don't have a slide with the results, but the STAR result is, there is no evidence of any benefit in our patients with ARDS, so again, incredibly disappointing.

One final story, and then we'll go onto some good news—this is simvastatin, and statins, as many of you will be aware, really about 10 or 15 years ago, there was a huge interest in statins across a wide range of diseases for their potential immuno-modulatory and anti-inflammatory effects, and again, like the story with KGF or with aspirin, really very compelling animal data that simvastatin or other statins have beneficial effects in animal models with both sepsis and ARDS. And so, for example, in this one, animals treated with [simvastatin] had reduced amounts of neutrophils in the alveolar space, the interstitium, and reduced mobilization of neutrophils from the bone marrow; reduced markers of permeability, reduced markers of elastase, which is a protease that damages lung tissue, and again very encouraging. Similarly, reduced numbers in a different experiment, looking at markers of neutrophil activation, and reduced evidence of histological damage in response to statins. So we used simvastatin again in our healthy volunteer model, we pre-treated for three days with high dose simvastatin, 80 mg. On day four we gave them a further dose under supervision so we knew at least everybody had had one definite dose of simvastatin, and when we assayed for simvastatin in all of

our volunteers, those who had been randomized to it, had actually taken it. We challenged with LPS and undertook our own plasma sampling as before, and in these figures, the white is the placebo one, the grey colours are the simvastatin treated groups. Again, we found reduced markers of neutrophil activation, reduced levels of myeloperoxidase, reduced inflammatory cytokines, reduced evidence of neutrophil protease activity, MMP-8 is a neutrophil specific protease, and reduced systemic inflammation reflected by a reduction in CRP. And we did a number of mechanistic studies looking at this, and there was clear evidence in a number of follow-up studies, that we had reduced NF-κB activation in our pulmonary space, and then particularly in the alveolar macrophages in the persons who were randomized to simvastatin, and the NF-κB is sort of a master regulator of many inflammatory cytokines, so it is a transcription factor that binds promoter and aids the production of inflammatory cytokines including IL8 and many of the proteases that we discussed.

So, the next study [HARP], in our cohort, and, in fact, one of our earlier studies in the cohort, was to undertake a small phase 2 study of simvastatin in patients with ARDS, and so this was a safety study primarily looking for evidence of efficacies. We randomized 60 patients, half to simvastatin, 80 mg, and half to the placebo. And the simvastatin was well tolerated in the critically ill group, and there was a range of markers suggesting benefits, so the suggestion that people's oxygenation index was getting better in the simvastatin treated group at an earlier point in time, that they had less stiff lungs to reduce plateau pressure, reduced systemic organ dysfunction, reduced lung injury scores in the simvastatin treated group, and some reduction in the inflammatory cytokines that we know are important in ARDS, and again a greater reduction in CRP in the simvastatin treated group than the placebo treated group.

So that phase 2 study was quite encouraging, and of course phase 2 studies like this are not practice-changing, nor should they be, but they are the next step to the larger study, and so HARP 2 was born, and HARP 2 was a 540-patient study of patients with ARDS across multiple sites within the UK, and again randomized to either simvastatin, or a placebo. And we looked at a range of clinical outcomes in our HARP 2 study, and our primary outcome was around duration of ventilation. It was negative for that from a statistical point of view. There was a suggestion, when you look at the Kaplan–Meier curves, that people who are treated with simvastatin tended to come off the ventilator slightly earlier, but that did not reach statistical significance. Again, in the survival data, at 28 days, a suggestion that, in the simvastatin treated group, there was a trend towards an improved survival, but again not significant. This is reported as a negative study, and that's how it should be reported.

Interestingly, and I'm not a health economist, but there was a health economic analysis as part of this study, and even though the primary and the secondary outcomes that were measured within the clinical study were all non-significantly altered, when you looked at follow up a year after their admission to ICU, there was a health economic benefit in the patients who were treated with simvastatin. And it was always hard to explain, because we weren't able to show a clinical benefit in the ICU patients at that time, but again, just that signal that simvastatin was maybe beneficial in that group to some extent, but wasn't proven by that trial.

So why, when we have what we think are good models, are the trials not showing a difference? There are lots of potential reasons for that, one that the drug actually genuinely doesn't work in ARDS, but increasingly, in the field of ARDS, we're starting to ask the question about the patient group that we have. It's a syndromic definition, and this is a paradigm that we are all familiar with in our own clinical practice, so even when a drug has been shown to work in a clinical trial, when we actually give it to our patients, afterwards we have had very low responses, so the best outcome for many of our licensed drugs is that the drugs appear to improve outcome for our patients, or work, and are not toxic. We do have people who appear to derive some benefit, but then develop a side-effect that limits its use. We have people who don't appear to have any benefit from the drug, and don't have side-effects, and people who don't have any benefit and who do have side-effects.

Ultimately, there are people, even when a drug, so when a drug is being shown very clearly in a clinical trial to have an effect on that disease, so people do have varying responses to that, and there has been a big question over the last number of years about the patients that we are recruiting in our ARDS studies—are they all the same? We have a colleague in the States, in San Francisco, Carolyn Calfee. She's a good friend, who has really been leading the field in actually trying to look at this, and she looked at two retrospective cohorts of patients who'd been in clinical trials for various different ventilatory strategies in ARDS, and undertook what's called a latent class analysis. I'm not a statistician, and I hope nobody here is, because I can't tell you how to do a latent class analysis, but what it is meant to do, or what it does do, is help you find hidden subgroups within a population, and latent class analysis was undertaken in both these groups of patients, looking at a variety of clinical parameters and biomarkers from the blood collected during these large studies, and she identified in both of these studies two groups, one that was called hypo-inflammatory, or non-hyper-inflammatory might be a better description, and patients who had really very profound hyper-inflammatory response, and they have very different outcomes in terms of their trajectory, and so the hyper-inflamed groups were much more likely to die. They were more likely to die, they were more likely to have a longer degree of ventilation, and have other organ dysfunction.

So when Carolyn had shown this in these first two studies, we asked her to look at, could she reproduce this latent class analysis in our ARDS cohort, in our

HARP 2 study. She did do this and was able to show again the presence of both hyper-inflamed and non-hyper-inflamed subtypes in our HARP 2 study, our statins study of 540 patients, she was able to show a marked difference in their duration of ventilation and their survival.

When we looked again at our HARP 2 data, our 540-patient data, and stratified the patients according to whether they were in the hyper-inflamed or hypo-inflamed group, and looked at their responses to simvastatin, there were interesting findings. So essentially our non-hyper-inflamed group didn't appear to derive particular benefit from simvastatin, but our hyper-inflamed group did, so the duration of ventilation in our hyper-inflamed group who received simvastatin was less than in those who received the placebo, and likewise there is a suggestion that people who were in the hyper-inflamed group who received simvastatin were more likely to survive. So is there a group of people who response to simvastatin better?—but there seemed to be no particular benefit to simvastatin in the non-hyper-inflamed group.

But there are limitations to this, so it's a post hoc analysis, and although this has been shown now in a number of different cohorts of ARDS patients, it has never been shown prospectively. A full latent class analysis depends on a wide range of biomarkers and clinical data, and can only be done retrospectively, so very interesting for us to look back at, but how would it be practically applied for clinical practice? And really we have spent some time over the last couple of years of working with Carolyn and her colleagues—can this be simplified, and is there some way we can apply the knowledge we've received from this, or gained from this, to improve our trials for patients with ARDS?—and in particular, could we prospectively identify our hyper-inflamed and non-hyper-inflamed cohort?

Again, Carolyn's group have worked very hard on this, and have come up with what they call a parsimonious 4-variable model, and this is really incredibly interesting, so they have found that specific cut offs for two biomarkers, soluble TNF receptor one and IL-6, and a baseline bicarbonate level, and the need for inotropes at the time of presentation with ARDS, can predict whether you're likely to fall into the hyper-inflamed or non-hyper-inflamed [group]. Again, these are retrospective data but those four markers appear to be enough to predict or assign people to the right class in up to about 95% of cases.

So all well and good. Bicarbonate's easy to measure from your blood gas. Whether you need inotropes, I guess, is slightly subjective, because there might be different thresholds for using that, but it's relatively easy to define. Defining the levels of these biomarkers, however, is a little bit more challenging, because they're both currently measured by immunoassays, and they're not something you can pop off to the lab and get an answer back on in an hour. I'm not going to talk at length about immunoassays, but just to explain that immunoassays are complex and quite crude assays at times, but they are time-consuming and while they can be cheap to do in bulk, they're actually very expensive if you wanted to use an immunoassay plate for one simple sample, and they require relatively experienced laboratory personnel. And actually getting any of the assays to function at high GLP level, or at a good laboratory practice level, is really quite challenging.

There are some proposed faster solutions that exist commercially. There's a machine that can turn this immunoassay around for IL-6 or sTNFR1, within a couple of hours, but this is laboratory-grade equipment, and again is designed for multiple samples rather than for testing one sample at a time, and are hugely expensive. This is where one of our current studies comes in, this is called a PHIND study, or Phenotypes in ARDS study, and this is a collaboration that we have with Randox Diagnostics, around trying to develop a point-of-care assay that we can measure IL-6 and sTNFR1 really at the bedside in intensive care, and can we use that to stratify our patients, at the outset of illness.

So PHIND, the assay, is just being finalized at the moment, and the machine are being distributed to the clinical sites at the moment, so we're looking at 20 clinical sites. We will look at blood from 480 patients, and use this assay along with their blood gas bicarbonate level, and their need for vasopressors to assign a sub-phenotype at the outset, and we'll follow them up to assess the clinical outcomes, and show, can this so-called concept of two classes be defined at the outset, is it a real prospectively validatable finding? At the end of the study, we will validate the point-of-care assays against traditional lab analysis, to show whether there's a correlation between the two.

So ultimately, we're aiming to try and see if this parsimonious model, allows us to classify patients at the outset, and if so, that will allow us to have an enriched population to test at a trial for an intervention that we think works well in the hyper-inflamed cohort, and I hope that PHIND will also explore the potential for other [?] studies in both, and phenotypes not just for the statin itself, and hopefully we'll get some further understanding of the mechanisms at play that drive the different responses in our patients.

So really this is near the end of my talk, and I think what will be the subject of a lot of talks during the course of lectures in the Ulster Medical Society this year, is about trying to help us sub-phenotype and characterize our patients better, and within these sub-phenotypes, are we able to identify what the mechanisms at play are? When we do identify the mechanisms at play in these different groups of patients, can we then identify traits or biological targets that we can hit? And the aim ultimately is, for example, in our statin group, that we have our hyper-inflamed patients identified, and within that, be able to identify mechanisms of inflammation and targets that we can treat, and the aim is to enrich our clinical trials with populations of patients that are most likely to benefit from the intervention that we're

treating, because clearly in our statin group, for example, we were treating a whole series of patients who were unlikely to derive any benefit from it. Again, that concept of stratifying medicine, and improving or enriching our practise in our clinical trials, is a well-recognized paradigm across a range of disciplines now, and I think in my own practice, in respiratory medicine, we see this really exemplified in asthma, for example, where there's a lot of work done to try and stratify our asthmatic patients into those who have a highly eosinophilic driven process, who respond to eosinophil inhibitory treatments, whereas we know that patients with neutrophilic asthma don't respond to those, and why would we want to give these potentially toxic and incredibly expensive medications to a group of people who are unlikely to have any benefit? That's the strategy across other disciplines too, and again well seen recently in cystic fibrosis, where patients can be stratified according to their genotype that predicts their response to different molecular inhibitors or molecular activators of their CF and channel. Breast cancer and lung cancer stratification really drives the direction of treatment for individuals, and I think it's the way forward for many of us within medicine.

There are challenges though, and that's really about us trying to define what the pre-clinical models and human models are actually assessing, and so do our pre-clinical models. Do they reflect a hyper-inflamed or hypo-inflamed phenotype, and can we model those well?—and to really see if the models we use for testing sub-phenotypes, or in modelling the different sub-phenotypes, will we improve our pre-clinical testing?

All research, like all clinical care, is a team effort, and although I'm presenting tonight, this is work that reflects our whole critical care research group, and I just wanted to acknowledge in particular two of the PIs in our research group, so Danny, who many of you know, and most of you probably know I'm married to, Danny leads much of our clinical trial work, or most of our clinical trial work, and I lead the biomarker and laboratory work. I also wanted to acknowledge Mary, who is a PI within our centre, who was really instrumental in setting up the LPS challenge and model in Queen's here, and that's been a huge investment to us, and a huge resource to our lab. The research nurses, who helped facilitate the clinical trials, are a key asset to us, and we wouldn't get anywhere without them. And I really also want to acknowledge the work being led by Carolyn Calfee in driving phenotypes, and while we want to try and claim it was us who was defining those, it's definitely Carolyn's work. So thank you very much.

Professor McMullin:

Thank you very much, Cecilia. That was absolutely wonderful. Would you be happy to take some questions?

Professor O'Kane:

Yes, of course.

Professor McMullin:

Anybody want to kick off? Perhaps we could take it a little bit further, about these latent case classes assignment. Is there any relationship to the aetiology of the ARDS?

Professor O'Kane:

Yes, so you sort of think, that seems logical, that people who have a different insult, or people who have trauma, people who have pneumonia, will have a different response, but the biological responses that we see in those in terms of the biomarkers are actually unrelated to the etiology, and that, I guess, is very surprising to us as clinicians, because you would imagine that a patient's trauma would have that potentially very different inflammatory profile to a patient with pneumonia, but it's not the case.

Professor McMullin:

And is the hyper-inflammatory response in any way driven by anything?—and I say that because we have things that we see in haematology, like haemophagocytic lymphoproliferative disorder [HLH], where this huge and inappropriate inflammatory response occurs, and in my book we're looking for the wrong thing, because you're looking for the cause, and what is driving this, but why does one person do that and one person not do that?

Professor O'Kane:

Yeah, so that is the golden question, and I think, I mean we know this from practice as well too, some people get a flu infection and get incredibly sick, in response to the same organism, which is relatively innocuous in most people. I like that you asked about HLH. I didn't actually have that question, because it's a particular favourite topic of Danny's at the minute, and it's interesting that about 10% of our patients in ICU seem to have a HLH type of response.

They have very high ferritin, and very high IL-18 and IO1 beta responses, and I know Danny's very keen to try and test that specific cohort with R1Ra in due course, but again …

Professor McMullin:

But it's what is the response? If we look, I'm interested in your models, where you're going from the point of view of the models, because you must have had a major ethical issue going through all of this?—so we all want a treatment, and to get a treatment, you have to start off with the animal model and go from there. You're suggesting the animal models are not much use?—but if you had a drug, would you ever get it licensed if you didn't … ?

Professor O'Kane:

No, so I don't think the human models will ever replace animal models, so they have an important role. I think where we think that human models are important is that they're a stop, so that I don't think we would, for example in the ARDS one, to progress a treatment that worked in an animal model, but didn't

work in the human model, I don't think we'd want to progress that to a clinical trial. We have had examples where, I haven't shown them, but we have had examples, some of our colleagues, some really very interesting work around macrophage depletion, where there are effects in animal models, but not in human models, so definitely not, and you won't get licences without animals. We need them for toxicology, we need them for pharmacology.

Dr S Hawkins:
Just a question about the simvastatin studies, I guess you were using people who had not been using simvastatin previously?

Professor O'Kane:
Correct, yes.

Dr Hawkins:
Was it difficult to find such people?

Professor O'Kane:
Yes! I think, and again with the aspirin study, obviously we were studying people who were already on aspirin too, and those have both been big barriers to recruitment for both studies, so we lose huge numbers of patients.

Dr Hawkins:
When you use the simvastatin acutely like that, did you continue its use in the patients who survived?

Professor O'Kane:
No, we stopped it at 14 days, unless there was an indication that arose in the interim for a statin, such as a stroke or MI.

Dr J Logan:
Thirty years ago, I had a man who took paraquat and got lung damage, and I remember my reading at the time, seemed to suggest that the type II, is it macrophages in the lung? No, type II …

Professor O'Kane:
Epithelial cells.

Dr Logan:
Whatever they are, it was a bad thing to have, because they were thicker and didn't allow oxygen to diffuse, but I think you were suggesting that you needed the type II to develop into the type I?

Professor O'Kane:
Yes, so that is correct, and so the type I cells do arise from type II cells in the lung, and clearly you need the thin type I cell to allow gas exchange, but the process of trans-differentiation, certainly in vitro, is actually quite short within a day or two, and so we would be expecting that our patients, we would support them on ventilation, and as they recovered, and when we got the trans-differentiation into the type I cell, that we'd be able to wean back their ventilatory support.

Professor McMullin:
So I'm asking, which will be a stupid question, but back to the Hammersmith Hospital, and we had a group of patients who used to appear with sickle cell disease, and we were taught that, if they whited out their lungs, which they did, you exchange them, got the respiratory people to ventilate them, and in five days they got better, and they always got better. Is that the same process, and why did they all get better?

Professor O'Kane:
I can't answer that. There is this phenomenon of what we call an early recovering ARDS, and again, they might be a group of people who are on the trajectory to a faster resolution, so people who fulfil the criteria for the ARDS but within 24 hours, or two days, are really vastly rapidly better. I can't tell you a huge amount about the sickle cell, sorry.

Professor McMullin:
But it was a simple, their lungs, x-rayed them, white lungs, you're in trouble, get the guys to ventilate them, exchange them, and hang on for five days, and they all recovered. The big thing was that if you didn't do it, and get them ventilated, they were in trouble, so there was something about that.

Professor O'Kane:
And that probably, to actually learn from that in terms of recovery.

Professor McMullin:
Anybody else? Okay, well thank you very much, with what was a fascinating talk. I think you've totally fulfilled what I was told the Robert Campbell orator should be, somebody from a Queen's or Northern Ireland background …

Professor O'Kane:
Not a man!

Professor McMullin:
Not a man! No, definitely not a man, there's only men in the … who were certainly making their mark within the scientific community, so I'm delighted you came today, and I would like to give you a little token, the Robert Campbell medal, which is designed by Rosalind Praeger, so congratulations, and thank you very much.