Paul

Hello, my name's Paul Klenerman. I'm a professor at Oxford and I'm the host of this podcast on immunology called To Immunity and Beyond. So this is just to say that what we're putting forward with the podcast is a scientific discussion and it's really just for information and it isn't in any way medical advice. So if it's medical advice you're after, please go and talk to your doctor or some other medical professional. Meanwhile, enjoy the podcast.

So, welcome to another edition of To Immunity and Beyond, and today's guest is Fadi Issa from the Department of Surgery. And welcome, Fadi. So we're going to talk today about a really cool paper on regulatory T-cell therapy. So it's got all the best bits about basic science, and clinical translation and everything in between. So, congratulations on the paper in Med. We'll put the link in with the podcast. But first of all, Fadi, perhaps you could tell us a little bit about your backstory, how you got here and how a plastic surgeon ends up studying kidney transplants.

Fadi

Yeah, thanks very much. It's a question I have to answer all the time, actually, justify myself and why I'm here. But it actually started, it was when I was still training and people were doing the first hand and face transplants. If you remember that, 20 years ago. And I was in plastics, I was training in plastics and I thought, this is very interesting. This is probably going to revolutionize plastic surgery. I need to find out more about it. And I realized that the biggest barrier to those transplants was all immunological. So I went to see Kathryn Wood. And she very kindly agreed for me to do a DPhil with her. And I stayed on, stayed on with a postdoc and then took over the lab and here I am.

Paul

Okay, great. And actually on the subject, so what's happened with all the hand and face transplants?

Fadi

They've kind of plateaued a bit, so there's still a few happening, worldwide. We're into sort of three digits at the moment. Mostly upper limb transplants happening at the moment. In the UK, Leeds is the biggest centre. They've published their experience just this year of about a dozen patients who've had upper limb transplants. They are the only NHS commissioned centre. In fact, probably the only centre worldwide that performs funded VCA transplants as opposed to research transplants elsewhere. So we refer to them, they do transplants for our patients when needed, et cetera, very successful, very good, protocolized. And then the field otherwise is kind of, I'd say plateauing. People are realizing now, 10 to 20 years afterwards that the immunosuppression is a big problem. These patients are having complications. They're having renal failure from the nephrotoxicity of the immunosuppression. Some have had to have kidney transplants afterwards. Many have lost their transplants. In fact, the first few, so the first face and the first hand, one patient's dead and one's lost their transplant. So the long-term issues are as we would have expected of any transplants.

Paul

Right. In contrast, obviously, to kidney, where there's a wealth of experience and the expectation is that the grafts will do very well. So where does the idea of giving regulatory T-cell therapy come from? And just give us a little bit of the history, that underpinned the trial.

Fadi

Yeah, so the origins of this actually go back to some of the work that Fiona Powrie did in Oxford a long time ago, looking at autoimmune disease in mice and finding CD45 RA cells rescuing autoimmune disease in mice, but also for the transfusion effect in transplantation where patients having multiple transfusions actually became tolerized to their donor and part of that was due to an expansion of regulatory T-cells. So people have known that T Regs can promote tolerance therapeutically. And the route that we've taken here is to take them from patients, expand them ex vivo and return them to the patient as a cell therapy as opposed to in vivo expansion.

Paul

Good. So the paper is based on a clinical trial called the ONE Trial. And I know there's the TWO trial that's going on. Imaginatively. So just explain the kind of idea of the trial and what the kind of outcomes are and what the, not outcome, well, what the aims were really and any outcomes that have generally emerged from the trial because it's quite an old trial.

Fadi

It is now. So we're about 10 years follow up now of the ONE study. So it's an old trial. But at the time it was the first in human trial of T Regs in transplantation. The only preceding trial was a colleague in Poland who was actually a postdoc in a lab before who injected himself with some T Regs to show that they were safe. But then this was the first trial. And it was a European consortium trial where each different centre looked at using a different type of cell therapy, not just T Reg, so tolerogenic DCs, regulatory macrophages, et cetera. And it was all under a standardized protocol in living donor kidney transplantation as a feasibility and safety trial. So everyone did the same immunosuppression protocol across the centres, which was actually very difficult to achieve to get every different centre to do exactly the same protocol. So that's why it's called the ONE study. Everyone was doing the one protocol. And we here did regulatory T-cell therapy. And what we infused was polyclonally expanded T Regs. And it was a dose escalation trial, a conventional 3 plus 3 trial where we gave 1, 3, 6, and 10 million cells per kilogram to three patients. And there was a reference group, so not necessarily a control group, but they were patients who were recruited prior to the cell therapy, who underwent the same sort of protocol of immune monitoring, and then the cell therapy group afterwards. The cell therapy group here did not have induction immunosuppression, because the standard of care that was agreed upon was that patients would normally have induction immunosuppression with basiliximab, which is an anti-CD25 antibody. And there were concerns that giving that and T Regs would deplete the T Regs. So in fact, the cell therapy group did not have any induction immunosuppression, so they had already had a slightly lower background of immunosuppression. And then five days after transplantation, were given a dose of T Regs. And then they were monitored after that. There is a protocol biopsy, which we can, I'm sure we'll come to talk about. And in a cohort of the patients, and it was clinically driven, some patients were taken off MMF afterwards. So about 1/3 of these patients now continue without MMF.

Paul

Great. And so once you transfer the cells, is there any way of figuring out what happens to them? I mean, you sort of indicate they're not really traceable. Did you find any clever tricks to sort of work out how long they last for or anything?

Fadi

It would have been nice to have some way of tracing them. The only thing that we could do was look in the blood for FOXP3 or CD25 high cells. And what we found was after infusion, there was a sort of spike in both those signals, both by flow and RNA. And that then came down and plateaued, but remained slightly higher than the reference group patients. And it was dose dependent. So when patients had more cells, you could detect more cells afterwards. So it gave us a good...

Paul

So you'd actually think they might hang around quite a long time then?

Fadi

Well, it's difficult to know, but in other trials that have given T Reg therapy, particularly in diabetes in California, where they deuterium-labelled them, they found that they could detect those cells a year later. So probably.

Paul

Okay, and so what you've sequenced the cells. What do you think you're actually giving? It's not quite the same as what you started off with, but explain what the mixture of T Reg, it's not just one T Reg, is it?

Fadi

No, it's lots of different T Regs. So first of all, I'll just say what the protocol is for making these cells. Usually, we take a unit of blood from patients. They're not luca freezed. And then we volume reduce that. And then we just do magnetic bead isolation. So we deplete CD8, and then we sort negative, and then we do a positive selection on C25. And then we expand those with rapamycin, which reduces effector contamination, and with IL-2 to help expand. And that happens over, it's about a month of expansion. And then afterwards, they have some QC checks to check that they can be released. The single cell sequencing that was done, in fact, the CITE seq that we've done here on the product shows that it's quite heterogeneous. So there are lots of different populations. And I'm sure people can have a look at the UMAP there. But lots of different populations of cells are activated or naive. Helios-enriched or not, migratory, cytotoxic, all sorts of different populations. But really, they all express the core T Reg modules. So they are all Tregs, they express FOXP3 and high levels of CD25 both by RNA and protein.

Paul

And just from the practical side, how did you make a GMP product? Because I mean, obviously things have moved on a lot with CAR-Ts and things, but it's 10 years ago. So what was the protocol? Did you have a set up a specific lab for this or how did you do it?

Fadi

Yeah, it was a challenge and actually a lot of that work was, we were talking earlier about Andrew Bushell, he helped drive a lot of that work. So it was taking our lab protocols and seeing how they can be translated to GMP conditions. And at the time, the only way of doing it was with CliniMACS and then expansion in bags and then doing a centrifugation to isolate the products at the end, remove beads and then QC. All that is actually done in a GMP unit in London at Guy's Hospital, which was initially BRC funded. It would be nice to have a GMP unit in Oxford.

Paul

Yeah.

Fadi

Well, we could do that.

Paul

Okay, if anybody's listening.

Fadi

Yeah, because a lot of this cell therapy work is outsourced really.

Paul

Yeah, and I think it's only going to get better, so.

Fadi

I hope so, yeah. So, we initially, and up til now, we employ post-docs in that GMP unit. So, they're Oxford's post-docs, but work in the GMP unit.

Paul

Wow, okay. So that's really brilliant background. And maybe just run through the kind of highlights of the study that from your perspective, I mean, there's a lot of data in the paper, people can pore over it, but what do you think were the most interesting findings?

Fadi

So let me highlight the clinical findings first, because I think they're really interesting. We're about 10 years post-treatment. None of the patients who've had cell-therapy, and there's 12 patients here, have lost their grafts. Or had DSA, so donor-specific antibody development. And the ones who've had their immunosuppression reduced are all doing very well. So the ones who are on tacrolimus monotherapy are all doing very well. And the levels of tacrolimus are not higher than control patients. And they all have not had induction immunosuppression. And rates of infection are much lower in the cell therapy group as well, likely because they're having lower levels of immunosuppression.

Paul

Okay, well, that's very interesting.

Fadi

So things like CMV, BKV…

Paul

and potentially skin cancer and stuff like that?

Fadi

Hopefully, but we'll find that out later.

Paul

Okay, and so as you sort of indicate in the paper, the stats don't kind of yet stack up, but it's smallish numbers at this point. And there was one patient where things didn't quite go to plan that you've highlighted.

Fadi

Yeah, so there was a patient who had a recurrence of IgA nephropathy.

Paul

Yes. And I guess that's difficult to say much about that. You weren't really aiming to prevent the recurrence of…

Fadi

No, but it's interesting that there was a recurrence and that the T Reg therapy that we gave maybe didn't have any impact on the IgA nephropathy. So it's difficult to know whether in an IgA nephropathy… it’s difficult from that one patient.

Paul

OK, but generally the patients did well in ways which are not so black and white in terms of graft survival, but certainly indicate that…

Fadi

And the rates of infection are definitely significant.

Paul

That's really, really interesting. OK, so that's good because it, you know, that's such a critical outcome for these patients. So then, what was the sort of, what did you think was sort of your take on the kind of mechanism there?

Fadi

So what was very interesting is that we did protocol biopsies on these patients and patients don't normally have protocol biopsies after transplantation in Oxford. So it's not on the clinical protocol. And so we don't necessarily always know what a biopsy would look like, post-transplantation unless they're having a rejection episode. So they had these biopsies and they looked odd because they had these very focal infiltrates of cells, interstitial infiltrates of cells. And at the time, the pathologists were a bit worried, and the nephrologists were a bit worried as well. But actually the patients were doing very well, so there was no evidence of rejection otherwise. No DSA, no rising creatinine, eGFR was maintained, et cetera. So everyone held tight, which was good. So no one gave any more immunosuppressants. And some of those, so it's actually in all the patients who were biopsied, they all had these infiltrates, and the reference group don't, and the control group, any other control group that you look at, don't. And we then looked at rejection infiltrates, and they're very different. Rejection infiltrates are diffuse lymphocytic infiltrates. Here in cell therapy, they're very focal, and they're sometimes perivascular infiltrates. So they look histologically, morphologically, very different. And so we were quite interested in knowing what they were. And I later on dug into the literature and found old papers that described something called a Quilty effect in heart transplant. So patients who had heart transplants who were doing well maybe even moving towards a tolerogenic state, had similar infiltrates. So we were interested in knowing what they were. And so we decided to do some spatial profiling on them. And at the time, NanoString from GeoMx had just come out. That was the big technology which allowed us to do sort of broad, almost like bulk sequencing of regions. And we took those infiltrated regions and sequenced them to see what was in them, both, actually both protein and RNA at the time. And actually that would not have been possible really on these clinical biopsies. We didn't have any frozen biopsies either. FFPE biopsies, was the only way to look at them.

Paul

So what do you think they are?

Fadi

So if you look in the paper, you'll find that they're very B-cell enriched. There are lots of T Reg markers there, or at least markers of what looks like immune regulation. There's helios expression, IL-10, PD-L1, TIGIT. And then there's this very clear CD20 signal and confirmed also by immunohistochemistry. What do I think they are? I'm not entirely sure. There have been some studies, more recently, coming from America, where they do a mouse model of kidney tolerance, it's a kind of spontaneous tolerance model, and they see similar things as well, and they're all B-cell enriched as well. I think we need to go back and do some animal experiments to find out what's going on. If we deplete B-cells or if we have B-cell deficient mice, do they still form? Can you still promote induced tolerance? I think there is some big questions that we need to...

Paul

You sort of indicate that maybe based on the mouse stuff, the T Regs in that other model, not yours, the T Regs are important early, but later on something else is taking over their role. So do you think that this is a sort of B reg kind of little hive that's somehow doing that?

Fadi

Maybe. So in those animal models, if they deplete T Regs, they can't promote tolerance. And these infiltrates don't form. So certainly the T Regs seem to be important there. I don't know whether you are creating tolerance within the tissue and then the cells that infiltrate become tolerized. And that's what we see. What is not clear to me is whether those cells that become tolerized then perpetuate the effect.

Paul

So nowadays, of course, you could do, you're the kind of one of the people in Oxford who's done the most in terms of spatial transcriptomics. So presumably you could do a much deeper look at these.

Fadi

Yes, and we are. So following the ONE study, we've now moved on to a phase 2B trial called, as you said, the TWO study. And that's a bigger trial where we're aiming to recruit just under 70 patients. And we're at 58, 59 patients at the moment. It's a randomized controlled trial. So we actually have a proper control group, with biopsies as well. And so they're matched in time. So they're all both in the control group and the cell therapy arm. And we also have a pre-cell therapy biopsy. So we have a post-transplant pre-cell therapy and post-transplant post-cell therapy biopsy. So we can actually compare pre and post-infusion in the cell therapy.

Paul

The infusion is still at day five, is it?

Fadi

So it is. It's a complicated story. So initially when we started the TWO study, we wanted to give the cells late. Because what we wanted to do was give Campath, deplete everything and then give T Regs six months later into empty hosts as things start to repopulate. And that was based on previous data that we had from Campath patients where we showed that at six months, when repopulation happens in these patients, it seems to be T Reg enriched. So we were trying to kind of boost that. And we did that. We recruited 7 patients and randomized them. And then there were changes in the use of Campath, worries about Campath in virus infections, and then sort of some signals from Europe that they weren't happy about Campath in MS and so on. So we actually reverted back to a five-day infusion, sadly.

Paul

But overall, it sounded like there was a hint that if you gave T Regs, even though they are immunosuppressive, you might have less infection overall, but you're just sparing worse immunosuppression. So it's sort of a bit of a balance, perhaps.

Fadi

It is a bit of a balance, absolutely. And actually, I haven't answered your question about the spatial transcriptomics. So on the TWO study patients, we're now doing more detailed Xenium analysis. And also we've done some GeoMX whole transcriptome analysis, and IPA, which is the big protein panel.

Paul

And can you get down to the detail of T-cell and B-cell receptors in the spatial transcriptomics at this point?

Fadi

That's the aim. We've only done a few in which we're doing those, I'd say their neighbourhood analyses at the moment.

Paul

That would be fantastic to kind of really work out what the clonal…

Fadi

Yeah. We have done some TCR sequencing on product and then from the TWO study, because we had an extra core, we did some single cell sequencing of, so we isolated CD45 cells from the biopsy and we flow sorted them and then we did single cell on them and TCR sequencing just to see if there's any clonal overlap between the cell therapy that we're giving and that in the biopsy. And it's not clear that there is.

Paul

It's quite a big range, I guess. It is very round. It's a difficult experiment. Yeah, but it's still very interesting. So that's fantastic perspective. And does that sort of take us to the end of the conclusions from the study?

Fadi

Yeah, I think so. I mean, this is kind of observational. We found this. It looks immunoregulatory. And now the questions are, why, what is it? And can you induce it in other ways? Does it have to be giving cell therapy?

Paul

Okay. And what do you think the lessons would be for people doing either other transplants potentially or obviously in autoimmunity where similar approaches could be tried or are being tried?

Fadi

Yeah. So first of all, I'd say that if you're doing any kind of advanced clinical trial like this, the likelihood that you'll see any clinical effect is low. And so this immune monitoring is absolutely key to understanding what's happening. It gives you an insight into mechanism and maybe helps you understand whether your therapy really is working. So that's a sign. In terms of thinking what the next steps are, we, so CAR T regs are there, people are starting to use them in trials. There's, there are a couple of trials running of CAR T regs, one by Quell in London, who are a company spun out from KCL, where they're giving HLA-A2 directed CAR T regs to liver transplant patients. So we'll see what the results are of that. The most recent thing that I've seen in conferences when they present is that they show, again, survival, and they've done some biopsies in their liver transplant patients and the few that they've done. And because they can detect the CAR, it can actually show that some of those cells are actually in the liver. So that's...

Paul

Anybody spotted any little sort of... infiltrates?

Fadi

Yes. Yeah, it doesn't look like they form the same sort of focal infiltrates in the liver, they're different dynamics. Different dynamics there. Yeah. So CAR T Regs, probably the next thing. We're now starting to work on allogeneic T Regs, so these are all patient-derived, which is logistically complex, so we're now trying to see whether we could use third-party T Reg, that you can just bank and then give to any patient. That would be ideal.

Paul

Would they not be sort of kicked out quite quickly?

Fadi

They are. So we've done some animal experiments and we've shown that they die in the animals, of course, so they won’t work. And that seems to be largely CD8 mediated. So we've now knocked out class 1, class 2 upregulated HLA-E on these T Regs. And we show that those do survive in the animals and that they can regulate at least as well as autologous cells.

Paul

Okay. So there's plenty of biotech that you could explore in this area. That sounds really fantastic. As well as I understand, it's a really, I really appreciate the study because as I said at the beginning, it sort of crosses so many boundaries. And as you said yourself, if you do a clinical trial, you can sort of work your way backwards to the basic fundamental immunology as well. So congratulations. And so we'll put the paper up. And Fadi, if you've got other papers that you want to stick on there as well so that people can sort of get the context, that'd be brilliant. And it's a pleasure talking to you.

Fadi

Likewise. Thanks very much, Paul.