# Audio file

[Kirsty and Carolyn final.mp3](https://unioxfordnexus-my.sharepoint.com/personal/admn1202_ox_ac_uk/Documents/Transcribed%20Files/Kirsty%20and%20Carolyn%20final.mp3)

# Transcript

Paul

Hello, my name is 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.

Paul

Hello and welcome to another episode of To Immunity and Beyond and it's a pleasure to have two researchers here today, I’ll get them to introduce themselves, actually.

Kirsty

Hi, I'm Kirsty McHugh. I'm a researcher in Simon Draper’s Blood-stage Malaria Group here in Oxford. And with me…

Carolyn

Hi, I'm Carolyn Nielsen. I'm also a senior postdoc in Simon Draper’s group, currently in the Department of Biochemistry. Until Monday, when we move to Paediatrics.

Paul

Great. I didn't know about that last bit. Very exciting. So anyway, so we're going to talk about your paper that was published a few months ago now, in September time, but a fantastic paper on malaria in *Cell*, and we'll have the link available for people to look at. But, I mean, as we were talking earlier, this is a kind of culmination of, you know, many years of work. So perhaps you could just start by giving us a sort of history, and how you came to do these particular experiments?

Kirsty

Should I pass it to you to start with the history?

Carolyn

Yeah, some vaccine history. So we had our first in-human trials with RH5, which is the blood stage vaccine antigen that we're working with in this paper here. So that was first for the viral vector study, and then in this study we've moved on to look at characterizing the antibody response with protein and adjuvant regimen. And so we have a variety of different groups and different dosing regimens and we've been able to pull out and characterize the mAb response to each of those.

Kirsty

Yes. So I think I joined the group nearly over 10 years ago now, but it's only been in the last five years that I've been working in the malaria team. And I sort of joined the malaria team at this stage where we'd finished this VAC063 clinical trial, which as Carolyn's just said was a protein in adjuvant trial that used UK participants. It had a number of groups as Carolyn has mentioned and really the focus of what we wanted to do with these vaccine volunteer clinical trials samples was to isolate a large number of antibodies and then use this information to better understand the vaccine-induced antibody response. So to do that, you want a large number of antibodies, and this isn't something that had really been done that much before in the group. So the first couple of years of the antibody work that we were carrying out, it involved a lot of optimization of the pipeline to try and make sure that we could isolate the antibody sequences from these vaccinated volunteers and then the next stage was to set up the facilities so that we could carry out high throughput production of these antibodies and then characterize them.

Paul

Great. So the paper’s about the characterisation, which is fantastic. Maybe just take a step back. So RH5 is your target. That’s a pretty special antigen I think. And maybe you could explain to people why it's such a kind of good target for you.

Carolyn

Yeah. So the historical issue with blood-stage malaria vaccine development has been sort of on several fronts. One of them has been genetic polymorphism. Lot of variation, genetic variation, in the antigen. So it's been difficult for vaccine development purposes. So RH5 is very appealing because it's really well conserved. So we expect responses we induce with a vaccination program to be protective against strains in endemic countries. And I guess the flip side to that, that has made our lives a little bit more difficult is probably one of the reasons it's so well conserved is that it's exposed to the immune system for a really short period of time. So it's not really under a lot of immune pressure. But that means from a vaccine development perspective, we have found that we need to induce really high concentrations of antibodies, to I guess stochastically have enough antibody present at the time when RH5 is being used for invasion of red blood cells.

Kirsty

Yes, I'll just carry on from the last point. So to actually just explain what RH5 is. So RH5 is part of this pentameric complex on the merozoite, which is the blood stage form of the plasmodium falciparum parasite. And it's only really been recently discovered that it's part of a pentameric complex. So this involves four other antigens, and RH5 is the highly conserved antigen that is involved in the actual receptor binding. So these merozoites invade red blood cells through this essential interaction between RH5 and basigin. So as well as it having very low polymorphism, it's also an essential protein for the invasion to happen.

Paul

So is it the case that even if you developed some natural protection, it’s not complete immunity, but sort of functional protection against malaria, you’re not using RH5 antibodies in the part of your natural response?

Kirsty

Yeah. Yeah, that's absolutely right. So there's very low titers seen to RH5 in field studies.

Paul

Great. So that makes it a really super interesting target, of course. And there was this clinical trial, exposing healthy donors to the protein and then measuring antibodies. So just take us through quickly - so then it's a decent number of antibodies. It's the sort of thing that people were doing during the pandemic in COVID-19 to develop these panels, but it's still a large number. And it's a very large number screened with all these biochemical assays. So before we get into the biology, would you just tell me about the sort of technical approach to it, because it almost seems industrial scale.

Kirsty

Yeah, so as I mentioned earlier, one of the challenges we first faced was just isolating these numbers of antibodies from the vaccine samples. So we used single cell sorting using the RH5 as a probe and so we took PBMCs from 15 volunteers in the trial and those volunteers spanned the different regimens across the trial. And then we isolated the B cells from those volunteers and sorted them based on their binding to the RH5 probe. And then we set up a pipeline to do RT-PCR to isolate the antibody sequences. The challenge then became how you can make 200 antibodies using the protein expression tools that we have in the lab. And so we worked on that and we've now got a very high throughput system to produce antibodies. We have an ÄKTA Pure expression system. In order to purify a number of antibodies we linked it up to an auto sampler and this auto sampler allows you to run 84 samples in parallel on the purification system. You can set up 84 antibodies overnight and the next day you have purified antibody.

Paul

Great. And then what about the screening. You know, we've got all these fairly high tech on-off rate antibody studies. To do that at scale is quite something.

Kirsty

I think I'll take over because actually, yes, that was one of the, the bottlenecks that we were facing once we had these large piles of antibodies. We did sort of two or three main things, and the first was we tested them in functional assays. So we have a parasite growth inhibition assay and we have two very important parasitologists in our group and they carried those assays out. So even though those are quite hard to do high throughput, they manage to churn through the 237-odd antibodies and get the functional data. But we were really sort of stuck at how we were going to get kinetics and how we were going to understand the relationship that these antibodies had between each other and the epitopes that they bind to, because traditional SPR methods really limit you to smaller panels of antibodies. So we invested in a Carterra LSA platform, which is a high throughput SPR machine. And this allows you to do 384 antibodies at a time using sort of traditional gold surface SPR. And so what was great about this was we could take the full panel and actually in a single experiment get the kinetics data for all of our antibodies and then in a separate experiment, we were able to run what's called a binning experiment, where you look at the competition relationships between all of the antibodies. And in this paper you can see some really nice network plots that show you which antibodies bind to similar epitopes. And really, that's sort of a step change in actually the way that we can characterise antibodies because up till now you haven't really been able to do that with such large panels of antibodies.

Carolyn

Just to add to that, Kirsty's made it sound nice and straightforward, but that that was a ton of work. And like you said a big technical challenge, being able to work with such a huge panel of antibodies and just as a bit of a comparison, there's a former PhD student in our group, Dan Alanine, who also had a RH5 MAb paper in *Cell* in 2019 I believe. And he was working with samples from a previous trial and did some really nice characterization work, but I think was working with a total panel of just under 20 antibodies and that was a lot of work, especially when he started to find really interesting interactions between the antibodies. And so when you started wanting to look at them in combination these synergy experiments are just completely unwieldy when you're starting to work with numbers in the hundreds. So this was really a big step forward for us when Kirsty got the LSA.

Paul

Great. So, a massive piece of work, huge challenges. Let's sort of just look at some of the conclusions. So I really like the … you put them into, sort of, communities which was a really nice way to describe them. And one of the features that you were looking at was on and off rate and how they behave differently. So you've identified the on rate as being the big deal. Which, I mean, it's very clear, but … this is probably not very biochemically minded, but … the on rate and the off rate you would have thought would be related to each other. Maybe you just explain that a little bit and why you think, you know, biologically the on rate is such an important thing.

Kirsty

Yeah. So as Carolyn alluded to earlier, the RH5 as part of this invasion complex is only on the surface for a very short time. And so this invasion window for antibodies to be able to act and actually bind to RH5, is really short. So the fact that we found that the KA correlates well with antibody potency perhaps wasn't so surprising to us, because what's really important is the speed of these antibodies and how fast they can get to bind to RH5. And if they don't have a fast enough on rate, then it doesn't matter how good their off rate and overall affinity is because they're just not getting to the RH5 complex in sufficient time.

Paul

And what would make an antibody fit into that category where it had a particularly high on rate? Did you identify that? I know you've got lots of really cool genetics and so forth, but what's just in simple terms, what would make it really stick quickly?

Kirsty

Yeah, so there were a number of antibodies that had these fast on rates, but if you looked at the community map, we have three main, what we call, super communities that contain these, what we call GIA positive antibodies. So these are the antibodies that have the growth inhibition activity. And within those communities are where you find the antibodies that have the higher K on rates and those are the antibodies where the K on rate associates with potency. Outside of those communities, so we have a community 4 which we might talk about again later on. These community 4 and community 5s, they have no GIA activity. And so actually we found that those communities didn't have as strong a correlation with the on rates.

Paul

But maybe we should talk about those now. You did find, I thought this was one of the best bits, that you got these sort of, I guess for a virologist it’d be like a non-neutralizing antibody, but they're still playing an important role in the overall impact. So what was going on there? Have you got a sort of molecular explanation for how that works?

Carolyn

Yeah. So I think this was really exciting data and I think the first observations of this were just in the way things go just by chance. I think someone, I think this was Dan early on, included a non-neutralizing antibody as a negative control and then saw some of these exciting synergy results, which really sends us down this path.

Kirsty

Yeah. So actually it goes back to the *Cell* paper from 2019, the Alanine paper, and they isolated only a small number of antibodies and again we didn't have the technologies that we have now. So he managed to get just under 20 antibodies. But surprisingly, within that panel he had antibodies from a number of our communities and one of these antibodies was called R5.011. And we carried out some microscopy experiments in that paper with these antibodies and that was when this mechanism of how these antibodies in this green community appear to work, and it looks like they slow the invasion pathway down. So on their own they don't have any GIA potency, because ultimately the parasite can invade the red blood cell. But that invasion window is widened. So if you combine one of these antibodies from this community with the antibodies that we know are GIA positive, so community 1 for example, that gives those antibodies more of a chance to bind to RH5. And so that's why we're seeing the synergy.

Paul

So the idea would be that those ones might bind first, just increase the window, which is very short, and then once you've got the really good ones on, then it's game over for the parasite. OK, good. One of the other striking features was about this particular, essentially public structure, the heavy and light chain combination that you found. So was that expected? Obviously there are structural features that would make these antibodies look similar. But is this the first example or is it sort of something that you might have been looking for anyway?

Kirsty

Yes, we went into this with all of these sequences kind of not really knowing what to expect. I mean in other fields, public clonotypes have been identified and it's a feature of, I think both flu and HIV antibody repertoires. So for us it wasn't something that we were expecting to find, but it was something that we began to look for and were quite nicely surprised that we found these 4 antibodies from different donors. Two from a delayed factional regimen and two from just the monthly dosing regimen. And they all had this HV3-7/LV1-36 gene combination.

Paul

Good, excellent. So I had a question about that, do polymorphisms in that region in a host genome impact on your ability to make these antibodies?

Kirsty

We haven't really looked into that yet, so it's probably something we should look into. What we have now started doing though is, we have a number of other clinical trials where we're isolating sequences from, and so this is from different populations and again different regimens. So we now have a clonotype that we can start looking for, and perhaps we'll be able to understand it better.

Paul

So I'm a big fan of the delayed regimen because we got similar findings in COVID-19 and it sort of makes lots of sense. So perhaps could you just elaborate a little bit on what you found with the people who had a longer interval between the doses and how that relates to all these things?

Carolyn

Yeah. So we're very excited about the delayed regimen as well. That's progressing to further clinical trials and we have some exciting data coming out there. But prior to this paper, what we'd observed in terms of the B cell and antibody response with this delay boosting regimen, so the vaccinations are given at 0, 1 and 6 months, rather than at 0, 1 and 2, and in this case the final vaccination was fractionated, so given at a lower dose. We’ve previously seen that in these delayed factional boosting vaccinees, that the serum antibody response had higher avidity, and this was done with the sodium thiocyanate displacement assay. So this was before we were starting to do fancier things with K on and K off and the more comprehensive kinetics. And we've also seen higher frequencies of antigen specific B cell responses using the same probe based dinging we've done for the B cell sorting here. But it was really interesting to have enough vaccinees and enough mAbs isolated from these vaccinees to not just include in the full repertoire analysis here, but actually be able to do comparisons between the groups. And there were some differences in terms of changes that the dosing regimen could mediate in terms of the K off rate, I think.

Kirsty

Just as Carolyn said, we have some nice supplementary data in the paper that shows that there are statistical differences between the antibodies that are isolated from the volunteers that received the monthly dosing regimen compared to those that got the factional dosing. And so those with the factional dosing or delayed dosing had statistically significantly better off rates. Which I guess when we're looking at our previous finding that on rates are determinants of potency, it's quite interesting that the affinity maturation potentially that's happening in these individuals is sort of skewing the responses to have better off rates. So that's something we're also interested in following up on.

Paul

OK, so you need to find a regimen that enhances the on rate. Good, so it's a huge paper. I’d encourage everybody to read it, it’s really fantastic. So at the end of it you show protection in a in a mass model, and you've got a lead monoclonal. So the end is quite brief, the implications bit. So maybe you could just elaborate on two things. Firstly, the kind of use of that monoclonal and secondly that the kind of next wave, the next generation, of RH5 vaccine. So you could take it in turns with those.

Kirsty

Yeah. So, as well as using this information to understand the vaccine responses and to design the next generation of vaccines, which I'll let Carolyn take on next, we are interested in getting the first blood stage antibody therapeutics into clinical trials. And so what was really nice in this study, was we isolated, identified, the most potent antibodies to date, and they happen to have this public clonotype. The HV3-7/LV1-36 antibodies. So we took one of those, our lead candidate in the paper is R5.034, and we took that into this humanised mouse model to confirm that you could get protection against challenge. And so that's given us some really positive data, but the next steps and what we're now looking to fund is taking one of these antibodies into clinical trials. So we haven't really in the past looked at developability of antibodies. So that's something we're now doing. And I mentioned we've got antibodies now from actually more than just this clinical trial. So we now have a much larger pool of antibodies to pick from and the plan is to identify those that are most potent but also have the best developability to put into a clinical trial fairly soon.

Paul

And would that have to be combined with some other prophylaxis as well? Or can you just give a blood stage monoclonal and hope to essentially reproduce what you saw in the mouse? Is that the plan or do you plan to combine it with other things?

Kirsty

These sort of decisions are still happening and we're having conversations with different funders on how we want to do this. But, it might be, one thing we're really interested in is combining it with an antibody to the liver stage. So there's been some really, really nice data out in the last couple of years on these couple of antibodies that target the CS antigen, which is the lead, it's the vaccine that's out there now in, in the field, the R21 and the RTSS vaccines. The two antibodies I think that have gone into clinical trials are L9 and CIS43 and they've shown some really promising data. But of course, when we're focusing on the liver stage, if any of those sporozoites evade the vaccine or an antibody and get into the hepatocyte those mechanisms are no longer functional. And then ultimately, you'll get merozoites coming out of the liver and people will go on to develop malaria, the clinical symptoms being associated with the blood stage of the disease. So if we can combine a liver stage antibody with a blood stage antibody to sort of pick up anything that escapes that first wave of protection, then that should be really powerful therapeutic that can be used.

Carolyn

And just to add to that, a sort of comment on general dynamics in terms of malaria prophylactic testing is that we're in a really exciting context now in the malaria public health space where there are in fact two licensed malaria vaccines that target the pre- erythrocytic stage. So our RH5 approaches come after that. But that becomes a really important consideration when we're moving into efficacy studies in endemic settings, is that if there is already a malaria vaccine that has been rolled out, it's important to take that into account because we need to be working alongside the standard of care in that country. You couldn't remove a licensed malaria vaccine to test a mAb on its own. So that's definitely making trial design much more interesting, but it's a really good place to be moving towards for the malaria vaccine and mAb community more broadly.

Paul

And what about your next generation ones?

Carolyn

So I think I actually think from both the 2019 mAb paper from Dan, and then this one here, one of the takeaways I have is a sort of interesting cautionary tale about not getting too focused on your epitopes that seem to have the greatest neutralization capacity that you don't actually want to remove those, because you’re perhaps missing some of these synergistic effects. And so I think this paper is really interesting in terms of what you can take away, both to guide vaccine development and also to guide mAb development, and they might be different. So the dosing regimen is a really interesting angle because that is a modifiable vaccine parameter that we can change. And so we have gone into Phase 2B efficacy studies. So there's one running in Burkina Faso right now with our collaborators led by Halidou Tinto at the Nanoro clinical research facility, looking at comparing monthly and delayed booster regimens in children with RH5 protein, so the same protein antigen, and we've seen some really interesting efficacy data with the delayed regimen. So I think that's our sort of most interesting candidate at the moment and there's other antigens coming down the track to be combined with RH5.

Kirsty

Yeah. And I'll just add to that just this this concept of doing the high throughput epitope landscape mapping of antigens. I think this is a really powerful method to better understand vaccine candidates. And from this paper again, I'm sort of reinforcing, I guess, what Carolyn has just said. You might be inclined to remove these community 4 region of RH5 because at face value those antibodies look to be negative. But actually when they combine with some of these other antibodies and they're showing these really powerful synergistic behaviors, it's important to not overlook that. So what we are doing for our next generation of RH5 vaccine is we're looking to see which epitopes we want to keep in the vaccine and which ones we can remove or mask by sort of glycosylation sites, for example. But as things stand, we're looking to keep these synergistic regions in our next vaccines.

Paul

Excellent. So well, I look forward to next generation stuff. It will maybe take a little while, but you’ll maybe bring it back to the podcast. Many congratulations on the paper and also on what sounds like your new environment. We look forward to hearing more from the group. So I think we'll stop there and we'll have more throughout 2025. Thanks for listening.