**Steve Lewis** 00:10
Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis, and today we welcome Dr. Felipe Galvez-Cancino to the show. Felipe is a group leader at the Center for Immuno-Oncology at Oxford University. He and his team of immunologists' study T cells and macrophages with the hope of developing innovative treatments for cancer. Felipe has also been recognized as a Gibco Cell Culture Hero, a program that highlights the work of scientists driving research that can change lives. We hope you enjoy our conversation. We begin by asking Felipe about his early career and how he arrived at his current work.

**Felipe Galvez-Cancino, PhD** 00:59
After my PhD, I decided to move to the UK, to London, at the UCL University College London, the Cancer Institute, to do my postdoctoral work in monoclonal antibodies as therapeutics. In particular, developing antibodies in understanding the mechanistic action of depleting antibodies in glioblastoma targeting, which is a very aggressive type of brain cancer. Part of the results that I got from my postdoc led me to try to understand how phagocytosis works. So, we were working with these depleting antibodies that target a particular population of cells called regulatory CD4 T cells that promote cancer, so they are bad when they are inside tumors. So we had an antibody that allow us to deplete these regulatory T cells, and I found that this depletion, this elimination of these regulatory T cells, was mediated by a particular population of macrophages that were highly phagocytic, and they express a particular set of receptors that we call FC gamma receptors. So now, following my postdoctoral work, I moved to the University of Oxford to start my research lab. And here, one of the key things I want to understand is the phagocytic landscape of tumors, of solid tumors. So basically, this idea that if you can solve phagocytosis, you can probably manipulate this and make these phagocytic cells that we found, these macrophages, eat the cancer cells, or eat the regulatory T cells, or maybe take this concept beyond cancer and apply it to other diseases. The other thing I'm doing is going back to my roots in my PhD. So, regarding cancer vaccines, so we want to launch and develop the first cancer vaccine for a rare type of childhood sarcomas called rhabdomyosarcomas.

**Steve Lewis** 03:14
So you've had an extremely diverse background in really all areas of cancer, I would say on the preventative side, right, with vaccines and then the therapeutic side with your monoclonal antibody research. I'm curious. It sounds like you've come full circle. Starting working in cancer vaccines and then ultimately moving back to it. What led you to come full circle?

**Felipe Galvez-Cancino, PhD** 03:40
When I was doing my PhD in cancer vaccines, it was the time cancer vaccines were not cool. So we were studying these vaccines. And people used to ask us, "Why are we studying that if they don't work?" Which is true, like actually, for many years they haven't worked. Even today, there's still not huge evidence that they work. I think COVID and open a lot of possibilities around cancer vaccines, and today, there is a lot of hope, more than data that suggests that they could work. So there is very strong data that they could work, but we still need to run the biggest trials. Beyond that, I think it's very promising in certain cancer types, rare type of sarcomas, that there's no way to make a personalized therapy. You can basically sequence the tumor, go and understand which mutations these tumors

have, which mutations could be seen by the immune system, and may personalize cancer vaccines. The good thing about these sarcomas, and many sarcomas, is that they respond very well to chemotherapy and surgery. And what we want to do is, at that time, like a minimal residual disease come and intervene with our vaccines, that we want to design. So basically, why I'm back to that, no particular reason, more than I have the expertise and probably in the right place to do it.

**Steve Lewis** 05:09
I noticed that your laboratory is called the Immune-Regulation Laboratory, and one of the things that I found really fascinating about that is you're trying to understand, really, the regulatory mechanisms that are restricting the activity of effector cells and then making it so that that restriction is removed, so that you can actually have an impact on a, I'm assuming, a broad swath of tumor types. Is that an accurate understanding?

**Felipe Galvez-Cancino, PhD** 05:40
Yeah. So basically, when you think of an immune system, you have to think of two mechanisms. Like regulation, which is everything related to suppressing a potential immune response. And then you have immunity, which is actually what we think about most of the time, which is, if you're infected with a virus, you will develop an immune response. So you have to think of cancer, that cancer is basically your own cells, and our body has been taught not to attack itself. Very difficult, in general, to mount an immune response against cancer. So it does happen. So you do generate these effector cells that can attack cancer. So I'm particularly interested in T cells, which is the most classical type of effector cells, and macrophages, as I said at the beginning, which is you can also think of them as effector cells. And these cells, when they go and try to attack cancer, because there is spontaneous responses against cancer, they get suppressed. They become regulated at the tumor bed. And the idea is to understand how that regulation works. So, we work with these two concepts of intrinsic regulation, which is what happens uniquely to the cell, and extrinsic regulation, which is which other cells within the tumor are affecting the activity of these effector cells.

**Steve Lewis** 07:08
You're programming pathways that exist naturally but making it so that they can impact cells that are maybe masked as your own cells, but they're actually cancer cells?

**Felipe Galvez-Cancino, PhD** 07:24
Yeah. So what we know is that in cancer, for super melanoma tumors or lung cancer, the cells that become cancer have accumulated mutations over many years. So those mutations can eventually be seen by the immune system so you have an immune response against the cancer, yeah, that recognizes these, these mutated proteins. The problem is that those T cells that can see those mutations, or in the case we target a macrophage towards the cancer cell, they can start killing. They will kill, but they will get suppressed. And there's been some advancements on understanding that suppression, which is the current therapies that are have been approved by the FDA today, for example, the Anti-PD-1 antibodies, Anti-PD-L1, so the PD-1, PD-L1 access is key at suppressing the T cell responses in the tumor. So what people have done is they make therapies that block this access, and T cells become reactivated, and they start killing the cancer. Likewise, what I found in my postdoctoral work, it's basically that I can target macrophages to the cancer cells, but they also won't be

very good at killing cancer. Yeah, they also get regulated. And what I found, if I co-deplete this population of regulatory CD4 T cells, these macrophages become more phagocytic, they start eating. So that's kind of the core concept of understanding regulation.

**Steve Lewis** 09:02
Is it fair to say that your philosophy is the combination approach of, maybe the Anti-PD-1 coupled with the, I guess, upregulation of macrophage attacks or otherwise? Is that what, essentially your lab is looking at? How can we modulate up certain protein expression and down regulate others?

**Felipe Galvez-Cancino, PhD** 09:28
Yeah, to some extent. So we want to go into new things. So PD-1 and PD-L1, we look at it from a point of view as standard of care therapy, because that therapy that is approved by the FDA is used in the clinic, but yeah, we want to find new things. So, in our understanding of phagocytosis, so we know that macrophages are in the tumor, we know we can target them against the tumor, but we know that they are not very efficient at killing the tumor. So we want to find new molecules or new proteins that are expressed on the macrophages. Or we want to understand which cell types that surrounding them and interacting with them are suppressing the phagocytic capacity and target those mechanisms to make them more phagocytic and make them better at killing cancer.

**Steve Lewis** 10:17
Antibody dependent, cellular phagocytosis?

**Felipe Galvez-Cancino, PhD** 10:20
Yeah. Macrophages will kill very differently from T cells. So macrophages won't see necessarily the cancer cell as the T cells see it. So, the cancer cell will express certain proteins on the surface. One of the most well-known proteins is, for example, CD20. So you can make an antibody that binds to CD20. And antibodies, by definition, are B functional, so they can bind to whatever molecule you want, but they have what we call the Fc portion, or the isotype, and through the Fc portion, antibodies will bind to Fc gamma receptors, which are expressed on macrophages. And macrophages, when they bind to the Fc portion of the antibody through their Fc gamma receptors will emit antibody dependent cellular phagocytosis and will eat the cancer cell.

**Steve Lewis** 11:18
Focusing for hepatocellular carcinoma, it sounds like that's where you're starting, but this kind of a treatment could be used for other types of solid tumors as well, right?

**Felipe Galvez-Cancino, PhD** 11:31
Yeah. So the question is, okay, so I know that I can redirect macrophages to a cancer using an antibody, but then I think there is a very fundamental question here, which is, if we look at the tissue, in this case, a tumor, but it can be applied to any tissue, and we look at all the cells that live in that tissue, and we ask, "Which one of these cells is the most phagocytic?" We really don't know which one is it. We know macrophages are phagocytic, but you have different types of macrophages, as I just said. You have this embryonic derived, this monocyte derived, but then you have others, and we really understand very little about the phagocytic capacity of these cells. So then that's kind of the

fundamental question. But then the question we can use to develop therapies is like, okay, so if we understand which ones are the most phagocytic and we understand how they're regulated, so we we find a receptor or a cell that is modifying their phagocytic capacity, we can probably make new therapies.

**Steve Lewis** 12:36
Walk me through your discovery pipeline for something like that. I assume you probably model it, maybe on a computer, and then work your way in vitro, and then maybe to mouse models beyond that. What is what does that pipeline look like to explore some of those ideas?

**Felipe Galvez-Cancino, PhD** 12:55
When I started my postdoctoral work, we wanted to study glioblastoma, and again, I was very interested on glioblastoma, because, as I said, in the case of liver, the brain also has these two types of macrophages. You have the microglia that arise early during development. They are embryonically-derived from progenitors that reside in the in the brain, and they are macrophages, microglia derived type of macrophage. And then you have a monocyte input, so cells that come from the blood, and they become a macrophage. And then you have this deadly disease that at that time, we knew very little about the microenvironment and things like that. So, within glioblastoma, within the tumor, you have the two cells living together. You have the microglia and the monocyte-derived macrophages, these two types, these two lineages of cells, they reside within the tumor. And we started very naively, so we our original question is, I was in a lab that developed a therapy that was being tested in clinical trials at that time to eliminate regulatory T cells, yeah, in cancer. Regulatory T cells, we know they make the tumor grow, so what you want is to get rid of them. Yeah, that's the concept. So we had this antibody. It was a very good opportunity, because we had the mouse version of the antibody, to testing mouse models, and the human version of the antibody, because the theory was in clinical trials, so we had access to it. I started setting up mouse models of brain cancer, and then we were treating these animals with this antibody to deplete regulatory T cells, and we found that strikingly, anti-T was very good at regulating T cells in the brain, and that around half of the mice were surviving. When we analyzed what was going on, we found that this depletion of regulatory T cells was modulating the killer T cells that kill the cancer, but at the same time, it was making the macrophages more phagocytic. What we did is basically so we found that very interesting, and we knew how to redirect macrophages towards the cancer. So we used an antibody to direct those macrophages that were becoming more phagocytic against the remaining cancer cells. So remember, the antibody was working, but not in all the mice. And what we found is when we combine the two antibodies, so the antibody that directs the macrophage towards the cancer, plus antibody that eliminates regularly T cells, we found that we were curing all the mice in one of the models of breast cancer and in other models from zero responses. So in preclinical models of cancer, you will have variability, so some will respond better than others. So we tested in multiple models, but we always found that increasing the phagocytic capacity of macrophages, by eliminating regulatory T cells and at the same time redirecting them against the cancer, work really well. I believe that sceme can be used as the basis for developing novel therapies. But more importantly, what we found is a regulatory mechanism of phagocytic cells. So we know that T-regs are not just these regulatory T cells. Are not just suppressing T cells, if not, they also control the phagocytic capacity of macrophages.

**Steve Lewis** 16:36
Are you or someone you know pushing the limits of what's possible in the lab? Thermo Fisher Scientific Cell Culture Heros Program celebrates scientists driving breakthroughs in immuno-oncology, cell therapy, regenerative medicine and beyond. Research that can change lives deserves the spotlight, and the scientists doing that work deserve the spotlight as well. I encourage you to visit thermofisher.com/cellcultureheroes, to meet past heroes and find out how to nominate yourself or colleague today. Because science needs more heroes like you.

**Steve Lewis** 17:12
How do you go about designing and then ultimately, I guess, expressing and acquiring these antibodies that you utilize?

**Felipe Galvez-Cancino, PhD** 17:29
I think we are more of immunology lab. So antibodies these days, you can easily, if you know the sequence, antibodies will bind to your target antigen, which is your target protein at the end through the Fab portion. And that's basically a sequence of amino acids. If you know that sequence, these days, it's very simple. So you, you just basically pay a company to do it, and they, 20 days later, you get a bottle in the lab with that antibody. You can make the antibodies, in this case, to engage this antibody induced phagocytosis,, you can make them to not engage with different modifications. So we don't make antibodies in the lab, and I don't want to make antibodies in the lab because, yeah, it's not my main priority. I'm more interested in the biology. Yeah, that's how you bring these antibodies, and it's pretty nice, because, similarly, you can also make antibodies for humans. So just to run experiments in the lab but using human antibodies. So all the sequences these days of most antibodies that have been tested or used in the clinic are available through patents. So you can access that. You find this Fab sequence, this sequence of amino acids, tell the company to make it for you, and 30 days later, you have the antibody in your lab. So it's much easier than how it sounds.

**Steve Lewis** 19:08
Yeah, so de novo gene synthesis, going through the cloning and plasmid prep and expression and all that can be very meticulous work to do on your own, for sure. Do you mostly start at the biomarker and kind of work backwards? So you maybe, do you have, like, a topological design that you're like, “I would like to inhibit this,” and then you go speak to a partner and try to design it and based, based on the concept that you provided?

**Felipe Galvez-Cancino, PhD** 19:46
Yeah, most of the time you use the normal antibody, which is two Fab portions, and the Fc portion, that is the one that engages Fc gamma receptors. So we keep the Fab constant most of the time. But as we're interested in this antibody dependent phagocytosis, we play around with the isotype or subclass, the Fc portion, so we can change that to manipulate the ability of the antibody to engage antibody-dependent phagocytosis. So for example, you will have Fc portions that will bind strongly to Fc gamma receptors and will drive phagocytosis. But you also have Fc portions that won't do it and you won't have any phagocytosis. You can also have Fc portions that will be completely silent, yeah, so you can introduce mutations. You can ask the company to put the mutations and that antibody won't bind at all

through Fc gamma receptors. With the new constructs we are proposing to make, we're working with a protein, yeah, with a guy that knows about these things. We are making these trispecifics, and ultimately we've come up with the idea they test many variants and then we, we asked, we outsource this for to scale it up.

**Steve Lewis** 21:08
Now, when you do the in vitro applications, what, what does that look like within your laboratory? Are you receiving samples and then, I guess applying them to those human cell lines within tubes. Or do you have a different kind of protocol for how you do things in the laboratory?

**Felipe Galvez-Cancino, PhD** 21:32
So, yeah, no, the lab is will do two things. So on one side, we have our pre-clinical testing of our therapeutics or molecules we are interested. So that means orthotopic mouse models of liver cancer. And at the same time, we are working with clinicians here at the John Radcliffe Hospital in Oxford, and we are getting a human sample from real patients with hepatocellular carcinoma. And there we can get the biopsies or the blood. And with the biopsies, what we want to do, rather than tricking them or testing our molecules there, the question is again, so if we look at this biopsy, where are these Fc gamma receptors that define these phagocytic populations. Where are the, where are the most phagocytic populations? How do they look? If we want to understand regulation, we want to identify this phagocytic cell and see who is this phagocytic cell surrounded with. With that which you could hypothesize that those cells are probably regulating that cell. And at the same time this cancer type, we're also interested, not just on the macrophages, we also want to understand the T cells. So we also on the on the samples, we want to see where T cells are localized, if they are close to the cancer cell, and the same are they surrounded by cells that may be suppressing that activity.

**Steve Lewis** 23:00
Very systems kind of focus, not just any one area.  For solid tumors, you have intratumor heterogeneity that is always a challenge. How do you view that in the context of emerging therapies?

**Felipe Galvez-Cancino, PhD** 23:21
It's a big challenge. For sure, I'm up for designing and developing new therapies that can at least increase survival. Yeah, not, not that you sometimes you if you aim to cure cancer, might be too much. But now, if we think of how I could address heterogeneity, or cancer heterogeneity, or intratumor heterogeneity. So for example, these macrophages that will eat cancer, the cancer cells. If I can efficiently make the macrophages eat the cancer, I could first maybe make them eat all the cancer. That's a very unlikely though. So then the other thing we I'm very interested in is to, in this concept of antigen presentation, yeah. So we know that macrophages, certain populations of macrophages, like dendritic cells, are very good at inducing a T cell responses, yeah. And these T cells can then go and kill the cancer. I'm very interested in understanding if these macrophages that eat the cancer can then teach T cells through antigen presentation, to go and kill the cancer, and that it becomes a concept of antigen spreading. So basically, you can target the macrophages to one particular thing on the surface of the tumor cells. They will eat the tumor cell, but then that tumor cell will have many proteins that might be seen by the immune system and those macrophages will teach T cells to recognize those proteins that are also present in other cancer cells. So I work on this before, and I think that can be a

straight to address intratumor heterogeneity. Yeah, because basically you start by attacking one or a few kinds of cells. But as you do that, you teach T cells, these killer T cells, to go and kill the cancer, and as they keep killing, maybe you prime new clones of these killer T cells.

**Steve Lewis** 25:37
It's really advanced conceptualization, as well as technology. As somebody who does work with preclinical and clinical applications, what would you need from research and development organizations? What are some gaps that you might want to see close to help you at your stage in the development pipeline?

**Felipe Galvez-Cancino, PhD** 26:03
With molecular biology, so for example, with the models we are using where we want to transfect in vivo the hepatocytes in the liver, yeah. So your efficiency of transfection is very low, yeah. And we know that you don't want to use a virus necessarily. I haven't followed completely the literature there, but viruses will induce a lot of inflammation, and anyways, how we do it is basically injecting a high dose of plasmids in the tail vein of the animal that then transfect the liver of these animals, and then the animals develop cancer. So I think there, there is a big gap, for example, if you want to study liver cancer, how do we in vivo, modify cells. In general, so you might want to modify brain cells. I also, I try to use some mouse models that also were induced mouse models of brain cancer that were induced by transfecting neuro stem cells, and they were also very difficult, yeah, better transfection systems, better viruses, better transduction systems. Yeah, there are big limitations these days.

**Steve Lewis** 27:25
Moving a bit more into molecular biology techniques that you utilize in your laboratory, tell me a bit about how you leveraged today's molecular technologies in your work.

**Felipe Galvez-Cancino, PhD** 27:39
So, yeah, what we are using in the lab is a mouse model of hepatocellular carcinoma, and this model works by in vivo transfecting hepatocytes. So we do a lot of molecular biology related to plasmid purification. So we have a set of plasmids that you can inject into the animals a through a technical hydrodynamical vein injection and transfect in vivo the hepatocytes of these mice. And that is quite important, because what you do, you basically, you can, in the plasmid you can encode the Cas-9, or guide RNAs, or a just an antigen that you want it to be overexpressed, and then the hepatocyte will get transfected and will express this. So using cloning techniques, you can probably change the whatever you can express anything you want. You can knock out anything you want. So we can easily change the guide RNA, that is in our plasmids, and then knock out different genes, in vivo, in these hepatocytes. That's kind of the strength of the model, and that's why it becomes so relevant. So we are highly reliant on plasmids, cloning, etc.

**Steve Lewis** 29:00
Tell me a bit about your plasmid prep that you do in your labs.

**Felipe Galvez-Cancino, PhD** 29:06

So I did my PhD on DNA vaccines. And DNA vaccines are plasmids. So I spent my whole PhD and undergrad thesis purifying plasmids with midipreps and gigapreps. So I'm an expert on midipreps and gigapreps. And now these days, I think we are mostly doing gigapreps because we need a huge amount of plasmid to transfect in vivo and deliver to animals.

**Steve Lewis** 29:36
I think that's a, that's a perfect example how early in your career can really lead you to even more advanced, advanced research. And I imagine there are quite a few listeners who maybe lament how many maxi- and gigapreps that they do. And may not be enjoying it right now, but I think you're a great example of where you get to go with that kind of work.

**Felipe Galvez-Cancino, PhD** 30:02
Yeah, no, well, at the end of the day, they're not that boring, right? Yeah, no, I used to like it. So I used to also I have a student, so in Chile, our education is very theoretical. So yes, so I had in Uni, I had to learn how it works every step. So remember reading like the protocol from the company, like very thorough when I came to Europe, sometimes students don't know because they're more used to just using the kit. So I remember, like, spending a lot of time teaching the students how actually the protocol works, where you use certain reagents, etc., and until today with my student now, I still try for her to learn those things.

**Steve Lewis** 30:53
That's fantastic, and I think that really speaks to how valuable it is to kind of pass along your expertise and knowledge to researchers and scientists who may not have as much experience or maybe early on in your overall scientific journey. That leads me to another question that we love to ask. Here is, what would you recommend to somebody who wants to follow in your footsteps?

**Felipe Galvez-Cancino, PhD** 31:21
I guess you have to really like it, no? I always tell my students, yeah, the pay is not as great as in other industries and it, you have to spend a lot of time, but it gets very rewarding to make discoveries and to be surrounded by smart people. And I guess here in Oxford, everyone is kind of smart and nerdy in the, in the good way, in the good sense. So you can always have interesting discussions with people. So you have to really enjoy those things. You have to be really drawn by wanting to know things, because otherwise will become a bit painful. So I guess people should take all alternatives, and even if they think they like it and then they don't like it, they should be able to change. But if someone really wants to become a scientist and do like great science, make new discoveries, you have to really like it and enjoy it. It's going to be very frustrating, for sure, but when it works, it gets very rewarding.

**Steve Lewis** 32:39
That was Dr. Felipe Galvez-Cancino, group leader at the Center for Immuno-Oncology at Oxford University. If you would like to learn more about Philippe's research, as well as that of other Cell Culture Heros, visit thermofisher.com/cellcultureheroes. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti and Matthew Stock. Join us next time for more fascinating discussion about the wide world of molecular biology. Until then, cheers and good science.