Podcast: Immunity by Design

Episode Title: *Decoding T Cell Recognition at Scale* by Dr. Stephanie Gaglione

Host: Dr **Dr Hashem Koohy**

Duration: 47 minutes

Transcript

Dr Hashem Koohy

Welcome to Immunity by Design, where we speak with leading scientists, biotech innovators, and policymakers about how disruptive technologies are shaping the future of immunology and opening new ways to detect the rules of immunity. My name is Hashem Kouhi, and I'm delighted to host this service. Today, we will be discussing a seminar paper published in Immunity, titled Scalable TCR Synthesis and Screening Enable Antigen Reactivity Mapping in Waitiligo. To briefly highlight the importance of this work, the ability to map T-cells to their target antigens is a central challenge in immunology. It holds enormous promise for both fundamental discovery and translational applications. However, the vast diversity of T cell receptor repertoire combined with the combinatorial complexity of antigen space makes comprehensive experimental mapping extremely difficult. Computational approaches have long been expected to help bridge this gap, yet progress has been very limited, largely due to insufficient high-quality data. This study from Professor Michael Birnbaum's group at MIT introduces a breakthrough strategy that could enable the generation of large-scale, high-quality data sets, potentially paving the way for more generalizable computational models. I am honored to be joined by Dr. Stefani Gaglioni, the first author of this seminar paper. Stephanie, thank you for joining me. It's a pleasure to have you on board on this podcast.

Dr Stephanie Gaglione

Thank you, Hashim. Delighted to be here.

Dr Hashem Koohy

To begin with, could you please introduce yourself and tell us about your background?

Dr Stephanie Gaglione

Sure. So I'm currently a postdoc in Alex Marson's group at Gladstone. I'm originally from Toronto, did my PhD with Michael Birnbaum at MIT, where I worked on this project. And my

earlier scientific training was actually at the University of Oxford with Omer Dusak and at MIT with Bob Langer. And although I'm a chemical engineer by training, my undergraduate training, my PhD training, I fell in love with T-cell biology while working with Omer Dusak. And working with Michael allowed me to pair the engineering mind with the immunology mind to work on what I've discovered to be one of the most fascinating problems. in T-cell immunology.

Dr Hashem Koohy

That's a fantastic background. You have already had the opportunity to work with some outstanding groups. So with that experience in the back, what gap were you aiming to address in this study?

Dr Stephanie Gaglione

No, it's a great question. So, you know, for the listeners who are less familiar, T-cells are the adaptive immune systems. precision sensors. So each T cell has a unique T cell receptor that recognizes antigens on the surface of cells. And these antigens are short peptide fragments that are presented on something we call MHCs. So when TCRs recognize these peptides on the MHCs, these antigens, T-cells activate, proliferate, and in the case of CD8, T-cells kill. But the combinatorics of these interactions are incredibly challenging. We have up to 10 to the 8 of these receptors per person, limitless possible antigens. And these engagements are, very weak, and each T-cell receptor can recognize thousands of possible antigens. So, some of the main, the fundamental basis of some of the questions in this area are, how can we identify what a T-cell receptor recognizes? And the critical importance of that is so we can understand the mechanistic basis of an immune response, we can see what a T-cell is seeing, and we can manipulate those antigen-specific interactions to design highly targeted therapeutics. So at the highest level, that's the motivation of the work. The vast majority of T-cell receptors and antigens that we've identified, we don't really have a good sense of how they map to one another.

Dr Hashem Koohy

That's great, Stephanie. Thank you. Earlier, when we were discussing the challenges of mapping T cell receptors to their target antigens, you mentioned the enormous diversity on both the TCR and antigen sites. Beyond that diversity, what other technological or methodological hurdles do you think are currently limiting progress in this space?

Dr Stephanie Gaglione

Yeah. So, I'll back up and say one. I wanted to say one thing. It was actually one of your papers, one of your reviews, that was actually a source of inspiration many years ago that I

have been referencing for years. And the 2023 paper where you showed, you know, you had a review where you explained that a group of 100 antigens composes 70% of all the known TCR antigen pairs, that 97% of the antigens are of viral origin, and that we have less than a million unique known TCR antigen pairs, and less than 4% have both the TCR chain information. And so, yeah, I think when I looked at this, we, you know, Michael has been in a large inspiration determining what the core challenge is there. And the core challenge we believe is really screening tools, that the vast majority of screening tools one T cell receptor against many antigens or many T cell receptors against one antigen, but not many versus many. And even more, many of these tools are just not accessible. They're difficult to execute. They require specialized training. So although clinical and research groups have mastered the ability to generate very high throughput TCR sequencing data, it's very challenging to translate that TCR sequencing information into knowing what those T-cell receptors recognize, and it's far easier to focus on known viral antigens due to the reagents that we all have access to.

Dr Hashem Koohy

Given the gaps and hurdles you have just described, what technologies were available prior to this study? I mean, in practice, the experimental strategy often depends on the starting point. Sometimes you are interested in a single TCR and trying to identify its target antigens. Sometimes you are working with a group of TCRs. And in another case, you are interested in capturing all antigen-specific TCRs in a given repertoire. Each scenario requires a different technical approach. However, this different situation typically addressed before this work.

Dr Stephanie Gaglione

Yeah, so I think we can roughly, we can categorize them, right? So on the, many TCRs where we're screening primary samples, we're screening PBMCs from blood. In that context, usually you might use something like tetramers, right? DNA barcoded tetramers. different multimer strategies, dextrimers, and these are exceptional, gold standard, but they are quite difficult to scale beyond the hundreds. beyond hundreds. And even at that point, you have challenges in using them across different MHC alleles. We have challenges with protein aggregation. They can be ordered, but they're very difficult to synthesize on an individual lab basis, even though we can, you know, exchange the peptides out. There's always going to be a bit of a scale challenge there. But that would be a great example of a many TCR lower number of antigen efforts. You can also use peptide-pulsed antigen-presenting cells. Those are phenomenal for looking at functional responses, but can be challenging in the demultiplexing. How do you do them in pool where you can then extract

what the mapped pairs are? Then on the flip side, we have approaches like yeast display or tools where you can use a single TCR receptor against many antigens.

Dr Hashem Koohy

Sorry to interrupt. What number of TCRs here we are talking?

Dr Stephanie Gaglione

Number of TCRs. And so, that was a motivation for a lot of the work of Raptor that was done by, Connor Dobson, Enterseek, V. Karma from Howard Chang's group and Steve Elledge that have been phenomenal efforts at creating these many versus many. And we pinpointed a particular problem as we were trying to execute our proof of concept demonstration of some of these many versus many screens. And that challenge is that it's very difficult to reconstruct T-cell receptors. So, I know when I mentioned that we can screen many TCRs, that's usually from a primary sample. But if you don't have a primary sample, you know, you have a tumor sample or a tissue sample, something that's precious that you can't control. You can't conduct repetitive tremor staining on, or you can't conduct repetitive antigen presenting cell experiments with, or LSPOTs, that you need, you've often opt to single cell sequence it to immortalize that information and extract as much as you can out of those cells, but you can't easily study them. And so the vast majority of groups until this point, for most of us, would select a short list of the TCRs, based on expansion or phenotype or how prevalent they are between donors. And you really have a few choices. You could synthesize that T cell receptor gene for $100 a TCR as a gene fragment. You could manually assemble the TCR from oligos using the variable portions. but you would have to use robotic liquid handling or do it manually, or you could use some pooled assembly approaches for TCR construction, but they're pretty challenging to access. So the summary here is that, you know, many versus many challenging, It's even more challenging when you have rare samples that you can't easily screen without some way to go from sequence to a screenable reagent.

Dr Hashem Koohy

Yes, thank you. That makes sense. Going back to your work, one of the strengths of your work is the way you address scalability without compromising efficiency. while still keeping the cost remarkably low, as it is demonstrated to your robust validation. So my question is, how do you achieve that balance in practice?

Dr Stephanie Gaglione

So I think the way we viewed it is that this paper for us is a demonstration of an end-to-end workflow, where you can take TCR sequences, reconstruct them, functionally screen

them, and link that specificity to phenotype from the single cell data. And so that workflow is something we see as being a foundation for a deeper and future efforts to do more expansive antigen screening and to link specificity to function, you know, for a broad number of samples. But this really is sort of the first demonstration we think of a, you know, a good foundation to do that type of high throughput screening and even more so to introduce an accessible tool for reconstructing TCRs that's high fidelity, that is maximally accessible and that's very scalable. So I can talk a little bit about how we decided to construct that approach and why, you know, why we embarked upon it. Yeah. Yeah. So I think we can maybe we'll talk about Tea Craft a little. So that's sort of the first part of the paper.

Dr Hashem Koohy

Oh, yes, exactly. For people like me with a theoretical background, this elaboration will be very useful.

Dr Stephanie Gaglione

Yeah, sure. So just as a little backup for a second there. So T cell receptors have variable regions, constant regions. You have two chains, the beta chain, the alpha chain. Each one consists of a variable and a constant region. Those variable regions, there are 45 on the alpha side and 48 on the beta side. And there's also a unique region for each TCR that we call the CDR3. So to think about this as one linked construct, you have a TRBV, meaning the variable beta. You have this unique CDR3, as we call it, that goes with that variable chain. Then you have a constant. And then you have the exact same construction on the other chain. So this is something where there is regions of moderate variability, something that is completely unique to each and every individual TCR, and then a constant region. And so when we were thinking, we need an approach to be able to reconstruct these vitiligo TCRs that we were interested in looking at that doesn't require us to reconstruct this entire, you know, long fragment all at once. Instead, we can leverage just the variable portions that are conserved and the constant reagents without having to reconstruct those every time we wanted to make a TCR. And so we established some base design criteria for what we wanted out of this tool. We wanted it to be low cost, so something that was less than a dollar per TCR, something that's easy to execute and freely available so that, you know, beyond us, any group can reconstruct a library of TCRs using common reagents. We wanted something highly scalable, so in the thousands of TCRs, and compatible with antigen screening assays, sequencing workflows, just with some thoughtfulness about how these TCRs might be used downstream. And so to do that, you know, there have been a number of phenomenal previous papers that have shown the utility of using these variable regions as reproducible reagents. And Our critical note was that we could order

both of the CDR3s in a single oligo and use this highly variable piece that's unique for every T cell receptor. By encoding it in one oligo, we could then do a two-step, relatively simple golden gate reaction where you take that oligo and it matches with the correct beta variable, and then you do a second one that matches it with the correct alpha variable. And the simplicity of this was that by putting both of those CDR3s, both of the pieces that are unique to a TCR in one oligo, you don't need to do any kind of pairing step to create what one TCR is. Versus if you had, you know, if you had separated them, you would have to do some kind of first step to go, okay, which of the beta CDR3s goes with which of the alpha CDR3s. And that creates complexity that can be challenging on the scale that scales with the number of TCRs you're reconstructing. So to bring that back out, to simplify it, essentially the innovation here for us was recognizing that we could set up a very simple system that does not scale with the number of TCRs you put in. that every single reaction looks the same, uses the same overhangs, has the exact same composition, whether you have 1000 TCRs or a million TCRs, that from the perspective of the Golden Gate reaction is the same. And so that was, I think, the moment we said, okay, this is, you know, this type of strategy makes sense. And the way we did it was using, you know, orthogonal overhangs that we designed using NEB's publicly available ligase fidelity data, where they show how four-base overhangs interact with other four-base overhangs. And that proved to be a phenomenal resource for identifying the way we would pair each of these CDR3s with its appropriate components.

Dr Hashem Koohy

That is excellent. So in this way, you are able to capture a substantial number of antigen-specific TCRs, each linked to their respective target antigens, and that is across a broad panel of antigens. But then the question is, where does the cellular phenotype come into play? Because as we both know, that is also a critical component of the overall picture.

Dr Stephanie Gaglione

Yes. Yeah, that is exactly where we were headed with this. The goal of this work at its core before we devised the technological advancements that would help us execute it were to be able to de-orphanize TCRs to then use that as an ID that you can look back at single cell data and say, okay, this particular T cell recognized this antigen. Can we learn anything from the populations of cells that recognize different antigens? And as we went through the effort of executing Raptor, we found the vast majority of T cells. And at least in vitiligo here, we're recognizing MR1, which in some ways wasn't overly surprising. But you know, when you go fishing, sometimes you find what, you find the data is what it is. You find what the biological truth was in the situation. And so what we were looking for was what is the T-cell state of these antigen-specific cells that might be driving vitiligo? We found that the vast

majority of them were in a cytotoxic state, that they had high levels of granzymes, that they generally adopted a tissue resident memory phenotype, and that we could easily identify which T-cells were Mart-1 reactive by looking at the variable regions that they had.

Dr Hashem Koohy

And these are all in this vitiligo system that you were showcasing the model, yes.

Dr Stephanie Gaglione

Yeah. So that's, you know, that is essentially what we managed to do with the data.

Dr Hashem Koohy

Yeah.

Dr Stephanie Gaglione

But it was, yeah, there were some takeaways from it, which were that from our efforts, the vast majority of, not the majority, but a lot of the clonally expanded cells did recognize MART-1 that we could, in contrast with previous efforts, in a unique way, having profiled all of the TCRs in the sample, not just the clonally expanded ones, we could at least confirm that the clonally expanded ones were indeed driving the response. Perhaps not overly surprising for me, in thinking about it, but something that has yet to be validated in the data in the same way that we can if we reconstruct every TCR from tissue. But it also enabled us to demonstrate that we were capturing, you know, majority of the clonally expanded ones that were reactive to at least HLA-A2, you know, binding antigens.

Dr Hashem Koohy

How interesting. I found the phenotypic commonality you report between T cells in cancer and autoimmune disorders particularly intriguing. I have often thought of these two settings as almost opposite sides of a coin. In cancer, T cells can become trapped and functionally dampened by the tumor microenvironment, whereas in autoimmune disorders, T cells seem to became hypersensitive and dysregulated. Given this perspective, could you elaborate on how you interpret this apparent overlap in phenotype? What do you think it tells us?

Dr Stephanie Gaglione

Yeah, so I think that this part of our work was a little bit surprising and, you know, leads to some open questions. On the one hand, we examined autoimmunity because we feel it's an area of major clinical need. These are samples that could not be easily examined because of tissue localized T-cells and that antigens are often shared between patients.

We also noted that vitiligo often indicates a positive prognosis in melanoma treatment. And we know that there's a link in antigen specificity between the diseases. from some of the foundational work by Rosenberg and others that were characterized MART1 and GP100. But looking at the signatures, it can be challenging to say how the cells behave, both their cytotoxic, there's some very similar effect or T-cell programs, there's overlapping antigen targets. And this leads to some questions that I don't have good answers to, but we do think that some of this distinction could be in not necessarily in the effector programs, but in tissue, immune tissue specific tolerance, tumor immune suppression, that there could be a similar response, but the cells in that environment are impeded by something related to antigen presentation persistence. the ability of T-cells to respond even in this immunosuppressive environment. There's something about the environment that's determining how the T-cells have a functionally different outcomes on disease, but yet display sort of, yeah, similar cytotoxicity. And even though we detected some slight differences between them. So, you know, for example, in melanoma tumor, reactive T-cells tend to be have Lag3 up antigen processing components, HLA2 genes, whereas the vitiligo reactive T cells had some upregulation of TCR signaling components and IL-7 receptor. But overall, yeah, we were surprised by that. But we also wanted to say, you know, we're very grateful to Kathy Wu and Jacomo Olivera for sharing, you know, these signatures. from antigen-specific T-cells in melanoma that allowed us to do some of this comparison at this preliminary stage, at least in providing A roadmap for how we might all in the future. consider looking at relationships between diseases using the specificity as relevant information. But yeah, Ashim, we don't really have a great answer to that question. And I think this is a, just an initial effort to see what you can learn from signatures and whether there's some biological significance there.

Dr Hashem Koohy

Well, absolutely. I mean, it's really important, you know, when it comes to understanding rules of T cell response. to take some first disease specific strategies to find out, what of those rules perhaps are disease or context specific, but also look into the commonality between, different conditions and different diseases. And yeah, I'm a little bit, going back and forth, but one question I remember when I was reading your paper and thinking about your technology, In this paper, T graph, you basically capture thousands of T cells with about just more than 100 antigens, yes.

Dr Stephanie Gaglione

So we used Raptor for 101 antigens, very limited snapshot, A2 only. And then we also used the same TCR library using peptide-pulsed antigen-presenting cell, T2 cells, as an orthogonal way, not only in validating, but our our integrated Raptor TCraft approach was

pulling out the TCRs that we thought we were as a validation means, but also as an approach that we think others will likely use these TCR libraries for. But yeah, there are some limitations there and we are very cognizant of that.

Dr Hashem Koohy

Sure. We will come back to limitations shortly. But before that, I would like to explore something related. How does the type of data generated by your approach compare to other techniques, such as dextromer-based strategies that capture antigen-specific TCRs at the single cell level alongside gene expression or even proteomics for a number of antibodies? And therefore, my question is, from the perspective of training computational models, what would you say are the key similarities, differences, and perhaps the pros and cons of these different data modalities?

Dr Stephanie Gaglione

Sure. So at the basis, TCraft is great for reconstructing TCRs. And so that means that you could reconstruct these TCRs, create, put them in primary cells, or put them in put them in an immortalized jerk hat library. And the advantage of that is that each clonotype is now represented roughly equally or so, right? It's no longer just whatever's most clonally expanded in a primary sample, and even more so what you can't get from a tissue. Then you can take those TCR libraries and screen them. So we used Raptor completely correct on, you know, on 101 antigens as our first pass here. But you could equivalently use dextromers or tetromers or other, I know, the diverse of approaches that have been constructed to do TCR antigen mapping. One of the principal differences, and I think there's actually two principal differences. One that many of the dextromer and tetromer based approaches are binding based. They're identifying what binds a TCR, not necessarily, and it is a subset, an important subset, not necessarily what activates, what generates A functional response. And the literature is ripe with examples of TCRs that bind to an antigen, but don't necessarily, aren't necessarily activated by that particular PMHC. And we're still learning to understand some of the rules of those engagements. But the approach that we took here using our PMHC pseudotype viruses, these viruses are potent activators of our TCR expressing cells. And so that allows us to capture activating events that they TCR and interacting with this antigen pseudotype virus, that TCR internalization that results from that engagement is what results in a permanent encoding in the genome. And that was, you know, the innovation that Michael and Connor Dobson brought to the table in constructing Raptor. And we feel that Raptor is a foundation for significant future expansion, where dextromers and tetromers may hit the limits on the thousands of 10s of thousands scale, even though this paper doesn't directly address that, it's the future basis that this is a tool that can theoretically scale beyond hundreds, and that groundwork was

what will allow, at least in principle at present, this type of approach to become the become the first step in an eventual tool that will be able to do the many on many, 10s of thousands versus 10s of thousands in a way that will probably not be easily achievable with dextromers in the near future. So I'm going to summarize it, say, you know, binding versus activation, and it's a foundation for the future of scaling beyond hundreds into that unbiased antigen space that we're all so keen to finally work on.

Dr Hashem Koohy

So going back to the limitations you outlined here in the paper and also briefly mentioned here, the approach is currently demonstrated with a single HLA and roughly 100 antigens. While, of course, 100 antigen is already a substantial step forward, A broader challenge in the field is that these technologies typically require us to define the antigens a priori. In many real life settings, however, we don't actually know what the relevant antigens are. So the question is, how do you think about this limitation and how do you envisage addressing it moving forward?

Dr Stephanie Gaglione

Yeah, great, great question. I think very timely. I do think that is the core next piece of where the TCR antigen discovery world is headed. I think there's many groups, you know, converging on these core questions of how do you screen any TCR that you can find in tissue against, you know, a proteome-wide potential of what antigens could be presented. And so, even though this paper didn't I wasn't able to touch on that core question, and we focused on an autoimmune disease where we do have some ground basis of what antigens might be presented, and we could effectively screen against known melanoma antigens as well. You know, we did make an attempt in our screen of hundreds of antigens using peptide-pulsed APCs to screen genes that were upregulated in skin, And we found almost no hits there. But I think that speaks to just the scale that's really required, that this isn't going to be something we're solving by, some incremental advance into the hundreds of antigens, but something that's going to require screening in the thousands, 10s of thousands, potentially millions of antigens in order to be able to do that unbiased screening. And so we expected and hope that the groups that adopt TCRAFT and in our strategic design to make this accessible, that many groups can use this with other immunopeptidomic approaches to nominate good candidate antigens, to screen, at least begin screening those using both Raptor or other phenomenal antigen discovery approaches, but also that there will be some future advances in this realm. And also just, to add that I do think the field as a whole is challenged by, as far as data, is challenged by the diversity of different types of data and the difficulty in data science to understand where that data is originating from. Is it binding data? Is it activation data? Was it generated

in primary cells or not primary cells? You know, as an experimentalist, some of these differences seem obvious in how they might alter what the final data output is. But if we're using this data collectively to model TCR antigen interactions, you'll run into some inconsistencies where, you know, one data set shows that a certain antigen pair is a positive, whereas another data set will show it's a negative. Or this is a binding data set. Do we use binding and activation data sets together? Do we, you know, how do we consider what true and true positives and negatives are. And I think that's something the field is just starting to grapple with. So, even though in our paper, we feel that we use some orthogonal methods so we could truly define that our hits, our positive hits were positive and our negative hits were negative insofar as the particular candidate set of HLAA2 binding peptides were, it's just scraping the surface of this of this question of how do you define what a true positive and true negative is to produce good quality data for models of TCR antigen interaction, which I think is something, you know, that's of interest to you.

Dr Hashem Koohy

Yes, absolutely. As we discussed earlier, the diversity in both TCR sites and antigen space is huge, far beyond experimental mapping of T cell receptor to target antigens. Of course, in silico, strategies has long expected to come to rescue, but the success has been very marginal, largely due to lack of data, both in terms of quality and quantity. Now my question is, from your point of view and under the light of your technology, how do you see the role in silico strategies can play here?

Dr Stephanie Gaglione

Great question. I think at the baseline one, it's improving the diversity of what TCRs and which antigens we're studying. So the vast majority of data is HLAA2 for a particular allele. The vast majority of data is viral, and not just viral, but predominantly against the same EBV, CMV, and flu epitopes. Because due to reagent availability, that is often the first set of antigens that we screen for when we look at TCR data sets. So at a baseline, it's can we vastly expand with paired TCRs the number and type, the number and diversity of TCR antigen pairs to generate, you know, a stronger base data set of TCR antigen pairs that can be used, not just positive, but also negative, what TCRs do not bind in constructing better models. And the second piece of it, and I think something that I've learned and haven't overlooked in working with the phenomenal team that put together this paper and with the counterparts that I've met in groups that do modeling, is that to generate good quality data requires accessible tools. And what that means is it can't be one group that's trying to build all of the TCR engine data that's ever existed with a, you know, with a tool that no one else can use. That the large, to me, the large part of the success of the dextramer tetramer story is that any group can do it, that you can order these reagents, that you can use them, you

can generate, you know, high-quality data without a steep learning curve, and so the point of... particular excitement for us in building this paper is that we've given, we've given TCraft to a handful of groups right at the outside who were able to execute it without any type of special training. And what that means is that if other groups can easily use these types of tools, they can generate at scale, we can collectively generate better data and at scale in a way that will allow us to build, you know, deeper data sets beyond what a single group's going to be able to do. So I think the answer to that the question is twofold. It's one, accessibility. So many groups are generating data. It's a collective effort where if it's easy to screen for TCR antigen pairs, then we will build deep, large data sets. And the second being that if we build tools to enable comprehensive screening on the antigen side and on the TCR side, that's where the truly diverse data that's going to inform better models will come from, not not varying a single TCR, 100,000 different ways and varying that antigen, 1000 different ways and looking, even though that does generate, a deep data set of that interface, it's not really helping with this core question of how do you take a TCR that has a sequence that doesn't have an antigen that we know and map it to, you know, a completely unknown side on the antigens, even though we've started making progress in that direction, of course, with TCR Dest and Glyph, that it is pretty difficult to do without truly diverse TCR energy data.

Dr Hashem Koohy

Well, Stephanie, this is clearly a topic I found deeply engaging. As we are reaching to the end though, what three key insights would you highlight from this study?

Dr Stephanie Gaglione

Great, great question. Three key messages. I think we would say one, that we can build, we can reconstruct TCRs from sequences accessibly and inexpensively, and that this tool will enable any group to reconstruct rare, you know, T-cells from rare samples for further study. Two, that this can be integrated into a, you know, complete workflow where you take TCRs, examine them functionally, and link that data back to the back to the phenotype using single cell data, and three, that in examining vitiligo, we were able to derive signatures and look at dominant V-region usage across antigen-specific cells. connect the data to what degree those cells were clonally expanded, identify an interesting and, leading many open questions, correlation between signatures in vitiligo and melanoma, just showing what this type of data can be used for. And so the big takeaway, I think, for me at least, is that we have produced an adoptable workflow for others and a foundation for deeper future work on TCR antigen pairing that is accessible to the community.

Dr Hashem Koohy

I should say congratulations. You guys have already laid strong foundations with this work. Earlier, you spoke about the importance of scientific collaboration and multidisciplinary approaches. And in that context, you also mentioned the Matchmaker Consortium, which is one of the largest and most systematic efforts to unravel T-cell recognition. More proudly, reflecting on this project, what did you enjoy most about working on it?

Dr Stephanie Gaglione

I'd say working, the people that I was working with. So this was not an individual effort, and that is true of, I think, almost all modern great science today is a team effort. And so TCraft, I did not develop this alone. It was developed with, co-developed with Rakhum Kamala, a phenomenal, at the time, undergraduate who's now an excellent PhD candidate. And I think from that, you know, great science can come from anywhere, and we were very much a dynamic duo in building. building that approach out. The single cell work was largely spearheaded by Chirag Growa at Pfizer. There's such a diverse set of skill sets and capabilities that are required to put together projects like this one, and I think that was rewarding. Learning from my peers, having the creativity to sit around a whiteboard and pinpoint what the problems were, to solve them, to meet others and say, what are your pain points? Can we solve them? I think we're very much brought an engineering mindset to this, and it was brilliant to spend time working with. as colleagues and as co-developers, tools that we're excited to share with the community. I think that for me is the most exciting piece of it.

Dr Hashem Koohy

Very good. And similarly, what was the thing that you perhaps disliked the most, if it was any?

Dr Stephanie Gaglione

Oh, wow, that's actually quite a hard question. I think the PhD life cycle is tough, right? In fact, it is surprising how much of the work that we put together here was done within a year, right? It was five years of laying the dominoes, so to speak. And, you know, we had been screening the clonally expanded TCRs. We had reconstructed genes, the old-fashioned way of ordering them as gene fragments. We had spent time trying to troubleshoot how Raptor could be used at the scale of this number of TCRs with a good degree of sensitivity, there's a lot of failure, right? And I think you can have a long time where you go, I don't know if any of this is going to work out. I have a vision that these pieces will slot together. I'm working together with the most phenomenal people to execute this. Will the pieces slot together? Is there going to be any reactivity that we're going to detect here? In some ways, I think tool development is difficult because if your

tool doesn't work, Nobody really cares. But if it, when it, if it does, you're hoping that you can find something and apply it to a great context. And Vitiligo is a good first application. But we weren't, we weren't 100% sure what we were going to find. And I think a lot of that uncertainty can be very difficult when you're in training and you're five years into your PhD and you're working with a great team, but you're not so sure that the pieces you have are going to slot together. But once you have that moment and those pieces are there and you can see how they're going to stitch together as a team, you go, okay, this is the moment, we're going to lock in, we're going to execute and bring this to the finish line, that I think that was the turnaround moment.

Dr Hashem Koohy

You just mentioned how often things feel like familiar setbacks until a breakthrough finally comes along. I would like to ask you what advice you would give to a PhD student who so often feels that nothing is working and everything is going against them. I usually try to remind them that setbacks are part of the process, that if you are not failing, you are probably not pushing the boundaries. And if you are not pushing the boundaries, you are not growing. So how do you think about this?

Dr Stephanie Gaglione

Yeah, and that's a great point. I think it's, it is tough in that phase. Every phase had its own separate difficulty, right? There's the stress of trying to put your... put your work into a thesis or, collectively get your data together in time to put a paper out. But I think for some of us, the stress is tougher in that middle phase where you're just going, I don't think, I don't know if this is going to work. There's no taking a bad question and making it into a good product, right? And sometimes you go, I don't know if I've approached this question correctly or if I have the tools that I need. And I think it was, you know, I think it was, it's the the people that I was fortunately working around who were, we were all in it together. And there's something about camaraderie in some of this, in this suffering. And there's something about just having a, having a bit of grit and persistence through that final phase where you, you've got to have just enough optimism that you know when, you know, that you have a sense of when things will work out. But also that how to let go when things aren't working. So I think during the journey of this, there were one or two moments earlier on where I'd abandoned a project I'd spent two years on. I had not, we weren't working at all on this TCR assembly work until I had met RAC and this, phenomenal scientist. We sat down and we said, okay, I actually think together we can, we can do this. is not something that was on the horizon. The project did not involve this. But when we sat down and said, you know what, I think it's worth killing this other project to focus on this because I think we can get this done. And when we stitch that together, it will, it will result. So it, and a long-

winded answer here. but perhaps it's the balance of shrewdly knowing when things are not working, it's okay to let them go. And when you see the, when there's no, when there's less resistance in some categories of your work, you meet great people, you have some momentum, not being afraid to pursue that and let go of those other strands that might be holding you back. So it's a bit of luck, a bit of willingness to push through those tougher times. But it's also the knowing when to let go and when to pursue different threads that I think brought this project together as a.

Dr Hashem Koohy

Team. Thank you, Stephanie. I'm afraid this is all we have time for. Any final thought or comment to conclude with?

Dr Stephanie Gaglione

I think I just want to say that we should all be watching this space, looking at how T-cells recognize, and even just other immune receptors, that this is an exciting time to be working in this area, that I think we're on the cusp of being able to design de novo. excellent binders to antigens of interest and that there's a lot of open space for innovation here. And I hope we can all collaborate together to solve some of these big outstanding problems.

Dr Hashem Koohy

And with that, I hope you enjoyed this conversation as much as I did. A sincere thank you to Stephanie for sharing her insights and thank you for listening. I look forward to having you join us for the next episode of Immunity by Design.

Note: This transcript has been lightly edited for clarity and readability.