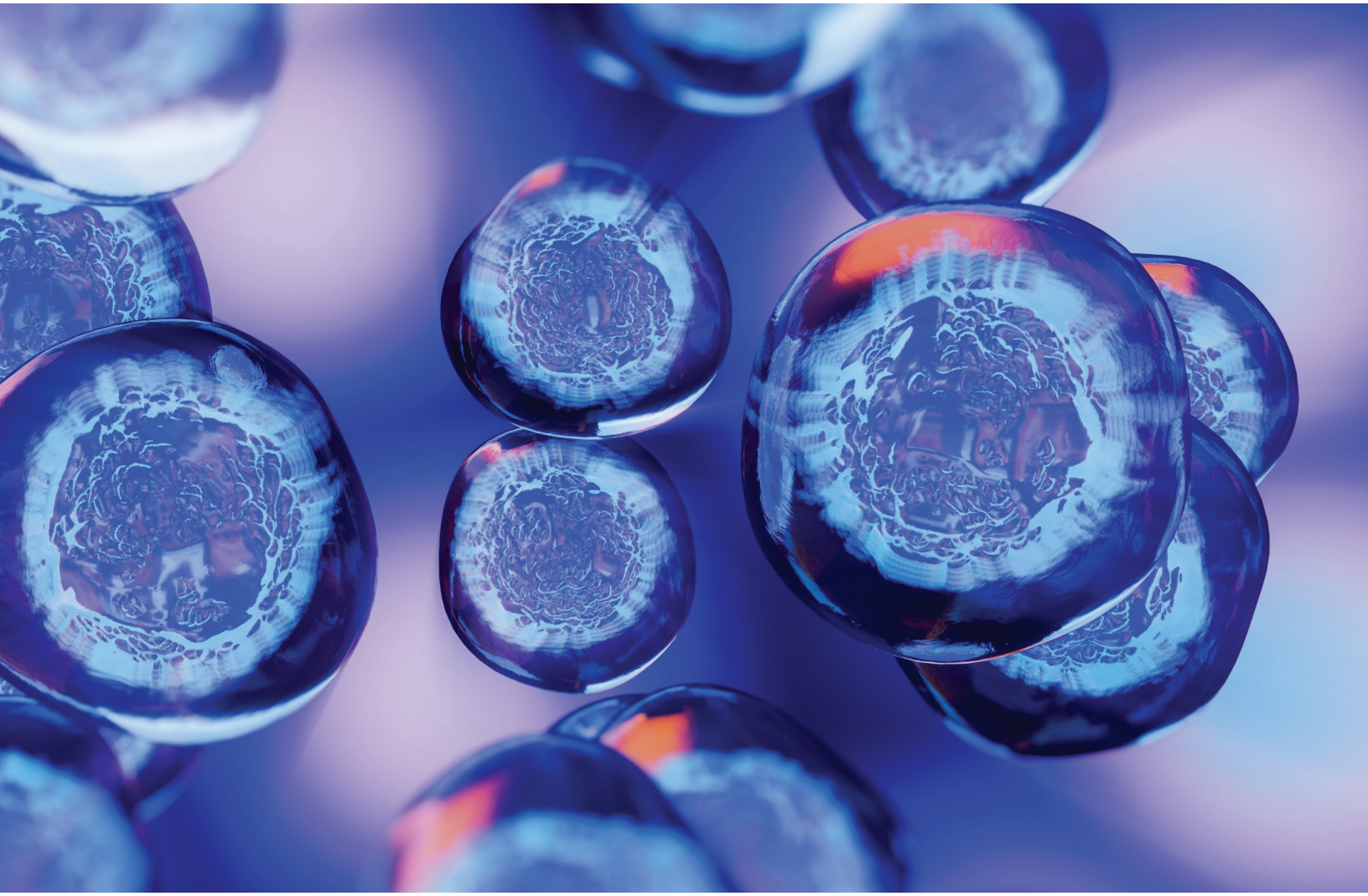
ROUNDTABLE

# Understanding the Role of Polyfunctional Immune Cells in Cancer and Other Diseases



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# Understanding the Role of Polyfunctional Immune Cells in Cancer and Other Diseases

Rare subsets of superpowered immune cells have the ability to execute diverse functions in orchestrating the immune response. These cells can coordinate defenses against a variety of diseases, or conversely, drive inflammation, toxicity, and disease progression. The capacity to decipher dynamic shifts in the proteomes of these superpowered polyfunctional cells provides granular insights that could help develop new cell and gene therapies and biomarkers for diagnosis or assess disease progression and therapeutic efficacy.

This roundtable highlights how scientists are mining single-cell proteomic data to identify and understand dynamic proteomes in high-functioning immune cells and their immune impact. Our expert panel will discuss the development of new cell and immune therapies that target tumors, based on the insights gleaned from proteomic profiles of multifunctional immune cells. In addition to tumor cell heterogeneity, the unique features of the tumor microenvironment, which are playing a critical role in developing effective immunotherapies, are explored by the panel.

*GEN's* Editors

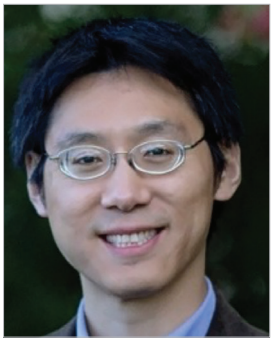
## Seth Pollack, MD

Director of the Sarcoma Program at the Lurie Cancer Center, and the Steven T. Rosen Professor of Cancer Biology at the Feinberg School of Medicine at Northwestern University

Seth Pollack, MD is the Director of the Sarcoma Program at the Lurie Cancer Center and the Steven T. Rosen Professor of Cancer Biology at the Feinberg School of Medicine at Northwestern University. As a physician-scientist, he is developing novel immunotherapies for patients with advanced sarcoma. His work targeting the cancer-specific protein NY-ESO-1 using adoptive cellular therapies and vaccines has led to important breakthroughs in our understanding of the sarcoma immune microenvironment. These discoveries have shaped dozens of clinical trials, each with the potential to impact patient care. As a clinician and sarcoma expert, he has twice been featured in the widely followed audio/CD/podcast series *Research-to-Practice* and he wrote the invited review on sarcoma immunotherapy for the *Journal of Clinical Oncology*. He has chaired sessions at the annual meetings of the American Society of Clinical Oncology (ASCO), the Japanese Society for Medical Oncology (JSMO), and the Connective Tissue Oncology Society (CTOS), and gave the keynote talk at the 2019 Annual Meeting of the British Sarcoma Group. He is on the National Leiomyosarcoma Foundation executive committee and sits on the NCCN guidelines panels for both soft tissue sarcomas and gastrointestinal stromal tumors. He received the Sig Kohl Legacy award from the Northwest Sarcoma Foundation. He is now thrilled to be at the Lurie Cancer Center serving the sarcoma patients of Chicago.

## Rong Fan, PhD

Professor of Biomedical Engineering, Yale University
Professor of Pathology, Yale School of Medicine



Rong Fan, PhD, is Professor of Biomedical Engineering at Yale University and of Pathology at Yale School of Medicine. He received a PhD in chemistry from the University of California at Berkeley, completed the postdoctoral training at California Institute of Technology, and then joined the faculty at Yale University in 2010. His current research interest is developing microtechnologies for single-cell and spatial omics profiling to interrogate functional cellular heterogeneity and inter-cellular signaling networks in human cancer and the immune system.

Dr. Fan was a co-founder of IsoPlexis, Singleron Biotechnologies, and AtlasXomics. He is the recipient of a number of awards including the National Cancer Institute's Howard Temin Career Transition Award, the NSF CAREER Award, and the Packard Fellowship for Science and Engineering. He was elected to the American Institute for Medical and Biological Engineering (AIMBE), the Connecticut Academy of Science and Engineering (CASE), and the National Academy of Inventors (NAI).

## James R. Heath, PhD

President and Professor, Institute for Systems Biology

James R. (Jim) Heath, PhD, is President and Professor at Institute for Systems Biology in Seattle. Heath also has the position of Professor of Molecular and Medical Pharmacology at UCLA, and he has directed the National Cancer Institute-funded NSB Cancer Center since 2005. Formerly, he was the Elizabeth W. Gilloon Professor of Chemistry at Caltech, and served as co-director of the Parker Institute for Cancer Immunotherapy at UCLA until 2017.

Heath received his PhD in 1988 from Rice University, where he was the principal graduate student involved in the discovery of C60 and the fullerenes—work that resulted in three senior members of his group winning the Nobel Prize in Chemistry in 1996. He was a Miller Fellow at UC Berkeley before joining the research staff at IBM Watson Labs in 1991. He took a faculty position at UCLA in 1994, and moved to Caltech in 2003.

He has received several awards and honors, including the Irving Weinstein Award from the American Association of Cancer Researchers, and the Sackler Prize in the Physical Sciences. He was named one of the top seven innovators in the world by *Forbes Magazine*.

Heath has founded or co-founded several companies, including PACT Pharma, Integrated Diagnostics, Indi Molecular, CTI Molecular Imaging (acquired by Siemens in 2005), Sofie Biosciences, IsoPlexis, and NanoSys.

***GEN:*** Single-cell functional proteomics has revealed unique subsets of highly functional immune cells in a variety of published studies. What does it mean for a cell to be functionally "superpowered," and what is the impact of these cells on the overall immune response?

**HEATH:**

This goes back to some of the first work that we did on looking at the polyfunctionality of immune cells. When we talk about the functionality of immune cells, that functionality is best measured in the proteins that are secreted by that cell, i.e., those proteins are functional. They can recruit other cells, promote inflammation, and promote proliferation. So, when you capture a lot of proteins that are being secreted from an individual cell, you can ask how many functions that one cell has and compare it to the population of cells.

What we found early on is that the cells that had the highest numbers of different functions, meaning they were secreting multiple different types of cytokines, were also secreting by a lot the largest absolute numbers of any of those given cytokines. What that tells us is that even though these are the most functional cells, they may only be a handful of five or ten percent of your population by almost an order of magnitude. Yet, they're actually dominating the functional immune response.

So they're a small fraction, but their footprint is a hundred times larger than the other cells. Being able to capture those cells in many ways, depending on the question you're asking, allows you to determine the status of the immune system at that moment.

This was an enlightening moment for us because this was back early in the days of what's now called multiomic analysis. We found that those polyfunctional T cells, if tracked over time, were pretty accurate reflectors of how patients were responding to, in this case, an adoptive cell transfer cancer immunotherapy.

Rong can talk about this work as well because he was involved in those early days as my postdoc. We did not have access to a lot of other metrics. We looked at cell phenotype, which is the standard thing you do with flow cytometry, but that didn't track response. We looked at plasma proteins that also didn't truly track response.

But with the polyfunctionality of T cells, you could follow patient responses pretty well with that, which goes to tell you that you're looking at the functional status of the immune system, at least in that T cell compartment.

**FAN:**

Polyfunctionality or polyfunctional strength index has turned out to be a very useful clinical index and potentially could be developed to predict patient outcome. I rather like the superhero cells terminology Jim defined. However, different superheroes, like Aquaman and Iron Man, have different superpowers and it seems that the polyfunctional strength index or polyfunctionality also comes in different flavors. For example, my laboratory has been looking into the question of who can respond and who cannot respond to a CAR-T therapy even before the therapy is given to the patients. Polyfunctionality of all the CAR-T cells together predicted the initial response in patients, but the polyfunctionality of a specific subset of CAR-T cells turned out to be more critical in terms of long-term remission versus relapse in five years.

Single-cell functional proteomics technology allows you to look at the different flavors of CAR-T cell function or the different types of superpowers they have, which can give you an unbiased view of the functional landscape of CAR-T cells, thus facilitating preclinical development of novel T cell therapies. I think that will also be transformative down the road when you are applying these tools to either pre-infusion product characterization or clinical patient monitoring.

**POLLACK:**

We've been using this single-cell proteomic assay to look at the changes that happen in patients' blood and in the T cells that infiltrate their tumors over time as they change with different treatments. So we had one trial where we

were injecting a toll-like receptor agonist into patients' tumors. We were characterizing the changes that happened in our good responders in comparison to those that did not respond so well. It's informative when you see the changes in these intracellular cytokines. For a long time, people have been interested in characterizing immune cells, and the best way we could do this was by using cell surface markers. That's the classic way that you characterize immune cells. But when you're looking at the functionality with respect to the cytokines that the immune cells make, you're looking at something integral to the way the cells function.

In the past, we've done a type of flow cytometry called intracellular cytokine staining. We could look at one, two, or even a few cytokines in the cell, but what's new is that you're now able to look at a lot of different cytokines in the cell. The polyfunctional strength index is just a great metric for how you can quantify and describe the changes that are happening in these cell populations before and after treatments. When we saw that there was a change in our patient's T cells after treatment using the polyfunctional strength index, we were able to dig down into what cytokine changes cells were having, and then we were able to use some other assays to sort off the cells of interest and do other in-depth studies to try and do further characterization.

***GEN:*** How have these proteomically active "superhero" cells uncovered insights into the development of novel therapies such as cell therapies and cancer immunotherapies?

**FAN:**

I think that superheroes are rare. You have technology that can detect, in a relatively unbiased manner, and profile these very rare superhero cells. If you make an assumption as to what superpower these cells should have and the one most critical trick they can play, then you try to sort them out to make the best therapeutic product, it is very likely that you may end up with the wrong cell types or wrong cell subsets. I think the new insights you're gaining from extremely high-plex single-cell cytokine profiling is that now you can see the different superpowers these cells may develop and the different superheroes you have in the cell therapy product such that you can link the abundance and combination of these superpowers together to identify the right team of superheroes to work together to improve therapeutic outcome.

And so when we develop something called a polyfunctional principle component analysis, you see different clusters of those polyfunctional cells in the whole CAR-T population. It's never just the one polyfunctional CAR-T cell subset in an infusion product but, actually, you always have multiple subsets and they have different combinations of polyfunctional profiles. Then you can see what superpowers are present in each subset of those polyfunctional cells. You have some cells in there that can have a combined type 1 & 2 response, and you have some cells that produce regulatory T cell function molecules in addition to effective function molecules. Now, you have a much more complete picture of the cells in your therapeutic product at the whole population level across the full spectrum of functions. I think that's where new insights can be obtained. To achieve this fine scale T cell function characterization, first you need a single-cell resolution and second, the full picture of all functional outputs. That's a whole new type of data you can generate and new insights to be generated.

**HEATH:**

I can comment here because Rong made some good points. By and large you will find that polyfunctional cells are going to have a particular direction. They're going to be stimulatory or they're going to have an inflammatory suppressive effect. They're going to have some overall direction that you can capture out of the polyfunctionality. In other words, you're measuring all this stuff but directly out of the functional measurements comes a sort of method to the madness, a direction of the immune response.

As long as I've been doing biology, function trumps other measurements. So when you do, let's say transcription measurements or something else like, you always want to

know "Am I actually looking at self-function or not? Or is this just a transcriptional network that happens to be active, but maybe it's not functionally active?" With these proteomic measurements where you're looking at the secreted proteins, you're directly capturing function. That's why it's so informative compared to some of these other assays just because, again, function trumps other measurements.

**POLLACK:**

Rong spoke to the fact that if you have a cell product that you're going to be infusing into a patient and there are more polyfunctional cells, those patients seem to do better after the cells are infused. There have been some other similar studies with other immunotherapies. For example, there was a study of patients who were going to be receiving a checkpoint inhibitor and when they looked at patients' tumor infiltrating lymphocytes, if the patients had more polyfunctional T cells, those patients were more likely to do well.

When you look at the polyfunctionality of a cell, you're looking at how it functions rather than trying to use some sort of substitute to just represent a certain type of cell. Focusing on polyfunctionality, you're actually looking at what cytokines these T cells make. We're also discovering that the polyfunctionality of monocytes is important as well. Conversely, a lot of times monocytes have an inhibitory function, both in infectious disease and cancer. You may find there are more polyfunctional monocytes that may indicate these are going to work against you in terms of your treatments and against the patients when you're trying to have an active immune response.

***GEN:*** How have highly proactive cells acted as supervillains versus superheroes in the context of disease and cancer progression?

**POLLACK:**

That's exactly what I was talking about when it comes to these monocytes. When you look at monocytes with the polyfunctional strength index, a lot of times those will be inhibitory. There are also studies in the context of autoimmune disease showing that these functional T cells can be related to adverse outcomes for those patients. Single-cell proteomic analysis is a tool that needs to be applied to specific circumstances and situations. I think that even as we learn more, we're going to be able to parse the data further. Right now we're categorizing things with the polyfunctional strength index. We may even get a little bit more granular over time, looking at the specific cytokine profiles of the different cells as we learn more.

**HEATH:**

There are supervillain cells but it's all context—like every dog's a good dog—except in the bad context. In particular, innate immune cells, like monocytes and NK cells, are typically a class of the immune system where one doesn't think you have memory. But in fact there is memory in the innate immune department and we're just beginning to understand it. For example, if you look at patients that have recovered from COVID-19, but are still having post-acute sequelae COVID, you find that their innate immune system remembers it, and this probably relates to the autoimmune phenomena that Seth was referring to at least at some level. But these immune cells are functionally active. It's not obvious looking at the single cell transcriptome that you actually have a lot of activity in that immune compartment, but you see it in the functional analysis. It comes out clearly.

**FAN:**

I have two specific examples I can give. As Seth mentioned, you can get a little bit more granular. Polyfunctionality can show the overall fitness of CAR-T cells but still more details should be included for different functional subsets. There was a paper published by Kite Pharma about four years ago in which they were looking at the polyfunctionality of CAR-T cell fusion products. Overall, for the entire population of the CAR-T cells, if there was more function, then the patient ended up doing better. But when they were looking at whether or not one specific CAR-T cell subset might lead to higher risk of neurotoxicity, they found one subset of Th17-producing cells that were producing a specific cytokine, IL-17, but were also highly polyfunctional, which

means they were the "bad" CAR-T cells. Once equipped with superpowers as defined by functionality, they were now leading to severe toxicity. So, in the infusion product, if you saw those CAR-T cells that had a combination of the superpower cells but also produced IL-17, the patients who received those CAR-T cells may show much higher risk of developing neurotoxicity.

The second example is what we have worked on with Memorial Sloan Kettering for many years. We found that not just immune cells, but cancer cells such as leukemia cells also produce a lot of inflammatory cytokines to mess up the tumor microenvironment or the bone marrow micro-environment, and hijack the nonmalignant hematopoietic cells to do bad things. Those non-malignant "normal" hematopoietic cells then contribute to pathogenesis and disease progression. These non-malignant cells also produce proinflammatory cytokines to make the entire bone marrow environment worse. I think that's a good example showing that not just the immune cells but also cancer cells, once they have superpowers, they're going to do something much more deleterious to our body.

***GEN:*** These unique superhero and supervillain cell types characterized by functional proteomics and published in peer review studies have been organized into a publicly available functional cell library. How does functional proteomics and insights into these superpowered cells complement the existing data that has been gathered for the genomic cell atlas?

**HEATH:**

That's a good question. Over the past five or six years, there've been a lot of atlases developed. There's a peptide atlas, a cell atlas, there's a polyfunctional atlas, etc. In many ways these are giving us a treasure trove of domain knowledge in which we can begin building models that look across different areas. For a functional atlas, it's relatively young. If you look at cancer cells that someone advanced, they become differentiated, they become mobile, they actually will secrete, and they'll actually become polyfunctional themselves in terms of their secretomes. We know almost nothing about that. That has a profound effect on the tumor microenvironment. It's going to have a profound effect on how tumors respond to various therapies, especially things like immunotherapy and how they develop resistance.

**"While those atlases are designed initially by asking questions about where the keys are, we're looking under the streetlight because we're only doing polyfunctional analysis of the cells that we actually know are important."**

**— Jim Heath**

That's also the case for solid tissues where there is a lot of functional activity at the security protein level. We know little. The atlas that is being developed of single-cell functionality is still pretty incomplete. If you look at NK cells, for example, that are highly polyfunctional after someone's recovered from the disease, we don't yet know quite how to interpret that. But that's why people are collecting all these atlases so that you can actually begin to query components that you see in one database against a different database.

These tool sets are just becoming available to us. While those atlases are designed initially by asking questions about where the keys are, we're looking under the streetlight because we're only doing polyfunctional analysis of the cells that we actually know are important. But that will expand just as it's expanding for the other types of atlases that are being developed. The usefulness of it right now is probably a bit limited. The usefulness will actually be much higher in a few years. It'll be in the vernacular.

***GEN:*** How do these new proteomic insights in superpower cells complement what you are already working with? And how is it impacting the genomic cell atlas?

**POLLACK:**

This is a great new technology. For the problems we're working on right now, like CAR-T cells and checkpoint inhibitors, there's been a big rush to look at the cells we already know are important for these therapies and to see how the technology can characterize them. But as we look at all the different situations where in normal physiology cells are making proteins and cytokines, we're going to be able to observe differences in various disease and treatment settings. These efforts to characterize the normal physiology in cancer and other diseases are going to be important, and we may see important applications in clinical situations that are not currently obvious. We know that cytokine secretion, for example, is important in cancer metastasis and how cancer cells that are circulating find out where they're going to go. I'd like to see some of this work done in that setting. That's exciting.

**FAN:**

I think that to some degree, this concept has been well recognized in a very specific subdiscipline of immunology research, and that's T cell polarization. We know for decades that T cells polarize to Type 1 or Type 2 T cells, and each type has a unique signature of cytokine secretion, like interferon Gama and TNF alpha for Type 1 cells, and IL-4, -5, and -13 for Type 2 cells. That's what we have known for decades about T cell "polyfunctionality." But I think this concept is much broader and potentially ubiquitous, and not limited to T cell polarization. For example, by assessing the protein secretion profile of cancer cells during metastasis, we may see different protein secretion profiles that can define distinct phenotypes of cancer cells at different stages and detect the ones that can secrete a range of proteins to break down the extracellular matrix, migrate over a long distance, attract blood vessel growth toward them, and then enter the blood stream to initiate metastasis.

We're very interested in senescence-associated secretory phenotype (SASP), which is an important topic in aging research but could be broadly implicated in many different fields. For example, in cancer, SASP cells might generate an inflammatory environment to predispose the tissue to higher risk of neoplastic transformation, which means they are going to develop cancer or an early stage of primary tumor. Some stromal cells, once they become senescent, are able to induce inflammation and may promote metastasis. Different cell types when becoming senescent may have totally different SASP signatures. How different senescent cells impact the local tissue microenvironment remains largely unknown. When you build a catalog of different senescent cells, single-cell protein secretomics is one of the most important tools to use to directly characterize different senescent cells and the SASP signatures in order to build an atlas of senescent cells across all different cell types and different organs.

***GEN:*** The articles recently published in *Cell* looked at COVID-19 at different stages of disease—one in the context of disease severity, and another looking at early factors and mechanisms contributing to long COVID, also known as PASC. What are the similarities and differences in immune cell function of patients among these stages?

**HEATH:**

We identified several factors that could predict the development of long COVID in patients. Then we tried to understand what long COVID meant in terms of immunological relationships. That picture is one that's not going to be painted well by any single analytic measure. We can see risk factors for long COVID. You could even see them before the patients got COVID and they have to do with autoantibodies or certain co-morbidities. There are a few things that get activated when you get COVID-19, such as reactivation of latent viruses, and these things lead to ongoing immuno-

logical activity that has given you some sort of neurological or respiratory viral at recovery. It's pretty straightforward to detect that ongoing immunological activity using polyfunctional type analysis.

However, it's challenging to interpret it. This ties in with our previous cell atlas discussion. You can take a functional index and you can identify which patients are going to respond to this CAR-T cell therapy and which ones don't, even before they get the T cells. That is basically a manufacturing metric for preparing CAR-T cells. It's great. You don't actually have to know too much about the mechanism of why that's the case to use that. However, for trying to dissect an autoimmune disease, how you get functional T cells from the fact that you had preexisting in certain levels of autoantibodies, that's a challenging pathway to resolve. And so in this case, the functionality gives you an end result that you can read out through the blood, at least that associate with symptoms.

And it gives you maybe a glimmering of insight into hypotheses for why this all may be happening, but it's incomplete at this stage. That's where the polyfunctionality needs to be integrated with some of the other types of analytics, including clinical labs and symptoms and things like this, to try to resolve mechanistic insights. This functional strength index is useful generically as an engineering metric. When you're trying to optimize cells, it's useful as a mechanistic insight into complicated biology. It's a piece of the puzzle, and it's a puzzle that we are only now beginning to collect some of the most salient pieces of.

***GEN:*** What did Jim's paper suggest to you and how does it go about being used in your daily practice, trying to understand what's going on with COVID-19?

**POLLACK:**

We're seeing some of the same themes that we've seen in some of the cancer-related work mirrored in the infectious disease setting. We're seeing that this is a way to look at T cell response, but also this is a way to look at these myeloid cells that may have inhibitory function.

**FAN:**

We're studying COVID-19 but are focusing on slightly different things. We are looking at COVID-19 serology and the cytokines in blood, and not as much about the cells. But similar technologies that we have developed for single cells can be tweaked to detect a large panel of COVID antibodies, including variants, all together on the same assay panel. It would be nice if we could eventually see the correlation between some of those circulating cytokines, including effector molecules or inhibitory factors, and those produced by single immune cells from the same patient. That would be, I think, a perfect clinical correlation study that we should pursue which could eventually reveal the cellular mechanisms implicated in COVID-induced hyperinflammatory pathology.

***GEN:*** One of the hot topics that we cover a lot in *GEN* is the tumor microenvironment. What are the challenges that exist today in better characterizing the tumor-immune environment and how can single-cell functional proteomics be applied to developing better therapeutics for especially difficult-to-treat cancers?

**POLLACK:**

So far, we've looked at things in a relatively simple way, where we're looking at the T cells and we're trying to characterize the polyfunctionality of the T cells in the tumor. We've looked at the monocytes and the tumor and tried to characterize the polyfunctionality of those. Some people have started to look at the tumor a little bit as well, but where things will get really interesting is once we start looking at all the different components together and try to see how the different components relate to one another. That's going to allow for some new insights, which will move things forward.

**HEATH:**

One of the challenges, and I agree with what Seth said, is that there's a host of new technologies coming out that will capture, at the single-cell level, a lot of aspects of the microenvironment, at the proteomic transcriptomic, epigenomic, and metabolomic level. We've done some of that. One of the things that we need to get past is correlative analysis because that's taken us to a certain level of distance. Most of us are writing papers where we almost have to cherry pick the data we put in the papers because there's so much data you can collect right now.

**"Where things will get really interesting is once we start looking at all the different components together and try to see how the different components relate to one another."**

**— Seth Pollack**

It's actually hard to tell a story and use that data, but some of these methods are getting relatively complete. And what I mean by that is when you measure the whole transcriptome, you're measuring proteomic methods that can, in some cases, be relatively complete. For a particular biological process, you're measuring multiple components of that process. You can take that process, let's say it's glycolysis, something that's common to everything. You can estimate the glycolytic flux through that cell and through that tissue. Now you can look at that from the transcriptomics point of view, you can look at that from the functional protein point of view, and you can look at it from the metabolic point of view.

All those can reinforce each other to give you a pretty compelling model of the system, where you're not doing correlative analysis, you're making a steady state approximation during what's called flux analysis. In the world of measurements that we're entering into are now, for the time, allowing us to begin to think about biology in that way, as opposed to just correlative analysis, which is what's taken us to where we are today. There's always a lot of mechanistic studies, but those are oftentimes one protein, one gene type of thing. I look at something like the tumor microenvironment and how we can develop a model that is predictive. That's one of the grand challenges of biology. Function prompts everything. Function's going to be a big part of it, but you're going to have the other parts as well. That's kind of a big goal, but that's where the world is headed right now.

**FAN:**

I agree with Jim as to where the field is going and to utilize all kinds of tools at the whole genome scale as well as in the spatial tissue context because they're looking at the tumor microenvironments, which means you have to look at the spatial relationship between the cells in the tumor microenvironment. That's where the field is heading. I want to emphasize one more thing. If you see immune cells, especially T cells, in a tumor microenvironment, you would like to know whether or not they are tumor-reactive and can eradicate tumor cells once activated. And, if they are, you can take them out, expand them, and potentially develop something like a tumor-infiltrating lymphocytes therapy. Thus, being able to directly measure tumor reactivity in connection to the functions a T cell may have is critical, and this can only be accomplished through imaging the live cell interaction between tumor and T cells while simultaneously detecting all immune function proteins this T cell produces.

You can assess tumor-efficient T cells and functional level by putting the patient's tumor cells in a microchamber and seeing how they interact or how they fight each other. You can't really see whether those T cells are tumor-reactive. At a single-cell resolution you can characterize those cells. If we can do that in an extremely informative manner, I think that's transformative right now, and that's the missing piece to connect. Once you gain the knowledge

of the tumor microenvironment versus clinical translation, you can learn how to turn that into future therapeutics discovery, for example.

***GEN:*** As our knowledge of the role of the immune system in various diseases expands, what applications and diseases do you envision will be the next frontier?

**HEATH:**

I'll take an academic perspective here. Some of the hardest problems that we have right now involve chronic diseases, autoimmune diseases, things like this, that we have a low understanding of. We need all the technologies we have to understand those. If we think about the problem that Rong just described, where you're looking for the Superman killer T cells that are in the tumor, that's actually analogous to how people look for neutralizing antibodies from B cells to try to find that B cell clone that produces the ultimate antibody. It's a harder problem because you're looking inside a tissue. But what I'm talking about is relatively near-term, when we can imagine approaches towards finding those Superman killer T cells, taking them out, and cloning them into a therapy.

Those are some of the near-term things, but this idea of integrating these multi-omic pictures together that involve some of these more nebulous or less defined diseases is one of the big challenges. And, going back to this idea of can we develop sort of computational models that can integrate this data? There are people who want to measure everything, and I don't blame them. A year ago, the deep mind of one of these big computer houses had two equal computers play each other in chess. One learned how to play by looking at all the grand master games throughout history. And the other one was just told the rules. The one that taught itself the game won by a lot. It wasn't even close. To compare this to biology, this danger of looking under the streetlamp for the solution involves using your domain knowledge, as opposed to trying to understand what the rules are. People have said for a long time, maybe there aren't rules in biology. Maybe there are, and they are hard to resolve, but that's the big challenge coming up. Those kinds of approaches may be necessary for solving some of these challenging diseases.

**POLLACK:**

Thinking off the top of your head, what are the diseases and clinical settings where the immune system plays a huge role? Transplant biology, probably, is a place where we're going to learn interesting things from this technology. The immune system plays an important role in cardiovascular disease and diabetes, and these are areas where there's a lot of opportunity to learn a lot with this novel technology.

**"What I am super excited about is how immune cells play a critical role in aging."**

**— Rong Fan**

**FAN:**

I think Jim and Seth covered this quite well. To look at it in a broader sense, I think this is applicable to two different human diseases—cardiovascular and diabetes. As I alluded before, what I am super excited about is how immune cells play a critical role in aging. My colleague, Deep Dixit, published a paper in *Science* a few weeks ago. He was looking at calorie restriction and how it changes the aging process and how it benefits lifespan. Subtle changes in those immune cells over an entire lifespan and understanding how those cells change other parts of your physiology and how at the collective level it might contribute to the gradual development of all kinds of chronic diseases. That's largely unknown. I really think that will be a huge impact down the road for cancer and many other human diseases. Hopefully one day we can revert this process and slow down aging and ultimately improve lifespan and health span. ■

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