Stephanie Xie: Thank you for coming in on such a gorgeous day to hear about my work around hematopoietic stem cells. I'm an expert in manipulating hematopoietic stem cells and leukemia stem cells in a xenograft model because we can't really do experiments on you and I and the vigor that many of us would like to do. I really care about what distinguishes them across our lifetime and whether that heterogeneity that's inherent in our hematopoietic stem cell pool could underlie many of the age-related disorders that we are affected by. Just to get us all on the same page, I know a lot of you come from a blood field. A hematopoietic stem cell resides in the bone marrow. That pool has two remarkable abilities. It can self-renewal, it can also differentiate into all of the blood cells. But they are in polar opposites in terms of quantity, and those hematopoietic stem cells are quite rare. In the context of the human system, from seminal work from Peter Campbell, Lisa Lorenti, and now others, through normal hematopoiesis in various diseases, we know that genetically, we only have about 50 to 200,000 of these clones in any given individual. But they have the ability to really proliferate so that there is such rampant daily turnover for immune cells, about 10 billion daily in the context of the red blood cells and platelets that really prevent us from dying every time we bump into something. It's in the hundreds of billions. With that enormous proliferative potential, they also go everywhere in our body. This is, I think, when we're in the thick of things, and we're really going asking what makes a stem cell, how they go bad, we start forgetting that outside of the bone marrow, they could be doing a lot of good or bad things, outside of what are the daily aspects of clotting, carrying oxygen, immunity. Why does this matter? Well, in 2020, when the bulk of the story that you're going to hear about, it was a pivoting point for all of us. Of course, the COVID pandemic, but the one that really struck me was that that year, the World Health Organization reported that there were more people over the age of 60 than children younger than five years of age. We are living in an aging epidemic, and we also know from work in the last couple of years that that HSC pool that is polyclonal, tens and hundreds of thousands of clones, by the time we get to age 70, there's 100 or less. There was this beautiful paper, a case study published outside from Spain where the oldest living individual had three HSC clones. You know what, all of them carry mutations, but she was healthy. What is going on in our HSC pool that leads to a situation where we're contracting that pool, but some people have amazing healthspan, others do not? Can we parse that out? If we can do that, can we then figure out, frosts are lifetime, where in this case, I'm showing you some data where Rafaella, Dimicos, Serena Scala, and I did an aging compendium, where this is bone marrow and just immunohenotypic analysis, frost lifetime. You see all these outliers. There are people that across this aging spectrum, where, yes, there is a decrease of stem progenitor cells as we age. There is a bit of cytopenia, especially a decrease in B cells and anemia. Monocytes stay the same, and others there's really high counts, but on the surface, they look healthy. Does that impact the blood production and risk for disease, and do we need to start thinking about both quality and quantity, and how do we measure that in our stem cell pool? Something that I've been really thinking about, we see these wheels a lot, is that within the hallmarks of aging and the hallmarks of cancer, there's one that has stood out to me and a lot of us. We hear about it a lot, inflammation, but that association of inflammation with aging and cancer is correlative. To most extents and purposes, at least in us. In mouse models, it's beautiful work from many different groups. Over the last ten years or so, says the HSC pool, fitness goes down with age, it goes down with inflammation, but is this actually happening in us? How does this happen, and is erosion of stem cell fitness perhaps in response to inflammation at the root of heterogeneous health outcomes when we look in the general population? With that introduction, I'm going to just get into a little bit of this concept of HSC heterogeneity, looking at clonal hematopoiesis how the response to something called inflammatory memory may really matter about how we rethink about what CH is, and to really go to using the xenograft model to ask about clonal expansion and the relationship with aging and inflammation, and then really asking about the translational implications of this story that I'm going to tell you about in the next steps. This is one of the most pivotal functional experiments, I think, in the HSC field from the late Connie Eaves. Her group just took single stem cells, sorted from mice, and then transplanted and looked across 24 weeks. The remarkable thing is at any given stem cell will give a different pattern of engraftment. There is so much functional heterogeneity. Now, there

are specific patterns. She brought it down to four. In the context of the hematopoietic blood system of seminal work from Luca Biasco, we've done some. We know that, in general, in the human system, we have a population of cells that will give early engraftment, pit her out. There are others that are slow but persist, and we have some idea of what's going on there, but we have to reconcile this functional heterogeneity with, up until recently, we still used to really that spa and stick model of hierarchical organization of hematopoiesis. On top of that, in the last five, 10 years, and more and more now, right, every single paper has some single-cell compendium. The single cell lens suggests how do we reconcile this with this functional heterogeneity, where there is a continuum, whether we look at chromatin accessibility or the transcriptome. Measuring and interrogating that in a functional manner is really what I've been going after for the last 15 years in the context of human hematopoiesis. What we have found is what distinguishes a stem cell is its ability to stay quiescent and maintain its self-renewal, but upon certain stimuli, it will activate, and then it has to make that decision. Does it stay a stem cell? Does it differentiate? Across this just basic question of what regulates stem cell function, what makes a stem cell a stem cell, I've been privileged to be part of many stories that say, it's a lot of things that integrate. Everything from cell cycle regulation, the epigenome is absolutely pivotal, but then other things that, when I set out to do some of this, Rob was on the same page with me, but we've turned, I think the field and others have joined us that proteostasis, organelle biology, metabolic stress, and inflammation could all be tied together to help a stem cell decide if it's a stem cell or it's not. In doing these projects, I kept coming back to why is there an underlying inflammatory program in normal cord blood stem cells? In fact, where we see if we take these stem cells from umbilical cord blood, and we also know this is occurring in adult stem cells, they look, by all intents and purposes, quiescent. You put them in culture, within six hours, there's a dramatic transcriptional change. The three gene sets that come up are my most favorite lipids in the world, these sphingolipids, a reactome around TNF signaling, and essentially a fake decision around life or death. What is going on? Why is there inflammatory signaling here? It took a while to decide to really go after it. I had to come up with a way of doing so, but with that in mind, with all the work that was coming up, around this phenomenon called clonal hematopoiesis, where somatic mutations of what were previously thought before seminal work from Ben Ebert and others have really said that these are found in high prevalence as high as one in five over the age of 60 have mutations primarily in DNMT3A and TET2, but many others. These mutations prior to this were thought to be drivers within myeloid neoplasms, especially AML. How can this be occurring? On top of that, it was shown that if you carry a mutation, even though you don't have cancer at the time, that it really increases your risk for mortality, increases your risk for hematological cancer, over 10-fold more. But you can see clonal hematopoiesis in the general population, in fact, in super-agers. The connection here is perhaps systemic. There is something around inflammation, and what has really become even more interesting and there are very few hematopoiesis talks at AACR, but they have an entire session on clonal hematopoiesis is in recent years, the cancer community has found that not only does it raise risks for all these age associated disease, if you are, in this case, a lung cancer patient and you carry a mutation, in one of these clonal hematopoiesis genes, your risk of death is much higher than those that do not carry this. More interestingly, they find that myeloid cells with a higher prevalence of the CH mutation. The variant allele frequency that's in the blood is found to be higher within the tumor, and it's the myeloid cells that are infiltrating. But we know myeloid cells are among the most short-lived of hematopoietic lineages. How can this be? I have been quite struck by work from the incomparable Elaine Fuchs, now ten years ago, and she just had this beautiful paper again in science, showing that this phenomenon upon wound in mouse epithelial stem cells actually changes the stem cells, and they acquire something called inflammatory memory, and it allows them to respond better the second time. In the context of the skin, that means better repair, faster repair. In the context of the immune system, they call this train immunity, and a lot of the work underlying childhood vaccination with BCG, and Maziar Divangahi, Mihel, Netea, and others have really shown that BCG, as well as essentially any inflammatory stimulus, can educate and cause a faster response from the myeloid cells through education of hematopoietic stem cells. This is all in the mouse

settings. Inspired by this, I set out to ask, is this a phenomenon that occurs in the human system, and how does this integrate with the heterogeneity that we know is inherent in the hematopoietic stem cell pool, and by really modeling inflammatory response and recovery, can we ask if this is what distinguishes some individuals who are healthy from those that are not? We did this by doing these models, and it was a giant endeavor. Three co-first authors, Andy Zeng, Murtaza Nagree, Asger Jacobsen. Authors. There's so much computational analysis here that's going to blow your mind. I've tried to condense it. It will come out in a few weeks, but you're welcome to look through the 10 extended data figures and five supplemental notes, as well as everything else. But to boil it down. Why did I do this? For over a decade, we knew that there was heterogeneity, and when quite a few years now, we did this experiment where we just purified CD49F long-term HSCs from umbilical cords, and we asked, Let's look at the single-cell level. If there is heterogeneity in those quiescence programs, inflammatory programs, and others that we've been dancing around for a long time. It turns out there are, and in fact, they are polar opposites from each other. Using machine learning here, we're just showing you that there are some that are highly quiescent and there are some that are highly inflammatory, especially around TNF signaling. This is happening in neonatal cord. It is, of course, not immune naive, but it's the cleanest we can get in our system. If we take that core blood enriched for their stem progenitor cells and put them into the xenograft and then ask, how did they respond to acute inflammatory stimulus? I had lots of work to draw from from the mouse system to decide. But what I did decide to do was to ask not just about a recovery from bacterial infection, but human TNF because it kept coming up again and again. There weren't comparable experiments for human TNF other than Manuel Pasagy and Masa Yamashita's data, and it was very compelling to me. I wanted to ask, is this the same in the xenograft? Can we model it? To show you, we did this at an early point in transplantation because everything is undergoing xenograft transplantation. That itself is stressful. We did it at a time point where we know the stem cells are cycling. From a paper that Lisa, Catherine, and I did in 2015. Also at 20 weeks, when we know most of the stem cells have gone back to dormancy. In both cases, blue is TNF, red is LPS. You can see that engraftment marked by CD45, as well as CD34, to mark the stem progenitor pool, there is a decrease. That's great. They respond to acute inflammation. Things go down. This is what happens in the mouse. Now, what's happening to the cytokine environment? Can we recapitulate the cytokine storm that we see in us? We did the plasma cytokine profiling with a company where we can look at one cytokine here at TNF, just and we used LPS to show, yes, we can see it go up. We looked, in fact, at 23 human cytokines, 18 mol cytokines. In the xenograft setting, we are creating a cytokine storm situation, and that graft can generate human cytokines in abundance. Now, this is the real experiment. This was actually the first experiment I did, which was to take that same xenograft model, but now we do one challenge, and then we take some of the animals through, and we repeat the challenge, and then give them a chase period of 10 weeks. With a single challenge, yay, they recover because we don't die and animals don't die either. Wonderful. Everything, at least at the level of graft, is the same, but lo and behold, with a second challenge, now it looks like, at least grossly, like acute inflammatory stimulus. There's durable change, and this has to come from the stem cells because that's at 20 weeks. We've done lots of work. It's only a stem cell graft. Now was the big thing. It's just a lot of money and technical issues here. But we sorted 427 million cells from all of these animals, put them through purified out those rare stem progenitor cells, the 10x single-cell multiome, so that we can look at both the chromatin accessibility and the transcriptome. Andy has this beautiful paper. He had to create a reference map so we call all the populations out of this. That set of cells are both stem and progenitor cells. For anyone doing xenograft analysis, marker fidelity is not as tight as out of primary sources. But we can use this reference map to call the HSCs and everything else and to boil it down. This map embedding of all three conditions, whoa, yes, there's a continuum here in blue of a stem cell population and everything else. But we got the psi population, another HSC population marked in green, and some progeny. By all intents and purposes of signatures that are out there, including ones we generated here at the transcriptome and chrome accessibility level, these are stem cells. They're bona fide stem cells by these molecular signatures. But unlike out of neonatal cord, now, out of the xenograft, this

second HSC population are actually both inflammatory and they are quiescent. What's going on? To really ask, and this is just to show you that, yes, there are two HSC populations, but they differ. Once from the ones that recover from TNF or LPS. We went after the gene regulatory networks using this machine learning tool called Senic Plus, where we can really connect transcriptional changes and epigenetic changes together and all this fancy stuff that I don't understand, but fortunately, Andy's a whiz at. What it boils down to is that one of these, the HSC-I population, when he looks at comparing PBS, in this case, TNF, he also did it with LPS. Look in the paper and look at all the GRNs. There are essentially no significant changes. The changes are underlying HSC-II. It's NF-CAP-B and AP-1, Phos June. Phos and June that has now come up in pretty much every case looking at inflammatory memory from Elaine Fuchs and very recently, from Jason B. Rostrls Group, pancreatic cancer. Nikola Valley in 2019 also shows that as well. There's something about this program, and it's durable. There are two populations. This is just a plug for a paper that's going to come out. We spent three years. I'm not done yet, but so far we have not found any prospective isolation markers for HSEIM to distinguish it from HSC-I. But one of the top candidates is this calcium exporter called ATP2B1. There is an antibody for it, and we spent a couple of years characterizing it because it turns out that we can separate CD49 F long-term HSCs and fractionate it into different populations. CD9F-positive cells can be positive for ATP2B1 or negative, and we did primary-to-secondary transplants. The double positive cells here are more functional, and in fact, even if you just take them and then put them in, we have not been able to, at least with this limited set of mice, all of them engrafted. This seems to be marking a superior new population of HSCs, which we hope will be a useful tool in various human disorders. That's coming out in the proofs we looked at, so it should be out online very soon. No markers. But what we have found is that there are actually two HSC subsets. When you put in the empirical model to really ask about stress recovery, both from inflammation and proliferative stress. Only one of them, this HSC-II, which renamed it HSC inflammatory memory, has durable transcriptional epigenetic changes. Who cares? It was done in the xenograft. What does this mean? We started first at what is the best-known situation about memory T cell memory. There's this beautiful real-world data set where volunteers took yellow fever vaccination. They also drank deuterated water, and the investigators were able to get both naive effector and long-term T cells from some of these volunteers 4-13 years out, and they did 10x Motium as well. We didn't have platform challenges. Andy generated, among the various ways he looked at it, was to create a long-term stem cell signature memory, or T cell signature, and it shares many properties with our HSC-iM. He also generated a chromatin-based signature, as well as the top 200 genes. You'll hear me talk about this one a lot. Following is what we call the HSC-iM signature, and he showed that both at the chromatin level and the transcriptional level, there are shared properties between our HSC-iM state and long-term T cell memory in people. I care about aging. We pulled together from 41 donors, including six that we did in-house, and this represented 23,000 transcriptional HSCs, and across these datasets, we asked, does HSC-iM enrich for HSCs? These are lollipop plots looking at normalized enrichment score. I hope you can see how strong he is. Yes. Accumulates with H. Then we went about it and looked in the scenario of, yes, aging. We created an aging magnet signature. I will come back to one of them. It's called CD83. That in itself might be a good resource for some people. It isn't rich in HSC-iM out of the xenograft. We use this beautiful paper from my colleague and now friend, Steven Josefowitz, through this. He shared this data before. It was published, and we looked, and in the context of post-COVID recovery, individuals that were hospitalized for severe COVID infection also is likened to what we see out of the xenograft. What is most intriguing to us was in partnering with Parish Vias, where they have this data set that I'm going to talk about more, where we can look at stem cells that are mutated versus those that are not from individuals out of bone marrow. We could generate a CH-associated signature. It's enriched in HSC-iM. As part of the long process of getting this out to press to you guys, we were asked, Well, all those scenarios are cross-aging. Is it specifically around inflammatory stress? We went into sickle cell anemia, where it's associated with a premature aging inflammatory state. There was, fortunately, a paper that just came out recently where we can interrogate and ask. We found that those pediatric patients that have sickle cell disease does in fact

have enrichment of HSC-iM versus HSC-I, but it's heterogeneous. You can appreciate that. If we have more data and we can go into each individual ask, could this be a biomarker, perhaps for things like gene therapy? We don't know, but certainly it's there, but it's heterogeneous. This is all correlative. Are there really two HSC populations in us? We turn back to this Colonial Humano Posis paper that we collaborated with Parish on, where there was 13 donors, 14. Often, I don't even remember. Asgard and Sven did Target Seek, this protocol from Adam Meade, and they made it better. They were able to look at the transcriptome, the immune phenotype, as well as do genotyping on this. We did a bunch of work to say, Hey, mutant stem cells out of people with CH actually have attenuated response to inflammation. But that dataset also allowed us to do machine learning to ask, within there, are there two HSC populations? I can hope you'd appreciate, yes, there are. When we took our HSC-I and HSC-iM programs from the xenograft and projected onto this, that RROC score, they're essentially identical. Yes, this can happen in people. Now it gave us the possibility, knowing that HSC-iM, that state, is created, in fact, in response to recovery from acute inflammation, to ask, is this occurring in these individuals, and perhaps to ask about what comes first? Is it an inflammatory environment that's generating the state? This is what we did here. But we can see that when we compare the stem cells with CH from MUT, that is there, and in fact, whether you're CH MUT, CH WT, when we compare it to the controls you have, especially of DNMT3A, an equal enrichment, less so in the context of tattoo. We can discuss why that might be the case. But this is suggesting that, hey, this state may be preceding any selective advantage of that CH mutation when it first forms. But that mutation could actually be changing how the stem cells respond to the inflammatory environment. We have evidence of this because we can see that there is a down regulation of, among other things, TNF Alpha V and NF Kappa B signaling when we compare the mutant stem cells to the wild type. If this is the case, then we would expect that there would be decrease in aging signatures. We do. We also see that there is a nice correlation of enrichment for that HSC-iM state relative to the clone size across this limited dataset. Is this really occurring? Well, we can start asking about the outcome of HSC-I and HSC-iM in this cohort. That's what we did. The first thing we had to do was to take that set of samples and classify what proportion of their stem cell pool is HSC-I and HSC-iM? This is what is shown here. Some of them are HSC-I dominant, some of them are iM dominant, some of them are mixed, and it's not dependent on mutation status. Now we have this scenario to then do pseudo time analysis. What Andy did was to take just look at the wild type cells and across from stem cells down to progenitors, who has better output? I hope you can appreciate the green cells, those HSC-iM, have decreased output in this analysis relative to the HSC-I enriched cells. More importantly, when we look in either HSC-I dominant, HSC-iM dominant, now we can ask, does the mutation do anything? If you're coming from a HSC-iM state, you would imagine that now you should have increased output. If you carry a mutation, in fact, that's what we see here. This suggests something around clonal advantage, that the mutation is taking advantage of the inflammatory aging state that is occurring. It's not positive clonal expansion, in fact, could be a negative selection of the wild type stem cells. Then we asked, is it heritable? In that CH dataset, we have cells down to few, but we have a mature cells as well. Andy generated a signature of 257 genes, if I can recall, and then asked, looking at HSC-iM versus HSC-I dominant cells, taking that signature across the hierarchy, that there is enrichment of iM signature all the way down to the monocytes. There's also a retention of this quiescent state, at least to GMPs, and it's still there a little bit in the monocytes and they're inflammatory. There seems to be some transmission of this state down to the progeny. But this is a limited dataset, and every individual, we don't know the exosmic circumstances of their life. There could be lots of potential for this to occur. We went back to the xenograft model, where we sorted out the stem progenitor cells, but we also sorted out within that 427 million cells, the mature myeloid cells and the progenitor cells. We used the trick called supercell that for the computational biologists, you're typically using this pipeline to call doublets. But it can see small nucleotide polymorphisms, and this set of experiments was across three different pools of cord blood with over 62 donors. We're able to classify some genetic clades, clonal groups, and ask, again, in the same way we did with the individual bone marrow samples. Some of them are HSC-I dominant, some of them are HSC-iM dominant, some are in between. But this

lets us essentially have a trick of genetic lineage tracing. From this, we know that iM stem cells have enrichment, and they will pass down to monocytes. If we then go through the entire hierarchy from stem progenitor cells through the precursor state, including B like cells to monocytes, to DCs, we can see that there is actually transmission all throughout the state if you're iM versus I. They're transmitting these programs. Again, this is great. It's in a state where everybody was in the mouse. They're suggesting that there is something to heritability, that's intrinsic. But does this have any application? We partner and really took advantage of a cohort epidemiological study in Canada called Campath. This is from one of the provinces of mine in Ontario from individuals that have volunteer to enroll in this, they had a blood sample, and they used a mortality risk score based on just common blood parameters that's been out there for over 10 years that says that depending on how these parameters are, it's intermountain risk score. If you are greater than five, your risk for five-year mortality is much higher than if you're below three. They classify these 400 individuals across a lifespan from their 20s into their 80s. They fractionated across some that are young and low risk or high risk and young or some that are age over 65 that have a low risk and high risk. We asked, does this in any way correlate in this Punnett square here? What we found was in the young individuals, there was an enrichment of this HSC-iM program. This is no stem cells here. We're just asking, could this be a biomarker? We are finding that there is this correlation with all cause mortality, and we're seeing it most interestingly in dendritic cells, B cells, and NK cells, which are becoming of great interest in the context of immune signaling, immunotherapy, the immune micro environment. Could that stem cell signature be passing down to various immune cells that are infiltrating into tumors or other diseases that can be leading to the heterogeneous outcomes. With this, I would like to summarize. We found using just xenograft models that there are two HSC subsets. One of them retains molecular memory upon recovery from prior inflammatory stress. It's quiescent has limited hematopoietic output. But with aging where somatic mutations build, there's now a selective pressure within HSC-iM for these CH mutations to relieve that differentiation decrease to allow for increased differentiation. This could be one reason why DNMT3A is so prominent because if our stem cell pool is so quiescent, we're not making very much blood. That could be the selective pressure. They transmit these HSC-iM. They're pro-inflammatory properties to downstream progenitors, as well as it seems to be all immune effector cells. This has implications for inflammatory associated diseases across lifespan. What's the implication here? One way to interpret this is actually those red dots that that CH variant allele frequency that we see increase may actually be selective advantage. We're not seeing those clones that may have become very dormant. This is perhaps due to that inflammatory niche in each individual. If that's the case, then we need to think about targeting the inflammatory niche, and perhaps we can take lessons from obesity. Is there something that we can use to target inflammation and mitigate CH expansion? Well, everybody and their mother. I was reading an article opinion piece in New York Times right now. It appears that the bros on social media that are tied down to this case study of an individual concussion are taking these, whether off label or on label, and some of the indications are outside of weight. It seems to be for their anti-inflammatory properties. We know that obesity induced inflammation exacerbates clone hematopoiesis. Could we co-op the properties of GLP-1 to mitigate inflammation? Luckily, Daniel Drucker, one of the prominent experts on this is literally next door to us at Princess Margaret. He had this beautiful paper from 2023 where he showed that is on target with GLP-1 receptor, you require it, but you can dampen TNF output with GLP-1 of these syngeic mice models. Recently, Steven Chen, and we helped them show that using another common diabetic drug, metformin, reduces competitive advantage, at least for DNMT3A R882H. We decided to think about this, and at the time we wrote the grant, this also came out. There were multiple epidemiological studies saying that in diabetic patients, that there was a decrease of colorectal cancer, of liver cancer relative to insulin in these cases, as well as blood cancer. We created this team initiative. Remarkably CIHR ticket for cancer prevention, and we've started doing pilot studies to really ask, can we alter clonal bath size, in this case, in the mouse model, where we took TET2 mutant cells transplanted one to one, and we've been giving them daily injections of semaglutide between four individuals. There's a lot of mouse handling, and we're seeing some

efficacy at a relatively low dose. That's heartening to us, and we're doing more pilots and really going in full force now on this. I'm a hematopoietic stem cell expert. I have this acute inflammatory model. I'm not going to look at that first. I want to see if there's a cytokine change. We did this experiment. We piggyback on my acute inflammation model, and we asked, can we see changes with pre-treatment of GLP-1, followed by LPS stimulus relative to PBS control. We used a marker that we have found coming out of the HSC-iM story. We found to be exquisite biomarker in long term HSCs, highly sensitive to TNF as shown here. When we did this, relative to animals that were transplanted and then just treated with vehicle or with LPS, either with another vehicle LPS subcue or GLP-1 pre-treatment, we're seeing a decrease, not just in CD83 as acute inflammatory marker, we're seeing a decrease in TNF as well. There could be something to this. We've really gone full force now. We're going across the next five years to really ask mechanistically, can GLP-1 or some of the other agents really target CH varying o frequency in genetic models? If so, how? We're doing xenograft models with CRISPR mutations to DNMT3 and tattoo. The human setting. We've partnered with an endocrinologist around Toronto to do observational study from the endocrinology clinic to see who has CH, and then they're going to be funneled into our CHIP clinic and to really ask, can we guard against clonal hematoposis with GLP-1 receptor agonists? Thank you so much for your time. If you're almost there, I'm still looking for postdocs. Come and talk to me. Thank you.

Speaker 1: Thanks, Stephanie. Really terrific talk. Really cool. Thinking about how our HSCs are changing. I've have lots of questions, but we'll start with one or two. With the HSC-iM, why, as we get older, aren't we all just filled predominantly with HSC-iM? We have lots of acute inflammatory stress over the course of our lives, and yet you show this remarkable heterogeneity of that heterogeneity where some people are still dominant with HSC-Is and some with these HSC-iMs. Any explanation or ideas?

Stephanie Xie: No explanation. This is where I am continuing the partnership with Philip, and hopefully Cancer Research UK will give us a bunch of money. Because it's hard. This is the only study we have right now where we can really ask and also that round mutations. With all the work that we've shown, mutations don't need to occur. KJ Patel, with his beautiful paper that just came out and sell a stem cell says there are other ways to get clonal expansion that is not somatic mutation driven. If we can use the IM signature or other ways to boil it down, use it as a biomarker, can we perhaps not do it on stem progenitor cells, but just out of our blood, given that it's transmitting and classify people as one or the other is, I think, something to be of use. But it doesn't answer your question. The question is, why is it there in the first place? I was very careful to say that neonatal cord is the most immune naive we have. But there is plenty of inflammation in utero. In fact, especially with all these cases of preeclampsia and others, could there be ready selection in the womb? I don't know, but I would love to find out. I've been trying to track down individuals, obstetricians who could give me some samples or at least out there, but that's something that I really wonder about.

Speaker 2: Are we predestined to fall into one of these categories?

Stephanie Xie: I wonder. We saw the GLP-1 receptor agonist story as cancer prevention, but it's really to see if there is a predestiny, can we swap? Because there's a mixture. There's never not one or the other. This individual had the low, like it was 98%, but we always see the other population in every scenario we look at. Is it just the time when we sample them? How dynamic it is, I think that is something we don't completely understand. But we have some evidence, at least from five of these individuals. I didn't show it because our reviewers asked, we transplanted and is transmissible.

Speaker 2: You focus on the downside of these HSCIM. Is there a good side to them?

Stephanie Xie: I sell it as such, but I think that it's actually pivotal. I think a lot of the anti inflammation treatments, at least after specific cytokines, we don't have enough evidence to go after them because a TNF signaling at least on the human side, it's a underlying feature of what makes

them stem cells. They have to respond quickly. I think, especially with the prevalence of DNMT3A, nature is smart. Nature is doing this to allow for that lifelong blood production. If we're always responding to every agent coming through like we are neonatal cord, we'll be gone. Our blood source will be gone. I think the training at the level of hematopoietic stem cells is to go more dormant. But as we are aging, our population expands but maybe that's why we're seeing clonal hematopoiesis. That's the flip side of the evolutionary selection.

Speaker 3: Beautiful work. I'm really curious about the relation between the cytokine signatures and the cytokine storm and inflammatory IMHSC and cytokine storm in patients receiving CRT therapy? Are there ways that these signatures could be leveraged or as biomarkers of predicting inflammatory reactions in patients in the clinic like that?

Stephanie Xie: Mouse and humor are interspersed here, at least with LPS all goes down. Among the supplemental data, we actually show at 20 weeks we captured the bone marrow aspirates, and a handful of them are still up, even in the PBS setting. Transplantation is a little bit stressful. Which of these are important? This is a xenograph model. We can only say that the grafts are making these and they are responding as they should. As you say, it's critical. Whenever I talk about this, I see clinical colleagues who are on trials, and I'm like, do you have plasma bank? Can we stratify and start asking with outcomes? We may or may not need mutation. I think we just need outcomes. Then can we go back to the patients and sample their blood to ask how much of this is from their blood and not the tumor itself? I'm so HSC centric. We love the blood. But one possibility from this is and the immunotherapy revolution has said so much of cancer could be just the immune system not responding the way they should. The other part of the immune system is they shouldn't be acting up. At times, they should be flexible. I think our immune systems as we age become inflexible. They're not toggling back and forth as quickly as they should. Trying to figure out exactly which cell types, which of the signaling pathways, which level that's at, I think is taking me down to speak to a lot of immunologists.

Speaker 2: I'm wondering in this chronic expression of inflammatory genes, have you looked whether or not there's any negative regulator of expression of AP-1 mediated expression of these genes, which is lost in the IM cells?

Stephanie Xie: I'm looking for a computational postdoc to really, really delve into. Our chromatin data, we used it. But to be perfectly frank, we didn't go into as deeply as we could have. Part of it is hard, and we're locking a critical component, methylation. I have been thinking for years that this was required, but we needed to get this across the line, but also in the context of these rare stem cells, like it requires some potential single cell methylation or at least key marks. Now, from Elaine Fuchs' recent paper, it's narrowed down to a handful that is, I think, doable to at least cherry pick and do in the human setting. I'm working with some colleagues on that, but yes. I think that's the wave. If I may add, I think part of it is actually the binding partners. That all cases, AP1 is there, but at least in pancreata cancer versus the beautiful study from Rostrels Group and this latest one from Lane Fuchs, one's mouse epithelial, normal epithelial stem cells. The other is a KRAS colorectal cancer model. Sorry, it's an APC. Now I forget what the driver is. But it's the transcription factors that actually modifies and says, which of the open areas are transcribed further down and respond quickly. In that case, I think it's going to be tissue specific.

Speaker 4: Hi. Thank you for your beautiful talk. I have a very basic question about the origin of the IM2 type of hematopoietic stem cells because they have the similar functions with the type I and regulatory programs that are equal. Have you done some like transcriptional trajectories, like analysis to see where these cells are coming from and the origin of the cells?

Stephanie Xie: If I understand the question, we have, that's what all this pseudo time analysis is and that's just a couple of examples. We've also done it across everything from VJ Sangart his mito tracing

paper where we also had done this thing. At least with the datasets that are available out there, we've done it. Have we done actual lineage tracing? We tried. Marking with something like Larry and then being able to transplant and pull back clones is very, very inefficient in the human setting. That doesn't mean that I'm giving up. It's gotten better. I keep in close contact with some of the best out there. Let Hill, Rodriguez Particlli is great at this. But we may need other tricks to do it. The other issue at play is all of the lineage tracing right now, at least in the hematopoietic system primary requires lentiviral barcoding. That's very stressful. With my CD83 marker, anytime you put any stem cell into culture starts lighting and you're selecting.

Speaker 2: Great talk. Quick question. HSCIM's obviously nicely showed many inflammatory signatures obviously are associated in them. I was curious to try and bring it back to some of your earlier work and how you were mentioning you and Rob were the forebears of looking at metabolism and HSC, etc. I'm wondering what other stuff was associated with HSCIM like any sphingolipids or proteostasis, or I'd also be curious about ribosomal biogenesis factors.

Stephanie Xie: That ATP2B1, that new marker, it's a calcium exporter. We trace the mechanism for its increased functionality to endolysosomal functions. They're marking the cells that have the most nuclear TFEB. At least there, that's a big part of it, that dormancy state is, in fact, probably up regulation of a lot of the programs we know that retain that inherent self renewal. Now, your main question around my beloved finger lip is, I put them aside for now almost six years. That wasn't the intention. I thought I would just have a signature, and I would use this set of xenographs and those are ongoing. I'm going back to it. I didn't talk about it because it's published work. But especially this blood cancer discovery paper, I did all this. I gave almost nine years of my life trying to disentangle sphingy metabolism and AML and it was all around inflammation. That's one of the reasons why you need a postdoc. We have a system. We have a phenotype, and now we need someone to really do it. Thank you.

Speaker 3: Thank you so much for your great talk. Your comment briefly about sickle cell disease brought up some questions for me clinically. I was wondering with this HSEIMF, it's something that could be used to stratify the cells that are edited for gene therapy or stratify cells that are transplanted.

Stephanie Xie: We have an ongoing collaboration with Juliana Ferrari, not in sickle cell, but in Beta thalassemia in the context of gene editing and outcome. Some of her patients responded to the editing successfully, and others did not. We can use the HSCIM and other genes encased in that to serve we're trying to disentangle it. Some of it programs went up. I mean, there's a ash abstract out there. HSCIM is not one single state even there. We can see that a subset of cells are reliant on more interferon and another subset is reliant on a TGF-beta program. What that may mean, we don't know. But across various diseases across aging, we can further classify some individuals that have a stronger IMA versus IMB. That one, I don't know how long it will take to disentangle. But thank you.

Speaker 2: Thanks, Stephanie again for a terrific talk.