Transcript of PRF Webinar with Amanda Brandow and Kate Sadler

Francie Moehring (FM): Hi, my name is Francie Moehring, and I'm a postdoc in Dr. Cheryl Stucky's laboratory at the Medical College of Wisconsin in Milwaukee. I'm speaking with Dr. Amanda Brandow and Dr. Kate Sadler about their symposium titled *Translational Models for Investigation of Non-Opioid Based Pain Therapies for Sickle Cell Disease* at the American Pain Society annual meeting here, in Milwaukee.

Dr. Amanda Brandow is an associate professor of pediatrics in the section of hematology/oncology and in bone marrow transplantation at the Medical College of Wisconsin. In addition to being a clinician, Dr. Brandow also received a Master of Science in epidemiology from the Medical College of Wisconsin where her thesis was focused on functional outcomes in children with sickle cell disease after treatment for painful events. To this date, Dr. Brandow is interested in developing better ways to assess and treat pain in children with sickle cell disease and to provide those pediatric patients with better compassionate care.

Dr. Kate Sadler received her PhD from Duquesne University where she trained under Dr. Benedict Kolber where her dissertation was focused on characterizing the divergent roles of the left and right central amygdala in rodent models of visceral pain. Dr. Sadler joined the Stucky lab as a postdoctoral fellow at the Medical College of Wisconsin in January 2017 when she started working on investigating the underlying causes for acute and chronic pain in a mouse model of sickle cell disease.

Kate and I do see each other quite a bit these days. We hang out outside of work, but we also work in the same laboratory. But we work on very different projects. I'm really excited to be here today to have a chance to learn more about what she's up to these days in terms of science and also what she's thinking about in terms of her future work and how to communicate to non-science audiences.

Let's get started. Can either one of you tell me a little bit about the disease that both of you are investigating.

Amanda Brandow: So this is Amanda Brandow, and as you said, I'm a pediatric hematologist. I'd be delighted to talk about sickle cell disease, which is something that I'm really passionate about.

So, sickle cell disease is the most common inherited blood disorder in the United States, and it's been estimated that approximately 100,000 Americans live with the disease, and 1 in 400 African American people. It's inherited in an autosomal recessive pattern, and approximately 8% of African American people carry sickle cell trait. Fortunately, in the US, it's diagnosed on newborn screening. However, this is not routine throughout the world. So an estimated 3 million people live with sickle cell disease worldwide. However, this is probably an underestimation of the true prevalence of the disease.

So what causes sickle cell disease? Well, it's a DNA mutation in the beta chain of hemoglobin where valine is substituted for glutamic acid. This mutation results in abnormal hemoglobin that polymerizes under certain conditions and results in the pathognomonic shape of the red blood cell as the sickled cell.

The sickled cell has shortened red cell survival, so patients experience a chronic hemolytic anemia. This sickled cell also has a very rigid membrane, and so it causes obstruction of blood flow in multiple vessels which can lead to tissue ischemia and many of the multi-organ system dysfunction of sickle cell disease including strokes, pulmonary complications, renal complications, among other things, but it is also thought to be one of the contributing factors to the very severe pain that patients with sickle cell disease experience.

Sickle cell is also a very chronic proinflammatory disorder, and it's been repeatedly shown that many cytokines and chemokines, among other things, are elevated in patients with sickle cell disease and likely contribute to both the acute pain and the chronic pain that accompanies the disease.

FM: Kate, how do you model sickle cell disease in mice?

Kate Sadler: Yeah, hi, this is Kate Sadler. We use two different transgenic mouse models in the Stucky lab to investigate sickle cell disease pain specifically. Both of these models were developed in the late 1990s. So they're called the Berkeley mouse model developed at UC Berkeley. And then the Townes model which was developed by Tim Townes.

So these models have the exact same point mutation in the beta hemoglobin gene that Amanda talked about. So these mice recapitulate many of the phenotypes that Amanda just described including the sickled red blood cells characteristic of the disease, the massive proinflammatory state that the patients are typically in—we see the same exact thing in our mice. And then, we can talk a little bit more about the pain that these animals have, but the phenotypes of the mice largely recapitulate [the phenotypes] seen in the humans.

So I don't know if Amanda wants to chat about the pain that the patients in sickle cell have.

Amanda Brandow: Sure, I'd love to, Kate. So, patients living with sickle cell disease experience two types of pain, which makes the pain seen in this disease very unique. Patients with sickle cell disease have very severe acute intermittent pain, and this pain is really unpredictable and extremely severe and brings patients into the hospital and the emergency department for care.

Some of the triggers of pain include cold temperatures, among other things like infection, fevers, and stuff that we're still trying to understand. This acute, severe, intermittent pain

starts as early as the first year of life and increases throughout patients' lifespan, and this frequency oftentimes increases with age.

There's also the emergence of chronic pain that we're just beginning to understand over the last several years. And what we're finding is that adolescent and young adult and into the adult age group develop chronic pain for reasons that aren't entirely clear, but some of the biology we're beginning to understand that's been done eloquently in Kate's lab among others is that there's certain contributions of the central and the peripheral nervous system that really mediate this pain. And it's been estimated in patients that approximately 30 to 40% of adolescents and adults with sickle cell disease suffer from chronic daily pain.

The mechanisms of this pain are really important to understanding future therapies, and that's why the translational collaboration that Kate and I have studying this model is really vital.

FM: Kate, can you model both this acute and chronic pain in your mice?

Kate Sadler: Yeah, we can. So, as Amanda said, about 30% of patients will develop chronic sickle cell disease pain. So 100% of our mice develop chronic sensitivity or hypersensitivity to both touch, so mechanical stimuli, cold stimuli, and heat stimuli.

So that's the chronic pain that these animals are exhibiting as early as 6 weeks of age, and the intensity of these pain-like behaviors, we'll call them, increases with age. So there's been work published from our lab and others that show as both male and female sickle cell mice age the hypersensitivity to these stimuli becomes even greater. So that's the chronic part of the pain.

The acute pain we can also model in these animals. So we can place either transgenic strain into a hypoxic box, so basically an environment in which the oxygen concentration has been lowered for several hours. We take the animals out of the hypoxia chamber, and they become very sensitive to, again, cold, heat, and mechanical stimuli, which is really interesting that we see hypersensitivity in all three of those modalities or applications of stimuli. It's sort of the one time that our data haven't exactly matched up with the human data.

So I think, Amanda, you recently just published a really nice paper in *PAIN* that shows during a vaso-occlusive event that patients don't report heat hypersensitivity. Is that correct? But we see that in our mice. So that's maybe one of the interesting divergent points between the mouse model and the human model, but largely, everything else is basically one for one the human to the mouse, which is pretty cool.

FM: And I'm assuming we don't know the reason for that difference.

Kate Sadler: Not yet, no. But a great biological question.

Amanda Brandow: Always more questions.

Kate Sadler: Yes.

FM: Amanda, what's the current standard of care for adult sickle cell disease patients or even your pediatric patients?

Amanda Brandow: The standard of care in children with sickle cell disease, at least in our center and many other centers throughout the country, is that when patients have inherited the most severe form of sickle cell disease, which is a genotype called hemoglobin SS or hemoglobin S beta zero thalassemia, is that we initiate a disease-modifying drug called hydroxyurea at 1 year of age. This is an oral drug taken once a day, and one of the mechanisms that we think it works through is inducing the production of fetal hemoglobin, which is a type of hemoglobin that has been shown to be effective against preventing some of the complications of the sickle hemoglobin.

Children stay on this drug throughout life essentially. The drug was initially approved in adults and studied in a randomized controlled trial and was found to decrease the number of acute pain events or pain events dramatically, which is why it led to its widespread use, and then there's some additional work done in the young pediatric patients that has also shown the effectiveness of the drug for prevention of pain.

So that's really our standard of care as far as trying to prevent pain events. However, the treatment of pain events right now is not as well standardized. I think one of the problems is that we have a very small, small, if any, evidence base to support the use of pharmacologic and non-pharmacologic interventions for pain. So the mainstay right now for pain is opioid-based therapies in addition to some non-steroidal therapies. However, it is certainly important to look beyond that, and we're in the process of developing some evidence-based guidelines right now through the American Society of Hematology that can help provide some structure and some guidance to providers on ways to most optimally treat both acute pain and chronic pain.

Kate Sadler: Can I ask a question about that, Amanda?

Amanda Brandow: Of course.

Kate Sadler: So, hydroxyurea, has anyone looked to see if longitudinal administration of this compound is preventative or decreases chronic pain in patients with sickle cell disease?

Amanda Brandow: That's a fabulous question. It's actually a question that I hope to answer with some of the work that I'm doing in my laboratory – to look longitudinally over time to see if early initiation of the drug decreases some of the longitudinal poor outcomes that we see in regards to pain. Because in addition to induction of fetal hemoglobin, which probably reduces sickling, it also has some other potential effects immunologically and other things that we need to understand better.

Kate Sadler: We should do it in our mouse too—to give our baby mice hydroxyurea in their water and see what happens over time.

Amanda Brandow: Yes, I agree with that.

Kate Sadler: Everyone listening, don't take that idea. Kidding!

FM: So what are really the biggest challenges then that these sickle cell disease patient populations are facing?

Amanda Brandow: That's a great question. I think the treatment of pain is a huge challenge for these patients, and I think the challenge is magnified in the current state of our country in the context of the opioid crisis and the opioid epidemic.

Patients with sickle cell disease are predominately of African descent, and so there's a lot of stigma and negative provider attitudes around the treatment of pain in our patients, and that causes significant suffering both emotionally and psychologically, and physical suffering when they present to emergency departments for care.

So I think that's a big challenge right now in trying to insure that our patients continue to receive compassionate care and are not labeled as drug seekers and have their pain treated appropriately when they seek treatment.

Kate Sadler: And I think from a basic science standpoint, I think that the prevalence of sickle cell disease in the general population has made it, I don't want to say less attractive for people to study, but I think it hasn't necessarily recruited as many people as should be studying it. But I think that I would argue right now as the basic scientist here that the mouse model that we have for sickle cell disease is one of the very few actual naturally occurring models. Modeling the acute-to-chronic pain transition is a big deal right now for NIH funding initiatives and just because we don't understand how so many different acute pains can turn into a chronic pain condition. But I think that we have a really important tool in these transgenic sickle cell mouse models to study not only how that happens in sickle cell disease but take what we're learning from the sickle mouse and then seeing if we can apply that to other chronic pain conditions. So I think they're actually a really valuable tool for studying that specific type of question.

Amanda Brandow: I agree wholeheartedly because really your mouse recapitulates the exact biological transition without an artificial induction of some sort of pain state.

Kate Sadler: Right. And there are not many of those models. You can count on one hand how many of those exist.

Amanda Brandow: And I think one of the other challenges that Kate just brought up, Francie, is the lack of research and the lack of people wanting to delve into the biology of pain in sickle cell research. And I think pain and sickle cell disease has suffered from that

lack of people really taking it on as something that they want to study because it's hard to study both in humans and in animals. And so a lot of the other complications of sickle cell disease such as stroke and pulmonary or cardiovascular complications have been much better studied because in a way there are more concrete outcomes that can be used for research.

Kate Sadler: It's super messy, right? It's clearly a chronic proinflammatory state. So I mean, neuroimmunologists are probably a great set of people who should be doing more of this work, I think.

Amanda Brandow: I 100% agree.

Kate Sadler: I think there's so much neuroscience wrapped up it in as well. It's so multifactorial coming in, all of these things that complicate the condition in the mouse model. But I think just starting at ground zero and finding the thing that you're good at in relation to this mouse model, all of that can help with the questions of pain and how to treat it.

Amanda Brandow: Agreed.

FM: So why did you two really decide to apply to present a joint session here at the American Pain Society meeting? I'm just curious.

Kate Sadler: So I saw the call that was put out from American Pain Society for people to submit proposals to present their data here, and I thought it would be fun to highlight this really unique translational relationship that the Stucky lab has with Amanda and her colleagues at the Medical College [of Wisconsin]. And I think that we set a good example for the field. The questions that Amanda is asking in her patients, we can also directly ask in our mice and then take what we learn in our mouse model and then bring that back to the clinic.

And so, I thought highlighting that here at a national pain meeting would be a great thing to do. So we submitted a proposal, and it was, thankfully, accepted by the committee that was reading over them. The American Pain Society, I think, is interested in sickle cell disease. They have been for a while. There's a special interest group that's focused solely on sickle cell disease pain here at the American Pain Society. So I'm glad that the Society, as a whole, really respects the work that people are doing in relation to sickle cell disease pain and wanted to support advocacy almost of that research by having us present here.

Amanda Brandow: I concur with all of Kate's very well-articulated comments. When she reached out to me initially, I was 100% on board and very grateful for the opportunity to be able to present our work and really highlight, like Kate said, this unique and fun collaboration that we have to try to learn from each other – from human to mouse and mouse to human and back and forth because that's really the only way that the field's going to move forward. Because my research in humans has limitations, and I need

partners and collaborators that actually can dissect the biology and literally dissect down to nerves and cells that we can't obviously do in humans. And then that just informs research questions that I can ask too.

Kate Sadler: As the basic scientist, are we doing things that clinicians care about? So I think so often we get – I know I get – really into the nitty gritty molecular mechanisms of what I'm studying. But if that doesn't translate into a potential therapy for a clinician or one that's going to be accessible in the next few years, it's obviously still worth it because science for science is wonderful. But at the same time, I also want to do science that will be relevant to clinical populations, and so making sure that my preclinical research goals are aligned with clinical research goals is something that's really important to me.

FM: That sounds really good. So, can you tell me a little bit about how you two interface on a daily basis because maybe other people in the field would like to use you two as a model of how to form these collaborations between basic science and a clinician?

Amanda Brandow: So I actually attend many of the lab meetings that Kate and Francie and Cheryl Stucky have that are focused on sickle cell disease and also pain mechanisms outside of sickle cell disease because I love to learn. It helps me learn is there something you're doing in this area of pain research that can also be explored in the area of sickle cell disease. And so, that's been one of the ways that we keep the momentum going. And Kate is actually going to come join me in clinic and actually see some of the human side of things because I think having brilliant scientists who think about pain mechanisms on a daily basis see patients may trigger more ideas that either they can bring back to the lab at the bench or I could study in the humans.

Kate Sadler: Lab meetings are the main way that Amanda and I interact most regularly, but then obviously just going out for drinks too and talking about science.

Amanda Brandow: It's got to be fun too, right?

Kate Sadler: Yeah, exactly.

FM: So that was a really great introduction in to the topic. So let's talk a little bit about the research both of you are doing right now. Amanda, what is your lab currently working on in terms of sickle cell disease?

Amanda Brandow: Over the last decade or so, I have really always focused my work on sickle cell disease pain of course, but I started initially looking at outcomes, and then got much more interested in also trying to understand the biology. And so, one of my initial projects was looking at how patients perceive pain, looking at some pain sensitivity studies comparing baseline health to race-matched controls. And then doing some work around substance P in patients with sickle cell disease and then trying to further

understand how pain sensitivity changes during disease states, so during sickle cell disease baseline health as compared to admission for pain.

And some of the main outcomes we've seen is that patients with sickle cell disease have increased sensitivity to cold stimuli during their baseline health as compared to healthy controls, which correlates with some of the anecdotal reports of patients having onset of acute pain during exposure to cold. When we looked at this between two disease states, baseline health and acute pain, we also that their cold hypersensitivity increased during acute pain, which sort of confirmed some of the anecdotal findings and also very well capitulated what was found in the mouse model.

And now, my lab right now is really interested in looking at neuroimmune interactions and how the chronic inflammatory state of sickle cell disease contributes to the pain biology, and this spectrum of pain from acute to chronic pain and this sensitization that occurs because if we can understand potentially some of these neuroimmune interactions better, there may be targets that can be used for modulating the immune system that are very specific that may treat either acute pain or chronic pain.

FM: How do you investigate those neuroimmune interactions?

Amanda Brandow: It's complicated in patients, right? And so right now, we're doing primarily some interesting work looking at inflammatory assays through gene expression. And so the work in humans is primarily correlative work because that's sometimes the best we can get at in humans. But the cool thing is that if we find strong correlative interactions with some of these neuroimmune components and pain, then Kate can investigate it further in the mouse model. At least that's my plan, I'm not sure if it's your plan.

Kate Sadler: I've got lots of pharmacological tools to do those things in animals.

FM: What has really been the most exciting finding for you to this date?

Amanda Brandow: Wow, that's a good question, Francie. All of it, no.

Kate Sadler: Everything we learn is important.

Amanda Brandow: That's right. I think probably the work I just recently published in *PAIN* might be some of the most exciting work that I presented yesterday during our symposium really looking at the difference in sensitivity between baseline health and acute pain where we found exacerbation of cold pain sensitivity and mechanical pain sensitivity and actually no changes in heat pain sensitivity. And from what I see [in] these patients, that recapitulates what they actually are feeling when they're coming into the hospital. So that, to me, seems to be the most exciting work so far.

FM: Kate, can you tell me what the Stucky lab is doing right now in terms of sickle cell disease?

Kate Sadler: Sure. So Cheryl Stucky's lab got interested in sickle cell disease I think about 10, 15 years ago. So Cheryl Hillery, who used to be at the Medical College of Wisconsin, initiated this collaboration between the two Cheryls, as I affectionately refer to them.

Amanda Brandow: Cheryl squared.

Kate Sadler: Cheryl squared. Yeah, exactly! So they started working with the mouse model of sickle cell disease and were the first lab to really look at the peripheral nervous system specifically and specific ion channels that are expressed on these peripheral sensory nerves and how those could potentially be driving both the acute and chronic pain that our mouse model experiences.

And so they originally identified TRPV1, transient receptor potential vanilloid 1. It's the capsaicin receptor or the hot chili pepper receptor. That channel is sensitized and drives a lot of pain in sickle cell disease. So all of that work started like 10, 15 years ago, and we've been following up in the Stucky lab on mechanisms of sensitization of that channel. So what are things floating around in circulation that could be sensitizing that ion channel and a number of other ion channels that Cheryl has lots of experience with. We're trying to get very mechanistic in the questions that we're asking in the Stucky lab. How does that relate to firing of these peripheral nerves, and then ultimately, how does that increased peripheral nerve firing lead to changes in central neuron sensitivity or sensitization.

And so, right now, we're really, really interested in lipids that are circulating in our sickle cell disease mice that studies have also shown are circulating in patients with sickle cell disease, how these things changes during an acute pain episode versus baseline increases in these lipids in patients. So we're asking questions about those and very specific receptors that those lipids bind to, again in the context of the peripheral nervous system.

And then, we're also interested in non-neuronal cells that could be contributing to the pain that's going on in both our mice and in patients. And so, we're looking at keratinocytes, which are our skin cells. Your beautiful work, Francie, that you published last year that shows that they can directly contribute to our sensation of touch. So are keratinocytes and the signaling within those cells dysregulated in sickle cell disease models? And similarly, other non-neuronal cells the whole way from the periphery to the central nervous system. So we've got our feelers out in a lot of places, and we're working on a lot of, I think, really interesting biological questions in our mouse models right now.

FM: Yeah, I totally agree.

Kate Sadler: As you should.

Amanda Brandow: Me, too.

FM: Is there a specific direction either one of you hope to see the sickle cell disease research field going in?

Amanda Brandow: Yeah, I think two things. One, I hope people can figure out how to identify which patient is going to move on to have a very severe pain phenotype and which patient won't.

Kate Sadler: Like a biomarker, almost?

Amanda Brandow: Because as a pediatric hematologist I see infants for their first visit at 2 months of age, and so I'm talking with the families throughout their first year of life about different complications they can have. And patient parents will very appropriately ask me, which one will my child have? And I actually have no way right now of really truly answering that question especially in the context of pain. So if we could figure out, is there a genetic predisposition to certain painful conditions in sickle cell disease or are there certain other things that we can identify, that would be really helpful.

And then I also would like to see more mechanistic targeted pain therapies that can be used. And I know that's probably in any painful condition people will answer that the same way, but I think in sickle cell disease there's some very unique things about it that Kate has already eloquently talked about, about how the pain develops from the disease itself over the lifetime of the disease. And so, if there are certain things we can further understand, can we interrupt that early on in life in order to prevent some of the long-term effects?

Kate Sadler: I totally agree with what Amanda just said. So I think doing longitudinal studies in the mouse, because we can in a much shorter time frame than we can do in our human patients, I think is a really, really interesting question that we've been thinking about in this Stucky lab, how to do that. And then, I would also argue that there is a serious lack of information – basically none – in the mouse models looking at how the central nervous system, in particular the brain, is involved in acute and chronic pain in these mouse models.

So, as Francie mentioned at the beginning of this, I studied the amygdala in graduate school, and I know something is going on there in our sickle cell disease mice, and I just haven't had the time to look at it yet. But I think there's one beautiful paper from Don Simone's lab looking at central sensitization of neurons within in the spinal cord dorsal horn and how those things change in these sickle cell mice compared to controls, but outside of that, there's nothing really yet, to my knowledge, published brain-wise with these mice especially in the context of the chronic pain that these animals are experiencing. There are clearly lots of things going on. Sorry to sound generic, but we have no idea because no one's even looked. So I think that that, to me, is where I would eventually go with these types of things, to circle back to the amygdala to start and then look at other regions that are really important in pain processing.

Amanda Brandow: I agree completely as far as the brain. The little fMRI work that does exist in humans definitely confirms what you're saying. There's definitely CNS/brain abnormalities.

Kate Sadler: There's altered functional connectivity between some of these critical pain regions of the brain, and we just have not at all looked at those things in the mouse.

FM: So let's shift gears a bit. Both of you talk to non-science audiences on a regular basis. Amanda, you talk to them basically daily when you interact with your patients. And, Kate, I know you do a lot on the social media front, for example, the social media champion [program] here at the American Pain Society meeting. So maybe both of you can give the awkward scientists that have had a harder time talking to these non-science audiences some tips. What can we do better?

Amanda Brandow: So I guess there are two things. If I talk to my patients, I probably talk a different way, as far as non-science, than if I'm talking to colleagues that aren't really doing what I do or actually friends and family members who are non-medical and non-science. And so, I've had to explain my research in several different areas to multiple audiences. How I usually describe it is that I'm trying to understand how people feel pain. People feel pain differently, and is it possible that sickle cell disease patients feel pain because their disease makes them feel pain differently?

And if we can understand how they feel pain and what are the causes of their pain, then we might be able to better treat their pain whether it's with a different medicine or whether it's with a different way of thinking about their pain and accepting their pain and coping with their pain, also trying to get people to understand that pain is a whole-body experience; it's not just the true physical aspect. As you all know and everyone at this meeting knows that understanding the social and emotional aspects of pain are as important as understanding the true biology of pain and that it all works together. And so, I think trying to get people to think about pain in a multidisciplinary way is oftentimes challenging but important.

Kate Sadler: So how I can talk about science to people who are not scientists – I practice with my family. Neither of my parents has a college degree, but they're interested in what I'm doing. And so I find that describing what I do to them and having them ask me very real, very basic questions was a start. That's where I started refining how I talk to, we'll call them laypeople, about the science that I do. That started as early as graduate school, like explaining some of the things that I was learning about in classes, like what I was doing in my own research. I'm obviously still refining those skills; I'm clearly not an expert. I further refined those skills whenever I was teaching undergraduates as a teaching assistant when I was at Duquesne because they have like a high school level. I was teaching freshman biology majors and also non-biology majors and having to break things down to 19-year-olds who just have gen bio is challenging but also fun. I think it's great. I want people to be as excited about science as I am, and so I think that finding ways to articulate my intense joy of what I do and why I think what I do is so important is

a skill that all of us should have. I think that when we become less accessible or we make our science too hard to understand, that's when we lose interest from laypeople.

So I think practicing those things over and over again, having friends that were graduate students but not neuroscientists also helped me to really be able to explain what an action potential is or why people should care about it. So that type of thing. So that's very generically how I practiced getting my science out to laypeople.

And then I think social media, as you mentioned Francie, is a great way to do that even further. I think an issue with social media is that we can become wrapped up in the circles that we want to be in. So, for instance, I follow all of the big deal pain researchers on social media, and so if I use the word nociceptor in a tweet, they're going to get it. But I also have people from high school and college that follow me, and they don't know what a nociceptor is.

So I try to be very aware of the audience that I'm trying to speak to or trying to get my point across to and constantly self-reflecting on even the vernacular that I'm using; each lab has their own. We could have a conversation right now that very few people would understand if we decided to do that, right?

So just being constantly aware of that and checking in with that and again practicing with all sorts of people all the time.

FM: That's really cool. And I guess with social media, I never really thought about the people that are following me to really make sure that I write the post in a way that everybody who follows me understands them. So that's actually a really good point to make.

Amanda Brandow: Yeah, I agree.

FM: It's something I hadn't considered before.

Kate Sadler: So my sister was my roommate in college, and I hope Zoe is listening to this right now, but I would force her to listen to the talks that I gave while I was practicing them. And she's not afraid to ask questions or make me look like a fool because she's my little sister. So she would always ask the most basic questions, but sometimes those are the questions we forget about. Some of the things she asked me when I would practice, I was like, why didn't I think of that? Or I didn't have an easy enough answer for her to explain, and I tried getting too complex, and I think it really helped me see, don't get so caught up in the small details all the time when you're trying to articulate the importance of your science as a whole. We can do that at a professional meeting like this and talk nitty gritty about C-fiber nociceptors if we felt like it, but my sister doesn't care. And neither do people that have pain. They just want to know why should I care about what you're actually working on.

Amanda Brandow: Agree.

FM: I totally agree. Okay, so let's shift gears again a little a bit. So since both of you took a little different career path, I'm just kind of curious if either one might want to start to tell me a little bit about the hurdles you've personally faced going to medical school or graduate school and how did you overcome them because I think there might be some trainees listening that could take some helpful hints here.

Amanda Brandow: Thanks Francie, for that important and difficult question. I went to medical school, and then I completed a residency in pediatrics which was 3 years. I took 1 year as chief resident and then another 3 years as a pediatric hematology/oncology fellow. And I knew I really always wanted to work in hematology and sickle cell disease. So I started doing research during my fellowship and really fell in love with it and decided I wanted to make it my career.

And then my eyes were opened big and wide to rejection. Rejection of your grants. Rejection of your papers. Rejection of more of your papers. Rejection of more of your grants.

Kate Sadler: It never ends.

Amanda Brandow: But I still maintained my passion and my desire to do what I wanted to do, and I still had – this sounds so, probably, trite – but I really had a fire in my belly to want to try to really understand what was going in sickle cell disease patients who had pain because I witnessed so much suffering and I want to make it better. And so, I just kept going. And my mentors just kept encouraging me, you just resubmit, you resubmit, you resubmit.

And you just have to keep going. And I did and ended up eventually getting a K23 from the NHLBI that supported some of my early work, but that was after I had multiple grant rejections. And so I was fortunate to get that. And I also received an award from the American Society of Hematology, and that supported some of my early work. And then I have received some subsequent internal funding grants and some local foundation grants and now have a successfully funded R01.

So I think rejection is a part of our jobs. I guess you can think of it as a hurdle or you can just think of it as part of the job. And so, I choose to think of it as part of the job because if you think of it as a hurdle then it becomes something you have to overcome. If you accept it as, this is just what it's like being in science, it's not a hurdle anymore because you can always find another journal to submit your paper. I always start high impact and then go down from there because you never know, right? And if you start low, then you sell yourself short. And low-impact journal doesn't mean a bad journal. It just means that it was the right place for that paper.

Kate Sadler: Great advice, Amanda. I love that. So I went to graduate school at Duquesne, as Francie mentioned, and I think one of the initial hurdles that I had is that I did not know what to expect in graduate school. So I don't necessarily know that I had

the best idea of what to expect going into it, and I don't think that's anyone's fault. I think it was just the environment in which I spent the first 21 years of my life.

And so, getting used to being a graduate student was something that took me some time. How do you balance life commitments especially when those life commitments don't necessarily understand or appreciate what you're going through as a graduate student? I think that was something that was particularly hard for me to deal with. Sort of like explaining why I'm doing what I'm doing to people. And why I have to work on Saturday this week or why I'm going in Sunday night to prep something for Monday. Graduate school is not a 9 to 5, five days a week job by any means. At least I don't think it is.

So I think that was one of the initial hurdles that I had in graduate school. And then also I wasn't getting data for the first 2 years of graduate school. The technique I was using was difficult at best, and so, finally someone came in and helped me really turn that around. And then I started producing data more quickly after that, but for a while, I felt like, what was I doing here. Am I good enough?

I think imposter syndrome is something that we probably all experienced and could talk days' worth of conversations about if we so choose. But I think I felt that, again, more so at the beginning of graduate school, and it was something that my PhD advisor really helped me kick towards the end. Also, when you're getting data and feeling good about stuff and things are working. And I got a fellowship; I somehow was not rejected the first time.

Amanda Brandow: I still think it's crap [laughter]. Sorry, it's not crap.

Kate Sadler: Thank you, reviewers, for giving me my fellowship. But I didn't believe it, that that was happening. So I think it's been this really slow process of me really believing in myself and what I'm capable of doing. I still struggle with it. I think that another sort of a low point in my career trajectory so far was the first few months I was in Cheryl's lab because regardless of how supportive Cheryl is, because she's amazing and the other people in the lab, it's hard because you're on the top in graduate school and then your sort of bottom of the barrel again when you're a postdoc. And what projects do you want to work on? What do you feel passionately about?

I came to Cheryl's lab because I wanted to learn a specific technique, and the science that she does is really cool, but it wasn't that I had a very specific science question that I was asking when I joined. Now I feel like I've figured that out, but that took time, and I still sometimes struggle with [it]. I love all science. I think that's not a hurdle but maybe a problem is that I like too much science, and I can be excited by lots of different things. Yeah, I think that's a lot of it.

And then I think, emotionally speaking, dealing with the change of – so I was in Pittsburgh and I moved 9 hours away and knew literally nobody in Milwaukee outside of the people that I was working with on a daily basis. But not having that familial and

friends support network really close by was something that I really struggled with when I joined Cheryl's lab. And so, total note about mental health and making sure we're always checking in with each other and getting outside help if we need it as part of this process, I think, is something that I'd like to re-echo here. But I think those are some of the major hurdles that I have felt so far. I'm sure there will be way more to come.