**Transcript**

**Behind the ASO Design and Discovery with Konstantina Skourti-Stathaki, Ph.D.**

**Narrator:** Join us at the 2024 nano-rare patient colloquium hosted by Biogen on October 30th through 31st in Cambridge, MA. At this event, n-Lorem will review the foundations progress, present case studies, and host discussion panels with members of the nano-rare community. You'll also have the opportunity to connect with supporters, physicians, advocates, and of course, patients and their families. We hope to see you there. Visit nlorem.org to register today.

**Stan:** Hello once again everyone. I'm Stan Crooke. I'm Chairman, CEO, and founder of n-Lorem and your host for the n-Lorem podcast series. Just before we get underway, I want to remind everyone of our second annual nano-rare patient colloquium. We're very excited about the opportunity to share what we're accomplishing with our patients, our families, and all of the different colleagues who are contributing so much to our effort. It is in Boston, at the Marriott Cambridge Hotel, and it's October 30 and 31st. So, we look forward to seeing you there. And today we have a guest that I think you'll find very interesting, and I know reasonably well and getting to know better every day since she has chosen to come to work here at n-Lorem, and we call her Nadina, and I can't pronounce her name it's a hopeless Greek name. And so, I'm going to ask Nadina to introduce herself.

**Nadina:** Absolutely. I'm thrilled to be here, Stan. Thank you for having me. So, my full name is Konstantina Nadina Fusor Skourti-Stathaki. Yeah, these Greeks have sometimes lots of last names.

**Stan:** Yes, and many vowel and consonants. And I think we're going to have to talk it over and maybe change your name to a nice American one. I don't know what, but anyway, anyway we've managed our way through that and so Nadina welcome. Nadina and I, of course, talk very frequently multiple times a week, so this is just one of those interactions that it's a little different from what we normally do, but we normally focus on data and progress that we're making and that sort of thing. So, Nadina, I know that you grew up in Greece, and went to university in Crete. You know you've made a lot of transitions in your life and at the time you started school in Greece, I'm sure there were lots of good things, but there were also many challenges in Greece. So, I'm intrigued by how you got interested in science and what brought you here, I guess is a simple question.

**Nadina:** I think at school I was always drawn to more life sciences. So actually, biology and physics were my two favorite subjects. I like physics because I thought it was very logical and it made sense. And I like biology because I like imagining. I had so many questions, so much about the microscopic, and what you cannot see but you have to understand it, and to some extent imagine it that I found it fascinating. And when the time came for me to choose where I would go to university I was like, no, I think I'm going to go for biology, not for physics. I think I have more questions unanswered in biology than physics. So I chose biology, and at the time I like more the molecular side of it and Athens at the time was more zoology and plants and things like that aspects of biology, whereas Crete with more biochemistry and molecular biology, and they had a very, at the time, new build institute there. So, I thought, OK, I will go to Crete, I will go to Heraklion. So, it was a general degree of biology but with more focus on molecular biology and biochemistry, and I got into an internship program at the time where I was on my second year or something of my course and there I started working in a lab. The lab was about transcription in yeast, so this is where I started like at the age of 18, I think got acquainted with RNA. And I found it fascinating how, you know, it can be transient, it can be versatile, it can you know, it's the intermediate between the big guys, right, the DNA and the protein. And throughout that internship I liked a lot the lab I stayed there for my bachelor thesis and I just never left the field I guess I just you know from yeast I went to my mammalian cells for my masters and then from there I just expanded and expanded, you know, and learning more and more about RNA.

**Stan:** So, curiosity drove Nadina I guess is the simplest way to say it, huh?

**Nadina:** Absolutely, yeah.

**Stan:** And both your parents were teachers were they not?

**Nadina:** Yes so my father is a physics teacher and my mother is a chemistry teacher. So yeah.

**Stan:** The two most basic of the applied sciences right, the mathematicians consider all science applied except math, but and so I'm sure they encourage you toward a higher education as well.

**Nadina:** Yes, absolutely yes.

**Stan:** Yes, and then after you did your masters, you ended up at Oxford. Big step. Of course, it's a shorter trip from Athens to London than it is from San Diego to London, but it's still a big trip to take for a young person.

**Nadina:** Yeah, you see, now, looking back, I was like, oh, wow, you know, I was not even thinking about that. I was like, no, I'm going. I'm doing this. Yeah, I think I left Greece when I was 22 or 23 or something like that to go to Oxford. And yeah, I think I saw it was actually my masters boss at the time that he was saying oh you know, because he could see that I was interested in things and he was like, oh, you know, maybe you can look at programs outside Greece. And I started looking and looking and I came across at this studentship, this like, almost grant things for Ph.D. students in Oxford and now thinking about that, I was like, I don't even know how it came into my mind that I'm going to try it. And I did and then, yeah, is this so I think it just like happened in that way. Yeah.

**Stan:** Well, life does happen, doesn't it? So, and you were in some good labs at Oxford and Proudfoot and others. And good RNA labs as well and I guess there was where you first encountered R loops and RNase H and all that sort of thing, right?

**Nadina:** Yes, all these super cool, fascinating things, exactly. It was in my PhD that, yeah, I got to meet RNA/DNA hybrids and maybe the 2nd or the 1st best enzyme in the world right? RNase H1, maybe the second best RNA polymerase 2. But yeah. And I think life is very funny sometimes because I thought oh okay in my academic career I was studying a lot RNA/DNA hybrids and I thought okay, maybe I'm going to abandon them for a bit, but nope.

**Stan:** So, then you decided to leave academia and go to a company in the UK at the time. That's another often a decision that people stumble over.

**Nadina:** Yeah, yeah. It was definitely a well calculated decision. It was a logical decision. On one hand it was a no brainer for me, but on the other hand I had to convince myself also that that was the right decision because I was enjoying very much what I did, and I think I'm just I was very passionate about R loops and about the work that I was doing and I didn't have any, you know, regrets, I wasn't bored or anything like that. It's just that I think what I was missing was the impact, I think what I was missing was waking up every day and thinking I'm doing this because of that. And the so what? And I think this is you know my curiosity was definitely you know if we were to say that the two things are driving our curiosity and impact my curiosity was definitely full on, but the impact is what I started missing and I think this is what drove me to the decision to leave academia.

**Stan:** And so, you went to an RNA company, and is that pronounced Mina or Miner?

**Nadina:** MiNA

**Stan:** And was that in Oxford?

**Nadina:** That was in London.

**Stan:** And so what you worked there and then eventually you transferred to San Diego.

**Nadina:** Yes.

**Stan:** How did the work go there.

**Nadina:** So essentially what happened was I moved with my husband here in San Diego and then MiNA was very, very kind and told me no well, actually you can I had a team there and I was managing and they would say well, actually, you know, you can manage the team remotely as long as you come and see the team and us relatively frequently. And of course, the time difference was a challenge, but we worked it out. And you know the things that I was doing, you know, I had very, very early mornings. I was waking up very early and all of that to catch up meetings with the team and then work on, you know, on things that I had to take care of. And I think that that is maybe the transition towards the, you know, rare diseases more. When I joined, I was mostly involved in an immune oncology program. But then slowly I was more drawn to the to the rare diseases, genetic rare diseases like root cause. And yeah, I think that this is how the transition happened.

**Stan:** So, what are the most important things you learned in your first drug discovery and development experience?

**Nadina:** I definitely learned that the impact and the so what is a very important thing that this is what drives decisions. I also learned there is a very different mindset from academic research that there are times that you will have some questions that they are unanswered, but unless these questions are the ones that you need the answers to make a decision, you need to let go and move on. And go on to the next. For example for discovery. There might be some things that you don't understand and they're interesting to explore, but perhaps not relevant at the time, but you need to find the right experiments to do that will give you that decision making answer. I think it's very it's more specific focused and also, I think you need to be not necessarily an expert, but I think you need to have I find myself that I know more things about different things because you need to know a lot of different things to help you in these decisions. And being, you know, rational and reasonable and all of that rather than just have a lot of expertise on one particular subject.

**Stan:** And you were very comfortable at MiNA and yet you decided to leave what sounded like a comfortable spot to come to n-Lorem a new nonprofit with an impossible set of dreams really. Why?

**Nadina:** The mission is just something that is just indescribable to me. I really feel and this is not an exaggeration because I'm Greek, but I'm dramatic, but I really feel that all my life I was preparing to be where I am at the moment and doing what I do for n-Lorem and the mission. It taught me and it made me think that hope is possible, that I need to not think so much about the patient populations, but about that specific patient and their family and the fact that we are right there with them every step of the way and we are fighting a different battle, but we are fighting with them so that was one part that for me was yeah I mean, I had heard about n-Lorem and I was like, wow. And the other part was, of course, and meeting everybody here I was like, wow. I mean, this is amazing what n-Lorem does and what, you know, what a beautiful team you have assembled. And the other part was of course I knew your work, you know I know you're RNase H1 papers. I know all the fantastic things you've done with ASOs. I felt that that was close to my heart because of my, you know, my R loop connection. And of course I know what Ionis did and you know It was a time that now RNA is more sexy and everything after COVID. But you know we've been, you know what you've done was years ago. So, you know, it's very inspirational everything.

**Stan:** That's great and so I don't want to put you on the spot, but. As you think through that decision. What are the most fundamental benefits of having made that decision, what have you learned that you might not have learned at MiNA or another organization?

**Nadina:** The first thing that comes to my mind is that you need to look for that decision making process, I was talking earlier, you really need to come back to the patient. There are disease progression. There are specific situation, but the symptoms, this I believe and because the process for nano-rare is very different to what for example I would use as overseeing discovery programs at MiNA. You really need to look at the whole picture. It's a full circle. It's not just fragmented that you know, inevitably, if you have a biotech company, even a startup biotech company, a small biotech company like MiNA you still have this more fragmentation of discovery. You have the clinical development. Medical development, clean ups and all that now it's the full thing that you're seeing and I'm just learning so much, it's like I'm like a sponge, you know, every day. It's a new thing every day. I think this is something that you can't learn. Or it's difficult to learn in a commercial biotech.

**Stan:** I think what I want to do now Nadina is actually, you know, at a high level talk about the work you do every day and the challenges that are associated with ASO design, ASO discovery, whether it's for a single patient or whether especially if it's a single patient how you have to be even more precise than perhaps if you were developing an ASO for a broad patient population.

**Nadina:** Yes, it is absolutely. It's a very rewarding work, but at the same time, as you said, it is a very challenging one with very specific steps that I think that you know connecting these dots that's where I think experience of every step comes into play and this is why it's so important to have that every step of the way. Now, if we were to talk more about these steps, the very first step is, at least for me, and the work that I do every day is as soon as the patient's case is accepted through n-Lorem, what we do is I just see briefly the case. I just know what is the ASO strategy that we're going to go for? If it is an RNase H1 ASO if it is a splicing ASO and then I coordinate depending study a little bit the gene, how the gene is expressed, what are the best cells that we're going to do the screening on. So this is what I kind of coordinate, which are the fibroblasts, are they APCs are the neurons. What we need, and this is something we get from the physician and really the core of where the discovery starts is essentially the whole genome sequencing. And this is I think a part that is, for example, speaking about differences before this is different, right? Because to patient population because this specific whole genome sequencing is completely is about this particular patient. We need to look at their polymorphism without me going into too many details. Single nucleotide polymorphism, seeing their pathogenic mutation, seeing their nonpathogenic variations, and these are essentially the spots where we're going to design these ASOs so that that step is crucial and we have a very experienced team here where we as soon as we get this whole genome sequencing data in we assess them. Firstly we see how many snips we have do we have any go/no go decisions at any stage. If everything is good, then it goes through our normal discovery pipeline. We start designing these ASOs, we put the chemistry in and we order and this is the most crucial, I think step is the very beginning, but it's a very crucial one and this is the one that differentiates also what we do versus what a rare disease biotech would do, for example. And after this step and of course the more and you know this knowledge is transferable. So the more because now we have so many patients we every time you know we get this whole genome sequencing data we apply it to the next and the next and the next and we learn we learn so much. The next step then is we develop all the tools, so we order the ASOs in the meantime. As we had mentioned before about the cells, the cells arrive at the end Lorem lab without culturing them without banking them, we put them, let's say in a stage that they are ready for screening and in the meantime we also start developing all the tools that we need for the discovery and to really assess depending on the strategy for the ASO I'm going to speak about RNase H1 ASOs in particular, the readout that we want to see is we want to have a very allele selective ASO so we want to reduce the mRNA levels of the pathogenic allele where else keeping as steady as we can the wild type. In order to do that we have our tools and we have a toolbox where we essentially have primer probes, but they are very specific for each allele and for that it's not an easy and every case is completely different, but sometimes it's very challenging to actually find something that and this is, I think, where experience and expertise comes in. To find a set that we are very comfortable with, it has passed all our QC and we know that this is exactly what is going to give us the best readout.

**Stan:** And then we have to make the judgment about whether it's good enough and that and of course, we have formal systems to do that. How have you found those processes?

**Nadina:** I think that the this is the first thing that comes to my mind. I think you really are enabling and really inspiring a transparency system which I think that I personally value a lot and I think that this is something that is very also refreshing and nice. So, we all get together and this is like open to actually all employees where we make these decisions of like is this a so as you said good enough for this program that is essentially, we collect all the data and there we see the data and you know and where they have led us and what are the options that we have or not sometimes and then we need to make the decisions of do we continue, how do we continue. Do we need research? More research experiments to lead us the way or is there no way at this moment in time.

**Stan:** Well, and we are fortunate to have such a great team and a great team in your lab as well. And before we leave what you do every day, I thought maybe a minute on some of the basic research that we are also doing. On ASO mechanisms on various programs that require basic research.

**Nadina:** I will start with the first one on a little selectivity because this is such a crucial, as I said at the beginning, you know having an ASO that is very a little selective is really crucial for RNase H1 ASOs so obviously everything we can do from fundamental research, mechanistic ASO research to really improve this process is going to be advantageous for any program really for any program. So we have a program that we essentially focus on that focus on establishing ways of enhancing this allele selectivity that has built on from the work that you've done at Ionis. And now we are expanding this in order to be able to use it directly to discover. This is one aspect. The other aspect is of course sometimes we can have ASOs that they are, you know, pro inflammatory and they have an inflammatory response and of course the more that we learn about this response, the more this we can incorporate into our design. And essentially build and design ASOs that they are safest from the get go and so we also have a research program that focuses on that on us understanding and I think the goal here for all the mechanistic programs is the more you understand, the more you understand the fundamentals of how ASOs work, the more you can complete the circle on the design and discovery and the better and safest longest in duration ASO you will make. So, this is the mindset that we have.

**Stan:** I share your excitement about all that and you and I share that every week. So, it's been a passion of mine now for almost 35 years, so glad to share it with you. Thank you for all that. I suppose I should close by seeing if there's any question that I haven't asked that you wish I had.

**Nadina:** Why antisense?

**Stan:** Why antisense? Now that's a long conversation long ago, but it certainly had to do with finding a means of making new medicines that would be vastly more efficient and make better medicines than the technologies that were available at the time. And so it's that effort, founding Ionis and persevering through all the challenges that we faced, certainly is one of the great experiences that anyone could have, certainly a great experience for me and then to spent all those years scaling antisense up and then now downscale it to treat single patients properly is just another privilege that I've had and get to share with others. And in the end, advances are always driven by new technologies and the beauty of antisense is still advancing as you mentioned, it's advancing every day in our lab, and we'll make better drugs tomorrow. And for me, that's what more could what more could a person ask than do great research with an important goal and see it realized. Well, Nadina, thank you so much. Now I want to bring this to a close so you can get back to work absolutely. Thanks so much.

**Nadina:** Thank you very so much Stan, thank you.

**Narrator:** n-Lorem is a nonprofit committed to discovering and providing personalized, experimental treatments for free, for life to patients with genetic diseases that affect 1 to 30 patients worldwide referred to by n-Lorem as nano-rare, many of these patients progress and die without ever achieving a diagnosis. This is where n-Lorem comes in. They do the impossible by providing hope, and for those that they can help free lifetime treatment. For more information about n-Lorem or today's episode, visit nlorem.org. Any questions can be sent into podcast@nlorem.org search and n-Lorem on Twitter, Instagram, YouTube, LinkedIn, and Facebook to connect with us. This video is hosted by Dr. Stan Crooke and produced with the help of the following professionals. Thank you for watching.