Pharming Group N.V.
Cell VUS Study KOL Webcast

June 30, 2025

Rewatch the webcast
Download presentation slides

PARTICIPANTS

Anurag Relan, MD – Chief Medical Officer at Pharming Group N.V.
Joshua Milner, MD – Columbia University Irving Medical Center; Professor of Pediatrics and Director of the Division of Pediatric Allergy, Immunology, and Rheumatology

Anurag Relan, MD – Chief Medical Officer:

Thank you. Good morning. My name is Anurag Relan. I am Chief Medical Officer at Pharming. I'd like to welcome you to this webcast where we'll be discussing results from a new study that will advance the classification of variants of uncertain significance in APDS.

I'm pleased to be joined today by Dr. Joshua Milner, Professor of Pediatrics and Director of the Division of Pediatric Allergy, Immunology, and Rheumatology at Columbia University in New York. During the call, we will be making forward-looking statements, and I draw your attention to the details on this slide.

The goal of today's call is primarily to discuss the findings from a recent publication and provide context for these results. In this study, there was a significant expansion in the number of gain-of-function variants identified, which we will be reviewing.

And Dr. Milner will review the data, which can help reclassification of patients who previously received a VUS test result, as well as aid in the diagnosis of APDS patients tested in the future. In addition, we'll review how the study proposes a new understanding of a much greater prevalence of APDS.

Because of the significance of these new findings, we wanted to give all investors the opportunity to hear directly from Dr. Milner and Pharming to provide further context for understanding the results.

While these results are important, there are also key next steps that need to be performed by genetic testing labs to determine exactly how many patients have APDS. We continue to expect that over time, 20% of the 1,300 VUS patients identified in the United States will eventually be reclassified as having APDS. Dr. Milner will now present the results, followed by a discussion of questions that we have received from analysts and investors. And with that, I'll hand it over to you, Josh.

Joshua Milner, MD – Columbia University Irving Medical Center:

Thank you so much, Anurag, and thanks to everyone and good morning. Thank you for your attention. I'm a Pediatric Allergist, Immunologist, and a Physician Scientist, and I'm happy to talk about a study which we recently published in collaboration with Dr. Ben Izar's lab. He is an adult hematologist, oncologist, utilizing a very interesting and novel way of approaching the problem of how to understand when a variant comes back on a patient in a particular gene, how do we know if that variant is actually causing the disease that they have?

So what I wanted to start with is, is really to lay out the problem. The problem is that when we incorporate genetics into precision medicine, that is that a patient comes to us and we think actually that genetics might be able to give us information that allow us to treat this patient based on what's underlying, what's driving their particular symptoms.

The first step though, of course, is we need to actually get that genetic testing done. And it's certainly, a major issue that we don't often know that there's a genetic disease of the immune system right in front of us when a patient comes in with autoimmunity or with infections, or where their immune system is turned on and the cells are dividing too much, or a bad allergy.

And so, what we essentially rely on is what people have reported in the past. Well, Syndrome A tends to look like this because we saw, X number of patients with these different types of symptoms. But in the end, what we're looking at is a referral bias. We're looking at the most extreme cases that were brought to a very highly specialized physician, who then performed genetic testing and found that this was a new disorder. And very often, the first reports of that, even if it was a small number of people, tend to get canonized as to what is it that this genetic disease might look like.

Experience has certainly shown us that as more patients are found with mutations in a given gene, there can be more in different clinical presentations, including milder presentations where there's only one symptom that comes up, and those patients are almost never thought of as someone who should get genetic testing. But we know these things because, for instance, in families, we have what's called variable penetrance. Some family members could be very severely affected, and other family members may have very mild disease. Some family members have no disease.

That also creates problems because, in classic ways of understanding genetic syndromes, when there are people who carry the mutation but don't have symptoms, that tends to lead a geneticist to interpret that to mean that that mutation may not be as relevant to disease as one where everyone who carries the mutation has symptoms.

This is a very important point that is evolving in our field right now, where we are realizing that these, what we assume to be rare disorders, have a far more broad way of manifesting.

And where that really matters is that even if it's a mutation that only mildly changes the protein, that mild change might be enough to cause a single symptom, which could be treated the same way that someone who has multiple severe symptoms and has a more severe impact that is caused by the mutation that they have. So ultimately, what this means is that even though it's a more mild issue, there's still the same precision treatment that could help a wide variety of patients beyond just what we conceive of as the rarest of the rare.

Now, the problem is once we've gotten past rate limiting step point number one, which is to do the sequencing and to look for genetic issues. The second thing is, is once we get our results, there is a very good chance that no one has seen that mutation before in that particular gene.

So you'll see a rare mutation in the gene, and it changes the protein sequence, let's say, of that particular gene. However, because it hasn't been seen in the past or it's been seen in the past, but no one has ever done further research to decide if that mutation might actually be causing disease, it is then called a Variant of Uncertain Significance or a Variant of Unknown Significance or VUS.

And so, if we just look across many different disorders, it tends to be that a Variant of Unknown Significance is just as likely to be found as a variant that either is known to be benign, doesn't cause disease, or a variant that we do think causes disease. That's a huge number of variants. And in fact, some clinical sequencing companies don't even bother reporting those Variants of Unknown Significance because they don't change care from the perspective of a clinical geneticist.

In our field, in the field of genetic diseases of the immune system, there's no doubt that we want to see those Variants of Unknown Significance, because we do have tools to better evaluate the function of those genetic changes. We also may have patients who carry those variants who have symptoms similar to others who are coming in, which gives us a better idea that perhaps if we have more than one person with significant symptoms and they have the same variant, that gives us an idea that it might be causal.

But then, the work does need to be done to then functionally assess that variant and see if it does the thing that we expect it to do, if it were to be causing the disease. This is done, it's painstaking. It's done one by one, generally speaking, it's done usually by maybe one or two or three research labs, like mine and others in the country or in the world. It is not covered in any way clinically to do this type of investigation. So there's no clinical reimbursement for this type of work.

And the research-based evaluation of these variants is often not funded because that's just not necessarily where someone's focus was, to write a grant to do more research-based analyses of variants. That's really the state of the art when it comes to a variant showing up that's a Variant of Unknown Significance. It's a lot of hard work and it's difficult to get done, both because of the small number of labs that are able to do it and because there's no real way to do it quickly, because it's not necessarily funded to do that type of work.

And so, what we have tried is to apply a new method where you actually can, what's called multiplex, which means that you test many different variants at the same time as a screen. And I'll describe exactly how that's done. But essentially, you're looking for a particular biological effect that we expect to see, that we know is associated with the disease, and we look to see if that biological effect is happening in cells that bear different mutations. And those in mutations can be introduced at larger scale.

This type of method where you're not just testing one variant, but you're testing tens or hundreds or thousands of variants at the same time, is becoming a new method. It's done gene by gene. It's been done in cancers quite a bit and it has been done very, very minimally in the field of genetic diseases of the immune system. And we thought this would be a good way to apply this method for reasons I'll explain.

And importantly, in order to reclassify that Variant of Unknown Significance to determine if it was benign or if it is pathogenic, the American College of Medical Genetics and Genomics, and also the Association for Molecular Pathology, these are the parent groups, the umbrella groups that set the standards for how to determine what types of data are acceptable to help determine if a Variant of Unknown Significance may actually be pathogenic or benign.

They now accept well-performed experiments using these multiplexed functional assays, looking at multiple variants at the same time, so that, that which we produce can meet the criteria that are set out by these organizations, so that a genetic counselor, a molecular geneticist at a clinical

sequencing company can say, okay, I now see that this functional data exists for this particular variant and I can make a decision on the pathogenicity that I couldn't make before. Very important point. And that's why we wanted to go ahead and try this in this setting.

So, of course, activated P110δ or PI3Kδ syndrome, APDS is a very good example of a genetic disorder of the immune system. It causes immune dysregulation, infections, autoimmunity, allergy, lymph proliferation, risk for lymphoma, and a variety of other symptoms, and it's caused by mutations that render the enzyme PI3Kδ gain of function. It increases the function of the enzyme.

And that increase in function can actually be measured in cells from a patient, or if we take cells and edit a mutation or introduce a mutation into that cell, we can see that increase in function that we know correlates with causing disease when we see that increase in function.

So, the way that we actually introduce these mutations into cells is we actually take a healthy donor's peripheral T cells, just from a blood draw. We isolate the T cells, which are a major driver of the immune system, and obviously, a major driver of the abnormalities seen and the symptoms that are caused in APDS. These T cells really do cause the trouble for most of these patients. And so, they are a good model, and we can see in a T-cell from a patient, who has APDS, we can see an abnormality that can be measured that tells us there's too much P110δ activity. I'll show you that in a second.

We actually can take what are called base editors. They literally go and edit a single base pair, a single letter in the genetic code for the gene of interest. And they can introduce these mutations one by one, one mutation in each cell, using an enzyme called a base editor. It uses CRISPR technology, which is well-established. But it uses CRISPR technology to actually introduce a simple switch of the base to, I mean, it's not simple to get it done, but once it's done, there's no other disruption that's really happened to the cell except for to change to that one letter.

We're not over expressing the enzyme, which can cause difficulties. We're not knocking the enzyme out. We're simply changing the genetic code where it is and so that the regulation of that gene is as it is normally. It's just expressing a slightly different protein because we've made an individual change.

And we actually then can essentially print thousands and thousands of different templates, what we call, guides that go into the cell and then the guide actually causes the edit in the presence of an enzyme. So, we make many, many, many, many different guides and put them into millions and millions of T cells, so that any given T cells only expresses one of these mutations, the guide that's gone in there. And then we allow those T cells to expand.

Now, the next piece that we do after editing the cells is, what is the readout that we look for. So, again, let's imagine that these are millions and millions of cells, on the left-hand side, of T cells. We've introduced thousands and thousands of guide RNAs to make as many individual edits as we can, as we possibly can. And then, we put in the editor, which is the enzyme that actually makes the change that those guides bring to the DNA. And then we allow the cells to expand.

And then, we essentially look for that, which we want to look for. We can look to see after cells have divided a lot, which variants are found more or less, or we can take the cells out and perform a stimulation, in this case of the T-cell receptor, and look for these two outputs, AKT and S6, which

are measures of PI3 kinase activity.

When AKT and phospho-S6 are high after T-cell receptor stimulation, that implies a gain of function. In certain cases, we actually can see them elevated even without T-cell receptor stimulation. That for sure tells us that there is a gain of function.

When AKT or S6 are absent, after T-cell receptor activation, that is what a loss-of-function mutation can look like. And so, with these many millions of cells, each of which bears a mutation, we then sort and for enrichment of those that are, let's say, divided more or bear a marker, which I'll show you in a second, more, or less, we sort them.

And then we just perform sequencing and we sequence to see which variants show up more in the population that we are evaluating. So, and that's, we can do this with fluorescent activated cell sorting, where we actually can select the cells at the extremes to see what variants are enriched in that population, and compare them to each other and to the baseline.

So, this is actually the test that we are doing. This is actually data from fluorescent activated cell sorting and it looks exactly like someone who has a gain or a loss of function. And so, in this particular case, we have cells, we have an activation where we are looking at PI3 kinase as measured by AKT along the X-axis and phospho-S6 along the Y-axis.

And you can see that there are those that are higher and those that are lower. This is in a control where we haven't made an edit. This is in a loss of function control where we've put in a mutation that leads to inability to activate PI3 kinase. And as you can see up here, in the top right corner, there's very little.

And then, on the right-hand side is the gain-of-function mutation where you see that many more of the cells are high for phospho-AKT and phosphor-S6. And that is what a gain of function looks like. So if we sort these different boxes, we can see what the relative frequency of variants are that are contributing when we put all the variants together.

This is exactly what the data would look like if I did this assay on a patient who has a gain-of-function mutation where I haven't edited anything on the right-hand side, or if I did it on a patient with a loss-of-function mutation in PI3 kinase. It would look like one in the middle, whereas the normal looks like the one on the left. So, the edits are actually recapitulating exactly the test that could be done on patient's blood. And we do that in the lab now.

So these are the data that we got. This is looking at PIK3CD, one of the two variants that causes APDS and starred on the top. So if you look above the line, these are all variants going across the gene. Anything that's above the line is a gain of function, and that which is highlighted in red is what we've essentially set as a cutoff for significant gain of function because these are the sequences that we found more frequently. We've starred several variants, which we already know are gain of function from previous work, of that painstaking one by one type of work that I had mentioned before.

And it actually turns out that we did successfully find those two variants. We did not miss any gain of function variants using this method, but then we found many scores, new gain-of-function variants across the gene.

And then also, if you look below the line, those are loss-of-function variants. Those are variants that are dropping off, that are not being found in the activated compartment, and they're enriched in the unactivated compartment. Those are loss of function variants, that may help us diagnose other genetic diseases that are associated with decreased PI3 kinase activity.

The same story in PIK3R1, again, we were able to identify known gain-of-function variants, including the most common one, which is, what's called, a splice variant that takes out exon 11. That is the original PIK31 variant that was described. And then we found multiple others again across the protein, gain-of-function variants as well as loss of function variants.

So that was extremely exciting to us. We made thousands of different variants, about 10% of all variants that could possibly be made using the particular technology that we used. That's way, way, way more than has ever been evaluated before. But it also tells you that there's still 10 times as many that could be evaluated with additional changes to the technology that we're working on.

The next point is that we were able to take variants which we found, known and new, and we were able to evaluate one by one. So, once we knew that we could make the variant, we actually could take an individual variant, put it into healthy T cells, and make a line of cells that only expressed that particular variant, so that we could study it a little bit more.

And with all of these variants, we then stimulated in the presence or absence of leniolisib to demonstrate that these gain-of-function variants could be inhibited by the addition of leniolisib. And so, here, what you're looking at, the dotted red line is a normal PI3 kinase activity, measured by AKT on the top and phosphor-S6 at the bottom. Again, these are just measures of PI3 kinase activity. The dotted line tells you where wild type or normal is, and then the pink above the dotted line is showing you the gain of function. And then, the green is where that particular variant, that T-cell was stimulated in the presence of leniolisib and the green shows you how it has dropped down to near normal.

There were a few variants that showed not as complete inhibition with leniolisib and that may be of importance because it may mean that the dosing of leniolisib may need to be altered in those types of patients. We don't know yet. But it also could be that it's telling us that only that much inhibition is necessary to treat clinical disease. We'll only know that as more patients with those particular variants are found and treated with leniolisib.

But it's an incredible, essentially microscope on how the cells whose relevant abnormality is being corrected by leniolisib, can be evaluated with each variant one by one in those cells, having successfully picked them out from the screening. And then just to show what loss-of-function variants look like.

Again, if we put those variants in and we stimulate the cells, they don't get activated hardly, at all. And leniolisib, there's not much for it to inhibit when that ends up happening. So that just sort of validates that our method is working in the scores of variants which we just validated to show that our screen is actually telling us exactly what would happen if just a single variant were made into primary cells.

We then looked further and saw that leniolisib normalizes a number of different problems, beyond just that increased signaling and activity of PI3 kinase, there are a variety of different markers of T-

cell dysfunction that are abnormal in APDS. All of those markers essentially normalized in the presence of leniolisib.

And I'm highlighting here just two of them, because essentially, these are really telling us exactly there's an abnormal expression of these cells. And some of the cells that have this abnormal expression can be, what's called the T follicular helper cell. That is one of the main drivers of the autoimmunity and potentially the allergy in activated P110δ syndrome in APDS, is too many T follicular helper cells that are activated abnormally.

And what's nice to see is that the leniolisib can actually correct the surface markers that are seen and more work can be done and we can actually now study directly what happens, how does the mutation cause this increase in T follicular helper cells, and how does leniolisib inhibit, and to what degree does it inhibit?

We can get far more accurate information than if we try to take cells from a patient and control and compare it that way, because we can control the system much more carefully. So it's not just that we're measuring this increased PI3 kinase, which we know is what drives the disease. We're also measuring how that actually impacts the cells to actually cause the different types of symptoms like autoimmunity, immune deficiency, and such.

Okay. Now, the next step that we wanted to do having identified a number of these variants, was to now look back and look into databases of patients who have undergone genetic testing because they had immune symptoms that made one worry about an inborn error of immunity or a genetic disease of the immune system. This is important because we could actually take the data that we got, and send it to the clinical labs who performed that testing and say, Hey, those patients carry a variant, which we think is gain of function. Here is our data. Could you please reclassify it?

The second thing that we wanted to do is look into, now, we have very large databases in the general population of hundreds of thousands of people, where the patients have consented or the volunteers have consented to have whole genome sequencing done and then link that sequence to their medical record, so that associations can be made between genetic variants and clinical outcomes.

So, we did that and first, we looked in, again, the database of patients who've been sequenced for APDS and other genetic diseases of the immune system, and we actually ended up finding 27 patients who had variants that we now believe are gain of function that previously were described as a Variant of Unknown Significance.

And we looked in those patients and indeed, many of them had the very symptoms that patients with APDS were expected to have. We also found even more patients, who had a mutation at the same amino acid, but a different amino acid change. And that's important because, generally speaking, mutations at individual amino acids can be gain of function, even if it's a different change that ends up happening. And we're validating that as well.

But it essentially massively expanded the number of patients almost immediately, who could now be reclassified as a gain-of-function mutation and whose symptoms, like the ones that are listed on the far right here, could be treated successfully with leniolisib, which is a huge benefit to patients to now be able to receive the medication that would treat the underlying cause of their disease.

Until now, no one knew whether the mutation they had, the variant that they had really was causing the underlying disease. Now, we are providing this new data across many different variants that allows for a reclassification. So, that's what can be done there.

Now the second thing I mentioned to you is looking into these large databases. So, the U.K. Biobank is adults who volunteered for this study. There are about 570,000 participants in this study, matching genetic changes to phenotype. And then the "All of Us" database, which is in the U.S., about 270,000 patients where there's genetic information anonymously linked to clinical outcomes.

We looked in these databases and actually found that several of our variants, several of the gain-of-function variants we found, were encountered in these databases in the combined, let's say, 700 or 800,000 individuals who volunteered, generally speaking. Again, these are adults, and generally speaking, they skew towards healthy, in these studies, because they're volunteering, they're usually not acutely ill, They're just volunteering for a study.

So keep that in mind. But what we found is that, any of the gain-of-function variants we had, one in 5,000 of the volunteers carried one of the gain-of-function mutations, which we had newly discovered.

So in aggregate, that was an enormous number of people. In aggregate, that means that where I am in the New York metropolitan area, there may be thousands and thousands of individuals carrying a gain-of-function mutation. We then went in and looked to see what these individuals had. 12%, one in eight of the individuals who carried a gain-of-function mutation had multiple immune mediated disorders. So, autoimmunity plus infection, colitis plus infection or allergy.

Not just a single immune diagnosis, but multiple different immune diagnoses, one in eight of those carriers. So, if there are thousands and thousands of individuals carrying these gain-of-function variants, one in eight of them is symptomatic and could, as we've shown, in vitro in the tests earlier, they have too much PI3 kinase activity, which is inhibitable by leniolisib.

We can actually dig even a little bit deeper and look at individual symptoms that are enriched in people who carry versus those who don't. This is not to say that there aren't other symptoms that the carriers have, but here we're actually doing a comparison. What symptoms do people who carry one of these gain-of-function variants have compared to the hundreds of thousands who don't carry the gain-of-function mutations.

And the symptoms fall, generally speaking, into the categories that are seen in APDS patients. But a number of the symptoms like biliary cirrhosis, or biliary cholangitis, or sarcoidosis, those were things which are not gout. Those are not necessarily seen in patients with that are known, who've been referred to us with APDS. But those are not uncommon things that can happen.

And carrying the gain-of-function variant conferred a many, many fold increased risk for developing those clinical outcomes. Of course, things like lymphoma, which we know about, things like warts or other infections are also encountered as well enriched in those who carry versus those who don't.

Again, I don't want to make in any way, say that this is an exclusive list. This is just the list that are of captured clinical outcomes, which is enriched in the carriers compared to those that are not.

There are probably many more clinical outcomes that are in those carriers, but they're either not being measured correctly, or they're not rising to the top of the list of things which are enriched.

And so, really what we think this shows us, is that there's really a different model for how to think about patients who come in with immune disorders and using genetics to point to the pathway without even necessarily basing it on some strict clinical criteria.

So, in the past, if we wanted to have a genetic based precision outcome, we'd have a severe presentation. There'd be genetic testing, we'd find the variant, there would be lots of investigations to see if that variant actually causes the problem. In the case of APDS, look to see if AKT and S6 is increased in the patient's cells, you have to publish it. That variant then gets reclassified after the publication.

Now, what we can show is that someone presents with an APDS-like presentation, they have symptoms which we think are associated with APDS, we then just do the sequencing, we see the variants. We already have reclassified variants. Even the variants that we introduced many of them hadn't even been seen in a patient yet, but they are probably out there.

But now, we can just look it up as a patient can get approval for leniolisib to treat their symptoms. But as we begin to dig more and more and more and realize that APDS is probably more broad than what we actually understood, it's that essentially too much PI3 kinase, which can be inhibited by leniolisib, is what we're looking for, right?

So, we have symptom presentations derived from the many, many carriers of now gain-of-function variants. Those symptoms could paint a very different picture and point towards an intervention, like leniolisib, based on the genetic testing and just looking up, is that a gain of function or not.

So just to summarize, the massively parallel high-throughput screening allowed for functional classification, gain or loss, or neither,– for thousands of variants in the genes that cause APDS. We were able to pick up and detect all of the known gain-of-function variants, and then uncover many, many more gain-of-function variants.

These variants can be inhibited with leniolisib. And really I think, one of the biggest points here is that APDS is a continuum of severity of activation of PI3 kinase, of disease severity, and even a continuum of population prevalence, where some of these are actually more prevalent than we would tend to think of as in a "rare disease."

And so, we think that symptomatic APDS is many orders of magnitude more prevalent than were previously estimated, and that the heterogeneity of this disease is much more broad than had been understood beforehand. And so, there could be many other immune mediated symptoms caused by even other genetic problems, but included in other genetic findings like I said, we've only checked 10% of these genes, possible mutations, that could be treatable.

And we really have to essentially create new ways of defining genetic disease of the immune system, even like APDS, in order to hasten getting the precision therapy to the right person.

So, I just want to acknowledge, again, Ben Izar's lab, who was a major collaborator of ours, and then, Pharming was an amazing partner as well, With Anurag and Heather McLaughlin, of course,

the patients and families who we take care of with these diseases, who hopefully can benefit from this as well.

Anurag Relan:
Thank you, Dr. Milner, for the very clear presentation. What I thought we would do is I would just mention a few of the next steps now that we have planned based on these results that the team at Columbia have generated.

So, really the first step is, as you mentioned, for genetic testing labs now, to utilize this data, to take this data to independently now reassess those Variants of Uncertain Significance that they've already issued reports on. So these are patients who've had symptoms, who went and got a genetic test done, but got a VUS result.

Josh, and I think you nicely also pointed out that the data from this experiment could help patients who have both, what we call, these exact matches, as well as patients with a different amino acid substitution, but at that same amino acid residue, that led to a gain-of-function variant. We expect that this reassessment work will be completed in the second half of this year by these genetic testing labs. And this will again increase significantly, the number of patients with APDS who are diagnosed.

I think the other thing that we can spend a little time talking about, in a bit, is how do we answer the questions about the other variants and how do we generate additional variants to be able to help more patients who, let's say, whose results are still uncertain. And then lastly, I think the really unexpected finding in the study was around the prevalence and how do we now explore that further and what are some of the things that we can do in terms of understanding what the prevalence is, but also understanding what that phenotypic spectrum of disease is.

Because, as you say, it is not only heterogeneous and with regard to spectrum and severity, but also there's new things there, new symptoms, I think biliary cirrhosis is the one of the most prominent ones where it wasn't really on our radar. So, with that, maybe what I'll do is I'll now switch over and we can start discussing some of the questions that we received and as in the presentation, you actually addressed many of the questions, but maybe we can revisit some of these important topics.

QUESTIONS AND ANSWERS

Anurag Relan:
And I think the first question really is for you, Dr. Milner, in terms of, you've generated this data, but why do you think it's robust and why do you think it should enable the labs to be able to do their part now in terms of reclassifying these variants?

Joshua Milner:
So, we designed, and then, reported our study in such a way that it met the criteria and recommendations issued by those parent organizations for genetic reclassifications, so that when we produce the data, we can then state with a certain degree of confidence that a gain of function is a gain of function, a loss of function as a loss of function, and a benign one is likely to be benign.

And that's essentially by, again, by designing the entire study with that in mind, so that those

criteria would be essentially automatically met when the clinical genetics companies are reevaluating the pathogenicity of a mutation.

Again, also having published it in "Cell" and been under peer review, that also helps quite a bit. So, that robustness was built into the study, doing a technique which hasn't been applied as much to immune disorders. But we did so in such a way that it would be able to meet the criteria so that reclassification can happen. The other thing that we've done is by showing that there are people who carry those variants who have symptoms consistent with the disease that actually meets other types of criteria for reclassification.

So, that's even additional bonus is by actually pointing to people who carry those variants and saying that they have symptoms, that helps tremendously in assigning a change in the pathogenicity so that they can be diagnosed officially with APDS.

Anurag Relan:
Now, that makes a lot of sense. And I think, you actually touched on it in your very first slide that even before we come to issue of VUSs, is we have the problem of not performing enough genetic testing. So, what's being done about this in terms of boosting genetic testing awareness amongst physicians to perform this, also to perform genetic testing, and maybe in not only in the syndromic type of patient, but also to a wider population.

Joshua Milner:
So, I think in our field, in our field, in clinical immunology, there is just a rapid understanding that these rare disorders, that many of these rare disorders are not so rare, and that they look a lot different.

And so, the major focus of many of our continued medical education, of our national meetings is on conveying these messages as much as possible. Now, that's to the clinical immunologists.

So again, you're still talking about the more severe end when someone has finally made it to a clinical immunologist, it's the more severe end. But at the same time, we've also, all of us generally speaking, in major academic medical centers, have been partnering more and more with our subspecialty colleagues who take care of the organ system that's being impacted by the mutations.

Our gastroenterologists, the hematologist oncologists for the lymphomas, even if they don't necessarily have infections, and they're beginning to see more and more that there are precision interventions that are possible when a patient is not behaving the way that they expect them to behave.

So we've had a major educational push and outreach to both our pediatric and our adult colleagues to recognize that these are not so rare anymore, and that there are actual things that can be done in these patients. And I think that is more now to some extent, and there's way, way more sequencing that does get done. And that is not just - forget about genetics - it's not even sent by genetics. It may not only be sent by clinical immunologists, but now we do indeed have hematologists, oncologists and GI docs, and others who are doing the kind of sequencing needed to find these things. It's part of their workup and they are understanding that it needs to be part of the workup. But there, that's straight up for more education and more demonstrations like what we just did here to say, it's not just one in a million, it's a lot more common than that.

It's critical to be getting this word out, for many, many different disorders that we all follow. And we even have in New York, we've created a New York Regional Consortium to help interpret Variants of Unknown Significance by combining the clinical and research ends in disorders of the immune system, so that one can get essentially a consult, a virtual consult from experts in the field on every sequence that comes back, that has Variance of Unknown Significance.

And I can tell you that submissions to that system have only accelerated since we started it in the past couple years. And it's being sort of modeled and trying to expand throughout the country in order to promote more sequencing being performed, promote awareness of when you can find these genetic disorders.

Anurag Relan:
Now, that makes a lot of sense to advocate for more genetic testing. But I think we also value how you have this interesting finding here in the study of the increased prevalence in these databases. What can be done now to understand that further and how can that in sort of an orthogonal way, you can also potentially drive more awareness of genetic testing and sort of the results here?

Joshua Milner:
Yes, so I think there are several layers. One is that we indeed need to evaluate more variants because the odds are extremely good that we will find other variants that are more common, that are still gain of function. And so, that number, that one in 5,000, that's sort of a rough estimate, that may well be an underestimate. That's piece number one.

Piece number two is that we need to develop more sophisticated ways of going into medical records and going into these large databases. You're never going to find someone, you're rarely going to find, it will be one in a million or more to find a code for activated P110δ syndrome. It's not the way you're going to find it, it's going to be a combination of symptoms or a pattern of symptoms.

And that's going to take some advanced bioinformatic work to begin to create clusters of symptoms, which are highly enriched, in carriers so that we could, almost create like a scoring system of different types of symptoms that could trigger someone who should be thought of for APDS, or frankly, other immune disorders where the sequencing is done to look for all immune disorders, APDS being one of them to look for.

That takes some advanced looks. It also means going into even more databases to do this kind of work. And we have a number of colleagues who are doing this type of thing, to be able to really paint the picture. But of course, the sort of the keystone, the cipher is being able to define gain of function and loss of function.

I can tell you right now that there are thousands and thousands of carriers of yet undefined variants who have weak associations with clinical outcomes. But those associations are based on the assumption that any individual variant is the same as any others. When we grouped, as we just showed you here, if we can group together people who just have gain of function, just from the data we're staring at right now, in the 800,000 individuals, we'll be able to show significant enrichment for many more clinical either symptoms or constellations of symptoms.

Anurag Relan:
And I think this is really one of the things that I'm most excited about coming out of this work is

trying to understand sort of this, what this new definition, this broader definition of APDS really could look like.

But turning back to sort of the classic definition, I think one of the important validation points you did was when you looked for these new variants that you discovered, you looked for them in this database of patients who had underwent genetic testing and they had symptoms that were consistent with APDS, sort of the classic definition of APDS.

I want to just highlight that that was a sort of a subset of the overall population of patients who have a VUS result. So, it was really a subset of the ones that were, when you mentioned, were 27 patients, this came from a subset of all patients who underwent testing. But, can we try to extrapolate then from this in terms of what this result could mean from that group to that broader population?

Joshua Milner:
Yes, I mean, I think that the particular technology that we used, it's a very robust way of doing the editing. It only makes certain edits, but the edits that it makes are essentially random, right? So, it's not making edits that are more likely to be gain of function than any other type of way of doing it. And so, it's not at all a stretch to say it's likely, that we'll find 10 times as many new gain-of-function variants, perhaps more, we'll see, perhaps a little less, by going through the rest of the genes in this way, using different methods that are literally getting perfected as we speak.

And so, those who are carrying them, we will get a greater sense of how many of them are causing an issue or gain of function and are treatable by an order of magnitude, essentially, and when some of them are more common, and we already did see that there were a few of those variants that are more common in that group of people who were sequenced because of concern for APDS, we saw that some of them carried some of these more common variants.

And again, that immediately speaks to the fact that we'll likely see more folks with that. And again, when we look into the bigger databases of carriers of those variants, we'll see that the patients who are referred perhaps have more severe cases, but others have milder cases.

Anurag Relan:
And I think this is another question that comes up commonly, it's maybe more of a question for us, is the question of, okay, you've taken a patient who underwent genetic testing, who had a VUS result. The lab has now incorporated the data from the study and reclassified that patient, that patient now has a diagnosis of APDS and is eligible for leniolisib.

So, it doesn't require any further clinical trials per se or any other regulatory work. And I think you've actually had some experience here with patients who you've done sort of some testing on, again, on the one-by-one basis, but also I think from one patient in the study who was eventually, already taking the results from your study and the physician was able to reclassify that patient. So maybe just speak a little bit to this concept of how this can really impact patients.

Joshua Milner:
Yes, I mean, I can just, again, just personally, I recently was referred a patient who had infection autoimmunity, cytopenias, allergy. And it had gotten worse as she had gotten older. She had a family history of B cell proliferative disease. And she was found to have a variant in PIK3CD, one of

the APDS causing variants with the Variant of Unknown Significance.

And it was, I don't want to say ignored, but less was thought of it because of the fact that it was present in perhaps one in 15,000 individuals. And so, but because of her presentation, we were able to work up her primary cells and then introduce the variants well, show that it was a mild gain of function.

And I actually had to then, essentially write directly to the insurance in that particular case, and say that, my lab has done this, it's on a research basis, but it looks like gain of function. And then it would respond to leniolisib and she got it approved and her symptoms massively improved.

So, that's an example of the process there. I think there was another patient, who was, not one of mine, who was carrying a variant that we picked up in our screen, and could go on leniolisib now and have it approved. And again, it can be life changing when you actually know that's what's driving it, but it was held up until someone could actually show that that variant was gain of function.

Anurag Relan:
And I think actually, the way you've described it is, actually illustrates the problem too, right? In terms of this occurring on a one-by-one basis, and now, with these results, that not only the testing but the also the interpretation of results can also happen at scale so that this can be processed faster?

Joshua Milner:
Right, right. So that the next time they won't even ask me is this causing the problem? They can just look it up and see that it's there.

Anurag Relan:
Exactly. Exactly. So, and then, another question that comes up for us is, okay, so we've done this work, what happens next? And as I said, we expect the genetic testing results, or genetic testing labs to be able to incorporate these results into testing that they've already performed and to provide reinterpretation.

And we expect that really to happen over the course of the second half of this year. And we expect that because of that, this will actually increase the number of patients who are diagnosed with APDS and also then, eligible for leniolisib. I'll end with an open-ended question here for you, Dr. Milner is, what were you most surprised by when you saw these results and what was most significant for you?

Joshua Milner:
Well, first of all, I was surprised by the speed and the accuracy with which the entire study was done. This was less than a year's worth of work. I might have expected something like this to take way, way longer. So it's really extraordinarily how well this method worked, and how much the yield was, even knowing a priori that we were only going to hit a certain fraction of the possible variance.

But the second one indeed is that we could pick up this heterogeneity of frequency, right? That we could pick up a variant that's more common, that maybe look different in different people, And

those people are in general volunteer databases. These aren't even, again, when we look in those big databases, those are not people who are coming in because they're sick necessarily. And yet, we are seeing people out there who could actually benefit at a decent number, a decent frequency. I think I was surprised at how successful you were able to see that from there. That 's probably the biggest one.

Anurag Relan:
Well, that's great. And maybe with that, we will wrap up today's webcast. I thank you all for attending, your interest and engagement, and of course to Dr. Milner and the team at Columbia for leading this important work. We look forward to providing you further updates in the near future and have a nice rest of your day. Thank you.

Joshua Milner:
Thank you.

[END OF TRANSCRIPT]