Ben: Good afternoon, everyone, and welcome to this GenomeWeb webinar. I'm Ben Butkus, Editorial Director at GenomeWeb, and I'll be your moderator today. The title of today's webinar is *Correlation of a Non-Alcoholic Steatohepatitis Proteomic Test with Clinical Outcomes* and is sponsored by SomaLogic. Our speakers today will be Anne Minnich, biomarker consultant at Bristol Myers Squibb; and Joe Gogain, Director of Clinical Research and Development at SomaLogic.

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With that, I'll turn it over to Anne Minnich of Bristol Myers Squibb. Please go ahead, Anne.

Anne: Thank you. And thank you for the opportunity to present to you some recently generated clinical biomarker data from a study in non-alcoholic steatohepatitis, which I hope you agree is very exciting data. This is the title.

Next. Okay, so just to introduce the disease for those of you not familiar. Non-alcoholic steatohepatitis is generally abbreviated and referred to as NASH. And I'll certainly use this term in the rest of the talk. NASH is a progressive form of steatohepatitis. Let me just get this. That's shown here in this diagram. It starts off really as fatty liver, and the term for this is steatosis. And the medical term for this condition is non-alcoholic fatty liver disease or NAFLD. This is really a very prevalent condition, estimated to be present in 34% of the population.

Then steatosis can progress to steatosis combined with inflammation. Furthermore, can progress to combination with inflammation and ballooning, which is essentially hepatocyte death. This condition is what really defines NASH, and that condition is a subset of NAFLD and estimated to be in about 12% of the population. Furthermore, NASH can progress to NASH with fibrosis, and this actually becomes quite serious. NASH with fibrosis is associated with increased cardiovascular disease mortality and increased mortality from liver disease. At worst, NASH, which is present in 20% to 30% -- sorry, advanced fibrosis present in 20% to 30% of NASH patients can progress to cirrhosis, which is 2% of NASH patients. Cirrhosis is very serious and can result in hepatocellular carcinoma, liver transplant, death, and other clinical outcomes.

The unmet medical need is really in these later stages of the disease. The ultimate goal is to reduce morbidity and mortality. Another unmet medical need in this field, as is the topic of this presentation, is to really validate noninvasive diagnostics with these clinical endpoints.

This slide just shows more of the multifactorial features of NASH. As I mentioned, there are all these features. The disease is commonly associated with diabetes and obesity or metabolic disease. The onset is initially with metabolic disease and this progresses to hepatic inflammation, eventually to fibrosis. The reason I'm emphasizing that is that the current thinking in the field is that therapies with multiple combinations of therapies, with multiple mechanisms of action which address each

of these components, are considered to be optimal or necessary to best treat all of the patients.

Again, severe NASH is estimated to affect 3% of the population, but precise estimates of prevalence are difficult to come by. That's because of low diagnosis rates, which is unfortunate for a drug developer. The reason for that is that diagnosis is currently based on the necessity to obtain and evaluate liver biopsies. There are no drugs currently approved for NASH, but there are many ongoing clinical trials. The clinical development in NASH, again, is made difficult by the lack of validated prognostic and diagnostic biomarkers, which complicates trial design.

I need to explain the biopsy scoring system to you, because this biopsy scoring system is used not only for diagnosis, but also for endpoint monitoring in late-stage clinical trials as per the guidance of the FDA. In this system, NAS is defined as the NAFLD Activity Score. This is scored separately from fibrosis, according to the NASH CRN system. NAS is composed of the three components of NASH, which are steatosis, inflammation, and ballooning or hepatocyte death. The biopsies are scored with scores ranging from zero to two or zero to three. Eventually, these scores are all added up to form the NAS score, which can range from zero to eight.

On the other hand, fibrosis is staged separately from NAS on a zero-to-four scale. Zero is none, the subsequent stages are shown here. Stage 2 NASH is considered the stage which predicts further progression of the disease or the stage at which the patient is at risk for developing more serious disease, but Stage 3 and 4 NASH are really the areas that are considered to have the most medical need because of their severity.

All right. With that introduction, I'm going to be describing to you the data from a recently completed Phase IIb clinical trial of a drug called Pegbelfermin in Stage 3 NASH. In particular, today we're going to focus on the SomaScan pharmacodynamic analysis and the effects of the drug on this test.

Here we're introducing the drug. The drug is called Pegbelfermin. I have abbreviated this in subsequent slides as PGBF. Pegbelfermin is a long-acting FGF21 mimetic and targets the key drivers of NASH. FGF21 itself is a non-mitogenic hormone. It regulates energy metabolism. Endogenous FGF21 has a short half-life, and various mimetics have been shown to have positive effects in NASH in numerous clinical trials and preclinical studies. This diagram shows the actions of FGF21. Its receptors are present on liver and adipocytes, so the effects are on these tissues.

In liver, FGF21 inhibits gluconeogenesis and lipogenesis and promotes fatty acid β-oxidation, all of which would favor decreased steatosis in the liver. In adipocytes, it promotes increased insulin sensitivity and also production of adiponectin. Adiponectin itself is a hormone which reduces steatosis, inflammation, and fibrosis. Importantly, adiponectin is considered an important downstream regulator of our drug, and it's considered an important target-engagement biomarker for the drug for that reason. That's FGF21. Pegbelfermin is a PEGylated version of FGF21, and this PEGylation is designed to prolong the half-life. The result is that the drug can be dosed once a week for maximal pharmacodynamic effects.

The rationale for the clinical trial consisted of animal studies and a Phase IIa study conducted by BMS. The effects seen were increased adiponectin, increased hepatic fatty acid β-oxidation, decreased steatosis, decreased hepatic inflammation, and ballooning. In addition, there were anti-fibrotic effects: decreased liver stiffness as measured by imaging, and decrease in an important biomarker of fibrosis. That was the rationale for the Phase II trial.

Here is the design of the trial. The trial is called FALCON 1. It was a trial of Pegbelfermin in NASH and bridging fibrosis. Again, this is severe Stage 3 fibrosis. This is somewhat different than previous trials. This diagram here shows the study design. There were about 200 patients enrolled in this trial. They were randomized equally to receive three different doses of Pegbelfermin: 10, 20, and 40 milligrams Sub-Q every week and compared to placebo.

What's really important to point out here is that the primary endpoint was taken at Week 24, so after 24 weeks of dosing, and the primary endpoint, per the guidance of the FDA, consisted of liver biopsy endpoints. However, the drug continued dosing through Week 48, at which time other types of endpoints were measured. The inclusion criteria are shown here, the exclusion criteria.

The study occurred in Japan and the US. Primary endpoint was biopsy-based. All of the secondary endpoints were also biopsy-based, and these were at Week 24. However, we did measure a number of exploratory endpoints. These were measured throughout the study. I'll go into more detail about these, but suffice to say, these exploratory endpoints are going to be the topic of today's presentation. Of course, we measured PK and safety endpoints in this study.

I should add-- we're not discussing this today, but I should tell you that in parallel with FALCON 1, we also conducted a study called FALCON 2, which is a similarly designed study but in cirrhosis. That was a parallel study that was performed.

Now the data, the results. I should tell you that most or all of the data in this presentation are published. I don't have the references on this slide, but I can provide these on request. These data were published in JHEP and Gastroenterology quite recently in 2023. This slide shows the primary endpoint. The primary endpoint was defined as NASH CRN fibrosis improvement of greater than one stage with no worsening of NASH as measured by the NAS score, or NASH improvement, that is to say NAS score improvement, with no worsening of fibrosis at Week 24.

The data are presented here in a categorical way. The y-axis is the percent of subjects who achieve the primary endpoint. What you can see is that the drug-treated subjects, although there was an increased proportion of drug-treated subjects that achieved the primary endpoint relative to placebo, this was considered not statistically significant, as the design was to test for a dose-response and a dose-response was not observed, and so the study was considered not to have met its primary endpoint. This is true if you look at the ITT population or the less conservative Completer Analysis, and so the primary endpoint was not met.

However, as I mentioned, we measured a wide range of what we call NITs, or noninvasive tests, in addition to the primary and secondary endpoints. These NITs can be used to evaluate disease progression and drug effects, and they can,

hopefully, be supportive of biopsy endpoints and/or the different mechanistic aspects of the disease and the drug. The tests listed here predict additional -- liver biopsy was primary endpoint. Adiponectin was our target-engagement biomarker. These are pretty popular traditional biomarkers that are used in NASH clinical trials. I will describe them later.

Imaging is an important technology in NASH clinical trials. MRI measures liver fat or steatosis. MRE measures liver stiffness or liver fibrosis. The advantage of these imaging modalities is that they really measure the disease burden in the whole liver in contrast to liver biopsy. Further down, we measured PRO-C3, ALT and AST, CK-18. We also employ digital pathology of the biopsies as a potentially more sensitive assessment compared to manual scoring, and these composite fibrosis biomarkers. The last row here shows a couple of biomarkers that are sort of more novel and have not been well studied in many clinical trials. These are PC3X, which measures collagen III crosslinking and is thought to reflect severe fibrosis. Finally, the SomaSignal NASH bundle, which is really the main topic of today's presentation.

I'm going to proceed to show you the data. Generally speaking, the data are presented according to dose. The placebo is in gray, and the increasing doses of Pegbelfermin are depicted by these shades of blue-green of increasing intensity. In this slide, we have liver steatosis is measured by MRI-PDFF. This is proton density fat fraction. The y-axis is change from baseline, and the x-axis is time. You can see that the drug modestly decreased steatosis at Week 24, particularly in comparison to the placebo in which group it went up. These decreases were statistically significant only in the 10mg group or the combined group and really only at Week 24. Just to give you an idea, a decrease of about 30% from baseline in MRI-PDFF is considered clinically significant, so this is a modest decrease.

On the right side of this slide, you have liver fibrosis is measured by MRE elastography, change from baseline. The 40mg dose of Pegbelfermin did significantly reduce. Liver fibrosis is measured by MRE at Week 48. But again, there is no dose-response really clear from these data. In this test, just to give you an idea, a decrease of 15% is considered clinically significant.

Now I'm going to go into the blood biomarkers. I'm showing you all those data in order to try to put the eventual data from the Soma's test into context of the other biomarkers or the usual biomarkers measured in NASH clinical trials. As I have mentioned, on the top left, adiponectin is our target-engagement biomarker. In the y-axis shows change from baseline. The 40mg dose really increased adiponectin to a maximal level at Week 4. This increase waned over the course of time, but was still considered statistically significant at Week 24. Again, there's no clear dose-response amongst these data. These are in contrast to the placebo in which adiponectin was unchanged.

CK-18, it measures cytokeratin-18 on hepatocytes. It's considered a biomarker for hepatocyte apoptosis or cell death. All doses of the drug decreased this biomarker by Week 8 in contrast to the placebo, and this decrease was maximal at Week 8 and maintained through Week 24, and this decrease was statistically significant. Similarly, with AST and ALT, these liver injury biomarkers, the placebo remained constant or increased slightly, whereas in the drug-treated subjects, AST and ALT were decreased, and this decrease occurred over the course of the study and was

considered statistically significant. However, there was no apparent dose response to this effect.

The top row here are composites, and these are commonly used in NASH clinical trials. FIB-4 and APRI basically measure platelet and AST composites. FIB-4 in particular is a very promising biomarker in the area of diagnostics. You can see that Pegbelfermin compared to placebo did decrease FIB-4 and APRI. ELF test is interesting. It's more of a hypothesis-driven biomarker of fibrosis, in particular, composed of hyaluronic acid, PIIINP, and TIMP-1. This biomarker has recently received FDA approval as a prognostic biomarker. In this study, the placebo subjects had increased ELF over the course of this study, whereas the 40mg dose and other doses significantly decreased the ELF score, and this was the dose-responsive effect.

On the bottom half of this slide, you have these are fibrogenesis biomarkers. These are biomarkers that are specifically measuring fibrosis or collagen production in the liver. PRO-C3 is very well-characterized in the field of NASH. It's a biomarker in which a lot of people place a lot of stock and confidence. You can see that in the placebo, this PRO-C3 increased over the course of this study, whereas in the drug-treated subjects, PRO-C3 was decreased. This was a non-dose responsive, but highly statistically significant effect.

This PC3X is a more novel, interesting biomarker. PC3X basically measures the crosslinking of collagen III, the formation of crosslinks. I believe this is the first NASH clinical trial to show a pharmacodynamic effect on PC3X, shown in this slide. Again, all doses of Pegbelfermin decreased PC3X relative to baseline and relative to placebo at Week 24, and this effect was not dose-responsive.

Although Pegbelfermin, the trial did not achieve its primary endpoint, you can see that it has effects on some of these important blood and imaging biomarkers.

Now we're going to get to the SomaScan technology. SomaScan is a composite protein biomarker blood test, and it is composed of biomarkers that are trained on the specific features of NASH. This is really unlike all the previous biomarkers that I've shown you. It's trained on steatosis, inflammation, ballooning, and fibrosis in NASH and NAFLD. The cohorts used for development of this test, which is currently marketed as NASH bundle, the cohorts used were three different cohorts, one natural history cohort and two interventional study cohorts; PIVENS, which study Pioglitazone; and FLINT, which studied obeticholic acid. The combination of the samples and the biopsies were used to develop this model.

Again, the model is designed to reflect distinctly and directly the different features of NASH. Basically, the sera from these subjects were scanned for 5,000 different proteins, and the set of proteins which best matched the biopsy features was derived. That's shown on the right part of the slide. Again, there's a separate signature for each feature, ranging from 5 to 14 biomarkers. It seems a little blurry here, but what I want to say about this is that although these composites were derived empirically, if you look at the individual proteins, they do suggest biological plausibility. This is really interesting in and of itself. I think some of these are going to be further studied, which is not the topic of this discussion. There was very little

overlap amongst the features, and those analytes that do overlap are shown in purple and blue here.

The scores that are ultimately applied to a given patient are probability scores. The probability score is from zero to one or zero to 100%. A patient with a probability score of greater than 0.5 for any one of these features is at risk for having more severe fibrosis or more severe component. It's important to understand that this is a probability score that's generated, and for me, this took a while to understand.

I'm showing the baseline values. This is data from the FALCON trial, and I'm showing the baseline values of the scores here in this slide. The Soma's Soma test baseline in each of the different treatment groups were similar, although they weren't randomized for this, and you can see that the baselines are high. 75% for steatosis, over 50% for inflammation, high '60s for ballooning, and mid-'70s for fibrosis. These are high. This is compared to, again, the definition that a probability score greater than 50% is considered predictive of greater disease severity. Therefore, the probability score of greater than 50% is considered at risk for further developing more serious disease.

I just want to make the point, using these data, that the patient population in the FALCON 1 study was really Stage 3 NASH, yes, but even for Stage 3 NASH was more severe than many clinical trials that have been conducted in NASH.

These are the data from the SomaScan. Again, doses of Pegbelfermin and placebo: steatosis, inflammation, ballooning, and fibrosis. The steatosis, basically, the magnitude of this effect, I just want to comment, was in good agreement with the biopsy quantitation of stained fat determined morphometrically. You can see that for inflammation, ballooning, and fibrosis, there was a dose-responsive, statistically significant decrease in the scores relative to the placebo and relative to baseline. These data were just among the clearest biomarker data that were generated in the trial.

Again, these are probability scores on the y-axis. Basically, they translate into these percentages relative to placebo of lower relative probability of having more severe disease as reflected by these different features of NASH. I hope that's clear. These decreases were relatively modest. I don't think that they would have achieved the 50% threshold of having lower probability of progressing to more severe disease, but they were definitely sensitive, and quantitative, and significant. We measure all these biomarkers. How do we know which one is right? How do we know which one to believe? How do we know that the biomarkers are reflective of the mechanistic components of NASH that we think they should be? Really to address those kind of questions, we look at this correlation matrix.

This is like a heat map where we cluster the different correlations amongst the different biomarkers. I need to clarify that this data are derived from the week 24 time points. They are not changes. They are simply the week 24 snapshot data values for these subjects. You can see that we have tried to color code. We have color-coded the different mechanistic components as shown on the legends here. We have orange reflecting metabolic, purple reflecting fibrosis, et cetera.

Then the color-coding for the correlation coefficients is such that increased intensity of yellow or orange reflects a higher correlation coefficient. The white or blank spaces with no numbers in them are non-significant correlations or lack of correlations. We can really see using this heat map four clusters. The most prevalent marker cluster is in the metabolic-related cluster, as shown by these biomarkers here, and these correlate with SomaScan, NASH steatosis, and HDL and LDL, and really all of the metabolic-related biomarkers, and liver fat as measured in biopsy.

The next cluster is fibrosis as shown in these purple legends in the upper left corner. This cluster includes SomaSignal NASH fibrosis and ballooning, PRO-C3, PC3X, MRE, ELF score, and FIB-4 score, but not biopsy. That is important. The middle part is really the cluster of the biopsy-related endpoints. The biopsy-related endpoints cluster together, but they don't cluster with other biomarkers. One exception is the SomaSignal ballooning and fibrosis score, which exhibit moderate correlation with the primary endpoint, as shown here.

This was really the only biomarker test that exhibited correlation with a primary endpoint. Maybe this isn't surprising given how the test was derived, but we found this to be very encouraging. This slide shows the correlations between the changes of the week 24 biomarkers and the changes in the biopsy ordinal scores. This graph here shows mean with these whisker plots showing 95% confident intervals and medians are shown with a line.

The left side shows the NAS scores. These are changes in total NAS which correlated with week 24 changes in SomaSignal ballooning, and this was a highly significant correlation. We also saw correlation of other biomarkers such as MRI, adiponectin, and PC3X with the NAS score components, or changes in NAS scores. But on the other hand, fibrosis was really much more difficult.

The SomaSignal ballooning was the only biomarker that correlated with changes in NASH CRN fibrosis scoring, as shown here. Other biomarkers that you might expect to correlate, such as PRO-C3 and ELF, did not at all correlate. Incidentally, this kind of analysis, some people use it as an analysis that can reflect fibrosis regression. We thought this was very interesting.

Finally, we looked at concordance. In this analysis, we are looking at concordance within an individual patient. The individual patients are shown at the top here. The dose of drug that they received is color coded as usual with these shades of blue. The Y-axis shows all the different tests, all the different non-invasive tests, and biopsy-related tests. Basically, we're looking for response and non-response.

In the heat map here, the black indicates no change, the blue indicates a worsening, and the yellow indicates a positive improved response. Using this analysis, we identified basically four clusters. Group one was considered to be primary endpoint non-responders but with inconsist-- That's shown up here at the top, but with inconsistent biomarker responses as shown with this messy-looking bunch of different colors below.

This group really consisted mostly-- I should say most of the placebo subjects were in this group. The second group, on the other hand, was group four. This was a group of patients who were primary endpoint responders, but who also had

concordant biomarker responses as shown by the yellow here and the yellow here, a preponderance of yellow color. I will say that the SomaSignal Tests did exhibit concordance with biopsy for both of these groups.

More confusingly we saw that in group three there was a group of primary endpoint non-responders who, however, seemed to have, on the whole, positive biomarker responses. There was group two who was primary endpoint non-responders who, however, had inconsistent biomarker responses. I just want to point out that there's some concordance, but there's also some discordance between the bulk of the biomarkers and the biopsy primary endpoint, but that the SomaSignal generally was in concordance with the biopsy readouts. That's shown here in this yellow color for these Soma tests. I hope that's clear.

That was my last slide. Just to summarize, despite the fact that Falcon 1 did not meet its primary endpoint, we found that Pegbelfermin modulates several pharmacodynamic biomarkers relevant to NASH across all of the mechanistic components. We feel that possibly greater consideration should be given to the overall collection of data when evaluating NASH drugs to lower the risk of false negative conclusions, conclusions that can occur when evaluation is based on biopsy alone. This is not a new idea, but it's an idea which I think is well illustrated by the data in this study. To our knowledge, this is the first trial or one of the first examples of the use of the SomaSignal NASH Bundle to monitor drug activity in a NASH clinical trial. We obviously feel that it performed very well.

SomaSignal Tests were the only NITs to correlate with biopsy-based outcomes. Drug PD effects on Soma ballooning, in particular, appear to be more sustained than on other blood biomarkers of liver injury. The SomaSignal Tests appear to be a more sensitive and maybe relevant test to reflect drug effects on hepatic histological features of NASH. I want to just say that this has been shown to be true in a publication from a SomaLogist published this year in JHEP using the pivots in the Flint cohorts.

This cohort, I think, adds the additional information that the test can be used in a population of subjects with more severe NASH than in those populations. I think that's an important addition to the functionality of these tests. Not only diagnosis, but also monitoring. Not only in mild NASH, but also in severe NASH.

These are my acknowledgments. I really want to acknowledge the Falcon study investigators and the patients because, in particular, the latter part of this study was conducted during COVID, and the patients and the investigators made the extra effort to come to the centers for their evaluations at much risk to themselves during the epidemic.

I want to thank the Falcon study team and the Falcon development team. Diane Shevell was the person who initially designed the biomarker strategy for this trial. She had the vision to include SomaLogic's tests. Pete Schafer is our boss and was very supportive of this biomarker plan. Elizabeth Brown did all the statistical analysis or most of the statistical analysis that I showed you today. Again, this has been recently published in JHEP. Finally, I want to thank our collaborators from SomaLogic who were really very helpful in interpreting the data. I think that's all. I'll turn it over to Joe.

Joe Gogain: Great. Thank you, Anne, for going through those exciting results. I just wanted to touch briefly on the general development of SomaSignal Tests, and how we're using those tests to assess the at-risk NASH population, so the NASH population that has significant fibrosis. I'll start with SomaSignal Tests in general, and talk about how we're translating protein measurements into these clinical predictors into these tests that we call SomaSignal Tests.

Similar to the development of a NASH test you talked about earlier, we are working with collaborators around the world that have samples that come with clinical true standards. Some of these are long-term observational studies. Some of them are specific therapeutic studies. Some are in combination with consortiums, like LITMUS consortium and NASH CRN that went into the development of a NASH test.

These clinical true standards represent future outcomes, current states, impacted behaviors. The sample samples that we're using are representative of the intended use populations for which we're developing the test. We combine those clinical true standards with the proteomic measurements. We've been doing this for the test we've developed since we launched the 5k version of the assay in 2018 and have continued through to 7,000 [unintelligible 00:42:47]. We combine the clinical and proteomic measurements in machine learning applications, where we are identifying patterns of proteins that relate to those true standards.

We've run over 250,000 samples between those two assay versions, with greater than a million participant years of clinical follow-up data and we validated 22 tests of health status. In running all the samples, we've developed unique tools and datasets that allow us to account for the impact of model civility, robustness, interference, tools that allow for us to create robust tests that are transferable from population-to-population. Each of these tests are just really software products, then to ride on top of the broader proteomic measurement platform.

Every single sample we run, we collect all 7,000 data points, whether it's test development, validation, or delivery of the results to clients or to actual patients from our LVT, CLIA, CAP-accredited lab in Boulder. It tests are typically in the size of tens of proteins similar to the NASH component tests we talked about earlier. Adding new tests in silico was possible because we're collecting all 7,000 data points. If we develop a new test six months from now, a year from now, we could go back to that data and apply that algorithm. As you can imagine, hundreds of tests are possible.

This is a snapshot of the test that we've developed so far. Many of these are unique, prognostic for major adverse health outcomes such as cardiovascular disease, heart failure, chronic renal insufficiency, kidney prognosis, dementia risk, lung cancer risk. Some of them monitor current metabolic state like glucose intolerance, visceral fat, cardiorespiratory fitness in terms of your max resting energy rate. We can assess the impact of social behavior on the bottom right here, in terms of alcohol impact or tobacco exposure. Then of course, where we have the ability to assess these NASH component scores, steatosis, inflammation, ballooning, and fibrosis.

In addition, we're combining the results of each of those tests to identify a population that we call at-risk NASH, so the NASH with significant fibrosis. When we do that combination to identify at-risk NASH, and then we look at the combination of those other tests that I just introduced, we see that people who have at-risk NASH as

compared to early NASH or even NAFLD, they have significantly worse health profiles. This data was collected from the LITMUS metacohort where we've assessed those other tests in terms of at-risk NASH versus early NASH. You can see that they have significantly worse liver fat.

This test is slightly different than the steatosis test that we talked about a minute ago, and this one was trained on ultrasound. These subjects, at-risk NASH subjects, have additional impaired glucose tolerance, higher percent body fat, decreased cardiorespiratory fitness. They have increased risk of renal insufficiencies, they have greater risk at cardiovascular events, and increased visceral fat. The test that didn't have significant differences between those populations is shown at the bottom.

As Anne showed us earlier, in identifying that the NASH tests are sensitive to change moving over the course of the therapeutic invention, Pegbelfermin, we've seen this with other SomaSignal Tests too. When we think about the application of these tests in this broader overlap between NASH, metabolic disease, cardiovascular disease, and here specifically example in diabetes, we see that many of the tests that showed significant differences between at-risk NASH population and the early NASH population, they also changed significantly over the course of a year in this case upon going in intervention.

This intervention was a caloric restriction and weight loss in type 2 diabetes, where we see that we have significant differences from the intervention groups, the ones that lost less than or greater than 10 kilograms in the teal and the purple compared to the blue control. We see the significant difference in glucose intolerance. We have a reduction in glucose intolerance, reduction in visceral fat, excess liver. A reduced risk in cardiovascular disease, percent body fat, increase in VO_2 max.

When we think about therapeutic interventions in the type 2 diabetes population, as shown here from the Excel trial of the Exenatide, the GLP-1 receptor agonist, we see similar type of reductions here in cardiovascular risk at the top as you compare placebo versus the treatment group. Again, glucose tolerance, lean body mass, percent body fat, resting energy, and visceral fat. We believe that the use of this SomaSignal Test can give you really a broader holistic view of the changes occurring in these subjects upon therapeutic intervention. Especially as the prevalence of these broader NASH, cardiovascular, metabolic conditions start to increase over time, and as Anne pointed out, the importance of developing therapies for these conditions.

I'd like to switch gears briefly and talk about a recent publication from the LITMUS consortium. Here, they're looking at biomarkers for staging fibrosis and NASH in NAFLD. They've determined here through this comparison where they looked at 17 individual biomarkers or combination of biomarkers that the diagnostic accuracy for the NASH SomaSignal Test outperformed all of those biomarkers. We have the biomarkers here listed along the left side, and we're looking at, in the middle, NASH with clinically significant fibrosis, and at the far right, NASH with advanced fibrosis, and you can see the AUCs for the SomaSignal Test outperform all other single or multi-biomarkers.

Sorry, I lost my mouse here. When we think about moving that into looking at the lowest number of tests needed to identify individuals that are going into our trials that

have NASH with clinically significant fibrosis due to those performance numbers, you can see on the top right here of this chart that the number of individuals needed to be tested in this population was lowest for SomaScan to identify someone that would later go on to be positive for biopsy and able to be enrolled into trial.

Conclusions in the research and context section of this paper in Lancet Gastroenterology Hepatology Journal is that furthermore, our proposed strategy showed that some tests would substantially reduce the number of individuals who would need to have biopsy in future drug trials as only those testing positive for the marker would require further evaluation. Because of high screen failure rates in current trials, such pre-selection could facilitate trial recruitment and accelerate drug development.

Finally, I want to conclude with some of our thoughts regarding the use of these SomaSignal Tests in clinical trials. Certainly, these tests could be used and are being used in retrospective studies but could likely be written into protocols just like any other biomarker evaluation. In addition, we're continually discussing the potential of moving some of these tests through the FDA's biomarker qualification program using the drug development tools pathway. In order to do so for each of the tests, you would need to define a specific context of use.

Today we've identified two potential contexts of use for the NASH test and showed us the test could be used for pharmacodynamic or response monitoring, nicely displaying that the SomaSignal Test showed good dose response to the Pegbelfermin over time. Then through that LITMUS manuscript, we see that the SomaSignal Test could be used for patient selection prior to biopsy in order to reduce screen failures. We've also discussed the potential of using the cardiovascular risk test in similar contexts of use. I'd like to end there, thanking Anne and the organizers for having us here today and for the exciting presentation, and happy to take questions.

Ben: Thank you very much, Anne and Joe, for those excellent presentations. We'll now begin the Q&A portion of the webinar. One moment while we gather questions. Our first question is for Anne in regards to the study you described. What cause of liver disease was eligible for the study? The person mentions referencing the key exclusion criteria.

Anne: The study was entirely focused on NASH, so alcoholic steatohepatitis, viral hepatitis, any other cause of hepatitis other than NASH was not in the study. Does that answer your question?

Ben: I believe so. We can move on to the next question. For ELF and Pro-C3, what are the units of measure? Are they percent change?

Anne: Let me double-check. Sorry. Can you see this? I believe they're just change in the units, not percent change from baseline.

Ben: Okay.

Anne: Yes, I can't seem to amplify the slide. These are taken from the publication. I believe they're just straight change from baseline in the units.

Ben: Okay. If there's any further info on that, we can follow up with the attendee afterwards. [crosstalk]

Anne: We analyze them both ways. Personally, I prefer percent change from baseline, because it corrects for differences in baseline better. But the data look pretty similar regardless.

Ben: Okay. Great. In regards to the first question, the attendee said that, yes, that did answer the question. We'll move on to the next question. In the SomaSignal data, steatosis and inflammation dip at week 12, but rise again at 10 and 20 milligrams by week 24. This is frequently seen in other studies for fibrotic markers. Do you have any conjecture as to why this occurs?

Anne: Yes, we've thought about this a lot. I didn't show a lot of the week 48 data where the pharmacodynamic effect starts to disappear with later time points. We call that attenuation. The attenuation seems to occur only with the steatosis, inflammation biomarkers. The effect on the fibrosis biomarkers seems to be more sustained with time. That attenuation we saw starting to occur at week 24 and in some cases, it really got worse at week 48, particularly in adiponectin, for example, and some of the liver injury biomarkers.

I can tell you it's not due to immunogenicity. It was not associated with immunogenicity. It could be associated with the fact that because of COVID, some people tended to miss their visits, such that they were off drug by the time they got to the final visit. They were outside of the window of drug efficacy when these effects that occur at week 48. There's an enzyme that actually cleaves FGF 21. It's possible that this enzyme kicked into gear as the study went on and reduced the efficacy of the drug, but we don't have any data to prove that. We've talked a lot about this attenuation effect, and we really do see it occurring. Not at all, I'd say in the fibrosis, but more in the other metabolic and liver injury biomarkers.

Ben: Great. Thank you. Our next question, this attendee says that they can see the concordance with biopsy, but biopsy has a high inter-observer variation. How many pathologists evaluated each biopsy and was biopsy grading by a single expert liver pathologist or by multiple with or without consensus?

Anne: In this study, we use a single central observer, histologist. There was no consensus or variability amongst observers.

Ben: Thank you. Our next question, how much of a SomaSignal NASH Test drop during intervention would be meaningful to see the histology response? I think I've interpreted that correctly. Does that make sense?

Anne: Well, by definition, in our study that a drop to the level of 50% as opposed to the baselines, which were up around 75%, would indicate that the threshold for lower risk of disease progression was reached. I don't know if that answers the question.

Ben: Okay, thank you.

Anne: I don't know if Joe wants to weigh in on that.

Joe: Yes, I can weigh on that. We saw from your data a significant change in the score. The score is related to a categorical output of above 50% individuals for that particular component, or more likely to be scored in the ones, twos, and threes in terms of biopsy NAS scoring result. Those below 50% were the zeros, or in the case of fibrosis, the one. A drop from stage one to zero, moving across that 0.5 or 50% threshold would be significant, but the output of the score does relate to the severity of the component. The output of the SomaSignal Test score relates to the severity of the component score. Seeing a significant change in those numbers is still significant.

Ben: Great. Thank you. Unfortunately, that's all the time that we have for today. If we didn't have time to get to your question, we will try to follow up with our experts. We'd like to thank our speakers, Anne Minnich from Bristol Myers Squibb and Joe Gogain from SomaLogic, as well as our sponsor, SomaLogic. As a reminder, please look out for the survey after you log out to provide your feedback.

If you missed any part of the webinar or would like to listen to it again, an archived version will be emailed to all attendees. Thank you for joining us for this genome webinar.