DePalma: It's a pleasure to have with us today, Dr. Ramon Diaz-Arrastia, Professor of Neurology and Director of Clinical TBI Research Initiative at the University of Pennsylvania And Pennsylvania Presbyterian Hospital. Ramon?

Diaz-Arrastia: Hello. Can everybody hear me?

DePalma: Absolutely.

Diaz-Arrastia: Great. Well, again, thanks so much, Ralph, and thanks so much for the invitation. And the topic today is Biomarkers of Traumatic Brain Injury: Implication for the Next Generation of Clinical Trials. So, I’m going to start with the introduction of an outline of what I’m going to tell you.

So, the first point--and I think those of you who maybe have heard me give talks before have heard me talk about endophenotypes and how important they are. And I think, bear with me, and you'll hear about it a little more again. I’m going to try to emphasize that that's ultimately why we need biomarkers; we need biomarkers to help us measure the endophenotypes because this is going to be the key to future therapies. We'll talk briefly about the context of use and how different biomarkers can be useful at different stages in the trajectory of a patient who has had a traumatic brain injury, all the way from the site of the accident or site of the injury through the emergency room, intensive care unit, even to the rehabilitation unit.

And then I’m going to finish off, I’m going to spend really a bulk of our time giving you some new data on three biomarkers that, in my view, are rising to the top in terms of finding particular value in traumatic brain injury research, and in particular, how they may be useful for informing the next generation of clinical trials.

So, what's an endophenotype and why is it important? Well, an endophenotype is a $25-word, but what it really tries to get at is that it is an internal or an intermediate phenotype that is closer to the underlying pathophysiology of the disease than a phenotype; a phenotype which is usually a categorical variable, usually, a patient has the disease or they do not. An endophenotype, in distinction, is a continuous quantitative variable, something that can be measurable and usually measured with a biomarker; and a biomarker can be a physiologic biomarker, a biochemical marker, or an imaging biomarker. Now others use these important concepts--and maybe not use the particular term that you may hear other literature refer to endotypes or subphenotypes. To me, these are totally synonymous terms and I think we can use any of them. I happen to like endophenotype.

So, why are endophenotypes important? Well, the reason the endophenotypes are important is that the therapies--when we ultimately have them--are not going to be directed at the phenotype, because the phenotype is too complex,  it's too heterogeneous, it varies a great deal from patient to patient, and therapy is closer to the underlying biology--the underlying pathophysiology of what is going to be causing the disease and causing the evolution of the disease than the endophenotype. So, what are the endophenotypes of TBI? Well, we don't necessarily know them all, but I think we know enough that we can say that things like inflammation, diffuse axonal injury, diffuse vascular injury, and potentially many others are endophenotypes of traumatic brain injury.

And in the future, when a patient with a traumatic brain injury presents to either an emergency room, or an intensive care unit, or a rehabilitation unit, what the physicians caring for that individual are going to have to do is they're going to have to do biomarker measurements--either imaging biomarkers or blood tests--to measure how much of a particular endophenotype and what mixture of endophenotypes are active in that particular patient, and the therapies that are going to be chosen will be informed by those biomarkers, will be informed by those in the phenotypes that are playing a predominant role in that particular patient, and that's really the only way we're ever going to find effective therapies here.

And how are we ever going to get there? Well, the first step is going to have to be to do careful prospective and sufficiently-powered observational studies in humans so we can understand the natural history of these endophenotypes and understand which are the better biomarkers to measure those endophenotypes. and hopefully, identify the subset of patients that are likely to merit further therapy, meaning patients that are not likely to recover spontaneously but rather likely to end up with substantial disability if they are not treated appropriately.

Once we have those sufficiently-powered observational studies that are going to make the case that over the last ten years, we've made a really big dent into that particular need; but once we have that, we're then going to have to go back into preclinical studies--and I think that's the stage where we are right now--and confirm that the preclinical models that are being used sufficiently mirror the clinical situation--and particularly mirror those endophenotypes that are prominent in the clinical situation, confirm the mechanistic benefit of whatever therapies you're going to be testing, and also establish the pharmacodynamic relevance of the particular biomarker. And then once we have that, is it then really reasonable to go forward then to biomarker-driven Phase 2 clinical trials which is how we're going to need to establish really important principles such as the optimal dose timing and duration of therapy.

So, that's perhaps a longer introduction, but I wanted to frame the intellectual perspective in the field of how biomarkers are really, in my opinion, going to be absolutely essential if we are ever going to get effective therapy.

Now, the brain is a very complicated organ, by far the most complicated organ in the body and obviously made up of multiple different cell types, and each cell has multiple different regions; an axon has the soma--the axon neuron has the soma--the axon, the dendrites, the proteins that are expressed are very different in these different parts of the neuron.

In addition, there are glial cells, there are oligodendrocytes, they're blood vessels--and I just want to make the point that unlike the heart, or the liver, or other organs where a single or a small number of biomarkers are really very informative as to the underlying biology and underlying pathophysiology that's going on in that particular organ, given that the brain is much more complicated, we're going to need more than one or two. I’m hoping we don't need a huge number, but I think we all anticipate that once we figure out what we need, it's going to be a subset--a handful--of biomarkers and we're going to need biomarkers that inform us about the biology of astrocytes, biomarkers that inform us about the biology like soma degeneration, microglia, neurogenesis, synaptogenesis, and certainly blood vessels as well.

I think we have a reasonable idea what some of these may be, and I think this table is from a grant proposal that we submitted really several years ago to the Department of Defense, this is a grant that was ultimately funded, we call it the TRACK-TBI Precision Medicine initiative and the idea being that we're going to use both imaging and molecular biomarkers, and we're going to be measuring at least these three endophenotypes that likely play a big role, although it may not be the full axonal inflammation, diffuse axonal injury, and diffuse vascular injury.

And the reason that's important is that we actually have candidate treatments for each of those endophenotypes--and I think I’m not going to belabor too much here, this is not going to be the topic of this particular talk, but based on really very extensive preclinical data over the last two or three decades, I think we know that there are drugs that work on one or another of these endophenotypes that appear to be attractive candidates for the treatment of traumatic brain injury. But we're going to have to use them in a smart way, we're going to have to use them by enriching clinical trials for patients in which that endophenotype is prominent.

And then the other thing that is really important about biomarkers is that, well they're not static, but rather they're highly dynamic and they differ in terms of when after the injury they are released, how long they stay elevated or potentially depressed after injury, are they playing a role primarily in the very acute period in the emergency room, in the ICU, are they playing a role in the subacute period--meaning for the first week or so after where the patient may be in the rehabilitation unit--or are they playing a role, are they going to be informing us about the biology that's going on in the chronic stage right after the patient has tried to reintegrate into society, go back to work, go back to school, go back to being with your other family? So, these biomarkers are likely to be informative throughout this whole process and we're going to have to know a lot about the dynamic natural history of how these biomarkers are present.

The final thing that I wanted to bring up also by way of introduction, is--and this was recently--this is a gnostology that was recently proposed by the FDA and the NIH, and I think it's pretty important to think about the different types of biomarkers and the different purposes that they may serve. So, a diagnostic biomarker--and frankly, most of the work so far has focused on diagnosis--the diagnosis biomarker is useful to determine if the patient has the condition or they do not. A prognostic biomarker, though, which is something that we really need, is going to be helpful in categorizing patients by the likelihood of risk of disease progression. So, it's going to be something that informs us about the natural history of the disease; and in the TBI setting, if prognostic biomarkers are going to be needed in order to select patients who are likely to not have a good recovery and therefore likely to warrant therapy.

Predictive biomarkers--and sometimes the words "predictive" and "prognostic" can be used a little loosely to be synonymous, but according to the FDA and NIH [gnosology], they're not. A predictive biomarker is a biomarker that helps categorize patients by their likelihood of response to a particular treatment. So, it's used to measure the presence of a mechanism or an endophenotype in a particular patient that is likely to respond to a particular treatment. And finally, pharmacodynamic biomarkers are biomarkers that can be used to show that a biologic response has occurred in response to a treatment or a particular intervention. And we're going to need certainly prognostic, predictive, and pharmacodynamic biomarkers in order to have efficacy in developing new drugs.

The other thing that is really critically important--and the FDA has made this very clear--is that we need to be clear as to what the context of use is in which we are going to be using these biomarkers. And that depends on the clinical setting in which we're going to be using, it also depends on the particular purpose and the particular goal by which that biomarker is going to be useful.

So, one potential context of use could be in the pre-hospital setting, so at the scene of the accident, or the sidelines of a sporting event, or in a combat theater, biomarkers would be very useful to inform the decision of whether this is an individual who needs to be transferred to the emergency room for medical evaluation or whether this is someone who can be just sent home and expected to recover within the next hours to days; it can also help inform the decision of whether to bypass the nearest emergency room and go straight to a facility that has neurosurgical capability--or in a combat setting, this can inform the decision of whether to call in a helicopter to medically evaluate that individual. So, that's critically an important decision that is often life-saving and frequently, the folks at the scene need help from a biomarker.

Now, for such a biomarker to be useful, it would need to have very, very high sensitivity because you certainly don't want to miss anybody who could be saved by a proper surgical intervention. The specificity can be moderate; so, as soon as you have very high sensitivity, you can get by with moderate specificity in this particular context of use. Additionally, it needs to be detectable really within minutes because otherwise, it's not going to be particularly useful.

And a different set of contexts of use would be in the emergency department. So, the patient's already made it to the emergency department and here, the need for biomarkers can inform the decision, "Well, is this a patient who needs a cranial CT or not?" We do many cranial CTs in emergency departments in the United States and I think we all know that a large number of them, we could probably do away with if we were smart enough to identify those patients who did not need a CT. It can help--potentially help--identify that small subset of patients who may benefit from a cranial MRI, who may not need a CT at all, but they need an MRI, right? Those are people who may have pathologies that CT is not going to be helpful in identifying, but an MRI would be; it can certainly help inform counseling or emergency room discharge.

One of the things we have uncovered in the TRACK-TBI studies is that frankly, we can do a much, much better job--and we should do a much, much better job in counseling and emergency room discharge. And if we could identify patients who are at risk of developing persistent post-traumatic stress syndrome, that is something that can be very helpful and that, ultimately, for the future, it can help us identify patients who may be good candidates for enrollment in clinical trials of neuroprotective or restorative therapies.

In the intensive care unit, this is a patient who we know from the emergency room evaluation may need a syncoma, may need care, and in an intensive care unit, biomarkers can help us identify patients who are at risk of developing secondary injuries such as ischemia, intracranial hypertension, and inflammation; we monitor these things, but frankly, we are often late and we can often identify them only after the horse has already left the barn; and if we had biomarkers that could inform people and give us even just a few hours of advance notice of when these neural worsening events were likely to happen, that would be a very useful thing. Obviously, they can inform decisions regarding intensity of care, decisions regarding benefit of rehabilitation services, and, of course, they can help select patients for enrollment in clinical trials.

But even beyond that--and we're now talking into kind of the sub-acute or early chronic stage--in the rehabilitation unit, biomarkers can also play a very important role, they can help us identify patients who are at risk of developing late complications of traumatic brain injury such as post-traumatic dementia, chronic traumatic encephalopathy, post-traumatic epilepsy, they can help identify mechanisms of post-injury morbidities such as headaches or neuropsychiatric disorders, and of course, help us identify patients for clinical trials as well.

Alright. So, that was by way of introduction and I hope I have impressed upon you that biomarkers are really, really important and that we are relatively in our infancy of understanding the natural history, understanding the particular endophenotypes that they measure, and particularly understanding their prognostic, predictive, and potentially pharmacodynamic role.

So, for the rest of my time, I’m going to talk about three biomarkers that have been the source of fairly intensive investigation over the past several years, and they probably have risen to the top in terms of being ready--or in the cusp of being ready for their inclusion into clinical trials and even clinical practice. So, the first one of which I’m going to talk about is glial fibrillary acidic protein, which is an intermediate filament protein, which is highly specific to astrocytes, it's induced upon neural injury and it's released into the cerebral spinal fluid as well as the blood, and there have been sensitive assays that have been able to measure these things in the blood in severe TBI and even mild TBI for really over ten years.

As I think many of you know of GFAP, as well as UCH-L1, in 2019, a little over a year ago, were cleared by the FDA and approved by the FDA for use in the emergency department to assist in the evaluation of mild TBI. And the data that supported that FDA clearance was obtained from the ALERT study. So, I’m going to spend a couple of minutes going through the conclusions of the ALERT study and what it shows us--this was published in Lansing Neurology back in 2018. So, the ALERT study overall enrolled just about 2,000 patients, there were a few exclusions, a relatively small number--and these folks had measurements of glial fibrillary acidic protein as well as another biomarker that I’m going to mention only tangentially, because turns out it's not quite as useful, meaning UCH-L1.

So, of those folks, about 1300 had what was classified as a positive biomarker test, about 676 did not. This is what the raw data actually looks like; as you can see, both for GFAP and for UCH-L1, there is actually a very substantial difference between the participants who had a positive trauma-related abnormality on their CT scan from those that did not. I mean there is some overlap, but overall, these are very good biomarkers for distinguishing between CT-positive and CT-negative.

The area under the curve is--and we actually have this here--is over 0.9; and depending on what cutoffs you choose, you're ending up with a very, very high sensitivity, sensitivity in the order of 97, 98 percent with pretty decent specificity--"specificity" meaning that if the test is abnormal, it actually will predict an abnormal CT scan. So, the specificity is sort of in the order of 34 to 35 percent.

Now, there are a couple of caveats to this study. First of all, the number of neurosurgical-manageable lesions was very, very low right, only eight. Now, remember, this study enrolled basically everybody who came to the emergency room needing a CT scan. So, the vast, vast majority of those people--and again, as everybody who was GCS 13 to 15, so everybody who was in a coma or attended was, of course, excluded; we knew those people needed a CT scan. So, there's a very small number of folks that have neurosurgical-manageable lesions in this particular cohort; and of course, in every single one of those people with neurosurgical-manageable lesions, the amino sensitivity was 100 percent; you did not miss any single one of those cases--but it's a small number, which means that the confidence intervals, as you can see here, are actually pretty broad right. So, it's a concern and this is clearly the people you really care about.

Now, the other limitation of the ALERT study--although it was a very large study and it was very well done--is that it did not compare the performance of the biomarkers versus available clinical guidelines. Available clinical guidelines are based on things such as age, or based on the medications that the patient is taking, some other particular findings in the history, and what have you.

So, overall, this is helpful. It has so far, not been adopted in emergency room management--and the reason for that is that the assay that was used in this particular study was a large instrument platform lab-based assay which had a turnover time of several hours; but as I think many of you know--and this is also with DoD support--Abbott has launched--or is launching--it's already been started in several sites--a study where they can assay these two biomarkers in a point-of-care i-STAT platform. So, Abbott is basically repeating the ALERT study; very, very similar sample size, very, very similar design, except that they're going to be doing it using their point-of-care platform which is going to be what we ultimately are going to need if these biomarkers are going to be incorporated into emergency room evaluation of TBI.

This is some newer data from the TRACK-TBI study that is submitted, is being evaluated for publication; and the point that I want to make here is that GFAP is not only useful in the first 12 hours--both the ALERT study and the newly-launched Abbott study are enrolling folks within 12 hours of the injury--but as we know, many patients don't come to the emergency room within the first 12 hours, and this is particularly an issue in military settings where it actually may take time; it may take longer than 12 hours to transport an injured service member to a site.

So, the data I’m showing here in these three bars are uninjured controls, CT-negative TBI in green, and CT-positive TBI in blue. And as you can see, obviously, the CT-negatives are showing mild elevation, but big elevation in the CT-positive TBIs; and obviously; that's very, very prominent in the first day. On the other hand, even on Day 3 and even on Day 5, there are still highly significant elevations in GFAP compared to the CT-negative and compared to the TBI. That's not so much the case for UCH-L1; UCH-L1 is helpful on Day 1 as I’ve shown you, but really not so much beyond that. And even at two weeks, there are still differences between the CT-positive and the CT-negative TBIs as I’ve shown you down here.

So, this is a useful biomarker, it stays around for a long time--or maybe it's not just that it stays around, but maybe there is some continued release of GFAP from reactive astrocytes that persist for the first several days, maybe up to a week or so, and they carry some information.

The other thing that we were able to establish in the TRACK-TBI cohort is that GFAP has some prognostic usefulness--and that's not so much in the Day 1 GFAP; Day 1 GFAP is, I guess, mainly helpful diagnostically to help settle the issue of, "Does this patient need a CT or not?" But particularly at two weeks, I think it has a pretty reasonable C-statistic area under the curve statistic for distinguishing those individuals who are going to make a reasonable recovery meaning that are here to have a Glasgow Outcome Scale score of greater than 5 or 5 or greater compared to those that actually have a poor recovery meaning, meaning a GOC of less than 5. So, this is useful information and it speaks to the potential usefulness of GFAP as a prognostic biomarker or potentially, even as a pharmacodynamic biomarker. If we had a therapy that reduced the GFAP level at two weeks compared to what it was initially, that would really help out. And again, UCH-L1, perhaps, not quite as helpful in this setting.

So, GFAP obviously, a very promising biomarker, is already FDA-approved, more to come on that particularly as we get the results of the ongoing Abbott i-STAT study.

So, let's move on now to C-reactive protein. So, C-reactive protein is a biomarker that has been widely studied in many areas of medicine. C-reactive protein is primarily made in the liver although there are some sites of extrahepatic synthesis as well. And whenever there is injury or inflammation, that stimulates hepatocytes to produce a native C-reactive protein which really circulates in the blood as a pentamer right, as these five subunits--and this is what the assay is actually picking up. The pentamer which circulates in the blood is what can be measured, it's relatively soluble, and it's been known for a long time that this is a non-specific--and certainly not brain-injury specific--but a useful marker of inflammation.

Now, it also has an important role so when C-reactive protein gets--when this pentamer structure gets into the tissues, it dissociates and it's the monomeric CRP that's actually the business end of this molecule. Now, monomeric CRP is not highly-soluble; this is present only in tissues and it's not something that's measured by the blood assay, but it's the business end, right? So, it stimulates the production of a large number of cytokines and other inflammatory mediators, both in endothelial cells as well as in the smooth muscle cells, it promotes adhesion of leukocytes, it has a large--it stimulates gene expression very broadly.

Now, in several areas of medicine, C-reactive protein has been found to be useful as a pharmacodynamic biomarker of therapies that are anti-inflammatory, or in some way, dampening the innate immune response. So, this is just one example from the stroke literature, and in this particular study published a couple of years ago, used statin therapy which was started soon after stroke and the goal was to see whether statin therapy would reduce CRP levels, and as a consequence of that, presumably reduce the risk of subsequent stroke. And as you can see within two months of starting statins, there was already a highly statistically-significant reduction of CRP levels, which is why statins, as you may recall from one of the tables I showed you earlier, is one of the drugs that we are excited about that could be useful as a repurposed drug for the treatment of traumatic brain injury.

I’ll just show you one more study--and this was published in the New England Journal a couple of years ago--and this is using a molecular antibody called canakinumab, which is a monoclonal antibody against IL-1β, is again an anti-inflammatory drug. And as you can see, C-reactive protein is a highly, highly sensitive pharmacodynamic biomarker for anti-IL-1β therapy, different doses reduce that. And what's very nice to show in this particular study is that that reduction was associated with a lowering risk of--the primary endpoint in this study was subsequent stroke or subsequent heart attack. So, it not only was anti-inflammatory, it had a clinical benefit by reducing the risk of a clinically significant event later on.

So, what do we know about C-reactive protein and TBI? And again, this was published a few months ago, again from the TRACK-TBI experience. So, one thing we know--and again, not unexpected--is that this is not a brain injury specific-biomarker. So, the blue line are the healthy controls, obviously at very, very low levels of C-reactive protein--even within one day of injury, there are dramatic elevations of C-reactive protein and it's basically equivalent in the brain-injured subjects shown here in red with the--actually, brain-injury subjects shown in green compared to the orthopedic trauma controls shown in red.

So, there's actually no difference at any of these levels, it's highly-elevated on Day 1, but it continues increasing over the first several days; and even two weeks later, it's come down compared to the Day 1 levels, but it's still highly significantly higher than the uninjured controls. And that's true for orthopedic injury as well as for TBI.

That's, again, as we expected. We know that this is not a brain injury-specific protein, and it also points to the fact that I think we did a pretty good job within TRACK-TBI in matching the extent of injury and this orthopedic injury control. As we anticipate, there's a strong relationship between CRP levels and the Injury Severity Score; the Injury Severity Score is a measure of polytrauma right. So, the higher the Injury Severity Score--and ISS of over 25 with pretty substantial polytrauma, they have much higher CRP levels, but overall, they behave pretty similarly.

Now, the reason that why we're excited about this--and what we've been studying in a TBI cohort--is that elevated CRP levels turn out to be a pretty good prognostic biomarker, and not just in Day 1, but also in the subsequent days after that--and again, this should not be as a surprise. Systemic inflammation is a bad thing and the brain is exposed to whatever is circulating in the blood; so, any inflammatory mediators, any inflammatory molecules that are circulating in the blood--even if they're coming from the liver or they're coming from an injury somewhere else--are actually going to make it into the brain and they could particularly have deleterious effects there.

So, what I’m showing you here is that in those individuals who make a good recovery--meaning that they have no or very minimal disability after their TBI-- their CRP levels, although they are elevated--they are dampened particularly over Day 3 and 5 compared to the folks that end up making a fairly poor recovery and that's true whether or not you're dichotomizing the GOS at 5 or whether you're by dichotomizing the GOC at 8.

So, looking at the data in somewhat more detail--and again, I’m including here the data I showed you earlier with GFAP as a prognostic biomarker, on Day 1 CRP doesn't really help that much for prognosis, but particularly in Day 5 and particularly at two weeks, CRP actually does substantially improve the AUC and provides helpful prognostic information. And in fact, in those unfortunate individuals who end up having a poor recovery, CRP is actually higher at two weeks than it is at Day 1. This is not a large number of people, but I think it does speak to the potential usefulness of CRP as a prognostic or even a pharmacodynamic biomarker. If we had a therapy that was targeting systemic inflammation, that was targeting an inflammatory molecule, CRP may be an attractive pharmacodynamic biomarker to use in order to demonstrate the efficacy of that therapy.

Finally, let's switch over to the last biomarker that I wanted to talk to you about, which, as many of you know, has received a lot of attention over the past several years, and that's the neurofilament light chain. So, neurofilaments are a set of structural proteins, cytoskeletal proteins, they're very enriched in axons; in fact, neurofilaments are specific to the central nervous system and specific to axons; they are a family of neurofilament light, or neurofilament medium, neurofilament heavy, as well as peripherin and α-internexin which are also members of the same family.

Neurofilament-light, because it's the smallest of the neurofilaments, has received the most attention and really for many years now--probably 15 years or more--it's been possible to measure neurofilament-light levels in the cerebrospinal fluid, and there have been some important studies that have been done over the past couple of decades measuring neurofilament-light in cerebrospinal fluid.

However, more recently--and this really goes back only in the last five to seven or eight years or so--it's been possible with highly sensitive immunoassays, and the assay that has primarily been used is the Simoa assay from the Quanterix Corporation. It's recently been possible to measure the neurofilament-light levels that are found in the blood which are obviously present at much, much lower concentration than is found in the CSF. But the nice thing that it turns out is that the correlation between the CSF NfL levels and the blood CSF levels is almost one to one, so what you're measuring--I mean it's obviously much lower in the blood, but it's a very good reflection of what's going on in the CSF and, as a consequence, likely very good reflection to what's going on in brain tissue itself.

The other thing that I would like to point out to this audience is that neurofilament-light has received a lot of attention recently and there's a lot of excitement in the general neuroscience field about neurofilament-light being useful as a biomarker in multiple sclerosis, being useful as a biomarker in Alzheimer’s disease, in dementia, in a number of different conditions--and partly because it can be helpful as a pharmacodynamic biomarker.

So, this is data from a study published a couple of years ago in multiple sclerosis where neurofilament-light appears to be very helpful as a pharmacodynamic biomarker. So, here--this is a large randomized control trial of patients who were placed on Fingolimod which is a disease-modifying therapy in multiple sclerosis; and as you can see, those people who were placed on placebo, there really is essentially no change in neurofilament-light levels, where there was a significant change in those that were randomized to the active treatment.

In this related study published in the same paper, Fingolimod was compared to interferon-β-1a which is another, but maybe somewhat less effective disease-modifying therapy; and again, they both reduce neurofilament-light levels, but much more significantly in the Fingolimod group.

So, it would be very interesting to know if neurofilament-light--how neurofilament-light levels behave after traumatic brain injury and whether they could be useful as a pharmacodynamic biomarker in an early phase clinical trial. And this is the initial paper that studied neurofilament-light in TBI. This study from Pashtun Shahim and then colleagues, he was the first author of this paper; he was a graduate student at the time with Kaj Blennow and Henrik Zetterberg In Gothenburg University and they were the ones that developed the ultra-sensitive Samoa assay for neurofilament-light.

And one very interesting thing--so these were all patients who were in the intensive care unit at Gothenburg University, either moderate or severe TBI, and then they were able to obtain almost daily neurofilament-light levels in a significant number of these people. And one very interesting thing about this molecule is that it is significantly elevated in Day 1 compared to the healthy controls here. However, it keeps increasing over the first several days; and in fact, by two weeks, it is almost ten times higher than it was on Day 1.

So, this appears to be a marker of the secondary injury--particularly the secondary axonomy. Now, a subset of these people had external ventricular drains where CSF was obtainable, and the very same pattern that you see in blood is also observed in CSF; and as I told you earlier, there's a highly, highly--very high correlation between the serum and the CSF NfL, to the point that we can, with confidence, assume that when we're measuring in the blood is a good indicator of what's happening in the CSF; and ergo, of what's happening in the brain itself.

This is a study that was published a couple of months ago. Dr. Shahim is now working at the NIH, and he had the opportunity to collaborate there with Jessica Gill and Leighton Chan, and other investigators who have been interested in biomarkers of TBI, particularly with the sub-acute and chronic phase. So, the interesting thing about this study is that a cohort of patients with TBI were seen at the NIH clinical center, and were then followed up--in some cases, up to five years, which is actually quite remarkable. And this is for GFAP, Tau, and UCH-L1--I’m not going to discuss those so much, I’m going to focus rather than the neurofilament-light data.

And obviously, even at 30 days, it is substantially elevated compared to the uninjured controls; but the important thing is that it stays elevated for a very prolonged period of time, even years--even two or three years after TBI, particularly in those who had suffered a severe TBI, the levels of neurofilament-light remained elevated--and that's actually even the case for those who had a mild to moderate TBI, although less so.

The other--and this is, again, showing the same data, but rather cross-sectionally; this is sort of a spaghetti plot and it's kind of trying to show you what the evolution of neurofilament-light levels in particular individuals. So, these are individuals who had more than one sample measured across several years and it's basically confirmatory of what I showed you in the prior slide.

Now, the other very interesting thing about neurofilament-light--and this is why we're very excited about how it may be useful as a biomarker of the axonal injury endophenotype--is that there's a strong relationship between plasma neurofilament-light levels and MRI measures of like [soma] integrity. So, in this case, we're looking at diffusion tensor imaging and these individuals had DTI measurements--had MRI and DTI measurements obtained at multiple time points. And as you can see, the neurofilament-light levels was well-correlated--well, anti-correlated, rather--with the fractional anisotropy in the corpus callosum, and that through whether it's the genu of the body; and rather than the anticorrelation positively correlated with the radial diffusivity and the mean diffusivity.

So, I think this tells us--and again, this is what we expect given where we know that neurofilament-light is expressed, this is then--this is the protein--the intermediate filament protein that's abundantly expressed in axons, and this kind of confirms the hypothesis that neurofilament-light if found in the blood, is a marker of axonal injury.

And again, this is another figure from that very same study; I won't belabor the point too much more. And the interesting thing is that it can be predictive of what's substantially going to happen? So, the neurofilament like level, at the first baseline visit, is actually predictive of the MRI changes over the subsequent year. So, the higher the neurofilament-light level at the baseline visit, the greater change in radial diffusivity, the greater change in volume atrophy in these brain structures over time. So, I think that is supportive of the view that this is attractive as a prognostic and potentially, even a pharmacodynamic biomarker.

Not surprisingly, the levels of neurofilament light in Day 1 are correlated--although loosely correlated--I would say a low-correlation--with neurofilament-light levels found in Day 10 to 12. So, again, data from the Gothenburg study. And if we're to use this data to try to power a clinical trial, meaning if we were to just--if we had a drug which--which we have some candidate drugs that we believe are going to be axonal protective, and are going to work by promoting resilience or promoting repair of axons, and we wanted to measure the change in neurofilament-light from Day 14 to Day 1, as the biomarker or surrogate outcome for that particular study, this tells us what the sample size we would need in each arm of the study to be able to know that.

I think what this is telling us is that, in fact, if we were to propose kind of medium to even low effect sizes on this biomarker--again, the standard deviation is around 0.2, 0.25, effect sizes of 0.3 to 0.5 are things that could be detected with a reasonably-sized Phase 2 study, something in the order of 50 to 100 participants in each arm of the study, should be enough to detect an effect on this biomarker, which we believe has a strong relationship with clinical outcomes.

So, I think this is belaboring that point as well, it may be actually that GFAP--Day 1 GFAP--is superior as a predictor of what Day 14 NfL will be like compared to NfL itself; and it may be that using GFAP as a prognostic biomarker and then perhaps NfL as a pharmacodynamic biomarker is going to be the most attractive way to go forward.

And this final slide--again, I’m presenting work from the CNRM cohort, but the data was mainly analyzed by Pashtun Shahim, also Jessica Gill did all the biomarker assays. It does appear that neurofilament-light outperforms other molecular biomarkers, and even other imaging biomarkers in terms of the power for a clinical trial. So, in this particular graph, those who show you we're plotting treatment efficacy, or this is potential treatment efficacy, postulated treatment efficacy against needed sample size; and for example, a reasonable power study where we would be postulating a treatment efficacy around 20 percent. Well, it could be done using somewhere around 70 to 80 patients per group if we were going to use neurofilament light. On the other hand, if we were going to use MRI, fractional anisotropy, mean diffusivity, we would need much larger sample sets.

So, I hope that this kind of data has convinced you about the direction through which these discoveries in the natural history of biomarkers are going to be informing the future generation of clinical trials.

So, in conclusion, I hope that I’ve convinced you that endophenotype-specific biomarkers are going to be needed to inform the next generation of clinical trials if those clinical trials are ever going to have any chance of being successful. I think I’ve convinced you that CRP shows promise, neurofilament-light shows promise particularly as a biomarker of common traumatic axonal injury, but then, obviously, there is much work that remains to be done.

And finally, this is my group at the University Of Pennsylvania as well. I’m speaking here not only for large and really very talented collaborators at Penn, but also collaborators at USHCS and CNRM; I’ve talked about Pashtun Shahim and Jessica Gill, but obviously, Kim Kenney, Harold Moore, and Leighton Chan had a very instrumental role in this study. Jeff Manley, Sonia Jane, and Jessica Puccio and Eva Puccio had a big role as well in the TRACK-TBI biomarker studies.

So, again, thanks for your attention, and I think we have a few minutes for questions.

Whitney: Great. Thank you, Dr. Diaz. So, we have one question here right now. Just as a reminder, please send your questions through the Q&A function of WebEx and I will be able to ask them. "Are you familiar with the military's effort to collect blood sample biomarkers as soon as possible post-trauma blast occurring downrange? I believe there was a contract lab to have medics trained to obtain samples as close as possible to the time of injury or TBI trauma. And is there a difference in the biomarkers for blast versus impact-blow-to-the-head-type TBI trauma?"

Diaz-Arrastia: Well, I am familiar with several attempts at doing that which actually go quite a while back, go back over ten years. And it actually has been enormously challenging; as you can imagine, when in a combat theater or even a training site, obtaining biomarkers is not necessarily the highest operational priority. So, I mean I am aware of attempts that have been made at that; I’m frankly not aware that any of those have been successful.

In terms of the question of blast versus other mechanisms of injury, that's been an issue that's been around for a long time, and what I’m most impressed by--and I think these were studies that were mainly led by Christine McDonald And David Brody--is that isolated blast is so rare as to be vanishingly almost non-existent. And certainly, when I was attending the neurology clinic at Walter Reed, every single one of these injured service members... yes, they were exposed to a blast, but yes, their head also hit a wall, their Humvee ran off the road and turned over; isolated blast TBI may be so rare as to be non-existent.

Obviously, the question is does blast TBI, in addition to a more traditional mechanical TBI, have a different consequence? Frankly, it has been very difficult to demonstrate that. And again, Dr. McDonald And Dr. Brody studied that very, very thoroughly, and that appears to be a difficult thing to demonstrate in terms of actual details of the brain injury whether they used MRI or they used a number of other conditions.

I think that question remains an open question. I know, in pre-clinical models, it is possible to isolate blasts compared to other mechanisms of injury--and frankly, the histopathology data and some even some of the biomarker data that's coming out from those preclinical models, probably does not--in my opinion, the reading of that literature does not support the conclusion that they differ all that much. But again, in the real world, there's a lot of interaction.

Whitney: Thank you. This one is more of a comment, "Thank you for your very informative presentation; I appreciate the neurological illustrations of accompanying the explanation." And then our next question is, "What effect does remote TBI have on the assay you presented on acute TBI?"

Diaz-Arrastia: Right. So, that's a very good question. And to be honest, we are only in the very early stages. And it appears--at least for neurofilament-light--but it may be the case for some other ones--that once you've had a TBI, your neurofilament-light level may be elevated forever, for the rest of your life. And this may be part of the explanation of the increased risk of late-life dementia that these individuals can have.

So, the question is what happens if someone who had a TBI say five years, and then they have an NfL level that's elevated, but say three years later or four years later, has another TBI? And I mean my suspicion would be that it's going to be even worse than if they had not had a prior TBI, that there's going to be a summation of the injuries, even if they are apart by several years; that it's not as if the brain ever gets back to normal after having had--certainly not a severe TBI, and that having a subsequent TBI--even a relatively minor one--on a brain that is still different as a consequence of a TBI even years before, it's going to have a different effect, and potentially, a much more serious one.

Whitney: Thank you. Our next question is, "Are microglial biomarkers useful?"

Diaz-Arrastia: We actually don't know that much about microglial biomarkers yet, but I am certain that they will be useful and important. Microglial plays a very important role in the pathology of mild TBI. But to my knowledge, there are no microglial-specific proteins that have been measured to date; there have been attempts to look at circulating monocytes, there may be some equilibrium between circulating monocytes and brain-resident microglia, but that that area of science is very much in its infancy.

Whitney: Thank you. This next question asks, "What impact will those levels of NfL have?" Not sure what this means.

Diaz-Arrastia: Excellent. Excellent question. We know that NfL--I mean, first of all, NfL is not going to be TBI-specific, right? So, we know that NfL is elevated in obviously multiple sclerosis, dementia, stroke, and a number of other conditions. We know that NfL increases with age, so we're going to have to have an age-specific marker. An NfL level that is elevated in a 30-year-old may be normal in a 60-year-old, so we're going to have to have age-related norms; and certainly, someone who has survived an earlier TBI, it's going to be somewhat more difficult to interpret what a particular NfL level may mean compared to someone who has never had a previous one. We know NfL is elevated in diabetes, we know NfL is elevated in renal failure--so, all of these factors are going to have to play a role.

Now, the one thing we do know is that most of the time, the Day 1--or the first few the first week or so--elevations in NfL is actually very, very robust, and several orders magnitudes higher than what you find in multiple sclerosis, or what you find in age-related dementia, or anything else. But I think that if you see an NfL level of 200 or 300, something--some acute brain injury occurred in that individual. It could have been a stroke, but that certainly could have been a TBI as well. That is not going to be due simply to age, or simply due to MS, or anything like that. However, if you're analyzing it years out, I think all of these factors in history are going to have to be evaluated and paid attention to.

Whitney: Great. Thank you. So, we are just at the top of the hour, so Dr. Diaz-Arrastia and Dr. DePalma, do you have closing comments?

DePalma: I would like to thank Ramon for this wonderful presentation. Perhaps it would be best to direct our attention to the primary blast injury studies going on with the breachers and heavy weapons exposures as a fruitful future area. This has been a remarkably well-attended, informative session.

Diaz-Arrastia: Alright. Thank you so much. I appreciate the questions and I appreciate your attention.

Whitney: Right. Thank you so much for taking the time to present for us today Dr. Diaz-Arrastia.