Protagenic Investor Virtual Science Review (9/8/2021)

Garo Armen: I'd like to begin by thanking you for joining us. Today is the Protagenic Therapeutics, Inc. science and clinical review. With me are Professor David Lovejoy, who is the scientific founder of PTI. Professor Lovejoy will tell us about his personal journey that led to the discovery of TCAP. We will tell you a bit about TCAP, a remarkable peptide found in the brain and the role that TCAP plays in modulating our responses to environmental stress. We will also tell you that PTI is developing a synthetic version of TCAP, which we have designated PTI-00114. PTI-00114 has remarkable pharmacological effects in animal models of significant neuropsychiatric diseases, including treatment-resistant depression, generalized anxiety, Post-Traumatic-Stress Disorder (PTSD), addictions, and potentially neurodegenerative conditions. Stress, which has become a fixture in modern life, contributes to most – if not all – of these conditions, and TCAP is the body's natural defense against the deleterious effects of excessive responses to pervasive, chronic stress. After Professor Lovejoy we will hear from Dr. Andrew Slee, a world-class pharmacologist and PTI's Chief Operating Officer. Andy has substantial expertise and experience related to drug discovery for neurological and neuropsychiatric diseases. Dr. Slee will relate his initial skepticism about TCAP, which seemed almost "too good to be true" and the myriad experiments which he has led over the last several years that have converted him from a skeptic about TCAP and PT-00114 into a firm believer in the potential of PT-00114 to become a major medicine. After Dr. Slee Dr. Jen Buell, a member of the Executive Committee of the Board of PTI. will tell us about our up-coming, highly innovative clinical trials designed with Dr Maurizio Fava, Psychiatrist-in-Chief of Massachusetts General Hospital and Director of Clinical Research at the MGH Research Institute. Dr. Fava is a world leader in the clinical development of neuropsychiatric drugs, having led trials leading to the approval of many of today's best medicines for patients with neuropsychiatric illnesses. Dr. Fava's trial design for PT-00114 could save PTI years in driving PT-0014 to registration for optimal use of the drug in patients with a variety of stress-driven neuropsychiatric indications.,

Garo Armen: And last, Dr Robert Stein, PTI's Chief Scientific and Medical Officer, will provide the big picture as well as some specifics about the role that stress plays in a wide range of psychiatric disorders and how PTI-00114 has the potential to uniquely address these significant and largely poorly treated diseases.

TCAP, as David Lovejoy will tell us, has played a very critical role over hundreds of millions of years of evolution in balancing and managing stress responses. This fundamental role of TCAP has persisted throughout the evolution for many different species. As we are about to advance PTI-00114, the synthetic TCAP, into human clinical trials, we will be validating in people the large number of experiments we and Dr. Lovejoy's lab have conducted to characterize the beneficial pharmacological effects we have observed in animal models of many significant neuropsychiatric diseases. Entering into human clinical trials of PTI-00114 will be the exciting next step for Protagenic Therapeutics.

David Lovejoy: It's a pleasure to have the opportunity to talk to you today. Most of you probably don't know me. I'm David Lovejoy, and it was our research team that discovered TCAP. This is the hormone that PTI's new drug PTI-00114 is based upon. Over the years, my team has discovered and contributed to understanding of a number of new peptide hormones associated with stress and reproduction. And since then, I've had the honor to be able to work with the very talented Protagenic Therapeutics team.. You'll be hearing a lot about peptide hormones during this webinar. If you're not familiar with them, there are small proteins consisting of a chain of different amino acids. They're special because, unlike many of the therapeutics used today, they're based on DNA sequences encoded directly by the genes and have been optimized for very specific functions over millions of years of evolution. I did my degrees in evolutionary biology and neurobiology in Canada, then spent three years at the Salk Institute in San Diego to investigate the role of stress-associated peptide hormones. Following that, I was appointed to the faculty at the University of Manchester in England, where I developed a method to discover the evolutionary ancestors of today's human peptide hormones. After that, I was invited to the University of Toronto in Canada to continue my work in neurobiology. Since then, I have focused on discovering and understanding the

biology of stress peptides. But why this focus on peptides? Let me tell you a quick story about why I wanted to do this and ultimately how it led me to the discovery of TCAP. As a young student, some highly stressful life events left me so incapable of thinking that my only way of dealing with this was to consider ending my own life.

David Lovejoy: I felt like a complete failure as a person. It was as if my sense of rationality had suddenly disappeared. Thankfully, after a while with the support of close friends, I realized I wanted to live. But I know not everyone has that support, and I was particularly fortunate. I suspect many of you listening to this have experienced profound stress in your life. This might be due to work-related stress or intense social relationships, perhaps a loss of fundamental beliefs or the tragic experience of a serious accident. It could be due to early childhood abuse or perhaps even combat or police-based situations. Clinically, the consequences of extreme stress can manifest as major depression, anxiety and panic disorders, obsessive compulsive disorders, addiction, and perhaps post-traumatic stress disorders. Yet in so many of these situations, we in the medical community have limited abilities to help ourselves or loved ones that are going through these extremely stressful experiences. And if we've been through this and if you've been through this, this sense of helplessness in the face of adversity is not a pleasant feeling. We can feel a lot of emotions such as guilt, shame, and worthlessness. And I've been through this myself. So it's likely that you and I may have this one thing in common, and ultimately we want to solve these problems. Right now, the major pharmacological approach to solving stress-induced conditions is to use a novel chemical compound that is created by medicinal chemists and then administer it to the most complex and intricate structure on the planet: the human brain.

David Lovejoy: As a neuroscientist with a background in evolutionary biology, this approach has always been confusing to me. But what if we could take a novel inverted approach and examine the stress response early in the course of evolution of animals, then trace that mechanism to humans. This was our basic philosophy moving forward. Now, stress is not unique to humans. Animals do not experience stress in the same way that humans do, though. Faced with an overwhelming challenge, stressed animals will try to escape, hide and then eventually recover. For the types of chronic stress we as humans encounter in the modern world, we humans can't do this. We have to continue

to work at our personal goals or support our families for a variety of reasons. Profound stress is nasty. Anybody who's been through it knows that it stops us from interacting with others, inhibits sexual activities, and interferes generally with the things we find pleasurable. But this is an adaptive evolutionary mechanism to protect ourselves. It forces us to withdraw from society to essentially reboot the brain. Yet we can't hide like animals. We have to continue to function. Sadly, endogenous human stress responses are not prepared to solve this response. Human society is advancing faster than we can deal with and respond to chronic severe stresses.. And yet we still have to be functional members of society. Because of this, I wanted to understand the biological options for ending this cycle of stress. In our research over many years, we concluded that human stress response, like those of all animals, is ultimately dependent upon hormones required for survival that appeared early in evolution.

David Lovejoy: The effective utilization of energy is required for survival, and it was those ancient, early evolving hormones that eventually helped lead us here. So, what I was looking for were these ancient hormones, and I discovered a new peptide hormone, which we call TCAP. It was distantly related to other peptide hormones involved with the stress and energy responses, and it's been around for about a billion years. Wow. A billion years. I mean, that's a long time! I mean, a billion years ago, there were no multicellular organisms. Only single-celled organisms are thought to have existed. So, imagine how important TCAP must be to still be around today. But we can only reach this conclusion by using the findings of many other researchers who came before us, who were engaged in the work on the evolution of animals and genomes. We sifted through many potential peptides and eventually we focused on TCAP. Importantly, this peptide was likely active before the development of the nervous system. If so, such a peptide would have been utilized by the brain as it developed in complexity. Thus, the nervous system evolved in part around the presence of TCAP and not the other way around. So, returning to the laboratory, we showed that this peptide affected brain cell activity and reduced the anxiety response by inhibiting the actions of a number of established stress peptide hormones mediating the responses to stress.

David Lovejoy: These hormones have been the target of numerous therapeutics in the past to try to reduce stress-associated pathology, but unfortunately few of them have

met with clinical success. So recognizing TCAP's importance, we contacted Dr. Armen about developing TCAP into a therapeutic to combat the deleterious consequences of stress. The concept was simple. Our work and inferences from the work of others led us to conclude that TCAP is one of the evolutionarily oldest peptides to regulate cell health and metabolism. So how could we show this? This began a collaboration between Dr. Armen and our laboratory. Dr. Armen advised us to “Follow the Science”, and this is what we did. Gifted with a number of bright, energetic young scientists in my laboratory, these talented individuals helped me establish that TCAP possessed all of the hallmarks of a peptide that regulated brain cell health and vitality. But each time that we had a success, we asked the question of how we could prove that TCAP was an essential stress protecting peptide system? We designed more complex experiments to establish the actions of TCAP in depression, anxiety and addiction. With each experiment, TCAP showed its ability to moderate the stress response in numerous behavioral and metabolic studies. And Dr. Slee and Dr. Stein will be discussing this in detail in a little while. We then turned to investigate the potential side effects of TCAP by increasing the dosage of TCAP in rodents in some of our earlier studies. Dr. Dalia Barsyte in my laboratory at the time, who holds two PhDs, one in genetic toxicology and another in molecular and cell biology, was struck by the low toxicity profile of synthetic TCAP.

David Lovejoy: As she stated at the time, the animals were particularly relaxed and remained healthy, even after taking doses of over 10 times greater than the effective dose. This gave us considerable insight. So how could TCAP have this action? So let me ask you this question: How do you solve complex problems? What is the one thing that all complex problems have, right? These are strenuous mental tasks that might take hours, days, weeks, or even years to figure out. But is there something common to all of these elements, all of these different conditions? Well, as it turns out there is, and this is energy. The brain consciousness and decision making require energy. You've likely noticed that when you're engaged in a strenuous mental task, you crave particular sweet or fatty foods, or you may indulge in intense exercise. These activities increase brain activity, and generally you feel better. Well, cells and brain cells in particular are no different. The more energy that you have available, the better they have to protect themselves in stress, so stress acts like a switch to change our energy reserves to focus from normal day-to-day action on being ourselves to a situation where the priority

is now to challenge the stress or to escape from the stressors. We have a finite amount of energy that can be used to develop ourselves or predict against an attack. And in most cases, unfortunately, it's one or the other.

David Lovejoy: But TCAP, on the other hand, provides more energy resources to ward off these stressful attacks by increasing the uptake of glucose into brain cells. And in doing this, it manages communication among brain cells to help restore normal thought processes. Well, since then, Dr. Slee has developed a greater understanding on the mechanisms of stress and TCAP in animals. And he's going to be discussing this with you shortly. And in the meantime, we continue to work on the molecular actions of TCAP on brain cells by identifying all of the essential molecular and cellular mechanisms that regulate TCAP action. So now we have a working understanding of how TCAP acts on the brain and on the organism and how it can moderate stress responses. Now Dr. Slee has taken this to the next level with a number of behavioral and pharmacological findings that support the evolutionary origins of TCAP and its role in stress response and regulation of energy utilization. So, from my perspective as the discoverer of TCAP, this has been a very exciting journey into some of the most crucial intrinsic elements of the human stress response, and I want to thank you for all of your support over the years as you've led to the development of a new technology that is now at the forefront of our understanding in the treatment of stress-related disorders. I think the upcoming clinical trials are going to lead to some very exciting findings indeed. So, thank you all for listening.

Garo Armen: Thank you very much, David. And next is Dr Andy Slee.

Andy Slee: David, thank you so much. That was a fabulous introduction. We got involved with the quest to understand the pharmacology of TCAP several years ago, when we were offered the opportunity to study the pharmacology of our drug candidate PT-00114, synthetic TCAP. When we embarked on our efforts to look at what David had done over several years with his whole lab, we came in basically as pharma skeptics. The discovery mode in the University of Toronto has been that of academic discovery. We came in and say, this is rubbish, we're big pharma. We're going to actually destroy it. And oh boy, did they prove us wrong, because there really is something there in this

naturally occurring brain peptide that is very, very beneficial. I came out of 41 years in the pharma and biotech,, and in my role in DuPont-Merck Pharmaceuticals we did a lot of work in the CNS arena, particularly pursuing small-molecule antagonists of Corticotropin Releasing Factor (CRF), one of the critical peptide hormones involved in the response to environmental stresses. We were looking for small molecule CRF antagonists, so we were very familiar with this particular area. Our goal when I joined the PTI team was to examine the work on TCAP that come out of 15 years of TCAP research of the Lovejoy lab at the University of Toronto, where they have presented to us some activity within animal models where they had shown that when they synthesized TCAP, which is a 41 amino acid residue peptide and administered it to rodents, it altered mood and behavior in animal models which had face value, which means that one can project from the results obtained in the animal model to what you might expect to see in humans. A particularly intriguing finding the Lovejoy lab observed was a stabilization in dendritic spine density, an indication that TCAP stabilizes synaptic connections between neurons, which could counteract the effects of chronic stress to lead to loss of neurons and disruption of neuronal connectivity.

Andy Slee: This is something we are following up on, and this also opens up a great avenue for looking at a whole host of diseases, such as loss of sense of smell in patients with COVID-19 infections. As David mentioned, one of the startling effects when you administer TCAP is an increase in glucose utilization by brain cells.. Decreased glucose utilization in the brain is a feature observed in depression. This suggests that administered TCAP would be therapeutic in depression. When it has been tested in animal models of depression, TCAP reverses the depressed behaviors. We are excited to see the results of testing PT-00114 in the clinic in depressed patients.

Another results that David and Dr Susan Erb had generated in drug-addicted rodents suggested to us that PT-00114 would be useful in treating drug-addicted people. Doctors Lovejoy and Erb studied rodents addicted to cocaine. These rodents were then deprived of cocaine and subjected to pharmacologically-induced stress (by injection of CRF into their brains). This resulted in the rodents frantically seeking cocaine. However, if the animals were administered PT-00114 either before or after the CRF-induced stress, they remained calm and the frantic cocaine seeking was

prevented.This supports a potential for PT-00114 to useful in the prevention of recidivism to drug use in addicts trying to break the habit. It took very small doses of TCAP to produce this effect. Importantly, the response to TCAP was NOT accompanied by signs of sedation or reduced movement. The very measurable effect of TCAP in these experiments was to reduce the drug-seeking behavior in a dose-dependent manner. We realized that this animal pharmacology implied that TCAP could be administered to human drug addicts to deal with one of the greatest challenges to getting addicts off drugs: recidivism. Even those addicts who can beat their physical dependence upon a drug like cocaine or opioids or alcohol retain a psychological dependence on their drug of choice: when encountering stressful situations, the impulse to blunt the stress by using their drug of choice plagues them.

So, we said, well, this is a great place to start. Let's look at some simple studies to confirm these findings and what we came up with was a study in mice which was based on a opioid testing paradigm developed by J.K. Saelens and colleagues. In the Saelens testing protocol one addicts mice to an opiate such as morphine, or we have also adapted it so that the mice become addicted to fentanyl. This can be done by administering seven doses of the opioid over two days. By doing this, the animals become addicted! Parenthetically, this sheds light on the ease with which people can become addicted to opioids prescribed to relieve pain or used “recreationally”.

Andy Slee: In this Saelens protocol you then induce these opioid-addicted mice to undergo sudden withdrawal by administering the opioid antagonist Narcan (naloxone), another product which we helped introduce to combat opiate overdoses when I was ran of pharmacology groups at DuPont Pharmaceuticsls. This produces the experience of opiate withdrawal in these mice, accompanied by extreme stress, The mice respond to this extreme stress by exhibiting a so-called stereotypic response: the begin to repeatedly jump up in the air, perhaps about 5 or 6 inches. This stereotypic jumping occurs very frequently, perhaps about once every 15 seconds. The mice can be placed in a covered beaker about 7 inches deep and they will jump repeatedly, nearly hitting the cover! The number of jumps over 20 minutes can be counted, usually about 75.. This turns out to a be a very quantitive assay, having been originally developed to assess the ability of other opiates to substitute for the original opiate to which the mice

were addicted. Amazingly, when we treated them with TCAP, what we saw was remarkable: while the control animals jumped about 75 times in 20 minutes, animals treated with TCAP jumped many fewer times. Those dosed with two doses of TCAP at 10 micrograms/kg (a VERY low drug dose of approximately 200 ng per injection) subcutaneously jumped only about 20 times in 20 minutes. There was a clear dose response which was quite reproducible, and importantly the more TCAP we administered, the greater its benefit on the stress response. This is important because many CNS-active drugs display a “inverted U-shaped” dose response: the response increases as the dose increases UP TO A POINT, then higher doses actually show reduced benefit. This is very challenging, since it makes it difficult to administer effective doses in the clinic. We were very pleased to see that TCAP does not have this drawback. Furthermore, we discovered that dosing with TCAP was effective when administered (1) before the protocol to get the mice addicted, (2) when administered after the addiction was established but before the Narcan was administered, or (3) after both the addiction was established and the withdrawal was initiated by the dosing with Narcan. This suggests that clinically TCAP could be used as (1) prophylaxis to protect against addiction, (2) as treatment to blunt the impact of withdrawal, and (3) as maintenance therapy to prevent recidivism, i.e. return to drug use when struggling with the stresses of daily life. .

One of the puzzles I have tried to figure out over the past two decades is why small molecule CRF antagonists, which were so efficacious in animal models of anxiety and depression turned out to be so disappointing in clinical trials. Based on experiments which we have conducted comparing TCAP to one of the best of the small molecule CRF antagonists (a Pfizer compound), I think we now understand why they failed and why we expect TCAP to succeed as a treatment for anxiety and depression. As background, the small molecule CRF antagonists act by blocking the binding of CRF to one of its receptors implicated in mediating the stress response and prevent activation of that receptor. TCAP also blocks the effects of CRF, for example as described above in the Erb cocaine addiction rodent model. However, it does NOT do so by preventing binding of CRF to its receptors or preventing the activation of those receptors. TCAP has its own receptors, which the Lovejoy lab has shown to be a type of G-Protein Coupled Receptor called Latrophillin, expressed in brain regions involved in emotion

and memory and learning. TCAP “cuts CRF off at the pass”, acting downstream of the CRF receptors and moderating the CRF-driven responses to stress. We decided to compare the Pfizer small molecule CRF receptor antagonist to TCAP in the Saelens test for effectiveness to block the stress response to opioid withdrawal. The CRF receptor antagonist could blunt the stereotypic jumping behavior indicating the level of the stress response to opioid withdrawal. However, at the maximally effective dose of 20 mg/kg subcutaneously administered (2000-fold higher than the maximally effective dose of TCAP), the mice still jumped about 50 times in 20 minutes (>2x the number of jumps in TCAP-treated mice). Furthermore, the effect of the CRF receptor antagonist was lost at doses both below AND ABOVE 20 mg/kg! These results showed us that TCAP is more potent, more efficacious, and better behaved pharmacologically than direct CRF receptor antagonism with a small molecule drug.

Andy Slee: The results in the Saelens test and the Erb experiments highlight the potential of TCAP in the setting of various forms of addiction. We believe that TCAP could help in a wide range of stress-augmented addictions, both chemical (like opioids, alcohol, nicotine) and behavioral (gambling, sex, eating, *etc*.)

Based on numerous other rodent experiments we and the Lovejoy lab have conducted, we are excited about TCAP for treating anxiety, depression, and Post-Traumatic Stress Disorder (PTSD). There are experimental designs that are known to elicit a stress response in mice or rats. If the rodent is placed in a tube that restrains it, the animal finds this stressful. This stress drives an increase in the CRF in the brain, which in turn triggers the release of the stress hormone cortisol from the adrenal glands, This rise in cortisol is measurable in both the blood and in saliva. When rodents are tube-restrained and then released, the stress influences not only their cortisol levels but also their behaviors. There is a test called the open field test which quantitates a rodent’s anxiety. The animal is placed into a box about the size of a bridge table, open on top and bounded on four sides by walls. Anxious animals will move around but hug the walls, while non-anxious animals will also explore the open spaces, traversing the central area of the open field. By monitoring their walking trajectory for both ambulatory response and speed of motion, one can measure both the level of anxiety and the degree of sedation. These measurements are all made by overhead video recordings, so no humans are present to interfere with the readouts. Benzodiazepines like Valium reduce

anxiety but also reduce locomotor activity, indicating that they are sedating at anxiolytic doses. When the tube-restrained rodents are tested in this open field model, they display anxious behavior, staying close to the walls and avoiding the exposed central areas of the field. However, we found that animals treated with TCAP showed non-anxious behavior in the open field test. Furthermore, much as we saw with the Saelens test, we could either treat with a single dose of TCAP before of after the tube restraint and either (1) prevent both the rise in cortisol and the anxious behavior or (2) treat the anxiety after the stress response had developed and the rise in cortisol had been triggered. After either TCAP dosing regimen, the animals were not anxious judged by their open field behavior. We established the dose response curves for this anxiolytic effect of TCAP in both tube-restrained mice and rats after subcutaneous administration of TCAP. We then were able to examine the effects of TCAP given either buccally or intranasally and compare the dose response curves to those obtained when we administered the TCAP subcutaneously. Significantly, TCAP reduces cortisol rise and blunts anxiety when delivered by any of these three routes: subcutaneously, buccally, or intranasally. The doses of TCAP required when administered intranasally are logs lower than the very low doses required subcutaneously to maximally reduce the biochemical and behavioral hallmarks of the stress response.

Andy Slee: Having been part of the intense efforts to discovery and develop small molecule CRF receptor antagonists while at DuPont Pharmaceuticals, it dawned on us that all the animal models where these compounds had displayed very promising pre-clinical pharmacology were models of acute stress. By contrast, the clinical settings in which stress plays a central role are much more often chronic settings. We had already determined that both TCAP and a CRF receptor antagonist worked in acutely stressed rodents and that TCAP was significantly more efficacious in this setting. We wondered about how they would fare in the setting of chronic stress. So we decided to create a model of chronic stress in rodents and test both TCAP and a small molecule CRF antagonist to see if their efficacy was still detectable. Interestingly, while tube restraint acutely stresses rodents, if you repeatedly tube restrain them, they habituate to the setting and no longer display the biochemical or behavioral signs of stress-induced anxiety. To achieve chronic stress, you need to keep subjecting the rodents to changing environmental perturbations, such as noise, tube restraint at unpredictable intervals,

and the like. When we tested TCAP in chronically stressed rodents, we were gratified to find that it retained its full efficacy. By contrast, a chronically dosed small molecule CRF receptor antagonist lost its efficacy in the setting of chronic stress. Now, we felt that we had developed significant insight into the clinical failures with the CRF receptor antagonists and significant confidence that TCAP in the form of PT-00114 will succeed in the clinical settings driven or exacerbated by chronic stress, including anxiety, depression, PTSD, and addictions. .

Andy Slee: In order to determine whether or not we should expect PT-00114 to have any safety or toxicity inssues, we have conducted extensive characterization of the compound in IND-enabling 28-day GLP toxicology studies in rats and non-human primates. PT-00114 has a very benign safety profile, even at doses logs above what we anticipate to be the clinically effective dose range. The findings in these toxicology studies were restricted to mild changes at the sites of injection, which we suspect are due to the formulation we had to use to administer the very high doses of PT-00114 tested.

To recap TCAP, PT-00114 has remarkable preclinical pharmacological effects in moderating responses to stress, very much better than CRF receptor antagonists. It is also remarkably safe and well tolerated. We are anxious (in the best sense of the word) to see what it does in the clinic. Fortunately, we won't have to wait too much longer to begin to find out!

Jenn Buell: The rest of the Protagenics team shares Andy's excitement as PT-00114 moves closer to human clinical trials. I think it is really worth noting that for many non-psychiatric diseases, animal models are poorly predictive of human clinical outcomes in subsequent drug trials. In contrast, for neuropsychiatric diseases, animal models have been found to be highly predictive. And that's likely why this robust preclinical data, some of which we have just shared with you has generated so much enthusiasm from our clinical collaborators: Drs. Maurizio Fava and Michael Murphy, who are designing and will be conducting our clinical trials with PT-00114.. I will tell you more about these two world-class experts and our planned clinical trials shortly.later. First, let me emphasize some of the key features that will enable us not only to get early signals of activity with PT-00114 but also allow us to validate the mechanism of

action that Garo, David and Andy have shared with you today. These key features will also allow us to get very early signals from correlative biomarkers for the disease modulating benefits of TCAP. Based on the very clever, rapid, and affordable trial design, we expect to obtain early clinical readouts in a number of different disease settings. So just to recap a couple of things: TCAP is an endogenous brain peptide evolved over many hundreds of millions of years to become a central governor of the responses to stress. PT-00114 is our synthetic version of TCAP, synthesized by solid-phase Merrifield chemistry. We have demonstrated that PT-00114 can be administered peripherally by subcutaneous, buccal, sublingual, or intranasal routes and successfully crosses the blood-brain barrier (BBB) to exert its effects on the brain and behavior. Difficulty crossing the BBB to gain access to the CNS has proved to be challenging for many other peptidyl agents which have struggled in development. With PT-00114, there's rapid onset of action after peripheral administration in animal anxiety and depression models, as Andy just shared with you. This rapid and sustained onset of efficacy in depression models is in sharp contrast to the slow onset of action (over weeks to months) observed for the various neurotransmitter reuptake inhibitors such as Prozac and Zoloft, currently used to treat depression.

Jenn Buell: PT-00114 is potent and its effects are long lasting: we can observe the benefits of a single dose of TCAP in animal models for three weeks or more. These lasting effects occur despite the fact that administered TCAP is rapidly cleared from animals: the half-life of administered TCAP in the plasma is 5 to 10 minutes after intravenous administration and 20 to 30 minutes after subcutaneous administration. As Andy indicated, it is extremely safe in animal toxicity testing, which reflects the fact that it is composed of naturally occurring amino acid residues which don’t produce unnatural metabolites, in contrast to many psychoactive small molecule drugs. We anticipate that this will allow us to avoid liver toxicities that often hamper development of compounds for chronic neuropsychiatric uses. So, it gives us a lot of development flexibility as well as dosing flexibility. We have no evidence of dependency development. The biologic features of this compound, though most critically, is that given that stress is a surrogate marker is elevated in diseases that we're pursuing, that biomarker, namely cortisol in this case will serve as a tool that will allow us to enrich for patients with elevated stress through the chronic elevation of this particular biomarker. And it also allows us to

evaluate the disease modifying behavior of the compound early in quickly and throughout the course of dosing. So, these features and the data that Andy generated really garnered the enthusiasm of world experts in the space. Dr. Maurizio Fava, who Garo introduced at the beginning of the call, is a chief of the division of Psychiatry at Mass General Hospital.

Jenn Buell: He's the head of the Clinical Trial Institute there. He's a trained endocrinologist, and when he saw the preclinical data here, he was so enthusiastic that he committed to personally develop the program. And what we're going to share with you today is a really elegant design that will allow us to get information about the efficacy of PT-00114 across a host of different disease indications very quickly. Now Maurizio's trial design for PT-00114 that I'm just about to highlight is effectively a multipronged strategy that allows us to evaluate sequential dose escalation of PT-00114 with cohort replication to evaluate dose and biologic response across a number of different diseases. So, we'll will be enrolling (1) patients with Major Depressive Disorder (MDD) who have had suboptimal responses to prior therapies or have poorly tolerated prior SSRIs, (2)patients with Generalized Anxiety Disorder (GAD) who have had suboptimal response or poor response prior therapies, (3) patients struggling with Addiction Withdrawal Disorder (AWD), and those with Post-Traumatic Stress Disorder (PTSD). Our pre-clinical data suggest that PT-00114 has the potential to help with each of these indications.. We'll be looking at a 28-t day exposure to PT-00114, and we'll be looking at approximately 45 subjects across these diseases of interest. The biomarker signatures in the study will be read out, and they will also serve as a selection criterion for enriching the patient population for those with chronic stress response.

Jenn Buell: We'll be looking at salivary cortisol both before and after dosing, as well as a number of psycho-physiological markers, wearable bracelet measurements of skin conductance, *etc.* This will give us quite a bit of depth of detail and early readouts of the mechanism of action of PT-00114. The design is outlined here: we will start with the healthy volunteer cohort, a handful of patients evaluating safety in healthy volunteers. We'll also be interrogating the biomarkers in the healthy volunteers as a baseline for the disease-specific cohorts. And then we'll start with what amounts to a "pick the winners" approach in which patients with MDD, GAD, AWR, and PTSD will be evaluated across a

range of escalating doses. And as we continue to advance across these cohorts, we'll be further enriching and building out the cohorts where we're seeing the most evident responses. Dr. Fava has pioneered this “pick the winners” strategy while leading the clinical trials of a large number of potential medicines, and it has proven to be highly effective in rapidly and accurately generating clinical efficacy signals. We look forward to the results that Maurizio and Mike will soon be generating with PT-00114 as it enters Phase 1 / 2 clinical trials early in 2022. With that, I'm going to turn it over to Dr. Bob Stein, Protagenic’s Chief Medical Officer.

Garo Armen: Bob is one of the world experts in medicine, clinical development and also neurological pathways, and he has been intimately involved from the beginning in the design of our pharmacology models and also, very importantly, the design of our clinical trials, along with Dr. Mauricio Fava. And he will be talking about the role that stress plays - a very important role indeed - in the manifestation of many of these diseases and how uniquely TCAP is positioned to address them with its remarkable mechanism, a mechanism more central to natural stress control than anything that has been tested previously. Bob, thank you.

Bob Stein: As Jenn and David Lovejoy and Andy Slee and Garo have indicated, we're incredibly excited about the potential utility of PT-00114, which is TCAP in its synthetic form, as an intervention in a broad range of psychological and even physiological disturbances. Just to remind everybody, this is a brain peptide that David Lovejoy discovered and characterized and showed that it has existed as a natural control mechanism to optimize the responses to environmental stressors for hundreds of millions of years across many species throughout the course of evolution. TCAP is produced naturally within each of our brains. As Andy outlined, when TCAP is synthesized and administered as a drug, it readily crosses the blood brain barrier, meaning you can deliver it in the periphery, and it'll get to the brain where it needs to do its work. There's a rapid onset of action, and Andy has done a good job of showing it. Within 30 minutes of the administration of PT-00114 to rodents, you see profound effects on behavior. Its effects are, interestingly, very long lasting, and even though the peptide itself is relatively rapidly cleared from the body, the pharmacologic actions are persistent and can be seen for three weeks or more after a single dose of PT-00114. It's

rapidly metabolized, and its actual physical half-life is measured on the order of minutes to under an hour. Yet it causes changes in the brain that are very stable, including some of the things that David Lovejoy and Andy have discovered. It increases glucose utilization. It also stimulates the outgrowth of what are called dendritic spines, which are attempts by nerve cells to find and connect to other nerve cells, which is extremely significant because one of the effects of stress is to cause the loss of synaptic connections over time, meaning nerves stop talking to each other and the isolated nerve cells can die off, leading to diminished mental capacity and eventually to dementia.

Bob Stein: These effects of TCAP to enhance the energy utilization of nerve cells and their connectivity have profound implications not only for things like anxiety and depression, but also possibly for neurodegenerative illnesses, like Parkinson's or Alzheimer's. It's been definitively shown that chronic stress can produce neurodegenerative changes in the brain. One of the very interesting things that has become progressively more apparent over the last 20 years is that stress contributes to a wide range of illnesses, and “stress” is not just a threatening environment. It's the brain's responses to that environment and how it influences internal processes. A stress signal increases the level of certain hormones in the brain, including Corticotropin Releasing Factor (CRF), also sometimes called Corticotropin Releasing Hormone (CRH), which was intensely studied target by drug companies about 15 years ago, and also something called Arginine Vasopressin (AVP). Stress-induced increases in CRF and / or AVP lead to increased anxiety, disrupted sleep, overall dysphoria or discomfort, and key CNS related symptoms. They also trigger the release of cortisol from the adrenal glands through an indirect mechanism involving the pituitary gland. What we've shown as a team is that TCAP administered as PT-00114 blunts or abrogates this stress response in a broad range of animal models of neuropsychiatric conditions. PT-00114 not only moderates the psychological responses to stress but also changes the physiologic responses.

Bob Stein: You can identify people who are chronically stressed because they have elevated levels of cortisol in the blood. And we're going to use that feature to identify which patients are most likely to respond to TCAP administered as PT-00114. Dr. Slee has done a very good job of showing that he can stress animals by certain mechanisms,

and they have a rise in their circulating cortisol in the blood. The rise in plasma cortisol is a highly useful biomarker of the stress response. We have shown that plasma cortisol can be lowered by the pre-administration or coadministration of PT-00114 ( ). Earlier, Dr. Slee made an important point. Over 15 years ago, small molecule CRF antagonists were very much touted as a potential treatment for anxiety and treatment-resistant depression. A number of small molecule CRF Receptor antagonists were taken into the clinic by companies such as Pfizer, Glaxo SmithKline, Bristol Myers Squibb, and Neurocrine, and although they had impressive animal pharmacology preclinically, they didn't really do what they had been hoped to do in the clinic. And we think we understand quite a bit about why that happened. What we have discovered preclinically is that small molecule CRF antagonists work in acute stress, but they lose their efficacy in settings of chronic stress. Furthermore, CRF antagonists don't work as well as PT-00114 even in acute stress. And the CRF antagonists have what Andy Slee referred to as a "U-shaped dose response": as you administer increasing doses of these compounds, their beneficial effect increases up to a point, and then when you continue to increase the dose, the benefit decreases, rather than showing a plateau at the maximal benefit. It is really sort of an inverted U. With a drug like a small molecule CRF antagonist with a "U-shaped" dose response, if you're not careful, you don't end up with the right dose because as you go up on the dose, you go from more activity to less activity. PT-00114 (synthesized TCAP) has none of these limitations. And the other important thing about PT-00114 is that in sharp contrast to and unlike benzodiazepines like Valium and Xanax, PT-00114 is *not* sedating. PT-00114 does not "dull out" brain function and yet it reduces anxiety and improves mood. Animals treated with TCAP move around normally, and if they aren't stressed, they don't really show much of a response. Interestingly, some strains of mice are genetically more anxious and difficult to handle than others. When TCAP is administered to these anxious strains, they become calmer but remain fully active. By contrast, benzodiazepines administered to these anxious strains can reduce their anxious behaviors, but also make the animals distinctly sedated and generally less responsive to their surroundings.

Bob Stein: TCAP as a brain peptide hormone has evolved as an endogenous is a governor to prevent stress response overshoot, so it prevents animals and people from becoming "deer in the headlights", but – importantly - TCAP doesn't blunt their

appropriate response to increasingly threatening environmental stimuli. We are confident that we are going to be able to identify the right patients in whom to assess the activity of PT-00114 (administered TCAP) by the inclusion of patients with elevated plasma cortisol. We think we can look at blunting the elevation of cortisol by PT-00114 as a biomarker in conjunction with the very excellent tools that Dr. Fava has developed and validated for assessing anxiety, depression, addiction, and PTSD. And we also believe that there is the potential for this intervention to block many of the peripherally undesirable effects of elevated cortisol, which can cause derangements of metabolism, such as insulin resistance. Cortisol-induced insulin resistance can cause obesity and difficulty in losing weight. And we also believe it could have an impact in settings where stress leads to neurodegeneration. So we're very enthusiastic about PT-00114 and its mechanism of action. We're looking forward very much to the results of the clinical trials, which will be starting soon, and it really could represent a tremendous new approach to treating a broad range of diseases. We have an initial cluster of neuropsychiatric diseases that will be trying to treat with administered TCAP in this very exciting basket trial design that Dr. Fava has pioneered. There are many future opportunities beyond that.

Garo Armen: Thank you very much, Bob. Thank you all for listening, and we're ready for any questions you may have. The first question is from Tim. Go ahead, Tim.

Tim McInerney: Garo, good morning, how are you?

Garo Armen: Very fine.

Tim McInerney: Long time. Hope all as well. The question I have is could you address the CMC issues and where you stand with the IND and maybe a timeline going forward? Are you on track with that? When can we expect data readouts and some meaningful milestones going forward? Thank you.

Garo Armen: Sure. So what Tim is referring to is the fact that we had filed an IND for PT-00114 and the FDA commented that they wanted more characterization of how we had formulated PT-00114. This resulted in the need to refile the PT-00114 IND. Tim's

question is “Are we on track with that refiling based on a formulation that the FDA requested from us?” And the answer is yes, we're very much on track. We expect the refiling to occur certainly by the end of the year, possibly before that and the clinical trials to commence in the first quarter of 2022. And so other than that, we expect no real major changes in our projections and forecasts. Dr. Beull, would you like to add any comments today?

Jen Buell: Thank you, Garo. You covered it: We are on track; we don't expect any changes to those timelines.

Garo Armen: Thank you very much.

Tim McInerney : Thank you very much. Good luck going forward.

Garo Armen: Thank you.

Garo Armen: Other questions. We have Kumar Raja. Go ahead, Kumar.

Kumar Raja: Hi, thanks for taking my questions. Maybe update on the intranasal formulation. What's going on there and how soon could we see the intranasal formulation and at what stage do you plan to shift to the intranasal formulation?

Garo Armen: Ok, so I had just answered the question about the FDA's request of a subcutaneous injectable formulation which was on target to submit with our refiling of the IND certainly by the end of this year. With the IND we had submitted, we had planned to provide each trial site with lyophilized PT-00114 to be suspended by the hospital pharmacies in the vehicle for subcutaneous injection. The FDA expressed concern about potential variations between different centers in reconstituting the PT-00114 and requested that we provide the hospitals with ready-to- inject products. This was the reason for the about three-month delay in the process. And that's now the subject of the IND resubmission, which is very much on track. Kumar, does that answer your question?

Kumar: Yes, it does, thank you.

Garo Armen: Thank you.

Andy Slee: Anyone else who has a question, you can indicate that by either raising your hand or sending a message in the chat box?

Garo Armen: If there are no more questions, let me make a few concluding remarks and after those remarks if there are still any outstanding questions, we're happy to take them. To reflect on what prompted the discovery of TCAP, as Dr. Lovejoy articulated, it was the interest in exploring What is stress? How is it induced? How does it affect more downstream conditions, including fight or flight reactions? And living in stressful environments and developing effective responses to stressful stimuli has been a challenge for animals beginning early in the course of our evolution. Over the past decades, working with Dr.Lovejoy and his team we have very methodically explored and demonstrated that stress responses are moderated by endogenous TCAP and that when synthesized. TCAP can be administered to successfully eliminate the harmful effects of stress without abrogating the useful aspects of the stress response. The central role that stress plays in a broad range of both psychological and physiological ailments has been the subject of growing interest as we find ourselves in an increasingly stressful world. We have very clearly demonstrated TCAP's ability to blunt the harmful effects of stress and demonstrated its potential to benefit patients suffering from MDD, GAD, AWR, and PTSD, amongst other stress-induced illnesses. It is not unreasonable to believe that PT-00114 may also provide benefit in other diseases to which stress contributes, such as metabolic disease, neurodegeneration, and immunosuppression, which can contribute to susceptibility to infection and malignancy. We are very excited about TCAP and about PT-00114 progressing into the clinic in the near future. We have teamed up with the world's best players, including Dr. Fava, who was attracted by TCAP’s remarkable pharmacological activities, to optimize our chances to demonstrate the utilities are PT-00114 quickly and effectively in the relevant clinical settings. So, thank you very much for your time. Thank you, our panelists, for participating, and we will update you with some frequency to make sure that anything that is reportable comes to your attention. Thank you again.