Hello and a very warm welcome to everyone joining us online and in the studio for this Science/AAAS webinar. My name is Sean Sanders and I’m the editor for custom publishing at Science. The webinar today will explore how translational neurobiology research is being conducted in the Intramural Research Program of the NIH in a broad variety of disorders including depression, age-related macular degeneration, and Gaucher disease. Our panelists all conduct translational research across the full bench-to-bedside continuum, with the ultimate goal of developing novel paradigms for the treatment of a range of diseases and improving quality of life for patients. Today, they will share their experiences and how they have applied their basic research in a clinical setting.

It gives me great pleasure to introduce these three exceptional scientists joining me today all from the Intramural Research Program here at the Bethesda, Maryland campus of the NIH. They are Dr. Carlos Zarate from the National Institute of Mental Health, Dr. Ellen Sidransky from the National Human Genome Research Institute, and Dr. Anand Swaroop from the National Eye Institute. Many thanks to you all for being with us today.

Dr. Carlos Zarate: Thank you, Sean.

Sean Sanders: Before we get started, I have some important information for our audience. Please note that you can adjust the size or hide any of the windows in your viewing console. The widgets at the bottom of the console control what you see. Click on these to see the speaker bios, additional information about the NIH Intramural Research Program, or to download a PDF of the slides.

Each of our speakers will give a short presentation about their work. After which we will have a Q&A session during which our panel will address questions submitted previously via email or from our live
studio audience. We unfortunately will not be accepting questions from our online viewers today.

You can also log in to your Facebook, Twitter, or LinkedIn accounts during the webinar to post updates or send tweets about the event, just click the relevant widgets at the bottom of the screen. For tweets, you can add the hash tag, #sciencewebinar.

Finally, thank you to the NIH Intramural Research Program for sponsoring today's webinar.

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Now, I’d like to introduce our first speaker, Dr. Carlos Zarate who is chief of the Experimental Therapeutics & Pathophysiology Branch and of the Section on Neurobiology and Treatment of Mood and Anxiety Disorders at the National Institute of Mental Health. His research focuses on the pathophysiology and development of novel therapeutics for treatment-resistant mood disorders as well as the study of biomarkers and neural correlates for treatment response. Welcome, Dr. Zarate.

Dr. Carlos Zarate:

Thank you, Sean, for introducing me and giving me the opportunity to present our work in today’s webinar. So the topic today will be depression specifically focusing on two major points: one, how we go about developing treatments that work in hours instead of six to eight weeks, which conventional antidepressants take. The second is what are those cellular, molecular, and neural correlates of this rapid antidepressant effects in the hope of developing better treatments that work similarly within a very short period of time.

Slide 4

But first let’s talk about the impact of depression. Depression is a major mental disorder that is associated with significant impairment in ability to function. Patients experience guilt; anhedonia; lack of pleasure, drive, motivation; and have significant impairment in their ability to work, to function and carry out their normal duties. This goes on for weeks if not longer for years at a time. It is one of the leading causes of disability worldwide, much more disability than major medical disease such as cardiovascular or cerebrovascular disease. It’s estimated that approximately 10% of the American population suffers from depression and there is an increased rate of death even after controlling for risk factors such as suicide and smoking.
It’s estimated that not only is there significant morbidity associated with depression but significant mortality. There are over 30,000 suicides per year in the United States alone and individuals who experience depression and also have had severe medical illnesses such as cancer describe the angst and the suffering that go with depression much worse than when they experienced the cancer.

Now we have over 20 to 30 different antidepressants and it’s fair to say that they do benefit many individuals, but for those with more moderate to severe depression, recent studies have found that patients do not benefit as much as previously believed to. It’s estimated that if one takes a course of an antidepressant first line treatment, we’ll refer to as citalopram for example, only 1/3 of individuals achieve remission within 10-14 weeks. Remission means absence of depressive symptoms, only a few depressive symptoms. It often takes two antidepressant drugs or six months for half of the people to have a significant improvement in antidepressant treatment. So in my view that’s unacceptable. We’ve got to do much better. Other areas of medicine can intervene very rapidly within a matter of hours and so we should also try to do the same in major mental disorders particularly in depression.

Now one of the limitations has to do with that most of our treatments are monoaminergic based, have been developed based on serotonin and norepinephrine. On the left side of this panel, we see what are the number of mechanistically distinct drug targets in the 1950s. In yellow and green, we see depression and schizophrenia targets, respectively, and after nearly five decades, we remain about the same number of mechanistically distinct drug targets. Whereas in other areas such as cardiovascular disease, there has been a significant increase in the number of mechanistically distinct drug targets, which has resulted in a decrease in mortality from major cardiovascular diseases. That has to do with novel treatments but also perhaps exercise and preventive measures whereas we have not developed a new group of medications. That’s not because the industry has not tried or government or academics. They have tried. It’s just that the etiology of all major mental disorders is not really that clear.

Towards the right are drugs that we use for bipolar disorder and in essence, we have not developed a single agent for bipolar disorder based on an understanding of the molecular underpinnings of the
Most of the drugs have been repurposed from epilepsy, anticonvulsants, or from schizophrenia, the antipsychotic drugs.

Slide 6

Now one of the limitations of our current medications is the lag of onset of antidepressant effects and it’s highlighted in this figure. Towards the bottom, we can see the natural course of the illness without receiving treatment, particularly in the beginning of the illness it’s about 6 to 12 months. But as we give an antidepressant in the middle yellow line on the bottom of the graph, we see we shift the time curve a little bit sooner in terms of improvement where one achieves remission within 10 to 14 weeks, only 1/3. That’s what I already mentioned.

The goal with our next generation of treatments would be in the beginning of a major depressive episode when symptoms begin to start specifically those with a high risk of relapse, we would intervene with a next-generation antidepressant that produces a response in hours.

If we’re able to do that, one can see the top line of the figure, yellow is euthymia, stabilized mood, blue refers to major depressive episodes, and each one has a toll or produces disruption on personal, family, occupational role and also there’s a considerable risk for suicide. But if we intervene with a treatment that works rapidly, we will decrease the length and severity of the depressive episodes and we’ll decrease the impact on one’s personal/social life and increase yellow, the euthymic periods where they stay well.

Slide 7

Now another area where we can explore besides the monoaminergic based system is the glutamate complex system. Glutamate is an excitatory amino acid, abundant in mammalian CNS and we could see it’s a rich area to pursue targets and already we and others have pursued targets on regulating glutamate. It’s believed that in mood disorders, major depression, there is a disruption of the glutamate-glutamine cycling pathway and we may be able to regulate this with a host of compounds.

There is indirect evidence that glutamate is involved in depression. We see regional reductions in brain volume such as prefrontal cortex, hippocampus that translates into reductions of spine densities in the pyramidal neurons that are involved in glutamate function.
This figure in the top left illustrates a summary of the presumed mechanism of current treatments. We see that serotonin, norepinephrine, when I give an antidepressant serotonin reuptake inhibitor, you can change intra-synaptic levels of serotonin within minutes yet it takes weeks if not longer for antidepressant effects to take effect. Why is that? Well there have to be these intracellular signaling cascade changes, changes in gene expression and what perhaps ultimately matters is changes in a protein of brain called BDNF (brain-derived neurotrophic factor), which is involved in synaptic plasticity.

Most of our antidepressants do increase that BDNF but it takes a long time to do so. Whereas with treatments that act more directly closer to BDNF either at two targets, a mammalian target of rapamycin, mTOR, or eukaryotic elongation factor 2, are more proximal to what is believed to be involved in the BDNF release, would result in a more rapid antidepressant effect.

Now in order to do that, one can target the ionotropic postsynaptic glutamate receptor NMDA particularly at the synapse. One drug that does that is ketamine, which is a dissociative anesthetic. It’s derived from PCP and it’s referred to as a noncompetitive NMDA antagonist.

Now when ketamine binds to the NMDA receptor particularly at the PCP site, while individuals receive ketamine, they experience psychomimetic or dissociate side effects. They’re temporarily disconnected from their body senses. There’s a long history of safety with this agent. It’s used in emergency rooms for diagnostic treatment procedures and it’s also used as an anesthetic.

So we and others came up with the hypothesis that if you target NMDA receptor directly with an NMDA antagonist, would you bring about a rapid antidepressant effect and the answer is yes.

Towards the left, we see a depression scale. Down means greater improvement. Towards the bottom left, we see the time course in minutes. So within two hours, we see rapid antidepressant effects. Towards the right are the percentage of individuals having a 50% improvement, what we refer to as response. We can see in the right graph the bars for current antidepressants. If one gets an antidepressant, it takes about 60%-65% response rate in six to eight
weeks. We see comparable response rates to ketamine within six hours to one day. So we see rapid, robust, and relatively sustained antidepressant effects with a single infusion of ketamine.

Slide 10

This is to highlight, towards the left, there are now several trials but we also found the same in bipolar depression. There’s rapid onset antidepressant effect within hours lasting most of the week with one infusion and towards the bottom right, lower right, we see not only are there rapid antidepressant effects but we see rapid anti-suicidal effects within one hour. This could have major impact on public health because we could produce rapid antidepressant effects and rapid anti-suicide effects within a very short period of time.

Slide 11

Now this figure or this cartoon illustrates that ketamine not only blocks NMDA receptor, but it enhances glutamate release. We could see that it’s really, since NMDA receptors are blocked, that there’s enhanced throughput through AMPA receptors, which ultimately is involved in BDNF, brain neurotrophic factor, production.

Towards the bottom right on work by Yale, Ron Duman suggests that mTOR, mammalian target of rapamycin, is involved in the rapid antidepressant effects of ketamine, one can see within one hour, very rapidly within a few hours behavioral effects in animals, increased synaptic activity and within 24 hours increased spine density. There is synaptogenesis within 24 hours.

Slide 12

So there are a number of compounds that are now in development because ketamine produces these dissociate side effects. Some are looking at other targets within the NMDA receptor complex I mentioned.

Slide 13

One compound that we tested here at NIH is AZD6765. It’s a low-affinity NMDA-trapping blocker. We see towards the left ketamine that has high-trapping blockade believed to be involved in the psychomimetic dissociate side effects and towards the right, we see this compound that’s associated. So in theory one would predict that there are lower dissociative side effects.

Slide 14
So in this proof of concept with one single intravenous infusion in people with treatment resistant depression, we see onset within hours, an antidepressant effect as illustrated by the decrease in the symptoms, depressive symptoms.

**Slide 15**

More importantly, we didn’t find any evidence of dissociative or psychomimetic effects. So this proof of concept study suggests that it’s possible to develop rapid antidepressant effects without these dissociative side effects.

The second point in mentioned in the beginning was we want to understand drugs or treatments that are radically different than existing treatments. Ketamine as I mentioned is one tool and scopolamine is another agent, which is a muscarinic antagonist produces response within a couple of days. So with a biological systems levels approach using a multitude of technologies to do very deep biophenotyping anywhere from the genes on the left all the way to rapid reversal of complex behavioral phenotypes such as depression, we can look for intermediate phenotypes.

**Slide 16**

This is a summary of some of the work that’s taking place.

[0:15:04]

At the genetic level, we know that BDNF a SNP within the – if one is largely a Met carrier towards the right we see that you have a lower degree of response to ketamine than if you have ValVal BDNF.

**Slide 17**

This is to show that one of the limitations of psychiatry or studying brain is we don’t have a window into the brain and so these are tasks, noninvasives such as top left, where you can see fearful faces that activates anterior cingulate cortex and it’s a measure of plasticity. The more activity you have, the better the response to ketamine.

Here we can see when individuals exposed to fearful tasks there is a greater chance of responding to ketamine and we can see pretreatment Rostral ACC predicts antidepressant effects to ketamine. On the right is a cognitive task. We see the reciprocal patterns with some examples of using measures to be able to measure response.
Yet another is cortical excitability. We can stimulate fingers with a pneumatic device, applies pressure. One can see in the bottom right that the sensory cortex is activated. When one gives ketamine we can see to the top right, which is the power spectra and in the middle is gamma, which gamma rhythms are important to connect in different brain regions precisely at the same time.

Towards the left, we can baseline, in yellow nonresponders, in green responders. The greater the gamma activity to the simple sensory task, we can predict response to ketamine and encircled is the difference.

So in the last slide, I’d like to give you a summary of the work that’s been taking place. On the right, we see we can produce very consistently, reliably a reverse of a complex behavioral phenotype within a couple of hours much radically different than existing treatments. Towards the left, we see some very preliminary evidence that genes might be involved in the response. In the middle at the cellular level, increased spine density appears to be important for response to ketamine that’s evidence at the cellular level. Towards the right of that on a circuit level, because we believe that mood disorders are disorders of circuits and synapses. So we hope that by filling in the gaps of a systems level biologic approach, we may be able to come up with a better understanding of treatments that are radically different than existing treatments. Still gaps remain but this is very promising work.

To conclude, I think using ketamine, scopolamine as tools as a new paradigm of research to develop across a systems biological level. Evidence of these effective treatments understand the cellular, molecular and neural correlates that impart this dramatic response very rapidly in anti-suicide effects and hopefully with that understanding be able to personalize treatment for our patients and to come up with a better understanding of our signatures that are involved in this rapid onset of antidepressant effects. Thank you.
pediatrician and clinical geneticist in the Medical Genetics Branch of the National Human Genome Research Institute.

Slide 24

Her work covers clinical and basic research aspects of Gaucher disease and Parkinson disease as well as studies of genotype/phenotype correlation and genetic modifiers, clinical insights from mouse models, and the development of new treatment strategies for the lysosomal storage disorders. She also focuses on understanding the complexity encountered in simple Mendelian disorders, the association between Gaucher disease and parkinsonism, and the development of small molecule chaperones as a therapy for Gaucher disease and related disorders. Welcome, Dr. Sidransky.

Dr. Ellen Sidransky: Thank you very much, Sean. It’s my pleasure to be here to tell you about our work that’s being conducted here at NIH. I’ve chosen to focus on our projects on Gaucher disease and parkinsonism, which you’ll see is an evolving story.

Slide 25

What I hope to show you today is how studies of rare recessive disorders can provide a window into more complex disorders. Where in our case by focusing a detailed examination of a single gene disorder, Gaucher disease, we've come up with insights that are applicable first to monogenic disorders but ultimately might help unravel complex disorders like Parkinson disease.

Slide 26

So just to introduce, the two disorders that I’m focusing on are really quite different. Gaucher disease is a rare recessive single gene disorder. It’s the deficiency of an enzyme leading to the accumulation of lipid. There’s variable age of onset, multi-organ involvement, and symptoms include enlarged livers and spleens, low platelet and blood counts, and at times blood and brain involvement.

[0:20:20]

In contrast, Parkinson disease is a common disorder affecting 1.5% of the population over age 65 and it’s a complex multigene disorder with a late onset. It results from a loss of dopaminergic neurons in the brain and we see the accumulation of aggregates of proteins including one that you’ll hear about, α-synuclein, within bodies inside the brain that are known as Lewy bodies. The symptoms of parkinsonism and Parkinson disease include bradykinesia, which is slow movements, rigidity, tremor, and sometimes dementia. It’s a
disorder that primarily affects the substantia nigra and brainstem regions.

Slide 27

So how are these two disorders associated? I’m going to show you that by using an integrated translational approach with pathologic studies, clinical studies, genetic studies, imaging, cell biology, etc., we’re beginning to gain some insight into this.

Slide 28

So to begin with Gaucher disease, it’s the inherited deficiency of the enzyme glucocerebrosidase, which cleaves the glucose moiety off of lipid glucocerebroside. It’s the most common lysosomal storage disorder and the most common inherited disorder among Ashkenazi Jews. It’s a disorder primarily of the reticulo-endothelial system where lysosomes within macrophages become engorged with the stored lipid giving rise to what you see on the left, the characteristic appearing Gaucher cell. On the right, you’re looking at an electron micrograph of a Gaucher cell and the distorted organelle that you see there is actually a lysosome, which is engorged with this tubular storage material.

Slide 29

There’s vast clinical heterogeneity encountered in this single gene disorder. It’s classically divided into three types, type 1 being non-neurologic, type 2 being acute neuronopathic, and type 3 being chronic neuronopathic. But having studied patients with this disorder for more than two decades now, I really come to see it much more as a spectrum ranging from asymptomatic octogenarians to fetuses that succumb in utero with wide range of associated manifestations. One of the groups that we started to appreciate was patients that developed parkinsonian manifestations.

Slide 30

So this association between what I’ll call GBA or the gene for glucocerebrosidase and parkinsonism was a story that really began here at the NIH clinical center with the observation of actually one particular patient who we were seeing for Gaucher disease who had pretty progressive parkinsonism. We then noted that these two phenotypes were encountered sometimes in other rare patients and then we also started to appreciate that Parkinson disease was seen in relatives of our patients with Gaucher disease more often than we might expect. Then we and other groups around the world started to appreciate that there was an increased incidence of GBA mutations
in patients with Parkinson disease and with associated Lewy body disorders. However, actually many of these initial studies were greeted with skepticism because of limitations of power and controls and also because large genome-wide association studies had not identified this gene.

**Slide 31**

But these associations persisted and now glucocerebrosidase is considered the most common genetic risk factor for Parkinson disease. In fact, if you just look in the last decade doing PubMed scans, the number of papers and studies on this gene related to Parkinson disease is growing exponentially. Though I do want to emphasize that the vast majority of the patients that we see with Gaucher disease and the majority of Gaucher carriers, people with GBA mutations, never develop Parkinson disease. So it’s a risk factor but not a predictive gene.

**Slide 32**

Well one of the reasons why this began to become more accepted was several years ago we spearheaded a multicenter study of glucocerebrosidase mutations in large groups of patients with Parkinson disease. We collected genotypes from 16 centers spanning four continents and ultimately had over 5000 genotypes from patients with Parkinson disease and about the same number from controls.

[0:25:00]

The bottom line was we determined that subjects with Parkinson disease are over five times more likely to have a mutation in glucocerebrosidase giving an odds ratio of over 5.4. We also noted that patients with Parkinson disease that carried mutations tended to have a little bit earlier Parkinson onset, about four or five years, and we had the impression that there were more cognitive deficits.

**Slide 33**

Just recently, we’ve actually gone back and done a very similar analysis with 11 different centers participating where we looked for the frequency of glucocerebrosidase mutations in patients with an associated disorder, dementia with Lewy bodies. Here there’s a much more rapid progression of cognitive impairment and it’s a rarer disorder so in this series we collected about 700 cases. Actually, the odds ratio was greater than eight suggesting that mutations in this gene play an even larger role in the dementia with Lewy bodies.

**Slide 34**
At the clinical center, we’ve been following these patients for about a decade now and our studies focus both on clinical features and PET imaging. We collaborate with Karen Berman’s group in the National Institute of Mental Health. The goals of the study are to look at fluorodopa uptake and to evaluate PET as a surrogate marker in subjects that have glucocerebrosidase mutations and to see if we can find the earlier signs of Parkinson disease in this at-risk cohort.

So in our studies, we recruit patient that have both Gaucher disease and Parkinson disease. We’re also looking at Gaucher patients and Gaucher carriers who have a positive family history of parkinsonism. Patients come to the NIH and undergo fairly routine physical, neurologic, and neurocognitive evaluation each time. We do olfactory testing and screens for nonmotor symptoms of parkinsonism to see if we can find some signs of early involvement. The imaging studies include MRI, fluorodopa PET studies for dopa metabolism. We do radioactive water studies to evaluate cerebral blood flow and we’re evaluating transcranial sonography.

We just recently published the results in our first 40 patients that we’ve studied and basically, we found that patients with Parkinsonism that also had Gaucher disease had fluorodopa uptake that was very similar to patients that just had sporadic Parkinson disease. Where we did see differences were in the cerebral blood flow studies where we see some changes that are more characteristic of disorders with cognitive impairment and this study is ongoing.

Slide 35

So how can mutations in a metabolic enzyme lead to Parkinson disease? Well the verdict is not out but there are certain hypotheses to consider. One is that we know that the formation of insoluble α-synuclein aggregates contribute to the neuronal cell death that occurs in Parkinsonism. So the gain of function hypothesis is that having this mutant enzyme around could somehow lead to an increase in aggregate formation as you see on the right or it could lead to organelle dysfunction particularly the lysosome leading to decreased aggregate clearance both cases contributing to this aggregates that contribute to neuronal cell death.

Slide 36

But another hypothesis would be that this is a loss of function. That having the mutant glucocerebrosidase around leads to unstable or deficient protein that’s degraded and then you don’t have enough
enzyme so the lipid accumulates and then accumulation of this lipid could lead to neuronal cell death.

Then another theory that was recently published by our group in collaboration with the group at Mass General is that it could even be more involved. There could be something like what we call the bidirectional feedback loop where there’s indications that having increases in this lipid level glucosylceramide lead to increase in soluble α-synuclein oligomers and fibrils and having these around would contribute to α-synuclein aggregates and neuronal cell death. The same time having these insoluble aggregates around seems to block the ER Golgi trafficking of the enzyme, which again would lead to increased lipid accumulation and compounding the problem in a vicious cycle.

**Slide 37**

In collaboration with Jennifer Lee’s group at NHLBI, we’ve also done some biophysics studies and we feel that there is likely a molecular link between alpha-synuclein and our enzyme glucocerebrosidase. This was shown by several different techniques including fluorescence spectroscopy, NMR, and co-immunoprecipitation studies. Though the association only appears to occur at pH 5.5 and not pH 7, the interaction between the two proteins appears to occur at the C-terminus of α-synuclein. So this binding at lysosomal pH could facilitate α-synuclein degradation or prevent aggregation. Also, this GBA story implicates the lysosome in PD pathogenesis.

**[0:30:33] Slide 38**

Now I’m going to move on a little bit towards some of the work that we’ve been doing in therapeutics and one approach that we’ve been looking at as a therapy for Gaucher disease is chemical chaperone therapy. The protein glucocerebrosidase is synthesized in the ER and it’s glycosylated and folded but it doesn’t reach its tertiary functional structure until it’s actually in the lysosome as you see the top panel on the right. If you have a mutation in the enzyme, it’s likely that it will not fold correctly and it will be degraded and none of it will get to the lysosome. So our strategy is to come up with small chemicals that are known as chemical chaperones that can bind to the mutant protein, stabilize it, so that it’s at least partially corrected and it can get to the lysosome where it can still function.

**Slide 39**
So in collaboration with the NCGC, the National Chemical Genomic Screening Center, we’ve been conducting high throughput screening of large libraries of small molecules to see what might impact the enzyme activity in glucocerebrosidase. In fact, recently we took a new approach and we actually used a patient’s spleen sample as our source of mutant enzyme. In this high throughput screen, we evaluated 250,000 compounds at seven different concentrations, fortunately, there’s robots to do this work, and identified 30 new noninhibitor chaperones that we’re very excited about. Our lead chaperones looked like they can improve the translocation of the enzyme to the lysosome in patient fibroblasts and in macrophages I’m going to show you the compound seems to reverse storage. So the small molecule therapies like this may stabilize mutant glucocerebrosidase and also be used to treat Gaucher disease as well as possibly Parkinson disease.

Slide 40

Well one problem that we had with this drug development is that we didn’t have a really good model for showing reversal of storage, which is what you’d target it in Gaucher disease. So in the last few years, we’ve been working to develop induced pluripotent stem cells as a model for Gaucher disease beginning with patient fibroblasts. We generated the appropriate embryoid bodies and then showed that our cells make the appropriate markers, have the right karyotype, it can go on to form teratomas.

We differentiated them first into monocytes and then macrophages and to our excitement, we were able to determine that Gaucher macrophages can show the storage, which we were never able to demonstrate before. We think this model will be very useful for drug development and for understanding pathophysiology.

To demonstrate this, so what we do if you see the two fluorescent images below, one is control macrophages generated from induced pluripotent stem cells and on the right is Gaucher. When you feed these cells with labeled erythrocyte ghosts, you can appreciate that only the fluorescent storage is much, much greater in the Gaucher macrophages.

Slide 41

Then we take these macrophages and we treat them with our best chaperones. So the top panel is the control and the two lower panels are macrophages from patients. In the very last column to the right, we’ve added our lead chaperone and you can see that we’re seeing a
reversal of the storage indicating that this does seem to have promise.

Slide 42

So I hope that I’ve shown you that understanding the links between these two disorders can prove to be quite fruitful teaching us about the pathogenesis of both disorders, providing some clues into the role of lysosomes and the development of Parkinsonism, and then ultimately it may yield improved genetic counseling and new therapeutic strategies.

Slide 43

I just want to briefly acknowledge all the people in my group who have done this work, my close collaborators here at NIH and around the world and of course to give special thanks to patients, family members, and the referring physicians who have contributed to these studies. Thank you.

[0:35:13]

Slide 44

Sean Sanders: Wonderful. Thank you so much, Dr. Sidransky. We’re going to move on to our final speaker for this webinar and that is Dr. Anand Swaroop. He is chief of the Neurobiology and Neurodegeneration and Repair Laboratory the National Eye Institute. His laboratory primarily focuses on photoreceptor development and retinal/macular degenerative diseases, including elucidation of transcriptional regulatory pathways involved in cell fate and homeostasis, the genetic basis of retinal defects, and the development of treatments using cell-, gene-, or small molecule-based approaches. Welcome, Dr. Swaroop.

Slide 45

Dr. Anand Swaroop: Thank you, Sean, and I’m delighted to be here. Blindness generally ranks second or third among all the feared or scary diseases in this world. In many, many surveys after cancer or cardiovascular diseases, people are very scared of going blind. What I’m going to tell you today is some of the research that we are doing on transcription regulatory networks to produce a photoreceptor cell that captures light and I’m also going to tell how some of this research is leading to new paradigms for finding treatment for retinal and macular degeneration.
The retina in fact is our window to this visual world and also to brain. Dr. Zarate earlier said we have no window to the brain, actually retina is. It’s the most approachable part of the central nervous system. At any point in time and space, you can look at thousands, in fact over a hundred thousand individuals of different size, shapes, color. You can look at the location in the visual field, you can see them moving around and despite all these objects, you’re able to focus on a single individual if you chose to. All of this visual information is processed through cells in the back of our eye called retina. In fact, light is focused through various optical elements called cornea and lens that many of are aware of and that focused light goes to retina, which is relatively simple but architecturally a beautiful stratified part of the central nervous system.

There are six major types of neurons as shown on the right side of this slide. These neurons are organized in three layers of cells. The layer, which is at the bottom actually is an epithelial layer called retinal pigment epithelium or RPE, which is extremely important for supporting the cells next to that and they are photoreceptors. I’m going to talk to you more about the photoreceptors a little later. The information that is captured by photoreceptors goes through a bunch of different kinds of neurons, different types of interneurons, and then these neurons convey information to ganglion cells and axons of these ganglion cells form optic nerve and that takes information to different parts of the brain. All of this information actually is captured, integrated, processed to a certain extent at least in the retina. In fact, 30% of our brain is devoted to processing of visual information.

As one can imagine, degeneration of these cells, which are post mitotic, will lead to blindness. Even though we have treatment for certain kind of blinding disorders like cataract and to a certain extent glaucoma, retinal and macular degeneration are still a major cause of untreatable blindness. They’re highly heterogeneous both clinically and genetically.

If you look at the left, rather right, the top part shows you the picture of a fundus. If you look in the eye of an individual, this is what you will see, a beautifully, nicely colored, uniformly colored part. You can see optic disk, which is where optic nerve goes through and then optic vessels come in the retina. The center of this retina is fovea. That’s where highest visual acuity is and that’s where the light actually gets focused. The area around fovea is called macula and
degeneration of photoreceptors and underlying pigment epithelium cells will lead to macular degeneration and as you can see in the picture below, degeneration of photoreceptors in the macular region will lead to loss of central vision and you will not be able to see or drive or watch TV. Whereas on the right side of that you have another picture of the fundus where there is degeneration of photoreceptors in the peripheral retina and that leads to loss of peripheral vision. Even though your central vision is okay, you will not be able to see on the periphery.

[0:40:22]

There are many, many different genes that can lead to retinal and macular diseases. As written here, over 200 genes have been mapped and more than 150 have already been identified. Many genes can lead to same phenotype and sometimes the same gene and even the same mutation in the family can lead to distinct phenotypes for a variety of reasons. Retinal degeneration is also observed as part of numerous syndromic diseases and in fact there are many diseases like nephronophthisis and others where you have kidney disorders or other neurological disorders along with retinal degeneration and then you have multifactorial diseases like age-related macular degeneration.

Slide 48

So in majority of these diseases, dysfunction or death of photoreceptors leads to loss of vision. There are two kinds of photoreceptors; rods, which allow you to see in the night and cones which are actually much less in number. In humans, it’s only 5% of all photoreceptors but they allow you to see in bright light. They’re responsible for high resolution and also color vision. Color vision is mediated by different kinds of cone receptors because they include different visual pigments, short wavelength like S cones, M cones have medium wavelength, visual pigment and long wavelength are L cones in human. In mice, we have only two kinds of cones, S cones and M cones.

Photoreceptors are highly active, metabolically active cells and the reason is that these highly polarized cells have got this membrane disk as you can see on top of these photoreceptors. 10% of these disks are shed every day. That means the whole outer segment is degenerated every year or rather every ten days. Even though these cells are post mitotic and they do not regenerate, the outer segment part, which is where the light is captured, has to be replaced every ten days. These outer segment disks are like membrane disks, which contain phototransduction material.
Slide 49

Now my lab over the last 20+ years has focused on all aspects of photoreceptor biology. We look at photoreceptor differentiation, look at aging of photoreceptors, many, many different diseases that are caused by defects in photoreceptor function, and then eventually trying to look at the treatment for these photoreceptor diseases. Today, however, I will briefly focus in this short duration on networks that are involved in differentiation of photoreceptors and how we’re trying to identify treatments.

Slide 50

Photoreceptors and in fact all retinal neurons and glia are generated from common pools of progenitor cells and as on the left you can see large photoreceptors would dominate the retina. There are over 70% of all cells in the retina. Their birth overlaps with the birth of all other cells. There is an order of birth and as you can see on the right, this photoreceptor differentiation like other differentiation of different cell types proceeds in a very sort of simple manner. You have dividing multi-potent progenitor cells. At some point in their differentiation, they become lineage restricted then when they exit cell cycle they have their fate specified and then through a variety of regulatory pathways, these photoreceptors acquire function.

Multiple transcription factors are involved in generating in this pathway, however, let us focus towards the right only on transcription factors that are involved in photoreceptor cell fate determination. The primary factor there is Nrl. Along with that you have a bunch of other of factors Crx, which are the homeodomain transcription factor, very critical for both rod and cone photoreceptors. TRβ2 is primarily for cone differentiation, and Nr2e3.

Slide 51

Several years ago, an excellent post doc in my laboratory, Allan Mears, made a knockout for this Nrl gene and showed that if you knock out this gene, a loss of function of this specific gene leads to a cone only retina. You have no longer any type of rods. There is complete fate switch. At the bottom of the slide, you could see the ERG or electroretinogram that shows the functional characteristics of these photoreceptors. In wild-type, you can see the dark-adapted ERG is very high but in knockout it’s flat. Dark-adapted ERG shows the response of large... Light-adapted is for cone cells and you could
see there is a huge increase in cone response in this Nrl knockout retina.

[0:45:32] Slide 52

What is even more exciting for us was when this graduate student—what he did was he took Nrl and expressed it under the control of Crx promoter, which is both in rods and cones and showed that now all cones become rods. So Nrl alone is sufficient to convert cone photoreceptors to rod photoreceptors. In fact, if you drive another expression under the control of S-opsin promoter, which is when the cone cells are actually even at a different stage in differentiation, even then some of these differentiating cone photoreceptors can get converted to rods, as much as 40% of these cells.

Slide 53

Something which is even more exciting is for us working with Douglas Forrest here at NIDDK, we showed that TRβ2 and Nrl these two transcription factors are present in certain photoreceptor precursors at the same time. As I mentioned earlier, TRβ2 is responsible for M cone differentiation whereas Nrl is for rod differentiation. What are they doing in the same cell? What we believe is happening is that there is some sort of tug of war going on between different transcription factors and they can then sort of determine what they’re going to be.

Slide 54

This particular slide shows us the transcription regulatory network. I’m not going to go in the detail of that, but what it shows is that the default pathway is the S cone pathway. If you have Nrl you’re going to make a rod photoreceptor, if you have no Nrl you’re going to go towards the S cone or if TRβ2 is there, you’re going to make an M cone.

Slide 55 to Slide 56

Now another post doc in the lab showed that if you can take the Nrl promoter and drive GFP, you could label the rod photoreceptors as soon as they are born. This is extremely important because now we can flow sort these cells and these flow sorted photoreceptor cells can be utilized to develop gene regulatory networks during differentiation and disease processes you could use these purified photoreceptor for cell replacement and for drug discovery.

Slide 57
In this brief slide, I’m going to give you a whole lot of background or a whole lot of information, what we have been doing is we have been trying to look at the networks that guide the differentiation of newborn photoreceptors to functional photoreceptors by doing RNA-Seq and other profiling, global profiling. We’re doing ChiP-Seq using different transcription factors, histone modification studies, and DNA methylation and I’ve listed the names of postdocs and fellows who have been involved in this particular work.

Slide 58 to slide 59

Now several years ago, we collaborated with a group in London and showed that we could take these photoreceptor precursors, the newborn Nrl+ photoreceptors and we can transplant in degenerating retina. These cells will then not only differentiate but also integrate within the retina and could give you some function but you need to have these immature developing rods. Once the cells differentiate, that means fully differentiate, they have outer segment, they can no longer function or integrate within these degenerating retina. This has been a very exciting development. A large number of labs have now been trying to use this technology to approach stem cell based therapy for retinal repair.

Slide 60

Here is another slide working with David Zacks. We showed that you could put that in a degenerating retina and these cells can integrate as shown here and they are viable for several months. Kohei Homma in the lab have got a paper now in press, which showed that these Nrl–GFP positive developing rod photoreceptors when you integrate them in a degenerating retina, they are functional and their function is very similar. Their membrane properties by using patch-clamp and other studies are very similar to native rods.

[0:50:03]

Slide 61

Now how do you make these rod photoreceptors if you want to do transplantation therapy? You could do that from human embryonic stem cells, IPS or induced pluripotent stem cells and you could develop these immature photoreceptors for a variety of different uses at a later stage.

Slide 62

Now this is just one of the slides that I wanted to put in here. Pioneering work in the lab of Dr. Sasai in Japan showed last year or in 2011 actually that you could make eye in a dish from both mouse
and later on, he showed it from human ES cells. And we have been using their protocols to develop the human retina in a dish and Nasonkin and Kohei and Jessica in the lab had been working on these strategies to look or to generate neural retina.

**Slide 63**

Now what we have also discovered very recently is that the retinal photoreceptors if you make them in a dish do not have outer segments and they will not respond to light. For that you require retinal pigment epithelium integrity and this was another work that we have published very recently in *Development* where we show RP is critical for only outer segment morphogenesis. Cell fate is still conserved but outer segments are not there if RPE is not properly polarized.

**Slide 64**

So what do we need to have cell-based replacement therapies for retinal and macular degeneration? We need to generate photoreceptors but we can make these cells but we still need to do a little bit more work on epigenetics and other characteristics of these cells. We should also be able to purify these cells without using GFP. Otherwise, it will be hard for us transplant these in humans. Transplanting methods have become pretty good these days but we need to find ways so that the cells do not clump and we might require some sort of biomaterial or a scaffold. Cell integration is still relatively poor. Only a small number of cells get integrated in the retina. We need to figure out ways to improve that. We also need to find fundamental methods to generate their connections and we are also working on many different methods for better assessment, for efficacy in animal models. Eventually, we believe that we need to have some sort of 3D reconstruction of outer retina if we want to have treatment for retinal and macular degenerative diseases.

**Slide 65**

I’m going to stop there. This is our group. A large number of people have been involved in this work. Along with that, we have several collaborators.

**Slide 66**

I’m going to stop with this quote from Helen Keller: The only thing worse than being blind is having sight but no vision. Thank you.

**Slide 67**
Sean Sanders: Thank you so much, Dr. Swaroop, and many thanks to all of our speakers for their excellent presentations. We’re going to move right on to the Q&A portion of the webinar now. We have probably about ten minutes so I’m going to get through as many questions as we can. So the first question I’m going to put to all of you and maybe we’ll start with Dr. Swaroop and we’ll work our way back down the table, is how might some of the processes and techniques that you’re developing right now in your research be applicable to some other fields of biomedicine?

Dr. Anand Swaroop: So just three very quick points, as I mentioned earlier, retina is part of the brain. In fact, retinal disease research has been at the forefront as a poster child for human genome project. The first disease that was mapped by using GWAS was age-related macular degeneration. Gene therapy has been highly successful in case of, you know, this retinal degeneration caused by defects in RP65 gene. So what we are hoping is that some of the work that we are trying to do in discovering transcriptional regulatory network, some of these will be directly applicable to research on other neurodegenerative diseases, specifically Alzheimer’s and Parkinson.

Sean Sanders: Dr. Sidransky?

Dr. Ellen Sidransky: Yes. I think that as we’re trying to understand the genetic basis of many different disorders, we’re beginning with work on Mendelian disorders, can give us sort of an anchor. Whereas if we can try to understand how we get this great spectrum of variability in a single gene disorder looking at modifiers or other contributing factors, it will be helpful when we go to tackle complex disorders that have multi-genes involved. I also think that some of the strategies and techniques that we’re looking for also will have wide applicability. First of all things like the IPS models and also high throughput screens for small molecule targets.

[0:55:23]

Sean Sanders: Dr. Zarate?

Dr. Carlos Zarate: With regards to understanding more about the circuits or synapses, we find that there is a lot of comorbidity with certain of our disorders. Within the psychiatric disorders, one may see more substance abuse, one might see more comorbid medical conditions, neurological conditions. This dysfunction at the synapse may apply not only to mood disorders but might apply to PTSD, might apply to other disorders. The more we understand how we stabilize a
synaptic dysfunction at the synapse level and also at the circuit level might be applicable to other disorders.

Some of our medications for example lithium is neuroprotective and it’s being studied in Alzheimer’s disease and other neurodegenerative disease and it’s also been studied in eye conditions where as a model for... So we can see that some of our drugs are being applied to other disorders, in particular its neuroprotective properties.

Sean Sanders: So a question I’m going to stay with you, Dr. Zarate, that I think is very interesting and hopefully not too controversial, is have you encountered any barriers or biases in your research amongst colleagues or other people in the same area who might not appreciate the molecular underpinnings of mood disorders?

Dr. Carlos Zarate: Well I think that one of the limitations we have in psychiatry is that we don’t have a clear etiology and there’s certain assumptions on what might be the causes. The issue is that the lack of targets has been a hindrance in terms of drug development for psychiatric disorders. When we go from to target to hit, target to lead, we’re assuming that it’s based on some obscure notion of how our disorders work, mental diseases.

The other aspect of that is most of our research was based on animal models, which were largely developed to identify compounds that modulate serotonin, norepinephrine monoaminergic systems and that’s led to development of the two drugs over the years. So now that there is a move to kind of more social affective models or animal models, other ways of assessing, you know, developing more sophisticated models than we previously had.

The other is to work backwards. I mean if we find treatments that are radically different then we can come to understand, you know, what might be the circuit synapses or genes involved. That perhaps will lead to an understanding of pathophysiology of illness but if not then at least we might be able to develop better treatments. So I think there’s a considerable progress in recent years, it’s quite exciting and where industry has been moving out of psychiatry now, there seems to be a greater interest of going back particularly in the area of mood disorders.

Sean Sanders: Great. Dr. Sidransky, I’ve got question for you. What kind of therapy for Parkinson’s do you predict from your research on Gaucher?
Dr. Ellen Sidransky: Well I think that our research is helping us focus, appreciate the role of lysosomal pathways in the etiology of Parkinsonism. I also think that there’s now more and more evidence in the last few years that because of this association between the glucocerebrosidase and α-synuclein that if we can find ways to enhance glucocerebrosidase it may ameliorate the aggregation you see with α-synuclein. So strategies like the chaperone therapy or other ways to increase enzymatic activity in the brain may have a role in Parkinson. Of course, we have a long way to go and unfortunately, we still don’t totally understand the mechanisms and I think that a lot of basic science work is needed before we can extrapolate totally.

Sean Sanders: Dr. Swaroop, what do you think is the biggest breakthrough that will be needed to occur before we are capable of growing complex organs for replacement such as eyes or possibly brains?

Dr. Anand Swaroop: Yeah. I think we still do not understand the basic physiology of each of these cells and how they behave in vivo. We look at biochemistry or we do the biology in isolated cell cultures and many times it does not reflect the biology in vivo. So what we have been trying to do is to develop sort of systems in vitro like we could do ex-plant cultures of the retina. We had been thinking to sort of combine biomaterials and nanotechnology based methods with the cell culture protocols in order to sort of generate these tissues in vitro, in 3D so that they can then be used to study biology first.

[1:00:38]

What we are lacking is really a collaboration among different scientists unfortunately and, you know, I came from extramural side, until very recently I was at the University of Michigan. In extramural science, we are always concerned about an RO1 grant, our own funds, how to do research but what we are not able to do many times is come together as a group and write large projects and many times those large projects are thought to be oh, you know, it’s impossible to do this.

Fortunately, NIH has taken note of that and now we are having larger projects or program projects that we are thinking about. NEI has simply started this Audacious Goals project and trying to sort of bring in people from many, many different areas together in order to really solve the problem. I think that’s what we need. We need for people to come together from different areas in physics and engineering to biology and sort of do work together to solve the
problem and not focus on a very tiny part of the big picture. We should look at the big picture.

Sean Sanders: Right. Any other comments, Dr. Sidransky, Dr. Zarate? Any thoughts on –

Dr. Ellen Sidransky: I think one of the strengths that we have here at NIH is the ability to collaborate with so many people in so many different fields. I think it’s greatly helped all of our individual work.

Dr. Carlos Zarate: And it’s also given us an opportunity now that there’s initiatives to collaborate with extramural and have these joint partnerships with the clinical center so I think that’s going to go a long way in facilitating discovery.

Dr. Anand Swaroop: Just one comment that that was the primary reason for me to come here. I mean NIH is a wonderful place. I mean if I don’t know anything, I can go around to different institutes even and different people. I collaborate with folks in seven different institutes already and there are topnotch scientists there. Sometimes that’s not easy to do in extramural and that’s why NIH is a great place to come.

Sean Sanders: So talking of interacting with other people in different fields, we have a question on the role, the potential role that epigenetics might play in neurodegenerative diseases and how these therapies, how therapies can maybe help to overcome such epigenetic alterations. Dr. Zarate?

Dr. Carlos Zarate: I’ll let the others –

Sean Sanders: Okay. Who would like to go at that? Dr. Swaroop?

Dr. Anand Swaroop: Epigenetics right now is still in very early stage and what we are trying to do is understand what it really means in many cases. So right now, what we are looking at is that we can manipulate the histone methylations or DNA methylation and we can change patterns of gene profile, but we don’t still have much control over that phenomena of epigenetics. So I think it will be a few more years from phenomenology that we move to really real benefits of understanding epigenetics. So I think we’ll take another few years to understand how epigenetic changes are really giving you a specific phenotype and how you can alter or manipulate that to give you a very distinct treatment or a different phenotype.
Sean Sanders: Dr. Sidransky, any thoughts?

Dr. Ellen Sidransky: I agree. I think we’re now well poised to look at the contribution of specific genes to phenotypes and as we start to appreciate the limitations of this, I think that’ll give us openings into probing for other epigenetic components of these disorders.

Dr. Carlos Zarate: I must admit at least in the mental illness, it’s going to be much more challenging. There are interesting findings already how you’re reared might affects or aspects of abuse and trauma might reflect later in greater suicide rates for example in bipolar disorder but that understanding epigenetics is really early on.

Sean Sanders: Great.

Dr. Anand Swaroop: And you need to do epigenetics in the specific cell type. You cannot use the whole tissue for that because there will be multiple different -- there are a lot of differences among different cell types and for many diseases particularly in psychiatric and neurological disease it’s hard to get those cells. If you had the cells in culture, the epigenetic landscape is going to be very different than in vivo. Again, I’m going to say retina hopefully will provide some of the early sort of conclusions for that kind of studies.

[1:05:15]
Sean Sanders: Right. So we’re pretty much out of time so I’m going to just fire one more question at you. Let’s start with Dr. Zarate, what do you see as the biggest challenge in moving proof of concept basic research into the clinic and how can this process possibly be improved? I know the NIH is very focused on this right now.

Dr. Carlos Zarate: Yes and I think that there’s initiatives at least by our institute in NIMH and also by NCATS, wonderful initiatives where most of the trials that were taking place were either industry run for many years and of course a lot of times they’re refinements over existing treatments, which I had mentioned. It’s not that industry, academia, and government has not tried coming up with new targets and testing them through. But we can see that the process of drug discovery and development is quite costly, it takes a long time and at least in mental health or CNS disorders, the targets aren’t clear and so the models as we talked are imperfect. But I think there’s enthusiasm now where one can in places like the clinical center do very specific hypothesis-driven questions where with drug target x or y if we go after this, would that lead into an improvement in
symptoms and several examples I talked about today. I think that’s possible here.

A lot of times these studies are done in patients taking many medications, with comorbidity and it’s unclear if you’ll find something. Not only that, the clinical center does permit a heavy biophenotype and integrated translation, these are multimodalities not only to test certain hypothesis but hypotheses generated and this is a wonderful place. You can find a lot of questions that you can pursue and that could be rapidly -- we can do collaborations with our colleagues here you know, go in the eye or at a metabolic level. So this is a very wonderful place to do that. I think part of our view, our mission in Intramural is to come up with a signal, some kind of spark through our work, which is very difficult to do outside. But once you have that spark, you can ignite discovery and development out there. I think this place does very wonderfully.

Sean Sanders: Dr. Sidransky?

Dr. Ellen Sidransky: I concur. I think there are certain things that are very special about the clinical center that help us with translational research. One is it’s the opportunity to become really expert at one thing and to also do natural history protocols where we longitudinally follow patients and really get to understand the disease. Through that we can find targets or biomarkers that can be used when we eventually have therapeutics. I also am very excited about the new NCAT Institute and the concept of programs like the Trend program, which will enable us to develop some of the new drugs and targets that we’re working on directly through people here. I also want to emphasize the clinical center gives us an opportunity to do therapeutic trials so that you can really actually go from the bench to the bedside and then learn something, go back to the bench and it really facilitates this evolution.

Sean Sanders: Dr. Swaroop?

Dr. Anand Swaroop: Yeah. So I mean we have come a long way. I think basic research still has to be forming the trigger or forming the basis of all the translational studies that we do. Translational studies are very expensive and therefore as it was pointed out earlier by Dr. Zarate, we need to have a good collaboration with industry whether it’s a small biotech or large pharma. There are certain things we cannot do in academic institutions whether it’s a university or even at NIH. However, NIH offers a very unique environment here that you have
people from various areas who can come together. I do hope that those sort of personnel are available at other institutions as well. Sometimes the money comes into play and we can do that. But I do hope that other universities and institutions take note of that and try to create the right environment where basic scientists can work very closely with clinicians and also with folks in industry.

01:10:00

I mean I was in clinical department and I can tell you in Michigan both in ophthalmology and then in genetics, many times everyone is so busy in whatever they do, it’s very hard for them to find time to collaborate with each other even within a department. I think it’s up to the institutions to create that. In NIH, we don’t have that sort of problem as much. We can go to our colleagues and it’s much, much easier to do. I do hope that NIH becomes a trigger or helps in creating that kind of environment for people to come together.

Sean Sanders: Fantastic. Well a lot to discuss but unfortunately, we are out of time for this webinar. So on behalf of myself and our viewing audience, I wanted to thank our speakers for being with us today, Dr. Carlos Zarate from the National Institute of Mental Health, Dr. Ellen Sidransky from the National Human Genome Research Institute, and Dr. Anand Swaroop from the National Eye Institute.

Slide 68

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Again, thank you to our panel and to the Intramural Research Program at the NIH for their kind sponsorship of today’s educational seminar. Goodbye.

01:11:43  End of Audio