

**Frontiers in Research Seminar Series**  
***Postdoctoral Data Blitz on Translational Research in Epilepsy***  
**(Transcript)**

- Priya Balasubramanian: [00:00](#) Welcome everyone. I'm Priya Balasubramanian, I'm Associate Director of Research at CURE Epilepsy and I want to thank you all for joining us today. Today is our next virtual seminar of 2021. This is part of our Frontiers in Research Seminar Series. Today's seminar is special: it's a postdoctoral data blitz. We are going to have three short talks from postdoctoral researchers and then a Q&A at the end.
- Priya Balasubramanian: [00:30](#) This is an opportunity for them to share their work with other epilepsy researchers, so we're really excited about these talks today. Although the topics for today's talks are quite diverse, they all focus on highly innovative translational epilepsy research that will hopefully lead to a cure.
- Priya Balasubramanian: [00:51](#) Our Frontiers in Research Seminar Series is supported by the Nussenbaum-Vogelstein Family. It aims to help and educate and expose researchers, clinicians and students to exciting epilepsy research and also provide opportunities for young investigators to interact with leaders in the field. Lately, we've had to do these virtually because of COVID and we will continue to do so until it is safe to get back together.
- Priya Balasubramanian: [01:22](#) Our next seminar is scheduled for May 7th, and that is a seminar on mTOR-dependent epilepsies by Angelique Bordey.
- Priya Balasubramanian: [01:37](#) CURE has been proud to be a leader in the epilepsy research community for over 20 years. We have funded over 260 projects spanning 16 countries. We have three different funding mechanisms currently, and our key research priority areas include the acquired and pediatric epilepsies, treatment resistant epilepsies, sleep and epilepsy and SUDEP.
- Priya Balasubramanian: [02:02](#) All of our grant applications, they go through a letter of intent phase and full proposal review by scientific reviewers as well as members of the community who are touched by epilepsy. The newest of our awards is our Catalyst Award. This is intended to fund translational research with the goal of advancing new discoveries and new therapies into clinical application. We will announce the RFA for this award very soon, so please be on the lookout for that. Of course, you can always contact us if you have any questions.
- Priya Balasubramanian: [02:41](#) And then our other two awards, the CURE Epilepsy Award is open to established investigators and our Taking Flight Award is intended to support junior investigators who have at least three years of postdoc training but have yet to attain significant funding. The 2021 cycle is already, we're in the middle of the 2021 cycle already so the next cycle will be announced in 2022 for these two awards.
- Priya Balasubramanian: [03:14](#) Today's hour-long seminar will highlight work from these three postdoctoral researchers who will get 10 minutes each to present their

work. Our presenters today are Maria-Belen Perez-Ramirez, who is from Stanford University. Then we have Lena Nguyễn from Yale, and Raji Banerji from the University of Colorado. Following all of these presentations, we will have time for some Q&A for our speakers today.

- Priya Balasubramanian: [03:50](#) I want to mention that our virtual seminar, today's seminar, and all of our future seminars are recorded and you can view them later on our CURE Epilepsy website as well.
- Priya Balasubramanian: [04:02](#) Finally, before we get started, I want to encourage everyone to ask questions. You can submit your questions anytime during this presentation, just type them into the Q&A tab located at the bottom of your Zoom panel and click send. I do request that you indicate which of the three speakers your question is addressed to, and we'll try to get through as many as we can.
- Priya Balasubramanian: [04:28](#) With that, our first presenter is Lena Nguyễn. She is a postdoctoral research associate in Doctor Angelique Bordey's lab at Yale University. Her research focuses on alterations in intracellular signaling pathways and how these contribute to epilepsy in neurodevelopmental disorders. Today she will be talking about targeting translational dysregulation in mTOR related epilepsy. Thank you, Lena. You're on mute.
- Dr. Lena Nguyễn: [05:10](#) Can you hear me now?
- Priya Balasubramanian: [05:12](#) Yes. Thank you.
- Dr. Lena Nguyễn: [05:17](#) Yeah. I'd like to thank CURE for their support in our epilepsy research endeavors, and for this opportunity to share some of my work with the epilepsy community. Today I will present to you some of our newest findings that we think have an exciting translational potential.
- Dr. Lena Nguyễn: [05:35](#) My work focuses on a group of neurodevelopmental disorders associated with aberrant mTOR signaling known as mTORopathies. The prototypical mTORopathy that many of you may be familiar with include tuberous sclerosis complex, focal cortical dysplasia-type II, and hemimegalencephaly. There are also others.
- Dr. Lena Nguyễn: [05:56](#) While these are distinct disorders, they share a set of characteristic features that include hyperactive mTOR signaling, cortical malformations with laminar disorganization, and the presence of enlarged dysmorphic neurons, and intractable epilepsy that is difficult to treat and that often requires surgery. The mTOR pathway is a complex intracellular signaling pathway that controls cell growth through multiple downstream processes, some of which are shown here in green.
- Dr. Lena Nguyễn: [06:27](#) In mTORopathies, this cascade is hyper activated due to what we now know are genetic mutations in components of this pathway. Many of these mutations were only identified within the past several years, and so this is an emerging field and there is a lot of interest in trying to

understand which mechanism contribute to epilepsy in order to develop more effective treatments.

- Dr. Lena Nguyễn: [06:52](#) In my talk today, I will show data that supports targeting one specific protein downstream of mTOR, namely 4E-BP, is sufficient to attenuate neuronal defects and seizure in an mTORopathy mouse model. We think that this protein could be a promising therapeutic target.
- Dr. Lena Nguyễn: [07:09](#) 4E-BP is a translational repressor that is directly regulated by mTORC complex one. When mTORC1 is activated, it phosphorylates 4E-BP one and two, which in turn alleviates the break on eukaryotic initiation factor eIF4F allowing for cap-dependent mRNA translation to occur. In contrast, when mTOR is inactivated before 4E-BP one and two is disinhibited, leading to repressed translation. Consistent with mTOR hyperactivation, me and others have found increased phosphorylation of 4E-BP in brain tissue from patients with TSC and FCDII.
- Dr. Lena Nguyễn: [07:58](#) Here, we stained for DAPI, which labels cell nuclei in blue. SMI-311 which labels dysmorphic neurons in green, and Phospho-4E-BP1 in red, and we found strong staining for Phospho-4E-BP1 that co-localized with SMI-3 positive dysmorph neurons in all samples we looked at. These findings support that 4E-BP repressor activity is inhibited in human TSC and FCDII.
- Dr. Lena Nguyễn: [08:30](#) To model mTORopathies in mice, we used in-utero electroporation to express an activator of mTOR complex one, namely Rheb, in the developing mouse cortex. We used a constitutive active form of Rheb, which leads to persistent mTOR hyperactivation. We performed in-utero electroporation at embryonic day E-15, where we inject Rheb and a tdTomato reporter plasmid into the embryonic ventricles, and then we electroporate to transfect the plasmas into progenitor cells that will eventually become cortical layer 2/3 neurons.
- Dr. Lena Nguyễn: [09:08](#) By post natal day zero, there is clear expression of tdTomato in the targeted area which in our case is the medial prefrontal cortex, and this diagram shows the targeted area in [inaudible 00:09:20].
- Dr. Lena Nguyễn: [09:25](#) In the control animals, the electroporated cells are found in layer 2/3, where they should be. Whereas in the Rheb expressing animals, the electroporated cells are found misplaced across all the cortical layers. In addition, the cell size are significantly larger and these enlarged cells display increased Phospho-4E-BP1 staining similar to in the human tissue, and the quantification is seen here on the right.
- Dr. Lena Nguyễn: [09:53](#) Given the finding that 4E-BP is dysregulated in both human and mouse model, we asked whether restoring 4E-BP activity could improve the neural defects and attenuate epilepsy phenotype that we observe in these mice.
- Dr. Lena Nguyễn: [10:11](#) To answer this question, we expressed the Rheb with a constitutive active 4E-BP1 plasmid that is resistant to mTOR phosphorylation, so that it

cannot be inhibited by mTOR activity. This is a conditional plasmid that is preceded by a lock [inaudible 00:10:29] flanked stop code on, which suppresses 4E-BP1 expression until a [inaudible 00:10:37] is expressed that removes the stop code on. With this, we included a Tamoxifen inducible Cre plasmid which allows us to temporarily control when 4E-BP1 is expressed. tdTomato was also included to label the target itself.

- Dr. Lena Nguyễn: [10:55](#) The effects of 4E-BP expression was compared to a group that received all of the same plasmid, but with a conditional GFP instead of 4E-BP. Mice were electroporated at E-15, and Rheb is shortly expressed after that leading to mTOR hyperactivation in-utero. Then at P-28, we administered Tamoxifen to turn on 4E-BP1 expression. By this age, the mTOR induced cellular abnormalities are well in place, and mice begin to exhibit a robust seizure phenotype. We selected this age because we wanted to see whether we could reverse the established phenotypes, which would be important from a translational aspect. Then, we performed EEG and histology in adults around two and a half months of age. For patch clamp electrophysiology, we shifted the timeline a little bit earlier for technical reasons.
- Dr. Lena Nguyễn: [11:50](#) We found that 4E-BP expressions significantly decreased neuron size without rescuing ectopic placement of neurons. At the functional level, membrane capacitance which is higher in the Rheb neurons compared to the control neurons, were partially reduced by 4E-BP expression, consistent with a decrease in cell size.
- Dr. Lena Nguyễn: [12:15](#) The resting membrane potential which is more depolarized in Rheb neurons was also restored to control levels, and so was the abnormal hyper-polarization activated H-current. I don't have time to go into the details of this, but we recently reported that Rheb neurons acquire an abnormal HCN4 channel expression that gives rise to this abnormal H-current that contributes to depolarized resting membrane potential. We find here that 4E-BP restores this abnormality.
- Dr. Lena Nguyễn: [12:48](#) Finally, in terms of seizures, the Rheb mice displayed frequent daily seizures with tonic-clonic activity as seen here in this EEG trace, and 4E-BP expression significantly attenuated this. More than 40% of the mice were seizure free during this seven day monitoring period, compared to only 10% in the mice that received GFP. This is very powerful, because these mice were having seizures already prior to 4E-BP expression. As a group, the average seizures frequency was reduced by more than 50% in the Rheb plus 4E-BP1 mice.
- Dr. Lena Nguyễn: [13:27](#) In summary, we have shown that 4E-BP is dysregulated in human mTORopathies, and in a related mouse model, and restoring 4E-BP activity via constitutive active 4E-BP1 expression improves neural defects and attenuates seizures in mice. Overall, these findings support that targeting 4E-BP1 is a potential therapeutic strategy for epilepsy, and we are excited about these findings.

- Dr. Lena Nguyễn: [13:54](#) Briefly, where we're going next with these data is that we are working to develop an AAV-based gene therapy approach to focally deliver 4E-BP into the cortical malformations in mice. What you see here is targeted intercranial injection of an AAV-eGFP virus into the mPFC of a wildtype mouse. We have now developed a protocol to successfully keep the virus spread focal, while at the same time cover enough of the targeted area and controlling for efficient transection of neurons. We are next looking to inject AAV-4EBP1 into the Rheb mice, and so we are very excited about this and we hope that this data that we obtain next will provide some insights into the feasibility of a 4E-BP targeted therapy for epilepsy and mTORopathies.
- Dr. Lena Nguyễn: [14:52](#) With that, I'd like to end by acknowledging my advisor Dr Angelique Bordey, members of the Bordey Lab, as well as our collaborators and my funding sources. Thank you.
- Priya Balasubramanian: [15:07](#) Thank so much, Lena. That was a great presentation. Again, I'd like to remind everyone we're doing Q&A after all the talks are done, but please do send in your questions through the Q&A tab.
- Priya Balasubramanian: [15:23](#) Next up, we have Maria-Belen Perez-Ramirez, she is at Stanford University at David Prince's lab. She obtained her PhD in biomedical sciences at the National University of Mexico, and she is now in the neurology department at Stanford. Thank you, Maria.
- Dr. Maria Belen Perez-Ramirez: [15:44](#) Thank you. Hi everyone. My name is Belen. Yeah, I'm going to present some of the results that we have from this project that is called Focal status epilepticus and Gabapentin effects in the neocortex.
- Dr. Maria Belen Perez-Ramirez: [16:03](#) Status epilepticus is considered any seizure that lasts more than five minutes, and unfortunately it is a life threatening neurological emergency. Then epilepsy may occur in 22 to 40% of the people with status epilepticus. Today, there is no treatment to prevent epileptogenesis after status and in this project, we are going to study the consequences of a single episode of status epilepticus in the neocortex of mice, and whether Gabapentin treatment can prevent these consequences.
- Dr. Maria Belen Perez-Ramirez: [16:43](#) Here I show you a time course of our experiments. What we do is we give a focal application of convulsant drugs, in number three, well, in somatosensory cortex. And then we put some electrodes to record the electrographic EEG activity. This is what you see here. This is an example of the electrographic activity, and this is an animal in control in electrodes in ipsilateral. Then contralateral, in three and four on side of the application of the convulsive drugs. This is before, and this is during the focal status epilepticus.
- Dr. Maria Belen Perez-Ramirez: [17:30](#) As you can see, there are [inaudible 00:17:33] charges on the side of the application drugs. Then, we let the status epilepticus go for two hours and after two hours are done, we stop the behavioral seizures

with diazepam. Three hours after the seizure activity detection, we inject or we start the status Gabapentin treatment. Then, we give 100 milligrams per kilogram per day during seven days. Then the animals are let to recover, and at 10 days and at 30 days we use cytochemistry and in-vitro electro-physiological experiments to obtain and to record what are the changes for status?

Dr. Maria Belen Perez-Ramirez: [18:23](#)

We wanted to know whether this induced epilepsy from activity persisted beyond the induction of status epilepticus. To do so, we recorded the electrical potential in layer five neo-cortical slices, at 10 days post focal status epilepticus and these abnormal or epileptiform activity can be seen up to 100 days post status epilepticus. We also found that some slices had spontaneous epileptiform events.

Dr. Maria Belen Perez-Ramirez: [18:58](#)

Then we found that 63% of the mice had at least one slice that show epileptiform burst discharges, and there were 53 slices that had epileptiform bursting discharges. But what's going on in the brain that is able to generate these bursting activity? In other animal models, there are increases in excitatory activity. We wonder whether this can be one cause.

Dr. Maria Belen Perez-Ramirez: [19:32](#)

Thanks to Eroglu, [inaudible 00:19:34] and other labs, we know that active astrocytes can produce thrombospondins. And then these thrombospondins are proteins that bind to the alpha-2 delta-1 receptor that is another protein, and then this interaction induces the formation of excitatory synapses. We wanted to study whether or not after focal status epilepticus there were these proteins present. We use immunocytochemistry and we labeled GFAP to identify astrocytes in red, and TSP 2 in green, and you can see that in control there is some expression of these proteins but then up to 30 days after the induction of focal status epilepticus, there are increases in both astrocytes, and TSP.

Dr. Maria Belen Perez-Ramirez: [20:40](#)

Here in these zooming image, you can see in yellow how these proteins merge. Then, when we gave the Gabapentin treatment for seven days and waited up to 30 days, there was a ... Less expression of these two proteins, and the same happened for alpha-2 delta-1. These protein was expressed in control, but then after 30 days there were increases and with Gabapentin there was a decreased expression of alpha-2 delta-1.

Dr. Maria Belen Perez-Ramirez: [21:25](#)

Here I'm showing you the plot of the results, and what you can see is that Gabapentin was able to decrease 10 days the thrombospondins, and the GFAP [inaudible 00:21:34] activity, and then there was a tendency to decrease it up to 30 days and then there was a significant decrease of the alpha-2 delta-1 protein after the Gabapentin treatment. Then we wanted to test whether the increases in synaptogenic proteins also results in increases of structural excitatory synapses.

- Dr. Maria Belen Perez-Ramirez: [21:59](#) To do so, we used dual immuno-staining of VGLUT-1 and PSD95, and then the co-localization of these two proteins means that there is putative excitatory synaptic connection. We did this and with help of confocal imaging, we could obtain the results in neocortical layer five of the cortex, and in control, and we can notice that 30 days after the induction of status epilepticus, there were increases of these excitatory synapses. In the examples, you can see them circled in these white circles.
- Dr. Maria Belen Perez-Ramirez: [22:47](#) Gabapentin, up to 30 days, was able to decrease the expression of synaptic excitatory connections. Then this plot shows that these changes were significant, but this structural changes in structural connections are also functional. To do so, we made in-vitro electrophysiological recordings of miniature of excitatory co-synaptic current which they can reflect the increases in the excitatory activity.
- Dr. Maria Belen Perez-Ramirez: [23:30](#) We did this in control, and you can see the currents and these are the currents 30 days after the induction of focal status epilepticus. These are the currents with seven days of Gabapentin treatment, and after 30 days of the induction of focal status epilepticus. As you can see in the plot, the increases 30 post status were significant, but then the frequency was significantly decreased with Gabapentin and this was up to 30 days after the induction.
- Dr. Maria Belen Perez-Ramirez: [24:08](#) So far, I've shown you the results in the neocortex at the area of focal status epilepticus. But then we wondered whether the consequences could reach other interconnected areas such as the hippocampus or thalamic structures like the nuclear reticular thalamus.
- Dr. Maria Belen Perez-Ramirez: [24:24](#) To do so, we did electro-physiological recordings of spontaneous excitatory postsynaptic current, and then we recorded the currents in control. And 10 days after the induction of status epilepticus, and yeah, please remember that this was made in neurons from the [inaudible 00:24:49] thalamic. And then, the increases in the frequency of the spontaneous currents, was observed up to 30 days after the induction of status epilepticus.
- Dr. Maria Belen Perez-Ramirez: [25:05](#) With these results that I show you, we can conclude that the focal status epilepticus increases the density of excitatory synapses as well as GFAP-, TSP2-, and a2d1-IR in the neocortex. And then, we think that this increases in the excitatory synapses can be as a consequences of the increases in these three proteins.
- Dr. Maria Belen Perez-Ramirez: [25:32](#) Then, status epilepticus induces chronic increases in excitatory synaptic transmission and epileptiform field potentials. Also, we found that prophylactic Gabapentin treatment prevents status epilepticus induced increase in synaptogenic proteins, excitatory synapses, and synaptic transmission. We also found that a single episode of focal status epilepticus in the neocortex increased the spontaneous excitatory synaptic activity to nRT. With this, we are thinking whether



or not Gabapentin treatment should be used to prevent complications of status epilepticus in humans.

- Dr. Maria Belen Perez-Ramirez: [26:21](#) Thank you to all of the support we have, thanks also to Isabel Parada that helped me with the immunocytochemistry, and the co-focal imaging, and that now she is teaching me to do so. Also, thank you very much for the support my mentor, David Prince, and of course thank you very much to the CURE Foundation that is giving us this magnificent opportunity to show our results. And then in case you didn't have time, and you have further questions, you can send over questions to these emails. Thank you.
- Priya Balasubramanian: [26:56](#) Wonderful. Thank you so much. We can move onto our final presenter of the day, Raji Banerji, who is at Manishi Patel's lab at the University of Colorado. Raji is a senior postdoctoral trainee, she is at the Department of Pharmaceutical Sciences, and her research involves understanding and developing metabolism based therapies for neurological disorders, especially epilepsy. Thank you, Raji.
- Dr. Rajeswari Banerji: [27:25](#) Okay.
- Dr. Rajeswari Banerji: [27:40](#) [inaudible 00:27:40]
- Dr. Rajeswari Banerji: [27:40](#) Thank you for the introduction. Good afternoon everyone, and thank you for joining in. Today I'm going to discuss a different aspect that is metabolic defects identified in a zebrafish larval model of a pediatric epilepsy known as Dravet syndrome. I'm going to tell you how normalizing metabolism via a mitochondrial specific [inaudible 00:28:01] could be therapeutic in this [inaudible 00:28:04].
- Dr. Rajeswari Banerji: [28:07](#) Dravet syndrome as many of you know is a catastrophic childhood epilepsy associated with de-novo mutations in the voltage gated sodium channel SCN1A. Patients have intractable seizures along with cognitive, motor, and behavior concerns. Here, I'm going to introduce you to the pre-clinical larval zebrafish model of Dravet syndrome known as scn1lab.
- Dr. Rajeswari Banerji: [28:32](#) This larval model has dark pigmentation as shown here, and recapitulates key phenotypes of Dravet syndrome patients. It has loss of function in the sodium channel snc1ng, and the mutants exhibit spontaneous abnormal electrographic seizures, hyperactivity, and convulsive behaviors. In fact, the two translation value of this model has recently been demonstrated. The drug Clemizole identified in Dr Scott Baraban's lab in UCSF was tested in this model, and is currently in phase two clinical trials.
- Dr. Rajeswari Banerji: [29:10](#) There are two important questions. Why is it important to study metabolism in Dravet syndrome? A major research gap exists between metabolic function and neuronal excitability. It is widely accepted that



glycolysis and mitochondrial respiration are key energy producing pathways in the brain.

- Dr. Rajeswari Banerji: [29:31](#) The second question is, do metabolic pathways play a role in seizure control? In fact, it may be true because there are scientific evidence that supports this notion. First, as many of you know, Dravet syndrome patients respond positively to ketogenic diet, which is based on providing alternate to mitochondrial [inaudible 00:29:52], and also mitochondrial defects and glucose hypermetabolism has been reported in Dravet syndrome patients. Additionally, altered metabolism and mitochondrial dysfunction has been studied in a wide epilepsies in our lab for a long time.
- Dr. Rajeswari Banerji: [30:07](#) Taking all these scientific evidence into consideration, one major question arise. Can any of these metabolic alterations be harnessed to improve seizure control in these mutant larvae? Interestingly, there are evidence of some additional therapies such as branching amino acids, ketogenic diet, serotonergic agent that have been useful for Dravet syndrome patients. In all these case, there's an increased flux of gluconeogenesis pathway and expression of the rate limiting enzyme phosphoenolpyruvate carboxykinase, or PCK indicated here, has been observed.
- Dr. Rajeswari Banerji: [30:49](#) Our lab in collaboration with Dr Scott Baraban has developed a mobile platform to [inaudible 00:30:57] in live Zebrafish larvae using the extracellular flux Analyzer. Both glycolytic and mitochondrial respiration rates were lower in these mutants, also we found that a downregulation of cytosolic PCK1 and mitochondrial PCK2 genes were down regulated in these mutants. My research builds on these previous findings of metabolic deficits in these models.
- Dr. Rajeswari Banerji: [31:23](#) I started by first validating mitochondrial dysfunction in the mutant larvae. This plots show defective maximum respiration, proton leak, and spare respiratory capacity, which are considered as the hallmark of the mitochondrial function. Additionally, I also observed significantly lower glucose levels in these mutants and thus establishing for the first time that hypoglycemia [inaudible 00:31:49] the mutants.
- Dr. Rajeswari Banerji: [31:51](#) Will enhancing glucose metabolism in these mutants rescue the metabolic defects and also suppress seizure? Will that identify a metabolism based therapeutic target for Dravet syndrome? In order to test my hypothesis, I adapted a strategy to pharmacologically upregulate the gluconeogenesis pathway by increasing the expression of the gene called PCK. For that, I used a metabolism based small shelf library to first validate PCK activators, and used them in a behavior and metabolism based assay. And finally, using the most promising drug for testing its anti-epileptic property.

- Dr. Rajeswari Banerji: [32:39](#) Coming to the first step was to validate potential compounds that upregulated PCK genes in the mutants. Interestingly, the QPCR data shown here shows majority of the drugs upregulated the cytosolic PCK1 gene whereas little effect was seen in the mitochondrial PCK2 gene. For this reason, I focused my further studies using the validated PCK1 activators.
- Dr. Rajeswari Banerji: [33:05](#) Next, I performed behavior based phenotyping using validated PCK1 activators from my previous step. By using an automated low pollution tracking software, I tracked larvae movement to get a quantitative read out of seizure like swim behavior. I'm showing you the heat maps which represent average swim velocity. Two independent screens were performed in one, in our lab. Another in our collaborator, Dr Scott Baraban's lab. And both independently identified a drug called PK11195 indicated by red arrows here, that significantly suppressed mean velocity by at least 40% in these mutants.
- Dr. Rajeswari Banerji: [33:48](#) Stiripentol, also present on the screen, as you know is a known anti-epileptic drug, was used as a positive control which also suppressed seizure-like behavior in these mutants.
- Dr. Rajeswari Banerji: [34:02](#) Next, I performed metabolic screening. I used the extracellular flux analyzer and determined in the PCK1 activators would improve the metabolic defects, that is glycolytic respiration deficits and the glucose in the mutants. For that, I can show you briefly that this drug, PK11195 was one of the drugs that significantly rescued both glycolytic and respiration rates. Additionally, this drug could also correct the glucose levels in the mutants. But it's important to point out that Stiripentol, our control, did not show any effect in these metabolic acids. Taking together, this drug PK11195, was identified as a top candidate which it was the most prominent drug that was positive hit in our small shelf screen.
- Dr. Rajeswari Banerji: [34:57](#) We went to the next step using this drug, because we selected this drug for the final step, we verified for its anti-seizure activity by performing electro-physiology. As you can see here, the local field potential recordings show a significant reduction in the frequency of ictal in interictal-like electrographic seizure events, thus confirming the anti-epileptic property of this drug.
- Dr. Rajeswari Banerji: [35:25](#) I keep telling this drug PK11195, so what do we know about this drug? It is a well-known synthetic translocator protein ligand located at the mitochondrial outer membrane. It plays different regulatory roles, for example in neuro [inaudible 00:35:42] synthesis, apoptosis, cancer progression, neurodevelopmental diseases, inflammation and also in anxiety disorders. This drug has shown to improve glucose tolerance in obese mice, and have also shown anti-seizure effects in epileptic mice.
- Dr. Rajeswari Banerji: [35:59](#) Additionally, low [inaudible 00:36:02] levels of this drug is widely used commercially as a neuro inflammation marker in humans.

- Dr. Rajeswari Banerji: [36:07](#) Coming to the last slide, I would like to summarize my findings. What I told you today, that I identified a therapeutic potential of a mitochondrial translocator protein ligand called PK11194, in a translatable larval Zebrafish model of Dravet syndrome. My findings suggest that the strategy of improving gluconeogenesis may potentially rescue metabolic defects and also suppress electrographic seizure in this mutant larvae.
- Dr. Rajeswari Banerji: [36:38](#) Of course, further in-depth studies are needed to establish the novel finding. But the most important message I would like to present is that these significant metabolism based findings suggest that targeting energy producing pathways is a viable and a novel therapeutic avenue for the treatment of genetic epilepsies such as Dravet syndrome. With that, I would like to acknowledge my mentors and also the funding agencies and all work shown today was recently published in Brain Communications.
- Dr. Rajeswari Banerji: [37:13](#) And at last, special thanks to CURE Epilepsy for giving me this great opportunity. Thank you.
- Priya Balasubramanian: [37:22](#) Thank you so much, Raji. That was a great talk, and again thanks to all three of you for really exciting, wonderful talks. We've had some questions come in, and I just wanted to remind everyone if you have questions, please do continue to send them in through the Q&A tab.
- Priya Balasubramanian: [37:42](#) Let's get to the questions.
- Priya Balasubramanian: [37:46](#) Let's start with a question that came in for Lena. They want to know, why did you focus on 4E-BP1 and not 4E-BP2?
- Dr. Lena Nguyễn: [37:58](#) Yes. That's a great question. There are three sub-types of 4E-BP, there's 4E-BP1, 2, and 3. 1 and 2 is expressed in the brain, with 2 being the most highly expressed. At the time that we started this study, the tools for targeting 4E-BP1 was more readily available to us since this is the most common studied subtype in the cancer field. That is why we used it.
- Dr. Lena Nguyễn: [38:28](#) Functionally, 4E-BP1 and 2 are very similar. The sequence homology, I think is more than 60%. In our case, we over-expressed 4E-BP1 which would compensate for both the inhibited activity of 4E-BP1 and 2. I think that expressing constitutive active 4E-BP2 could potentially have the same effect.
- Priya Balasubramanian: [38:56](#) Great. Thank you. And then here's a question for Belen. There's a couple of questions for you, but let's start with this one first. They want to know, how do you analyze your EEGs? And what criteria need to be met to establish that there is seizure activity in your models?
- Dr. Maria Belen Perez-Ramirez: [39:17](#) Yeah. Thanks for the interest, and yeah. To analyze the EEG, we use Power Analysis. And then, please remember that we induce the

seizure activity at the beginning but we haven't still studied later EEG activity. But then to establish that there is seizure activity, what we do is ... There needs to be interictal electrographic discharges, and then I also expect to have behavioral contralateral jerkings from the animal. When the animal has these contralateral behavioral seizures together with the interictal seizures, then that's when I start counting the time. The two hour period of seizure activity.

- Dr. Maria Belen Perez-Ramirez: [40:16](#) Then, something we notice is that after their interictal spikes, they can develop to ictal activity. But yeah, and then we just let the seizure activity for two hours and then we stop the seizure. But there should be both behavioral and electrographic interictal spikes.
- Priya Balasubramanian: [40:41](#) Excellent. Thank you. I'll come back to you for the other question. I'm going to move onto Raji. There's a question here, and they want to know please tell us more about PK11195? Do we know anything about the structure? Does it get into the brain? Has it been tested in other models of epilepsy?
- Dr. Rajeswari Banerji: [41:04](#) Yeah. That's a great question. And yes, we know a lot about this drug. This has been discovered in 1977, so a lot of research has been done and so this drug is a synthetic GSP11, so it binds to the mitochondrial ligand that is the translocator protein. It is also known as the peripheral benzodiazepine receptor, so it does have a lot of information on how it mines and how it producing neuro [inaudible 00:41:38], so there's a lot of research going on.
- Dr. Rajeswari Banerji: [41:41](#) As I mentioned in talk, there are a few papers which have shown an anti-epileptic property of this drug. We are really excited to try this in our new model, and also try it in mice models also.
- Priya Balasubramanian: [41:57](#) Then I was also wondering, Raji, do you know if the efficacy of this drug, this anti-seizure activity, is that dependent on its improvement of the metabolic activity or are they two separate things?
- Dr. Rajeswari Banerji: [42:17](#) [crosstalk 00:42:17]
- Dr. Rajeswari Banerji: [42:17](#) No, what we think, certainly after doing three years of research on our Zebrafish model, that a metabolic deficit is required for this drug to work. We think this drug may work as a censor, actually, because we don't think it always improves. It may reduce the glucose levels, also. A better term will be normalize, so it senses any kind of defects, metabolic defects, and then corrects it. We still don't know how it corrects a seizure phenotype, and that's something which we have to understand using other models maybe. But that's a good question also. Thank you.
- Priya Balasubramanian: [43:02](#) Thank you. Lena, back to you. The high variability in your seizure rescue finding is really interesting. Do you have any clues on why many saw complete reduction in seizures, while others looked similar to the

control group? Did you find that seizure frequency correlated with any of your other measures?

- Dr. Lena Nguyễn: [43:25](#) Yeah. That's a great question. Several things could contribute to this, one of which is a technical consideration for in-utero electroporation. We have been pretty good at getting consistent with the area that we target and the size of the targeted area, but when electroporating multiple plasmid as we did, not all cells are going to end up with all of the four plasmid. The majority will, but not all. These differences could contribute to the large variability.
- Dr. Lena Nguyễn: [43:58](#) In addition to that, differences with combination efficiencies could lead to different levels of 4E-BP expression, which could also contribute to this. In our case, cell size is a good readout of whether 4E-BP activity worked the way we had intended it to, and so we have correlated cell size with seizure activity and we found a very strong correlation where those with no or low seizure activity following 4E-BP expression had smaller cell size, and those with higher seizure frequency had larger cells, suggesting that 4E-BP might be insufficient.
- Dr. Lena Nguyễn: [44:47](#) It is interesting that we still see such a strong effect despite this variability, and we might actually be underestimating the effects of 4E-BP on seizures.
- Priya Balasubramanian: [45:01](#) Lena, have you looked at or has anyone else looked at different models of cortical malformations to know is there 4E-BP1, is it also hyperphosphorylated in other models? Is that known?
- Dr. Lena Nguyễn: [45:20](#) Other groups have shown that [inaudible 00:45:23] is increased in human mTORopathies, like TFC and FCD2, and increased phosphorylation is expected as well in other mouse models.
- Priya Balasubramanian: [45:44](#) Thank you. Next question is back to Belen. What would your next steps be to translate your findings to people who have experienced status? Have you tried Gabapentin animal models of epilepsy? Great question.
- Dr. Maria Belen Perez-Ramirez: [46:04](#) Yeah. Thanks. Yeah, that's a complex question, the first one. Yes. A bit, first, I think we need to find what will be the best regime of Gabapentin, or Gabapentin treatment. Meaning the concentration and timing, because also there is a time window as far as we know where this is synaptogenic activity. We still don't know in this model, what is this time window and the latency? That's also another issue we need to address.
- Dr. Maria Belen Perez-Ramirez: [46:42](#) Also, it will be important that once we know the most efficient Gabapentin treatment regime, then how this translates to human. How? Because Gabapentin is already an FDA approved drug, so we just need to understand how the doses even after status epilepticus can work in humans. Yeah, and then as I was telling, the window will be very important as also Gabapentin, it's as far as we know, the efficacy

happens also with newly formed synapses. That's why it is important to know the latency, or the synaptogenic period.

- Dr. Maria Belen Perez-Ramirez: [47:37](#) About the second question, which was in Gabapentin trialed in other models? In the lab here, in the lab it was already tested on the undercut model of epilepsy, and they found similar results with decreases in alpha 2 delta 1, and decreases in synaptic activity. And also, decreases in synaptic connectivity. And then, there is the group from Rossi, and they tried Gabapentin in the pilocarpine model of temporal epilepsy, and they found, I think they found also that Gabapentin was able to decrease astrocytic activity and the latest article, they test this with animals that were treated with PILO and then treated with Gabapentin, and then given a second dose of PILO, and they found that there was decreased mortality, decreased racine scale achieved, and decreased animals that get to actual status epilepticus on that second dose.
- Dr. Maria Belen Perez-Ramirez: [48:59](#) And then, there is the group of [inaudible 00:49:01] that they tried Gabapentin on a model of cortical malformation, and they also found decreased epileptiform activity with Gabapentin and I think also decreased expression of thrombospondins. But, yeah, it's been tried in different models.
- Priya Balasubramanian: [49:24](#) And you mentioned about timing, so have you looked at, I think you're doing it at three hours after status, the Gabapentin treatment you initiated? Have you tried other time points, like starting it later?
- Dr. Maria Belen Perez-Ramirez: [49:40](#) That sort of thing, we are interested in looking at. Yeah.
- Priya Balasubramanian: [49:47](#) Awesome. Thank you. I had another question for Lena, just a technical question. In the samples from the humans where you showed the 4E-BP1 hyper-phosphorylation, what did you use as controls for those?
- Priya Balasubramanian: [50:10](#) We can't hear you.
- Dr. Lena Nguyễn: [50:13](#) Sorry?
- Priya Balasubramanian: [50:14](#) I can hear you now.
- Dr. Lena Nguyễn: [50:15](#) Okay. Because they were human samples, [inaudible 00:50:20] come across, we have not been able to get control human tissue samples. But there have been other studies where 4E-BP at this level is not found in the control tissue. We also-
- Priya Balasubramanian: [50:42](#) Thank you.
- Dr. Lena Nguyễn: [50:43](#) Sorry, to add to that, we ... Looked at surrounding cells just to see. It's not the best control, but those cells did not have increase. It was very specific to the dysmorphic enlarged neurons, the increase.

- Priya Balasubramanian: [51:06](#) Great. There are no more questions, and I'd like to conclude this seminar and take this opportunity to again say thank you to the three of you for your time and for sharing your research with us. Really wonderful presentations. I also want to thank the Nussenbaum-Vogelstein family for their generous support of this Frontiers in Epilepsy Research Seminar series. I'd like to thank everyone who joined us today, and thank you for participating and asking questions.
- Priya Balasubramanian: [51:39](#) Of course, if you'd like more information about possibly hosting a seminar series, just you can apply and you can let us know. Or if you want to apply to one of our CURE Epilepsy grants, you can visit our for researchers page on our website, [CUREepilepsy.org](http://CUREepilepsy.org), or you can also write to us at [reseach@CUREepilepsy.org](mailto:reseach@CUREepilepsy.org). Finally, to register for our seminar on May 7th. Thank you.