7th European Rett Syndrome Conference Presentation
Jeffrey Neul
Title: Gene discovery to clinical trials: How clinical and basic research have intersected to develop and test new therapies for Rett syndrome.

Slide 1:
Hello, and good afternoon. I want to thank the French Association of Rett Syndrome for inviting me, and all of you for attending my presentation today, entitled “Gene discovery to clinical trials: How clinical and basic research have intersected to develop and test new therapies for Rett syndrome.”

Slide 2:
Rett syndrome was originally characterized by an Austrian pediatrician, Andreas Rett, in 1966. He published the initial report in German in a Viennese weekly medical newsletter and was not widely read. Broad awareness of Rett syndrome occurred after the publication in 1983 by Bengt Hagberg and colleagues, who named the condition “Rett syndrome” in recognition of Dr. Rett’s work.

Slide 3:
As I think this audience know, Rett syndrome is characterized by a typical disease pattern and progression. After apparently normal initial development, there is a developmental delay followed by regression, specifically of acquired hand skills and spoken language. There are also gait problems, or the inability to walk, and repetitive hand movements. After regression there is a stabilization period and other features such as seizures and breathing problems start. Later, in the teen-age years or young adulthood, people with Rett syndrome have a change in motor skills, with increasing stiffness, scoliosis, and some parkinsonian features that can impact mobility. Rett syndrome primarily affects girls, at a rate of about 1 in 10,000 live female births.

Slide 4:
In 1999, Dr. Huda Zoghbi and colleagues found that genetic mutations in the X-linked gene Methyl-CpG-binding protein 2, or MECP2, are present in the majority of people with Rett. 95-97% of people with Rett have a mutation in MECP2, meaning about 5% of people who clinically have Rett syndrome do not have a mutation in MECP2. My group and others have done genetic sequencing in these people with Rett who do not have MECP2 mutations and found mutations in other genes that cause other neurodevelopmental disorders, showing clinical overlap between Rett syndrome and these other neurodevelopmental disorders.

While the majority of people who have Rett are girls, we are increasingly identifying boys with MECP2 loss of function mutations, and they have a much broader range of clinical features than we previously realized. More work is needed to better understand boys with MECP2 mutations.

There are also people who have an extra copy of MECP2, which causes a severe neurodevelopmental disorder called MECP2 Duplication Syndrome, mainly in boys.

Slide 5:
The identification of the genetic cause of most cases of Rett syndrome allowed the development of animal and cell models of Rett syndrome. Groundbreaking work in 2007 by Jacky Guy and Adrian Bird demonstrated that turning MECP2 on in mice that did not have MECP2, even after the mice started becoming sick, reversed the problems. This reversal has been reproduced by other groups in both male and female mice, and provided hope that therapies could be developed to make meaningful changes for people with Rett, or even reverse the disease.

Slide 6:
In 2009, Daniela Tropea and Mriganka Sur treated Rett mice with a tripeptide, which is three amino acids that is a natural cleavage product from the growth factor Insulin-like Growth Factor 1. The structure of this tripeptide, called glypromate, is show in the middle. This treatment improved breathing, heart rate, movement, and survival of the mice.

Slide 7:
The ability of glypromate to improve feature in Rett mice lead to clinical trials using a drug that is a synthetic analog of glypromate, called trofinetide. Trofinetide has the same basic structure as glypromate, but with an extra chemical modification that makes it a better drug because it can be taken orally and lasts longer in the body. Two phase 2 trials were done with trofinetide in Rett syndrome, the first in adults and the second in children. Both of these phase 2 clinical trials found that trofinetide was safe and tolerated, and showed signals of efficacy in improving features of Rett syndrome. The positive results from these two phase 2 trials led to the initiation of a phase 3 trial of trofinetide in people 5-20 years old with Rett syndrome in the US, called the LAVENDER trial. The results from this phase 3 trial found that it improved symptoms in people with Rett syndrome, and these results led to the approval of trofinetide by the US Federal Drug Administration for the treatment of people with Rett syndrome.

Slide 8:
Here is the design of the Phase 3 Lavender Study of trofinetide in Rett syndrome. 187 young females with Rett syndrome, aged 5-20 years old were enrolled into a randomized, double-blind, placebo-controlled, multi-center study. Participants were randomized 1:1 to either trofinetide or placebo at a weight-based dose and treated for 12 weeks. The treatment as taken by mouth or through a gastrostomy tube twice a day. Double-blind means that the people participating and the clinical investigators in the trial did not know if they were on trofinetide or on placebo. There were two co-primary endpoints, a caregiver scale called the Rett syndrome Behavioral Questionnaire, or the RSBQ, and a clinician rated scale called the Clinical Global Impression of Improvement, or the CGI-I. There was also a key secondary efficacy endpoint, which is a caregiver rated communication scale. Because there were two pre-specified co-primary efficacy endpoints, for the trial to be a success both had to show improvement in the trofinetide group compared to the placebo group. At the end of the 12 week trial, participants were

given the opportunity to enter an open-label extension study, which means that everyone gets trofinetide.

Slide 9:
This slide shows the results of the primary efficacy endpoints in this trial. The caregiver rated RSBQ is shown on the left, and the clinician rated CGI-I is shown on the right. In both graphs, people on placebo are show as black dashed lines, and people on trofinetide as blue lines. You can see on the left that at the 2 week visit after starting treatment, both the placebo and trofinetide group showed improvement (which is lower scores) on the RSBQ, but the placebo group moved back to baseline by 12 weeks, the end of the study, whereas the trofinetide group remained improved at the end of the study. The graph on the right side shows the change in the CGI-I, which showed improvement in the trofinetide group compared to the placebo group at week 12. People on trofinetide were also better on the key secondary endpoint, but I do not have time in this talk to show that data today.

Slide 10:
This slide shows the rate of treatment-emergent adverse events, or TEAE. These are issues that came up with participants during the study. You can see that more people had adverse events on trofinetide compared to placebo, with almost 93% on trofinetide having an adverse event compared to 54% on placebo. The rate of serious adverse events was similar between the groups. The most common adverse events were diarrhea, with 80% on trofinetide having diarrhea compared to about 20% on placebo, and vomiting, with 27% of the trofinetide group having vomiting compared to about 10% in the placebo group. The majority of the adverse events were mild to moderate in severity. Diarrhea was the most common reason people discontinued taking trofinetide.

Slide 11:
Based on the results of this phase 3 trial, as well as the other trial of trofinetide in people with Rett syndrome, the US Federal Drug Administration approved trofinetide for people with Rett syndrome over 2 years old. It is a strawberry flavored liquid taken twice a day by mouth or through a gastrostomy tube, based on weight. As mentioned the most common side effects are diarrhea and vomiting.

Slide 12:
Dealing with the side effects, especially diarrhea, is the biggest challenge. The recommendations to decrease diarrhea are to stop laxatives before starting treatment, and decrease or switch medications that contain sugar. Additionally, it is helpful to start fiber supplements. We have found that it also helps to start at a lower dose than recommended and increase the dose over a couple of weeks. Some doctors are seeing if splitting the dose into 3-4 per day might make things better. If diarrhea starts, I decrease the trofinetide dose at least for a while, and start loperamide, which is an antidiarrhea medication.

Slide 13:

A big question that gets asks is about the availability of trofinetide around the world to treat people with Rett syndrome. Currently, trofinetide is only approved for use in the US. This summer, Acadia acquired the rights for trofinetide for the rest of the world, and announced that they are working for approval in Canada and then eventually Europe and Asia.

Slide 14:
I want to introduce the US Rett syndrome natural history study for Rett syndrome, which ran from 2003 until July 2021. This longitudinal study has been very important in developing our understanding of Rett syndrome and important for the clinical trials that have occurred or are starting or being planned. Dr. Alan Percy was the head of this study, and it is due to his hard work and leadership that this was able to happen. We had 14 sites across the US, enrolled people from all the states in the US and from over 20 countries, and these sites have been the fundamental sites for the industry sponsored clinical trials. Ultimately we evaluated over 1800 people in total, with over 8700 visits. There were over 1250 people with classic Rett syndrome, with over 80% seen more than once and over 50% of participants seen over 4 times.

Slide 15:
I think it is important to put the natural history study into context of the other critical events in Rett syndrome. In 1999, Huda Zoghbi and colleagues found the genetic basis of Rett syndrome. In 2007 Adrian Bird showed that you could reverse the disease in mice, even after symptoms started. And then in 2009, Daniela Tropea showed that treatment of Rett mice with a drug could improve symptoms. This led to where we are now with a successful Phase 3 trial of trofinetide in Rett syndrome and the first FDA approved drug for Rett syndrome. What is important to see is that the natural history study started shortly after the discovery of the genetic cause of Rett syndrome, and before we knew that there could be disease modifying treatments.

Slide 16:
The main goal of the natural history study was to develop clinical trial readiness for Rett syndrome, which means getting important things together so clinical trials can be conducted.

Slide 17:
One thing important for clinical trial readiness is having a network of sites that have experience in Rett syndrome and are able to do clinical research. In the natural history study we were successful in this area by supporting a network of 14 sites across the US, enrolled people from all the states in the US and from over 20 countries, and these sites have been the fundamental sites for the industry sponsored clinical trials.

Slide 18
The next goal to develop clinical trial readiness is to characterize the clinical features and natural history of Rett syndrome. This is important because effective treatments should address clinical problems and be guided by the natural progression of disease.

The US natural history study was very successful in this, with over 50 published manuscripts and multiple additional manuscripts submitted and in preparation.

Slide 19:
Here are some examples of things we learned from the natural history study. We characterized the relationship between specific mutations in MECP2 and overall clinical severity in people with Rett syndrome. This found that people with some MECP2 mutations, such as R133C, are generally less severely affected than people with other MECP2 mutations, such as R168X. However, it is important to say that these genotype/phenotype relationships are only at the group level, meaning that they cannot predict the clinical severity of a specific person. There are people who have ”mild” mutations who are as severely affected as people with “severe” mutations, and the opposite is true as well. I think this is very important for families and caregivers to know, because in a single person we cannot say how affected they will be based on the MECP2 mutation.

Slide 20:
We also described a number of other clinical features, such as seizures, which are very common in Rett syndrome and are seen at some time over life in nearly everyone with Rett syndrome. But there is a lot of variability on how bad seizures are between people. We also evaluated other important features of Rett syndrome such as abnormal breathing, repetitive hand movements, sleep problems, scoliosis, and developmental delay.

Slide 21:
We know that people with Rett syndrome have a number of issues related to growth and nutrition, and developed growth charts for height, weight and head size for Rett syndrome. Growth problems are closely connected with nutrition and gastrointestinal issues that are common in Rett syndrome, such as problems chewing and swallowing, reflux, and constipation. We also looked at the connection between Rett syndrome and biliary problems like gall stones, low bone density and increased risk of bone fractures, and pubertal development.

Slide 22:
We also looked at behavioral features and found that problems with behavior like anxiety, aggression, self-injury and social interaction are more common in people with Rett syndrome who are less severely affected in motor and functional skills. Finally, we characterized the quality of life for people with Rett syndrome and their families.

Slide 23:
The final goals of the natural history study to establish clinical trial readiness was to develop biomarkers and clinical outcome measures. Let me first talk about biomarkers. A biomarker is something measured that can do things like indicate disease severity, identify people who will respond to a treatment, or show changes before clinical improvement seen after treatment is started. Ideally, a biomarker should be something that is easy to measure and not very invasive.

Slide 24:
One thing we were interesting to look at as a biomarker is neurophysiological signals. These can be measured by surface electrodes on the scalp, and EEG. One thing that can be measured with EEG is called an “evoked potential”. This cartoon shows and example of a visual evoked potential, in which a flashing grid of light is shown many times and the EEG signal is recorded from the part of the brain important for vision. From this we can see a characteristic brain wave signal, shown on the right, which has peaks that can be measured both in terms of the time from the light flash, or latency, and the height of the peak, or amplitude.

Slide 25:
In the natural history study we looked at both visual and auditory evoked potentials and found they were different in people with Rett syndrome compared with typically developing people. In both cases, the height of the peaks were lower, and correlated with severity in Rett syndrome.

Slide 26:
What is nice about these studies is we can see the same kind of changes in mouse model of Rett syndrome as what we saw in people with Rett syndrome. This gives an opportunity to be able to use similar measures in studies of mice and people to translate information from mouse studies to human studies.

Slide 27:
In the natural history study, we did the same type of EEG studies in people with CDKL5 deficiency disorder, MECP2 Duplication Syndrome, and FOXG1 Disorder and also found that EEG measures could be useful as biomarkers in those disorders as well.

Slide 28:
The final part of clinical trial readiness is the development of clinical outcome measures, which are critical for a successful clinical trial. One of the very most important things to think about for an outcome measure is that it measures what is most important to people with Rett syndrome and their caregivers.

Slide 29:
During the natural history study, at every visit we asked caregivers what their top concerns were. They could choose from a list of problems or enter something not on the list.

Slide 30:
Here is the list of the top concerns in order. As you can see, the top issues are functional skills that are lost or impaired in Rett syndrome, such as problems with communication, hand use, walking and repetitive hand movements. But things like seizures and constipation are also in the top 5 concerns. An outcome measure in Rett syndrome should assess these issues that are most concerning to caregivers.

Slide 31:
We also asked caregivers about their impression of function and well-being at every visit, asking if their child was improved, the same, or worse. If the caregiver said improved or worse, we asked them to choose the reason for improvement or worsening. At the top left you can see that in about 50% of visits caregivers said their child was unchanged, 27% of visits had improvement, and 21% had worsening. So, what are the top reasons the caregivers said there was an improvement or worsening, and what are the top caregiver concerns when they said their child had improved or worsened?

Slide 32:
When caregivers noted improvement, the main reason for improvement was communication. In contrast, when they noted worsening, seizures was the main reason the caregivers indicated led to worsening. Similarly, the top overall concern for caregivers when there was improvement was communication, but seizures was the top concern when the caregiver thought their child had worsened.

Slide 33:
This data has been accepted for publication and is available as a preprint at Research Square (the weblink is at the bottom of the slide). It is important to understand what caregivers would like to see change and how much change will be meaningful. This type of information is important as we evaluate existing or develop new outcome measures to make sure they are aligned with top caregiver concerns.

Slide 34:
Let's look at the outcome measures that have and are being used in clinical trials in Rett syndrome. The trofinetide trial used a caregiver reported measure, the Rett syndrome Behavior Questionnaire, or RSBQ, and a clinician assessed measure, the Clinical Global Impression of Improvement, or CGI-I. For the RSBQ, there are 8 subscales listed on the left, and the pros are that it is relatively easy to complete and understood by regulators. The cons is that when we compare to the top caregiver concerns in Rett syndrome it does not completely cover all the concerns.

Slide 35:
The CGI-I is the clinician's view of global change for a participant scored on a 7 point scale with 4 meaning no change, scores of 1-3 being improvement and scores of 5-7 being worsening. Regulators understand and accept this, but it is important to have disease specific anchors which have been developed for Rett. One problem is that it does not give information on what features specifically improved. We asked if we could develop other outcome measures using the natural history study data.

Slide 36:
To develop a clinician scale we used a measure that we captured throughout the natural history study called the motor behavior assessment, which had 37 items but had never been evaluated as an outcome measure. We went through a formal process called "psychometric evaluation" which created the Revised Motor Behavior Assessment (or R-MBA), which has 24 clinician rated items that create 5 factors plus 3 important clinical

features. The factors are important features in Rett syndrome: Motor dysfunction, functional skills like hand use, speech, and walking, social skills, behavior problems, and breathing problems.

Slide 37:
The R-MBA has a total score, with higher scores being more severe. We showed that the R-MBA increased with increasing severity on the Clinical Global Impression of Severity, to caregiver assessment of problems, and showed the same relationship to MECP2 mutation groups.

Slide 38:
If we look at how well the R-MBA captures important clinical issues in Rett syndrome we see that it does hit many of the top concerns in Rett syndrome, but still not completely. We asked if we could use the natural history study to develop a better clinician rated scale and also a new caregiver rating scale.

Slide 39:
We used the same approach to develop a new clinician rated scale, which was supported by Alcyone Therapeutics, and created a new measure which has not yet been named that has 31 items that create 6 factors, again that match nicely with clinical features in Rett syndrome. We also developed a new caregiver rated scale, called the Rett syndrome Caregiver Assessment of Severity and Symptoms, or R-CASS, which was supported by the Rett Syndrome Research Trust. This has 32 items with 4 factors, and correlates with clinician rated severity, age, and severity. This has been submitted and is now available as a preprint.

Slide 40:
Although these new scales have a number of strengths, there are some areas not as well covered like constipation or sleep. But other measures have been developed or evaluated in Rett that cover these areas and could be included in a clinical trial. Also, it is important to have performance measures, and work has been done for Rett syndrome to develop performance measures for walking, hand use, and communication.

Slide 41:
There are other drug studies ongoing or recently completed in Rett syndrome. Anavex 2-73, or blarcamesine, was shown to improve features in Rett mice, and the company reported last year positive results on a clinical trial and are conducting additional trials. Ketamine was shown to improve features in Rett mice, and we completed a small trial of oral ketamine in Rett syndrome, which was safe and well tolerated and we are analyzing if it had any effects on improving clinical features or biomarkers. Excitingly, a company called PharmaTher announced this year that they are interested in developing ketamine for Rett syndrome and received Orphan Drug Designation from the US FDA.

Slide 42:

But what about developing newer therapies in Rett syndrome? When Adrian Bird showed that it was possible to reverse the disease in Rett mice the potential of the idea of gene therapy for Rett syndrome came up. There have been multiple studies in Rett mice showing promise of gene therapy and now two companies have started work on gene therapy in people with Rett.

Slide 43:
Taysha started a trial in Canada for adults with Rett syndrome. In the Taysha trial they deliver a gene therapy vector through intrathecal dosing, which means injecting through a spinal tap. So far two people have received this treatment in Canada. Taysha announced that they are submitting an application to the FDA to start trials in the US this year.

Slide 44:
Neurogene is the other company starting gene therapy trials in Rett syndrome. This is in the US with children and it is delivered through intracerebral ventricular injection, which means that the virus is delivered through a needle directly into the ventricles in the middle of the brain. Three sites have been announced, Texas Children's Hospital, Children's Hospital Colorado, and Boston Children's Hospital. It is great to get to the point that these gene therapy trials are starting in Rett syndrome.

Slide 45:
And beyond gene replacement therapy, there has been a lot of work in animal models of Rett syndrome with different approaches such as X-chromosome reactivation, read-through therapy for nonsense mutations, and DNA or RNA editing. This is all very exciting, because there are a number of additional approaches that eventually might lead to new clinical trials in Rett.

Slide 46:
Finally, there have been a number of studies in Rett disease models showing potential benefit of drugs that have already been approved by the FDA. We just received a grant from the US Department of Defense to conduct an umbrella trial of 3 approved drugs, ketamine, donepezil, and vorinostat in Rett syndrome compared to a common placebo. The goal is to create a platform to accelerate early clinical trials in Rett syndrome, with the ability to add additional drugs in the future.

Slide 47:
So, what is the future? Well, the success of the phase 3 trial of trofinetide and approval in the US is really promising, because it shows that therapies can be developed for Rett and similar disorders. There are exciting opportunities for testing new therapies targeted towards Rett syndrome like gene therapy or the other advanced treatments I mentioned. The Natural History Study provided the critical foundation for these clinical trials, and we are continuing to use this information and gather new data to improve how we do clinical trials, so we can get more treatments that will make meaningful impact on people with Rett syndrome and their families.

Slide 48:
I want to thank the Rett consortium who did the work, especially Alan Percy who was the visionary leader of the study for nearly 2 decades.

Slide 49:
Finally, I want to thank all the people who participated in the Natural History Study, and all my friends with Rett syndrome who taught me the most about this disorder. Thank you for attending this lecture.