Report on the International Rett Syndrome Foundation Annual Scientific Meeting 5-7 June, Nashville, TN, USA

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Introduction.

The meeting was opened by Dr Dominique Pickard, Chief Scientific Office of the International Rett Syndrome Foundation (IRSF) who stated in her opening address that sixteen years ago, the Rett Community had hope, but now had action. This was emphasised in spectacular fashion by the announcement (https://ir.tayshagtx.com/node/8626/pdf), timed to coincide with the start of the meeting, that Taysha had recently treated the first patient by gene therapy and by a press release from Neurogene that provided a link to access details for their planned Phase 1/2 clinical trial (see-https://clinicaltrials.gov/ct2/show/NCT05898620?recrs=abdf&cond=Rett+Syndrome&draw=2&rank=3). These announcements generated a palpable sense of excitement even before any of the talks were presented.

The meeting covered aspects of the molecular biology of MeCP2, strategies to express a functional MeCP2 protein in the brain including gene and cell therapy, a strategy to treat Rett duplication syndrome and recent information on novel therapies to treat symptoms related to Rett Syndrome itself. While many of these basic science studies provide a valuable framework for more clinical and translational studies, we have attempted to present a summary of the presentations most relevant to the RSAA community.

Gene therapy.

There were several talks on gene therapy, including a keynote talk from Dr Katherine High from the Rockefeller Institute in New York in which the current status of gene therapy for genetic diseases in general and neurological genetic diseases in particular, including Rett Syndrome, was discussed. Dr High identified several key features necessary for therapeutic success, most of which are included in the strategies developed by Taysha and Neurogene. The difficulty of gene delivery which is currently by direct injection into the brain was noted as an area that could be improved.

To continue the theme of gene therapy for a neurological disease, Dr Richard Finkel, St Jude Children's Hospital, Memphis, TN presented details of the success of gene therapy in treating spinal muscular atrophy (SMA) as a model for gene therapy for Rett Syndrome. A presentation from Dr Vania Broccoli, IRCCS Hospital in Milan highlighted strategies designed to improve the specificity of MeCP2 expression in different cell types in the brain. Although different cells in a normal brain express MeCP2 at different levels, current strategies for gene therapy employed by Taysha and Neurogene fail to account for these differences. To address this, Dr Broccoli used cell type-specific microRNA (miRNA) to restrict the level of expression of MeCP2 in different cell types in the brain of Rett mouse model. (An article on how miRNA controls MeCP2 expression in gene therapy was posted on the RSAA website last year -see https://rettaustralia.org.au/blog/a-game-changer-for-gene-therapy-for-rett-syndrome/). This strategy could be considered to represent fine-tuning of the strategy used by Taysha and Neurogene, both of which use a single miRNA to restrict the level of expression of MeCP2 to ensure that patients are not overdosed; it remains to be seen if it is important to differentially control the level of MeCP2 expression in different cell types in the brain.

Finally, presentations from Taysha and Neurogene highlighted many of the preclinical studies necessary before gene therapy of patients with Rett Syndrome can proceed. Pre-clinical

studies were conducted in 3 animal species, mice, rats and non-human primates, to prove safety/lack of toxicity and determine biodistribution in the brain after delivery of the MeCP2 gene. Details of the design of the current Taysha Phase 1/2 clinical trial were presented that aims to recruit 20 adults with Rett Syndrome. The presentation from Neurogene highlighted a point of difference, as the Neurogene strategy is designed to deliver a full-length MeCP2 gene whereas the Taysha strategy uses a mini-gene which encodes the two most important regions of the gene that have been shown previously to correct Rett symptoms in the mouse model.
To conclude the presentations on gene therapy and to provide a family perspective on gene therapy, a study from the University of Arizona Health Services showed that most caregivers are aware of gene therapy and are optimistic that the therapy might cure the disorder or at least improve communication or gross motor skills. It will be some time before we know these answers, but the process has started!

Alternatives to gene therapy.

X chromosome reactivation. As an alternative to gene therapy, re-activation of the inactive X chromosome has been proposed as a strategy to express a functional form of the MeCP2 protein. In a keynote speech, Dr Rudolf Jaenisch, Massachusetts Institute of Technology, showed that demethylation (a chemical change) in the MeCP2 gene can result in expression of a functional MeCP2 protein. Details of this work have been published (a summary appears on the RSAA website-https://rettaustralia.org.au/blog/a-new-strategy-to-improve-the-expression-of-mecp2-by-reactivation-of-the-inactivated-x-chromosome/) and provides a powerful stimulus to develop this strategy further, as expression from a reactivated chromosome will not run the risk of over-expression of MeCP2 resulting in MeCP2 duplication syndrome. The strategy was developed further as shown in presentations by Dr Jeannie Lee, Harvard University and Dr Kathrin Meyer, Nationwide Children's Hospital, Columbus, Ohio, who used small stretches of DNA known as anti-sense oligonucleotides (ASO) in combination with a DNA methylation inhibitor and miRNA respectively to reactive the MeCP2 gene in the inactivated X chromosome in mice. Delivery of the components directly into the brain resulted in long lasting MeCP2 restoration of expression and improvements in symptoms in the Rett mouse model.

Biomarkers
A major difficulty in determining the outcome of treatment of Rett patients is the lack of objective biomarkers-biological signatures which reflect changes in body fluids or tissues in response to the therapy, in a similar way to the detection of Covid-19 antigens after infection with SARS-CoV-2. At present, responses to therapy in Rett patients are assessed by care givers or clinicians and are often subjective. An array of biomarkers which reflect changes in the symptoms and/or behaviour of Rett patients is urgently required. Dr Victor Faundez, Emory University, Atlanta, Georgia discussed how best to select biomarkers to address this problem and suggested that proteins found in the CSF were most likely to be useful in parallel with high and low density lipids (fats) which circulate in the blood. Dr Judith Armstrong, Sant Joan de Deu, Barcelona used a combination of molecular biology and protein chemistry in an effort to define useful biomarkers. Her studies compared Rett expression profiles with those associated with Rett duplication syndrome and noted that some markers were consistently affected in Rett patients. These studies are still ongoing but are becoming more important as additional patients are enrolled in different clinical trials.

Clinical trials

Trofinetide (DAYBLUE). Information was presented on the clinical trials of Trofinetide (DAYBLUE), a synthetic analogue of a natural occurring protein in the brain. The most recent LILAC study, a long term open label study of over 52 weeks demonstrated some improvements in the Rett syndrome Behaviour Questionnaire, particularly communication and there was an overall impression of improvement. Of note however a significant number of the study cohort experienced troublesome diarrhoea and / or vomiting and some experienced weight loss. As a result of these side effects several participants withdrew treatment. Further studies are now being planned.
In March 2023 Trofinetide received FDA approval for treatment of females with Rett syndrome over 2 years of age in USA. As yet it is not approved for use in Australia.

Medicinal Cannabis.
Associate Professor Carolyn Ellaway was invited to present information about an Australian study being planned using a novel, full spectrum medicinal cannabis in females with Rett syndrome. Preliminary data from a study of children with severe autism has demonstrated significant improvements in communication and anxiety in the cohort. In addition there are known benefits from other studies of medicinal cannabis in terms of improvements in seizure control, pain and inflammation. More information will be provided once the clinical trial has the appropriate approvals to proceed.

Summary.
There was a general feeling of excitement during the meeting related to the progress in new treatments and gene therapy. While we share this excitement, we also caution that progress is likely to be slow as the major aim of Phase I clinical trials is to prove safety. The companies are aware that premature action leading to any serious side effects will set the program back by several years, so that patience will ultimately be rewarded.