Translational Science of Rare Diseases 6 (2023) 137–150
DOI 10.3233/TRD-230063
IOS Press

IRSF 2023 - Rett Syndrome Scientific Meeting Report

Nupur Garg[a,*], Zhaolan Zhou[b,c], Eric D. Marsh[c,d], Colleen M. Niswender[e,f] and Dominique C. Pichard[a]
[a]*International Rett Syndrome Foundation, Cincinnati, OH, USA*
[b]*Department of Genetics and Epigenetic Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
[c]*Intellectual and Developmental Disabilities Research Center (IDDRC), Children's Hospital of Philadelphia, Philadelphia, PA, USA*
[d]*Department of Neurology and Pediatrics and Orphan Disease Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA*
[e]*Department of Pharmacology, Warren Center for Neuroscience Drug Discovery, Vanderbilt Brain Institute, Vanderbilt Institute of Chemical Biology, Vanderbilt University, Nashville, TN, USA*
[f]*Vanderbilt Kennedy Center, Vanderbilt University Medical Center, Nashville, TN, USA*

1. Introduction

The 2023 International Rett Syndrome Foundation (IRSF) Rett Syndrome Scientific Meeting was held in Nashville, TN, USA, from June 5-7. It brought together leading scientists, clinicians and industry partners to discuss recent progress towards advancing our understanding of methyl-CpG binding protein 2 (MeCP2) and Rett syndrome (RTT) biology, as well as to celebrate the significant strides that have been made towards finding treatments for RTT. A historic milestone was reached in March 2023 with the approval of Trofinetide, now marketed under the name DAYBUE™, as the first available treatment for RTT [1–3].

RTT is a severe X-linked neurodevelopmental disorder (NDD) caused by loss-of-function mutations in the *MECP2* gene [4]. Mutations in *MECP2* occur in both females and males but with much greater incidence in females [5]. There has been a growing appreciation of the clinical spectrum of RTT and variations of *MECP2* [6]. In female patients, MeCP2 mutations manifest primarily as RTT, which is clinically defined as an apparently normal early period of development, followed by developmental plateau and then regression of acquired hand, language and gross motor skills. While most females present with classic RTT symptoms, there is a spectrum of mild intellectual disability (ID) to more severe encephalopathy present. Males often present with neonatal onset encephalopathy and early death to a pattern of X-linked ID. Therefore, loss-of-function mutations in *MECP2* lead to a spectrum of

*Corresponding author: Nupur Garg, International Rett Syndrome Foundation, 4500 Cooper Road, Suite 204, Cincinnati, OH 45242, USA. E-mail: ngarg@rettsyndrome.org.

disease, with RTT being the most prevalent [5]. Duplications of *MECP2* on the X chromosome have also been identified to cause NDD, though mostly in boys with a pattern of hypotonia, global developmental issues and associated symptoms [7–9]. Though RTT primarily affects the central nervous system (CNS), involvement of other co-morbidities as the disease progresses highlights the systemic impact of MeCP2 dysfunction [10–13]. The conference, organized by IRSF and supported by several sponsors, covered topics ranging from basic research to clinical trials. The meeting featured 7 keynote addresses, 13 invited speakers, 9 selected oral abstracts, and 7 presentations on genetic and non-genetic treatments currently in trials or under development for RTT. In addition, 77 posters were presented over two poster sessions and a caregiver panel was organized on day two to provide an opportunity for the research community to hear directly from the patient community. This report summarizes the proceedings of the meeting and highlights new research avenues and treatments for RTT emerging from around the globe.

2. Session I: MeCP2 basic and surprising functions

The opening session featured talks on the breadth of molecular and cellular functions attributed to MeCP2, and how the molecular underpinnings of its absence may lead to the development of RTT.

The keynote address for the session was given by **Dr. Rudolf Jaenisch** (Whitehead Institute, USA) who emphasized the complex proposed role of MeCP2 as both a transcriptional repressor and an activator. Using techniques to map the genomic binding landscape of MeCP2 in human induced pluripotent stem cells (hiPSCs), his lab has demonstrated unique binding of MeCP2 to many promoter regions, and has shown that MeCP2 interacts with RNA Polymerase II through its Intrinsically Disordered Domain (IDR). This binding occurs at Transcription Start Sites (TSS) and CpG islands; depending on the target gene, MeCP2 can be involved in transcriptional activation or repression. Dr. Jaenisch's studies further showed a predominantly gene-activating function for the wild-type MeCP2 protein as evidenced by global repression of gene expression in RTT neurons [14]. Dr. Jaenisch also discussed efforts by his group to reactivate *MECP*2 expression on the silenced X-chromosome by utilizing dCas9-Tet1 mediated demethylation of the promoter [15]. dCas9-Tet1 is a fusion protein of inactive Cas9 nuclease (dCas9) and ten-eleven (TET) 1 hydroxylase. These studies have shown that the transient reactivation of MeCP2, induced by demethylation alone, can be maintained for longer periods by combining it with CCCTC-binding factor (CTCF) insulation.

Dr. Yehezkel Sztainberg (SKiP Therapeutics, Israel) presented his preclinical studies on antisense oligonucleotide (ASO) therapy to downregulate MeCP2 expression as a potential novel treatment for *MECP2* duplication syndrome (MDS) [16, 17]. In these studies, a humanized mouse model of MDS carrying two copies of human *MECP2* was generated. Intracerebroventricular (ICV) administration of ASOs resulted in normalization of *MECP2* mRNA and protein levels, and also reversed behavioral dysfunction in adult transgenic mice.

Dr. Michael Müller's (University Medical Center Göttingen, Germany) talk focused on the aberrant mitochondrial function seen in RTT neurons [18, 19]. In a mouse model of RTT, imaging studies using a redox-sensitive Green Fluorescence Protein (roGFP) revealed a systemic oxidative burden as evidenced by increased reactive oxygen species' release concomitantly with reduced scavenging. In addition, proteomic and metabolomic analyses revealed key alterations in the brains of MeCP2 mutant mice involving lipid metabolism, mitochondrial and extramitochondrial pathways, as well as amino acid and carbohydrate metabolism which have implications in furthering our understanding of the disease process.

Dr. Sameer Bajikar (Baylor College of Medicine, USA) presented his findings on how gene expression changes temporally if MeCP2 is turned off in adult mice to address if steady-state changes are proximal or secondary to the loss of MeCP2. These experiments have shown significant overlap in

transcriptional profiles between adult and germline loss of MeCP2, and that molecular changes precede behavioral changes in those mice. Additionally, for genes that show early mis-regulation following depletion of MeCP2, differences in mechanism were observed between targets that are down-regulated versus up-regulated by MeCP2.

Dr. Jing Zhang (University of Colorado Boulder, USA) discussed his research on the mechanism and implications of MeCP2's ability to bind methylated ribonucleic acid (RNA). Specifically, his studies have identified the long non-coding RNA, Rncr3, as a binding target of MeCP2. Mutational analyses have delineated the domain and residues in MeCP2 that mediate this binding, and ongoing work using iPSCs will help identify additional RNA targets that may be involved in neuronal dysfunction in RTT.

3. Session II: Control of MeCP2 (Over) Expression

MeCP2 is a dosage-sensitive gene, and achieving its optimum expression is necessary for the success of therapeutic approaches aimed at restoring it. This session delved deeper into this issue and also continued with exploration of molecular consequences of MeCP2 dysfunction.

Dr. Huda Zoghbi (Baylor College of Medicine, USA) gave the keynote address for this session, and discussed how varied expression levels of MeCP2 contribute to the spectrum of disease severity and the therapeutic implications this has on ameliorating the symptoms of RTT [20]. Using various approaches to modulate its expression levels, her lab has shown that the brain is very sensitive to changes in MeCP2 expression levels, and even partial restoration of the protein can result in improvement of subsets of outcomes [21]. Studies with a unique missense mutation, G118E, as well as with the e1 and e2 isoforms of MeCP2, have demonstrated how dosage and functional deficits in MeCP2 contribute to disease presentation. In addition, she presented recent data on MDS showing how different chromosomal rearrangements that result in *MECP2* copy number increases correlate with molecular and phenotypic severity. Finally, based on work with training protocols in RTT mice, Dr. Zoghbi drew parallels with considerations that should be taken into account for interventions in RTT patients [22].

Dr. Hume Stroud's (University of Texas Southwestern Medical Center, USA) presentation focused on emerging data from his lab that showed novel genomic binding sites for MeCP2. These sites, scattered across the genome, have revealed that, contrary to the well-known mCA residues that are bound by MeCP2, they are not methylated. These sites have been named MeCP2 Binding Hotspots (MBHs). Ongoing work is aimed at investigating their relevance and contribution to the functional repertoire of MeCP2 and development of RTT.

Dr. Vania Broccoli (San Raffaele Scientific Institute, Italy) described his group's efforts to achieve optimal expression of virally delivered *Mecp2* in mouse brains through the use of an instability-prone *Mecp2* transgene (*iMecp2*) packaged inside a neurotropic AAV-PHP.eB capsid [23]. The instability of MeCP2 messenger RNA (mRNA), achieved by engineering a short 3'-UTR, helps decrease protein amounts to physiological levels. When injected into symptomatic RTT mice, the PHP.eB-iMecp2 virus was able to increase lifespan and rescue behavioral deficits in both male and female mice. However, a strong immune response was observed in male mice and necessitated the concomitant use of immunosuppression.

Dr. James Ellis (SickKids, Canada) presented his recent findings on how global, steady-state mRNA changes seen in RTT are not solely attributable to the rate of transcription, but also involve post-transcriptional buffering that alters mRNA half-life [24]. His lab has used RATEseq on patient iPSC-derived neurons to show that microRNA and RNA-binding Protein (RBP) motifs in the 3'UTRs of genes are involved in this fine-tuning of steady-state mRNA levels. Dr. Ellis also discussed the characterization of extracellular vesicles (EVs) released from astrocytes, and the presence of specific miRNAs in these EVs that may potentially serve as RTT biomarkers [25].

Dr. Lisa Boxer (National Institutes of Health, USA) talked about the generation of knock-in mice harboring a tagged, degradable MeCP2 isoform to study the molecular consequences of MeCP2 acute loss. MeCP2 was tagged at the C-terminus with a degradation tag (dTAG) that allowed its degradation upon dTAG-13 treatment as early as 30 mins in cultured neurons and within 3 hrs *in vivo* in knock-in mice. Surprisingly, gene expression analysis in cultured neurons did not show any significant changes 1-72 hours post degradation of MeCP2, but possible reduction in nuclear size was observed. Gene expression analysis *in vivo* in the hippocampus revealed some gene expression changes 24 and 72 hours after MeCP2 degradation. Ongoing studies are aimed at exploring and clarifying other acute consequences of MeCP2 degradation.

Perspective: Presentations at these two sessions centered around the traditional question of "what does MeCP2 do", a seemingly simplistic question that has fueled more than 3000 publications since the MeCP2 protein was identified [26]. Every time a conclusion is drawn, it instantly elicits more questions. At one point, MeCP2 was demonstrated to bind to chromatin broadly across the genome [27–29]. At these sessions, distinct binding of MeCP2 at promoter regions, MBHs, and even methylated RNAs were presented, demanding additional work to address the discrepancies. The subtlety of gene expression changes, however, seems to stand, from the early microarray studies [30], examination at specific cellular context [31], to the new evidence presented at these sessions upon acute or adult loss of MeCP2, and even considering post-transcriptional compensation. Finally, the clinical report of MDS [32] has prompted numerous studies to understand the regulation of MeCP2 expression levels, and intriguing new avenues have been shared at these sessions to achieve an appropriate control of MeCP2 expression. MeCP2 remains a fascinating molecule to investigate and understand in the years to come.

4. Session III: MeCP2 Outside of its Comfort Zone

This session continued with presentations to understand MeCP2 function at molecular and cellular levels and beyond what have been discussed in the past, as well as studies that are looking closely at MeCP2's interaction with DNA and nucleosomes.

Dr. Nathaniel Heintz (Rockefeller University, USA) discussed his lab's long-standing interest in the study of 5-hydroxymethylcytosine (5hmC) in the neuronal genome, and its relationship to MeCP2 binding [33–35]. Work from his group has demonstrated a crucial role for 5hmC in DNA demethylation, and, more recently, in aging. It was found that 5mCG sites in the gene bodies of expressed genes, which have high-affinity for MeCP2, are oxidized to low-affinity 5hmCG sites, thereby causing MeCP2 to be lost from these sites, derepressing transcription of those genes. On the other hand, MeCP2 binds strongly to both 5mCA and 5hmCA, and conversion of 5mCA to this hydroxymethylated form does not alter MeCP2 binding and gene transcription. Therefore, Dr. Heintz suggests that the 5hmC modification sculpts MeCP2's ability to bind DNA and fine-tunes gene expression for proper neuronal differentiation. Further, single molecule imaging in live cells was used to investigate the nuclear dynamics of MeCP2, and it was found that its mobility is strongly influenced by the presence of 5mC sites and differs among distinct neuronal cell types. Finally, emerging data from his lab suggests 5hmC progressively accumulates in neurons during aging, which relaxes DNA and reduces MeCP2 binding. How this process is involved in RTT pathology remains to be studied.

Dr. Janine LaSalle (University of California Davis, USA) discussed the contribution of the gut microbiome and metabolome to disease progression in RTT [36]. Using an MeCP2-e1 mutant mouse model, analysis of fecal samples revealed perturbations in gut microbiota as well as metabolites. These changes showed a high degree of correlation with lipid deficiency in the RTT brain and the neuromotor deficits seen in mice. Further, distinct differences in gut profiling were observed between male and female mice, with the latter showing a pattern more consistent with what is seen in RTT patients.

Dr. Rocco Gogliotti (Loyola University, USA) presented profiling studies conducted in 40 autopsy samples from RTT patients and identified downregulation of the muscarinic acetylcholine receptor subtype 1 (M1) [37, 38]. To test if M1 is a disease-relevant target, an M1 positive allosteric modulator (PAM), VU595 was used to treat MeCP2 heterozygous female mice and resulted in phenotypic rescue in the animals. Further analysis on patient samples revealed that typical and atypical RTT cases share molecular changes in the Heat-Shock (HS) signaling pathway. Ongoing studies are aimed at determining whether up-regulation of the HS cascade is consequential or causative for RTT pathology.

Ms. Gabriella Chua's (Rockefeller University, USA) talk focused on the use of a single-molecule platform that employs correlative fluorescence and force microscopy to investigate how MeCP2 dynamically interacts with methylated DNA and nucleosomes [39]. These studies have shown that DNA methylation limits MeCP2's otherwise diffusive movement on unmethylated DNA and that nucleosome-binding is preferred by MeCP2, an interaction that is impacted by the co-binding of the histone H1 protein. Many RTT patient-associated MeCP2 mutations were found to interfere with MeCP2's ability to navigate DNA and bind nucleosomes.

Perspective: Sometimes it might be tempting to think, "What doesn't MeCP2 do?", and each year finds our scientific community uncovering new layers of regulation, pathways, and roles for this ubiquitous and important protein in cellular function. MeCP2's potential involvement in aging had the audience thinking beyond RTT, and new techniques to visualize and appreciate MeCP2's movements on DNA painted a picture that all could visualize and appreciate. As more scientists are drawn to the study of MeCP2, we anticipate that additional roles for this protein beyond those of traditional transcriptional regulation will be uncovered.

5. Session IV: Insights from affected families and other disorders

There has been remarkable progress in the development of genetic approaches for treating many complex neurodevelopmental and non-neurodevelopmental disorders. This session brought together speakers with expertise in these disorders and focused on sharing insights that can be applied towards development and advancement of RTT therapeutics.

In addition, the session featured a discussion with three caregivers of RTT patients, **Caregiver Panel: Living with Rett Syndrome**, that highlighted the day-to-day experience and challenges of individuals and their families living with RTT. Different ages and genders of patients were represented in the panel to enable a broad understanding of RTT presentation and exposed the basic science research community to important patient and caregiver issues. These included topics pertaining to diagnostic journey, navigating and managing the complexity of care, advocating for their loved ones, challenges with daily care and communication, as well as the expectations and preferences the families have for treatment avenues. Hearing directly about the lived experience of RTT and the impact it has on the families was an immensely valuable opportunity for the audience to gain a new perspective on the disease, and one that can be looked back upon to motivate future research and clinical trials.

Dr. Katherine High (Rockefeller University, USA) provided a comprehensive overview of gene therapies currently approved by the FDA and/or EMA for genetic disorders and highlighted how learnings from these studies can be used to inform similar approaches for RTT [40–43]. The talk also delved deeper into the challenges unique to CNS disorders, and particularly RTT. These include issues relating to crossing the blood brain barrier, achieving homogeneous viral vector transduction in brain tissue, the need for biomarker development to characterize the efficacy of transduction, as well as the need to regulate the level of expression of the MeCP2 transgene within a tight therapeutic range. To address this last issue, Dr. High went into further exploration of gene editing, base and prime editing and Xi reactivation as approaches that can leverage endogenous regulatory signals to modulate

expression of reconstituted MeCP2. These strategies are, however, currently not advanced enough for clinical application. She also recommended that, in order to achieve regulatory success, it is useful to adopt an iterative approach to testing new genetic therapies by carefully studying each patient and identifying the right subset of the patient population to enroll in a clinical trial.

Dr. Darcy Krueger (Cincinnati Children's Hospital, USA) talked about the possibility of utilizing preventative therapeutics in rare genetic disorders to ameliorate the progression of disease in patients and improve outcomes [44–46]. Ongoing trials with Tuberous Sclerosis Complex (TSC) patients are testing if early diagnosis, prior to onset of seizures, and coupling with other therapeutic interventions can make a positive impact on the clinical course of the disease, including cognitive function and management of otherwise often refractory epilepsy. For RTT, robust natural history data, such as data that IRSF has collected over years mainly through the NIH-funded US Natural History Study HD-0061222, are crucial for the identification of early symptoms to guide strategies for timely treatment options.

Dr. Richard Finkel's (St. Jude Children's Research Hospital, USA) presentation focused on strategies that have laid the groundwork for successful clinical trials in Spinal Muscular Atrophy (SMA), including robust trial readiness, flexibility during the trial process, and regular communication with the FDA to obtain timely and relevant guidance for achieving drug-approval [47, 48]. He emphasized the importance of selecting the right outcome measures, including capturing patient-reported outcomes, as they can be clinically meaningful. Dr. Finkel also discussed the post-approval landscape and the value of collecting longitudinal real-world data in a broader population of treated patients to learn the long-term impact of the treatment and safety profile on the natural history of the disease.

Ms. Keri Ramsey (University of Arizona Health Sciences, USA) described her work on gathering caregiver input on gene therapy as an emerging therapeutic possibility for RTT. She conducted a survey and convened a focus group to collect data on topics such as families' extent of gene therapy awareness and understanding, assessing the level of risk that would be acceptable, openness to considering gene therapy for the patient, and expectations from the treatment. Adult caregivers of RTT patients living in the USA participated in the study, and recruitment was supported by IRSF through its my Rett Trial Finder (mRTF) tool. Though a majority of participants were familiar with gene therapy, the study highlighted concerns about unknown side effects, cost and logistics amongst families.

Perspective: Therapies for RTT are at an inflection point with the possibility of many gene-based and small molecules going to clinical trial and, hopefully, to individuals with RTT to improve their functioning and quality of life. Session IV highlighted the costs of RTT/MECP2 related disorders by having parents describe their experiences living with children with the condition and how they are yearning for effective therapies. While all physicians, scientists, care givers want therapies, there is also a need to perform trials correctly and efficiently. In this session, we heard from 3 leaders about their experiences in developing gene-based therapies and small molecules. While neuromuscular and retinal conditions have been successful, these programs took time, on-the-fly adjustments and persistence by companies and physicians to see these through to the end. These talks put the entire meeting in perspective by showing the burden of RTT and the complexities of obtaining drug approvals.

6. Session V: Innovative Therapies

Recent years have seen the emergence of a variety of genetic approaches for the treatment of monogenetic disorders that aim to restore the WT gene product in cells. This session featured presentations on such strategies being developed for RTT, including X-chromosome reactivation, RNA editing and epigenetic editing.

In her keynote presentation, **Dr. Jeannie Lee** (Massachusetts General Hospital, Harvard Medical School, USA) talked about the feasibility of reactivating the silent copy of *MECP2* that resides on the inactive X-chromosome (Xi) in female patients as a treatment option for RTT [49]. By coupling decitabine-mediated inhibition of DNA methylation with ASO-mediated Xist RNA silencing in cells, robust upregulation of MeCP2 from Xi was observed [50, 51]. Dr. Lee is currently testing this strategy *in vivo* and preliminary evidence indicates a substantial MeCP2 reactivation leading to marked phenotypic improvements in female mice. To further clarify how much reactivation would be therapeutically beneficial, Dr. Lee has been assessing the range of normal MeCP2 expression levels across different cell types in the human brain in the general population. Her findings have revealed significant variability in expression across cell types and suggest that achieving low levels of reactivation of the normal copy of *MECP2* may be sufficient to ameliorate disease symptoms.

Dr. Ronald Emeson (Vanderbilt University, USA) described the approach his lab is undertaking to edit mutated residues in *MECP2* RNA to restore functional protein activity. This approach leverages the endogenous RNA-editing machinery of cells that employs enzymes called Adenosine Deaminases Acting on RNA (ADARs) that edit adenosine bases to inosine in RNAs, with the latter serving as a guanosine mimic during translation. The approach is advantageous because any observed off-target effects are temporary, it does not require packaging and delivery of large components, and can be used in non-dividing neurons. However, it is limited to repairing $G > A$ mutations, which converts missense amino acids back to the wild-type version; as a result, this approach is only applicable to a very small subset of commonly occurring RTT mutations. To expand the application of his approach, Dr. Emeson is assessing whether conversion of nonsense mutations (UGA) to missense tryptophan codons (UGI), would be a viable therapeutic option, as this would allow repair of many common RTT mutations where a stop codon is present.

Dr. Yuval Tabach (The Hebrew University, Israel) presented his group's work on identifying novel drug targets for RTT using a comparative genomics approach [52]. Phylogenetic profiling was performed to map the *MECP2* gene network and uncovered 33 proteins that have co-evolved with MeCP2. Further analysis into which candidates can be targeted by existing FDA-approved drugs identified IRAK, KEAP1 and EPOR as promising leads. 3 drugs, namely Pacritinib, DMF and EPO, that respectively target these proteins were found to rescue aberrant NF-kB signaling and phenotypic deficits *in vitro*.

Dr. Julian Halmai (University of California Davis, USA) discussed development of a modified Cas9 system to facilitate epigenetic editing of the *MECP2* promoter on the silenced X-chromosome. This approach has been tested in a cell line expressing CDKL5, mutations of which cause *CDKL5* deficiency disorder [53]. This system employs a novel, miniaturized version of the Cas editor to overcome size limitations of the payload for packaging into AAV capsids. By enabling editing of DNA methylation on the promoter, this approach aims to induce MeCP2 expression akin to endogenous mechanisms that allow expression of escape genes located on the Xi.

Dr. Andrew Dietz (Shape Therapeutics, USA) described the company's AAVid^{TM} capsid discovery platform, which is geared towards identifying next-generation AAV capsids that demonstrate novel tissue tropism and targeted biodistribution. The approach involves delivering massively diverse AAV capsid libraries directly to non-human primates (NHPs), recovering variants from dozens of tissues, and using machine learning (ML) to identify tissue-specific sequences. Secondary screening validation of empirically observed candidates and *de novo* ML-generated variants identified novel capsids that exhibit significant CNS targeting and liver detuning relative to wildtype AAVs when delivered systemically to NHPs. Shape Therapeutics aims to use this approach to help pave the way for the development of next-generation AAV capsids with improved targeting abilities for gene therapy applications.

Perspective: 2023 saw the approval of the first drug treatment for RTT, trofinetide (DAYBUE^{TM}) and the first patient dosed with gene therapy. It is not an understatement to say that the energy of this

conference was one of profound hope. The RTT pipeline extends beyond these encouraging approaches and complements them with multiple strategies spanning X chromosome reactivation, RNA and DNA editing, drug repurposing, capitalization on MeCP2's downstream targets, and harnessing pathways that might be exploited therapeutically. These are exciting times in RTT and MeCP2 research, and next year's conference promises to provide critical and important updates on all of these potential therapeutic approaches.

7. Session VI: Outcome Measure and Biomarkers

Developing and validating reliable and relevant outcome measures is a cornerstone of clinical trial readiness. Additionally, identification of robust biomarkers that can predict if a therapeutic intervention has the potential to succeed is highly desirable. This session focused on topics that revolve around outcome measure selection and exploration of novel biomarkers for RTT.

Dr. Michelle Campbell (FDA Center for Drug Evaluation and Research, USA) talked about the crucial role that patient-focused data play in shaping the drug development process for rare diseases. This involves collecting and utilizing patient input throughout the life cycle of the process, starting from engagement during the early research and discovery phase through clinical trials and post-approval. A patient-focused drug development (PFDD) approach ensures that a treatment under development addresses the patients' needs in the most meaningful way, which in turn is a key consideration during regulatory decision making. Dr. Campbell discussed PFDD guidance documents that address how to best approach data collection, including choosing participants and methodology, selecting and framing the right questions, developing appropriate clinical outcome assessments (COAs), and incorporating them into trial endpoints. She emphasized the importance of balancing patient expectations to achieve a clear understanding of a treatment's mechanism of action, its potential impact on the disease, and what is important to patients. Failure to do so can result in collection of irrelevant data and negatively impact the treatment's approval. Dr. Campbell also talked about the use of various biomarkers during different stages of drug development, such as for assessing proof-of-concept data, diagnostics, monitoring safety, and establishing efficacy endpoints including surrogate and supportive endpoints.

Dr. Michela Fagiolini (Boston Children's Hospital, USA) presented her studies on using the visual impairment associated with RTT as a tool to study the disease, and targeting the choroid plexus for therapeutic intervention [54–56]. Recent data from her lab have uncovered aberrant visual cortical activity prior to regression in RTT mice, and the hyperactivation of inhibitory circuits during early development [56]. Mechanistically, her findings implicate enhanced orthodenticle homeobox 2 (Otx2) production in the choroid plexus and cerebrospinal fluid (CSF) as a non-cell autonomous contributor to disease progression and suggest that targeting Otx2 could be a potentially new treatment avenue.

Dr. Victor Faundez's (Emory University, USA) talk focused on the insights gained from studying brain, other organs and CSF proteomes in RTT and identifying potential biomarkers for the disease [57–59]. His studies have revealed a surprising divergence between transcriptomic and proteomic profiles across brain regions in RTT mice and suggest that analyzing the CSF proteome as a means to investigate by proxy molecular changes in the RTT brain may be the most relevant process to identify disease biomarkers. Studying the CSF offers several advantages since it carries secretions from different regions of the CNS, is clinically accessible, can be sampled repeatedly, and its proteome can be harnessed for identifying different types of biomarkers. Finally, these studies have uncovered the contribution of aberrant synaptic function, lipid metabolism, and mitochondrial pathways to the systemic development of disease in RTT.

Dr. Jonathan Merritt (Vanderbilt University Medical Center, USA) discussed his research on developing a predictive model of RTT severity across different ages and *MECP2* mutations. For this,

data on Clinical Severity Scores (CSS) from the large, multi-year Rett Syndrome Natural History Study (RNHS) were utilized to build disease trajectories [60]. These trajectories, or "growth curves", allow meaningful comparison of disease severity in different patients, and also permit analysis of other factors that could be contributing to the progression of CSS, including the degree of mosaicism of X-chromosome inactivation in female patients and the BDNF genotype.

Perspective: These talks spanned clinical outcome measures (COMs) and biological markers of disease. They highlighted the need to have COMs that are ready for use in the clinic for trials but also the need to potentially adjust these if the granularity of any measure is not sufficient to track changes with disease. Similarly, having biological markers to supplement and support COMs is vital and both small molecules and physiological measures are being developed and need continued work to ensure they are ready for use in the next generation of clinical trials.

8. Session VII: Treatments on the horizon for Rett syndrome

This session featured presentations on the clinical trials landscape for RTT, including treatments in late stages of development, upcoming and ongoing clinical studies, as well as the keynote address from Acadia Pharmaceuticals on the journey to approval of DAYBUE™ (trofinetide) for the treatment of RTT.

Dr. James Youakim (Acadia Pharmaceuticals, USA) opened the session with his keynote address on the development of the first and only FDA-approved treatment of RTT, DAYBUE™ (trofinetide), from phase 2 studies through the approval. Trofinetide is a synthetic analog of the peptide GPE. GPE, which is naturally present in the brain, is generated by the cleavage of insulin growth factor-1 (IGF-1), and was shown to improve disease symptoms in RTT mice [61]. Phase 2 trials with trofinetide were conducted in 2 parts, and enrolled a total of 138 participants, including both pediatric and adult female RTT patients [62, 63]. The treatment was generally safe, with diarrhea being the most common side-effect, and provided evidence of efficacy as measured by improvements on the Rett Syndrome Behaviour Questionnaire (RSBQ) and Clinical Global Impression – Improvement (CGI-I) scales. This opened the door for the phase 3 study, LAVENDER™ [1, 64]. Based on learnings from phase 2 data, the dosing regimen was revised to fixed doses in order to achieve target exposure. The LAVENDER™ trial was a 12-week, randomized, double-blind, placebo-controlled study in female RTT patients 5 to 20 years of age. It employed RSBQ and CGI-I scores as co-primary endpoints, and the Communication and Symbolic Behavior Scales Developmental Profile Infant-Toddler checklist (CSBS-DP-IT) Social Composite as the secondary endpoint. The LAVENDER™ study was subsequently extended to the open-label LILAC and LILAC-2 studies that spanned a period of 40 weeks and up to 32 months respectively. The DAFFODIL study was designed for patients 2-4 years of age with the goal of assessing safety and drug exposure data. Efficacy measurements using RSBQ and other scales were deemed unfit due to the rapidly variable nature of symptoms during the regression period of the disease, and instead similarity of trofinetide exposure to the LAVENDER study was assessed to extrapolate efficacy. A New Drug Application for trofinetide was filed in July 2022, and received FDA-approval on March 10, 2023 under the name DAYBUE™. DAYBUE™ is indicated for the treatment of RTT in adults and pediatric patients 2 years of age and older.

Dr. Benit Maru and Ms. Emily McGinnis (Taysha Gene Therapies, USA) provided updates on the company's investigational gene therapy program in clinical evaluation for RTT. Recruitment and dosing for a phase 1/2 trial in Canada are currently underway (REVEAL study) to assess safety and preliminary efficacy in adult female patients with RTT. Named TSHA-102, the self-complementary intrathecally delivered AAV9 gene therapy candidate is designed to regulate MeCP2 expression through the incorporation of a novel miRNA-Responsive Auto-Regulatory Element (miRARE) platform, and

utilizes a *miniMECP2* transgene. Dosing of TSHA-102 in the first patient was announced on June 5, 2023 and marks a significant milestone in being the first ever gene therapy to be evaluated for RTT. The talk also emphasized the importance of educating the patient community on gene therapy and fostering engagement throughout the development process.

Dr. Stuart Cobb and Dr. Albena Patroneva (Neurogene, USA) presented preclinical data and an overview of the clinical trial design for the evaluation of NGN-401, a gene therapy candidate for RTT. NGN-401 uses Neurogene's Expression Attenuation via Construct Tuning (EXACT) technology to regulate MECP2 transgene expression. In multiple preclinical models, NGN-401 delivered well-tolerated levels of the vector genome to cells containing normal levels of MeCP2 and delivered efficacious levels to MeCP2 deficient cells, while avoiding overexpression. The construct incorporates a non-mammalian miRNA that enables controlled MeCP2 levels through feedback binding of the miRNA to its complementary recognition sites. NGN-401 contains full-length human MECP2 and is administered intracerebroventricularly (ICV), a delivery method that has been shown to maximize vector distribution to key brain regions underlying RTT pathobiology. Earlier this year, Neurogene received FDA IND clearance to initiate a phase 1/2, open-label study to assess the safety, tolerability, and efficacy of NGN-401 in female pediatric subjects with typical RTT. The study will enroll five subjects ages 4 to 10 and will be conducted at three sites across the US.

Dr. Carolyn Ellaway (Sydney Children's Hospital Network, Australia) talked about a phase 1/2 clinical trial being planned for RTT that will study NTI164, a full spectrum medicinal cannabis-derived treatment. Its low THC level is well-suited for pediatric use and is being developed by Neurotech International Ltd. for the treatment of neuro-inflammatory disorders in children. A Phase 1/2 trial was conducted in autism spectrum disorder (ASD) and showed that NTI164 is safe for long-term administration and resulted in significant reduction in ASD severity. The RTT trial will include female participants between 5-18 years of age and assess safety as well as improvements in the CGI-I scores for Part I of the study which is expected to begin in August 2023 in Australia. This open-label study will be followed by an adaptive, double-blind, placebo-controlled and randomized Part II study in the first half of 2024.

Dr. Jeffrey Neul (Vanderbilt University Medical Center, USA) discussed a novel umbrella design for a clinical study in RTT involving repurposed drugs [65]. The study, which is funded by a Department of Defense CDMRP grant awarded in 2023, will be a phase 2a, double-blind, randomized trial to evaluate the safety and efficacy of three FDA-approved drugs – ketamine [66], vorinostat [67] and donepezil [68], using a single placebo arm. By utilizing a common placebo treatment arm, the study allows simultaneous examination of three drugs while reducing participant numbers, cost, and time, an approach that is particularly advantageous for a rare disease like RTT. The first year of the study will involve a planning phase for finalizing the trial design and obtaining IND approval for the three drugs. This will be followed by a 4 year clinical trial phase that will enroll female patients between the ages of 5-12 years at 4 trial sites across the US.

Dr. Kathrin Meyer's (Nationwide Children's Hospital, USA) talk focused on ACTX-101, an X-chromosome reactivating approach invented by Dr. Sanchita Bhatnagar (formerly at the University of Virginia) and Dr. Meyer, which is now being developed by Alcyone Therapeutics for the treatment of RTT. ACTX-101 consists of a miRNA sponge payload packaged inside an AAV9 vector. The miRNA sponge, miR106sp, acts by binding to and sequestering miR106a, a miRNA that interacts with Xist and interferes with its function in X chromosome inactivation (XCI). XCI is an epigenetic mechanism that randomly inactivates one of the female X chromosomes. Inhibition of miR106a function in XCI leads to gene accessibility on the inactive X chromosome. Preclinical studies have demonstrated the ability of AAV9-miR106sp to express MeCP2 from inactive X chromosomes and positively impact behavior, breathing, and physical health in RTT mice. Additional studies conducted in mice and NHPs have demonstrated the safety and tolerability of the treatment over a period of several months.

Dr. Frederic Vigneault (Unravel Biosciences, USA) described a novel drug discovery platform that combines an AI-based prediction tool called BioNAV^{TM} with *in vivo* screening in a *Xenopus* model [67]. BioNAV^{TM} utilizes RNA network analysis and computational screening of a library with more than 40,000 compounds to predict compounds that will effectively restore the network back to health. A *Xenopus laevis* model, mosaic for MeCP2 expression, was generated and characterized for behavioral and motor deficits, including seizures. BioNAV^{TM} screening identified vorinostat, an HDAC inhibitor, as a potential target for treating RTT. Vorinostat proved efficacious when tested in both *Xenopus* and mouse models, both showing improvements in both CNS and non-CNS phenotypes. The company has developed a novel small molecule, RVL002, based on the metabolite of vorinostat. Mechanistic studies in mice have shown that RVL002 acts in an HDAC-independent manner and may work by normalizing acetylation and fatty acid metabolism. Unravel is planning an exploratory, proof-of-concept trial in Colombia in RTT patients, starting in the second half of 2023, to validate the new mechanism of action and enable RVL002 commercial development.

Dr. Nicholas Tonks (Cold Spring Harbor Laboratory, USA) talked about protein tyrosine phosphatase 1B (PTP1B) inhibition as a therapeutic approach for RTT [69]. PTP1B is a phosphatase that is negatively regulated at the transcriptional level by MeCP2 and antagonizes protein tyrosine phosphorylation-dependent signaling, including the activity of tropomyosin receptor kinase B (TrkB), the receptor for BDNF. Elevated PTP1B activity is seen in RTT and its inhibition with DPM-1003, a small molecule drug candidate being developed by DepYmed Inc, has been shown to improve various symptoms in RTT model mice, including enhanced rotarod performance, ameliorated apnea frequency and duration, and improvement of the QTc interval profile. DPM-1003 has been granted Orphan Drug designation in the US and Europe and a Rare Pediatric Disease Designation in the US, and DepYmed is preparing to move into the clinic with this mechanism-based therapeutic approach to treating RTT.

Perspective: The RTT field has come a long way since the first description of RTT as a distinct disorder by Dr. Andreas Rett in 1966. The blooming and expanding landscape of treatments under development presented at this session is a direct testament to the tireless and collaborative efforts of families, clinicians, and scientists over the past several decades. These efforts have resulted in orchestration of resources, accumulation of knowledge, and development of innovative strategies towards the common goal of finding solutions for all those affected by RTT. We hope that the infrastructure that has been created will continue to attract new ideas and players to the field ensuring that there is a robust therapeutic pipeline for RTT in the years to come.

9. Conclusions

The science behind MeCP2 and RTT has advanced tremendously since the genetic cause was discovered, with a dramatic increase in interest in research, drug development and gene therapy. These efforts have led to several firsts for the Rett community in 2023, including approval of trofinetide as the first treatment for RTT, initiation of the first gene therapy trial in RTT adult patients, and announcement of the first gene therapy trial to be conducted in children. As we recognize these energizing achievements, we must also remember that we are still far from a world without RTT and much more remains to be done. The complexity and variability inherent to RTT necessitate development of multiple therapies, each working in a different way to target the many changes that occur due to the loss of MeCP2. The 2023 IRSF Rett Syndrome Scientific Meeting underscored this urgency and helped bring together scientists and clinicians, particularly trainees, from around the world to share their work and brainstorm ideas that will streamline our knowledge of RTT and ultimately result in effective treatments, including cures, over the finish line.

Acknowledgments

The authors are grateful to James Eubanks (Krembil Research Institute, Canada) and Jean-Christophe Roux (Aix Marseille University, France) for their help with planning and organizing the conference. We thank all the speakers, poster presenters and participants for attending and contributing to scientific discussions. IRSF acknowledges the generous support from Acadia Pharmaceuticals, Taysha Gene Therapies, Anavex Life Sciences, Shape Therapeutics, DepYmed, EveryLife Foundation, Hive Networks, Neuren Pharmaceuticals, Neurogene, DiamiR and mejo for sponsoring the conference. IRSF is also thankful to its Board of Directors and all the donors and families for their support in making this event possible.

References

[1] J.L. Neul et al., Trofinetide for the treatment of Rett syndrome: a randomized phase 3 study, *Nature Medicine* (2023).
[2] Collins, B.E. and Neul, J.L., Trofinetide. Glycine-proline-glutamate (GPE) analogue, Treatment of Rett syndrome, Treatment of fragile X syndrome, *Drugs Future* **46**(1) (2021), 29, doi: 10.1358/dof.2021.46.1.3208246.
[3] S.J. Keam, Trofinetide: First Approval, *Drugs* **83**(9) (2023), 819–824, doi: 10.1007/s40265-023-01883-8.
[4] R.E. Amir, I.B. Van den Veyver, M. Wan, C.Q. Tran, U. Francke and H.Y. Zoghbi, Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2, *Nat. Genet.* **23**(2) (1999), 185–188, doi: 10.1038/13810.
[5] M. Chahrour and H.Y. Zoghbi, The story of Rett syndrome: from clinic to neurobiology, *Neuron* **56**(3) (2007), 422–437, doi: 10.1016/j.neuron.2007.10.001.
[6] B.E. Collins and J.L. Neul, Rett syndrome and MECP2 duplication syndrome: disorders of mecp2 dosage, *Neuropsychiatr. Dis. Treat.* **18** (2022), 2813–2835, doi: 10.2147/NDT.S371483.
[7] A.J. Sandweiss, V.L. Brandt and H.Y. Zoghbi, Advances in understanding of Rett syndrome and MECP2 duplication syndrome: prospects for future therapies, *Lancet Neurol.* **19**(8) (2020), 689–698, doi: 10.1016/S1474-4422(20)30217-9.
[8] D. Ta, J. Downs, G. Baynam, A. Wilson, P. Richmond and H. Leonard, A brief history of MECP2 duplication syndrome: 20-years of clinical understanding, *Orphanet J. Rare Dis.* **17**(1) (2022), 131, doi: 10.1186/s13023-022-02278-w.
[9] M.B. Ramocki, Y.J. Tavyev and S.U. Peters, The MECP2 duplication syndrome, *Am. J. Med. Genet. A* **152A**(5) (2010), 1079–1088, doi: 10.1002/ajmg.a.33184.
[10] Y.M. Vuu, C.-T. Roberts and M. Rastegar, MeCP2 Is an Epigenetic Factor That Links DNA Methylation with Brain Metabolism, *Int. J. Mol. Sci.* **24**(4) (2023), doi: 10.3390/ijms24044218.
[11] C. Fu et al., Multisystem comorbidities in classic Rett syndrome: a scoping review, *bmjpo* **4**(1) (2020), e000731, doi: 10.1136/bmjpo-2020-000731.
[12] J.C. Cronk, N.C. Derecki, V. Litvak and J. Kipnis, Unexpected cellular players in Rett syndrome pathology, *Neurobiol. Dis.* **92**(Pt A) (2016), 64–71, doi: 10.1016/j.nbd.2015.05.005.
[13] S.M. Kyle, N. Vashi and M.J. Justice, Rett syndrome: a neurological disorder with metabolic components, *Open Biol.* **8**(2) (2018), doi: 10.1098/rsob.170216.
[14] Y. Li et al., Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons, *Cell Stem Cell* **13**(4) (2013), 446–458, doi: 10.1016/j.stem.2013.09.001.
[15] J. Qian et al., Multiplex epigenome editing of MECP2 to rescue Rett syndrome neurons, *Sci. Transl. Med.* **15**(679) (2023), eadd4666, doi: 10.1126/scitranslmed.add4666.
[16] Y. Sztainberg et al., Reversal of phenotypes in MECP2 duplication mice using genetic rescue or antisense oligonucleotides, *Nature* **528**(7580) (2015), 123–126, doi: 10.1038/nature16159.
[17] Y. Shao et al., Antisense oligonucleotide therapy in a humanized mouse model of MECP2 duplication syndrome, *Science Translational Medicine* (2021).
[18] M. Müller, Disturbed redox homeostasis and oxidative stress: Potential players in the developmental regression in Rett syndrome, *Neurosci. Biobehav. Rev.* **98** (2019), 154–163, doi: 10.1016/j.neubiorev.2018.12.009.
[19] G. Golubiani, V. Lagani, R. Solomonia and M. Müller, Metabolomic Fingerprint of Mecp2-Deficient Mouse Cortex: Evidence for a Pronounced Multi-Facetted Metabolic Component in Rett Syndrome, *Cells* **10**(9) (2021), doi: 10.3390/cells10092494.
[20] H.-T. Chao and H.Y. Zoghbi, MeCP2: only 100% will do, *Nat. Neurosci.* **15**(2) (2012), 176–177, doi: 10.1038/nn.3027.
[21] Y. Shao, S.S. Bajikar, H.P. Tirumala, M.C. Gutierrez, J.D. Wythe and H.Y. Zoghbi, Identification and characterization of conserved noncoding cis-regulatory elements that impact Mecp2 expression and neurological functions, *Genes Dev.* **35**(7–8) (2021), 489–494, doi: 10.1101/gad.345397.120.

[22] N.P. Achilly, W. Wang and H.Y. Zoghbi, Presymptomatic training mitigates functional deficits in a mouse model of Rett syndrome, *Nature* **592**(7855) (2021), 596–600, doi: 10.1038/s41586-021-03369-7.
[23] M. Luoni et al., Whole brain delivery of an instability-prone Mecp2 transgene improves behavioral and molecular pathological defects in mouse models of Rett syndrome, *eLife* **9** (2020), doi: 10.7554/eLife.52629.
[24] D.C. Rodrigues et al., Buffering of transcription rate by mRNA half-life is a conserved feature of Rett syndrome models, *Nat. Commun.* **14**(1) (2023), 1896, doi: 10.1038/s41467-023-37339-6.
[25] S. Gordillo-Sampedro et al., iPSC-derived healthy human astrocytes selectively load miRNAs targeting neuronal genes into extracellular vesicles, *BioRxiv* (2023), doi: 10.1101/2023.04.29.538761.
[26] R.R. Meehan, J.D. Lewis and A.P. Bird, Characterization of MeCP2, a vertebrate DNA binding protein with affinity for methylated DNA, *Nucleic Acids Res.* **20**(19) (1992), 5085–5092, doi: 10.1093/nar/20.19.5085.
[27] H.W. Gabel et al., Disruption of DNA-methylation-dependent long gene repression in Rett syndrome, *Nature* **522**(7554) (2015), 89–93, doi: 10.1038/nature14319.
[28] L. Chen et al., MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome, *Proc Natl Acad Sci USA* **112**(17) (2015), 5509–5514, doi: 10.1073/pnas.1505909112.
[29] S. Lagger et al., MeCP2 recognizes cytosine methylated tri-nucleotide and di-nucleotide sequences to tune transcription in the mammalian brain, *PLoS Genet.* **13**(5) (2017), e1006793, doi: 10.1371/journal.pgen.1006793.
[30] M. Tudor, S. Akbarian, R.Z. Chen and R. Jaenisch, Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain, *Proc Natl Acad Sci USA* **99**(24) (2002), 15536–15541, doi: 10.1073/pnas.242566899.
[31] B.S. Johnson et al., Biotin tagging of MeCP2 in mice reveals contextual insights into the Rett syndrome transcriptome, *Nat. Med.* **23**(10) (2017), 1203–1214, doi: 10.1038/nm.4406.
[32] H. Van Esch et al., Duplication of the MECP2 region is a frequent cause of severe mental retardation and progressive neurological symptoms in males, *Am. J. Hum. Genet.* **77**(3) (2005), 442–453, doi: 10.1086/444549.
[33] M. Mellén, P. Ayata, S. Dewell, S. Kriaucionis and N. Heintz, MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system, *Cell* **151**(7) (2012), 1417–1430, doi: 10.1016/j.cell.2012.11.022.
[34] M. Mellén, P. Ayata and N. Heintz, 5-hydroxymethylcytosine accumulation in postmitotic neurons results in functional demethylation of expressed genes, *Proc Natl Acad Sci USA* **114**(37) (2017), E7812–E7821, doi: 10.1073/pnas.1708044114.
[35] F.M. Piccolo et al., MeCP2 nuclear dynamics in live neurons results from low and high affinity chromatin interactions, *eLife* **8** (2019), doi: 10.7554/eLife.51449.
[36] K. Neier et al., Sex disparate gut microbiome and metabolome perturbations precede disease progression in a mouse model of Rett syndrome, *Commun. Biol.* **4**(1) (2021), 1408, doi: 10.1038/s42003-021-02915-3.
[37] R.G. Gogliotti et al., Total RNA sequencing of rett syndrome autopsy samples identifies the M4 muscarinic receptor as a novel therapeutic target, *J. Pharmacol. Exp. Ther.* **365**(2) (2018), 291–300, doi: 10.1124/jpet.117.246991.
[38] M. Smith et al., Clinical and preclinical evidence for M1 muscarinic acetylcholine receptor potentiation as a therapeutic approach for rett syndrome, *Neurotherapeutics* **19**(4) (2022), 1340–1352, doi: 10.1007/s13311-022-01254-3.
[39] G.N.L. Chua, J.W. Watters, P.D.B. Olinares, J.A. Luo, B.T. Chait and S. Liu, Differential dynamics specify MeCP2 function at methylated DNA and nucleosomes, *BioRxiv* (2023), doi: 10.1101/2023.06.02.543478.
[40] C.E. Dunbar, K.A. High, J.K. Joung, D.B. Kohn, K. Ozawa and M. Sadelain, Gene therapy comes of age, *Science* **359**(6372) (2018), doi: 10.1126/science.aan4672.
[41] X.M. Anguela and K.A. High, Entering the modern era of gene therapy, *Annu. Rev. Med.* **70** (2019), 273–288, doi: 10.1146/annurev-med-012017-043332.
[42] K.A. High, Turning genes into medicines-what have we learned from gene therapy drug development in the past decade?, *Nat. Commun.* **11**(1) (2020), 5821, doi: 10.1038/s41467-020-19507-0.
[43] K.Z. Reape and K.A. High, Trial by 'firsts': clinical trial design and regulatory considerations in the development and approval of the first AAV gene therapy product in the united states, *Cold Spring Harb. Perspect. Med.* **13**(5) (2023), doi: 10.1101/cshperspect.a041312.
[44] D.A. Krueger et al., Short-term safety of mTOR inhibitors in infants and very young children with tuberous sclerosis complex (TSC): Multicentre clinical experience, *Eur. J. Paediatr. Neurol.* **22**(6) (2018), 1066–1073, doi: 10.1016/j.ejpn.2018.06.007.
[45] K. Kotulska et al., Prevention of Epilepsy in Infants with Tuberous Sclerosis Complex in the EPISTOP Trial, *Ann. Neurol.* **89**(2) (2021), 304–314, doi: 10.1002/ana.25956.
[46] Y.-W. Shen et al., Sirolimus treatment for tuberous sclerosis complex prior to epilepsy: Evidence from a registry-based real-world study, *Seizure* **97** (2022), 23–31, doi: 10.1016/j.seizure.2022.03.003.
[47] R.S. Finkel et al., Treatment of infantile-onset spinal muscular atrophy with nusinersen: a phase 2, open-label, dose-escalation study, *Lancet* **388**(10063) (2016), 3017–3026, doi: 10.1016/S0140-6736(16)31408-8.

[48] R.S. Finkel et al., Treatment of infantile-onset spinal muscular atrophy with nusinersen: final report of a phase 2, open-label, multicentre, dose-escalation study, *Lancet Child Adolesc. Health* **5**(7) (2021), 491–500, doi: 10.1016/S2352-4642(21)00100-0.
[49] N.-B. Grimm and J.T. Lee, Selective Xi reactivation and alternative methods to restore MECP2 function in Rett syndrome, *Trends Genet.* **38**(9) (2022), 920–943, doi: 10.1016/j.tig.2022.01.007.
[50] L.L.G. Carrette et al., A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders, *Proc Natl Acad Sci USA* **115**(4) (2018), E668–E675, doi: 10.1073/pnas.1715124115.
[51] L.L.G. Carrette, R. Blum, W. Ma, R.J. Kelleher and J.T. Lee, Tsix-Mecp2 female mouse model for Rett syndrome reveals that low-level MECP2 expression extends life and improves neuromotor function, *Proc Natl Acad Sci USA* **115**(32) (2018), 8185–8190, doi: 10.1073/pnas.1800931115.
[52] I. Unterman et al., Expanding the MECP2 network using comparative genomics reveals potential therapeutic targets for Rett syndrome, *eLife* **10** (2021), doi: 10.7554/eLife.67085.
[53] J.A.N.M. Halmai et al., Artificial escape from XCI by DNA methylation editing of the CDKL5 gene, *Nucleic Acids Res.* **48**(5) (2020), 2372–2387, doi: 10.1093/nar/gkz1214.
[54] S. Durand et al., NMDA receptor regulation prevents regression of visual cortical function in the absence of Mecp2, *Neuron* **76**(6) (2012), 1078–1090, doi: 10.1016/j.neuron.2012.12.004.
[55] J.J. LeBlanc et al., Visual evoked potentials detect cortical processing deficits in Rett syndrome, *Ann. Neurol.* **78**(5) (2015), 775–786, doi: 10.1002/ana.24513.
[56] A. Patrizi, P.N. Awad, B. Chattopadhyaya, C. Li, G. Di Cristo and M. Fagiolini, Accelerated Hyper-Maturation of Parvalbumin Circuits in the Absence of MeCP2, *Cereb. Cortex* **30**(1) (2020), 256–268, doi: 10.1093/cercor/bhz085.
[57] V. Faundez, M. Wynne, A. Crocker and D. Tarquinio, Molecular systems biology of neurodevelopmental disorders, rett syndrome as an archetype, *Front. Integr. Neurosci.* **13** (2019), 30, doi: 10.3389/fnint.2019.00030.
[58] S.A. Zlatic et al., Convergent cerebrospinal fluid proteomes and metabolic ontologies in humans and animal models of rett syndrome, *BioRxiv* (2021), doi: 10.1101/2021.11.30.470580.
[59] S.A. Zlatic et al., Systemic metabolic and mitochondrial defects in rett syndrome models, *BioRxiv* (2023), doi: 10.1101/2023.04.03.535431.
[60] A.K. Percy et al., Rett syndrome: North American database, *J. Child Neurol.* **22**(12) (2007), 1338–1341, doi: 10.1177/0883073807308715.
[61] D. Tropea et al., Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice, *Proc Natl Acad Sci USA* **106**(6) (2009), 2029–2034, doi: 10.1073/pnas.0812394106.
[62] D.G. Glaze et al., A Double-Blind, Randomized, Placebo-Controlled Clinical Study of Trofinetide in the Treatment of Rett Syndrome, *Pediatr. Neurol.* **76** (2017), 37–46, doi: 10.1016/j.pediatrneurol.2017.07.002.
[63] D.G. Glaze et al., Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome, *Neurology* **92**(16) (2019), e1912–e1925, doi: 10.1212/WNL.0000000000007316.
[64] J.L. Neul et al., Design and outcome measures of LAVENDER, a phase 3 study of trofinetide for Rett syndrome, *Contemp. Clin. Trials* **114** (2022), 106704, doi: 10.1016/j.cct.2022.106704.
[65] J.J.H. Park et al., Systematic review of basket trials, umbrella trials, and platform trials: a landscape analysis of master protocols, *Trials* **20**(1) (2019), 572, doi: 10.1186/s13063-019-3664-1.
[66] A. Patrizi et al., Chronic Administration of the N-Methyl-D-Aspartate Receptor Antagonist Ketamine Improves Rett Syndrome Phenotype, *Biol. Psychiatry* **79**(9) (2016), 755–764, doi: 10.1016/j.biopsych.2015.08.018.
[67] R. Novak et al., Target-agnostic discovery of Rett Syndrome therapeutics by coupling computational network analysis and CRISPR-enabled *in vivo* disease modeling, *BioRxiv* (2022), doi: 10.1101/2022.03.20.485056.
[68] E.C. Ballinger et al., Mecp2 Deletion from Cholinergic Neurons Selectively Impairs Recognition Memory and Disrupts Cholinergic Modulation of the Perirhinal Cortex, *eNeuro* **6**(6) (2019), doi: 10.1523/ENEURO.0134-19.2019.
[69] N. Krishnan et al., PTP1B inhibition suggests a therapeutic strategy for Rett syndrome, *J. Clin. Invest.* **125**(8) (2015), 3163–3177, doi: 10.1172/JCI80323.