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A Coordinated Attack: Rett Syndrome Therapeutic Development

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Abstract

Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the *Methyl CpG binding protein 2* (*MeCP2*) gene. This Science & Society article focuses on pharmacological strategies that attack RTT treatment from multiple angles, including drug repurposing and *de novo* discovery efforts, and discusses the impacts of preclinical study design and translationally relevant outcome measures.

Rett Syndrome (RTT)

RTT is a rare neurological disorder that affects one in 10 000 live female births. RTT patients undergo developmental regression beginning at 6–30 months, resulting in the loss of acquired social and motor abilities as well as the emergence of autonomic dysfunction, respiratory phenotypes, and repetitive behaviors that persist into adulthood [1,2]. Arguably, the two most important discoveries in RTT research to date are that: (i) RTT is caused by loss-of-function mutations in the X chromosome-linked *MeCP2* gene [3]; and (ii) genetic rescue of MeCP2 levels significantly improves the disease course in rodents [4]. Buoyed by these discoveries, researchers have undertaken extensive efforts to identify pharmacological and biological treatment strategies, several of which have either completed or are preparing to enter clinical trials.

The limited number of RTT patients, coupled with the complexity of the disorder, creates contexts in which target identification and preclinical development are often driven by academia or specialized biotechnology companies and then subsequently partnered with larger industrial companies for clinical progression. This places a burden on academics and small organizations to strive for enhanced understanding of the subtleties of the clinical development process, particularly for a rare disease. Here, we share our perspective on preclinical development of pharmaceuticals for the treatment of RTT, which largely fall into

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the category of drug repurposing, based on overlapping symptoms or shared drug targets, and *de novo* drug discovery efforts. In addition to the pharmacological options discussed here, gene therapy approaches, as well as DNA and RNA modification techniques, are also progressing into development and attack the features associated with RTT at the level of MeCP2 itself; we refer readers to [5] for an excellent review of these subjects. Finally, while the discussion herein focuses on *MeCP2*-based RTT, RTT-like symptoms can also result from distinct genetic mutations and often overlap with related disease states; for these reasons, it is anticipated that pharmacological impacts in classical RTT have the potential to extend to a number of other disorders.

Drug Repurposing

The majority of pharmacological approaches currently being explored for RTT seek to ameliorate core symptoms of the disease. Many of these strategies originated with other neurological disorders and rely on conserved mechanisms of cellular dysfunction. For example, the sphingosine 1-phosphate mimetic fingolimod was originally developed and is now approved for the treatment of multiple sclerosis [6], where it mediates neurotrophic effects, in part, by increasing brain-derived neurotrophic factor (BDNF) levels [7]. As increasing Bdnf signaling has beneficial effects in MeCP2-knockout animals [8], it has been hypothesized that fingolimod could have utility in RTT. In support of this theory, fingolimod administration improves motor phenotypes and prolongs survival in male *MeCP2*$^{-/y}$ mice [7] and, although results have not yet been published, a clinical trial for safety and efficacy has recently been completed (Clinical Trial Number[i]: NCT02061137). Likewise, the insulin-like growth factor 1 (IGF1) active peptide trofinetide, originally developed as a stroke medication, was also progressed to Phase II clinical trials, where it showed a favorable safety profile and exhibited efficacy in correcting several core RTT symptoms at the highest dose [9]. While these improvements did not completely correlate with trofinetide's robust preclinical data set in all symptom domains, these promising results have prompted Neuren and ACADIA Pharmaceuticals to announce their intent to advance trofinetide into Phase III studies[ii]. Sarizotan, an antipsychotic with serotonin$_{2A}$/dopamine D_2 receptor agonist activity, has also been administered to RTT patients (Clinical Trial Number: NCT02790034) for the treatment of apneas, although results from these trials have yet not been published. Another example of a repurposing attempt is exemplified by the antidepressant desipramine, which, despite strong preclinical results in mice, had only limited effects on respiratory phenotypes and presented with adverse effects in patients in a recent European clinical trial [10]. Disconnects between observed preclinical and clinical efficacy and safety suggest that limitations may exist in the translation of RTT animal model results to RTT patients for certain targets.

Perhaps the best example of repurposing efforts in RTT is that of the anesthetic and *N*-methyl-D-aspartate receptor (NMDAR) antagonist ketamine. As is extensively discussed and referenced in [11], subanesthetic doses of ketamine improve cortical function in *MeCP2*$^{+/-}$ and *MeCP2*$^{-/y}$ mice and may also impact repetitive movements (paw clasping) and

[i]https://clinicaltrials.gov/
[ii]www.acadia-pharm.com/pipeline/rett-syndrome/

respiratory phenotypes. Interestingly, efficacy is observed during times when ketamine exposure is observed as well as during periods after which the drug has been cleared. This suggests that acute effects on NMDAR function and sustained downstream signaling events may both impact RTT phenotypes. Relative to other potential treatments, ketamine is an example of a compound whose efficacy has been demonstrated in multiple laboratories, in both male and female *MeCP2*-knockout mice, and in acute and chronic dosing paradigms [11]. While an early trial with IV ketamine and RTT was terminated (Clinical Trial Number: NCT02562820), a Phase II clinical trial for the use of ketamine is currently enrolling (Clinical Trial Number: NCT03633058) and optimism remains high that efficacy will be observed in RTT patients.

Discovery Efforts

Studies in autopsy samples have demonstrated that the expression of ~2000 genes is compromised by pathogenic mutations in *MeCP2*, most of which are only moderately shifted up or down [12]. This increase in transcriptional noise is postulated to result in a series of interactomes of affected proteins that may additively contribute to the manifestation of RTT phenotypes [12]. *De novo* discovery efforts for RTT seek to identify nodes on these interactomes, which can serve as potentially druggable access points to modify specific symptom domains. For example, both glutamatergic and GABAergic signaling are compromised in *MeCP2*-knockout mice. Based on our experience with modulating synaptic plasticity using allosteric modulators of metabotropic glutamate (mGlu) and muscarinic receptors, we profiled human RTT and control tissues for expression of these targets and found significant changes in two metabotropic glutamate (mGlu) receptors, $mGlu_5$ [13] and $mGlu_7$ [14], as well as in the M_4 muscarinic receptor [12]. Using positive allosteric modulators that were originally under development for other indications, such as schizophrenia, we have built preclinical datasets for these novel targets in RTT model mice. Additional work has also exploited induced pluripotent stem cells (iPSCs) from RTT patients and controls; recently, this approach has provided a rationale for targeting chloride potassium symporter 5 (KCC2) in RTT [14].

In the field of neuroscience drug discovery, there is a staggering failure rate of therapeutics to cross the preclinical to clinical ‘Valley of Death’, often despite broad and reproducible efficacy profiles in animal models. For this reason, it is anticipated that the incorporation of clinical datasets, such as data generated by expression profiling of patient samples, genotype-phenotype correlations with known RTT clinical symptoms (i.e., seizures, preserved speech), findings arising from patient-derived iPSCs, and datasets integrating clinical neural imaging studies (i.e., labeled ligands to validate target disruption), early in the development process will heighten confidence in the translational relevance of the proposed targets.

Outcome Measures

In recognition of the numerous previous and ongoing clinical development programs for RTT, the National Institutes of Health (NIH), the RTT research community, and patient family organizations such as the Rett Syndrome Research Trust[iii] and rettsyndrome.org[iv] have adopted standards for preclinical research designed to improve translational success [15]. These standards include testing potential therapeutics across a range of concentrations in both male and female mouse models of various clinically represented *MeCP2* mutations, using sufficient sample sizes, and incorporating clinically relevant outcome measures. Additionally, the safety and efficacy of clinical development candidates should be replicated across laboratories and published in the peer-reviewed literature. While the merits of these standards are clear, adherence has been mixed, which may be reflected in the results observed in the desipramine [10] trial discussed above in which preclinical development relied heavily on male *MeCP2*$^{-/y}$ mice. Furthermore, there remains considerable debate regarding how distinct preclinical measures will translate to clinical trials. For example, RTT model mice exhibit reproducible impairments in learning and memory paradigms that are responsive to numerous pharmacological interventions [12,16,17]. However, it will likely be challenging to quantify changes in cognitive ability clinically in patients who are primarily nonverbal and have limited use of their extremities. A similar problem exists with measures of sociability, alterations in which can be observed in RTT mouse models and modulated with certain therapeutics, but which are challenging to quantify in patients [12,16,18]. In clinical trials for related autism-associated disorders, such as fragile X syndrome, both cognition and sociability have been assessed using caregiver-derived metrics, and this has historically been linked to increased placebo responses [19].

It is anticipated that driving RTT clinical trials using outcome measures that are more reproducibly identified through natural history studies designed to systematically phenotype RTT patients throughout life (e.g., Clinical Trials Number: NCT02738281) or quantified via functional biomarkers like neuroimaging, metabolomics, or electroencephalography (EEG) may enhance clinical success. For example, apneas have been linked to heart rhythm abnormalities and spontaneous death in RTT [20]. These breathing abnormalities can be objectively quantified via take-home wearable devices, as can other RTT phenotypes such as seizure activity. Likewise, motor symptoms are routinely assessed during clinical visits and can be quantified via distinct measurements (e.g., gait dynamics, fine motor ability, repetitive movements). EEG abnormalities were among the first deficits reported in RTT [1,2] and several measures of epileptiform activity and sleep appear to translate between *MeCP2*-deficient mice and RTT patients [5,15], providing a rationale for increased use ofEEG in preclinical studies. While outcome measures should integrate efficacy domains identified in animal models, it will be important to prioritize parameters that can be reliably quantified across a patient population with varying degrees of symptom severity.

Conclusions and Future Perspectives

Nearly 20 years of therapeutic development for RTT have resulted in a number of completed clinical trials and a bevy of compounds and biologics still in the clinical development process. The ‘preclinical research in RTT’ [15] best practices document is a strong starting point to improve the probability of clinical success, but fieldwide adherence must increase. Forwardthinking projects that incorporate clinical data sets early in study design and include

[iii] https://reverserett.org/
[iv] www.rettsyndrome.org/

female model mice with disease-relevant mutations, dose-response relationships, and clinically feasibly outcome measures will be needed for success. Increased incorporation of translational rigor in the grant submission and review process will be required to make these standards more commonplace and financially feasible in academia. With the shift of large pharma away from early-stage development, the onus is on individual researchers within the RTT research community to rise to the challenge of clinical development to ensure appropriate application of new approaches to matched patient populations.

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