Evaluating the effect of new readthrough drugs using a novel in vitro Rett Syndrome system, human neural cells carrying MeCP2 nonsense mutations

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Rett syndrome (RTT) is an X-linked dominant neurodevelopmental disorder with characteristic cognitive, motor, autonomic dysfunction and additional typical features. The primary cause of RTT is de novo germline mutations in the methyl CpG-binding protein 2 (MECP2) gene. MeCP2 activity is critical to early brain development and also in the adult and aging brain. Approximately 30%-40% of RTT cases arise from a premature stop codon into the MeCP2 gene. Recently, new semi-synthetic aminoglycosides were developed as readthrough drugs (ELX-01 and ELX-02), and shown in vitro to selectively induce ribosomal readthrough of MeCP2 nonsense mutants (R168X, R270X, R294X) and to exhibit significantly lower nephrotoxicity and ototoxicity to gentamicin, allowing for their chronic administration. Next, a novel in vitro RTT model systems was generated using immortalized human neural stem cells, neuronal and glial cells overexpressing the R168X and R255X, tagged to N-GFP. We have shown that 5-days of ELX-01 and ELX-02 treatment to human neuronal cells expressing a $MeCP2^{R168X}$ mutant resulted in a dose-dependent increased in the expression of the MeCP2-GFP protein (ELX-01 EC_{50}=139μM; ELX-02 EC_{50}=164μM), an increased nuclear MeCP2-GFP expression (up to 40% of positively stained cells) and in up to 2.9- (ELX-01), 3.9- (ELX-02) fold increase in relative BDNF mRNA levels. Similar observations were documented for glial cells expressing $MeCP2^{R168X}$ mutants. In addition, ELX-01 and ELX-02 treatments increased the number and arborization of cell neurites in $MeCP2^{R168X}$ neurons. In conclusion, ELX-01 and ELX-02 were shown to be promising drug candidates for readthrough therapy in RTT, using novel in vitro systems.