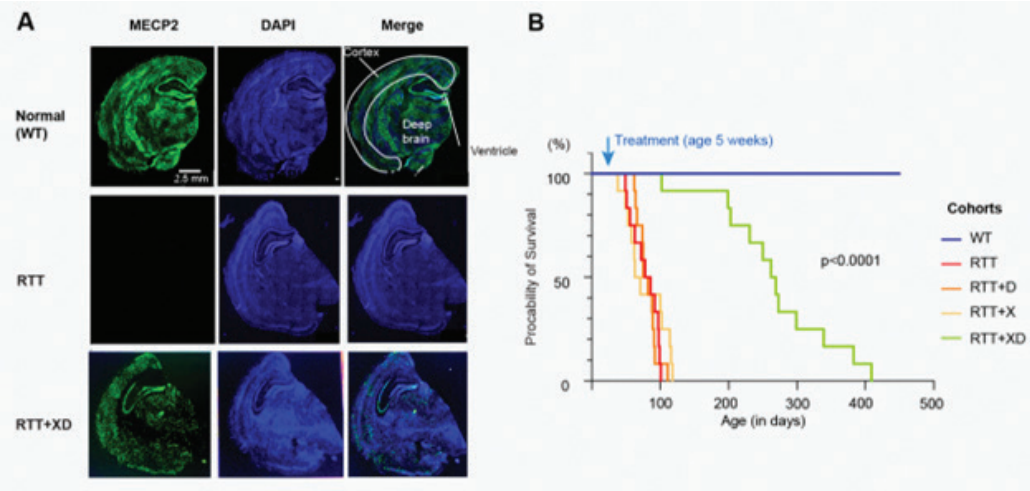

Figure A: MECP2 immunostaining (green) of coronal sections of hemi-brains from normal (wildtype, WT), untreated RTT, and RTT treated with the Xist ASO (X) and decitabine (D) at 5 weeks of age and tested at 10 weeks of age. DAPI counterstain (blue). Scale bars: 2.5 mm. N=5 animals for each cohort, with similar results within each cohort.

Figure B: Kaplan-Meier survival curves for each cohort (n=12/cohort). Single treatment was initiated at 5 weeks of age. P< 0.0001 (Mantel-Cox test) when comparing RTT+XD versus untreated RTT, RTT+D, and RTT+X. The differences between untreated RTT, RTT+D, and RTT+X are not significant.

Selective Reactivation of the Dormant MECP2 Gene: A Disease-Modifying Therapy for Rett Syndrome

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Rett syndrome (RTT) is a severe neurodevelopmental disorder that affects ~1:10,000 girls throughout the world. Affected girls inherit one defective copy of the X-linked MECP2 gene. Affected girls develop normally until about ages 1-3, at which point RTT leads to severe cognitive and motor impairments, seizures, and shortened lifespan.

The current standard of care involves treating symptoms rather than targeting the root cause of RTT. However, mouse models have shown that genetically restoring MECP2 expression reverses the neurological disease, raising hopes of a pharmacological treatment. While gene therapy to do this is under investigation, challenges with delivery and risks of MECP2 overexpression remain major limitations. We have developed a complementary epigenetic approach that selectively reactivates the normal MECP2 gene on the inactive X chromosome (Xi). Because affected girls retain one functional MECP2 allele lying dormant on the Xi chromosome in roughly half of their cells, our approach unlocks a genetic cure from within.

We demonstrated proof-of-concept for this approach in a female mouse model of RTT. We found that targeting XIST RNA — the master regulator of the Xi — with an antisense oligonucleotide (ASO) will selectively reactivate MECP2 from the Xi. Treatment is administered via direct CNS delivery at 5 weeks when animals begin to manifest symptoms. The restorative effects are most robust when animals are primed with a DNA methylation inhibitor, decitabine, at the start of treatment. Treated animals exhibit phenotypic reversal, transcriptomic restoration, and a nearly 4-fold extension of lifespan. Furthermore, we achieved a high level MECP2 expression in neurons with minimal off-target effects on other X-linked genes. Thus, we established that RTT can be pharmacologically reversed through selective Xi-reactivation using an epigenetic approach. This novel approach opens a new therapeutic modality for RTT and other X-linked disorders.