mTOR inhibitors decrease seizures and enhance survival in a mouse model of Tuberous Sclerosis Complex (TSC)

Mackenzie Catron[1], Zoë Fuchs[2], Geoffrey Tombaugh[1], Rishi Rakhit[3], Roy Y. Kim[3], Nadia Khan[3], Nicholas T. Hertz[3], Dean J. Aguiar[2], and Stephen Morairty[1]***

1. PsychoGenics Inc., Paramus, NJ, USA; 2. TSC Alliance, Silver Spring, MD, USA; 3. Montara Therapeutics, San Francisco, CA, USA

INTRODUCTION

TSC is a rare genetic disorder caused by mutations in either the *TSC1* or *TSC2* genes, leading to the loss of function and increased activity of the mechanistic target of rapamycin (mTOR). Approximately 85% of those affected with TSC exhibit epilepsy, which is either not treatable or only partially treated by current anti-epileptic drugs including rapamycin. To address this, preclinical mouse models of TSC-specific epilepsy have been developed to enhance translational drug development. In the *Tsc1$^{flox/flox}$; GFAP-Cre+* conditional knockout (Tsc1 CKO), Tsc1 is knocked out in glial fibrillary acidic protein (GFAP)-expressing cells, including astrocytes and some neurons. Convulsive seizures begin around weaning (P21) and worsen with age. Survival is highly impacted, with ~50% of mice succumbing to the disease by two months of age. The standard comparator/positive control used in preclinical studies is rapamycin at 3 mg/kg. In this study, we sought to define the dose relationship of two clinical mTOR inhibitors (rapamycin and everolimus) on seizure reduction and mortality. A better understanding of this relationship will facilitate the interpretation of the effects of novel compounds in this model.

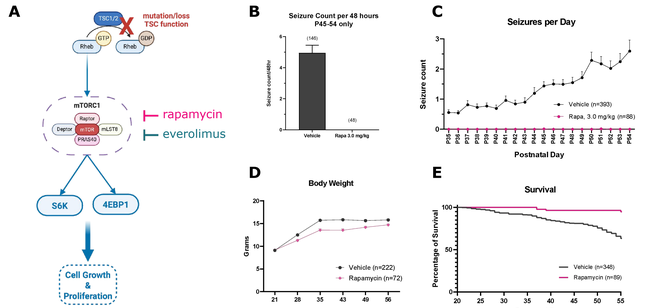

Figure 1. A) Molecular mechanism of the impact of TSC1/2 mutations. B-E) Historical data in vehicle- and rapamycin-treated Tsc1 CKO mice showing B) average seizures counts per 48 hours for P45-54, C) seizures per day for P35-54, and D) body weight and E) survival from P21-P54.

METHODS

Treatment groups: Tsc1 CKO mice were bred and genotyped at PsychoGenics using breeding pairs derived from the Wong Laboratory (Washington University, St. Louis). Tsc1 CKO mice were separated into seven treatment groups [rapamycin (R-5000, LC Labs) and everolimus (RAD001, MCE) at 0.03, 0.3, and 1 mg/kg, and a vehicle control (1.6% ETOH, 5% DMSO, 5% PS80, 88.4% H2O)]. All groups were balanced across sex. QD i.p. dosing began at P21.

Study design: Mice were implanted around P21 with a 3-channel head mount (Pinnacle, 8201-DEP) which has two cortical leads (frontal and parietal), and a hippocampal depth electrode. 16 animals per group were tethered, and ten days of continuous EEG (P45-P54) were analyzed for generalized tonic-clonic seizure (GTCS) frequency and duration. Seizures were detected manually by scorers blinded to treatment condition. Weekly body weights and daily survival data were collected, and tissue was collected at P56 for bioanalyses. Whole brains from n=3-4 mice per group were used to determine protein levels of p70S6K and S6 as well as other relevant biomarkers. Drug levels were examined by LC/MS in plasma and brain two hours post final dose.

RESULTS AND SUMMARY

Both test articles significantly reduced seizure frequency compared to the vehicle group (rapamycin and everolimus at 0.3 and 1 mg/kg). A dose-related decrease in mortality was also found for each test article. Decreased seizure duration was associated with decreased seizure frequency, but changes in duration were hard to statistically assess at the high doses due to the general lack of seizures. Brain and plasma drug concentrations were proportionately dose-related across compartments. Total protein levels of S6 and p70S6K remained unchanged across treatment groups, indicating that the effects were due to phosphorylation inhibition. Total 4E-BP1 levels varied in response to treatment, suggesting that prolonged mTOR inhibition may influence 4E-BP1 expression and stability.

IN-VIVO RESULTS

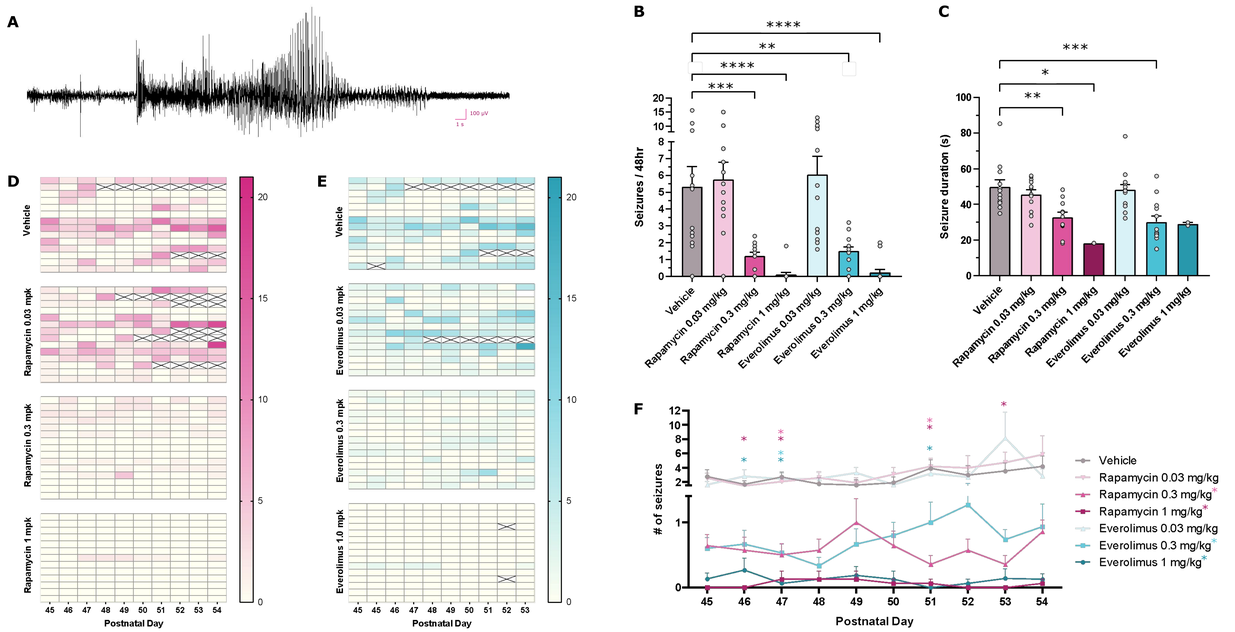

Figure 2. A) Sample trace showing a GTCS in the frontal cortex. Scored events are present in all three channels (frontal cortex, parietal cortex, hippocampus), are at least 10 seconds in duration, and there is at least 30 seconds of normal EEG between events. B) Seizures per 48 hours per group (a traditional reporting measure in the literature). One-way ANOVA, p<0.0001. Šídák's multiple comparisons test results shown on graph. C) Average seizure duration, excluding long terminal events. One-way ANOVA (p<0.0001) with Dunnett's multiple comparisons test results shown on graph. D,E) Heat map displaying seizure frequency in E) vehicle- and rapamycin-treated groups, and F) vehicle-and everolimus-treated groups. Crossed out cells are days without a seizure score (death of animal, disconnected tether, etc.). F) Seizures per day per group. Repeated-measure mixed-effects analysis, p=0.0043 interaction, p<0.0001 group effect. Dunnett's multiple comparisons test on group main effect comparing each treatment to vehicle shown on treatment shown in legend, * for all p<0.05. Dunnett's multiple comparisons test comparing each group to vehicle shown on graph as * for all p<0.05. All panels: mean ± SEM, α=0.05, n=13-16 (except C). B and C: *=<0.05, **=<0.01, ***=<0.001, ****=<0.0001.

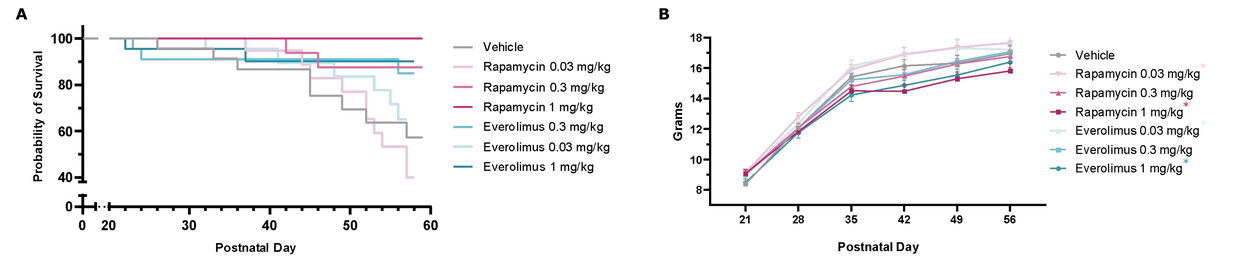

Figure 3. A) Survival analysis. Mantel-Cox test: all groups, p=0.0012; vehicle and rapamycin, p= 0.0010; vehicle and everolimus, p=0.1245. B) Body weight analysis as measured on a weekly basis. group. Repeated-measure mixed-effects analysis, p=0.0004 interaction, p=0.0021 group effect. Dunnett's multiple comparisons test on group main effect comparing each treatment to vehicle shown on treatment shown in legend, * for all p<0.05. Dunnett's multiple comparisons test on interaction, not significant. All panels: mean ± SEM, α=0.05, n=22-23.

MT1110 BLOCKS PERIPHERAL mTOR INHIBITION AND ELEVATES BRAIN:PLASMA RATIOS OF EVEROLIMUS

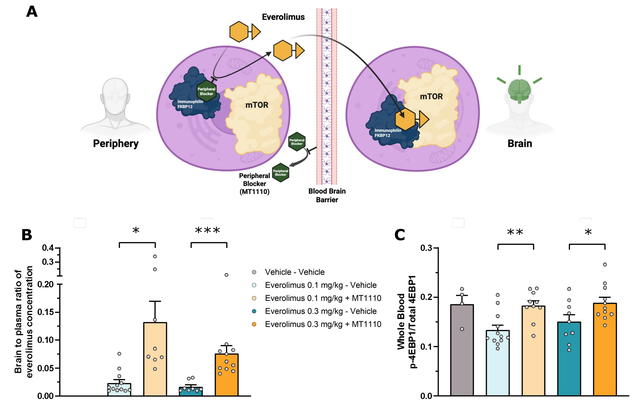

Figure 4. A) Schematic illustrating the brain-selective pharmacology enabled by Montara Therapeutics' BrainOnly Platform. B) In an additional study following the same design presented in the methods, co-treatment of everolimus and MT1110 at 200 mg/kg, a peripheral blocker of mTOR inhibitors, enhances everolimus brain concentrations as shown by increased brain:plasma ratios. C) Blood peripheral biomarker for mTOR inhibition is reversed with 200 mg/kg MT1110. B and C: 1-sided *t*-tests between everolimus and everolimus+MT1110 groups. Data shown as mean ± SEM, α=0.05. *=<0.05, **=<0.01, ***=<0.001.

MT1110 POTENTIATES SEIZURE REDUCTION AND AMELIORATES EFFECTS ON A MARKER OF IMMUNOSUPPRESSION

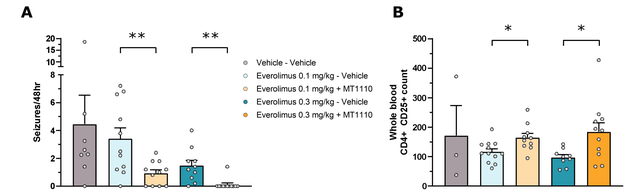

Figure 5. Tsc1 CKO mice were co-administered everolimus and vehicle or MT1110 (200 mg/kg) from P21 to P55. A) Seizure counts from P45-P54. 11/12 mice treated with 0.3 mg/kg everolimus + MT1110 had no recorded seizures; 1/12 had >3x reduction in seizures compared with vehicle. B) Whole blood CD4(+)CD25(high) (activated T-cells) terminal count. Everolimus causes immunosuppression and is reversed upon co-treatment with MT1110 as measured by increased CD4(+)CD25(high) cell counts. 1-sided t-tests between everolimus and everolimus+MT1110 groups. Data shown as mean ± SEM, α=0.05. *=<0.05, **=<0.01.

CONCLUSIONS

Both rapamycin and everolimus reduced seizure frequency and seizure duration, as well as improved survival in Tsc1 CKO mice. The effects for both drugs occurred in a dose-related manner. Seizure frequency correlated to three biomarkers: p-S6/Total S6 ratio, total 4EBP1, and GFAP (data not shown). Co-dosing with the Montara peripheral mTOR-inhibitor blocker MT1110 potentiates everolimus seizure reduction, while blocking clinically relevant adverse effects, such as immunosuppression. These data support the validation of this clinically relevant translational model of TSC for testing the therapeutic potential of novel compounds and validate Montara Therapeutics' BrainOnly approach for mTOR inhibitors in TSC. This work was funded in part by the TSC preclinical consortium.

For more information email *Stephen.Morairty@psychogenics.com.
For information on Montara's BrainOnly platform and MT1110, email **NHertz@montaratx.com.