Bi-steric mTORC1-selective inhibitors are superior to rapamycin and induce apoptotic cell death in tumor models with hyperactivated mTORC1

Abstract #: 4859

Heng Du[1], Yu C. Yang[2], Mallika Singh[2], Heng-jia Liu[1], David J. Kwiatkowski[1]

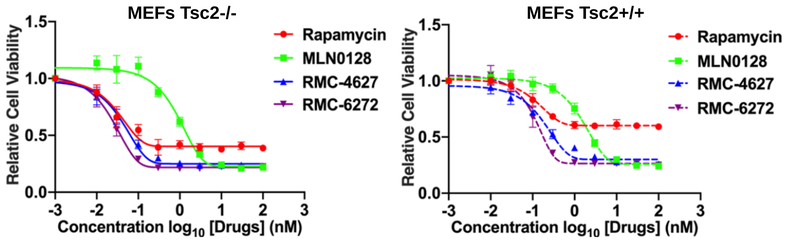
1. Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
2. Department of Biology, Revolution Medicines Inc., 700 Saginaw Drive, Redwood City, CA 94063, USA.

INTRODUCTION

- The PI3K-mTOR pathway is one of the most commonly dysregulated pathways in human tumors
- Rapalogs have been used extensively in human clinical trials but exhibit modest clinical benefit, possibly due to their lack of effect on 4E-BP1
- 4E-BP1 is a key target downstream of mTORC1, and can be inhibited by ATP-competitive mTOR inhibitors such as MLN0128; However, these inhibitors are poorly tolerated possibly due to their inhibition of mTORC2
- A new class of selective mTORC1 inhibitors has been developed and termed 'bi-steric', which comprises a rapamycin-like core moiety covalently linked to an mTOR active-site inhibitor
- RMC-5552 is the first clinical candidate of this bi-steric class of mTORC1 inhibitor, and clinical testing is currently ongoing (NCT04774952)
- RMC-4627 and RMC-6272 are representative bi-steric tool compounds that exhibit potent and selective inhibition of mTORC1 over mTORC2
- We hypothesize that the bi-steric mTORC1-selective inhibitors will demonstrate superior activity than rapalogs (e.g. rapamycin) in mTORC1 hyperactivated tumors

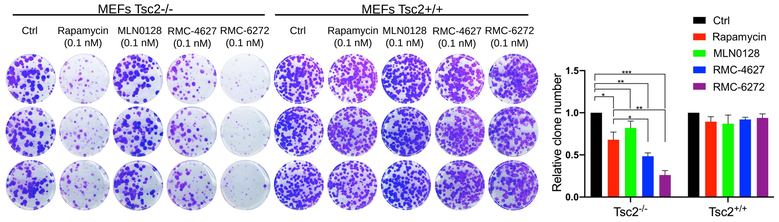
RESULTS

1. Bi-steric mTORC1 inhibitors showed a more potent inhibition of growth in Tsc2-null MEF cells than rapamycin and MLN0128.



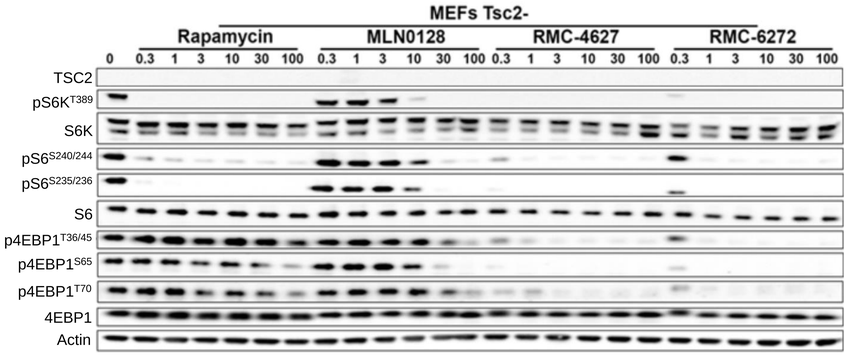
Above. RMC-4627 and RMC-6272 were tested and compared to rapamycin and MLN0128 in pairs of Tsc2-null mouse embryonic fibroblasts (MEFs) their wild type counterparts. The two bi-steric inhibitors showed a greater magnitude of inhibition of growth. Furthermore, the bi-steric mTORC1 inhibitors displayed increased potency in the Tsc1/2-null lines versus wildtype. Similar findings were obtained in Tsc1-null MEFs .

2. Bi-steric mTORC1-selective inhibitors significantly reduced the growth of Tsc2-null MEFs.



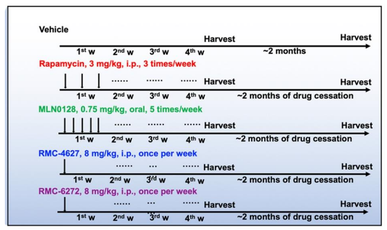
Above. Low dilution clonogenic assays showed that RMC-4627 and RMC-6272 caused a greater inhibition of clonal growth than rapamycin and MLN0128. No significant effect on the wild-type cells indicates a differential sensitivity for Tsc-2 null cells. Similar findings were obtained in Tsc1-/- and Tsc1+/+ cells. 200 cells were seeded in 10-cm dishes. Cells were treated for 14 days. The assay was done in triplicate. Student t test was used for statistical comparisons; *$p<0.05$, **$p<0.001$, ***$p<0.0001$.

3. Bi-steric mTORC1-selective inhibitors resulted in potent and complete inhibition of pS6 and p4EBP1, whereas rapamycin only inhibited pS6.

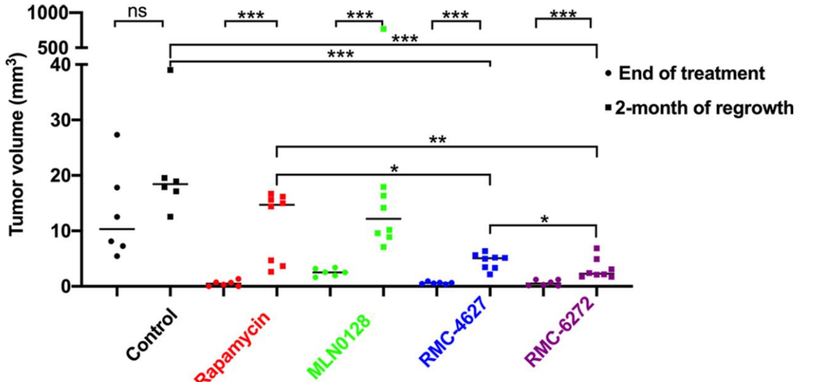


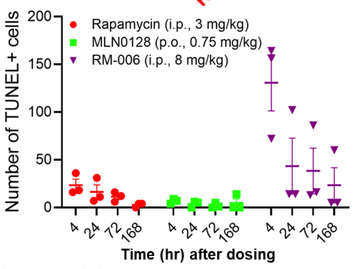
Above. Tsc2-/- MEFs were treated by indicated concentrations of rapamycin, MLN0128, RMC-4627, or RMC-6272, and cells were collected for Western Blot.

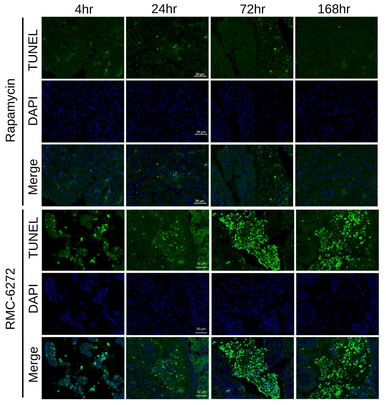
4. Bi-steric mTORC1-selective inhibitors showed profound anti-tumor activity *Tsc2*$^{+/-}$ A/J mice and induced apoptosis *in vivo*.



Below. Tumor volume was quantitated by tumor score based on histology. Tumor volume in rapamycin (0.493 mm^3), MLN0128 (2.514 mm^3), RMC-4627 (0.59 mm^3) and RMC-6272 (0.50 mm^3) was markedly reduced than the vehicle controls (10.32 mm^3) in 10-month-old *Tsc2*+/- A/J mice after four weeks of treatment. Two months after treatment cessation, both RMC-4627 (5.05 mm^3) and RMC-6272 (2.27 mm^3) treatment showed less tumor regrowth than rapamycin (14.7 mm^3) and MLN0128 (12.18 mm^3). Each symbol represents a single kidney; N ≥ 6 kidneys per group.

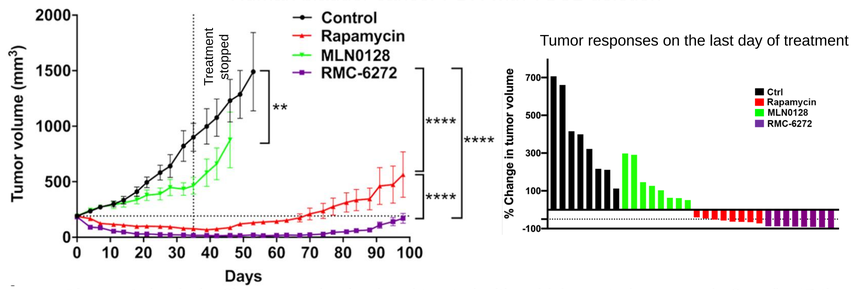






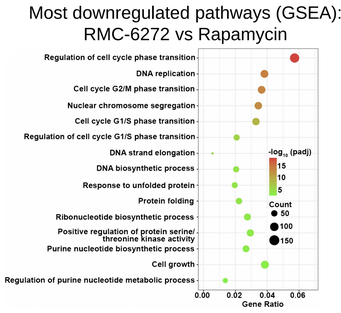
Above & Right. *Tsc2*$^{+/-}$ A/J mice were dosed once by Vehicle, rapamycin, MLN0128 or RMC-6272. Mice were sacrificed at indicated timepoints. Kidneys were harvested and TUNEL staining was performed on FFPE sections. RMC-6272 induced more apoptosis than rapamycin. Representative confocal microscopy images are shown.

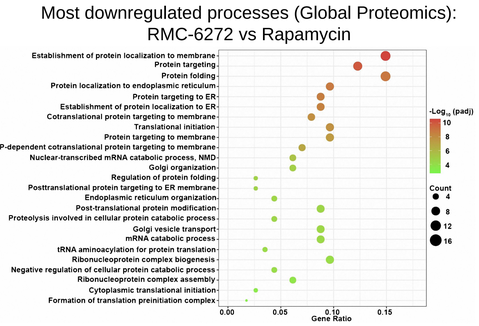
5. RMC-6272 drove deep tumor regressions and delayed tumor regrowth after treatment cessation in human bladder cancer tumors

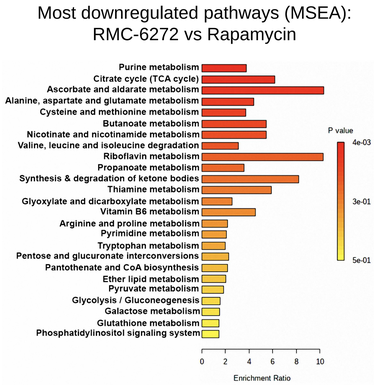


Above. Animals (n=8) were randomized and treated with vehicle control, rapamycin 3 mg/kg IP tiw, or MLN0128 0.75 mg/kg po 5d-on/2d-off, or RMC-6272 8 mg/kg ip qw.

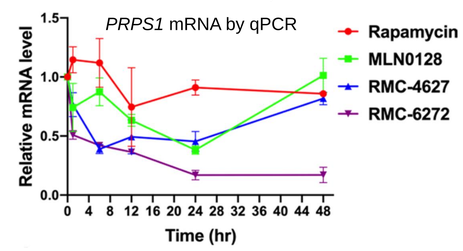
6. Integrative multi-omics analyses revealed differential global changes induced by RMC-6272 in comparison to rapamycin

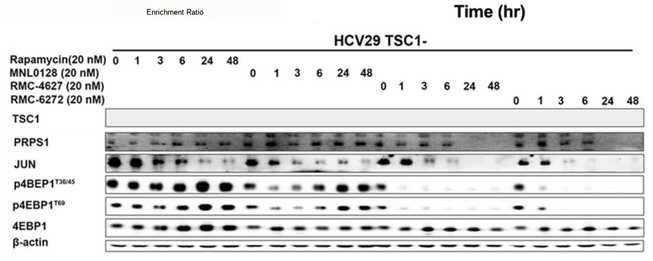






Left. Purine metabolism was the most decreased metabolic pathway by RMC-6272 compared to Rapamycin in HCV29 TSC1-null cells by MSEA.
Bottom. RMC-6272 suppressed PRPS1 expression through JUN (confirmed by knockout studies. Data not shown here).





CONCLUSIONS

- Bi-steric mTORC1-selective inhibitors demonstrate improved *in vitro* and *in vivo* inhibition of mTORC1 in comparison to rapamycin, and induced more cell death in TSC1/2-null tumors *in vivo*
- These preclinical data support the potential of bi-steric mTORC1-selective inhibitors (such as RMC-5552) as a novel therapeutic strategy to treat tumors with mTORC1 dysregulation