Title: Combination therapy may be beneficial for the treatment of lymphangioleiomyomatosis

Dr. Lyn 27281 Moir lyn.moir@sydney.edu.au 1,2, Mr. Ho Yin 27282 Ng patrickng@med.usyd.edu.au 1,2, Dr. Brian 27283 Oliver brian.oliver@sydney.edu.au 1,2, Dr. Janette 27284 Burgess janette.burgess@sydney.edu.au 1,2, Dr. Vera 27285 Krymskaya krymskay@mail.med.upenn.edu 3 and Prof. Judith 27295 Black judy.black@sydney.edu.au 1,2. 1 Cell Biology, Woolcock Institute of Medical Research, Sydney, NSW, Australia, 2037 ; 2 Discipline of Pharmacology, The University of Sydney, Sydney, NSW, Australia, 2006 and 3 Department of Medicine, University of Pennsylvania, Philadelphia, PA, United States, 19104.

Body: Dysfunction of the tuberous sclerosis genes (TSC), in particular TSC2, results in enhanced cell proliferation and migration associated with lymphangioleiomyomatosis (LAM). Although rapamycin has shown some clinical benefit cessation of treatment causes a decline in lung function. Rapamycin inhibits cell proliferation but has little effect on migration. Thus, targeting multiple processes may be beneficial for LAM treatment. We examined the combined effect of doxycycline and rapamycin on cell proliferation and migration and investigated the signalling pathways involved. Methods: Proliferation and migration of TSC2+/- and TSC2-/- mouse embryonic fibroblasts (MEF) were assessed by manual cell counts, transwell and wound closure assays. Phosphorylated p70S6K (p-p70S6K) was assessed by immunoblotting and RhoA activity by ELISA. Results: Doxycycline inhibited both RhoA activity and migration of TSC2-/- MEF (n=4, p<0.05) but had no effect on p-p70S6K (n=5, p>0.05) or proliferation (n=4, p>0.05). Rapamycin inhibited p-p70S6K and proliferation (n=5, p<0.05) but not migration. In combination, rapamycin and doxycycline significantly inhibited cell migration, proliferation, and RhoA activity, but the degree of inhibition was no greater than the individual drugs alone (n=4, p>0.05). However, combination treatment reduced the rate of wound closure by TSC2-/- MEF compared to individual drugs (n=4, p<0.05). Conclusion: Doxycycline inhibits the enhanced cell migration associated with TSC2-dysfunction through the RhoA GTPase pathway. LAM is a multifactorial disease, utilising multiple signalling pathways, thus combination therapy that targets these pathways may be beneficial.