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**Review**

# Tuberous sclerosis complex, mTOR, and the kidney: report of an NIDDK-sponsored workshop

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**Henske EP, Rasooly R, Siroky B, Bissler J.** Tuberous sclerosis complex, mTOR, and the kidney: report of an NIDDK-sponsored workshop. *Am J Physiol Renal Physiol* 306: F279–F283, 2014. First published November 13, 2013; doi:10.1152/ajprenal.00525.2013.—Remarkable basic and translational advances have elucidated the role of the mammalian target of rapamycin (mTOR) signaling network in the pathogenesis of renal disease. Many of these advances originated from studies of the genetic disease tuberous sclerosis complex (TSC), leading to one of the clearest therapeutic opportunities to target mTOR with rapamycin and its analogs ("rapalogs"), which effectively inhibit mTOR complex 1 (mTORC1) by an allosteric mechanism. Clinical trials based on these discoveries have provided strongly positive therapeutic results in TSC (Bissler JJ, McCormack FX, Young LR, Elwing JM, Chuck G, Leonard JM, Schmithorst VJ, Laor T, Brody AS, Bean J, Salisbury S, Franz DN. *N Engl J Med* 358: 140–151, 2008; Krueger DA, Care MM, Holland K, Agricola K, Tudor C, Mangeshkar P, Wilson KA, Byars A, Sahmoud T, Franz DN. *N Engl J Med* 363: 1801–1811, 2010; McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, Barker AF, Chapman JT, Brantly ML, Stocks JM, Brown KK, Lynch JP 3rd, Goldberg HJ, Young LR, Kinder BW, Downey GP, Sullivan EJ, Colby TV, McKay RT, Cohen MM, Korbee L, Taveira-DaSilva AM, Lee HS, Krischer JP, Trapnell BC. *N Engl J Med* 364: 1595–1606, 2011). In June 2013, the National Institute of Diabetes and Digestive and Kidney Diseases convened a small panel of physicians and scientists working in the field to identify key unknowns and define possible "next steps" in advancing understanding of TSC- and mTOR-dependent renal phenotypes. TSC-associated renal disease, which affects >85% of TSC patients, and was a major topic of discussion, focused on angiomyolipomas and epithelial cysts. The third major topic was the role of mTOR and mTOR inhibition in the pathogenesis and therapy of chronic renal disease. Renal cell carcinoma, while recognized as a manifestation of TSC that occurs in a small fraction of patients, was not the primary focus of this workshop and thus was omitted from panel discussions and from this report.

angiomyolipoma; cyst; mTOR; tuberous sclerosis complex

## *Angiomyolipomas*

*Background.* ANGIOMYOLIPOMAS can arise sporadically or as part of the tuberous sclerosis complex (TSC). Sporadic angiomyolipomas can be associated with translocations involving the TFE3 transcription factor (1) or the TSC genes (5). The panel focused primarily on TSC-associated angiomyolipomas. Angiomyolipomas are benign mesenchymal tumors composed of fat, smooth muscle, and abnormal vascular elements, all of which are known to arise from a common precursor cell (13). TSC patients typically have multiple, bilateral angiomyolipomas that can result in renal insufficiency, and these lesions have abnormal vasculature that can form aneurysms that can spontaneously hemorrhage, sometimes with life-threatening consequences (2). Clinical trials have clearly demonstrated that angiomyolipomas shrink in response to inhibitors of mammalian target of rapamycin complex 1 (mTORC1) (3, 4, 6, 8) and that the majority of the tumors return to original volume when the treatment is discontinued (4). Thus most patients appear to require continuous therapy to suppress angiomyolipoma size. Since the rapalog sirolimus typically induces a cytostatic response, it is likely that the decrease in tumor volume does not correlate with extensive apoptosis. However, because angiomyolipomas are highly vascular, mTORC1 inhibition could induce an apoptotic cellular response via an antiangiogenic mechanism.

The allosteric inhibitors (rapalogs) and catalytic inhibitors (that impact both mTORC1 and 2) are different with the

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mTOR activities based on in vitro studies. Preclinical work found equivalent benefit of allosteric and catalytic mTOR inhibitors in a murine model of TSC (10), but no clinical studies of the mTOR catalytic inhibitors involving TSC patients have been completed.

*Key unanswered questions.* The panel considered key issues that remain unaddressed related to the natural history and pathogenesis of angiomyolipomas, the evaluation/optimization of current angiomyolipoma therapies, and the development of novel angiomyolipoma treatment strategies, which were deemed essential to future progress. These issues are summarized in Fig. 1.

*Highest priority translational research initiatives.* Multiple translational approaches were discussed by the panel to address the most critical unanswered questions outlined in Fig. 1. The highest priority initiatives are the following: *1*) genetic analyses of angiomyolipomas from individual patients, including both indolent and rapidly growing tumors and including both the solid and vascular components; *2*) identification of the cell-of-origin of angiomyolipomas, which could facilitate the development of animal models of angiomyolipomas; and *3*) development of additional cell culture models of angiomyolipomas with bi-allelic *TSC2* inactivation to facilitate translational and preclinical therapeutic advances.

*Highest priority clinical research initiatives.* In parallel, multiple clinical research approaches were discussed to address unmet needs in the clinical care of individuals with angiomyolipomas. From these discussions, the highest priorities are the following.

*1*) Identification of biomarkers to quantitatively and sensitively measure angiomyolipoma size and composition. Current technology does not easily quantitate the percentage of fat or smooth muscle components so that imaging between time points or patients can be accurately compared. Both imaging and "liquid" (plasma or urinary) biomarkers could be pivotal in monitoring angiomyolipoma burden of disease and therapeutic response. Imaging modalities that measure the effects of mTORC1 activation on cellular metabolism are particularly attractive. Serum biomarkers such as VEGF-D, which is a diagnostic biomarker and an indicator of therapeutic response in lymphangioleiomyomatosis (LAM) (25), a disease that shares cellular and genetic features with angiomyolipomas (12), should be sought, and markers like collagen IV should be pursued (3). Sensitive and specific biomarkers would facilitate trials to determine optimal dosing and duration of therapy, and to test novel therapies that might result in durable responses, ultimately resulting in shorter treatment intervals with associated cost savings and a decreased risk of adverse effects.

| Natural History and Pathogenesis of Angiomyolipomas | Evaluation/Optimization of Current Angiomyolipoma Therapies | Development of Novel Angiomyolipoma Treatment Strategies |
|---|---|---|
| • Are angiomyolipomas "locally metastatic," such that a single lesion can seed other lesions within the kidney?<br>• Are there genomic or epigenetic differences between indolent and locally aggressive angiomyolipomas?<br>• Is there a developmental window during which angiomyolipomas initiate?<br>• What are the mechanisms of aneurysm formation in angiomyolipomas?<br>• Are there gender differences in angiomyolipoma incidence, size, rate of growth, timing of growth, or risk of bleeding?<br>• Does blood pressure correlate with the risk of hemorrhage from angiomyolipomas?<br>• Can angiomyolipoma cells be detected in the circulation, as has been already shown in LAM, providing a "liquid biopsy" allowing cellular features of angiomyolipomas to be monitored in "real time." | • Why is there such a variable response to mTORC1 inhibition?<br>Some angiomyolipomas shrink in size while others do not, even within the same kidney.<br>Does this reflect the composition of the tumor (fat-containing versus fat-poor) or its vasculature?<br>• Why do some tumors regrow when treatment is discontinued while others do not?<br>What are the kinetics of the response, and is the earliest response vascular or cellular?<br>• What is the mechanism of response?<br>Does the decrease in angiomyolipoma size with mTORC1 inhibition reflect primarily a reduction in individual cell volume, since mTORC1 is a key regulator of cell size, or a decrease in cell number?<br>• What is the minimum dose and duration of mTOR inhibitor therapy required to induce and maintain a reduction in angiomyolipoma size?<br>• What are the long-term benefits and risks of mTORC1 inhibitor therapy for angiomyolipomas, including the risk of hemorrhage, the impact on the aneurysmal vessels associated with angiomyolipomas, and the potential immunosuppressive effects? | • Can early intervention with Rapalogs prevent the development of angiomyolipomas?<br>• Can addition of other therapeutic agents to Rapalogs produce a more robust and durable therapeutic response?<br>• Can mTORC1 inhibitors be targeted directly to angiomyolipomas, thereby avoiding systemic toxicity and increasing the dose delivered?<br>• Will catalytic inhibitors of mTOR, which inhibit both mTORC1 and mTORC2, be more effective than Rapalogs for angiomyolipoma therapy?<br>• Does mTOR-dependent feedback to AKT and MEK impact the clinical response to Rapalogs?<br>• How does activation of autophagy impact the therapeutic response to Rapalogs? |

Biomarkers may also identify the subset of angiomyolipomas with a more aggressive clinical phenotype, prioritizing patients with these tumors for earlier therapeutic intervention.

*2*) There is an additional need for biomarkers that predict the risk of hemorrhage, which is a potentially life-threatening complication of angiomyolipomas. MRI analysis of aneurysm size/complexity may help define imaging biomarkers. Work with vascular-related markers may be helpful, and understanding the biology of aneurysms in the context of TSC will likely prove to be a critical step (3, 16, 24).

*3*) Genome-wide studies to identify factors that modify the risk of angiomyolipoma development, the risk of angiomyolipoma severity, the risk of angiomyolipoma hemorrhage, and/or the response to mTORC1 inhibitor therapy should be performed.

*4*) The immunosuppressive effects of mTORC1 inhibitors, when used alone in TSC and related diseases, are unclear. The risk of infectious complications appears to be low, based on available data, but needs to be clarified through longer term observational studies.

*5*) Natural history studies to identify the earliest development of angiomyolipomas, to determine whether spurts of growth can be defined in childhood, puberty, or young adulthood, whether there are gender differences in the timing of angiomyolipoma growth, and whether bleeding risk and/or growth are correlated with clinical parameters including blood pressure, will be pivotal to future prevention trials.

*6*) Designing and conducting a placebo-controlled, early-intervention study of mTORC1 inhibition for angiomyolipomas to evaluate its effectiveness as preventative therapy.

*7*) Conducting clinical trials to evaluate agents that could synergize with mTORC1 inhibitors to produce more effective treatment, e.g., Hsp90 inhibitors, autophagy inhibitors, and mTOR kinase inhibitors.

*8*) Determining the potential benefit of low-dose long-term therapies on inhibiting angiomyolipoma development, e.g., metformin, NSAIDs, and low-dose or intermittent rapalog therapy.

### *Renal Cystic Disease in TSC*

*Background.* Renal cystic disease in TSC is common, affecting ~50% of patients, ranging in severity from a single cyst to multiple, bilateral cystic disease (7, 9, 17). Individuals with the contiguous gene syndrome who carry deletions of both TSC2 and the adjacent PKD1 gene (<5% of TSC patients) can even develop severe very early onset polycystic kidney disease (18). The mTORC1 pathway has been implicated in the pathogenesis of renal cystic disease in autosomal dominant polycystic kidney disease (ADPKD) (21). To date, the use of mTOR inhibitors in treatment of ADPKD has yielded equivocal results (4, 5). The timing of mTORC1 inhibition may be pivotal in the response of ADPKD-associated cysts to therapy (20, 23). Defects in the primary cilium have been observed in TSC-deficient cells, including increased ciliary length (11). Together with the prominent cystic disease, these findings suggest that TSC may be a ciliopathy, yet the role of cilia in the initiation of, progression of, and therapy for renal cystic disease in TSC is not yet established.

*Key unanswered questions.* The panel considered key issues involving TSC renal cystic disease that remain unaddressed and are essential to future progress. These issues are summarized in Fig. 2.

*Highest priority translational research initiatives.* Multiple translational approaches to TSC renal cystic disease were discussed by the panel, with the highest priority initiatives being the following: *1*) comprehensive genetic analysis of a large cohort of TSC patients with the polycystic kidney phenotype to define in greater detail the spectrum of mutations that cause this manifestation and determine whether mutation of the contiguous *TSC2* and *PKD1* genes account for all of these patients; *2*) development of an animal model of the TSC2/

- The therapeutic response of TSC-associated renal cysts to Rapalogs is largely unknown because the angiomyolipoma trials were not designed to assess cystic disease. A particularly important consideration is whether mTORC1 inhibitor therapy can prevent chronic kidney disease in patients with the TSC2/PKD1 contiguous gene deletion syndrome.
- The origin, developmental timing and natural history of cyst initiation and progression in TSC are poorly understood. To what extent do cysts initiate pre- and post-natally, and what factors and mechanisms are involved in their expansion?

  Do cysts in TSC originate from different segments of the kidney at different developmental time points?

  How does cyst formation and progression relate to angiomyolipoma development?
- The optimal therapeutic endpoints for cystic response are not defined. How should proliferation vs. secretion be considered when considering the response of cysts to therapy?
- Factors that may promote cyst progression are not defined. Is hypertension in TSC correlated with cystic disease?

  Does renal injury contribute to cyst progression?
- What accounts for the severity of cystic disease in the TSC2/PKD1 contiguous gene syndrome?

  The impact of co-deletion of *TSC2* and *PKD1* on a single copy of chromosome 16 in humans seems strikingly different than mutational inactivation of each gene on different copies of chromosome 16 in mouse models. Can a mouse model be generated with loss of both genes on a single allele to address this?
- There is considerable phenotypic variation in the degree of cyst formation in TSC patients. What is the basis for this variability and is it possible to carry out genetic studies of patients at the extremes of the phenotypic distribution?
- What are the functional consequences of ciliary dysfunction in TSC kidneys, and how does this contribute to cystogenesis?

PKD1 contiguous gene deletion syndrome; *3*) definition of the developmental timing and nephron segment-of-origin of cysts in TSC; *4*) further examination of the connections between ciliary function and cyst formation in TSC; and *5*) identification of factors (hypertension, injury) that promote cystogenesis in animal models of TSC.

*Highest priority clinical research initiatives.* Multiple clinical research approaches to TSC renal cystic disease were considered with the highest priorities being *1*) design of clinical trials to specifically determine whether and how cysts in TSC respond to rapalog therapy, including individuals with the TSC2/PKD1 contiguous gene syndrome, as the therapeutic response of the cystic disease has not been evaluated as an end point in previous studies; and *2*) identification of factors including hypertension and renal injury that may promote cyst progression in TSC.

### *Intrinsic Renal Disease Related to mTORC1 Inhibition*

*Background.* Although rapamycin appears to be minimally nephrotoxic when used alone, most of the data from humans are from studies in which it was used in combination with cyclosporine. Rapamycin was not associated with a significant increase in proteinuria during the EXIST2 trial of the rapalog everolimus for angiomyolipomas (3). However, this trial was of relatively short duration and included just over 100 patients. Thus the long-term effects of rapalogs as single agents on the kidney are not entirely understood. Prolonged treatment with mTORC1 inhibitors reduces the total expression of mTOR, as well as the expression of rictor and thus mTORC2 formation (19). Podocyte expression of nephrin, transient receptor potential cation channel 6, and the cytoskeletal adaptor protein Nck are significantly decreased following prolonged exposure to an mTORC1 inhibitor (22). Furthermore, mTORC1 inhibition reduces podocyte adhesion and motility. Together, these effects may have a long-term impact on the glomerular and tubular structures and deserve attention.

*Key unanswered questions.* The panel considered key issues involving intrinsic renal disease related to mTORC1 that remain unaddressed and that are essential to be understood as treatment may be prolonged. Questions that were discussed included the following. Does prolonged rapalog therapy induce proteinuria and/or other glomerular or tubular effects in humans? Are there differential effects of mTORC1 vs. mTORC2 inhibition on the kidney that could be relevant to future clinical trials involving catalytic mTOR kinase inhibitors?

*Highest priority translational and clinical research initiatives.* Given that mTORC1 inhibitor therapy will be used in both children and adults with TSC and that there are many unknowns related to the long-term impact on the kidney, the panel concluded that renal function and proteinuria should be monitored in a standardized, prospective manner in individuals receiving long-term rapalog therapy.

### *Conclusions*

In summary, there was consensus that areas of high priority related to the roles of mTOR in renal disease include the following.

*Preclinical models of angiomyolipomas and renal cystic disease.* Priorities for which the panel had clear consensus included developing mouse models of angiomyolipomas, and developing of additional cell lines derived from angiomyolipomas. A mouse model that recapitulates the severe, early-onset cystic disease observed in the TSC2/PKD1 contiguous gene syndrome is likewise required. Further investigation of the nephron segment-of-origin, the developmental timing of cystic disease in TSC, and the role of ciliary dysfunction in TSC-associated cystogenesis is critical to the development of targeted therapy for TSC-associated renal cystic disease. It is also important to develop cell culture models for study of TSC using induced pluripotent stem cells (iPSC) from patients.

*Biomarkers.* The panel determined that future research focused on developing and refining imaging techniques to monitor disease progression, evaluating responses to therapy, and providing valuable natural history data would address key unmet needs. Focus areas included modalities that would allow more precise monitoring of tumor size, imaging with novel PET tracers that would allow differentiation between fat and other elements within angiomyolipomas, and serum biomarkers of disease burden and therapeutic response are crucial. Similarly, imaging and biochemical biomarkers that could prognosticate and monitor therapy for the renal cystic disease are critical.

*Future clinical trials.* The panel recommended that future clinical trials should include optimizing rapalog therapy by defining the minimum dose required that maintains a maximum response. Furthermore, identifying agents that could be combined with rapalogs or that could be used individually to induce a more complete and/or durable response could prove to be pivotal in allowing periodic or one-time, rather than life-long, therapy. In addition, determining whether catalytic mTOR inhibitors induce a more complete and/or durable response compared with rapalogs is a high priority.

*Prevention.* In general, current studies have focused on the treatment of large or enlarging angiomyolipomas. Future studies should focus on the prevention of angiomyolipomas, which would require additional natural history information, including defining whether there is a "window" of more rapid growth of angiomyolipomas during development. Although there is an anecdotal sense that angiomyolipomas can grow more rapidly during adolescence and early adulthood, this is not well defined. Epithelial cysts in TSC remain understudied, and the effects of rapalogs on cyst progression are unknown. Individuals with the TSC2/PKD1 contiguous gene syndrome, who are at risk for early-onset, severe polycystic kidney disease, represent a population in whom prevention studies are a high priority.

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#### DISCLOSURES

## AUTHOR CONTRIBUTIONS

Author contributions: E.P.H., B.J.S., and J.J.B. drafted manuscript; E.P.H., R.R., B.J.S., and J.J.B. edited and revised manuscript; E.P.H., R.R., B.J.S., and J.J.B. approved final version of manuscript.

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