MRI Assessment of Treatment of Tuberous Sclerosis kidney with Rapamycin and IFN-g in a Mouse Model

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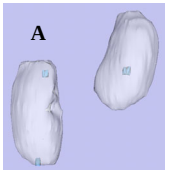
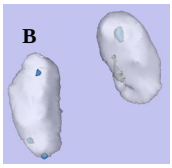
Introduction: Renal disease is an important cause of morbidity in tuberous sclerosis complex (TSC), a mendelian disorder with autosomal dominant inheritance. From the recent molecular understanding of this disorder, there is now hope that effective treatment can be developed. We have successfully created TSC mouse kidney model. This is an excellent disease model demonstrating the utility of developing targeted therapeutic approaches to investigate promising new strategies for treating human diseases. Rapamycin is a mTOR kinase inhibitor that is an approved drug (Rapamune; Wyeth-Ayerst) for immunosuppression following organ transplantation. Because rapamycin has been shown to normalize disregulated mTOR signaling in cells that lack normal hamartin or tuberin, mTOR kinase inhibition may be a useful approach to systemic therapy for TSC (1). Human IFN-γ OMIM is a homodimeric 34-kD peptide secreted by T lymphocytes and natural killer cells. It plays an important role in the coordinated regulation of expression of the immune response via the stimulation or repression of key genes (2). In this study, we used MRI in conjunction with histology to assess whether treatment with a rapamycin analog (CCI-779) or murine IFN- γ decreases severity of disease.

Method: To evaluate the utility of CCI-779 or IFN- γ for treating TSC renal disease, we used Tsc2+/- mice for a three arm preclinical study with an untreated control arm (n=7), a CCI-779 treatment arm (n=10), and an IFN- γ treatment arm (n=10). The CCI-779 cohort was treated with CCI-779 at 4mg/kg by intraperitoneal injection (IP) three times per week for 12 weeks (from 40-52 weeks old). The IFN- γ cohort was treated with murine IFN- γ at 20,000 units IP three times per week for 44 weeks (from 8-52 weeks old). The kidneys were evaluated via MRI at 8.5T using a T2 sequence with TR=3000ms, TE(effective) =60ms, slice thickness = 0.75 mm, matrix size=128.128 and FOV=3x3 cm^2. 3D T2 weighted images were generated with in-house 3D software and tumor volume was measured using a thresholding method. Severity of disease was determined in all animals at 52 weeks of age. To validate the utility of MR imaging for quantitating severity of disease, all MR images were obtained within two weeks of necropsy and histopathologic analysis. For histology analysis, we examined phosphorylated S6 (pS6) levels because elevated pS6 is a useful marker for the increased mTOR kinase activity that occurs in cells/tissues with defective hamartin or tuberin.

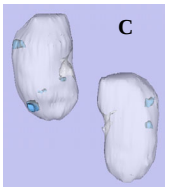
Results: Fig. 1 shows representative 3D-reconstructions of T2 weighted images, obtained at 10.5 and 12 months old with IFN- γ treated (Fig 1a and b) and untreated mouse (Fig.1c and d). It is quite striking that the IFN- γ treated mouse has no change in the total tumor volume (4 mm^3 at 10.5 months and 12 months) but the untreated mouse has a 3-fold increase in total tumor volume (8mm^3 at 10.5 months and 25mm^3 at 12 months). Fig 2 shows 2D T2 weithted images of the same mice imaged at 12 months old. One can see the untreated mouse has 5 times more cystadenomas than the treated one. Fig 4 shows the overall results of the treatment. We observed a significantly higher burden of kidney cystadenomas per kidney in the untreated control group than in either the CCI-779 treatment group or the IFN- γ treatment group. There was a 62-92% reduction in the number of cystadenomas per kidney in Tsc2+/- mice treated with CCI-779 and a 43-67% reduction in the number of cystadenomas per kidney in Tsc2+/- mice treated with IFN- γ when compared with an untreated control group. Immunohistochemistry and immunoblot analyses demonstrated a dramatic normalization of mTOR signaling with CCI-779 treatment and a modest normalization of mTOR signaling with IFN- γ treatment. We quantitated these results (Table 1) and found a lower frequency of pS6 positive kidney cytadenomas in both the CCI-779 group and the IFN- γ group (66%, *P*=0.002) compared with the untreated control group.



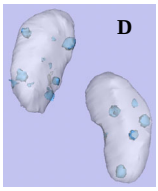
3D model of a treated mouse kidneys at 10.5 mos.



3D model of a treated mouse kidneys at 12 mos.



3D model of a untreated mouse kidneys at 10.5 mos.



3D model of a untreated mouse kidneys at 12 mos.

Figure 1: 3D-reconstructions of T2 weighted images, obtained at 10.5 and 12 months old Tsc2+/- with IFN- γ treated (a and b) and untreated mouse(c and d).

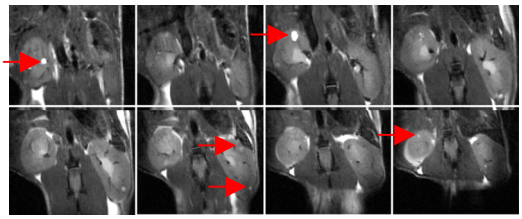


Figure 3: T2 weighted images of Mouse #200 at age 12 mons treated with IFN-g 3 times a week for 44 weeks. Images show a total of 5 cystic regions (red arrows).

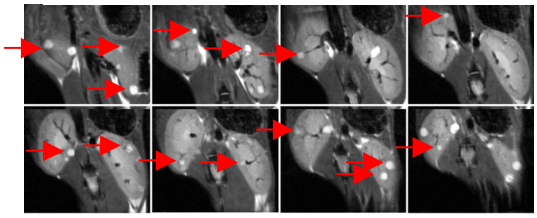


Figure 2: T2 weighted images of a 12 month old untreated Tsc2 +/- mouse. Images show a total of 15 cystic regions (red arrows).

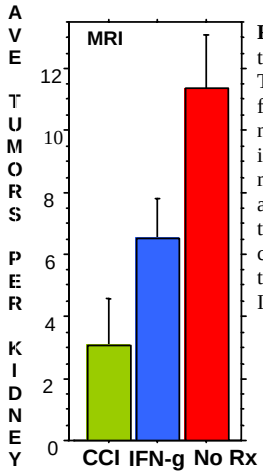


Figure 4: Average tumors per kidney. There is a three-fold increase in the number of tumors in an untreated mouse compared to a CCI mouse. A two-fold decrease comes with treatment by IFN- γ.

Table 1: pS6 Expression in Kidney Cystadenomas

Group	No Rx	IFN-g	CCI-779
N (mice)	7	10	10
Total Cystadenomas	99	53	17
PS6 positive cystadenomas	87	35	5
% pS6 positive	88%	66%	29
P value (vs untreated)		0.002	<0.0001

Discussion: Our findings that both CCI-779 and IFN- γ, when used as single agents, can reduce the severity of kidney cystadenomas in Tsc2+/- mice has direct relevance for the treatment of kidney angiomyolipomas in TSC patients. Because the duration of CCI-779 treatment was shorter than IFN- γ treatment, disease severity was lower in the CCI-779 group (by MR imaging and histopathology), and normalization of aberrant mTOR signaling with CCI-779 treatment is dramatic, it appears that CCI-779 (or rapamycin) is the best first choice agent for testing in clinical trials but that IFN- γ is an important alternative. MRI, a noninvasive method, that quantifies the *in vivo* anti-tumor activity of an experimental treatment, is a powerful tool to facilitate new therapeutic approaches.

References: 1. Goncharova, E.A., D.A., etc., J Biol Chem, **277**(34): 30958-67, 2002. **2.** Farrar MA, etc., Annu Rev Immunol 11: 571-611, 1993.