

Efficacy of a Rapamycin Analog (CCI-779) and IFN- γ in Tuberous Sclerosis Mouse Models

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Tuberous sclerosis complex (TSC) is a familial tumor disorder for which there is no effective medical therapy. Disease-causing mutations in the *TSC1* or *TSC2* gene lead to increased mammalian target of rapamycin (mTOR) kinase activity in the conserved mTOR signaling pathway, which regulates nutrient uptake, cell growth, and protein translation. The normal function of *TSC1* and *TSC2* gene products is to form a complex that reduces mTOR kinase activity. Thus, mTOR kinase inhibition may be a useful targeted therapeutic approach. Elevated interferon-gamma (IFN- γ) expression is associated with decreased severity of kidney tumors in TSC patients and mouse models; therefore, IFN- γ also has therapeutic potential. We studied cohorts of *Tsc2*^{+/-} mice and a novel mouse model of *Tsc2*-null tumors in order to evaluate the efficacy of targeted therapy for TSC. We found that treatment with either an mTOR kinase inhibitor (CCI-779, a rapamycin analog) or with IFN- γ reduced the severity of TSC-related disease without significant toxicity. These results constitute definitive preclinical data that justify proceeding with clinical trials using these agents in selected patients with TSC and related disorders. © 2004 Wiley-Liss, Inc.

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal-dominant tumor disorder that can affect multiple organs, including the kidneys, brain, heart, and lungs (Gomez et al., 1999; Online Mendelian Inheritance in Man, 2003b). The incidence of TSC is 1:6,000, and an estimated 1–2 million individuals are affected worldwide (National Tuberous Sclerosis Association, 1994). Sporadic pulmonary lymphangioliomyomatosis (LAM; Sullivan, 1998; Online Mendelian Inheritance in Man, 2003a) is a progressive pulmonary disorder that is genetically related to TSC because somatic mutations in *TSC1* or *TSC2* have been identified in abnormal lung tissue from LAM patients (Carsillo et al., 2000). Renal manifestations in TSC and LAM patients are significant because 60%–80% of TSC patients and 40%–50% of LAM patients develop kidney angiomyolipomas (tumors consisting of abnormal blood vessels, smooth-muscle cells, and fat cells; Sullivan, 1998; Gomez et al., 1999). TSC patients also can have a number of other medical problems, including epilepsy, cognitive impairment, behavioral problems, brain lesions (tubers and/or subependymal nodules), skin tumors (facial angiofibromas), cardiac tumors (rhabdomyomas), kidney cysts, renal cell cancer, and pulmonary abnormalities including LAM (Gomez et al., 1999; Dabora et al., 2001; Franz et al., 2001).

It is known that the *TSC1* and *TSC2* gene products, hamartin and tuberlin, form a complex that inhibits mammalian target of rapamycin (mTOR) kinase activity in a conserved cellular signaling pathway (the mTOR pathway) that regulates nutrient uptake, cell growth, and protein translation (Consortium, 1993; van Slechtenhorst et al., 1997; Gao and Pan, 2001; Potter et al., 2001). A schematic diagram of the mTOR pathway is shown in Figure 1a. Key proteins in this pathway include PI3kinase, Akt, TSC1/TSC2, Rheb, mTOR, p70S6kinase (S6K), S6 ribosomal subunit (S6), and 4E-BP1. TSC2 is negatively regulated by Akt via phosphorylation (Manning et al., 2002; Potter et al., 2002). More recently, it has been shown that under low-energy conditions, TSC2 is activated by LKB1 (a serine/threonine kinase tumor suppressor that is mutated in Peutz–Jeghers syndrome) through AMP-dependent protein kinase (Inoki et al.,

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