EUROPEAN RESPIRATORY journal

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Research letter

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Please cite this article as: El-Chemaly S, Taveira-DaSilva A, Bagwe S, *et al*. Celecoxib in LAM: Results of a Phase I Clinical Trial. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.02370-2019).

Celecoxib in LAM: Results of a Phase I Clinical Trial

Short title: Celecoxib in LAM

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Take home message: COX2 inhibition is safe in LAM patients with mild disease. In the subset of patients with high VEGF-D (>800pg/ml) COX2 inhibition appears to cause a decrease in VEGF-D levels and may provide clinical benefit.

Lymphangioleiomyomatosis (LAM) is a multisystem disease associated with progressive pulmonary disease that affects almost exclusively women. LAM is characterized pathologically by proliferation of abnormal smooth muscle-like cells carrying mutations in predominantly the Tuberous Sclerosis Complex (TSC) gene *TSC2*, and rarely in *TSC1* (1). LAM can occur sporadically or in association with TSC. Mutations in the *TSC* genes lead to activation of the mammalian/mechanistic target of rapamycin complex 1 (mTORC1) (2). In the landmark randomized controlled MILES (Multicenter International LAM Efficacy of Sirolimus) trial, the mTORC1 inhibitor rapamycin stabilized lung function and improved symptoms in LAM patients with moderate to severe changes in lung function (FEV1<70%) (3). Recent *in vitro* and preclinical evidence showed that loss of *TSC2* resulted in upregulation of COX-2 and prostacyclin synthase (*PGTIS)* expression, independent of mTORC1. Treatment of *Tsc2*$^{+/-}$ mice with Celecoxib, a COX-2 specific inhibitor, resulted in a 50% decrease in renal cystadenomas volume, which occur spontaneously in this model (4). In addition, LAM nodules were found to express higher levels of COX-2 in comparison with control lungs (4).

Based on these data we performed the COX-2 inhibition in LAM clinical trial (COLA) NCT02484664), a phase I safety study of a single daily dose of celecoxib (200mg) taken orally in treatment-naïve patients with LAM who were deemed not to require treatment with mTORC1 inhibitors. The primary endpoint of the study was safety and tolerability. Toxicities were graded using the National Cancer Institute (NCI) Common Toxicity Criteria (version 4). The study design consisted of a screening visit and 24-week treatment period, with scheduled visits at weeks 8, 16 and end of study. Secondary endpoints included effects on lung function, quality of life, renal angiomyolipoma size, serum vascular endothelial growth factor (VEGF)-D levels, and levels of *TSC2* mutation in cell free-DNA (cfDNA).

From June 2016 through November 2018, 41 subjects were screened, 26 did not meet inclusion/ exclusion criteria and 12 subjects provided written, informed consent to participate in the study and were enrolled on study drug. Mean age of study participants was 48 (range: 39-

63). 6 subjects were post-menopausal. No dose-limiting toxicities were encountered. A total of 75 non-serious adverse events were reported for 11 patients. No serious adverse events were reported; most non-serious adverse events (97%, 73 out of 75) were rated as mild (CTCAE grade 1). Nearly half (37/75) were thought to be related to celecoxib. The most commonly reported non-serious adverse events were stomach/abdominal pain (6 events from 3 subjects) and headaches (6 events from 3 subjects)

Three patients had their participation in the study ended early. One subject withdrew due to gastrointestinal side effects probably related to celecoxib; one subject was withdrawn due to a rash probably related to celecoxib; and one subject withdrew due to a cardiovascular event, likely unrelated to celecoxib.

A secondary objective of the COLA trial was to determine the effects of Celecoxib on lung function. The least square mean of post-bronchodilator FVC (ml), post bronchodilator FEV1 and DLCO did not change significantly during the study period. The results were similar when a subgroup analysis of pre-menopausal subjects (n=6) was conducted. This is not surprising considering the short course of the study, 24 weeks, and mild lung disease in the enrolled subjects. Whether this reflects an effect of celecoxib in reducing lung function decline requires studies with larger enrolled populations and longer-term treatment. Over the study period, we found no change in the SGRQ, a validated tool to assess quality of life in patients with LAM (5, 6) (Table 1).

Baseline MRI showed a kidney angiomyolipoma in 3 subjects, which remained unchanged at the end of the study. Additional findings on screening MRI included kidney cysts (n=5 subjects), hemorrhagic or preoteinaceous cysts (n=2), liver hemangiomas (n=3), liver cysts (n=5), vertebral hemangioma (n=2), and abdominal lymphangiomyomas (n=1). Seven subjects had a repeat MRI, and no significant changes were observed except in one subject with bilateral renal cysts, which decreased in size. Four other subjects had renal cysts that did not change in size during the treatment period.

At screening visit, median serum VEGF-D levels were 448 pg/ml (Range: 279-2232 pg/ml). Two out of 12 subjects had VEGF-D levels greater than 800pg/ml. Treatment with Celecoxib for 24 weeks did not result in significant changes in mean VEGF-D (Table 1). However, the two subjects who had VEGF-D levels higher than 800pg/ml (meeting diagnostic criteria for LAM) demonstrated significant reductions in VEGF-D levels at the end of treatment with a decrease from baselines of 2156 and 2232 pg/ml to 1408 and 1865 pg/ml, respectively. Further, DLCO and post-bronchodilator FEV1 and FVC remained stable in these two subjects. Serum VEGF-D levels have been shown previously to correlate with disease severity and response to mTOR inhibition (7). Previous studies have shown that VEGF-D can regulate prostaglandin biosynthesis and COX mRNA expression (8). Further, COX-2 inhibition has been shown to inhibit nucleolin, which results in decreased VEGF-D mRNA translation (9). Hence, these very preliminary findings (n=2 subjects) of a drop in VEGF-D suggest the possibility that COX-2 inhibition may have therapeutic benefit in patients with early-stage LAM and elevated VEGF-D levels. There was no difference in PGEM plasma levels between screening [7.5 (5.4-8.7) pg/ml] and end of study or early termination visits [7 (4.4-13.4) pg/ml] (paired t-test 0.7). This result could be due to potential under-dosing (10), or pharmacokinetics of Celecoxib, which has a half-life of 8-12 hours (11), since plasma was collected 24 hours after last Celecoxib dose.

cfDNA samples were collected from all COLA subjects and subjected to high read depth massively parallel sequencing (MPS) analysis of *TSC1*/*TSC2* (mean read depth: 1040x) (12). Although several candidate mutation findings were identified, they did not replicate in a validation analysis. Hence, there were no mutation findings in either *TSC1* or *TSC2* in plasma cfDNA from these subjects. These data are consistent with our recent analysis of a larger LAM cohort (n=61) (12).

The use of COX-2 inhibitors as preventive therapy for cancer in healthy individuals is counterbalanced by potential adverse cardiovascular events (13). However, as an adjunct therapy in cancer treatment or prevention of recurrence, COX-2 inhibition remains a subject of

intense investigation. The clinicaltrials.gov trial registry shows more than a thousand studies examining the COX-2 pathway in a variety of cancers, with more than 200 using Celecoxib, some with significantly higher doses than used in this study. This is the first study to examine COX-2 inhibition in lung disease of any kind, to our knowledge.

Our study raises important questions: 1) are women with LAM with low burden of disease and high VEGF-D levels candidates for Celecoxib treatment? Should this subgroup of patients be the target of subsequent clinical trials? 2) Could COX2 inhibition play a role in the prevention of LAM development in patients with TSC? 3) Is there a role for Celecoxib in addition to mTORC1 inhibition in the treatment of LAM and potentially other manifestations of TSC?

In summary, the COLA trial has established the safety of Celecoxib 200mg orally daily in patients with LAM. Larger phase II/III trials are needed to establish the effectiveness of this treatment, perhaps in a subset of patients with LAM and high VEGF-D levels.

Acknowledgment

We are indebted to patients and their families for their participation in this study. We also thank The LAM Foundation and the Tuberous Sclerosis Alliance for patient referral. We thank the Data Safety Monitoring Board Members and Medical Monitor, as well as the clinical trials support staffs at the BWH and the Office of the Clinical Director, National Heart, Lung, and Blood Institute, NIH. We are grateful to Saint George's, University London for permission to use the SGRQ.

Support

This study was supported by a Department of Defense Grant W81XWH-15-1-0511, TS140054 to DJK and in part by the Division of Intramural Research NIH/NHLBI and the Lucy Engles Program in TSC and LAM research.

References:

1. McCormack FX, Henske EP. Lymphangioleiomyomatosis and pulmonary disease in tsc. In: Kwiatkowski DJ, Whittemore VH, Thiele EA, editors. Tuberous sclerosis complex. Weinheim, Germany: Wiley-VCH; 2010. p. 345-368.
2. Kwiatkowski DJ, Manning BD. Tuberous sclerosis: A gap at the crossroads of multiple signaling pathways. *Hum Mol Genet* 2005;14 Spec No. 2:R251-258.
3. McCormack FX, Inoue Y, Moss J, Singer LG, Strange C, Nakata K, Barker AF, Chapman JT, Brantly ML, Stocks JM, Brown KK, Lynch JP, 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. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med* 2011;364:1595-1606.
4. Li C, Lee PS, Sun Y, Gu X, Zhang E, Guo Y, Wu CL, Auricchio N, Priolo C, Li J, Csibi A, Parkhitko A, Morrison T, Planaguma A, Kazani S, Israel E, Xu KF, Henske EP, Blenis J, Levy BD, Kwiatkowski D, Yu JJ. Estradiol and mtorc2 cooperate to enhance prostaglandin biosynthesis and tumorigenesis in tsc2-deficient lam cells. *J Exp Med* 2014;211:15-28.
5. Swigris JJ, Lee HS, Cohen M, Inoue Y, Moss J, Singer LG, Young LR, McCormack FX. St. George's respiratory questionnaire has longitudinal construct validity in lymphangioleiomyomatosis. *Chest* 2013;143:1671-1678.
6. El-Chemaly S, Taveira-Dasilva A, Goldberg HJ, Peters E, Haughey M, Bienfang D, Jones AM, Julien-Williams P, Cui Y, Villalba JA, Bagwe S, Maurer R, Rosas IO, Moss J, Henske EP. Sirolimus and autophagy inhibition in lymphangioleiomyomatosis: Results of a phase i clinical trial. *Chest* 2017;151:1302-1310.
7. Young L, Lee HS, 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, Downey GP, Swigris JJ, Taveira-DaSilva AM, Krischer JP, Trapnell BC, McCormack FX. Serum vegf-d a concentration as a biomarker of lymphangioleiomyomatosis severity and treatment response: A prospective analysis of the multicenter international lymphangioleiomyomatosis efficacy of sirolimus (miles) trial. *Lancet Respir Med* 2013;1:445-452.
8. Davydova N, Harris NC, Roufail S, Paquet-Fifield S, Ishaq M, Streltsov VA, Williams SP, Karnezis T, Stacker SA, Achen MG. Differential receptor binding and regulatory mechanisms for the lymphangiogenic growth factors vascular endothelial growth factor (vegf)-c and -d. *J Biol Chem* 2016;291:27265-27278.
9. Morfoisse F, Tatin F, Hantelys F, Adoue A, Helfer AC, Cassant-Sourdy S, Pujol F, Gomez-Brouchet A, Ligat L, Lopez F, Pyronnet S, Courty J, Guillermet-Guibert J, Marzi S, Schneider RJ, Prats AC, Garmy-Susini BH. Nucleolin promotes heat shock-associated translation of vegf-d to promote tumor lymphangiogenesis. *Cancer Res* 2016;76:4394-4405.
10. Yang YC, Lu ML, Rao JY, Wallerand H, Cai L, Cao W, Pantuck A, Dalbagni G, Reuter V, Figlin RA, Belldegrun A, Cordon-Cardo C, Zhang ZF. Joint association of polymorphism of the fgfr4 gene and mutation tp53 gene with bladder cancer prognosis. *Br J Cancer* 2006;95:1455-1458.
11. Werner U, Werner D, Pahl A, Mundkowski R, Gillich M, Brune K. Investigation of the pharmacokinetics of celecoxib by liquid chromatography-mass spectrometry. *Biomed Chromatogr* 2002;16:56-60.
12. Ogorek B, Hamieh L, Lasseter K, Bagwe S, Machado T, Herranz-Ors C, Thorner AR, Nag A, Gulleman P, Giannikou K, Young LR, Pujana MA, Darling TN, El-Chemaly S, Moss J, Henske EP, Kwiatkowski DJ. Generalised mosaicism for tsc2 mutation in isolated lymphangioleiomyomatosis. *Eur Respir J* 2019;54.
13. McCormack PL. Celecoxib: A review of its use for symptomatic relief in the treatment of osteoarthritis, rheumatoid arthritis and ankylosing spondylitis. *Drugs* 2011;71:2457-2489.

Table 1: Effects of Celecoxib on outcome measures

Variable	Baseline*	End of treatment*¶	p value
FEV1[&] (ml)	2583 ± 166	2518 ± 166	0.14
FVC[&] (ml)	3415 ± 197	3384 ± 197	0.45
DLCO(ml/mmHg/mn)	18.6 ± 1.1	18.4 ± 1.1	0.24
SGRQ	19.1 ± 4.9	21.3 ± 4.9	0.20
VEGF-D	755 ± 199	656 ± 143	0.17

*Least square means ± SEM

[&] post-bronchodilators

¶ End of study visit or early termination