Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Barnes BT, Procaccini D, Crino J, et al. Maternal sirolimus therapy for fetal cardiac rhabdomyomas. N Engl J Med 2018;378:1844-5. DOI: 10.1056/NEJMc1800352

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Maternal Sirolimus Therapy for Fetal Cardiac Rhabdomyomas

Table of Contents:

Additional clinical details

A 27-year-old gravida 2 para 0010 Caucasian female was referred for a fetal echocardiogram at 21 weeks' gestation, revealing multiple homogenous masses, most prominently right ventricular (RV) and left ventricular (LV) masses (Figure S1A) suggestive of cardiac rhabdomyomas. At 28 weeks, progressive mass size increase led to new left ventricular outflow tract (LVOT) and right ventricular outflow tract (RVOT) obstruction as well as mitral valve regurgitation (Figure S1B-1D). Mild hypoplasia of the aortic isthmus raised concern for evolving aortic coarctation.

Supraventricular tachycardia (SVT) with 1:1 atrioventricular conduction at 220-240 beats per minute developed in the fetus at 29 and 6/7 weeks. Signs of increasing fetal cardiovascular compromise -- ascites, polyhydramnios, reversed a-wave flow in the ductus venosus, and pulsatility in the umbilical vein with increasing RVOT and LVOT obstruction -- were noted. After multidisciplinary discussion as well as discussion with the mother and her family regarding risks of the proposed treatment, oral maternal sirolimus therapy was initiated. The potential for hypertriglyceridemia, hepatic insult and bone marrow suppression were discussed with the mother prior to initiation of maternal sirolimus therapy. Along with maternal risks, the lack of data on mechanistic target of rapamycin (mTOR) use in pregnancy and unknown fetal risks were reviewed, emphasizing that maternal sirolimus therapy may have no effect on fetal rhabdomyoma.

Maternal sirolimus therapy was initiated at 30 weeks for a target maternal trough concentration of 10 to 15 ng/mL. At the time of initiation of therapy, the echocardiographic dimensions of the

RV mass were 15.6 x 19.5 mm, and the LV mass measured 12.6 x 20.7 mm. A total of 12 mg of sirolimus (6.3 mg/m^2) administered during the first 48 hours of treatment resulted in a level of 2.1 ng/mL. An additional 22 mg (11.7 mg/m^2), resulted in a level of 10.8 ng/mL, 19 hours post-administration. Daily dosing was then initiated (average daily dose of 13 ± 2 mg or 6.8 ± 1.04 mg/m^2) for an average level of 9.2 ± 2 ng/mL over the course of therapy. Weekly maternal triglycerides, liver function tests and lymphocyte counts were monitored. No elevation in maternal liver function tests or lymphocyte suppression was present. An elevated triglyceride level of 731 mg/dL (normal <150 mg/dL) was noted at 31 and 5/7 weeks. Interpretation of this increase is limited since, to our knowledge, this can occur during pregnancy without sirolimus treatment[1], and normalized following pregnancy. Maternal digoxin and flecainide were initiated for fetal rhythm management.

Daily and then weekly fetal echocardiograms and obstetrical sonograms were performed. Two weeks after maternal sirolimus therapy initiation, tumor regression was appreciated at 31 and 5/7 weeks with significant improvement in biventricular outflow obstruction and normalization of biventricular systolic function. Further tumor regression was noted at 32 and 6/7 weeks as well as 34 and 6/7 weeks (Figure S2A-2D). Fetal SVT resolved within 24 hours of initiating maternal antiarrhythmic therapy. Ascites resolved on ultrasound 4 days later. No arrhythmia was present on follow-up monitoring with normal ductus venosus waveforms and normal umbilical artery Doppler flow characteristics including normal pulsatility indices for gestational age. No growth restriction was present on obstetrical monitoring.

Induction of labor was initiated secondary to intrapartum fetal bradyarrhythmia associated with uterine hypersystole, leading to Caesarean section at 36 and 3/7 weeks. The placenta appeared grossly normal. The maternal sirolimus level at delivery was 6.9 ng/mL. The cord blood sirolimus level was 11.3 ng/mL, and the follow-up infant sirolimus level within 22 hours was 9.3 ng/mL (Table S1). Postnatal cardiac electrocardiography and telemetry revealed sinus rhythm with frequent premature atrial contractions without tachyarrhythmia. An initial postnatal echocardiogram confirmed a RV mass measuring 8.9 x 15.8 mm and LV mass measuring 8.2 x 17.2 mm with normal biventricular systolic function and no outflow tract obstruction. Despite a mildly hypoplastic aortic isthmus, no coarctation was present.

Prenatally, the mother was counseled on the high likelihood of tuberous sclerosis complex (TSC) in the fetus. The family declined prenatal diagnosis via amniocentesis and opted to pursue testing on cord blood. *TSC1/TSC2* sequence analysis and deletion/duplication analysis revealed a pathogenic variant, c.1781delT (p.Val594Glyfs*35), in *TSC1*, confirming the diagnosis of TSC. Parental genetic testing was not performed, but a pedigree review revealed no familial clinical findings associated with TSC in parents or first-degree relatives.

Sirolimus was not administered to the neonate after birth given reassuring clinical findings and the absence of outflow obstruction, despite the presence of masses on initial transthoracic echocardiogram. At two months of age, however, outpatient follow-up while off sirolimus therapy revealed regrowth of the rhabdomyomas and moderate RVOT obstruction with a peak gradient of 58 mm Hg (Figure S3A and S3B). The infant was restarted on oral sirolimus therapy

at 1 mg/m^2 daily, for a goal level of 10 to 15 ng/mL. Weekly sirolimus levels, triglycerides, liver function tests and lymphocyte counts were monitored without abnormalities (Table S1). Transthoracic echocardiogram after three weeks on sirolimus demonstrated interval decrease in rhabdomyoma sizes without ventricular outflow obstruction (Figure S3C and S3D). Sirolimus was discontinued at 4.5 months of age after tumor regression, but at 5 months of age the patient noted to have interval tumor regrowth again. Sirolimus therapy was restarted with the plan to continue therapy and monitoring until complete regression. The goal sirolimus level was decreased to 5 to 8 ng/mL to decrease risks of side effects.

Outpatient follow-up with TSC specialists have revealed no clinical seizure activity and the infant has remained off of anti-epileptic therapy. Fetal as well as initial postnatal brain magnetic resonance imaging have shown no brain masses, with the plan for repeat brain imaging at 1 year of age. The only other clinical manifestations of TSC are hypomelanotic macules. As there are concerns about growth and development in infants exposed to mTOR inhibitors during fetal life and postnatally, these clinical parameters have been followed closely. At 9 months old the infant was at the 89th percentile for weight and the 99th percentile for height based on the World Health Organization girls, 0-2 years reference charts. All developmental milestones have been met.

Additional discussion

Rhabdomyoma is the most common cardiac tumor in fetal life, accounting for 60-86% of primary fetal cardiac tumors. Up to 96% are associated with TSC[2–4]. Most regress naturally within the first 2 to 4 years of life[2,3]. However, large tumors can cause arrhythmia or flow

obstruction. Prenatal predictors of poor neonatal outcome include cardiac tumor size (diameter >20 mm), fetal dysrhythmia and hydrops fetalis[3]. Treatment, both surgical and medical, has been limited to the postnatal setting.

The clinical challenge in this case was how to support the fetus or premature infant with severe hemodynamic impairment until natural regression occurred. When fetal cardiovascular compromise is present, management options during fetal life have predominantly been limited to prenatal rhythm control or early delivery. No medical or interventional options have been described prenatally to specifically target inhibition of tumor growth. Several options were considered given our patient's gestational age with possibility of ongoing tumor progression *in utero,* including the risks associated with prematurity if delivered. Our multidisciplinary team took into consideration that rapid rhabdomyoma growth mainly occurs between the second and early third trimester[4,5]. Nir *et al*. demonstrated in three subjects that growth of rhabdomyomas either plateaus or decelerates relative to ventricular apex-base length, which steadily grows throughout gestation[5]. Chao *et al.* demonstrated that of 77 cases with data on tumor size, 9 (11.7%) increased in size and only a single case regressed *in utero*[3].

No known fetal surgical interventions to date for rhabdomyomas are described in the literature. Postnatal surgical intervention is considered when tumors result in significant hemodynamic abnormalities, although this is not without risk[6]. Padalino *et al.* demonstrated overall good outcomes in patients who underwent postnatal rhabdomyoma surgical resection; however, a 31% postoperative complication rate was noted[6]. This complication rate is primarily due to difficulty

of large myocardial tumor debulking in a neonatal heart with risk to surrounding cardiac structures and the conduction system as well as hemodynamic instability prior to intervention in a premature infant[6]. Ex-utero intrapartum treatment (EXIT) to extracorporeal membrane oxygenation (ECMO) was considered given bilateral outflow obstruction, but posed considerable constraints due to patient size and potential length of cardiopulmonary support while awaiting tumor regression. Lack of medical utility of postnatal prostaglandin therapy or single ventricle palliative surgery for bilateral outflow tract obstruction was also discussed.

Recent case reports using mTOR therapy in infants as a pharmacologic alternative to surgery have demonstrated successful cardiac rhabdomyoma regression[7–9]. This postnatal medical therapy offered a possible prenatal intervention. Therefore, given the significant risk of mortality and lack of other feasible therapeutic interventions at 30 weeks gestation, maternal sirolimus therapy was initiated.

It is known that pathogenic variants in TSC1 and TSC2 genes cause hyperactivity of mTOR, which enhances cellular proliferation and growth. Sirolimus was chosen for its mechanism of action as an mTOR inhibitor and feasibility of drug level monitoring. Careful consideration was given to safety and pharmacokinetic profiles to maximize anticipated benefit. Sirolimus is currently a pregnancy category C[10] drug, with limited clinical data in pregnant women. The largest populations of obstetrical patients in whom mTOR inhibitors, and sirolimus specifically, have been studied are renal and liver transplant recipients. No adverse effects on fetal development or increased incidence of congenital anomalies are reported [11–16].

Maternal dosing was based on established dosing recommendations for solid organ transplant recipients[17,18] . The higher concentration trough goal of 10 to 15 ng/mL was employed given absence of significant adverse effects[17,19,20] and the need to intervene expeditiously. Dosing was also based on potential limitations of sirolimus placental crossing including a molecular weight of 914 daltons and high plasma protein binding of 92%[10] which may impede transfer through lipid-bilayer membranes, including the placenta. Additionally, we recognized that sirolimus is primarily metabolized via P-glycoprotein (Pgp) efflux and the cytochrome-3A4 (CYP3A4) enzyme, both of which are expressed in the full-term human placenta[21,22]. This placental and possibly fetal liver enzyme expression further confounds the pharmacokinetics of the fetus. We acknowledge that there is no known maternal dosing for any fetal indication, specifically cardiac rhabdomyomas. It is quite possible that based on the cord blood level in this case, lower maternal dosing could have had similar clinical response in the fetus. While we cannot state by which route the placental transfer predominantly occurred, the sirolimus cord blood level at birth at 163% that of maternal level makes it plausible that the transfer was preferentially by unidirectional enzyme transportation.

While we were able to demonstrate that sirolimus crossed the placenta, the correlation between tumor regression and initiation of maternal sirolimus therapy is based on observation alone. The fetal rhabdomyomas rapidly declined in size after initiating maternal sirolimus therapy, which presents a strong timing correlation. The observational evidence in this case is further strengthened by the regrowth of the rhabdomyomas while off sirolimus therapy after delivery and again at 4.5 months of age with regression while on therapy (see figure, printed

correspondence). With the decision to start postnatal sirolimus therapy, dosing was chosen at a lower initial dose than used in a similar postnatal infant case report[9] to avoid side effects. The decrease in tumor size *in utero* and postnatally with the initiation of sirolimus therapy obviated the need for surgical intervention, neonatal ECMO or other less ideal treatments including single ventricle palliation[23].

Our case lacks tumor histology due to successful avoidance of surgical intervention, although characteristic imaging findings and confirmatory *TSC1* testing make rhabdomyoma a likely diagnosis[4]. This is a single report and outcomes or safety profiles cannot be generalized. Further studies will need to be done to determine whether these results can be confirmed. With the knowledge that mTOR inhibitors can cross the placenta, it is also unknown what impact the medication could have on other TSC related tumors such as in the brain or renal locations. Our patient did not have tumors in either of these locations so we could not describe any impact here. While a favorable outcome was achieved, our observations remain limited to short-term follow-up.

In summary, we have demonstrated *in utero* tumor regression of giant, obstructive, TSC-associated cardiac rhabdomyomas (associated with a high mortality risk) using maternal sirolimus pharmacotherapy. Further study is required to confirm the effectiveness and safety of maternal sirolimus use for regression of fetal cardiac rhabdomyoma.

Supplementary Figures:

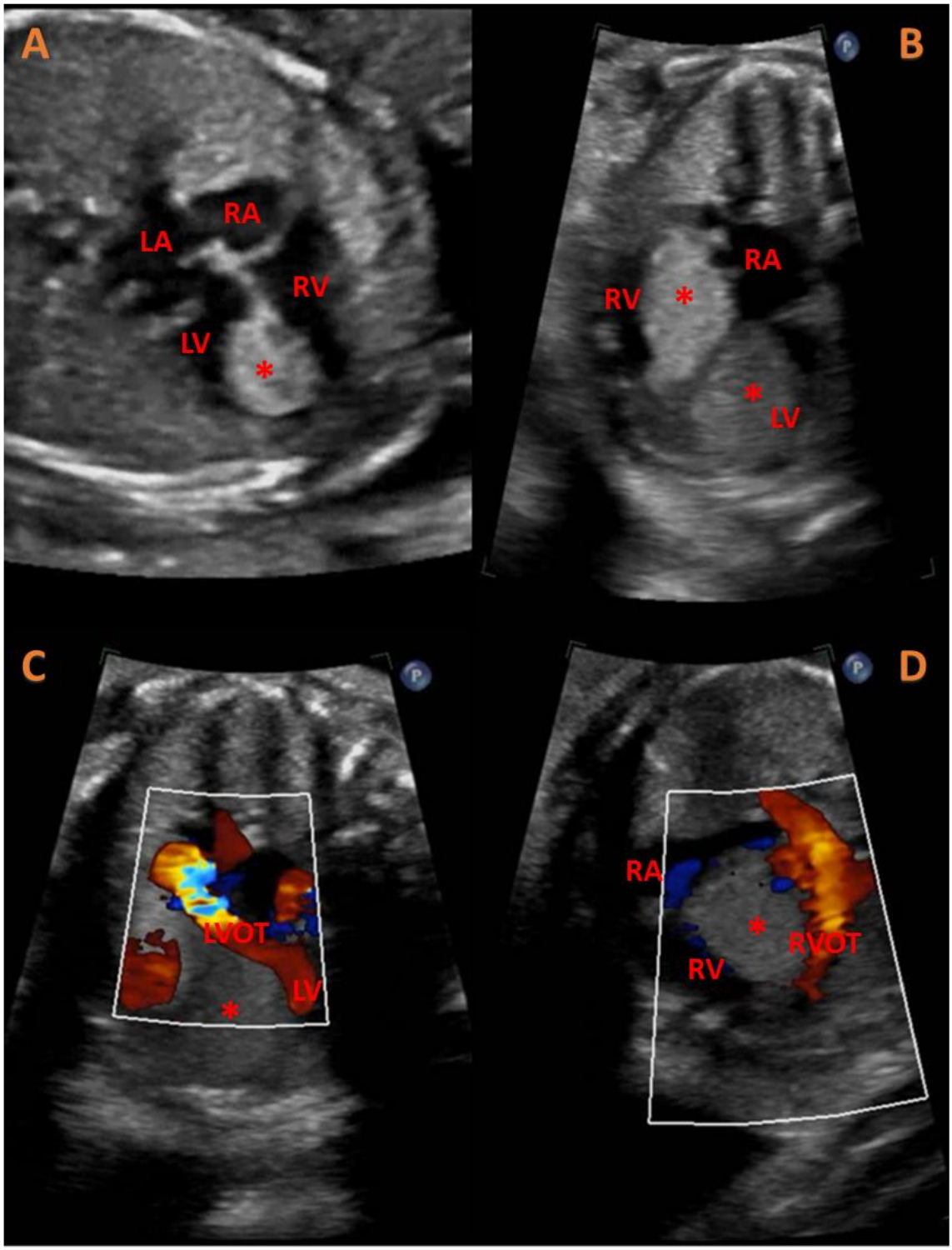

Figure S1. Fetal echocardiography of cardiac rhabdomyomas in this patient. A. Initial fetal echocardiogram demonstrating LV mass in a 4 chamber view, measuring 10 mm x 6 mm at

21 weeks' gestation. B. Fetal echocardiogram at 28 weeks' gestation showing a new RV mass and progressive growth of rhabdomyomas in apical 4 chamber view. C. Apical 5 chamber view with color Doppler demonstrating flow acceleration across the LVOT related to LV mass. D. Short axis view demonstrating obstruction by color Doppler in the RVOT due to mass. (RA: right atrium; RV: right ventricle; LV: left ventricle; LA: left atrium; LVOT: left ventricle outflow tract; RVOT: right ventricle outflow tract; *: rhabdomyoma).

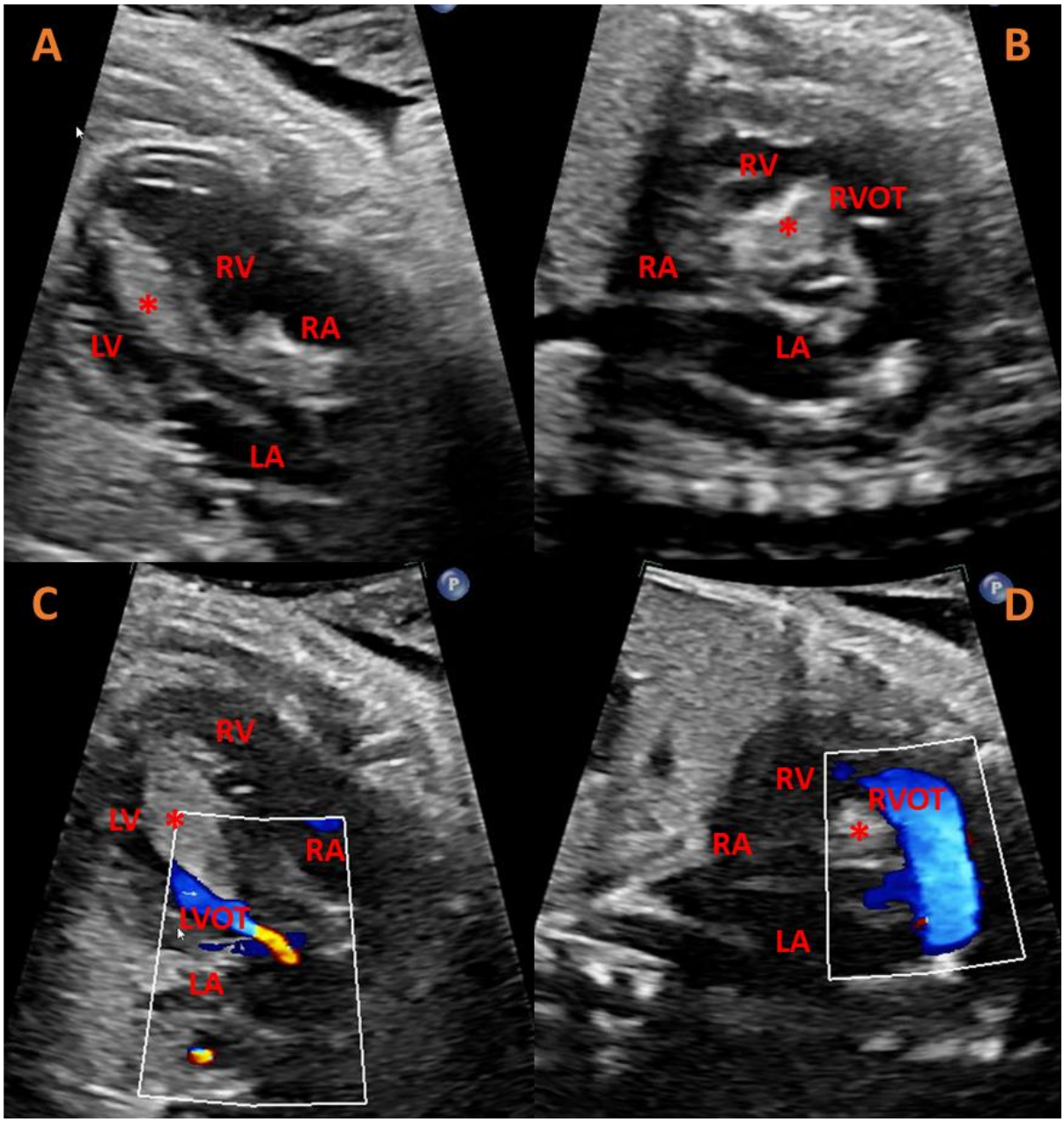

Figure S2. Fetal echocardiogram of cardiac rhabdomyomas at 34 and 6/7 weeks' gestation while on maternal oral sirolimus therapy. A. 4 chamber view demonstrating decreased size of the LV mass. B. Short axis view demonstrating decreased size of the RV mass. C. Apical 5 chamber

view with color Doppler demonstrating laminar flow across the LVOT. D. Short axis view demonstrating no obstruction by color Doppler in the RVOT. (RA: right atrium; RV: right ventricle; LV: left ventricle; LA: left atrium; LVOT: left ventricle outflow tract; RVOT: right ventricle outflow tract; *: rhabdomyoma).

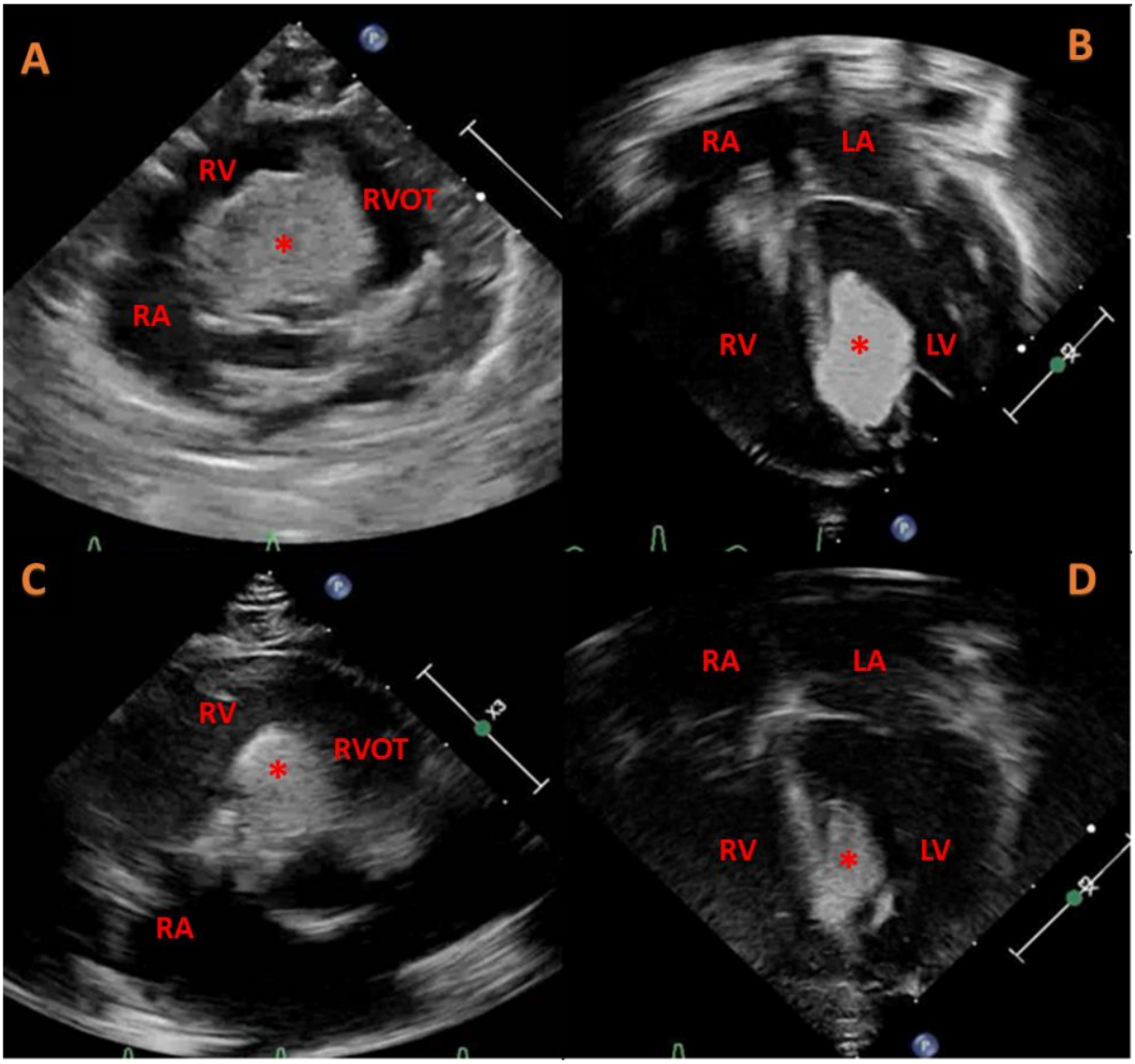

Figure S3. Postnatal transthoracic echocardiogram in the same patient. A-B: Regrowth of cardiac rhabdomyomas at 59 days of life while off sirolimus. A. Parasternal short axis showing the right ventricular rhabdomyoma. B. Apical 4 chamber showing the left ventricular rhabdomyoma. C-D: Transthoracic echocardiogram images at approximately 3 months of age after 2 weeks of sirolimus therapy showing a decrease in size of both rhabdomyomas. C. Parasternal short axis showing the right ventricular rhabdomyoma. D. Apical 4 chamber showing the left ventricular rhabdomyoma. (RA: right atrium; RV: right ventricle; LV: left ventricle; LA: left atrium; RVOT: right ventricle outflow tract; *: rhabdomyoma).

Table S1. Laboratory Values and Concurrent Sirolimus Doses – Infant

	Postnatal Age (Days)											
Laboratory Parameter (Reference Ranges)	**Birth**	**1**	**71**	**74**	**77**	**80**	**97**	**98**	**105**	**112**	**126**	**163**
Creatinine (0.5-1.2 mg/dL)			0.24				0.32	0.24	0.26	0.23	0.18	0.24
WBC (9-30 K/cu mm)	7.18	8.75	7.6				8.6	8.3	10.9	7.6	9.6	11.7
AST (16-60 U/L)			41				48	39	41	43	47	38
ALT (14-54 U/L)			36				53	50	44	44	47	32
Triglycerides (<150mg/dL)			173				138	124	94	130	192	106
Sirolimus Level	11.3	9.3	NA	5.9	13.4	11.5	7.6	5.3	6.6	9.7	8.1	8
Sirolimus Dose (mg/BSA)	0	0	0	1.1	1.1	1.1	0.97	0.97	1.29	1.94	1.71	2.1

References:

1. Pusukuru R, Shenoi AS, Kyada PK, et al. Evaluation of Lipid Profile in Second and Third Trimester of Pregnancy. *J Clin Diagn Res*. 2016;10(3):QC12-6. doi:10.7860/JCDR/2016/17598.7436.

2. Kocabaş A, Ekici F, Cetin İ, et al. Cardiac rhabdomyomas associated with tuberous sclerosis complex in 11 children: presentation to outcome. *Pediatr Hematol Oncol*. 2013;30(2):71-79. doi:10.3109/08880018.2012.734896.

3. Chao AS, Chao A, Wang TH, et al. Outcome of antenatally diagnosed cardiac rhabdomyoma: Case series and a meta-analysis. *Ultrasound Obstet Gynecol*. 2008;31(3):289-295. doi:10.1002/uog.5264.

4. Fesslova V, Villa L, Rizzuti T, Mastrangelo M, Mosca F. Natural history and long-term outcome of cardiac rhabdomyomas detected prenatally. *Prenat Diagn*. 2004;24(4):241-248. doi:10.1002/pd.825.

5. Nir A, Ekstein S, Nadjari M, Raas-Rothschild A, Rein AJJT. Rhabdomyoma in the fetus: Illustration of tumor growth during the second half of gestation. *Pediatr Cardiol*. 2001. doi:10.1007/s002460010287.

6. Padalino MA, Vida VL, Boccuzzo G, et al. Surgery for primary cardiac tumors in children early and late results in a multicenter european congenital heart surgeons association study. *Circulation*. 2012;126(1):22-30. doi:10.1161/CIRCULATIONAHA.111.037226.

7. Ahmet Demir H. Everolimus: A Challenging Drug in the Treatment of Multifocal Inoperable Cardiac Rhabdomyoma. *Pediatrics*. 2012;130:243-247. doi:10.1542/peds.2011-3476.

8. Bornaun H, Öztarhan K, Erener-Ercan T, et al. Regression of Cardiac Rhabdomyomas in a Neonate after Everolimus Treatment. *Case Rep Pediatr*. 2016;2016:8712962. doi:10.1155/2016/8712962.

9. Breathnach C, Pears J, Franklin O, Webb D, McMahon CJ. Rapid Regression of Left Ventricular Outflow Tract Rhabdomyoma After Sirolimus Therapy. *Pediatrics*. 2014;134(4).

10. Rapamune(R) [package insert]. Wyeth Pharmaceuticals Inc. Philadelphia, PA.

11. Danesi R, Del Tacca M. Teratogenesis and immunosuppressive treatment. *Transplant Proc*. 2004;36(3):705-707. doi:10.1016/j.transproceed.2004.03.017.

12. Framarino dei Malatesta M, Corona LE, De Luca L, et al. Successful Pregnancy in a Living-Related Kidney Transplant Recipient Who Received Sirolimus Throughout the Whole Gestation. *Transplantation*. 2011;91(9):e69-e71. doi:10.1097/TP.0b013e3182154267.

13. Fuchs KM, Coustan DR. Immunosuppressant Therapy in Pregnant Organ Transplant Recipients. *Semin Perinatol*. 2007;31(6):363-371. doi:10.1053/j.semperi.2007.09.006.

14. Jankowska I, Oldakowska-Jedynak U, Jabiry-Zieniewicz Z, et al. Absence of teratogenicity of sirolimus used during early pregnancy in a liver transplant recipient. *Transplant Proc*. 2004;36(10):3232-3233. doi:10.1016/j.transproceed.2004.11.102.

15. Veroux M, Corona D, Veroux P. Pregnancy under everolimus-based immunosuppression. *Transpl Int*. 2011;24(12):115-117. doi:10.1111/j.1432-2277.2011.01356.x.

16. Chu S-H, Liu K-L, Chiang Y-J, Wang H-H, Lai P-C. Sirolimus used during pregnancy in a living related renal transplant recipient: a case report. *Transplant Proc*. 2008;40(7):2446-2448. doi:10.1016/j.transproceed.2008.06.015.

17. Costanzo MR, Dipchand A, Starling R, et al. The international society of heart and lung transplantation guidelines for the care of heart transplant recipients. *J Hear Lung Transplant*. 2010;29(8):914-956. doi:10.1016/j.healun.2010.05.034.

18. Mancini D, Pinney S, Burkhoff D, et al. Use of rapamycin slows progression of cardiac transplantation vasculopathy. *Circulation*. 2003;108(1):48-53. doi:10.1161/01.CIR.0000070421.38604.2B.

19. Kushwaha SS, Khalpey Z, Frantz RP, et al. Sirolimus in cardiac transplantation: Use as a primary immunosuppressant in calcineurin inhibitor-induced nephrotoxicity. *J Hear Lung Transplant*. 2005;24(12):2129-2136. doi:10.1016/j.healun.2005.08.015.

20. Zuckermann A, Keogh A, Crespo-Leiro MG, et al. Randomized controlled trial of sirolimus conversion in cardiac transplant recipients with renal insufficiency. *Am J Transplant*. 2012;12(9):2487-2497. doi:10.1111/j.1600-6143.2012.04131.x.

21. Hakkola J, Pasanen M, Hukkanen J, et al. Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. *Biochem Pharmacol*. 1996;51(4):403-411. doi:10.1016/0006-2952(95)02184-1.

22. Hakkola J, Pelkonen O, Fasanen M, Raunio H. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto- placental unit: Role in intrauterine toxicity. *Crit Rev Toxicol*. 1998;28(1):35-72. doi:10.1080/10408449891344173.

23. Mir A, Veeram Reddy S, Ikemba C. Hypoplastic left heart syndrome secondary to intrauterine rhabdomyoma necessitating single ventricle palliation. *Ann Pediatr Cardiol*. 2014;7(3):207. doi:10.4103/0974-2069.140851.