

Sirolimus treatment of a PTEN hamartoma tumor syndrome presenting with melena

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ABSTRACT

Background. PTEN hamartoma tumor syndrome (PHTS) is an umbrella term including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and PTEN-related Proteus-like syndrome. One of the disorders in PHTS spectrum, CS is characterized by macrocephaly, mucocutaneous findings, gastrointestinal system (GIS) polyposis and an increased lifetime risk of GIS, breast, thyroid and other cancers.

Case. In this study, we report an adolescent patient presenting with recurrent life-threatening upper GIS bleeding as a result of hamartomatous polyposis. Genetic studies revealed a known pathogenic nonsense mutation confirming the initial diagnosis of CS.

Conclusions. Additionally, we describe our therapeutic intervention to improve the patient's clinical symptoms with sirolimus, which its use is infrequently addressed in the literature for pediatric age group harboring PTEN mutations.

Key words: PTEN hamartoma tumor syndrome, gastrointestinal system bleeding, PTEN mutation, sirolimus.

PTEN hamartoma tumor syndrome (PHTS) is an umbrella term including cancer predisposition and overgrowth syndromes such as Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS) and *PTEN*-related Proteus-like syndrome.¹ Cowden Syndrome (OMIM 158350) is the first of the PHTS spectrum disorders to be associated with autosomal dominantly inherited germline "Phosphatase and Tensin Homologous) *PTEN* mutations.²⁻⁴ Syndrome is characterized by macrocephaly, mucocutaneous findings such as trichilemmomas, papillomatous papules, acral keratosis and penile freckling, multiple hamartomas in

various tissues including gastrointestinal system (GIS) polyposis and an increased lifetime risk for benign and malignant tumors of breast, thyroid, genitourinary and GIS.^{2,5-7} Polyps are predominantly hamartomatous, but mixed histologic types including hyperplastic, inflammatory and adenomatous types could also be seen.¹ Polyps may be asymptomatic or complicated by bleeding, obstruction, invagination, and infarction.⁸ The prevalence of CS in the general population is thought to be 1 in 200,000, and although the cases are usually diagnosed in the third decade, there are rare cases in early childhood.^{8,9}

PTEN is a lipid phosphatase, a negative regulator of the phosphatidylinositol-3-kinase (PI3K) / protein kinase B (AKT)/ the mammalian target of rapamycin (mTOR) signaling pathway and plays an active role in cell cycle and apoptosis

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by controlling phosphoinositol triphosphate levels.³ Loss of function of this tumor suppressor gene leads to inappropriate activation of the PI3K/AKT /mTOR pathway, uncontrolled cell growth and proliferation causing benign and malignant tumor formations.^{3,10} In recent years, successful outcomes have been reported in the treatment of benign and malignant tumors by targeting the inhibition of PTEN-related PI3K/AKT/mTOR pathway with an mTOR inhibitor, sirolimus.^{11,12} Here, we report an adolescent CS patient presenting with life-threatening recurrent upper GIS bleeding. Additionally, we describe our therapeutic intervention to improve the patient's clinical symptoms with sirolimus, which its use is infrequently addressed in the literature for pediatric age group with PTEN mutations.

Case Report

A fourteen-year-old male patient with melena was referred to our hospital for the evaluation of GIS bleeding. From his medical history, it was learned that he had a stomach ache for the last two months, and malaise and darkening of the stool color for the last week.

His parents were first-degree cousins and he had no further significant finding in his family history. On his physical examination, body weight, height and head circumference were compatible with 25p, 10p, and >97 p, respectively. His skin and mucous membranes were pale. He had multiple skin-colored soft papules on his face, axillary region, upper extremity and back (Fig. 1a). Additionally, a hyper-pigmented macule with irregular edges in the glans penis was observed (Fig. 1b). Other system findings were normal. His laboratory examinations revealed iron deficiency anemia; Hgb 7.6 g/dL (9.5-13.3 g/dL), serum iron 24 µg/dL (45-182 µg/dL), unsaturated iron binding capacity 358 µg/dL (155-300 µg/dL), ferritin 9 ng/mL (23-70 ng/mL), white blood cell count $11.7 \times 10^3/\mu\text{L}$, platelet count $339 \times 10^3/\mu\text{L}$. In his upper GIS endoscopic examination, locally eroded, ulcerated and pedunculated or non-

pedunculated polyps with diameters ranging from 0.3-2 cm, localized mostly in the antrum-corpus junction and antrum were observed (Fig. 1c). Colonoscopy showed a decreasing number of polyps from rectum to sigmoid and no polyps were observed in proximal segments of colon. Histopathologic examination demonstrated that the polyps located in the stomach were compatible with mix histology consisting of both hyperplastic and hamartomatous types, and those in the colon were hyperplastic polyps (Fig. 1d).

Genetic studies were performed after obtaining written informed consent from the patient's parent. Genomic DNA of peripheral blood leukocytes was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Libraries were prepared using a capture-based target enrichment kit (Hereditary Cancer Solution™, Sophia genetics, Switzerland) containing 27 genes (Supp. File: *ATM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, MLH 1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PIK3CA, PMS2, PMS2CL, PTEN, RA D50, RAD51C, RAD51D, STK11, TP53* and *XRCC2*). Next-generation sequencing (NGS) was performed on an Illumina MiSeq System (Illumina, USA). After sequencing, data were analyzed using Sophia DDM software v.5.7.7. (Sophia Genetics, Switzerland). Analysis revealed a known heterozygous nonsense variant c.388C>T; p.Arg130Ter in the 5th exon of *PTEN* (NM_000314). No pathogenic variants were found in *APC* and *MUTYH* responsible for different polyposis syndromes. The identified variant in the patient was not observed in his mother. Segregation of the variant could not be done for the father, as he could not be reached.

Ultrasonography of the thyroid, abdomen and scrotum were normal. Cranial magnetic resonance imaging for the presence of AV-malformations was also normal. Upper GIS bleeding was treated with proton pump inhibitors (PPIs) and octreotide infusion therapy. Occult blood positivity in the stool continued, although his major symptoms

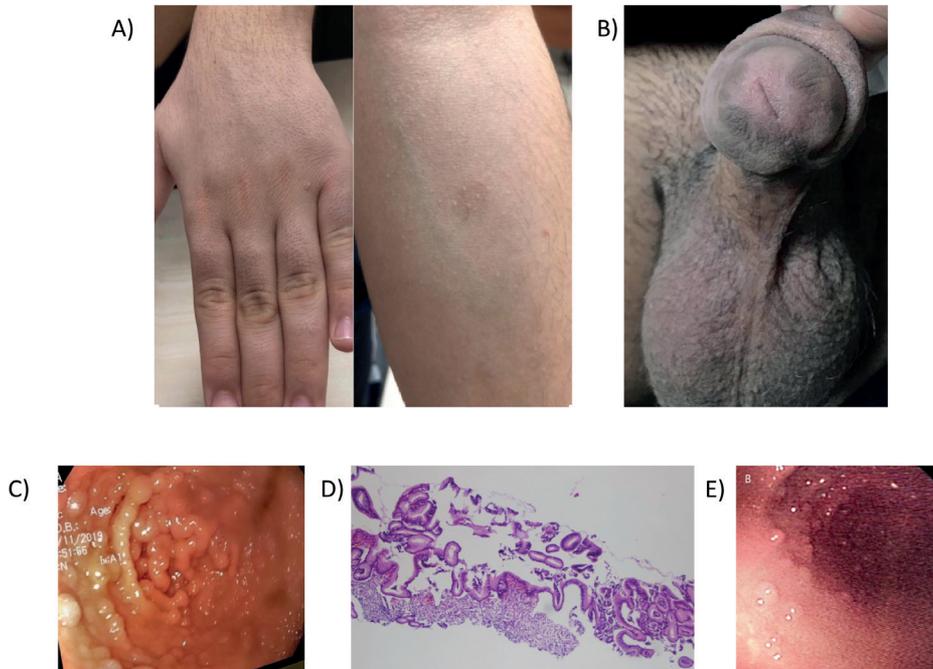


Fig. 1. A) Multiple skin-colored soft papules on the upper extremity. B) Hyper pigmented macule with irregular edges in glans penis. C) Upper gastrointestinal system endoscopic examination demonstrating locally eroded, ulcerated and pedunculated or non-pedunculated polyps with diameters ranging from 0.3-2 cm, localized in the entire gastric mucosa. D) Gastric hyperplastic polyp with visible elongated foveolar epithelium (Hematoxylin and eosin stain, ×20 magnification.) E) Significant decrease in the number of polyps and size of the polyps in the third month of treatment.

regressed. Sirolimus treatment was planned in the patient whose macroscopic bleeding recurred twice during the follow-up, as conventional treatments were insufficient. Sirolimus treatment was initiated at a 0.8 mg/m² dosage and was administered per oral twice daily. During the treatment, complete blood cell count, hepatic and renal function tests, lipid profile and serum levels of sirolimus were monitored regularly. Serum sirolimus levels were between 6-9 ng/ml during the follow-up. The upper and lower endoscopic examinations were repeated in the third month of the treatment. There was a significant decrease in the number and size of the polyps (Fig. 1e). He continued on a therapeutic dose of sirolimus for a total of 6 months without any major adverse effects. He had only transient mild hypercholesterolemia. Sirolimus was tapered down in two months. The patient was followed for two years and GIS bleeding did not recur.

Discussion

We report an adolescent patient with PHTS whose clinical, laboratory and endoscopic findings were successfully treated with sirolimus. His clinical features (macrocephaly, wart-like papules on his skin and hyperpigmented penile macules) and hamartomatous GIS polyposis were compatible with the clinical diagnostic criteria for PTEN Hamartoma Tumor Syndrome based on the National Comprehensive Cancer Network (NCCN) Guidelines.¹³ Clinical diagnosis is also confirmed with the identification of c.388>T p. (Arg130Ter) nonsense variant by molecular studies. PTEN protein consists of two important domains that are required for the tumor suppressor function. First domain is the phosphatase (catalytic) domain that is located between the amino acids 14 to 185, and the second is the C2 lipid membrane-binding

domain, which participates in membrane binding, placed in between the amino acids 190 and 350.¹⁴ Exon 5 is a hotspot for germline mutations as it harbors the tyrosine phosphatase signature motif H123CKAGKGR130 and within this loop, the C124 and R130 residues are essential for the physiological function of PTEN in tumor suppression.¹⁵

Identified variant in the present patient is located in signature motif of the catalytic domain at the 130th position. This variant has been observed in several individuals affected with PHTS.¹⁶ The truncating variant is expected to undergo nonsense-mediated decay, supporting haploinsufficiency as the cause of the clinical features of the present patient.

Main GIS findings of CS are non-neoplastic polyps. Although GIS polyps are predominantly of hamartomatous histopathology, hyperplastic or adenomatous polyps and mixed polyposis with malignancy risk are also reported.¹⁷ Multiple gastric, ileal and colorectal polyps were signs of major GIS involvement in our patient. Although the polyps detected in the patient were of hyperplastic and hamartomatous histopathology, long-term follow-up was continued due to increased risk for the development of malignancies, especially in the colon and thyroid. Thus, detection of *PTEN* mutation changed the management of the patient not only in terms of follow-up but also in terms of treatment options.

Although polyposis is rare in the pediatric age group, it is diagnosed more frequently with the more widespread use of endoscopic diagnostic examinations.¹⁸ Invasive procedures are often required for the treatment of polyps complicated by severe life-threatening bleeding and anemia. Although our patient's polyps were benign in nature, they caused severe and repetitive GIS bleeding, and profound anemia

requiring aggressive transfusion. Polypectomy was not considered as there were too many polyps to be resected.

PTEN alterations result in an enhanced PI3K/AKT/mTOR signaling, causing an uncontrolled cell proliferation and suppression of this pathway represents a rational therapeutic target in PHTS.¹⁰ In recent years significant success has been achieved in the treatment of PHTS patients with sirolimus, an mTOR inhibitor. Beneficial results using sirolimus were first demonstrated in 2008 in both experimental animal models and a 26-month-old male patient with *PTEN*-related Proteus syndrome (PS).^{12,19} In this patient with PS, oral sirolimus treatment at a dose of 0,1 mg/kg/d for 2 months was effective in reducing the size of hamartomatous masses with prominent clinical improvement.¹² Three years later, another report involving a treatment attempt with sirolimus, demonstrated that a 6-year-old male patient with BRRS regained pain free full mobility as a result of a reduction in the size of vascular masses, with only minor side effects.²⁰ Clinical improvements reported in abovementioned studies provide a rationale for sirolimus therapy in patients with disorders in the PHTS spectrum. As a result of this rationale, trials have begun to demonstrate the efficacy of sirolimus for patients harboring *PTEN* mutations with complex vascular anomalies. The majority of studies reported full or partial response to sirolimus such as an amelioration in pain scale scores, an improvement in the appearance and the cutaneous discoloration of the vascular lesions and an increase in patients' performance and quality of life.²¹⁻²⁵ In addition to vascular anomalies, there is a growing number of cases in the literature reporting successful outcomes with sirolimus in various clinical presentations associated with *PTEN* mutations including oral hamartomatous lesions, thymus hyperplasia, abdominal lipomatosis, isolated infiltrative soft tissue

lesions, ganglioneuromatosis, Lhermitte Duclos disease and hypoinsulinemic hypoglycemia.²⁶⁻³¹ Moreover, mTOR inhibitors were shown to inhibit polyp formation and prolong the process of the development of dysplasia in mouse models.^{32,33} Regarding the efficacy of sirolimus on GIS findings in patients, successful results in decreasing the number and size of polyps have been demonstrated not only for *PTEN* point mutations but also for intragenic *PTEN* deletions and larger deletions encompassing both *PTEN* and *BMRP1A*.^{26,34-37} In another recent interventional study by Komiya et al.¹¹, a 56-day course of sirolimus treatment was well tolerated in patients with PHTS and was associated with

some evidence of improvement in symptoms, skin and GI lesions, cerebellar function, and decreased mTOR signaling. Thus, mTOR inhibitor treatment was initiated in the present study, as a potent role of sirolimus was shown in several studies in patients with PHTS. Table I summarizes the comprehensive details of the main studies available in the literature to date.

During the treatment, hemoglobin and hematocrit values increased to reference values and significant decrease of the size and number of polyps was observed after a period of 6 months. Red blood cell transfusion was not needed during the 2-year follow-up.

Table I. Studies in which sirolimus was used for the treatment of PHTS associated clinical findings.

Study ^{Reference}	Disorder / Genetic result (NM_000314.8)	Sirolimus dose	Outcomes / Side effects
Komiya et al., 2019 ¹¹	PTHS (18 patients) / <i>PTEN</i> mutations were located in exons 1 through 8	A loading dose of 6 mg, followed by a 2 mg dose	Regression of skin and GI lesions, improvement in cerebellar function score at 1 month / Abnormalities in liver enzymes (39%), electrolytes (33%), and anemia (33%). Two individuals developed grade 3 toxicities (hypophosphatemia and lymphopenia)
Marsh et al., 2008 ¹²	Proteus syndrome (PTHS) / c.507delC (p.Ser170ValfsTer13)	0.1mg/kg/d, serum levels were maintained between 5–10 ng/ml	Increase in the patient's serum albumin levels and reduction in soft tissue masses. At the age of 5 years and 6 months the patient began walking independently / No side effects
Iacobas et al., 2011 ²⁰	BRRS (PTHS) / c.913_914insT (p.Ser305MetfsTer7)	0.8 mg/m ² /d aiming a serum level of 10–15 ng/ml	The pain was reduced and patient regained pain-free full mobility. Decrease in size of the vascular masses / Minor side effects: few oral ulcers and mild hypercholesterolemia
Adams et al., 2016 ²¹	2 patients with AVM, 4 patients with overgrowth + VA (PHTS) / NA	0.8 mg/m ² /d Target serum levels of 10 to 15 ng/mL	Partial response in 5 patients, stable disease course in 1 patient with overgrowth / NA
Triana et al., 2017 ²²	VA (PHTS) / NA	0.8 mg/m ² /12hour	Partial response / Insignificant side effects
Pimpalwar et al., 2018 ²³	Vascular anomalies (PHTS) (4 patients)	0.8 mg/m ² /d Target serum levels of 7 to 10 ng/mL	Pain improvement. Sirolimus did not prevent increase in the size of the hamartoma or development of VA / oral mucositis and elevated triglycerides
Sandbank et al., 2019 ²⁴	AVM (PHTS) / NA	NA	Improvement in appearance and cutaneous discoloration of lesion, betterment in pain scale score with increased performance capacity / Grade 1 mouth sores

AVM: Arteriovenous malformation, BRRS: Bannayan-Riley-Ruvalcaba syndrome, d: day, kg: kilogram, m²: square meter, mg: milligram, ml: milliliter, NA: Not assessed, ng: nanogram, PHTS: PTEN hamartoma tumour syndrome, VA: Vascular anomaly *Asterix represents the cases who did not receive oral sirolimus treatment.

Table I. Continued

Study ^{Reference}	Disorder / Genetic result (NM_000314.8)	Sirolimus dose	Outcomes / Side effects
Schmid et al., 2014 ²⁶	PHTS Heterozygous intragenic PTEN deletion of the exons 2–9	0.1mg/kg/d, serum levels were maintained between 5–10 ng/ml	Improved somatic growth and reduced thymus volume. After 1 year of treatment, the patient was able to walk. These effects diminished over the treatment period of 19 months / No side effects
Zak et al., 2017 ²⁷	Lhermitte Duclos Disease (PHTS) / NA	Via gastrostomy tube at 0.6 mg/kg/d*	Regression of the mass, improvement of clinical status and quality of life / NA
Rosenfeld et al., 2019 ²⁸	Ganglioneuromatosis (PHTS) / c.741dupA (p.Pro248ThrfsTer5)	NA	Improvement of rheumatologic manifestations. / NA
Botsali et al., 2019 ²⁹	Oral hamartomatous lesions (PHTS) / NA	Topical sirolimus 0.5% once daily*	Sustained benefit, lesions did not recur / NA
Siklar et al., 2020 ³⁰	Recurrent severe hypoglycemia (PHTS) / c.395G>T (p Gly132Val)	Beginning dose was 0.5 mg/m ² /d and increased to 4 mg/m ² /d to reach serum level between 4 to 12 mg/dl	Hypoglycemia was controlled / No side effects
Hill et al., 2021 ³¹	PHTS c.1028T>A (p.Val343Glu)	0.8 mg/m ² /d Target serum levels of 10 to 15 ng/mL	Marked improvement in pain, softening of the lesion, and decrease in neuropathy, no decrease in size of the lesion / NA
Busoni et al., 2019 ³⁴	Juvenile polyposis / Deletion of <i>PTEN</i> and <i>BMPRIA</i>	0.8 mg/m ² /d Target serum levels of 6 to 8 ng/mL	Scarce distribution and smaller size of polyps, improved severe protein-losing enteropathy / NA
Quaranta et al., 2019 ³⁵	Juvenile polyposis / Deletion of <i>PTEN</i> and <i>BMPRIA</i>	Blood concentration of 5 ng/mL	Decrease in number and size of polyps, weight improvement / No side effects
Taylor et al., 2021 ³⁶	Juvenile polyposis / Deletion of <i>PTEN</i> and <i>BMPRIA</i>	Target serum level of 5 ng/mL	Improved protein-losing enteropathy and chronic gastrointestinal bleeding / Well tolerated no side effects
Bell et al., 2022 ³⁷	Juvenile polyposis / Deletion of <i>PTEN</i> and <i>BMPRIA</i>	1.5 mg/m ² , targeting a serum trough level of 6–8 ng/ml	Improvement in protein-losing enteropathy, decreased intestinal blood loss, and improved weight gain. Decrease in gastrointestinal polyp burden / NA

AVM: Arteriovenous malformation, BRRS: Bannayan-Riley-Ruvalcaba syndrome, d: day, kg: kilogram, m²: square meter, mg: milligram, ml: milliliter, NA: Not assessed, ng: nanogram, PHTS: PTEN hamartoma tumour syndrome, VA: Vascular anomaly *Asterix represents the cases who did not receive oral sirolimus treatment.

Frequently reported side effects of sirolimus are abdominal pain, dyspepsia, mucositis, diarrhea, constipation, fatigue, headache, delayed wound healing, skin rash, lymphopenia, electrolyte imbalances, hyperglycemia and dyslipidemia.³⁸ No undesirable effects were observed in our patient, except for mildly elevated cholesterol levels, at the 24th week of the treatment.

There is no specific recommendation for the duration of sirolimus treatment in the literature as far as we know. Treatment period differs among previous studies ranging from 56 days to 40 months.^{11,39}

Due to the rarity of similar cases, information regarding all aspects of sirolimus treatment is limited. Improved post-treatment clinical,

laboratory and endoscopic findings contribute to the favorable outcome of sirolimus treatment for the GIS findings of PHTS patients. Nevertheless, monitoring the treatment outcomes of future patients will further delineate the long-term effects of sirolimus in PHTS patients and contribute to the establishment of standard therapy protocols.

Ethical approval

Genetic studies were performed after obtaining written informed consent from the patient's parent.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GES, FOH; data collection: GES, NGL, AEG; analysis and interpretation of results: GES, SY, NGL, AEG, GS; draft manuscript preparation: GES, SY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Yehia L, Eng C. PTEN hamartoma tumor syndrome. 2001 Nov 29 [Updated 2021 Feb 11]. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2021. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK1488/>
2. Pilarski R. PTEN hamartoma tumor syndrome: a clinical overview. *Cancers (Basel)* 2019; 11: 844. <https://doi.org/10.3390/cancers11060844>
3. Blumenthal GM, Dennis PA. PTEN hamartoma tumor syndromes. *Eur J Hum Genet* 2008; 16: 1289-1300. <https://doi.org/10.1038/ejhg.2008.162>
4. Lloyd KM, Denis M. Cowden's disease. A possible new symptom complex with multiple system involvement. *Ann Intern Med* 1963; 58: 136-142. <https://doi.org/10.7326/0003-4819-58-1-136>
5. Hammami S, Berriche O, Ali HB, Hellara O, Ansar F, Mahjoub S. Managing the risk of cancer in Cowden syndrome: a case report. *J Med Case Rep* 2012; 6: 225. <https://doi.org/10.1186/1752-1947-6-225>
6. Masmoudi A, Chermi ZM, Marrekchi S, et al. Cowden syndrome. *J Dermatol Case Rep* 2011; 5: 8-13. <https://doi.org/10.3315/jdcr.2011.1063>
7. Bubien V, Bonnet F, Brouste V, et al. High cumulative risks of cancer in patients with PTEN hamartoma tumour syndrome. *J Med Genet* 2013; 50: 255-263. <https://doi.org/10.1136/jmedgenet-2012-101339>
8. Cauchin E, Touchefeu Y, Matysiak-Budnik T. Hamartomatous tumors in the gastrointestinal tract. *Gastrointest Tumors* 2015; 2: 65-74. <https://doi.org/10.1159/000437175>
9. Smpokou P, Fox VL, Tan W-H. PTEN hamartoma tumour syndrome: early tumour development in children. *Arch Dis Child* 2015; 100: 34-37. <https://doi.org/10.1136/archdischild-2014-305997>
10. Dillon LM, Miller TW. Therapeutic targeting of cancers with loss of PTEN function. *Curr Drug Targets* 2014; 15: 65-79. <https://doi.org/10.2174/1389450114666140106100909>
11. Komiya T, Blumenthal GM, DeChowdhury R, et al. A pilot study of sirolimus in subjects with cowden syndrome or other syndromes characterized by germline mutations in PTEN. *Oncologist* 2019; 24: 1510-e1265. <https://doi.org/10.1634/theoncologist.2019-0514>
12. Marsh DJ, Trahair TN, Martin JL, et al. Rapamycin treatment for a child with germline PTEN mutation. *Nat Clin Pract Oncol* 2008; 5: 357-361. <https://doi.org/10.1038/ncponc1112>
13. Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Breast and Ovarian V.1.2022. ©National Comprehensive Cancer Network, Inc. 2021.
14. Hollander MC, Blumenthal GM, Dennis PA. PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer* 2011; 11: 289-301. <https://doi.org/10.1038/nrc3037>
15. Smith IN, Briggs JM. Structural mutation analysis of PTEN and its genotype-phenotype correlations in endometriosis and cancer. *Proteins* 2016; 84: 1625-1643. <https://doi.org/10.1002/prot.25105>

16. Bonneau D, Longy M. Mutations of the human PTEN gene. *Hum Mutat* 2000; 16: 109-122. [https://doi.org/10.1002/1098-1004\(200008\)16:2<109::aid-humu3>3.0.co;2-0](https://doi.org/10.1002/1098-1004(200008)16:2<109::aid-humu3>3.0.co;2-0)
17. Trufant JW, Greene L, Cook DL, McKinnon W, Greenblatt M, Bosenberg MW. Colonic ganglioneuromatous polyposis and metastatic adenocarcinoma in the setting of Cowden syndrome: a case report and literature review. *Hum Pathol* 2012; 43: 601-604. <https://doi.org/10.1016/j.humpath.2011.06.022>
18. Wang LC, Lee HC, Yeung CY, Chan WT, Jiang CB. Gastrointestinal polyps in children. *Pediatr Neonatol* 2009; 50: 196-201. [https://doi.org/10.1016/S1875-9572\(09\)60063-2](https://doi.org/10.1016/S1875-9572(09)60063-2)
19. Squarize CH, Castilho RM, Gutkind JS. Chemoprevention and treatment of experimental Cowden's disease by mTOR inhibition with rapamycin. *Cancer Res* 2008; 68: 7066-7072. <https://doi.org/10.1158/0008-5472.CAN-08-0922>
20. Iacobas I, Burrows PE, Adams DM, Sutton VR, Hollier LH, Chintagumpala MM. Oral rapamycin in the treatment of patients with hamartoma syndromes and PTEN mutation. *Pediatr Blood Cancer* 2011; 57: 321-323. <https://doi.org/10.1002/psc.23098>
21. Adams DM, Trenor CC 3rd, Hammill AM, et al. Efficacy and safety of sirolimus in the treatment of complicated vascular anomalies. *Pediatrics* 2016; 137: e20153257. <https://doi.org/10.1542/peds.2015-3257>
22. Triana P, Dore M, Cerezo VN, et al. Sirolimus in the treatment of vascular anomalies. *Eur J Pediatr Surg* 2017; 27: 86-90. <https://doi.org/10.1055/s-0036-1593383>
23. Pimpalwar S, Yoo R, Chau A, Ashton D, Margolin J, Iacobas I. Temporal evolution and management of fast flow vascular anomalies in PTEN hamartoma tumor syndrome. *Int J Angiol* 2018; 27: 158-164. <https://doi.org/10.1055/s-0038-1661377>
24. Sandbank S, Molho-Pessach V, Farkas A, Barzilay A, Greenberger S. Oral and topical sirolimus for vascular anomalies: a multicentre study and review. *Acta Derm Venereol* 2019; 99: 990-996. <https://doi.org/10.2340/00015555-3262>
25. Freixo C, Ferreira V, Martins J, et al. Efficacy and safety of sirolimus in the treatment of vascular anomalies: a systematic review. *J Vasc Surg* 2020; 71: 318-327. <https://doi.org/10.1016/j.jvs.2019.06.217>
26. Schmid GL, Kässner F, Uhlig HH, et al. Sirolimus treatment of severe PTEN hamartoma tumor syndrome: case report and in vitro studies. *Pediatr Res* 2014; 75: 527-534. <https://doi.org/10.1038/pr.2013.246>
27. Zak M, Ledbetter M, Maertens P. Infantile Ihermitte-duclos disease treated successfully with rapamycin. *J Child Neurol* 2017; 32: 322-326. <https://doi.org/10.1177/0883073816681340>
28. Rosenfeld EH, Chumpitazi BP, Castro E, Naik-Mathuria B. Diffuse intestinal ganglioneuromatosis causing severe intestinal dysmotility in a child with a PTEN mutation. *J Pediatr Gastroenterol Nutr* 2019; 68: e35-e37. <https://doi.org/10.1097/MPG.0000000000002072>
29. Botsali A, Caliskan E, Eşme P, Dündar Ö. Oral hamartomatous lesion of Cowden's disease treated with the combination of erbium:YAG laser and topical sirolimus. *Dermatol Ther* 2019; 32: e13002. <https://doi.org/10.1111/dth.13002>
30. Şıklar Z, Çetin T, Çakar N, Berberoğlu M. The effectiveness of sirolimus treatment in two rare disorders with nonketotic hypoinsulinemic hypoglycemia: the role of mTOR pathway. *J Clin Res Pediatr Endocrinol* 2020; 12: 439-443. <https://doi.org/10.4274/jcrpe.galenos.2020.2019.0084>
31. Hill LRS, Duis J, Kulungowski AM, Annam A, Siegele B, Nakano TA. A challenging diagnosis: PTEN hamartoma tumor syndrome presenting as isolated soft-tissue vascular anomalies. *J Vasc Anom (Phila)* 2021; 2: e011. <https://doi.org/10.1097/jova.0000000000000011>
32. Hung KE, Maricevich MA, Richard LG, et al. Development of a mouse model for sporadic and metastatic colon tumors and its use in assessing drug treatment. *Proc Natl Acad Sci U S A* 2010; 107: 1565-1570. <https://doi.org/10.1073/pnas.0908682107>
33. Hardiman KM, Liu J, Feng Y, Greenson JK, Fearon ER. Rapamycin inhibition of polyposis and progression to dysplasia in a mouse model. *PLoS One* 2014; 9: e96023. <https://doi.org/10.1371/journal.pone.0096023>
34. Busoni VB, Orsi M, Lobos PA, et al. Successful treatment of juvenile polyposis of infancy with sirolimus. *Pediatrics* 2019; 144: e20182922. <https://doi.org/10.1542/peds.2018-2922>
35. Quaranta M, Laborde N, Ferrand A, et al. Sustainable positive response to sirolimus in juvenile polyposis of infancy. *J Pediatr Gastroenterol Nutr* 2019; 68: e38-e40. <https://doi.org/10.1097/MPG.0000000000002179>
36. Taylor H, Yerlioglu D, Phen C, et al. mTOR inhibitors reduce enteropathy, intestinal bleeding and colectomy rate in patients with juvenile polyposis of infancy with PTEN-BMPRI1A deletion. *Hum Mol Genet* 2021; 30: 1273-1282. <https://doi.org/10.1093/hmg/ddab094>

37. Bell LD, Bernat JA, Rahhal R. Polygenic infantile juvenile polyposis syndrome managed with sirolimus and endoscopic polypectomy. *Gastroenterology Res* 2022; 15: 33-38. <https://doi.org/10.14740/gr1480>
38. Kaplan B, Qazi Y, Wellen JR. Strategies for the management of adverse events associated with mTOR inhibitors. *Transplant Rev (Orlando)* 2014; 28: 126-133. <https://doi.org/10.1016/j.trre.2014.03.002>
39. Yuksekkaya H, Yucel A, Gumus M, Esen H, Toy H. Familial adenomatous polyposis; succesful use of sirolimus. *Am J Gastroenterol* 2016; 111: 1040-1041. <https://doi.org/10.1038/ajg.2016.159>