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A NEW APPROACH TO CLOVES SYNDROME TREATMENT

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Abstract

Cloves syndrome (ICD Q87.3) is a rare disease included in PROS (PIK3CA-related overgrowth spectrum). This spectrum is formed by a heterogeneous group of rare disorders that overlap in several clinical aspects. In addition the patients within each group are also clinically diverse from each other. All this makes it particularly difficult to create standard diagnosis, follow up and classifications. The latter will become increasingly important now that for the first time clinical trials are being made with drugs that target this pathway.

Objectives:

Following on from a complete review of all Cloves in medical literature, the object of this paper is to propose a classification that will serve as a tool when taking therapeutic decisions. This classification takes into account the seriousness of the symptoms, progress and extension of Cloves. The diagram will select those children eligible for treatment with the new drugs that are being used in clinical trials. Categories will be defined which facilitate the taking of therapeutic decisions, distinguishing them from the degree of extension which may not be a factor of the therapeutic options. These categories will also be used as a much needed tool to establish the degree of disability.

Part one: a review of Cloves syndrome

Molecular base

Cloves syndrome develops due to a mutation in the 110-kDunit of pho catalytic alfa-subunit phosphatidylinositol-3kinase.

Phosphatidylinositol-3kinase/AKT/mTOR pathway promotes cell growth and regulates its maturation, angiogenesis, survival and metabolism. If the genes that encode the different aspects of this pathway are affected, the cell will be over-stimulated leading to competitive growth and proliferation and metastatic capacity. In oncology it is often a sign of cancer treatment resistance.

Thus, a *de novo* mutation in this pathway creates an oncogenic gene with the capacity to promote many different types of cancer.

However, when these mutations are present at birth they are related with an entirely different kind of pathology. These pathologies include a number of clinical diagnoses with different genotype and phenotypes. The clinical aspects of these diseases are extremely variable in their severity reaching from multisystemic diseases that affect vital tissues ranging from the central nervous system to an isolated macrodactylia. (1)(2)(3) (4)

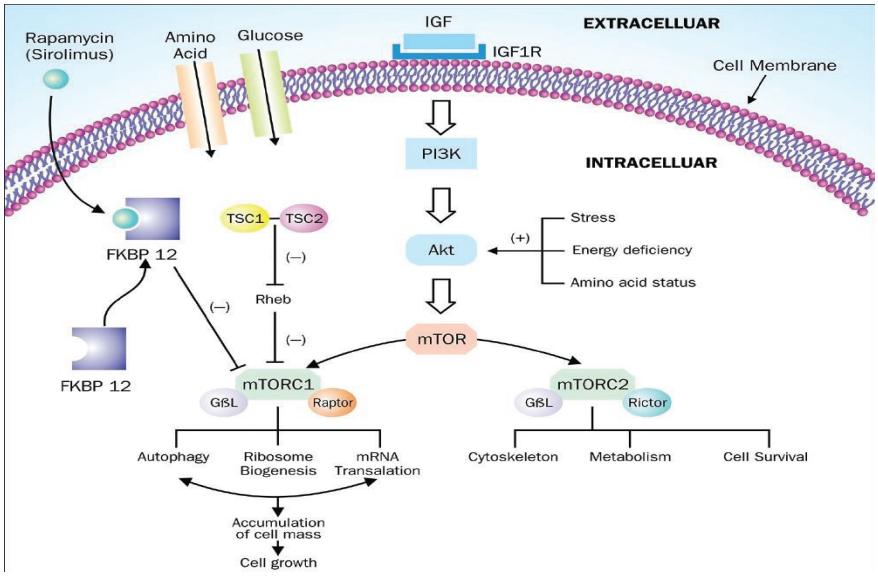


Image 1 PIK3CA signaling pathway (36)

Image 1: PI3K pathway is physiologically stimulated through the activation of the tyrosin kynasa receptor. These receptors will then phosphorylate the Insulin receptors substrate which in thir turn phosphorylates the subunit p85 of PI3K. This phosphorylation leads to this proteins conformational transformation which impulses the binding of the catalytic subunit (p110) to subunit (p85). Through phosphorylation PI3K will activate phosphtidil inositol 3,4 diphosphte (PIP2) which transforms into a second messenger: phosphatidil inositol 3,4,5 triphosphate (PIP3) which interacts in the cells membrane with Akt, thus activating it. AKT has various metabolic functions inside the cell and through the non direct mTOR activation it controls the cells proliferation, protein translation and autophagy.

PTEN regulates PIK3CA activity reducing its activation. This signaling pathway is extremely complex with multiple inhibitory feedback levels and interactions with the RAS/MAPK pathway. (37)(5)

Depending on which gene is mutated the pathway will be affected at one level or another leading to the following syndromes:

- **AKT mutation:** Proteus syndrome
- **mTOR mutation**: hemimegalencephalopaty, cortical dysplasia
- **TSC1, TSC2**: tuberous sclerosis
- **PTEN mutation:** Encephalic Autism, Cowdens syndrome, Bannayan-Riley-Ruvalcabas syndrome
- **PIK3CA mutation:** PROS spectrum, including CHHML (Hemihyperplasia-multiple lipomatosis syndrome), Infiltrating facial lipomatosis Fibroadipose hiperplasia,Macrodystrophica lipomatosa, Isolated lymphatic malformations, Isolated vascular malformations, Macrodactyly, Hemimegalencephaly, Macrocephaly-capillary malformation (MCM), Megalencephaly, polymicrogyria, and hydrocephalus syndrome (MPPH), Megalencephaly-capillary malformation-polymicrogyria syndrome (MCAP), Macrocephaly-cutis marmorata telangiectasia congenita (M-CMTC), Cloves syndrome (Congenital lipomatous overgrowth, vascular malformations and epidermal naevi, scoliosis and skeletal deformities), Klippel-Trenauney Syndrome (KTS), Complex epidermal naevus syndrome

Genetics:

PIK3CA (phosphatidylinositol—4,5-bisphosphate 3-kinase catalytic subunit alpha) (3q26.3) encodes for p110a, the catalytic subunit of phosphatidylinosotide-3-kinase (FP13k). The mutation of this gene occurs during embryogenesis. It is a post-zygotic mutations, that is to say it occurs in a cell that forms part of a multy-celular zygote. The genotype will depend on when this mutation occurs and of how many cells will be affected and the effect of the specific amino acid on the protein product. If it affects germline cells, somatic cells or both will depend on whether the mutation occurs before or after their separation during embryogenesis. Keppler-Noreuil (5) Describes two types of segmental mosaics forms of autosomal dominant genodermatoses

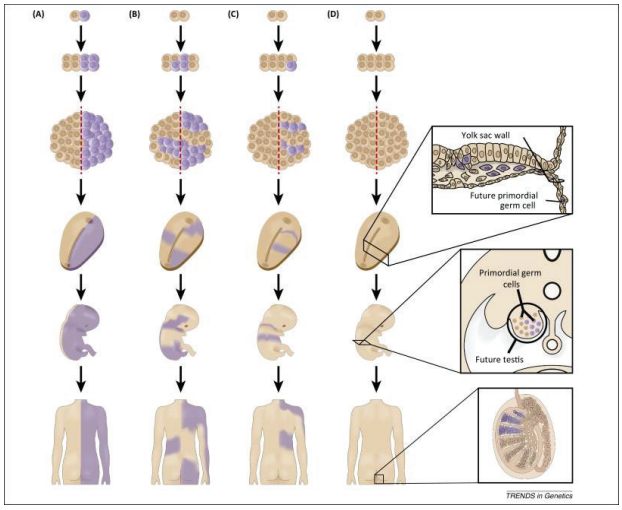


Image 2 Somatic Mosaicism (38)

- Type 1: the disease manifestations are limited to certain body regions. The mutation takes place on a normal genetic background. Type 1 can have solely somatic mosaicism or somatic and heterozygous mosaicism
- Type 2: the manifestations are more severe. It occurs after a second-hit mutation on a heterozygous germline background. Type 2 gonads will be heterozygous.

Genetic counseling: the risk of passing on the mutation to the descendents is very low. Until this day there is no confirmed case of vertical transmission. Because family members are not known to have an increased risk, prenatal diagnosis is usually not indicated for family members. (6)

Diagnosis:

Prenatal: to this day there is only one published case of prenatal diagnosis (7) This study took part in Texas where during an abdominal sonography and a further MRI scan a fetus was diagnosed with severe malformations probably related to Cloves. DNA was directly extracted from the amniotic liquid but the results for PIK3CA came back negative. Further tests were made cultivating DNA from amniocytes. Two test where made using ddPCR for the most frequent mutations in PROS and they both came positive for the mutation c.1624G>A (p.G542K) in the PIK3CA gene.

Despite these results a prenatal diagnosis is of questionable benefit. Firstly it is a test with very little sensibility, it is difficult to detect those fetuses that may have the mutation and even then when detected the mosaic nature of the mutation works in a way that not every sample we take will necessarily have the mutation. Secondly even if the fetus is correctly diagnosed with the treatments at hand (mainly surgical) the prognosis would not vary from a prenatal diagnosis to the one done on a newborn.

Childhood: PIK3CA mutation is established through a biopsy (except MCAP that can be diagnosed through saliva or blood samples). Due to the fact that there is a wide range of phenotypes it is a challenge to decide which patients to do a biopsy on and which not. Keppler-Noreuil have proposed testing eligibility criteria for somatic PIK3CA mutation (view Table 9). This table's key features are symptoms that are not only quite specific of the overgrowth spectrum but are also very sensitive.

Keppler-Noreuil et al, have also devised a table with diagnosis criteria (View Table 10). In it, it is stipulated that to establish the PROS diagnosis it is necessary to have a positive biopsy as well as the clinical diagnosis criteria. These clinical features are separated into two groups: group A (spectrum with two or more features) and group B (isolated features). These clinical manifestations must be present either at birth or during childhood.

Absence of a mutation in a DNA sample is insufficient to exclude diagnosis in individuals with suggestive features given that low-level mosaicism is observed in many individuals. If the patient meets PROS clinical criteria it is considered presumptive PROS.

Biopsy: due to its somatic nature it is necessary to choose which tissues are more likely to have higher rates of mutation. Therefore a fresh skin biopsy of overgrown tissue, vascular malformation or epidermal nevus or a surgical excision should be done in visually affected tissues.

Mutation detection techniques:

Less than 30 mutations in the PIK3CA gene have been reported of which five of them (p.C420R, p.E542K, p.E545K, p.H1047R and p.H1047L) are recurrent. (8)

Recurrent mutations: Hotspots are the most common sites for mutations related with this entity.

ddPCR: droplet digital PCR of the five most common mutations can be used with very low mutation levels (> 0.1%). This should be the first approach to PIC3KA mutation identification for it is quick and inexpensive.

Rarer mutations:

ELISA/Functional studies: its disadvantage is that it is only successful when there is more that a 15% rate of mosaicism.

Next generation sequencing (NGS): similar to SANGER method it is a practical way of detecting rarer mutations but only when the mosaicism rate is above 20%

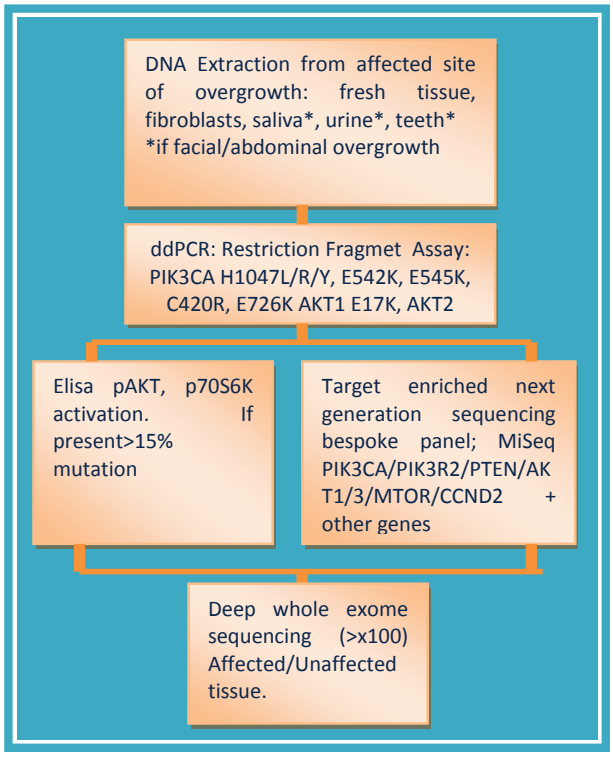


Image 3 diagram for PROS diagnosis

RFLP or digital droplet PCR: it is not as useful for rarer mutations as it can only sequence one fragment at a time but it can detect mutations in tissues with up to < 0.1 mosaicism.

Specific PROS diagnostic through biopsy testing will become significantly more important in the following years to come. As previously mentioned, clinical findings can sometimes overlap with similar syndromes such as Proteus syndrome. However although symptoms in these diseases are similar (for they affect the same pathway) the mutation is localized in different levels of this signaling pathway. As a result, these diseases will be treated with different and specific immunosuppressant drugs. Therefore an exact diagnosis will lead to a much more effective treatment.

Clinical findings in Cloves syndrome:

Cloves syndrome is an acronym formed by the clinical findings that define the disease: congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal anomalies, scoliosis, spinal anomalies and seizures. It was first describe in 2007. To this day over 200 people have been diagnosed with this syndrome. (4,6,9,10)

- **Lipomatous masses:** lipomatous masses are the most characteristic Cloves clinical sign. They are present at birth in one or both sides of the thorax and abdominal wall. Throughout the patient´s life they will continue to grow progressively extending to the groin, glutei, retroperitoneum or mediastine. It is related with vascular and lymphatic malformations (11) that can be found either covering the mass or inside it. Another possible location is in the lipomatous tissue of the face growing asymmetrically around the orbits, maxiliary bone o jaw line.
- **Musculoskeletal/acral abnormalities:** in Cloves syndrome any affected tissue can be subject to over-stimulation that will result in overgrowth of these tissues. This occurs typically in bone, muscular and lipomatous tissue which leads to clinical findings characterized by dismorphya and asymmetry. Certain anatomical regions will be affected more than others. Some patients with more severe overgrowth have striking lipoatrophy in areas not affected by overgrowth.
    - Bone structure overgrowth: it usually affects lower limbs. Its growth is not marked or progressive as lipomatous tissue can be. Its mosaic nature often affects only one leg and the asymmetry can often be invalidating requiring shoe rises and finally epiphisiodesis. Occasionally bones that had not been submitted to overgrowth can start growing after an important operation such as limb amputation.
    - Macrodactyly: due to the growth of methacarp/methatarsial bones as well as the subcutaneous lipomatous tissue it is characteristic to find palmar/plantar overgrowth in Cloves patients.
    - Sandal gap toe: increase of the interspace between the first toe of the foot and the rest of the toes (similar to the gap caused by a sandal). This is typical but also found in other syndromes such as Down syndrome.
    - Scoliosis: scoliosis can be congenital or secondary to lower limb asymmetry.
    - Pectus escavatum
    - Hip displasia
    - Spina biffida
    - Chondromalacia patellae

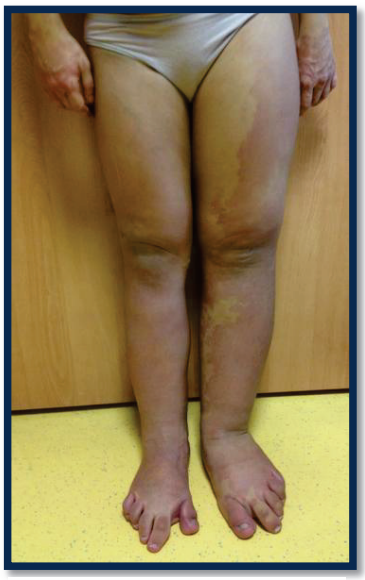


Image 4 Capilar malformation with dysmetria and sandal gap

- Dislocated knees

- **Vascular malformations:**
  - Vascular malformations are divided into three groups:
    - Low flow: venous, capillary, lymphatic (typically overlying truncal overgrowth). Present in most patients.
    - High flow: arteriovenous. Frecuently located in spinal, paraspinal region. These malformations are less frecuent in Cloves patients.
  - Capillary malformations are present at birth as port wine patches usually localized in extremities and lateral sides of the trunk and as previously noted occasionally covering lipomatous masses.
  - Venous malformations appear as bluish patches. The veins that are more often affected are those of the thorax and the central veins. Their tortuous trajectory facilitates the formation of cloths and the calcification of the veins. Central and thoracic phlebectasia is common in Cloves often leading to pulmonary embolism (2). There are also two report cases of spinal thrombosis and neonatal cerebral infarcts(4). It is important to do aggressive prophylactic measures and their diagnosis with Doppler ultrasound to prevent these episodes, for they can be fatal.
  - Other forms of venous malformations present themselves in the form of subcutaneous vein malformations that increase with valsalva maneuvers.
  - Lymphatic anomalies: they are to be found inside the lipomatous tissue or in the thorax and abdomen
  - These malformations can cause a vast number of symptoms ranging from gastrointestinal symptoms, nerve compression, radicular pain, muscular debility alteration in sphincter control or sexual dysfunction.
- **Cutaneous:**
  - The most common cutaneous affection is the epidermic nevus. Lineal epidermic nevus is characteristic of Cloves. It presents itself in the form of multiple papules with a hyperkeratotic and papillomatous surface that follow a linear path along Blaschko's lines (prints of the migration of the embrionary cells) or vascular or neural structures. These lesions turn verrucous and they continue growing until adolescence.
- **Visceral anomalies**
  - Renal: kidneys in Cloves are one of the most affected organs. Cases have been reported of agenesia/hipoplasia, renal cysts hidroureteral nefrosis, kidney stones and unknown origin hematuria as well as two cases of Willms tumor.
  - Esplenic lesions
- **Neurological**
  - The spinal cords affection is secondary to lipomatous masses or arterio-venous malformation. Lipomatous masses adjacent to the spinal cord can progressively grow until they come to compress the spinal cord, thecal sac and nerve roots or even infiltrate them. The clinical expression will depend on the level at which its affected and the number the level of infiltration/compression.

    - Hypertension in the venous paravertebral plexus can generate a pial vein reflux that can compromise the vertebral blood flow and cause congestive myelopathies though this is not as common as symptoms derived from compression.
    - Epilepsy**:** diagnosed in various patients.
    - Intellectual disabilities and behavior problems are present in almost fifty percent of patients.
    - Other observed central nervous system malformations include: megalencephalopaty, hemimegalencephalopaty, chyaris malfromation, polymicrogyria, spina bífida, spinal muscular atrophy polymicrogyria, aplasia or agenesys of the corpus callosum, meningoceles and mielomeningocele. These symptoms are found in Cloves but are more frequent in MCAP
- **Tumours**: there is a low frequency of cancers (1%). The reported cases of tumors in Cloves syndrome have been: choriocarcinoma, extraspinal medular tumor, hemangioma, multiple angiomatosis and two Wilms tumors.

Future directions:

To this day there is not a consensus over which are the clinical tests that must be done or with which frequency. With follow ups just as with treatment and classification it is difficult to establish fixed guidelines. The recommendations that have been published up to date encompass all the different clinical entities that form PROS. This is because although each entity has its own characteristic clinical spectrum, the significant overlapping between them implies that any of the complications could occur in any of the patients. As the treatment and future directions depend on the manifestations, a thorough examination should be done (physical and with images) to establish the extent of the disease at the moment of diagnosis Karen.W.Gripp et al propose a series of imaging recommendations for patients with PROS: (12)

Clinical finding	Follow up
• **Ventriculomegaly, hydrocephalus** • **Chiari malformation/cerebellar tonsiliar ectopia** • **Cortical brain malformations (Polymicrogyria)**	**Brain MRI** without contrast if there is macrocephaly (OFC>2 SO), developmental delay, epilepsy, facial or skull involvement.
• **Tethered cord,** • **Syringomyelia,** • **Lipomeningocele**	The initial imaging should be done at diagnosis if there is truncal involvement present. **Ultrasounds** in infants and **MRI** thereafter
• **Scoliosis**	**Spine radiographs** at presentation if spinal asymmetry or truncal overgrowth is noted
• **Overgrowth** • **Lymphatic or vascular malformations**	**Truncal whole body MRI** with or without contrast at diagnosis
• **Overgrowth** • **Asymmetry** • **Lymphatic or vascular malformations,** • **Thromboembolism**	**Radiographs, MRI,** consider **Doppler ultrasounds** of involved arms, legs or both at diagnosis.
• **Kidneys enlargement** • **Tumor (nephroblastomatosis or Wilms tumor)**	**Renal ultrasound** at diagnosis and then repeat every 3-4 months or until age 8.
All these tests should be repeated according on the patient's specific needs and manifestations. ***A published article on MCAP, (Ghayda Mirzaa et al 2013)**(6) **proposes a more aggressive follow up for MCAP syndrome consisting of a brain MRI at diagnosis and every six months until the age of 6, after this annually**	

Table 3 PROS follow up (12)

Treatment

Treatment of Cloves is complex and requires a multidisciplinary team. There is no cure for PIK3CA mutation and every complication has to be treated individually, sometimes recurring to aggressive surgery (debulking, liposuction and interventional radiology for vascular malformations). In Cloves syndrome most subjects will undergo several debulking and orthopedic surgical procedures. Capillary malformations can be treated with laser treatment, but more severe vascular malformations require sclerotherapy, embolism or in some cases surgical debulking. The surgery of paraspinal malformations is a very complex one and its treatment can cause iatrogenic neurological damage. Asymmetry of the lower limbs is treated with epiphysiodesis or in some very severe cases amputation. Lipomatous masses can be drained but usually require surgical debulking and recurrence is frequent. Anticoagulation is recommended and in some cases caval filtration, particulary in the perioperative period. Central and thoracic phlebectasia in patients with Cloves syndrome should be considered one of the few indications for placement of an SVC filter and coagulation should be closely monitored.

There has recently been a new approach to the treatment of PROS base on the idea that mTOR inhibitors reduce the over-activation of the PIK3CA/AKT/mTOR pathway. Drugs that target this pathway have been around for years but had mainly been used as immunosuppressants in transplanted patients and Tuberous Sclerosis .(13)

m-TOR inhibitors

Rapamycin (Sirolimus; ATC: L04AA10) is a macrolide compound that was first isolated and purified from the bacterium Streptomyces hygroscopicus in 1964. It was first used as an antifungal drug. Later, it was discovered that rapamycin also had antiproliferative and cystostatic activity in immune cells and tumor cells as well as a potent immunosuppressive effect.

Three other mTOR inhibitors and ramapycin analogs have been discovered since: everolimus (RAD001), temsirolimus (CCI-779) and ridaforolimus (MK-8669). Second generation mTOR inhibitors are being developed but are still to complete phase III trials. These analogs all share the same macrolide chemical structure, what differentiates them is the functional group added at C40 (14). Clinically this is important because the pharmacokinetics and pharmacodynamics vary between the different analogs. For example, there is evidence that everolimus distributes to brain mitochondria whilst this hasn't been proven in sirolimus.

Sirolimus binds to the immunophilin FK Binding Protein-12 (FKBP-12) to generate an immunosuppressive complex that binds to and inhibits the activation of the mammalian target of rapamycin (mTOR), a key regulatory kinase. This also results in inhibition of T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (IL-2, IL-4, and IL-15) stimulation and inhibition of antibody production.

Everolimus is also an effective antiproliferative and immunosuppressive agent that was developed to try to improve the pharmacokinetics of sirolimus.

The main clinical uses for these drugs are the following:

- Sirolimus: first approved in 1999 to prevent graft rejection in kidney transplant. Used in inmunosupresion in transplanted patients.
- Temsirolimus: advanced renal cell carcinoma
- Everolimus is also widely used in various diseases, mainly breast, neuroendocrine and renal tumors and solid organ transplant rejection. Also in the prevention of neovascularization of artificial cardiac stents.
- Ridaforolimus clinical trials have been started for advanced soft tissue and bone sarcomas as well as for hematologic malignancies

Use of mTor inhibitors in Tuberous Sclerosis (TSC)

TSC complex is a rare disease caused from aberrant hyperactivity of mammalian target of rapamycin due to a mutation in either TSC1or TSC2 which results in an increased cellular proliferation and tumor growth. mTOR inhibitors are already being used to treat several manifestations of TSC, including SEGA (subependymal giant cell astrocytoma) and renal angiomyolipomas (15). To date, only sirolimus and everolimus have been clinically tested and only everolimus is approved by the FDA as well as by the European Commission to treat SEGA

- **Sirolimus**: the first drug to be used in tuberous sclerosis. Many clinical studies in phases I and II have been conducted since the year 2006. It has proven in clinical trials to the treat angiomiolipomas and other tumors related with tuberous sclerosis as well as astrocytomas. It has also proven effective in the treatment of pulmonary fibrosis and skin manifestations(16)(17)(18)(19). The topical use of sirolimus is available in topical formulation and has proven effective in the treatment of facial angiofibromas(14). Most common adverse effects include mucositis, proteinuria, infections, diarrhea and aphthous ulcers. Most of the patients experienced adverse effects but these where mild. The more severe adverse effects were much less frequent. Roy et al. studied mice to find if seizures could be treated using Sirolimus (20). After trying the treatment for an hour seizures were reduced dramatically.

- **Everlonimus**: two mayor clinical trials have been conducted to prove its efficacy in the treatment of SEGA (21)(22). The second one (EXIST-1) and biggest clinical trial up to date recruited 117 patients. 49% of the patients experienced a reduction in more than 50% in SEGA volume. Everolimus has proven superior to sirolimus in the treatment of SEGA. These clinical trials also showed an improvement in the TSC manifestations related to the central nervous system, namely, behavior and cognitive development. Results regarding the response of epilepsy to everolimus are contradictory. Whilst a first clinical trial published in 2013 a response of over 50% of seizures in 12/20 patients (23), EXIST-1(21) showed no significant difference between everolimus and placebo. Adverse effects include upper respiratory infections, stomatitis, sinusitis, otitis media, mouth ulceration, convulsions, pyrexia and amonorrhoea.

Overall TSC patients have reported less mild/severe adverse effects than expected. This is probably due to the fact that these drugs are taken in monotherapy and in the minimum effective dose. (24)

Other uses: mTOR inhibitors are being explored in other rare diseases with mTOR dysregulation including preclinical studies in Leigh syndrome and Down syndrome. A phase 2 study evaluating everolimus in the treatment of Swchannomas in patients with Neurofibromatosis II concluded that it was not effective (25). Case series in patients with plexiform neurofibromas in treatment with sirolimus showed no shrinkage of the tumor but it did reduce pain(26).

	Sirolimus	Everolimus
Commercial names	Rapamune©	Afinitor© ,Votubia© Certican© Zortress© Evertor©
Biochemically functional form	Sirolimus is the active form	Active derivative (hydroxyethyl ester) of sirolimus
Route of administration	Orally, once daily	Orally, once daily
Protein binding	92%	75%
Bioavailability and distribution	Low oral bioavailability (~15%): 14% for solution and 18% for tablets. Large distribution (around 12 L/kg), ~95% into RBCs	Tablet: 20% Wide distribution into RBCs; good blood-brain partition coefficient
Metabolization	Hepatic CYP3A	Hepatic CYP3A
Terminal half-life	46-78h	26–30 h
Elimination	Feces (91%), urine (2%)	Feces (>90%), urine (2%)

Table 4 (24) **Pharmacological comparison between sirolimus and everolimus. Modified from Palavra et al 2017**

mTor inhibitors in PROS

The following trials have been carried to treat PROS syndrome with sirolimus

- In 2007 Deborah J marsh et al underwent the first case of the use of sirolimus (13) in an overgrowth syndrome. It was carried in a 2 year and 2 months old boy with Proteus syndrome and a de novo mutation in the tumor suppressor PTEN. The patient received oral rapamycin at a dose of 0.1 mg/kg per day, divided into two doses. Serum rapamycin levels were maintained between 5–10 ng/ml. Rapamycin was well tolerated and caused no side-effects. The results were very positive with a marked reduction in soft-tissue masses 14 months later. (13)
- From 2007 to 2016 several cases have over the use of mTOR suppressers in vascular and lymphatic anomalies have been published with positive results (27–32)
- In 2016 a trial was carried out by Adams DM et al with 61 patients (NCT02443818). It was the first prospective trial for complicated vascular anomalies. 57 patients were evaluable for efficacy. No patient had a complete response 47 had a partial response, 3 patients had a stable disease and 7 patients had progressive disease. Two patients were taken off the study secondary to persistent adverse effects.(33)
- There is currently a larger non-randomize phase II trial being carried between UK Cambridge, USA Bethesda and France Dijon (NCT02428296). This is an open trial and it expects not only to confirm the therapeutic effect of sirolimus over lipomatous masses and vascular malformations but also to investigate if sirolimus has any affect over somatic overgrowth. 30 patients of both sexes, with segmental overgrowth and aging from 3 to 65 will be included. The doses applied in these trials aim to maintain a concentration of 2-6 ng/ml of sirolimus. Recruiting started in 2014 and the trial started in 2016.

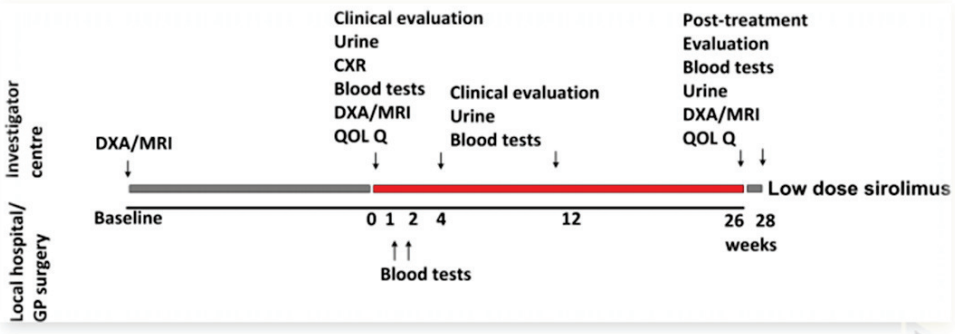


Table 5 Chronological outline of NCT02428296

Only the Dijon center has published a preliminary report. This report casts a troubling light over the effects of sirolimus. Until know, previous trials hadn´t encountered many major tolerance problems with the use of sirolimus. However, in this trial out of 13 patients 4 had to stop the treatment due to grade 3/4 adverse effects. They also recorded 8 infections, 4 thromboembolic episodes, one prolonged fever that revealed a Still syndrome and 4 isolated liver function test anomalies (34)

PART TWO

Up to this point the articles available in PubMed referring to Cloves, its management and classification have been reviewed to produce an up to date synthesis of this syndrome. In the following part of this paper I will endeavor to establish a correct classification as well as a diagram for treatment with sirolimus. Lastly I will establish the guidelines for the treatment with this drug.

The following pages must be submitted to reevaluation provided that new clinical trials come to light. It is highly possible that new evidence will make it necessary to modify the classification and treatment guidelines.

Classification of Cloves

This classification is divided into two different sections: The first one (I-V) establishes the treatability with new drugs (sirolimus) depending on the severity of the patient. The second one (A or B) relates to the extent of the syndrome and will aid the clinician to establish whether it is a localized or systemic disease.

I	II	III	IV	V
No lipomatous tumor, no Vas	Non progressive lipomatous tumor/Vas	Measurably progressive lipomatous tumor /Vas* or of significant size**	Lipomatous tumor /Vas with: • Affection of CNS or PNS Or the following*** • Distorting or restricting overgrowth	Lipomatous tumor/Vas that puts the subject´s life at risk.
Other than the degree of severity the symptoms will be classified as: **A: localized, if one of the following symptoms is present** **B: systematic: if more than one of the following symptoms are present** **Measurable asymmetry non related to lipomatous masses/VAs** **Macrodactily, plantar/palmar overgrowth** **Scoliosis, spina bifida** **Dislocation of knees, condromalacia patellae** **Renal affection** **Splenic affection** **Epilepsy** **Intellectual disability or CNS affection non related to lipomatous mass/Vas**				
Acronyms: Vas: Vascular anomalies				
• ***>1cm/year** • ****> that 10cm (5cm if it affects the face) or considered to affect psychologically/physically the subject´s daily life** • *****Subjects rely on external help for basic daily activities.**				

Table 6 Classification of Cloves

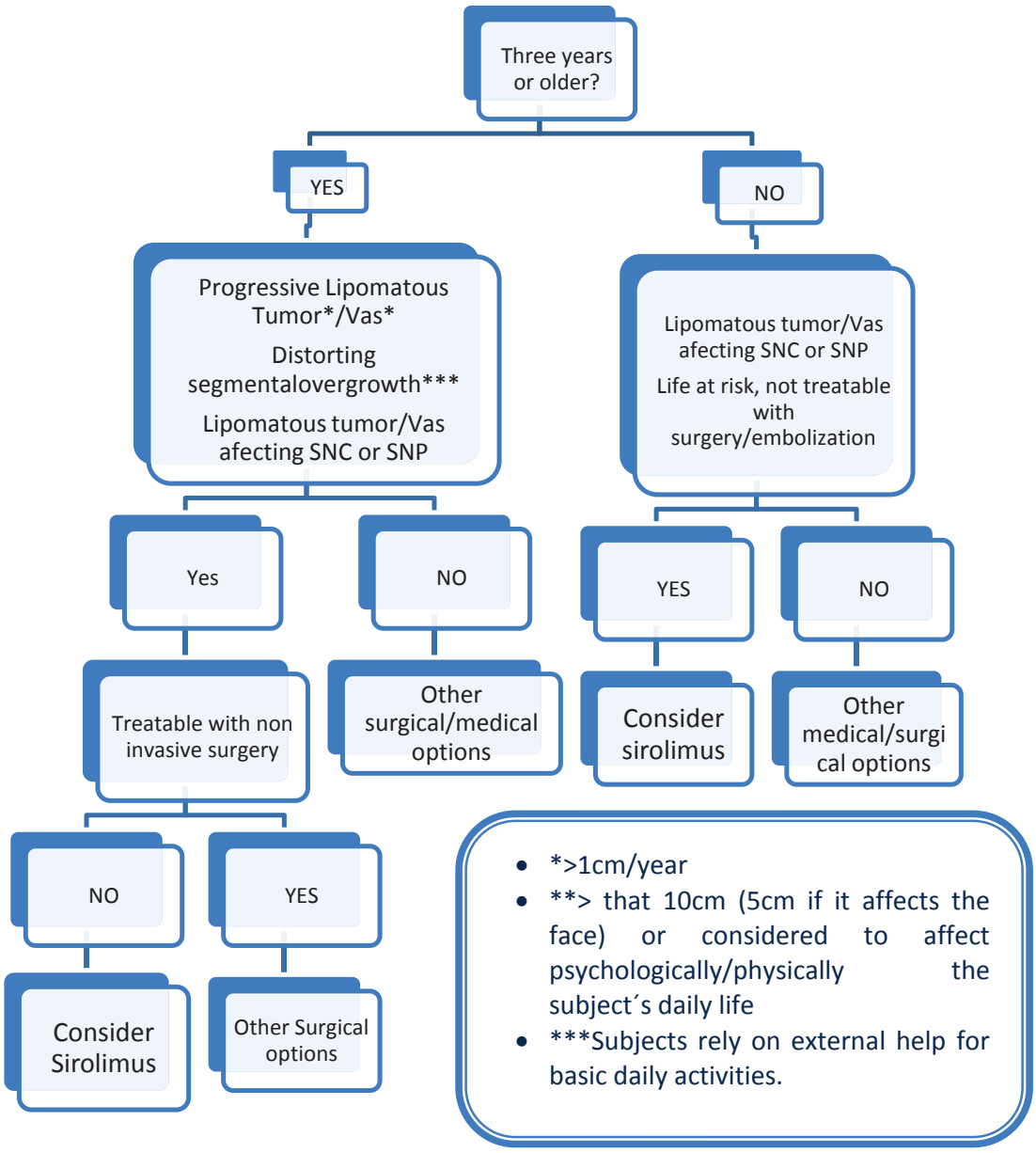
This classification intends to achieve the following:

- Compare patients throughout the world.
- Serve as a tool to establish disability degree.

This classification proposal is based on the clinical diagnosis criteria elaborated by Keppler et al (table 2). It integrates similar criteria to those established in the classification of tuberous sclerosis.

Treatment

Diagram



- Acronyms: Vas: Vascular anomalies
- This diagram only takes into account vascular anomalies, lipomatous masses and overgrowth. The reasons behind this classification are two: firstly, these are severe symptoms that are worth treating with a relatively new drug in this field as is sirolimus. Secondly, clinical trials have proven that these are the clinical features that can be reduced with the use of sirolimus.

- Patients with segmental overgrowth must be in critical conditions. There have been signs that segmental overgrowth is reduced when treated with sirolimus. However, the response of segmental overgrowth to sirolimus has never been specifically measured in a clinical trial.

Why Sirolimus?

Up to date Cloves syndrome has only undergone clinical trials with sirolimus. This raises the question of why sirolimus and not everolimus, for the latter has worked extremely well in Tuberous Sclerosis. Doctors who conducted these trials chose sirolimus for three reasons. Firstly, sirolimus has proven effective in reducing vascular malformations in the past. Secondly, sirolimus has a higher protein binding percentage that everolimus (see table 4). Finally, these are very expensive drugs and the company behind sirolimus has agreed to cover expenses for most of the trials that have been done up to date.

Doses:

Treatment should consist of a continuous dosing schedule of oral sirolimus starting at 0.8 mg/m2 per dose twice daily, with pharmacokinetic-guided target serum levels of 2 to 6 ng/mL These concentrations have proven effective whilst giving a low rate of mild/severe adverse effects (21). Target dosing may need to be individualized for some patients, especially those with coadministration of CYP3A4 inducers or inhibitors, compromised hepatic function or under three year olds.

The therapeutic index is narrow. Sirolimus concentrations should be measured every other day during the 7 first days after the start of the treatment and then every 2 weeks until stable trough concentrations are attained.

CYP3A4 activity modifiers:

Inhibitors (increase sirolimus blood concentrations): bromocriptione, cimetidine, cisapride, clotrimazole, danazol, diltiazem, fluconazole, protease inhibitors (e.g., HIV and hepatitis C that include drugs such as ritonavir, indinavir, boceprevir, and telaprevir), metoclopramide, nicardipine, troleandomycin, verapamil

Inducers (decrease sirolimus blood concentrations): phenytoin, carbamazepine, rifampin, rifabutin, rifapentine, or Phenobarbital St. John's wort

Image 7 CYP3A4 modifiers

Treatment with sirolimus criteria

- Adequate organ function (liver, bone marrow, and renal)
- An adequate lipid panel, Karnofsky/Lansky performance status ≥50
- No concurrent use of cytochrome P450 3A4 enzyme inducers or inhibitors.
- No present treatment with steroids, chemotherapy, or radiation and 2 weeks must have elapsed since undergoing major surgery.
- Chronic steroid use, known HIV, chronic severe or uncontrolled medical disease, uncontrolled infection, and previous use of an mTOR inhibitor.

- Pregnant and breastfeeding women cannot take sirolimus. Male and female subjects of reproductive potential are required to use effective contraceptive methods throughout the study and for 3 months after study end.
- Patients should be screened and treated for existing infections before starting sirolimus, and all immunizations should be current.
- Patients with uncontrolled infection and who were unwilling or unable to comply with the protocol were excluded from participation.

Effect

- Evaluation criteria should be the measurement of lipid tumor, vascular anomalies or segmental overgrowth at 6 months treatment. The evaluation should be done by volumetric MRI.
- HRQOL should be assessed by using the Pediatric Quality of Life Inventory 4.0 (3–18 years) and Infant Scales (≤2 years) and the Functional Assessment of Chronic Illness system (>18 years).

When to cease treatment with sirolimus

- The treatment should be maintained until the clinical finding that started the treatment (lipomatous tumors, vascular anomalies or segmental overgrowth) is no longer to be found on MRI. After this period sirolimus can be removed. If symptoms return treatment should be resumed without waiting to reach figures in diagram. If after two attempts to remove sirolimus, symptoms return it would be advised to maintain sirolimus for life.
- The treatment should be removed if the patient meets one of the following criteria:
    - At 6 moths there is <15% of tumor reduction or no clinical improvement
    - At 12 months there is < 30% tumor reduction
    - At 12 months health related quality of life has not changed.
- Severe adverse effects due to treatment.

Follow up:

- Patient should continue to have follow ups to control and avoid long-term adverse effects: hypertriglyceridemia, hyperglycemia, hypercholesterolemia, hipophosphatemia and potential risk of secondary malignancies. These controls should take place every 6 months for the first two years and every year from thereafter.
- Lipid abnormalities and hypophosphatemia should be treated according to guidelines if they appear. It must be noted that sirolimus has been reported to elevate unexpectedly CK levels and so statins effects on liver should be closely monitored. (16)

Vaccination

- Sirolimus is an immunosuppressant, therefore it may affect response to vaccination. The use of live vaccines should be avoided.
- Any patient who wishes to start treatment with sirolimus should be vaccinated up to date.
- Influenza vaccine must be taken once a year.

Under three year olds:

No study to date has evaluated younger infants treated with mTOR inhibitors using direct, detailed observational assessment tools for objective characterization neuropsychiatric deficits. This is a critical age when brain development is at the forefront, and early treatment exposure has the potential to exert dramatic effects on developmental trajectory and overall quality of life in patients that follow this treatment.

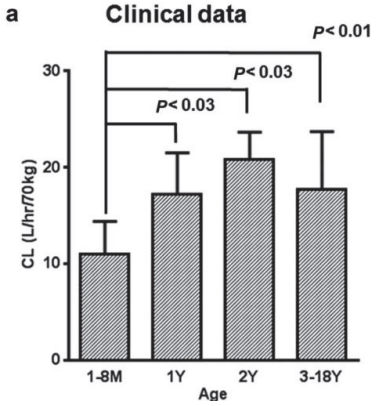


Table 8 Clearance in under three year olds (35)

Doses in under three year olds should be individually determined. This is due to the ontogeny of CYP3A pathway that affects sirolimus clearance.

C. Emoto et al estimated sirolimus clearance in 44 pediatric patients with vascular anomalies using concentration-time data collected as part of a concentration controlled trial. In patients younger than 1 year, the allometrically scaled sirolimus clearance was significantly lower than that observed in older patients (>3 years) indicating immaturity of clearance in these young patients. (35).

Testing eligibility criteria for Somatic PIK3CA mutations
PIK3CA mutation analysis should be performed, **If a patient has one or more of:** **Key features: Congenital Spectrum (S)* or Congenital Stand Alone (B)*** **Comined vascular malformation- Large Capillary, Lymphatic or Venous malformation** **Congenital Musculoskeletal Overgrowth** **Patterning defect** (e.g., Polydactyly, Sandal gap, Syndactyly)** **Congenital CNS (PMG/MEG/HC/Chiari/Syrinx)** **Congenital Epidemal Nevus (EN)** **Congenital soft doughy skin joint hypermobility** **+/- functional features: hydronephrosis/hydroureter, Urinary incontinence, Hematuria, constipation, gastrointestinal bleeding, Intractable Epilepsy, Seizures, Intellectual Disabilitym Autism, Hypoglycemia.**
Abreviations: CNS: Central Nervous System: HC hydrocephalus, MEG megalencephaly; PMG polycmicrogyria ***Refer these specific findings in Table II** ****** **Upper (UE) and Lower Estremety (LE) findings may include, UE: broad spade like hands with ulnar deviation of the digits, symmetrical overgrowth of I or more digits that does not usually follow a nerve territory-oriented pattern, laxity of collateral ligaments, furrowed palms and soles. LE: overgrowth of the feet, which presents as a large "sandal" gap between great and second toe, large bulbous toes, lipomatous masses on both dorsal and plantar surfaces, broad forefoot with wide gaps between the metatarsal heads; dislocated knees, leg length discrepancy, and patellar chodromalacia (Bloom and Upton, 2013)**

Table 9 Testing eligibility criteria for Somatic PIK3CA mutations, modified from (9)

Clinical Diagnostic Criteria for PIK3CA-Related Overgrowth Spectrum (PROS)
Required: **Presence of somatic PIK3CA mutation*** **Congenital or Early Childhood Onset** **Overgrowth Sproradic and Mosaic (Other terms: Patchy, Irregular)** **Features as described in either A or B**
Spectrum (two or more features)** **Overgrowth: Adipose, Muscle, Nerve, Skeletal** **Vascular Malformations: Capillary, Venous, Arteriovenous Malformations, Lymphatic** **Epidermal Nevus**
Isolate features **Large Isolated Lymphatic Malformations** **Isolated Macrodactyly*** OR Overgrown Spayed Feet/Hands, Overgrown Limbs** **Truncal Adipose Overgrowth** **Hemimegalencephaly (bilateral)/ Dysplastic Mealencephaly/Focal Cortical Dysplasia[1]** **Epidermal nevus[2]** **Seborrheic Keratoses[2]** **Benigh Lichenoid Keratoses[3]**
Abbreviations:+present,-absent; HC hydrocephalus; ID intellectual disability ***If no mutation identified, then consider as presumptive PROS** ****Typically Progressive. Can manifest as: Scoliosis (Kyphosis), Limb overgrowth, CNS (HC cerebellartonsillar ectopia, Chiari, Megalencephaly, Mega corpues callosum, Regional lipomatous undergrowth with overgrowth, Infiltrating lipomatosis, Wilm tumor/ovarian cystoadenoma** *****Other terms: macrodystrophia lipomatosa, macrodactylia fibrolipomatosis and gigantism** [1]**Dobyns WB,2014 (unpublished data)** [2]**Hafner et al. (2007)** [3]**Groesser et al (2012)**

Table 2 Clinical diagnostic criteria for PROS modified from 10

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