Rapamycin and treatment of venous malformations

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Purpose of review
The field of vascular anomalies has seen a fundamental change during the past 10 years. The identification of somatic genetic mutations as the explanation of sporadic vascular anomalies opened the doors to study prospectively and *a posteriori* the causes of various vascular malformations. This was helped by the rapidly evolving genetic techniques including the highly sensitive next generation sequencing. In parallel, knowledge on signaling alterations occurring in vascular endothelial cells because of the various mutations, development of in-vitro and especially the first in-vivo models, gave the possibility to test preclinically molecular therapies for vascular malformations.

Recent findings
One of the first molecules, rapamycin, showed clear evidence of interrupting lesion growth. As its safety profile had been established in other conditions, it was quickly accepted for clinical trials on vascular anomalies. Now, with a few trials published and others ongoing, it is establishing itself as a gold standard for molecular therapy for recalcitrant lesions.

Summary
Targeted molecular therapies are becoming interesting new additions to the management of vascular anomalies, and rapamycin is establishing itself as a gold standard for venous malformations.

Keywords
mutation, PIK3CA, precision medicine, rapamycin, somatic, tyrosine kinase endothelial, TIE2, vascular anomaly, venous malformation

INTRODUCTION

Venous malformations are the most common vascular malformations seen in multidisciplinary centers, with an overall incidence of one in 5000. They result from anomalies in the development of the venous network, which lead to the formation of thin-walled, dilated and dysfunctional vessels. These abnormal veins are composed of a flat endothelial lining surrounded by sparse, irregularly distributed vascular smooth muscle cells (pericytes) [1,2]. Venous malformations are usually present at birth, grow with the child, and slowly expand with time. They can affect any organ or tissue, but mainly occur in cutaneous, subcutaneous and mucosal tissues.

More than 90% of venous malformations occur sporadically and consist of a unifocal lesion. Rarely, lesions are multifocal, affecting at least two distinct sites. This multifocality can be seen in inherited forms that exhibit autosomal dominant transmission, such as cutaneomucosal venous malformation (VMCM) and glomuvenous malformation (GVM) [2,3] or in sporadic forms, such as multifocal venous malformation (MVM) and Blue Rubber Bleb Nevus syndrome (BRBN) [4▪]. Malformations combining venous with other vascular defects also exist, such as the capillary-lymphaticovenous malformations in the Klippel–Trenaunay syndrome (KTS), the complex vascular malformations in the PTEN hamartoma tumor syndrome (PHTS) and the variable lesions in the CLOVES syndrome (Congenital lipomatous overgrowth with vascular malformations, epidermal naevi and scoliosis).

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KEY POINTS

- Most venous malformations harbor activating mutations in TIE2 or PI3K.
- In preclinical murine models, inhibitors of the PI3K–mTOR axis reduce growth of venous malformations.
- In clinical trials, rapamycin increases the quality of life of most patients, by decreasing pain and improving functional limitation.
- Ongoing clinical trials are identifying venous malformation patients who benefit the most from rapamycin.
- New targeted agents should be evaluated in clinical trials in venous malformation patients.

Depending on their location and extension, symptoms are highly variable. Commonly, they cause deformation, pain, chronic anemia, bleeding, oozing and important functional restraint, which result in significant morbidity. Venous malformations can also threaten life because of extension into vital structures, airway obstruction and neurovascular dysfunction. Unlike other vascular malformations, venous malformations are associated with chronic consumptive coagulopathy (also known as localized intravascular coagulopathy), because of blood stagnation and repetitive cycles of thrombosis and thrombolysis. Approximately 50% of venous malformation patients have elevated D-dimers and some of them have low serum levels of fibrinogen [5,6].

Sclerotherapy alone or in combination with surgical resection is the gold standard procedure to manage venous malformations. However, these procedures are rarely curative or are unfeasible in patients with extensive and infiltrating lesions [7,8]. In this review, we describe the reported efficacy and safety of rapamycin, also known as sirolimus, the first targeting agent, for venous malformation treatment.

THE ROLE OF PHOSPHOINOSITOL 3 KINASE–PROTEIN KINASE B–MAMMALIAN TARGET OF RAPAMYCIN PATHWAY IN VENOUS MALFORMATION

The phosphoinositol 3 kinase (PI3K)–protein kinase B (AKT)–mammalian Target of Rapamycin (mTOR) is an important signaling pathway implicated in multiple cellular processes, such as protein synthesis, metabolism and survival. This pathway regulates also, through the angiopoietin/TIE2 system, maturation and stability of veins. Binding of angiopoietin-1 (ANGPT-1), secreted by pericytes, to the tyrosine kinase receptor TIE2 located on endothelial cells stimulates recruitment of the p85 regulatory subunit of PI3K to the membrane and activation of the p110 catalytic subunit of PI3K, which, in turn, phosphorylates AKT on Thr308, leading to a first, partial, activation of AKT. A second phosphorylation on Ser473, induced by one of the two complexes of mTOR (mTORC2), is required to fully activate AKT, which is thereby able to activate mTORC1 and its substrates, resulting in endothelial cell proliferation [9▪]. Activated AKT regulates negatively the transcription factor FOXO1, decreasing levels of pericyte attractant Platelet Derived Growth Factor-β (PDGF-β) and stimulating pericyte detachment. As the initiation of endothelial cells sprouting requires the detachment of pericytes, AKT activity is inversely correlated with PDFG-β levels. Conversely, cell confluence during tube formation induces pericyte recruitment, through a decrease in AKT activity and an increase in PDGF-β levels, to provide stabilization and maturation of nascent vessels [10–12] (Fig. 1a).

The majority of venous malformations are because of gain-of-function somatic mutations in the genes encoding TIE2 or PI3K. Up to 60% of them present an activating mutation in the tyrosine kinase endothelial (*TEK*) gene (chromosome 9) encoding the TIE2 receptor, resulting in its ligand-independent activation [4▪,13–16]. More than 20 different mutations have been described occurring alone or as double mutations and can cause all four subtypes of venous malformations: VMCM, venous malformation, MVM and BRBN, with the L914F mutation representing 60% of *TIE2*-mutated sporadic venous malformations [13–16]. Mutations in *PIK3CA*, the gene encoding the p110a catalytic subunit of PI3K, cause 20% of venous malformations [17▪]. More than 90% of *PIK3CA*-mutated venous malformations have mutations causing amino acid changes E542K, E545K or H1047R, which are also frequently found in cancer and other *PIK3CA*-associated malformation/overgrowth syndromes [17▪,18,19▪▪]. These *TIE2* and *PIK3CA* mutations result in an excessive and uncontrolled activation of AKT, even in confluent cells. The AKT-mediated inhibition of FOXO1 leads to inappropriately low PDGF-β levels, likely involved in defective and sparse pericyte coverage (Fig. 1b) [20].

Gene-specific effects can be observed clinically. *PIK3CA*-mutated venous malformations are deeper and not extending into the skin, in contrast to common *TIE2*-mutated venous malformations [17▪]. Furthermore, downstream signaling activation can be different. Both *TIE2* and *PIK3CA* mutations activate AKT *in vitro*, but only *TIE2* mutations cause phosphorylation of ERK1/2 and STAT [17▪,21].

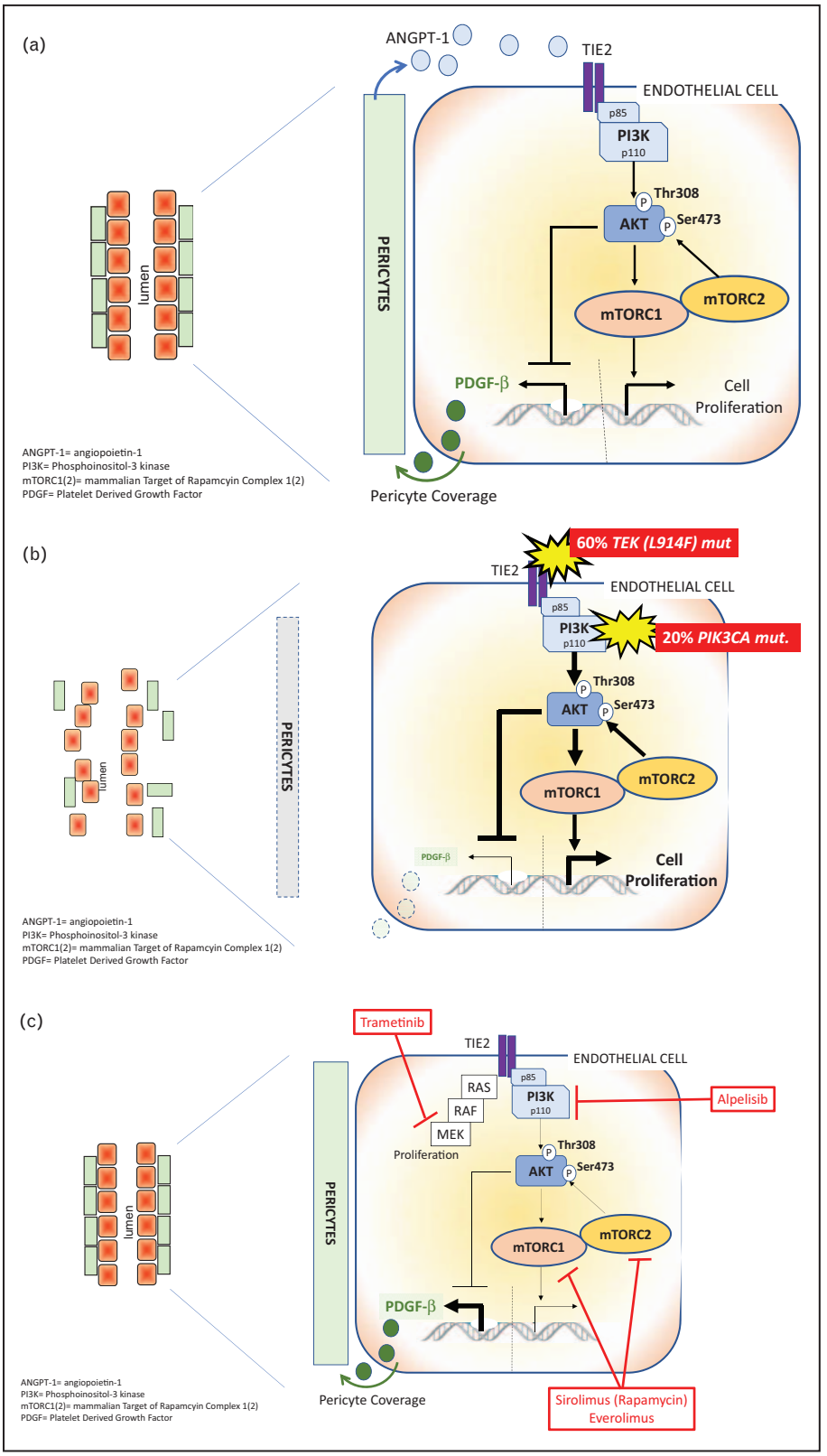

FIGURE 1. (a) The role of the phosphoinositol 3 kinase–protein kinase B–mammalian Target of Rapamycin (mTOR) pathway in venous homeostasis. Binding of angiopoietin-1 (ANGPT-1) secreted by pericytes to the tyrosine kinase receptor TIE2 located on endothelial cells stimulates the recruitment of the p85 regulatory subunit of PI3K to the membrane and the activation of the p110 catalytic subunit of PI3K. PI3K phosphorylates AKT on Thr308, leading to a first, partial, activation of AKT. mTORC2 induces a second phosphorylation on Ser473 to fully activate AKT, which is thereby able to activate mTORC1 and its

Murine models mimicking the human venous malformations have been developed and allowed to explore potential targeting agents. The first mouse model of venous malformation was generated through subcutaneous injection of L914F *TIE2*-mutated endothelial cells in mice, which resulted in development of vascular lesions closely resembling human venous malformations. Blood vessels were ectatic and surrounded by a thin, scarce, non-continuous layer of pericytes. Treatment with rapamycin reduced proliferation of endothelial cells and the growth of venous malformations. Blood vessels appeared smaller, less ectatic and showed an increased number of pericyte coverage [22▪▪]. This vessel 'normalization' was induced by a strong inhibition of p-AKT473 through rapamycin-mediated mTORC2 disruption. Indeed, even if rapamycin is an allosteric inhibitor of mTOR that predominantly inhibits mTORC1, a prolonged treatment with rapamycin has been shown to disrupt the mTORC2 complex, which is known to directly phosphorylate Ser473 residue of AKT [23]. A TIE2 inhibitor was unable to inhibit lesion growth.

Another two venous malformation mouse models were generated by a local expression of PIK3-CA^{H1047R} -activating mutation in murine endothelial cells. Two weeks of rapamycin treatment resulted in a 25% reduction in the volume of venous malformations in one of them [19▪▪]. In the other one, treatment with everolimus, a rapamycin derivative, blocked the progression of lesions, reduced vessel anomalies and re-established a normally vascularized tissue [24▪]. Finally, PIK3CA^{H1047R}-expressing cells were also injected into mice and they formed highly vascularized and proliferative masses, with a histology and appearance resembling that of venous malformations. Everolimus resulted in a decreased venous malformation size and proliferation, but the PI3K inhibitor alpelisib (BYL719) induced a greater response as measured by a more important decrease in venous malformation volume and increase of apoptosis [25] (Fig. 1c). This was also underscored in earlier in-vitro studies [17▪]. These findings show that a *TIE2* or a *PIK3CA* mutation in vascular endothelial cell is sufficient for venous malformation formation and that inhibition of the PI3K–AKT–mTOR pathway can decrease venous malformation lesions in in-vivo models.

CLINICAL EFFICACY OF RAPAMYCIN IN PATIENTS WITH VENOUS MALFORMATION

Pilot trial

A pilot phase 2A trial evaluated clinical efficacy and safety of rapamycin in six adult patients (mean 32 years, range 14–64) with highly symptomatic venous malformations (pain, bleeding, oozing, infection and functional limitation) refractory to standard treatments. All patients had elevated D-dimers and a poor quality of life (QoL). They received rapamycin 2 mg daily continuously and the dose was adjusted in order to reach a serum target concentration between 10 and 15 ng/ml. On rapamycin, QoL increased rapidly in all patients, with a self-assessment of this improvement ranging from 30 to 90% at 3 months and from 30 to 100% at 12 months. Pain, bleeding and oozing were rapidly controlled (within the first month) in all patients. The Visual Analogue Scale (VAS) score decreased from a baseline median value of 8 to a 3-month median value of 2, and a 12-month median value of 3. Correlated to these symptomatic changes, chronic consumptive coagulopathy also improved rapidly, with a decrease in median D-dimer values: 6700 in baseline and 1900 at 3 months and 1000 at 12 months. MRI at 12 months showed an approximately 20% reduction in the five evaluable patients. Even if benefits were maintained after a follow-up

substrates, resulting in cell survival and proliferation. Activated AKT also negatively regulates the transcription factor FOXO1, decreasing levels of pericyte attractant platelet derived growth factor-β (PDGF-β) and stimulating pericyte detachment. (b) Molecular anomalies leading to venous malformations. Sixty percent of venous malformations present an activating mutation in the *TEK* gene encoding the TIE2 receptor, resulting in a sustained and ligand-independent activation of TIE2. The L914F mutation represents the majority of *TIE2*-mutated lesions in sporadic venous malformations. Mutations in *PIK3CA*, the gene encoding the p110a catalytic subunit of PI3K cause another 20% of venous malformations. These *TIE2* and *PIK3CA* mutations result in an excessive and uncontrolled activation of AKT, even in confluent cells. The AKT-mediated inhibition of FOXO1 leads to inappropriately low PDGF-β levels, and defective and sparse pericyte coverage. (c) Promising targeted therapies for venous malformations. Rapamycin (as well as everolimus) is an allosteric inhibitor of mTOR. It inhibits mTORC1 and disrupts the mTORC2 resulting in decreased activation of AKT, resulting in increased levels of PDGF-β and stimulation of pericyte coverage. This leads to vessel 'normalization.' Alpelisib is a PI3K inhibitor that could result in a stronger effect than rapamycin. Other alternative pathways, such as the MAPK pathway, could appear as a 'survival pathway' responsible for resistance to PI3K–AKT–mTOR inhibition. Drugs targeting this pathway should also be evaluated in preclinical and clinical trials. PI3K, phosphoinositol 3 kinase; AKT, protein kinase B; mTOR, mammalian Target of Rapamycin.

reaching 5 years, no lesion disappearance was observed [22▪▪].

Phase 2B trial

The phase 2B prospective trial enrolled 19 patients (adult and children) with extensive slow-flow vascular malformations (lymphatic, venous and complex malformations) [26▪▪]. Ten patients had a venous malformation-type lesion (six venous malformations, one CVM, two KTS and one PHTS) with a median age of 19 years (ranging from 9 to 64). They all had a poor QoL with functional limitation ($n=9$), severe pain ($n=8$), severe physical deformation ($n=7$), bleeding ($n=3$), oozing ($n=2$) and/or infections ($n=2$). D-dimer levels were elevated in all 10 patients.

They received rapamycin 2 mg/daily continuously (or 0.8 mg/m^2 twice daily for children) with a serum target concentration between 10 and 15 ng/ml. Follow-up reached 48 months for some patients. All 10 patients experienced improvement in mobility or organ function, reduction of pain, bleeding or oozing, or reduction or cessation of infections, resulting in a 100% general improvement rate. Both the baseline continuous pain intensity and the frequency of painful crises decreased rapidly; the median VAS score for baseline continuous pain decreased from 7 to 2 at 3 and 12 months. The QoL of all patients increased within the first 3 months, with a 20–50% increase in 40% and a more than 50% increase in 60% of patients. This improvement continued at 6 and 12 months follow-up. D-dimer levels decreased in all patients, with a mean reduction of 58% (ranging from 25 to 86%) at 3 months and it persisted at 12 months. In some patients, D-dimer levels showed a temporary fluctuation that was correlated with resurgence of symptoms. When analyzing the 12-month MRI with the ITK-SNAP3-0 software, a size reduction was observed in five patients (mean reduction of 14%, ranging from 0.5 to 34.5%). For one patient, surgery became feasible after 1 year of treatment because of significant reduction in size of the venous malformation, allowing rapamycin arrest [26▪▪].

Phase 3 trial

A prospective multicentric phase 3 trial (VASE) is currently evaluating rapamycin in patients (adult and children) with complex slow-flow vascular malformations, including venous malformations that are refractory to standard treatment (EudraCT Number: 2015-001703-32). This study plans to enroll 250 patients. Rapamycin is administered for a 2-year duration, followed by cessation but may be reintroduced in case of symptom resurgence.

Preliminary results on the first 43 patients who reached at least 6 months of follow-up, were presented in May 2018 at the ISSVA Workshop (Amsterdam) and included 36 patients with a venous malformation-type lesion (29 venous malformations, 5 CVMs, 1 CMDV and 1 BRBN). Patients had poor QoL. The majority (98%) had strong pain and/or functional limitation (88%), and 19% had bleeding. Reduction in pain and/or in limitation (mobility or organ function) was observed in all but four (two venous malformations and two CVMs) patients with a venous malformation-type lesion, resulting in a general improvement rate of 89% (Fig. 2). Rapamycin effects were often observed rapidly and at 1 month, 22 and 38% of the 36 patients presented a 25–75% and a at least 75% decrease in pain intensity, respectively. At 6 months, 31 and 52% presented a 25–75% and at least 75% decrease in pain intensity, respectively. No conclusion could yet be drawn in regard to the specific sensitivity of the different venous malformation subtypes.

SAFETY PROFILE OF RAPAMYCIN

In all the studies that have evaluated rapamycin in slow-flow vascular malformations, rapamycin has been well tolerated with moderate and manageable adverse events from adults to infants, even neonates. Most common grade 1–2 adverse events (CTCAE version 3.0) reported in the phase 2B trial by Hammer and coworkers ($n=19$) were headache (58%), fatigue (48%), cutaneous rash (37%), mucositis (37%), gastrointestinal troubles (nausea/diarrhea) (37%) and flu-like syndrome (32%). Grade 3 adverse events were less frequent and included mainly mucositis (11%). There was no infection or hematological anomalies. One patient developed a basal cell carcinoma, but the causality was difficult to establish, as this patient had previous history of skin cancer. This toxicity pattern was consistent with other reports on rapamycin therapy for slow-flow vascular malformations, and with the preliminary data from the VASE trial [27–31].

Some rare occurrences of cancer during rapamycin treatment have been observed. Among our patients, one 11-year-old girl with a large lymphatic malformation extending from the supra-hyoidal region to the clavicular area, necessitating insertion of a tracheostomy, developed a non-Epstein-Barr virus (EBV)-related B-cell non-Hodgkin lymphoma after 34 months of rapamycin treatment. She had also a history of congenital cytomegalovirus (CMV) infection with ischemic lesions in the right posterior sylvian region as sequelae. No CMV reactivation was noted throughout rapamycin treatment [26▪▪]. Another 4-year-old girl with an unusual primary

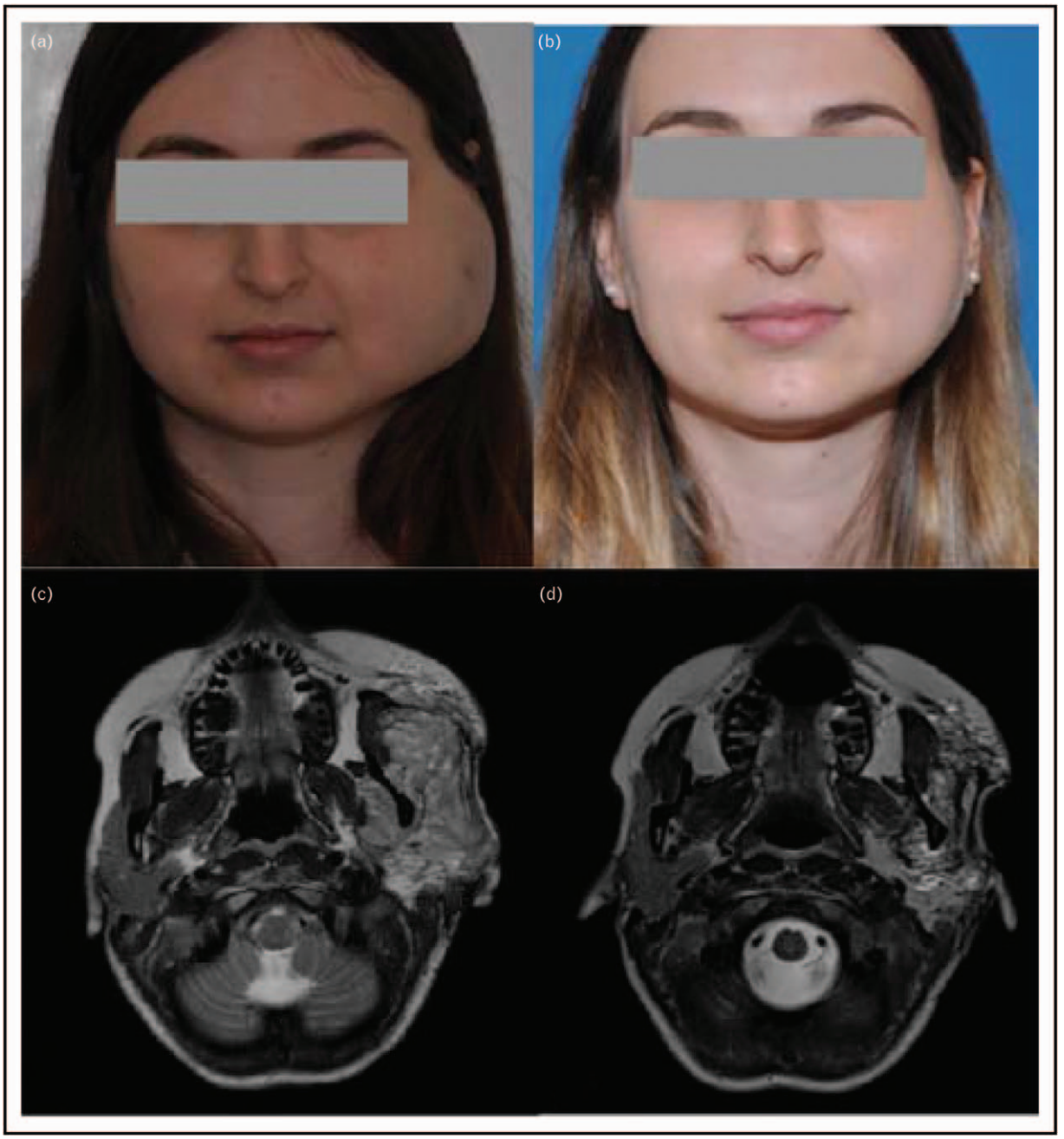

FIGURE 2. Decrease in size of left jugal venous malformation in a 29-year-old woman after 6 months of treatment with rapamycin. Bottom, MRI images.

upper extremity lymphedema with severe pleural effusions developed a lymphangiosarcoma at 3 months of rapamycin treatment [32]. In either case, a causal relationship with rapamycin could not be established, as only EBV lymphoma is associated with immunosuppression, and the second patient had important tissular chromosomal aneuploidy and only 3 months of rapamycin treatment.

In the trial that evaluated rapamycin in lymphatic malformations in children and young adults ($n = 60$; median age 8 years, ranging from 21 days to 28 years), a grade 3 bone marrow toxicity was observed in 27% of patients [27]. We have several children treated with rapamycin for venous malformations without such toxicity. The reason for this discrepancy is unclear, but could be related to vascular disease characteristics.

Patients should be well educated on potential adverse events, and because of the antiangiogenic and immunosuppressive properties, a close follow-up of patients treated with rapamycin is mandatory. At initiation, a blood control (including hemogram,

and liver, thyroid and renal function) should be performed at 1, 3 and 6 months, followed by a control every 6 months. An annual dermatological examination also needs to be done. To reduce the frequency of side-effects, the use of the minimal effective dose should be favored. In young children, antibiotic prophylaxis is helpful to avoid bacterial superinfections on common viral infections, hence avoiding treatment interruptions. Antibiotic prophylaxis is also important in children to avoid infections. The decision to initiate rapamycin should be based on individual patient characteristics and risk factors, and rapamycin should not be considered as first-line therapy for small or asymptomatic venous malformations that respond to standard therapies.

A NEW ERA... AND MULTIPLE DIRECTIONS

Rapamycin is the first targeted therapy that improves considerably the QoL of these patients. Rapamycin does not cure them, as no disappearance of lesions has been observed. However, by decreasing venous malformation volume, rapamycin renders lesions in some cases amenable to interventional procedures, initially considered unfeasible. Rapamycin could, thus, also play a role as a pretreatment before radical interventional procedures of large complicated lesions. A treatment period of at least 6–12 months should be envisioned, as the maximum of response often seems to be reached only after 6 months of treatment.

In the reported trials, rapamycin is administered in venous malformation patients who are refractory to standard treatments. However, as the main effect seems to be a halt in lesion development, it might be the best to start rapamycin therapy as early as possible, in order to prevent tissue infiltration by anarchic endothelial cells. This idea is supported by preclinical findings in mice in which rapamycin was efficient in preventing growth of new lesions but less potent in diminishing them once established [22▪▪]. Future clinical trials should thus evaluate efficacy of rapamycin on nascent malformations free of scarring, as it remains unknown whether sclerotherapy could affect rapamycin efficacy. This draws focus on treatment of children.

The action of rapamycin is rapid, particularly on pain, bleeding and oozing, as it appears within the first month and reaches maximal effect between 3 and 6 months, urging to consider it in life-threatening situations. It also suggests that if a patient does not show any signs of improvement within 3–6 months, rapamycin should be stopped, particularly if there is no decrease in D-dimer levels.

Some of our patients presented resurgence of bleeding and pain few days after rapamycin interruption; whereas rapamycin reintroduction resulted in a new symptom reduction within the next 3 days [26▪▪]. These events can occur even after several months from rapamycin initiation, suggesting that a minimal time is required to reach a significant efficacy. The optimal duration remains unknown but could be personalized, depending on venous malformation symptomatology, extension and the genomic profile. The VASE trial, by planning to treat patients for a limited 2-year duration, should allow to identify, which patient category will mostly benefit from rapamycin. Some patients have a high and rapid sensitivity to rapamycin whereas others do not respond even after a long exposure.

As rapamycin is unable to lead to a complete recovery of venous malformations, there is a need to develop new targeting agents and novel therapeutic strategies focusing on the TIE2-PI3K axis. Rapamycin has been shown to induce *in vitro* and *in vivo* an increased Erk1/2 phosphorylation through the mitogen-activated protein kinase (MAPK) cascade, which could potentiate some of the cellular abnormalities. Rapamycin can also induce negative feedback regulation leading to increased activation of PI3K or AKT, limiting its efficacy in cancer cells and maybe also in vascular anomalies. Combination with MAPK inhibitors, such as trametinib could thus increase the efficacy of mTOR inhibitors, with the caveat of also increasing toxicity [33,34]. Similarly, PI3K inhibitors, such as alpelisib could provide an advantage over rapamycin by avoiding the immunosuppressive effects of rapamycin while also targeting the signaling cascade upstream of mTOR, thereby mitigating hyperactivation of the PI3K-AKT pathway downstream of TIE2, as shown *in vitro* and *in vivo* [17▪,25]. Such efficacy was already demonstrated in PIK3CA-related overgrowth spectrum patients who have a somatic *PIK3CA* mutation with soft tissue hypertrophy [35▪▪]. Topical agents are also of interest, as they could abolish the need for systemic treatments that have wider toxicity.

In conclusion, rapamycin is a well tolerated agent that offers possibility to control venous malformation evolution. Over the years, its safety profile has also been well established, increasing the interest to use it, even in children. Clinical trials are needed to evaluate and compare the efficacy and safety of rapamycin to other targeting agents and strategies, in order to improve our capacity to manage venous malformations.

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Conflicts of interest

Pfizer (Belgium) provides sirolimus (rapamycin) for the clinical studies run by the authors.

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