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REVIEW ARTICLE

New and Emerging Targeted Therapies for Vascular Malformations

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Abstract
Vascular malformations are inborn errors of vascular morphogenesis and consist of localized networks of abnormal blood and/or lymphatic vessels with weak endothelial cell proliferation. They have historically been managed by surgery and sclerotherapy. Extensive insight into the genetic origin and molecular mechanism of development has been accumulated over the last 20 years. Since the discovery of the first somatic mutations in a vascular anomaly 10 years ago, it is now recognized that they are perhaps all caused by inherited or somatic mutations in genes that hyperactivate two major intracellular signaling pathways: the RAS/MAPK/ERK and/or the phosphatidylinositol 3 kinase (PIK3)/protein kinase B/mammalian target of rapamycin (mTOR) pathway. Several targeted molecular inhibitors of these pathways have been developed, mostly for the treatment of cancers that harbor mutations in the same pathways. The mTOR inhibitor sirolimus is the most studied compound for the treatment of venous, lymphatic, and complex malformations. Disease responses of vascular malformations to sirolimus have now been reported in several studies in terms of clinical changes, quality of life, functional and radiological outcomes, and safety. Other targeted treatment strategies, such as the PIK3CA inhibitor alpelisib for PIK3CA-mutated vascular malformations, are also emerging. Repurposing of cancer drugs has become a major focus in this rapidly evolving field.

1 Introduction

Vascular anomalies are a heterogeneous group of disorders characterized by abnormal growth and/or development of lymphatic and/or blood vessels. They remain a diagnostic and therapeutic challenge and are associated with very diverse symptomatology and morphology. Diagnostic and therapeutic progress for these disorders has been greatly facilitated by the classification and terminology initiated by Mulliken and Glowacki [1]. The International Society for the Study of Vascular Anomalies has updated and extended this classification since 1996 and published the current extended version in 2015 [2]. The classification was further updated in 2018 to include the most current information and

Key Points

Molecular and pathophysiological understanding of vascular anomalies has been immensely improved in recent years, establishing that most are associated with mutations in the phosphatidylinositol 3 kinase/protein kinase B/mammalian target of rapamycin (mTOR) or the RAS/MAPK/ERK pathway.

Several targeted compounds exist, and some are being planned to be used, or are being tested off-label to directly impact the appearance and symptomatology of these lesions.

Sirolimus, a direct mTOR inhibitor, has been the most extensively tested so far, including prospective clinical trials, yet other targeted inhibitors of these pathways are emerging as potential treatments for vascular malformations.

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is published on the International Society for the Study of Vascular Anomalies website (https://www.issva.org) [3].

The basis of this classification is the division of vascular anomalies into vascular tumors and vascular malformations. Vascular tumors are characterized by the abnormal proliferation of endothelial cells and blood vessels. They are subclassified as benign, locally aggressive, borderline, and malignant tumors [2]. Vascular malformations are inborn errors of vascular morphogenesis and consist of networks of abnormal blood and/or lymphatic vessels with weak endothelial cell proliferation. The recent update of this classification includes genetic knowledge that has rapidly accumulated during the past 10–20 years. In parallel, targeted molecular therapies have started to emerge.

2 Vascular Malformations

Vascular malformations are described by the main affected vascular component (lymphatic, venous, capillary, arteriovenous) and subdivided as 'simple', 'combined', or 'associated with other anomalies' [2, 4, 5]. Additionally, vascular malformations are further described as 'slow-flow' or 'fast-flow', depending on the absence or presence of an arterial component.

Vascular malformations are, by definition, present at birth and grow proportionally with the child. Nevertheless, appearance and symptoms are not static, with possible expansion or dilation of the affected vessels during growth spurts and puberty [6]. They do not regress or disappear spontaneously. They can be localized or diffuse, and appearance and symptoms depend on location, extension, and the involved anatomical structures. Common symptoms include pain, deformation, esthetic issues, and functional impairment.

Capillary malformations (CMs) represent the most prevalent vascular malformation and mainly occur in the skin as pink or red macules ("port wine" stains) [7]. They are present at birth and persist throughout life if left untreated. They can become thicker and darker with time.

Lymphatic malformations (LMs) consist of dilated lymphatic channels or cysts, lined with endothelial cells with a lymphatic phenotype. They can be subdivided into microcystic, macrocystic, and mixed subtypes. Generalized lymphatic anomaly (GLA) is a rare condition characterized by multifocally occurring LMs in the skin and soft tissue, as well as abdominal and thoracic organs and bone [8]. In Gorham-Stout Disease (GSD), LMs affect a single or multiple contiguous bones, leading to progressive osteolysis. Both entities can lead to pathologic fractures and abdominal and thoracic effusions.

Common venous malformations (VMs) represent the most frequent vascular malformation treated in expert centers [9, 10]. They are usually unifocal lesions. The overall incidence is estimated at 1/5000. They are soft compressible subcutaneous masses with bluish skin discoloration without bruit, pulsation, or redness. Recurrent thrombophlebitis in the slow-flow enlarged vessels is a typical feature, which can lead to the presence of phleboliths. More than 90% of VMs occur sporadically but familial forms exist [2, 4, 5, 9]. These are caused by germ-line mutations in the tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domain 2 (Tie2) gene, whereas most sporadic forms harbor somatic mutations in the same gene (see below).

Arteriovenous malformations (AVMs) are rare fast-flow vascular anomalies, composed of malformed arteries, veins, and capillaries [2, 7]. They are present as a warm painful pulsating lesion and can cause ulceration. Arteriovenous shunting can lead to cardiac overload and eventually decompensated heart failure. They may be associated with other vascular and non-vascular abnormalities and overgrowth.

3 Genetics and Pathophysiology

Extensive insight into the genetic and pathophysiologic origin of vascular anomalies is being accumulated. They are now mostly considered to be caused by abnormal signaling within vascular endothelial cells. This knowledge originates from the elucidation of the genetic anomalies behind some of the rare familial forms. Further studies demonstrated additional involvement of somatic tissue-specific mutations, which led to the hypothesis that a similar mechanism could be responsible for the more common sporadic cases. Moreover, understanding the dysfunctions caused by the mutations at the protein level has laid the basis for novel targeted therapies [8, 11–20, 22].

Patients affected by the inherited forms typically have multifocal small lesions, which increase in number over time. They are transmitted in an autosomal dominant manner and phenotypic penetrance, age at onset, and severity vary among mutation carriers. These characteristics seem to be explained by involvement of a para-dominant mechanism, involving a secondary somatic mutation in the second allele of the same gene, thereby abolishing normal gene function completely [11]. This has since been proven for almost all the 11 known inherited vascular anomalies. The importance of somatic mutations in the occurrence of vascular anomalies led to the hypothesis that the more frequently occurring sporadic forms could be due to somatic changes alone. Similar to oncology, tumor suppressor genes usually need two hits that both lead to loss of function (one eventually germline, the other as somatic) whereas oncogenes "only" need a single activating hit. The first confirmation of this hypothesis was the demonstration that 60% of sporadic VMs have a somatic activating mutation in TIE2/*TEK* [12, 13].

It is now established that most vascular malformations are caused by somatic or mosaic mutations that activate at least one of the two major intracellular signaling pathways: the RAS/MAPK/ERK or the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway [14] (Fig. 1).

The PI3K/AKT/mTOR pathway is implicated in many cellular processes, such as cell-cycle regulation, proliferation, protein synthesis, and cell survival. It is also called the "anti-apoptosis pathway". It is the canonical signaling pathway used by TIE2 and is thus involved in the development of VMs.

TIE2 (encoded by the *TEK* gene) is a tyrosine kinase receptor that is specifically expressed on endothelial cells. Upon binding of angiopoietin-1, recruitment and activation of PI3K, phosphorylation and activation of AKT, and mTORC1 and 2 are set in motion, resulting in endothelial cell proliferation [15].

Disturbances in the PI3K/AKT/mTOR pathway are associated with VMs, the majority (60%) being caused by gain-of-function somatic mutations in the *TEK* gene or (20%) the *PIK3CA* gene encoding the p110a catalytic subunit of PI3K [12, 13, 16–19]. All four subtypes of VMs (cutaneomucosal VM, VM, multifocal VM, and blue rubber bleb nevus [BRBN] syndrome) are associated with *TIE2* mutations. The L914F mutation is the most frequently occurring, representing 60% of *TIE2* mutations in sporadic VMs.

These mutations in either *TIE2* or *PIK3CA* induce an excessive and unregulated activation of AKT. *TIE2* mutations additionally cause phosphorylation of ERK1/2 and STAT [19, 20]. The most frequently observed amino acid substitutions in PIK3CA (E542K, E545K, H1047R) are

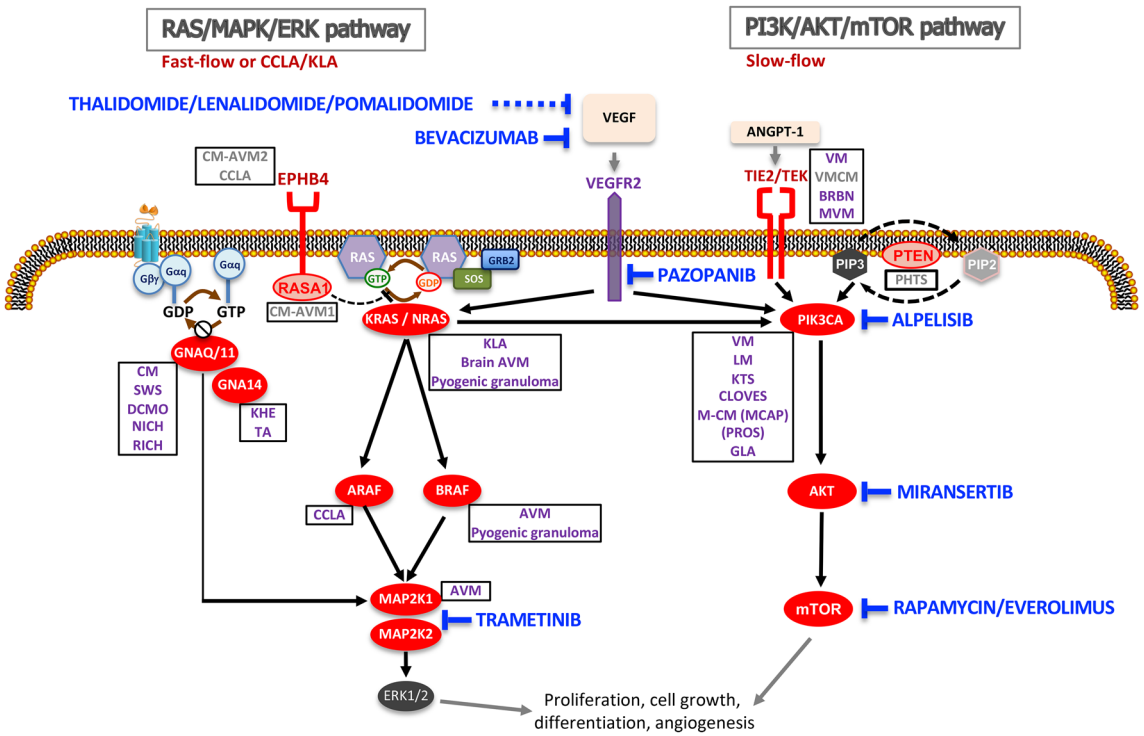

Fig. 1 Intracellular signaling pathways involved in vascular malformations and targets for therapy. *ANGPT-1* angiopoietin 1, *AVM* arteriovenous malformation, *BRBN* blue rubber bleb nevus syndrome, *CCLA* central conducting lymphatic anomaly, *CLOVES* congenital lipomatous overgrowth, vascular malformation, epidermal nevi, scoliosis/skeletal and spinal syndrome, *CM* capillary malformation, *CM-AVM* capillary malformation-arteriovenous malformation, *DCMO* diffuse capillary malformation with overgrowth, *EPHB4* ephrin B4, *ERK* extracellular signal-regulated kinase, *Gαq* guanine nucleotide-binding protein subunit alpha q, *GDP* guanosine diphosphate, *GLA* generalized lymphatic anomaly, *GNA14* G protein subunit alpha 14, *GRB2* growth factor receptor-bound protein 2, *GTP* guanosine triphosphate, *KHE* kaposiform hemangioendothelioma, *KLA* kaposiform lymphangiomatosis, *KTS* Klippel-Trenaunay syndrome, *LM* lymphatic malformation, *MCAP* megalencephaly-capillary malformation, *MCM* macrocephaly-capillary malformation, *MVM* multifocal (sporadic) venous malformation, *NICH* non-involuting congenital hemangioma, *PHTS* PTEN hamartoma tumor syndrome, *PI3K/AKT/mTOR pathway* phosphatidylinositol 3 kinase/protein kinase B/mammalian target of rapamycin pathway, *PIP2* phosphatidylinositol 4,5-bisphosphate, *PIP3* phosphatidylinositol 3,4,5-trisphosphate, *PROS* PIK3CA-related overgrowth syndrome, *PTEN* phosphatase and tensin homolog, *RAS/MAPK/ERK* Ras/mitogen activated protein kinase/extracellular signal-regulated kinase, *RICH* rapidly involuting congenital hemangioma, *SOS* son of sevenless homolog, *SWS* Sturge-Weber syndrome, *TA* tufted angioma, *VEGF* vascular endothelial growth factor, *VEGFR2* vascular endothelial growth factor receptor 2, *VM* venous malformation, *VMCM* cutaneomucosal venous malformation

also encountered in cancer and other *PIK3CA*-associated malformations, such as LMs and overgrowth syndromes, including Klippel-Trenaunay syndrome (KTS), congenital lipomatous overgrowth, vascular malformation, epidermal nevi, scoliosis/skeletal and spinal syndrome (CLOVES), and megalencephaly-capillary malformation [19, 21, 22]. Somatic activating *PIK3CA* mutations were also identified in patients with GLA [8].

The PI3K/AKT/mTOR pathway is inhibited by phosphatase and tensin homolog (PTEN). Loss of PTEN is another cause of abnormal stimulation of the PI3K/AKT/mTOR pathway. Germline loss-of-function mutations of *PTEN* cause PTEN hamartoma tumor syndrome, which includes vascular malformations as one of the minor clinical criteria [23].

The second pathway that is often implicated in the development of vascular anomalies is the RAS/MAPK/ERK signaling pathway, mostly in fast-flow vascular malformations. It is often called the "proliferation pathway" because of its role in many cellular processes such as cell-cycle regulation, cell proliferation, and migration. Upstream elements include the guanine nucleotide-binding protein subunit alpha q (Gαq) encoded by *GNAQ*, *GNA11*, and *GNA14*. These Gα-subunit proteins exchange GDP for GTP when their receptor is activated, ultimately leading to the downstream activation of the RAS-MAPK (Raf/MEK/ERK pathway) and the PI3K/Akt/mTOR pathway (Fig. 1).

Mutations in these genes are involved in congenital hemangiomas, including rapidly involuting congenital hemangiomas (RICH) and non-involuting congenital hemangiomas (NICH), in kaposiform hemangioendotheliomas (KHEs), congenital tufted angiomas, and pyogenic granulomas [24, 25]. Somatic activating *GNAQ* mutations are implicated in isolated capillary malformations and Sturge-Weber syndrome [26]. Fast-flow AVMs are also driven by mutations in the RAS/MAPK/ERK pathway. RASopathies are diseases caused by genes in the RAS/MAPK/ERK pathway resulting in uncontrolled activation, such as neurofibromatosis. Several isolated vascular malformations should thus be considered as RASopathies, including capillary malformation-AVM 1 and 2, intra- and extracranial AVMs, and pyogenic granulomas [27–29]. An overview of these entities is detailed elsewhere [30].

4 Targeted Treatment of Vascular Malformations

The discovery of the pathogenic involvement of the RAS-MAPK-ERK pathway and the PI3K-AKT-mTOR pathway in vascular malformations has paved the way for targeted drug treatment of these lesions. As the same pathways are involved in several cancers and other pathologies that occur at a significantly higher incidence than vascular malformations, drugs that specifically target these pathways have been developed.

4.1 Rapamycin (Sirolimus)

Sirolimus was first discovered as an antifungal drug, produced by *Streptomyces hygroscopicus* in the 1970s. It is a direct inhibitor of mTOR, and blocks downstream signaling and protein synthesis, resulting in antitumoral and antiangiogenic effects [31]. Its initial clinical use involved immunosuppression to prevent kidney transplant rejection and has extensively been studied in this context. It received US Food and Drug Administration and European Medicines Agency approval in 1999 and 2001, respectively, for this indication. Mammalian target of rapamycin (mTOR) inhibitors have further orphan indications in a large range of diseases such as soft-tissue and bone sarcoma, lymphoma, neuroendocrine tumors, and tuberous sclerosis. In tuberous sclerosis, patients with subependymal giant astrocytoma or renal angiomyolipoma, involving the PI3K/AKT pathway, respond to the mTOR inhibitors sirolimus and everolimus.

We and others have reviewed the preclinical evidence of rapamycin for the treatment of vascular malformations elsewhere [15, 32]. The first report of significant clinical response of sirolimus in vascular malformations was the description of six patients with complex life-threatening vascular anomalies in whom sirolimus was given in a compassionate use setting [33]. It concerned patients with KHE and Kasabach-Merritt phenomenon (KMP) with severe coagulopathy and high-output cardiac failure that resolved after treatment, a patient with capillary-lymphatic VM, and four patients with diffuse microcystic LMs with pleural effusions requiring chest tubes that could be removed after treatment with sirolimus. Since then, several retrospective series [34–40] and case reports [41–45] have confirmed these findings.

A small number of prospective clinical trials have also shown the efficacy of sirolimus in slow-flow vascular malformations [32, 46]. Boscolo and coworkers evaluated the clinical efficacy and safety of sirolimus in six adult patients (phase IIA) with VMs refractory to standard treatments with poor quality of life (QoL) because of severe symptoms such as pain, bleeding, and functional limitations [32]. In the follow-up phase IIB study, a larger group of 19 pediatric and adult patients with extensive venous, lymphatic, or complex malformations and poor QoL was described [47]. The QoL of all patients improved within the first 3 months of treatment. Improvement of coagulopathy could also be observed.

The largest prospective trial published to date reports on a phase II trial that enrolled 61 patients with complex vascular anomalies and at least one complication such as coagulopathy, chronic pain, recurrent cellulitis, ulceration, visceral and/or bone involvement, or cardiac dysfunction [46].

Fifty-seven patients were evaluable for clinical outcome and showed 83% of patients with partial response after 6 months and 85% of patients with partial response after 12 months of treatment. Overall, these patients had significant improvements in QoL measurements.

A prospective multicentric phase III trial (VASE) is currently underway, evaluating sirolimus in pediatric and adult patients with complex slow-flow vascular malformations, and VMs refractory to standard treatment (EudraCT Number: 2015-001703-32). Patients receive sirolimus for 2 years but may be retreated after the end of treatment in the case of relapse of symptoms.

4.1.1 Dosing of Sirolimus in Different Age Groups

Most studies propose an initial sirolimus dose of 2 mg per day for adults and 0.8 mg/m^2 twice daily for children. Dosages are subsequently pharmacokinetically guided and adapted based on sirolimus blood concentrations targeted at 5–15 ng/mL in most retrospective series, and 10–15 ng/mL in most prospective series (Table 1).

Significant inter- and intra-patient variability in dosing requirements has been described and requires therapeutic drug management [48, 49]. The fixed-dose strategy based on body surface area or weight often leads to drug exposures outside of the targeted range. Especially in infants and young children, sirolimus dosing experience is limited and dosing requirements may differ significantly between infants (aged < 2 years) and older children.

In children with vascular anomalies, a developmental trajectory of sirolimus clearance in neonates and children has been demonstrated, representing the age-dependent evolution in cytochrome P450 (CYP) 3A metabolism capacity [50]. As sirolimus is metabolized through CYP3A4 and CYP3A5 pathways, the authors used the sequential sirolimus clearance observations from patients with complicated vascular anomalies participating in a concentration-controlled sirolimus phase II study in parallel with measurements of sirolimus metabolites. They could describe a relationship between sirolimus clearance and a patient's age with a mathematical equation. They identified age-appropriate sirolimus dosing regimens, aiming at improving precision dosing for these very young patients and to improve the likelihood of early target attainment [51].

The same authors went on to describe a pharmacokinetic model-based strategy for precision dosing of sirolimus in patients enrolled in a phase II clinical trial [52]. The mean sirolimus dose needed to achieve a sirolimus concentration of ~ 10 ng/mL for patients aged older than 2 years was 1.8 mg/m^2 every 12 h (0.8–2.9 mg), and 0.7–1.6 mg/m^2 every 12 h for patients younger than 2 years of age. Their model allowed a detailed starting dose proposal from birth through adolescence as follows: 0.4, 0.5, 0.6, 0.7, 0.9, 1.1, 1.3, 1.6, and 1.8 mg/m^2 as identified for the age groups of 0–1, 1–2, 2–3, 3–4, 4–6, 6–9, 9–12, and 12–24 months and 2–18 years, respectively.

4.1.2 Safety and Toxicity of Sirolimus

Target of rapamycin is a protein kinase that regulates cell growth, metabolism, and proliferation. It is involved in numerous vital physiological cell processes such as protein and lipid synthesis, as well as cell-cycle regulation, proliferation, and cell survival [14, 53]. mTOR inhibitors present a safety profile that reflects this wide range of affected processes. Most side effects are dose dependent. A large proportion of the available evidence has been generated from solid organ or hematopoietic stem cell transplantation or treatment of cancer. These patients often require multiple immune-suppressive drugs, and therefore it is not clear to which degree the reported safety issues can be extrapolated to patients who receive sirolimus as monotherapy.

4.1.2.1 Tolerance and Cancer Risk Seront and coworkers reviewed studies that evaluated rapamycin in slow-flow vascular malformations. Tolerance was good with moderate and manageable adverse events in all age categories [15]. For instance, in 19 patients treated in the phase IIB trial by Hammer et al., headache (58%), fatigue (48%), cutaneous rash (37%), mucositis (37%), nausea/diarrhea (37%),

Table 1 Selected studies of sirolimus for vascular malformations

Authors	*n*	Dose	Target (ng/ml)	PJP prophylaxis	PJP	Neutropenia	Remarks
Prospective							
Boscolo et al. [32]	6	2 mg/day	10–15	N	N	0	All adult
Adams et al. [46]	61	0.8 mg/m^2 q12h	10–15	Y	N	30	Blood/bone marrow toxicity
Hammer et al. [47]	19	0.8 mg/m^2 q12h	10–15	N	N	NI	Children
		2 mg/day	10–15	N	N	NI	Adults
Retrospective							
Hammill et al. [33]	6	0.8 mg/m^2 q12h	10–15	Y	N	1	First retrospective series

NI not indicated, *PJP pneumocystis jirovecii* prophylaxis, *q12h* every 12 hours

and flu-like syndrome (32%) were the most frequent side effects [47]. We have observed two cases of cancer occurring during sirolimus treatment: one 11-year-old girl who developed a non-Epstein–Barr virus-related, B-cell non-Hodgkin lymphoma and one 4 year-old girl who developed lymphangiosarcoma in the context of a primary upper extremity lymphedema with severe pleural effusions [15]. It is not clear to what extent sirolimus treatment may have contributed to cancer development in these cases, as non-Hodgkin lymphoma in the context of immune suppression is mostly Epstein–Barr virus related, and lymphangiosarcoma has been documented to develop from underlying lymphatic malformations and lymphoedema, even without sirolimus therapy (Stewart-Treves syndrome) [54].

4.1.2.2 Wound Healing Wound healing complications associated with mTOR inhibitor therapy have been widely described in the setting of immunosuppression for solid organ transplantation and can be as high as 52% in some retrospective studies [55]. Treatment withdrawal has therefore been recommended before and after elective surgery until complete wound healing. However, we have seen better treatment results while maintaining patients with a vascular anomaly taking sirolimus during surgical management, specifically due to decreased lymph leakage post-operatively for lymphatic malformations. Thus, in the context of surgical vascular malformation management, sirolimus therapy seems to improve wound healing (L Boon, personal communication).

4.1.2.3 Hyperlipidemia The mTOR pathway is implicated in lipid synthesis and lipid homeostasis. Dose-dependent incidences of hypercholesterolemia and hypertriglyceridemia have been reported to be as high as 5% and 74% in clinical trials studying the efficacy and safety of sirolimus in patients undergoing a renal transplant, albeit in combined immune suppression regimens [56]. Treatment using statins and fibrates is often sufficient for controlling dyslipidemia, yet rhabdomyolysis has been reported in patients treated with statins and sirolimus [57]. Close monitoring is therefore advisable following treatment initiation.

4.1.2.4 Stomatitis and Cutaneous Side Effects Stomatitis and mouth ulcers are among the most frequently reported adverse events of mTOR inhibitor therapy. The incidence varies from 3 to 60%, depending on co-medication and indication [55]. The degree of stomatitis is usually mild and dose dependent. It rarely leads to treatment discontinuation and can be prevented by good oral hygiene, mouthwashes, and topical treatments with a variety of available formulations.

Sirolimus has also been associated with acne-like dermatitis and folliculitis, especially in patients with a history of acne. Exanthema, dry skin, pruritus, and cutaneous vasculitis have also been reported [55]. These skin problems rarely lead to treatment cessation.

4.1.2.5 Immune Suppression, Bone Marrow Toxicity, and Infection The mTOR pathway is recognized as a central regulator of the immune system, in which mTOR represents a key biologic "switch". The immunosuppressive properties of sirolimus are the result of its ability to inhibit T-cell proliferation. Because of the latter, the immunosuppressive effect of sirolimus is, at least partially, owing to promotion of T-cell anergy in the presence of a valid co-stimulatory signal. Mammalian target of rapamycin inhibitors have also been shown to modulate regulatory T-cell and dendritic cell activity [58]. Special attention is therefore warranted regarding the immunosuppressive and infectious side effects of sirolimus treatment.

Thrombocytopenia, leukopenia, and anemia have been reported in a dose-dependent manner. Most reports concern the setting of solid organ transplantation, where sirolimus was not used as monotherapy [59]. In addition, Adams et al. reported an incidence of 27% of blood and bone marrow toxicity (grade 3 and higher), as well as an infection rate of 2% [46]. Patients received *Pneumocystis jirovecii* prophylaxis with co-trimoxazole or pentamidine. It is not clear to what extent co-trimoxazole may have contributed to myelotoxicity. Its use for prophylaxis during sirolimus monotherapy remains controversial.

Several reports of *Pneumocystis* infection related to mTOR inhibitor treatment have emerged [60–69] (Table 2). They include case reports and retrospective studies, randomized controlled trials, and meta-analyses. However, all reported *Pneumocystis* infections occurred in the setting of solid organ transplantation or cancer, where patients are heavily co-treated with a variety of other immunosuppressive and/or myelosuppressive drugs. To the best of our knowledge, no documented case of *Pneumocystis* infection has been reported in the context of sirolimus monotherapy for tuberous sclerosis complex or vascular malformations, although a proportion of these patients had received prophylaxis.

A case of *Pneumocystis* infection was reported in a patient receiving sirolimus for KHE in combination with a prednisolone taper after a lack of adequate response to prednisolone, propranolol, and vincristine [66]. Two cases of mortality due to sirolimus-related pneumonia in infants with KHE have also been reported [67]. One of the cases was an infant, who died from proven *Pneumocystis* pneumonia, receiving a steroid taper after treatment with high-dose methylprednisolone (1 mg/kg/day), whereas the other case was a baby receiving sirolimus monotherapy for KHE with Kasabach-Merritt phenomenon, without demonstration of the micro-organism causing pneumonia. One case of

Table 2 PJP infection related to treatment with mTOR inhibitors

Authors	Study type	Indication	*n*	*n* PJP	PJP prophylaxis	Monotherapy	Co-treatment	Target (ng/ml)
De Castro et al. [60]	Case control retrospective	Renal Tx	33	11	N	N	Antiproliferative drugs, CNI, CS	
Saito et al. [61]	Case report	Renal cell Ca	1	1	N	Y	Previous sunitinib	
Kuik et al. [62]	Case report	Metastatic breast Ca	1	1	N	N	Antitumoral drugs, hormonal treatment	
Overwater et al. [63]	RCT cross over	TSC	23	0	N	Y	AED	5–10
Hu et al. [64]	Retrospective review	Heart Tx	38	6/13	Y 6 months	N	CS, tacrolimus, MMF	8–14
Krueger et al. [65]	Retrospective multicenter	TSC	45	0	N	Y	AED	
Russel et al. [66]	Case report	KHE + KMP	1	1	N	N	Prednisolone, VCR	8–15
Ying et al. [67]	Case series	KHE + KMP	2	2	N	N	Methylprednisolone, proprano-lol	
Li et al. [68]	Meta-analysis	TSC	671	0	?	Y	NI	
Ghadimi et al. [69]	Meta-analysis	SOT	35,597	NI	Y	N	NI	

AED anti-epileptic drugs, *Ca* carcinoma, *CNI* calcineurin inhibitor, *CS* corticosteroid, *KHE* Kaposiform hemangio-endothelioma, *KMP* Kassabach Merrit phenomenon, *MMF* mycophenolate mofetil, *NI* not indicated, *PJP pneumocystis jirovecii* prophylaxis, *RCT* randomized controlled trial, *SOT* solid organ transplantation, *TSC* tuberous sclerosis complex, *Tx* transplantation, *VCR* vincristine

Pneumocystis infection has also been reported in the context of lymphangioleiomyomatosis treated with another mTOR inhibitor, everolimus. The infection occurred in the context of disease-related lung damage and lymphopenia [70]. It is not clear from the report whether this patient received prophylaxis or concomitant immune suppressive treatments, such as steroids.

We did not encounter any case of *Pneumocystis* infection (or other severe opportunistic infections) in our own pediatric and adult series in the VASE study so far. These patients did not systematically receive *Pneumocystis* prophylaxis. Special caution is warranted, however, in patients who develop dose-dependent lymphopenia or neutropenia even when using sirolimus monotherapy, in very young children and in children with underlying diseases causing lung dysfunction, a poor general condition, or other co-morbidities. A reduction in sirolimus dose and/or pneumocystis prophylaxis should be strongly considered in these patients.

Mizuno et al. observed that patients experiencing infection display increased sirolimus concentrations [52]. This is explained by the fact that infection and inflammation down-regulate the expression and activity of CYP, leading to about 50% decreased sirolimus clearance. This justifies a 50% reduction in sirolimus dosage or treatment interruption during infectious episodes.

4.2 Other Emerging Targeted Treatments

4.2.1 Everolimus

Everolimus is an mTOR inhibitor derived from sirolimus. It has been in use for a shorter time, and thus our knowledge on its detailed effects in patients is more limited. It has been used sporadically for the treatment of vascular malformations, but no prospective clinical trial has yet been reported. Its off-label use was successful for the treatment of two patients with KHE [71, 72].

Everolimus was also efficacious in the treatment of a patient with "diffuse lymphatic, venous and arteriovenous anomalies" [73] and a case with "congenital segmental lymphedema" associated with tuberous sclerosis complex [74]. Whether everolimus and sirolimus have equal or differing benefits to patients remains to be studied. Although mTOR inhibitors have clearly revolutionized the therapeutic options for patients with complicated vascular malformations, a significant proportion of patients will need treatments targeting other signaling complexes.

4.2.2 PIK3CA Inhibition

CLOVES syndrome results from somatic, mosaic gain-of-function mutations of the *PIK3CA* gene and belongs to the spectrum of PROS (PIK3CA-related overgrowth

syndromes). A proportion of patients with PROS may respond to sirolimus treatment, although success rates have been rather limited [36, 46, 75, 76]. The use of low-dose sirolimus (2–6 ng/mL) was reported in a prospective, non-randomized open-label pilot study of 39 patients with PROS [77]. Some effect was observed on overgrowth, but without clear effect on QoL.

Inhibition of PIK3CA is a promising strategy for *PIK3CA*-mutated vascular malformations, such as PROS. Several PIK3CA inhibitors are under development for PIK3CA-dependent tumors. BYL719 (alpelisib) is currently being investigated in clinical trials and shows a favorable tolerability profile [78, 79]. Alpelisib (Piqray®) was also recently approved by the Food and Drug Administration for *PIK3CA*-mutated breast cancer treatment, opening doors for its wider use [80]. Alpelisib has been tested in a clinical study treating 19 patients with PROS, based on the preclinical observation that this compound could prevent and improve organ dysfunction in a mouse model of PROS/CLOVES [81]. BYL719 treatment decreased vascular tumor size, improved congestive heart failure, reduced hemihypertrophy, and attenuated scoliosis. No serious safety issues were encountered in these patients. Alpelisib may lead to peripheral insulin resistance and hyperglycemia, which could usually be managed with nutritional therapy during the 6-month follow-up period. Other side effects included discrete mouth ulcerations in the first week. They disappeared spontaneously.

Marked improvement of genital vascular malformation using alpelisib in a patient with CLOVES syndrome was also seen in a compassionate use case report. The patient did not experience any clinical or biochemical side effect [76]. Further investigation of this compound and possible other PIK3CA inhibitors is warranted in *PIK3CA*-positive complex vascular malformations with uncontrollable symptoms and poor quality of life. A formulation for topical use is also being developed. This could allow wider use in more localized vascular malformations, in parallel, by reducing side effects.

4.2.3 Protein Kinase B (AKT) Inhibition

Patients with PROS/CLOVES could also be candidates for trials with other PI3K/AKT pathway inhibitors, such as ARQ 092 (miransertib). It is a potent, selective, allosteric, orally bioavailable and highly selective AKT inhibitor, currently under clinical development for the treatment of cancer and Proteus syndrome [82, 83]. In an off-label Proteus syndrome case study, it had beneficial effects [84]. ARQ 092 is currently in a clinical phase I/II study in patients with PROS and Proteus syndrome in North and South America, and in Europe (MOSAIC study).

4.2.4 MAPK Inhibition

Other complex vascular anomalies, such as kaposiform lymphangiomatosis (KLA) and some central conducting lymphatic anomalies (CCLA) have been associated with somatic mutations occurring in the RAS/MAPK pathway (mosaic/somatic RASopathies). These devastating conditions may benefit from treatment with MEK inhibitors, such as trametinib, with or without sirolimus [85, 86]. This was underscored by the treatment of a patient with CCLA associated with a somatic gain-of-function A-RAF mutation who benefited from the off-label use of trametinib [87]. In addition, a patient whose AVM harbored an activating in-frame deletion of *MAP2K1* responded well to trametinib treatment with a reduction in volume and symptoms, and with good tolerance [88]. Only mild acne was reported as a side effect. A prospective phase II trial, TRAMAV, using trametinib to treat fast-flow lesions will soon start in Brussels, Belgium (EudraCT number: 2019-003573-26).

4.2.5 Vascular Endothelial Growth Factor Inhibition

Hereditary hemorrhagic telangiectasias (HHT), also known as Osler-Weber-Rendu syndrome, is a genetic disorder characterized by vascular anomalies, such as AVMs and telangiectasias [89–91]. It is an autosomal dominant disease, which presents mostly with recurrent epistaxis or gastrointestinal bleeding that can be difficult to control, secondary iron deficiency anemia, and high cardiac output failure. It is usually caused by mutations in the endoglin (*ENG*) or the activin receptor-like kinase (*ALK1*) gene. These genes are implicated in the transforming growth factor-β superfamily signaling pathway.

Vascular endothelial growth factor (VEGF) signaling acts in parallel to the transforming growth factor-β pathway and VEGF is elevated in the serum and nasal mucosa endothelial cells of patients with HHT. Bevacizumab is a recombinant humanized monoclonal antibody that binds and inhibits VEGF, making it a potential treatment option to control bleeding in these patients. It has been used as submucosal injections, intravenously, or topically as a nasal spray. Intravenous or intranasal administration does not seem to provide benefit to patients. Promising results have been obtained with intramucosal injections, although further clinical study regarding injection technique, dosing and interval, safety, and efficacy are needed [89–92].

4.2.6 Other

Other emerging treatment options for patients with HHT include thalidomides (thalidomide, lenalidomide, pomalidomide). They are thought to act in part via indirect inhibition of VEGF. Thalidomide has also been used to treat sporadic

AVMs recalcitrant to conventional therapies (Boon L, in preparation). In addition, pazopanib, which inhibits tyrosine kinase receptors including VEGF receptor-2, has shown promising results in patients with HHT with uncontrolled bleeding [93]. These and other emerging treatments for HHT are reviewed elsewhere [94].

5 Conclusions

Several insights have improved the outlook and treatment possibilities for patients with vascular malformations in recent years. Consensus regarding proper classification and terminology has permitted correct diagnosis and treatment guidance. Furthermore, the elucidation of underlying genetic and molecular mechanisms has led to better pathophysiological understanding of these lesions and has paved the way for the use of targeted treatments. Whereas vascular malformations historically were treated by surgery and/or embolization, targeted drugs are currently under investigation, based on the underlying cellular pathways involved. Medical treatment will likely be an effective addition to the therapeutic possibilities for these often difficult-to-treat entities.

The mTOR inhibitor sirolimus is the most extensively studied drug in this context so far. Promising results from several phase I and II prospective studies and from retrospective case series have led to a phase III clinical trial (VASE) that is currently underway in Europe. Evidence for the successful use of other targeted compounds in other indications, such as the PIK3CA inhibitor alpelisib, the MEK inhibitor trametinib, and the monoclonal anti-VEGF antibody bevacizumab is also accumulating, but needs further careful study.

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Compliance with Ethical standards

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