

# **PI 3-kinase: a new kid on the block in vascular anomalies**

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**Abstract (<300 words)**

Vascular anomalies are broadly divided in vascular tumours and malformations. These lesions are composed of abnormal vascular elements of various types, and mainly affect infants, children and young adults. Vascular anomalies may be painful, be complicated by bleeding, infection or organ dysfunction, and can have secondary effects on other tissues. Current treatment strategies include surgical excision, pulsed laser, and sclerotherapy, which are invasive with risk of recurrence. There are growing pharmacologic options for these vascular anomalies, but to date no specific targeted therapies have been developed. Phosphoinositide 3-kinases (PI3Ks) are a family of lipid kinases involved in signal transduction and vesicular traffic which modulate important cellular processes such as proliferation, growth and migration. Recent findings indicate that the PI3K signalling pathway is important in the pathogenesis of vascular anomalies. This provides an opportunity for repurposing PI3K inhibitors, already used in clinical trials for cancer treatment, for such lesions. Here, we provide an update on the classification of vascular anomalies, with their major features, and discuss the role of the PI3K signalling pathway in the pathogenesis of vascular anomalies and their clinical implications and therapeutic opportunities.

## Vascular anomaly classification

Vascular anomalies represent a broad spectrum of lesions, some with overlapping clinical features. Many of these entities were historically associated with several labels, used by both clinicians and pathologists, which created confusion and difficulty in management and evaluation of published data, since particular ‘diagnoses’ had different meanings and implications for patients and healthcare professionals. In order to address these issues, and to provide a common framework for both clinicians and researchers in this field, the International Society for the Study of Vascular Anomalies (ISSVA) proposed a classification system, which has since been widely adopted and recently updated [1,2].

In summary, the generic term “vascular anomaly” (VA) encompasses all such lesions, which are broadly subgrouped into vascular tumours and vascular malformations. Each of these categories contains a further range of entities, which are defined based on a combination of specific clinical, imaging and histopathological features (**Table 1**). This overall schema has now been generally adopted by clinicians, pathologists and radiologists [3-5].

Data from tertiary referral centres with specialist VA clinics indicate that by far the commonest type of vascular tumour is infantile type capillary haemangioma (IH), with much smaller proportions of congenital haemangiomas (CH), Kaposiform haemangioendothelioma / Tufted haemangioma (KHE/TA) and other rare entities. In contrast, the “vascular malformations” group consists of more similar numbers each of venous malformations (VM), arteriovenous malformations (AVM), lymphatic malformations (LM) and capillary or mixed-type vascular malformations [6] (**Figure 1**).

For some lesions, such as AVM, the clinical and imaging features are often characteristic, but for many other entities definitive diagnosis requires histological evaluation of tissue samples in conjunction with the clinical features [2]. In addition, such tissue samples may provide prognostic or therapeutic information based on immunohistochemical expression of specific markers which, in combination with other techniques such as molecular profiling, are increasingly likely to direct individualised care with development of specific small molecule therapies [7].

In order to develop rational scientific approaches to further understanding of such lesions, it is essential that both basic scientists and clinicians/pathologists understand and use appropriate terminologies, including, for example, when describing the findings of animal models of disease (see later section). To this end, we provide a brief overview of the main clinicopathological features of common VAs. More detailed clinical and histopathological details are referenced [3,8].

Correct classification and terminology by basic scientists and clinicians allows specific associations regarding possible genetic aetiologies and mechanisms to be determined, as subsequently illustrated in relation to PI3K. This may be in relation to development of specific entities in addition to subclassification of genetic types of morphologically similar lesions. For example, in the case of infantile type haemangioma, depending on the presence or absence of other malformations and the distribution of the lesions, specific syndromic disorders may be identified (for example PHACE and LUMBAR) [9]. Whilst genetic understanding of VAs is increasing, with additional associations described [10-12], at present

the classification system remains based primarily on clinical, radiological and histological features, but it is likely that further refinement will occur with additional distinction of cases based on underlying genetic mechanisms, as illustrated here.

## **Vascular tumours**

### *Infantile type capillary haemangioma (IH)*

This common lesion presents in infancy/early childhood, most often as a cutaneous mass lesion. It is histologically formed of numerous thin-walled vascular channels lined by bland endothelial cells with no atypia, which characteristically express GLUT-1 on immunostaining [13]. The precise appearance varies with the age of the lesion, with variably cellular, vascular and fibrous regions. The endothelial cell phenotype is thus similar to placental endothelium, raising several hypotheses regarding pathogenesis, in relation to endothelial/mesenchymal stem cells and vasculogenesis / angiogenesis [14-16]. In most cases there is spontaneous resolution but if treatment is required,  $\beta$ -blockers and corticosteroids are generally effective [17].

### *Congenital haemangioma*

Congenital haemangioma (CH) often appears histologically similar to IH on needle biopsy, being composed of thin walled vascular spaces and bland endothelial cells (in resection specimens, congenital haemangiomata, especially RICH, may demonstrate stromal fibrosis and sclerosis [north ref]). However, such CH do not express GLUT-1 [18]. Their further classification is largely based on the clinical history, as non-involuting (NICH) or rapidly involuting (RICH), which may show histological differences at later stages [13,19-21].

### *Kaposiform haemangioendothelioma (KHE) and tufted angioma (TA)*

These entities are grouped together here since they demonstrate distinct, but similar, histological and immunohistochemical features. In both cases lesions are composed of lobules of endothelial cells with slit-like vascular spaces. KHE usually exhibit a diffuse or nested histological appearance whereas TA demonstrate typical ‘cannonball’ distribution of nodules throughout the dermis. In KHE the tumours appear solid at low power, whereas TA demonstrate characteristic foci of cellular nodules protruding into larger vascular spaces. Both lesions demonstrate co-expression of vascular endothelial markers, such as CD34, with lymphatic endothelial markers such as D2-40 (podoplanin). In KHE, D2-40 expression is diffuse whereas in TA it is usually around the periphery of the nodules. This ‘dual phenotype’ appears to be associated with particular development of Kassabach-Merritt syndrome, due to platelet consumption [22-25].

## **Vascular malformations**

Vascular malformations usually also present in infancy or childhood but, in contrast to tumours, they progress but usually grow slowly with the patient, causing symptoms depending on site, size and subtype [1]. They are poorly circumscribed and often diffusely

infiltrating, with greater predilection to affect deep tissues / viscera. Vascular malformations are composed of collections of abnormal vessels, with simple lesions having a predominant vessel type, including arteries, veins, lymphatics and capillaries. Most lesions demonstrate background fibrofatty tissue, which may be infiltrated 'normal' tissue or part of the lesion in cases where there is evidence of a hamartomatous process. For example, the vascular (usually lymphatic) malformations associated with known overgrowth syndromes, such as Proteus, may be histologically indistinguishable from non-syndromic cases [26]. Vascular malformations are further classified according to the predominant vascular component, although it should be recognised that in histological sections most lesions may contain more than one component, in some cases with no one vessel type being prominent (mixed / combined type vascular malformations described according to the component elements).

The general simple subtypes therefore include arteriovenous malformations (AVM), demonstrating abnormal connections between venous and arterial components, venous malformations (VM), composed of abnormal 'dysplastic' veins, often associated with thrombi and calcification, and lymphatic malformations (LM), composed of predominant abnormal thin-walled lymphatic channels. A capillary component may also be present [8,10,11]. Recently a distinct further clinical subtype has been proposed composed of VM with a prominent adipose component, congenital lipovascular anomaly (COLA) or fibroadipose vascular anomaly (FAVA) [27].

The diagnosis of VAs can usually be made based on morphological findings in routine haematoxylin and eosin (H&E)-stained sections, but interpretation should always be in conjunction with clinical and radiological findings since sampling issues may hamper diagnosis in small biopsy specimens, particularly those with multiple elements. In some cases, distinction of specific components may be aided by immunohistochemical staining such as D2-40/LYVE1 for identification of lymphatic-type endothelium. [florez] It has also been suggested that expression of WT1 may be useful for distinction of vascular tumours from vascular malformations. ]Al Dhaybi]

A range of VAs are also reported affecting other animals, such as mice, but veterinary pathological terminology generally may not equate to the above ISSVA schema and therefore lesions may require secondary classification for comparative purposes. Vascular lesions have often been termed "haemangioma" or "cavernous haemangioma" to represent a wide range of entities which in reality represent a spectrum of haemangiomas and VMs. Nevertheless, such lesions can be classified according to a description of the major components present, and therefore mapped to the ISSVA schema.

### **Role of PI3K in vascular anomalies**

PI3Ks are a family of lipid kinases involved in signal transduction and vesicular traffic. There are eight catalytic isoforms of PI3Ks divided in three classes, based on protein structure and lipid substrate preference [28].

Of specific interest in the context of VAs are the so-called class IA PI3Ks. These are heterodimers consisting of catalytic subunits (p110 $\alpha$ , p110 $\beta$  and p110 $\delta$ , encoded by the *PIK3CA*, *PIK3CB*, and *PIK3CD* genes, respectively) in complex with a p85-type of regulatory subunit (of which there are five isoforms, encoded by the *PIK3R1*, *PIK3R2* and

*PIK3R3* genes). The p85 regulatory subunit keeps the heterodimeric p85/p110 complex in an inactive, cytosolic state. Class IA PI3Ks signal downstream tyrosine kinase- and G protein-coupled (GPCRs) receptors in the plasma membrane. Class I PI3K signalling can also be activated (directly or indirectly) through small GTPases, including Ras.

In endothelial cells, PI3K signalling can be triggered by many stimuli, including the tyrosine kinase receptors from the Vascular Endothelial Growth Factor Receptor (VEGF-R) family downstream of VEGFs, and the TIE receptors, binding to angiopoietins (ANG), regulating pivotal vascular processes such as cell proliferation and survival, morphogenesis, and maturation [29]. Binding of the p85 regulatory subunit to activated tyrosine kinase receptors and their substrates in the plasma membrane, brings the p85/p110 complex to the membrane, and relieves the inhibitory function of the p85 subunit, thus activating the catalytic class IA PI3Ks. Upon activation, class I PI3Ks phosphorylate the phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>) lipid to PI(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>), a second messenger that triggers signalling pathways that include, among other effectors, the Akt/PKB and mTOR protein kinases and FOXO transcription factors, which ultimately modulate important cellular processes such as proliferation, growth and migration.

*PIK3CA* (OMIM171834) was reported to be frequently mutated in cancer over a decade ago [30]. Cancer-associated mutations in *PIK3CB* and *PIK3CD* have now also been reported, but at a much reduced frequency compared to *PIK3CA* [31]. These mutations most often occur in ‘hot-spots’, leading to constitutive activation of the catalytic subunit. Activation of the heterodimeric p85/p110 complex can also be achieved by mutations in the regulatory p85 subunits, a common event in malignancies [32].

In 2012, several independent groups documented the presence of *PIK3CA* mutations, similar to those found in some cancers, in several congenital overgrowth syndromes with no associated malignancy [33-36]. Lower frequency mutations were also observed in other PI3K pathway components, such as *PIK3R2*, *AKT3* and *MTOR* [33-36]. These diseases have recently been grouped under the umbrella term of PROS “*PIK3CA*-Related Overgrowth Spectrum” [9]. These clinical entities cover segmental overgrowths composed by mesodermal tissues including adipose tissue, fibroblasts, muscle, and bone. Interestingly, a common feature of PROS diseases is the presence of vascular anomalies, suggesting a potentially important role of *PIK3CA* and other PI3K components in the pathogenesis of these lesions.

In contrast to malignancies, where these mutations are almost exclusively present in epithelial cells, PI3K pathway mutations in PROS are almost exclusively found in cells from mesodermal origin. The aetiology of PROS syndromes are consistent with an early, post-zygotic mutation that occurs early in embryonic development resulting in a mosaic presence of the *PIK3CA* mutation in the affected tissues.

In an attempt to model PROS pathologies, expression of *Pik3ca*<sup>H1047R</sup>, the most common *PIK3CA* hot-spot activating mutation found in cancer and in PROS, was induced in a mosaic pattern in the embryonic mesoderm of mice [37]. While no apparent overgrowth was observed, they displayed venous malformations (VMs), the most common type of vascular malformation in humans. Additional studies from several groups have reported that one in four human VMs carries somatic hot-spot *PIK3CA* mutations, with low level mutation in other PI3K pathway components, such as *AKT2*, *AKT3* and *IRS2* [37-39]. Taken together,

these observations strongly indicate a causative role of *PIK3CA* mutation in this type of vascular anomaly. Accordingly, PI3K pathway inactivation either by inhibition of p110 $\alpha$  (by small molecule inhibitors selective for this PI3K isoform) or mTOR (by rapamycin) leads to regression of *PIK3CA*-mutant VMs in mice [37,38], with rapamycin also documented to be effective in human patients with *PIK3CA*-mutant VMs [39,40].

Previous studies have indicated an exquisite sensitivity of endothelial cells to PI3K pathway alteration. Indeed, both genetic inactivation and over-activation of p110 $\alpha$  (ubiquitous or endothelial cell-specific) during mouse embryonic development lead to mid-gestation lethality due to severe vascular defects [41,42]. This may explain why *PIK3CA* mutations in human disease are somatic, since germline mutations would be expected to be incompatible with viable embryonic development.

Mechanistically, p110 $\alpha$  inactivation impairs endothelial cell migration whereas its overactivation leads to enhanced cell proliferation and possibly migration, defective mural cell coverage, and aberrant vascular network formation [37,38,41]. In addition, p110 $\alpha$  activation has been shown to dampen the expression of arteriovenous specification markers (Eph-B4, Ephrin-B2, and COUP-TFII) as well as production of the cytokines PDGF-B and ANG2 [37,38], probably as a result of PI3K-mediated inhibition of the activity of the forkhead box O1 (FOXO1) transcription factor, known to stimulate the expression of both cytokines. PDGF-B is essential for the recruitment of mural cells [43], and autocrine secretion of ANG2 favours vessel destabilization [44].

Thus, the overall cellular and molecular effects triggered by PI3K pathway activation are consistent with the pathogenesis of vascular anomalies, which are formed by immature vessels, characterized by endothelial cell hyperplasia and abnormal mural cell coverage.

### **Activation of PI3K pathway components in vascular anomalies**

As mentioned above, PI3K signalling is triggered by activation of tyrosine kinases, GPCRs and small GTPases in the plasma membrane. This generates a signalling cascade that engages a number of components and effectors depending on the cell type and tissue context. Taking into account the exquisite sensitivity of endothelial cells to PI3K alterations, alteration at any point in the pathway might also promote the formation of vascular anomalies. These pathway alterations are described in more detail below (**Figure 2**).

Twenty years ago, germline activating mutations in the *TEK* gene, which encodes the endothelial cell TIE2 tyrosine kinase receptor, were identified as the genetic cause of the inherited vascular anomaly mucocutaneous VM (MCVM) [45]. Similar mutations were also demonstrated in about half of sporadic VMs [46]. Thus, somatic *TEK* and *PIK3CA* mutations account for over 75% cases of sporadic VMs. Interestingly, in sporadic VMs, mutations in *TEK* are mutually exclusive with *PIK3CA* mutations [37,38]. This is consistent with previous observations showing that wild-type and mutant TIE2 signal through PI3K [40,47-49]. Also, the wild-type and mutant forms of TIE2 are known to signal partially through the mitogen-activated protein kinase (MAPK) signalling pathway in endothelial cells [40,48-50]. Of note, the MAPK signalling pathway has been also found to be over-activated in vascular anomalies by genetic mutation of its components or by indirect alterations [12], but this will not be described further in the present review.

PI3K and MAPK signalling pathways are also activated in cells derived from infantile hemangiomas, as a consequence of constitutive activation of the VEGFR2 tyrosine kinase receptor, in some cases as a result of missense mutations in the *KDR* gene which encodes VEGFR2 [51]. Likewise, VEGF-A and VEGFRs are over-expressed in angiosarcoma, the most aggressive type of vascular tumours [52].

The phosphatase and tensin homologue deleted on chromosome 10 gene (*PTEN*) encodes the PTEN phosphatase that dephosphorylates PIP<sub>3</sub> to PIP<sub>2</sub>, thus negatively regulating the activity of the PI3K pathway. Germline inactivating mutations in *PTEN* are the underlying cause of two disorders that predispose to cancer, namely Bannayan-Riley-Ruvalcaba and Cowden syndromes. Most of these patients show a spectrum of vascular anomalies with predominance of arteriovenous and venous malformations [53].

The Ser/Thr protein kinase AKT, the best characterized PI3K effector, has been also related to the pathogenesis of vascular anomalies. Upon PI3K activation, AKT binds to PIP<sub>3</sub> in the plasma membrane allowing its phosphorylation by other kinases and its subsequent activation. Antibodies to these phosphorylated forms of AKT (pAkt) are often used as proxy markers for PI3K pathway activation. There are three AKT isoforms (AKT1, AKT2, and AKT3), encoded by distinct genes, with the AKT1 isoform predominantly expressed in endothelial cells [54]. Overexpression of an active form of AKT1 in the murine endothelial cell line MS1 is associated with vascular malformations when these cells are injected as mouse xenografts [55]. Genetic mouse models with endothelial cell-specific AKT1 over-activation also leads to vascular anomalies with enlarged vessels with increased permeability due to deficient mural cell coverage [56]. Importantly, human vascular tumours including infantile hemangioma, Kaposiform haemangioendotheliomas, and angiosarcomas display increased levels of active AKT (pAKT) compared to adjacent normal blood vessels [57,58]. At present, it is not clear if this is due to mutations in *AKT* or other upstream PI3K pathway components in these diseases. Gain-of-function mutations in the *AKT2* and *AKT3* isoforms have also been also found in human VMs [38].

Proteus syndrome, a complex disorder with overgrowth and malformations of multiple tissues [59], is caused by activating somatic *AKT1* mutations that are mosaic in the affected tissues [60]. A major manifestation of this syndrome is the presence of vascular malformations, either capillary, venous or/and lymphatic [59], highlighting the role of aberrantly active AKT1 in the development of such vascular anomalies.

Mammalian target of rapamycin (mTOR) is a Ser/Thr protein kinase (downstream of AKT) that coordinates important cellular processes including cell growth and survival. mTOR phosphorylates and activates the p70-S6 kinase (encoded by the *RPS6KB1* gene) which phosphorylates the S6 ribosomal protein. The phospho-form of S6 (referred to as pS6) is often used as a cellular marker of mTOR activation. Endothelial cells from vascular tumours and malformations are often immuno-positive for pS6, therefore mTOR is expected to be active in these pathologies [61-63]. However, it is important to stress that the S6 protein can also be phosphorylated upon MAPK pathway activation at sites that are also phosphorylated by p70-S6K [64], thus it is difficult to distinguish whether these vascular anomalies show PI3K or MAPK pathway activation, or both. Currently, it is unclear whether mTOR activation in the vascular anomalies analysed in these studies is the result of genetic mutation in PI3K or MAPK pathway components upstream of mTOR,

mTOR is negatively regulated by the tuberous sclerosis complex (TSC), encoded by the *TSC1* or *TSC2* genes. Mice with endothelial cell-specific deletion of *Tsc1*, a negative regulator of mTOR, develop lymphangiosarcomas, a type of angiosarcomas with lymphatic differentiation [63]. This aberrant activation of mTOR also leads to autocrine VEGF production and signalling, which contributes to the development and maintenance of these tumours. The human tuberous sclerosis syndrome is a multi-system genetic syndrome caused by inactivating mutations in either *TSC1* or *TSC2* genes, leading to constitutive mTOR activation. Among other clinical manifestations, TSC patients show growths of vascular tissue such as facial angiofibromas and kidney angiomyolipomas [65]. Glomuvenous malformations are inherited vascular anomalies formed by abnormal vascular smooth muscle cells, called “glomus cells” caused by genetic alterations in *glomulin* (*GLMN/FAP68*) [66]. These mutations lead to up-regulation of the p70-S6 kinase activity, leading to the hypothesis that *glomulin* may play a part in the mTOR pathway [67].

### **Clinical implications and therapeutic opportunities**

Over the last decade, the development of targeted therapies has gained pace, mainly in the cancer field, largely as a consequence of the increased genetic and molecular understanding of the pathologies involved in malignancy. Such molecular pathophysiology has been lacking in vascular anomalies, explaining the relative lack of exploitation of targeted therapies. Vascular tumours are currently usually treated by chemotherapy, however, there are now growing pharmacologic options based on the use of anti-angiogenic drugs used for cancer therapy, including VEGFR inhibitors [68]. Vascular malformations are usually treated by surgical excision, pulsed laser therapy and sclerotherapy [69], but these treatments have limited efficacy and risk of recurrence.

Empiric observations have also led to the use of specific drugs in some types of vascular tumours and malformations for which the underlying molecular mechanism of their therapeutic effect is unclear. An example is propranolol, a non-selective  $\beta$ -adrenergic blocker that is now the gold standard therapy for infantile haemangiomas [70]. To the best of our knowledge, propranolol has not been reported to have a therapeutic impact in other human vascular anomalies and showed no benefit in a pre-clinical mouse model of mutant *Pik3ca*-induced VMs [38].

Progress in the sensitivity and speed of DNA sequencing techniques has identified causative genetic alterations of many diseases, even when present at low allelic frequencies, as in the case of some vascular anomalies in which mutant endothelial cells comprise a small proportion of the total lesional cell mass. This approach can provide specific diagnoses that ultimately will lead to personalised therapies. Since many of the genetic alterations in vascular anomalies are also described in a range of malignancies, drugs that are already used in the clinic or in clinical trials could be repurposed for these diseases.

Specifically, the PI3K signalling pathway is a major drug target in cancer therapy, with many ongoing trials using pan- or isoform-selective PI3K inhibitors or dual PI3K/mTOR inhibitors [31,71]. In addition, the mTOR inhibitor rapamycin and its analogues are already approved for clinical use. These drugs were first used as immunosuppressive agents to

prevent transplant rejection, and are now employed for other indications including cancer, based on their anti-proliferative and anti-angiogenic effects [72].

Pre-clinical studies have shown that treatment of mice harbouring *Pik3ca*-mutant VMs with BYL719, a p110 $\alpha$ -selective inhibitor currently in phase III trials, leads to a striking decrease in the volume of the VMs by diminishing endothelial cell proliferation [38]. Rapamycin and its analogue everolimus are also effective in the treatment of *Pik3ca*-mutant VMs in mice [37,38]. Rapamycin significantly reduces endothelial cell proliferation and blocks the loss of mural cell coverage of the vasculature in *Pik3ca*-mutant postnatal retinas [37]. Pre-clinical studies in mice have reported that rapamycin also efficiently blocks *TEK*-mutant-driven VM growth, whereas a TIE2 tyrosine kinase inhibitor was not able to prevent lesion growth, probably because the compound used appears not to inhibit the mutant form of TIE2 [40]. Importantly, in a pilot study, rapamycin treatment of human patients with either *TEK*- or *PIK3CA*-mutant VMs led to significant clinical improvement [39,40]. Rapamycin and its analogues have also been demonstrated to be clinically effective in vascular anomalies in which the genetic cause has not been identified [73].

Side effects of systemic administration of rapamycin mainly result from its immunological impact, including mucositis, stomatitis, oral ulcers and infections, with others including headaches, hypertension, insulin resistance and anaemia [74]. PI3K inhibitors have also been reported to be associated with various toxicities in patients upon systemic administration [75]; drug dose reduction of PI3K/mTOR inhibitors in this clinical setting may reduce such side effects while maintaining efficacy. In addition, topical administration in creams could also be a suitable alternative for the treatment of vascular anomalies since many of these lesions are present in the skin or subcutaneous tissue. In line with this, topical administration of rapamycin-based cream has been reported to offer clinical benefit in cutaneous vascular anomalies [62,76-78]. Similar strategies might be applied for PI3K inhibitors, as supported by preclinical evidence using topically applied PI3K inhibitors in *Pik3ca*-driven VMs in mice [38].

Unfortunately, lesions appear to recur upon cessation of rapamycin treatment, suggesting the need for continued drug administration in this disease. Indeed, following cessation of rapamycin treatment, symptoms recurred in human patients with *TEK* or *PIK3CA*-mutant VMs [39,40]. It is expected that targeting early components in the PI3K signalling pathway such as the PI3K isoforms themselves, rather than inhibiting downstream elements such as mTOR, might be more effective at pathway inhibition and result in a greater therapeutic impact. However, it remains to be determined whether PI3K inhibitors will provide effective lesion shrinkage with short-term treatment or also require continuous administration like rapamycin.

In summary, there is growing evidence for PI3K pathway activation in the generation and maintenance of vascular anomalies. Developments and improvements in highly sensitive techniques for the detection of specific genetic alterations have led to enormous advances in identification and understanding of new mutations present in vascular anomalies, since they are often present at low allelic frequencies. It is anticipated that such analyses of vascular anomalies will lead to new genetic discoveries that will inform and direct future classification systems, allowing both more precise diagnoses and also, more importantly, development of

targeted therapies. PI3K pathway inhibitors are potentially promising as specific “personalised” therapies for certain vascular anomalies based on promising early results from pre-clinical and clinical studies. However, systemic administration of these drugs may be associated with toxicities, thus topical administration presents a likely sensible alternative for therapy of cutaneous vascular anomalies.

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### **Competing financial interests**

B.V. is consultant to Karus Therapeutics (Oxford, UK).

## Figure legends

**Figure 1. Photomicrographs demonstrating:** (A) Combined type vascular malformation with venous components composed of dysplastic and dilated veins (two selected marked 'V'; H&E, original magnification x10). (B) Lymphatic malformation composed of thin-walled dilated lymphatic channels containing lymph (two selected marked 'L'; H&E, original magnification x20). (C) Infantile type capillary haemangioma composed of numerous capillary sized, thin-walled vessels (two selected marked with arrows; H&E, original magnification x40). (D) Infantile type capillary haemangioma with GLUT1 immunostaining showing lesional endothelial positivity (two selected marked with arrows; original magnification x40).

**Figure 2: Currently reported alterations in PI3K pathway components in vascular anomalies, and possible points of pharmacological interference.** TIE2 and VEGFR2 also activate the MAPK pathway, which is not shown. PROS, *PIK3CA*-related overgrowth syndromes.

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**Table 1**

Current classification of vascular anomalies (simplified from [www.issva.org](http://www.issva.org)). Asterisks indicate vascular anomalies in which mutational activation of the PI3K pathway has been reported.

<b>Vascular tumours</b>	<b>Vascular malformations</b>			
	<b>Simple</b>	<b>Combined</b>	<b>Of major named vessels</b>	<b>Associated with other anomalies</b>
<i>Benign</i>				
<ul style="list-style-type: none"> <li>• Infantile type capillary haemangioma*</li> <li>• Congenital hemangioma</li> </ul>	<ul style="list-style-type: none"> <li>• Capillary malformations</li> <li>• Lymphatic malformations</li> </ul>	<ul style="list-style-type: none"> <li>• Capillary-venous malformation</li> <li>• Capillary-lymphatic malformation</li> </ul>	<ul style="list-style-type: none"> <li>• Lymphatics</li> <li>• Veins</li> <li>• Arteries</li> </ul>	<ul style="list-style-type: none"> <li>• Klippel-Trenaunay syndrome*</li> </ul>
<i>Borderline</i>				
<ul style="list-style-type: none"> <li>• Kaposiform hemangioendothelioma</li> </ul>	<ul style="list-style-type: none"> <li>• Venous malformations*</li> <li>• Arteriovenous malformations</li> </ul>	<ul style="list-style-type: none"> <li>• Lympho-venous malformation</li> </ul>		<ul style="list-style-type: none"> <li>• Sturge-Weber syndrome</li> <li>• Proteus syndrome*</li> </ul>
<i>Malignant</i>				
<ul style="list-style-type: none"> <li>• Angiosarcoma</li> </ul>		<ul style="list-style-type: none"> <li>• Other combination</li> </ul>		