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Molecular Pathways and Possible Therapies for Head and Neck Vascular Anomalies

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Abstract:

Vascular Anomalies are a heterogenous group of vascular lesions that can be divided, according to the International Society for the Study of Vascular Anomalies Classification, into two main groups : Vascular Tumors and Vascular Malformations. Vascular Malformations can be further subdivided into slow-flow and fast-flow malformations. This clinical and radiological classification allows for a better understanding of vascular anomalies and aims to offer a more precise final diagnosis. Correct diagnosis is essential to propose the best treatment, which traditionally consists of surgery, embolization or sclerotherapy. Since a few years, medical treatment has become an important part of multidisciplinary treatment. Genetic and molecular knowledge of vascular anomalies are increasing rapidly and opens the door for a molecular classification of vascular anomalies according to the underlying pathways involved. The main pathways seem to be: PI3K/AKT/mTOR (PIKopathies) and RAS/RAF/MEK/ERK (RASopathies). Knowing the underlying molecular cascades allows us to use targeted medical therapies. The first part of this article aims to review the vascular anomalies seen in the head and neck region and their underlying molecular causes and involved pathways. The second part will propose an overview of the available targeted therapies based on the affected molecular cascade. This article summarizes theragnostic treatments available in vascular anomalies.

Keywords:

Vascular Anomalies - PI3K/AKT/mTOR - RAS/RAF/MEK/ERK - PIKopathies – RASopathies – theragnostic treatment

Introduction

Vascular anomalies are a heterogeneous group of pathologies involving the vascular system. They have been classified, according to the International Society for the Study of Vascular Anomalies (ISSVA) Classification[1], in vascular tumors and vascular malformations. Vascular malformations can be further subcategorized into slow-flow and fast-flow malformations. Vascular anomaliess are localized in approximately 60% of the time in the head and neck region. They occur in about 1 out of 22 children[2]. Their correct diagnosis is crucial to select the appropriate treatments, which classically consist of surgery, sclerotherapy, embolization, laser ablation or a combination of those. Treatments are rarely curative for extensive malformations.

Vascular anomalies are caused by germline or somatic mutations. The latter mostly occur in vascular endothelial cells (ECs). Most of the identified mutations are implicated in signaling that regulates angiogenesis, apoptosis, proliferation and/or vascular cell growth. Similar mutations are also often found in cancers[3]. The evolution of targeted therapies in cancer and the constant increase of our knowledge in the pathophysiology of vascular anomalies, open new therapeutic possibilities to manage these chronic and debilitating pathologies.

Genetic mutations and signaling pathways in vascular anomalies

We review the most frequent vascular lesions in the head and neck region regarding the known genetic mutations and involved pathways.

Vascular tumors

Congenital hemangiomas

Congenital hemangiomas (CHs) are rare pediatric tumors present at birth (Figure 1a). They present as a raised, gray-violaceous solitary cutaneous mass with fine telangiectasias and ectatic veins [4]. As opposed to infantile hemangiomas (IHs), the lesions are fully grown at birth and do not express GLUT-1[5]. Doppler Ultrasound can detect fast circulating blood flow. CHs present in three forms: Rapidly Involuting CH (RICH), Non-Involuting CH (NICH) and Partially Involuting CH (PICH). RICHs show rapid regression by 6–14 months, often leaving a patch of subcutaneous atrophy and dilated veins[4]. NICH stay stable after birth and do not regress. PICH, as their description states, partially regresses after birth.

Missense mutations in *GNAQ* and *GNA11* have been found in CH[4]. *GNAQ* and *GNA11* encode for proteins that are 90% similar to each other, with a guanine nucleotide binding protein(q) alpha subunit that hydrolyzes intracellular messenger GTP to GDP[4]. The missense mutations probably activate GTP-dependent signaling leading to constitutive activation of MAPK and/or YAP signaling. The same mutations have been found in RICH and NICH suggesting that other phenomena influence the postnatal behavior[4].

Infantile hemangiomas

IHs are the most frequent benign pediatric tumors and occur in about 4-10 % of newborns[5] (Figure 1b, 1c and 2). They usually appear during the first two weeks of life initially as a red spot, which quickly increases in size during the first three months. Following this proliferating phase, their growth stabilizes and spontaneous involution pursues from 2 to 6 years of age. Half

of IHs will leave significant sequalae after involution[6]. Around 10% of IHs will require treatment due to their size, location and/or ulceration.

The precise underlining mechanism for the aberrant behavior of ECs in IH remains unknown. Their origin, although still under debate, has been hypothesized to be due to embolic placental angioblasts or intrinsic endothelial progenitor cells with the ability to clonally duplicate[7]. The placental origin is supported be the placental markers found on IH : GLUT-1, Lewis Y antigen, merosin and Fcγ[7]. Another theory involves genetic mutations and decreased expression of VEGFR1 due to downregulation of NFAT. Variants found in *VEGFR2* and *TEM8* encode for mutated transmembrane proteins that sequester ß-integrin, decrease ß-integrin activity and NFAT transcriptional function, leading to decreased VEGFR1 expression [8]. With decreased VEGFR1, VEGF can bind to VEGFR2 which results in increased proliferation of ECs due to activated downstream signaling[2].

PHACE(S) syndrome

Patients presenting a large facial segmental IH (Figure 3a) must be screened for associated anomalies. Association of Posterior fossa brain malformations, segmental facial Hemangiomas, Arterial anomalies, Cardiac defects, Eye anomalies, and Sternal clefting or supraumbilical raphe is called PHACE(S) syndrome[9]. A third of patients with a large facial IH are diagnosed with PHACE(S) syndrome with the extent of the facial IH correlating with the number of affected organs with anomalies[10].

Considering the sternal anomalies that are observed, it is hypothesized that this syndrome starts in utero around 6 to 9 weeks of gestation[11]. The exact cause is not known. Skewed X-inactivation may be playing a role since there is a female predominance in this syndrome[12]. Copy number variations of specific genes could be another explanation[13].

Tufted angiomas and Kaposiform hemangioendotheliomas

Tufted angiomas (TAs) (Figure 3b) and Kaposiform hemangioendotheliomas (KHEs) (Figure 3c) are a spectrum of locally aggressive vascular tumors [14]. TAs appear as a solitary tumor or as a plane skin coloration, usually red or violaceous, whereas KHE is detected during the neonatal period in 60% of cases[14]. They regress partially after 2-6 years of age. Half of the patients with KHE develop Kasabach-Merritt phenomenon (KMP), characterized by a severe thrombocytopenia due to platelet entrapment in the lesion.

Three genes with a somatic mutation in some patients have so far been reported: *GNA14* in both TA and KHE[15], *NRAS* in a TA and *RAD50* in a KHE[16]. *RAD50* encodes for a DNA double-strand break repair protein. GNA14 belongs to the same GTPase family as GNAQ and GNA11. *NRAS* encodes a small GTPase which regulates cell proliferation. These data suggest that the RAS/RAF/MEK/ERK signaling pathway is activated in TA and KHE.

Pyogenic granulomas

Pyogenic granulomas (PGs) are acquired vascular lesions that often appear after trauma. They present as small solitary red lesions and tend to ulcerate and bleed (Figure 4a)[14]. Secondary PG are often seen above a capillary malformation (CM). They can shrink after healing of an ulceration but recurrence is frequent.

BRAF, NRAS and *KRAS* mutations have been found in primary and secondary PG[17]. Somatic *GNAQ* mutations have been found in secondary PG, causing a hyperactivation of the RAS/RAF/MEK/ERK pathway. Some secondary PG show mutations in *GNAQ* and *BRAF*. It

has been hypothesized that the *BRAF* mutations are a second hit, responsible of developing a PG, on top of an underlying CM caused by a *GNAQ* mutation[17].

Vascular malformations

Capillary malformations

CMs, or port-wine stains, are homogeneous red or violaceous congenital lesions (Figure 4b). They tend to darken over time and can acquire a cobblestone-like appearance (Figure 5a). They are frequently associated with other vascular anomalies or part of a more complex syndrome[5] Sporadic CM as well as classic Sturge-Weber Syndrome (SWS) are frequently associated with somatic *GNAQ* mutations [18-20]. The most common one is Arg183Cys mutation, which differs from the one identified in congenital hemangiomas[2]. Although they all result in activation of the RAS/RAF/MEK/ERK pathway, the ones seen in CMs seem to cause a more moderate activation than those in CHsendothe[19]. In one patient, a somatic *GNB2* mutation was reported[21]. Mutations in *GNA11* have been identified in CM of the extremity associated with overgrowth[22] as well as in atypical Sturge Weber syndrome, suggested to be called GNA11-SWS (Dompmartin et al, *under revision*).

Atypical CM can be associated in 30% of cases with arteriovenous malformations (AVMs) grouped under the name CM-AVM. These CMs are commonly small and multiple, disseminated all over the body with often a pale surrounding pathognomonic halo. CM-AVM1 is associated with mutations in *RASA1*[23]. Most mutations are germline (75%) and need a somatic second hit, but mosaic mutations have also been found[24]. All mutations cause loss of function. CM-AVM2 is associated with germline loss-of-function mutations in *EPHB4*[25].

RASA1 and *EPHB4* encode a membrane (p120RasGAP) and transmembrane protein (EPHB4) respectively, found in ECs [26]. Activation of the proteins result in RAS inactivation and reduced signaling via the RAS/RAF/MEK/ERK pathway. Hence, the loss-of-function mutations seen in CM-AVMs cause an activation of the downstream pathway.

CM of the lower lip in combination with head or neck LM, asymmetry and partial or generalized overgrowth are seen in CLAPO syndrome. PIK3CA missense mutations have been described resulting in an activation of the PI3K/AKT/mTOR pathway, described below [27, 28].

Lymphatic malformations

(LMs can manifest as microcystic (Figure 5b), macrocystic (Figure 6) or combined lesions. In the head and neck area, supra-hyoid LMs tend to be microcystic, as opposed to infra-hyoid LMs. Eighty percent of LMs are associated with activating mutations in PIK3CA, which encodes the p110*alpha* catalytic subunit of PI3K [29]. Genotype/phenotype correlations within LMs have been suggested. In one study, PIK3CA non-hotspot mutations appeared to be more frequent in head or neck region and in macrocystic LMs[30]. However, this was not seen in another study, which demonstrated non-hotspot mutations to be more frequent in syndromic forms (KTS and CLOVES) then in isolated LMs [31]. These differences could be due to disparities in cohorts and mutation classification. Variation in mutated allele fraction also correlated with the extent of the phenotype. A higher proportion of the mutated allele was found in more wide-spread phenotypes and syndromes[31]. This fits with non-hotspot mutations being more compatible with life even if affecting wider areas.

The timing during development of occurrence of the mutation also seems to determine the subtype of LM. In a murine model, activation of the mutated *PIK3CA* gene in early development was associated with macrocystic lesions. When the mutated gene was activated during late embryonic development or in the neonatal period, it caused microcystic lesions[32].

These gain of function mutations activate the P3K/AKT/mTOR pathway, involved in apoptosis, cell proliferation and growth, migration and angiogenesis.

Complex lymphatic anomalies (CLA) are multifocal and/or affect central lymphatic channels. They consist of Gorham-Stout disease (GSD), kaposiform lymphangiomatosis (KLA), generalized lymphatic anomalies (GLA) and central conducting lymphatic anomalies (CCLA). CLAs can be caused by mutations in *PIK3CA* but also in proteins implicated in the RAS/RAF/MEK/ERK pathway[29]. Mutations in *NRAS*[33, 34], *KRAS*[35, 36] and *ARAF*[37], have been associated with KLA, GSD and CCLA, respectively. All mutations hyperactivate the RAS/RAF/MEK/ERK pathway.

Venous malformations

Venous malformations (VMs) are congenital lesions, but may only become visible later in life (Figure 7a and 7b). They present as bluish compressible lesions, sometimes containing phlebolites, hard calcified nodules. They can expand during Valsalva's maneuver and their volume can increase over time. VMs can be associated with chronic consumptive coagulopathy (also called localized intravascular coagulopathy, LIC), reflected by high levels of D-Dimers and low fibrinogen[38].

Half of VMs have a somatic mutation in the *TEK* gene[2, 38, 39]. Up to 75% of mutations code for a L914F substitution[39, 40]. In the familial form (VMCM), a somatic second-hit is needed on top of the weakly activating germline *TEK* mutations [40]. Blue Rubber Bleb Naevus syndrome (Bean Syndrome, BRBN), a syndrome consisting of one usually large VM associated with multiple smaller VMs, typically in the gastrointestinal tract, is commonly caused by a combination of two mutations on one allele of the *TEK* gene. The same mutations are found in all the different lesions. Multiple sporadic VMs, patients who have multiple muco-cutaneous

VMs, but rarely in the digestive tract, are also caused by double mutations on the same allele, on the *TEK* gene. One of the mutations can be found in mosaic state in the blood, whereas the second one occurs later on the same allele. These two clinically different entities indicate a genotype-phenotype correlation [41].

TEK encodes for TIE2, a transmembrane tyrosine kinase receptor upstream of the PI3K/AKT/mTOR pathway. Mutations cause a hyperphosphorylation of TIE2 resulting in a constitutive activation of the downstream pathway[42].

Mutations in *PIK3CA*, coding for the p110*alpha* subunit of PI3K, are found in 20% of VMs[43]. They also cause hyperactivation of the PI3K/AKT/mTOR pathway[3]. PIK3CA mutated VMs tend not to involve the skin[43].

Arteriovenous malformations

Arteriovenous malformations (AVM) are rare congenital vascular malformations consisting of a direct communication between arteries and veins without interposition of capillaries[44] (Figure 7c). The abnormal communication can be through an arteriovenous fistula or a vessel entanglement called nidus. They tend to aggravate over time with invasion of adjacent tissues[45]. They can be (i) syndromic or (ii) sporadic.

Syndromic AVMs are encountered in Hereditary Hemorrhagic Telangiectasia (HHT), CMs associated with AVMs (CM-AVM1 and -2), Parkes Weber Syndrome (PWS) and PTEN-Hamartoma Tumor Syndrome (PHTS). HHT commonly presents as recurrent epistaxis, mucocutaneous telangiectasias and visceral AVMs[46]. It is caused by an inherited mutation in *ENG* (HHT-1), *ACVRL1* (HHT-2) or S*MAD4* (Juvenile Polyposis/HHT overlap syndrome, JPHT)[46]. These genes encode for endoglin, ALK1 and SMAD4, respectively, proteins involved in the TGF-ß signaling pathway. The known mutations cause loss of function and a

deregulation of this angiogenic pathway [47]. PHTS include patients suffering from the spectrum including Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. Up to half of the PHTS patients have an AVM[48] associated with tissue overgrowth, gastrointestinal polyps and/or macrocephaly[42]. The *PTEN* (phosphatase and tensin homologue) gene encodes for a tumor suppressor that regulates the PI3K-pathway [48].

Sporadic AVMs have been associated with mutations in genes, such as *MAP2K1*[49, 50], *BRAF*[50], *HRAS*[51] and *KRAS*[50, 52]. All mutations seem to cause an activation of the RAS/RAF/MEK/ERK pathway resulting in increased phosphorylation of ERK[51]. This pathway plays an important role in cell proliferation, differentiation, apoptosis, migration and stress response[3, 53].

New theragnostic developments

Vascular anomaly pathways

Looking at all the known genetic mutations found in vascular anomalies, a hyperactivation in two major molecular pathways can be observed: the PI3K/AKT/mTOR pathway (PIKopathies) in slow-flow malformations and the RAS/RAF/MEK/ERK pathway (RASopathies) in fast-flow malformations and CCLAs (Table 1). These two pathways communicate with each other complicating disease management (described in detail by Queisser at al. in 2021[3]) and yet have opened the door for theragnostic treatments[54] (Figure 8).

VEGF and VEGFR2 inhibitors

Thalidomide

Thalidomide is able to reduce the expression of VEGF, stimulate the proliferation of lymphocytes and downregulate expression of adhesion molecules[55]. It reduced epistaxis and corrected hemoglobin levels in HHT patients [56, 57]. A prospective study was conducted on 18 patients with severe symptoms related to isolated/non-syndromic AVMs with good clinical and radiological response (*Boon LM et al. 2022, Nat Cardiovasc Res, in press*). Out of the 18 patients, all experienced pain reduction, cessation of bleedings and healing of ulcerations. Main side effects were fatigue and peripheral neuropathy. Both appeared to be dose dependent and reversible after treatment cessation. A larger prospective trial is to be established.

Bevacizumab

Bevacizumab is a humanized monoclonal antibody that binds to VEGF-A and prevents VEGFR2 activation[58]. It was efficacious in reducing bleeding in HHT patients [59] but its use in other vascular anomalies is limited. No radiological effect was seen on brain AVMs, in a proof-of-concept trial with only two patients included[60]. Further studies are needed to prove its efficacy on vascular anomalies, but research is limited by the price (and side effects) of this humanized antibody.

Pazopanib

Pazopanib is a tyrosine kinase inhibitor that blocks VEGF, PDGF and c-kit receptors[61]. It was shown to reduce epistaxis in a study on 7 seven HHT patients. It did not cause severe adverse effects[62]. Further prospective trials are thus needed to clarify indications.

PI3K/AKT/mTOR inhibitors

Alpelisib

Alpelisib is a selective PI3Kalpha subunit inhibitor[63]. Its good tolerance in cancer patients has motivated its use in vascular anomalies, mostly in PIK3CA related overgrowth syndromes (PROS)[64]. A study with 19 patients showed a good clinical response without causing frequent major side effects[65]. Induced type II diabetes is the most worrisome side effect. Prospective trials need to be performed to establish the safety profile in long term use for vascular anomalies. An accelerated FDA review led to approval in April 2022 for the use of Alpelisib for PROS in the USA.

Miransertib

Miransertib is a selective AKT inhibitor [64], which was shown to improve clinical manifestations and symptoms in two children with PROS[66]. In an open-label study on 15 PROS patients, it showed radiological stability and pain reduction[67].

Rapamycin (Sirolimus)

Rapamycin is an mTOR inhibitor initially used in organ transplantation on long term. Its safety has been largely demonstrated and it is being used in clinical trials and off-label to treat slow-flow vascular anomalies [68, 69]. Prospective clinical studies have demonstrated its efficacy on venous and LMs[70-72]. A phase III clinical trial is ongoing on 250 patients with slow-flow vascular malformations (VASE; EudraCT Number: 2015-001703-32) (Seront et al, *in*

preparation). Preliminary results showed a general improvement rate of 89% on pain and functional limitations due to the malformation[68]. Side effects were mild and reversible. The main complaint was mouth ulcerations. Rapamycin has become the gold standard for medical treatment of slow-flow vascular anomalies, even if it has not yet been approved by EMA or FDA.

RAS/RAF/MEK/ERK inhibitors

Trametinib

Trametinib is a MEK inhibitor used in combination with dabrafenib, a BRAF inhibitor, to treat metastatic BRAF mutated melanomas[73]. It has since been tested to treat a few patients with a generalized lymphatic anomaly (GLA)[36, 37, 74], HHT [75] or AVM[76, 77] with encouraging results. A phase II clinical trial was started in 2019 including 10 adult patients with extracranial AVMs (TRAMAV; EudraCT number: 2019-003573-26). Preliminary results show a reduction in pain in all 10 patients and in clinical volume in 9 patients. All developed acne that appears to be dose related (Coulie J et al., *in preparation*).

Vemurafenib

Vemurafenib is a BRAF inhibitor and mostly used to treat metastatic melanoma[78]. It's utility in vascular anomalies was suggested by a *BRAF-V600E* mutated zebrafish model with blood flow restoration [50]. No result of human studies treating vascular anomalies have been reported.

Miscellaneous treatments

Propranolol

Propranolol is a non-selective beta-blocker used to treat IH. Its safety and ease of use have been largely demonstrated[79]. The precise underlying mechanism of action is partially unknown. Propranolol is a 1:1 mixture of R(+) and S(-) enantiomers. It appears that the R(+) enantiomer inhibits differentiation of hemangioma stem cells into hemangioma endothelial cells, through a ß-adrenergic independent effect, inhibiting the dimer formation of factor sex-determining region Y box transcription factor 18 (SOX18)[80, 81]. The use of propranolol reduces the need for surgical intervention, which is still part of the management of IH (Coulie J et al. *in preparation*).

Conclusion

Vascular anomalies are chronic pathologies with an important impact on quality of life. Their management is multimodal and multidisciplinary. Theragnostic management is a new tool for vascular anomalies and offers new possibilities. It is probably not realistic to imagine that medication only will cure patients, but it will become part of their treatment. Further research is needed to better correlate genotypes and phenotypes to be able to offer the best combinations of medical and invasive treatments to these patients.

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Tables & legends:

Table 1 : Vascular Anomalies, known mutations and affected pathways.

Figures & legends :

Figure 1 : (1a)Retro-auricular NICH. (1b) IH of the left upper lip. (1c) IH of the right nostril.

Figure 2 : IH of the nosetip.

Figure 3: (3a) Large segmental IH of the lower face in a PHACES syndrome. (3b) Left pre-auricular TA. (3c) Right cervical KHE.

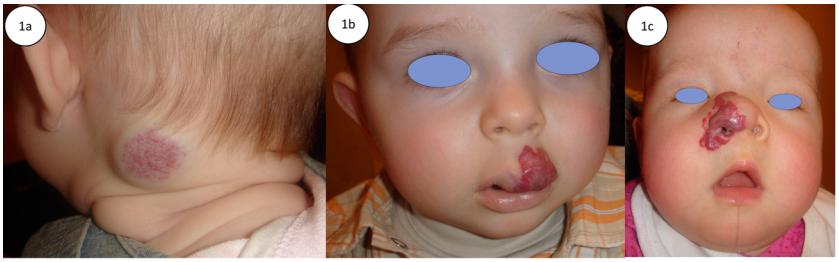
Figure 4a : (4a) PG of the right cheek. (4b) CM of the left cheek.

Figure 5 : (5a) CM of the right face and neck. See how the color tends to darkens over time. (5b) Microcystic LM of the tongue.

Figure 6 : (6a) Macrocystic LM showing only as a cervical swelling appeared in a few days during a upper airway infection. (6b) MRI of the same patient showing a much bigger LM then expected with axillary involvement and liquid-liquid levels visible inside some cysts, indicating a recent intracystic bleeding. This explained the sudden clinical swelling and resulted in a lateral upper airway deviation.

Figure 7 : (7a) Extensive facial VM. (7b) VM of the tongue. (7c) AVM of the central face.

Figure 8 : Figure showing the two main molecular pathways involved in Vascular Anomalies and were available medication intervenes.



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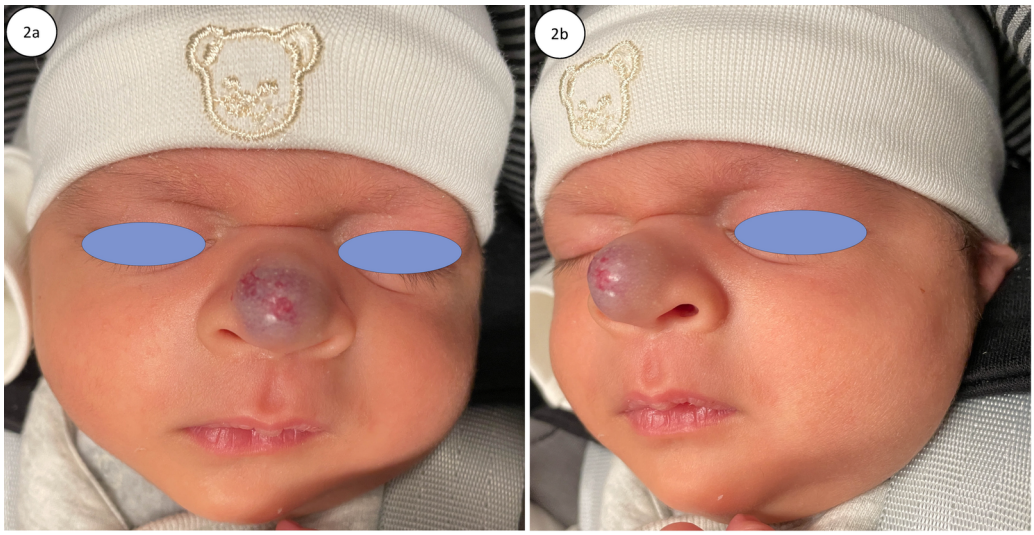

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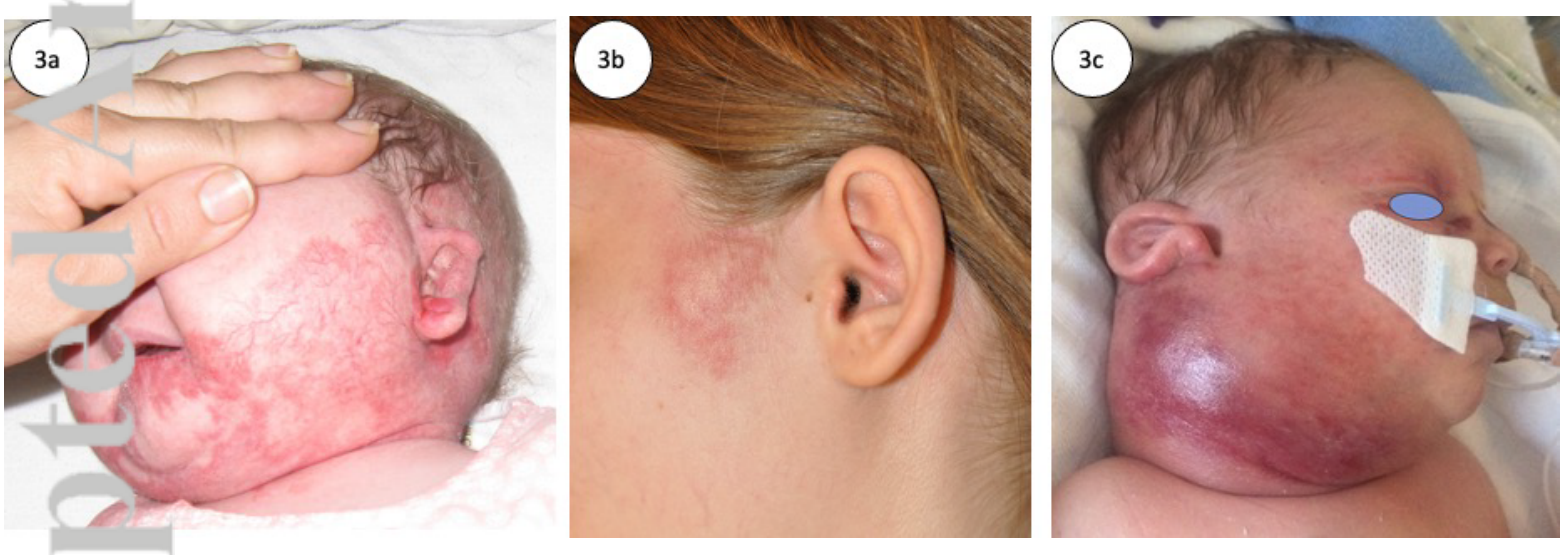

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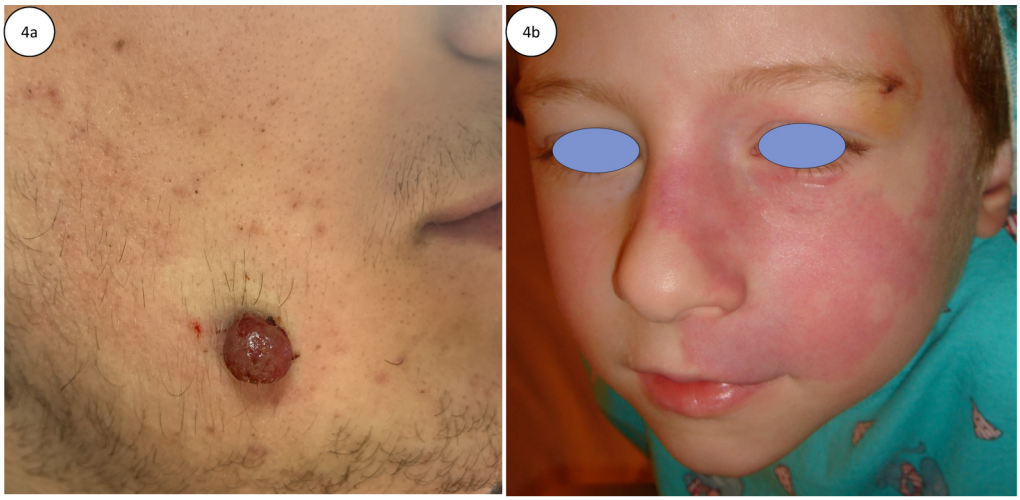

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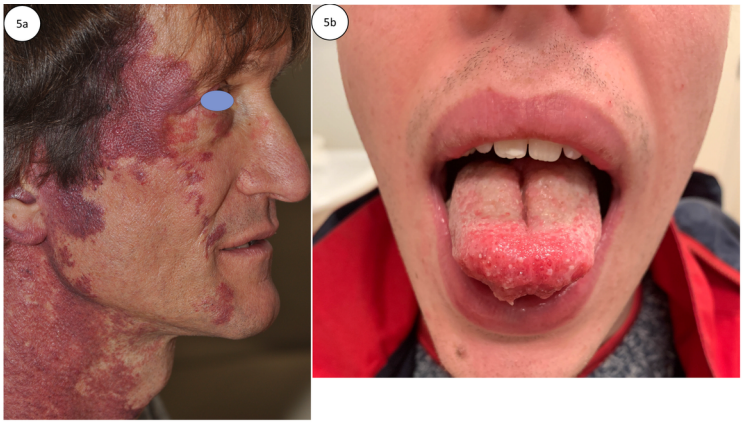

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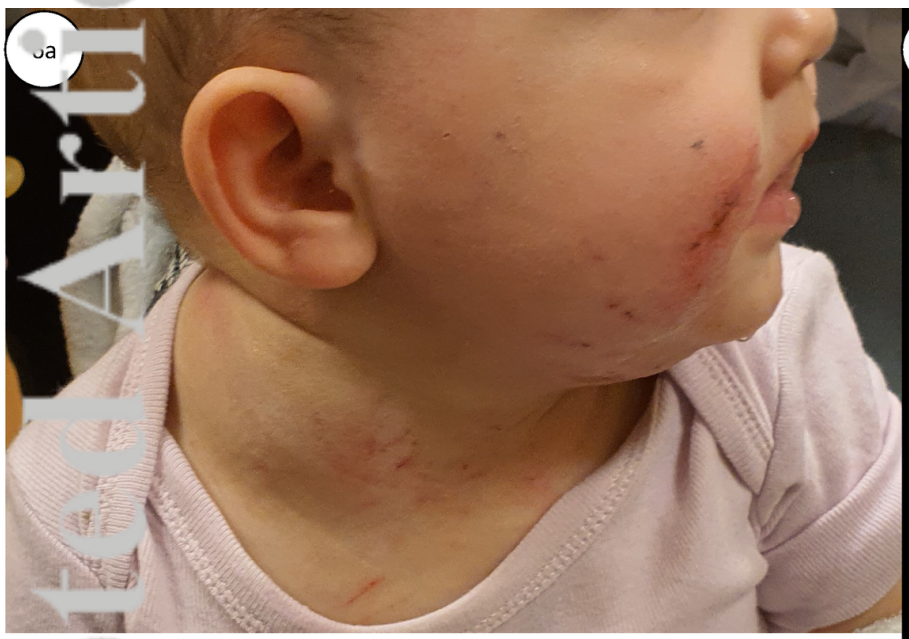

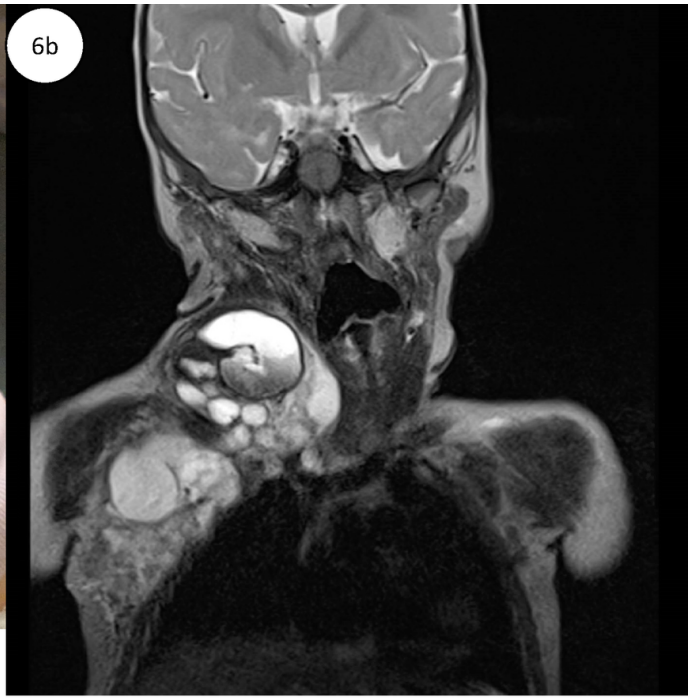

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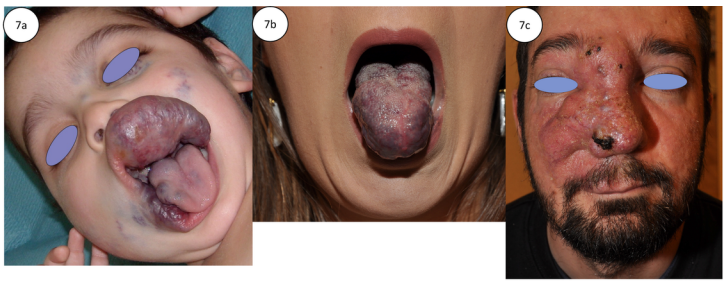

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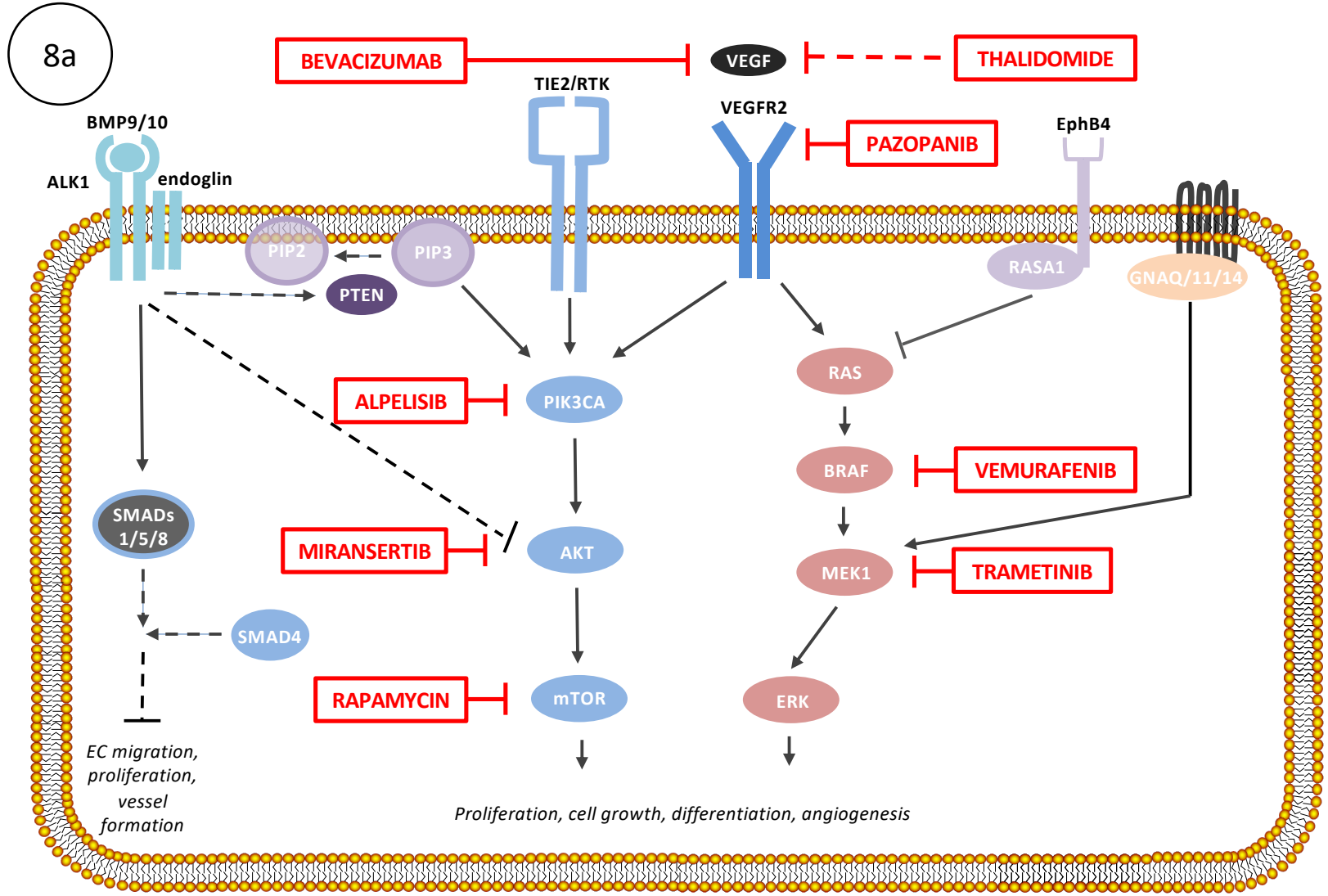

Vascular Anomaly	*Specific syndromes or subtypes*	*Known mutations*	*Affected pathways*
Vascular Tumor			
Congenital hemangioma		*GNAQ*	RAS/RAF/ERK/MEK
		GNA11	
Infantile hemangioma		*VEGFR2*	VEGFR1 & VEGFR2
		TEM8	
Tufted angioma		*GNA14*	RAS/RAF/ERK/MEK
		NRAS	
Kaposiform hemangioendothelioma		*GNA14*	
		RAD50	double strand break repair protein
Pyogenic granuloma		*BRAF*	RAS/RAF/ERK/MEK
		RAS	
Vascular Malformation			
Capillary malformation		*GNAQ*	RAS/RAF/ERK/MEK
		GNA11	
	Sturge-Weber syndrome	*GNAQ*	
	CM-AVM type 1	*RASA1*	
	CM-AVM type 2	*EPHB4*	
	CLAPO syndrome	*PIK3CA*	PIK3CA/AKT/mTOR
Lymphatic malformation		*PIK3CA*	
	Gorham-Stout disease (GSD)	*KRAS*	RAS/RAF/ERK/MEK
	Kaposiform lymphangiomatosis (KLA)	*NRAS*	
	Central conducting lymphatic anomaly (CCLA)	*ARAF*	
	Generalized Lymphatic Anomaly (GLA)	*PIK3CA*	PIK3CA/AKT/mTOR

Venous malformation		*TEK*	PIK3CA/AKT/mTOR
		PIK3CA	
Arteriovenous malformation	Sporadic	*MAP2K1*	RAS/RAF/ERK/MEK
		BRAF	
		HRAS	
		KRAS	
	Hereditary hemorrhagic telangiectasia (HHT) type 1	*ENG*	TGF-ß
	Hereditary hemorrhagic telangiectasia (HHT) type 2	*ACVRL1*	
	Juvenile polyposis/HHT overlap syndrome	*SMAD4*	
	PTEN associated hamartoma and tumor syndrome (PHTS)	*PTEN*	RAS/RAF/ERK/MEK