2 Pathogenesis, Genetics, and Molecular Developments in Vascular Lesion Therapy and Diagnosis

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Key Points

- Genomic research, Next-generation sequencing, and disease-specific therapeutic options to treat vascular lesions are the future due to genetic testing. Exploring genetic mutations of inherited or somatic types has opened up an understanding of the underlying molecular mechanisms.
- RAS/MAPK/ERK altered signaling due to mutations in GNAQ and GNA11 in congenital hemangiomas and capillary malformation (CM), in GNAQ, KRAS and BRAF in pyogenic granuloma (PG), in RASA1 in CM-AVM1, in EPHB4 in CM-AVM2, in KRIT1 in HCCVM, and MAP3K3 in verrucous venous malformations have established for the therapeutics.
- PI3K/AKT/mTOR pathway regulation alterations in hereditary hemorrhagic telangiectasia (HHT) are noted with mutations in BMP9/10, ALK1, and endoglin, in sporadic venous malformations (VM and MVM). Blue rubber bleb nevus syndrome (BRBN), inherited cutaneous-mucosal venous malformations (VMCM), all with mutations in TIE2, and in sporadic VM and lymphatic malformations (LM) with mutations in PIK3CA.
- Glomuvenous malformations (GVM)-have mutations in glomulin with a hyperactive hepatocyte growth factor/c-Met and TGF-ß is signaling.
- Infantile hemangioma (IH)- VEGFR1 expression is downregulated; VEGF-A/VEGFR2 signaling is upregulated, with mutations in TEM8 and VEGFR2.
- Acquired vascular lesions—paraganglioma of neural crest origin—APUD—DNES cells—cells—located in extra-adrenal locations like the carotid, vagus, orbit, tympanic, larynx, jugular, thyroid, nasal cavity, aortic arch, intracranial, etc. that are mostly benign.

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2.1 Introduction

Vascular lesions have been better understood recently with pathogenesis, mutational, and immune-expression with advances in immunohistology-chemical staining as well as genetics and mutational defects.

2.2 Pathogenesis

Blood vessels form consolidated aberrations in limited anatomic sites of the human body. They result in sporadic anomalies or hereditary occurrences. The classification helps differentiate the anomalies based on their morphology, histology, and dynamics of blood flow. The primary differentiating features divide these lesions into Vascular neoplasms and vascular malformations.

Histologically the vascular lumen is lined with young endothelial cells (Fig. 2.1). These are underlined by smooth muscle cells and pericytes that are more seen in a vascular network than lymphatic channels [1].

The formation of the blood vessel network begins with vasculogenesis. Hemangioblasts are the precursor cells forming an island of endothelial cells. The peripheral cells in the island differentiate into primordial endothelium and core cells as precursors of blood cells [2].

The primary capillaries are formed from tubular structures made of differentiated endothelial cells.

Arteries, veins, and capillaries are developed from the maturation and remodeling of the primary capillary plexus. This phenomenon is referred to as Angiogenesis. This represents the sprouting of newer capillaries, formation of transcapillary connections, and maturation of the vascular network [4].

Non-perfused blood vessels disappear, and the rest further modify and strengthen to form the mature vascular network.

Lymphangiogenesis follows angiogenesis and arises from endothelial cells. Theory that lymph vessels derive from lymphangioblasts and not vascular endothelial cells do exist. Various angiogenic and anti-angiogenic factors influence the formation of both vascular and lymphatic networks. Therefore, the occurrence of vascular anomalies may be consequent to their disharmonious behavior.

Hemangiomas are vascular neoplasms that have different phases of development. They go

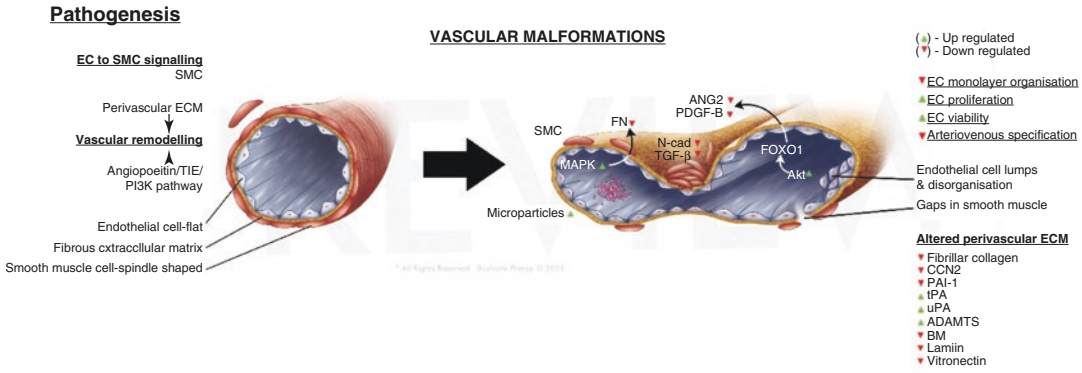

Fig. 2.1 Normal vascular lumen lined with endothelial cells with normal radiation, surrounding fibrosis extracellular matrix, and spindle-shaped smooth muscle cell layer. *SMC* Smooth muscle cell, *EC* Endothelial cell, *ECM* Extracellular matrix; Somatic mutations causing 'gain of function' in the tyrosine kinase receptor of -ANG (Angiopoietin) type, TIE and PIK3CA encoding genes activated cell signaling pathway leading to vascular malformations. In experimental mouse models of angiogenesis ANG/TIE/PIK3CA cause cell signaling and perivascular extracellular matrix remodeling, degradation, etc. Adapted from Kangas J, Nätynki M, Eklund L. Development of Molecular Therapies for Venous Malformations. Basic Clin Pharmacol Toxicol. 2018 Sep;123 Suppl 5:6–19. https://doi.org/10.1111/bcpt.13027. Epub 2018 May 29. PMID: 29668117

through the proliferative phase, quiescent phase, and finally involutory phase. Histologically they are characterized by the proliferation of plump endothelial cells with mast cell infiltration [5, 6]. The latter phases show flattened endothelial cells. Infantile hemangiomas show increased expression of glucose transporter-1 (GLUT-1).

Typically, most hemangiomas may not be visible at birth but present with telltale signs such as a pigmentation, a macule, or papular eruption. They attain prominence in the first month or two. Proliferation then occurs with a rapid enhancement of its clinical appearance. The phases to follow are quiescence and involution. The cellular activity during involution shows the reduction in angiogenesis with apoptosis of endothelial cells. The vascular channels now are replaced with fibrofatty tissue. Congenital hemangiomas in contrast are fully mature at birth and do not show a proliferative phase.

Pathogenesis is a complex interaction of genetic and environmental factors. The role of HPV-8 infection, chronic villus sampling, and hormonal, especially estrogen disturbances with possible hypoxia causing endothelial cell proliferation is another theory [4–6]. Multiple angiogenic factors play a role in proliferating hemangiomas in contrast with VEGFR1 being down regulated [8]. The role and efficacy of beta 2 blockers in the management of proliferating hemangiomas are documented [9–11]. Its effect on the VEGF, fibroblast growth factor, and matrix metalloproteinase are thought to be the method of action. Vasoconstriction and apoptosis of the capillary endothelial cells occur consequently.

Vascular Malformations are the result of errors in angiogenesis. The commonest among them is venous malformation. Depending on the involved vascular subunit, they may be capillary, arterial, venous, lymphatic, or a combination of these resulting in artery venous malformation or lympho-venous malformation. The differentiation can be picked up with an ultrasound doppler augmented with an MRA.

Venous malformations are thin-walled, and dilated channels with normal endothelial cell lining occurring on the skin or subcutaneous (Type 1/Type 2) (refer authors classification [11]). The VM vary in their clinical presentation. From small blush discolored, soft compressible swellings in the head and neck region to large vascular spaces invading deeper anatomic spaces in the head and neck region. They gradually expand over a period with enhancement associated with hormonal changes or trauma. Mostly isolated in appearance and rarely multiple manifestations occur. The lesions enhance with dependent positions like lying down or Valsalva maneuver. The increasingly static nature of flow causes the formation of phleboliths which are easily palpable as hard masses and result in pain. The plane radiographs or an US easily demonstrate the phlebolith. Local intravascular coagulation (LIC) shows a rise in D-dimer level in the presence of normal fibrinogen. The VM is often present unilateral and result in pressure on the surrounding structures. Skeletal deformity or hypo-plastic muscular tissue may be seen. Those within the orbital cavity cause exophthalmos globe as well as orbital dystopia. Intraorally large VMs of the tongue and floor of the mouth deform mandible or maxilla resulting in malocclusion of the teeth (see clinical figures in Chaps. 1, 3 & 5). Speech impairment and impairment of functions such as swallowing, respiration may be compromised. Histologically the venous channels are dilated with a lack of smooth muscle cells around the endothelial cells which can be demonstrated with IHC markers (Fig. 2.1). Thus, defective recruitment of SMC with lack of proliferation of EC probably result in dilation of the venous spaces. The molecular basis of the development of the anomaly is discussed later in this chapter.

Capillary Malformations because of their appearance are referred to as port-wine stains. They occur in 0.3 of newborns as flat, reddish, or deep red patches in the head and neck [1–9]. The histological picture is that of a dilated capillary network which is more in number. The primitive capillary plexus fails to mature or modify resulting in normally appearing endothelial cells on the vessel wall.

The significant reduction in innervation may be the cause of capillary vessel wall dilatation and resultant abnormal blood flow pattern. VEGF secreted by the cutaneous nerves reduces, leading

to an abnormality in the capillary network. CM occurs on the face distributed along the Trigeminal nerve dermatomes [12–14]. The presence of Sturge Weber syndrome must be ruled out. The maxilla, or mandible and the skin or gingiva may overgrow and appear deep red or purple in appearance as age advances.

Arterial Malformations and Arteriovenous Malformations are anomalies with absent or imperfect capillary intermediaries between arteries and veins. The presence of a 'nidus' which is a dilated vascular sac replaces the capillary network of vessels. This dilated sac acts as a pressure break between the arterial and venous systems and is comparable to a cardiac chamber. Transmitted pulsations from the arterial system is palpable over the nidus. Local warmth and increased coloration over the swelling are other features. Large AVMs show the phenomenon of vascular steal resulting in high output cardiac failure. Schobinnger [18] in 1990 has staged the AVMs clinically into [Ref. 18]:

Stage I (quiescence): Discolored skin with arteriovenous shunting demonstrated with an US doppler.
Stage II (expansion): Like stage I with pulsatile enlargement.
Stage III: Stage II with ulceration, necrosis, bleeding, and pain of overlying skin.
Stage IV: Presence of cardiac failure.

Histologically the endothelial cells are proliferated in the absence of sensitivity to inhibitory cytokines (IL-1 B, TNF- alpha, TGF- Beta, and interferon- Gama [4, 5, 7]. Their sporadic appearance, therefore, contradicts any genetic influence.

Lymphatic malformations are also common in the head and neck and extremities. Their development is unknown. Clinically they appear as venous malformations without the discolouration [19]. The spaces within the lesion differentiate them into macrocystic, microcystic, or combined. Preoperative imaging help differentiate the types of lymphatic lesions [7, 9, 11]. Cystic Hygroma is a variant of a macro cystic LM. The surface lesions appear as minute blisters that appear dark red because of intra-vesicular bleeding.

Like VM the LM shows pressure effects on the skeletal structure causing deformity [20]. Large lesions in the tongue and floor of the mouth result in the anterior open bite as well as macroglossia. Functional impairment and airway distress may necessitate even tracheostomy.

2.3 Genetic Basis of Vascular Lesions with Mutation and Inheritance

2.3.1 **Mutations** (Table 2.1, See Also Fig. 2.6 Consolidated Review)

The majority of Vascular anomalies are of a ***sporadic type***. There are a few rare reported types of familial inheritance (germline type) [15–17].

2.3.2 Cancer and Vascular Lesions

Mutations causing Malignancies and vascular lesions share similar pathways (Figs. 2.2, 2.3, 2.4, 2.6). However, surprisingly causation and phenotype are poorly understood between oncogenesis and vasculogenesis [18–23, 34]. The Growth and differentiation of vascular lesion and endothelium are driven by two major pathways-RAS/RAF and PIK3CA (Fig. 2.1 of RAF/RAS).

Table 2.1 Germline versus Somatic Mutation differences [24–31]

Germline mutation in vascular lesions	Somatic mutation in vascular lesions
Inherited	Nonheritable
Syndromes & malignancy association	Sporadic
Mutations present in egg/sperm	Non-germline tissue
All the offspring affected	Most vascular lesions

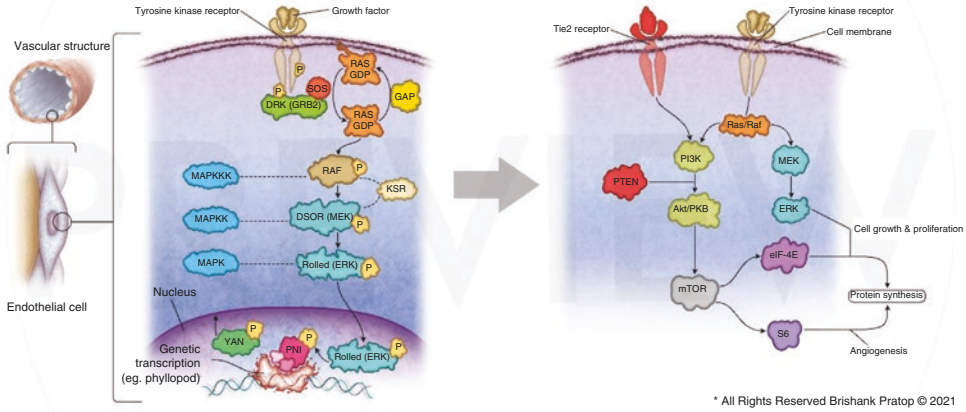

Fig. 2.2 Transmembrane tyrosine kinase receptors like ANG (angiopoietin) homologs TIE1 & TIE 2 (tyrosine kinase with immunoglobulin and epithelial growth factor (EGF) homology domain) are present on vascular endothelial cells. The activation of the Ras-Raf-MEK-ERK pathway leads to angiogenesis and cell growth and proliferation. Various growth factor receptor mutations in downstream have been identified in vascular and lymphatic endothelial cells leading to vascular malformation

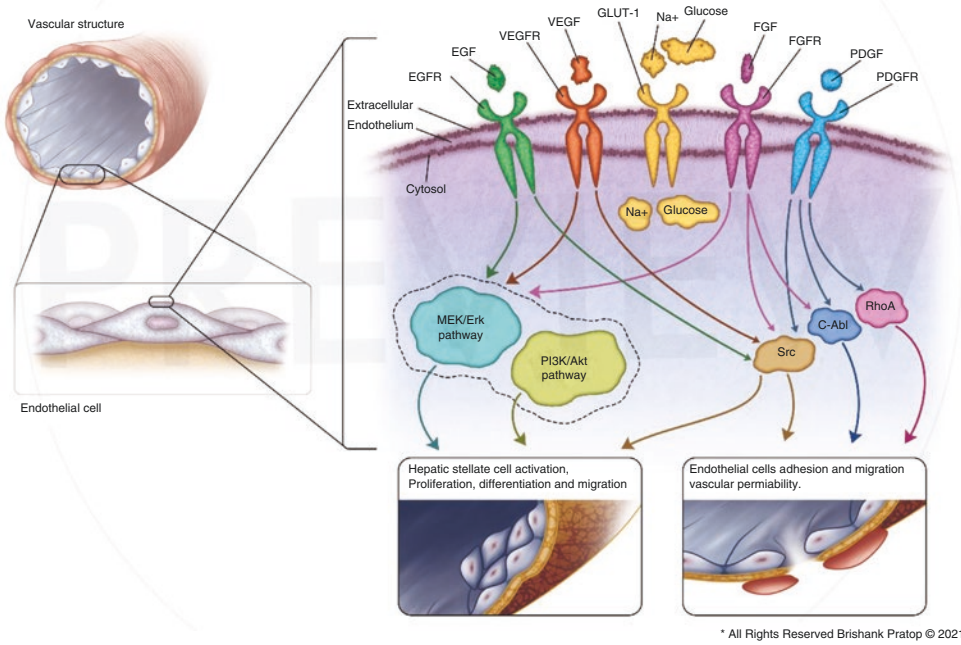

Fig. 2.3 Tyrosine kinase receptors picture. Receptor Tyrosine Kinases (R TKs): Cell surface expresses two peptide amino acid tyrosine class receptors of growth factors like EGFR: Endothelial growth factor receptor family (Receptor, TK class I), PDGFR: Platelet-derived growth factor receptor (receptor tyrosine kinase class III), VEGFR: Vascular endothelial growth factor receptor (Receptor, TK class IV), FGFR: Fibroblast growth factor receptor (Receptor tyrosine kinase class V). These polypeptide, cell surface, transmembrane, high-affinity receptors for cytokine, growth factors and hormones are responsible for angiogenesis and growth. This growth, proliferation, DNA synthesis by Ras–Raf–MEK–ERK pathway downstream is similar in vascular lesions as well as oncogenesis. The vascular endothelial cells proliferate once the surface RKTs get activated and can cause hepatic cell proliferation or endothelial cell migration and adhesion. In vascular lesions, the adhesion is defective leading to dilated and tortuous pools. The defect can extend to the smooth muscle cell layer. *EGF* Endothelial growth factor, *VEGF* Vascular growth factor, *GLUT-1* Glucose transporter 1, *FGF* Fibroblast growth factor, *PDGF* Platelet-derived growth factor

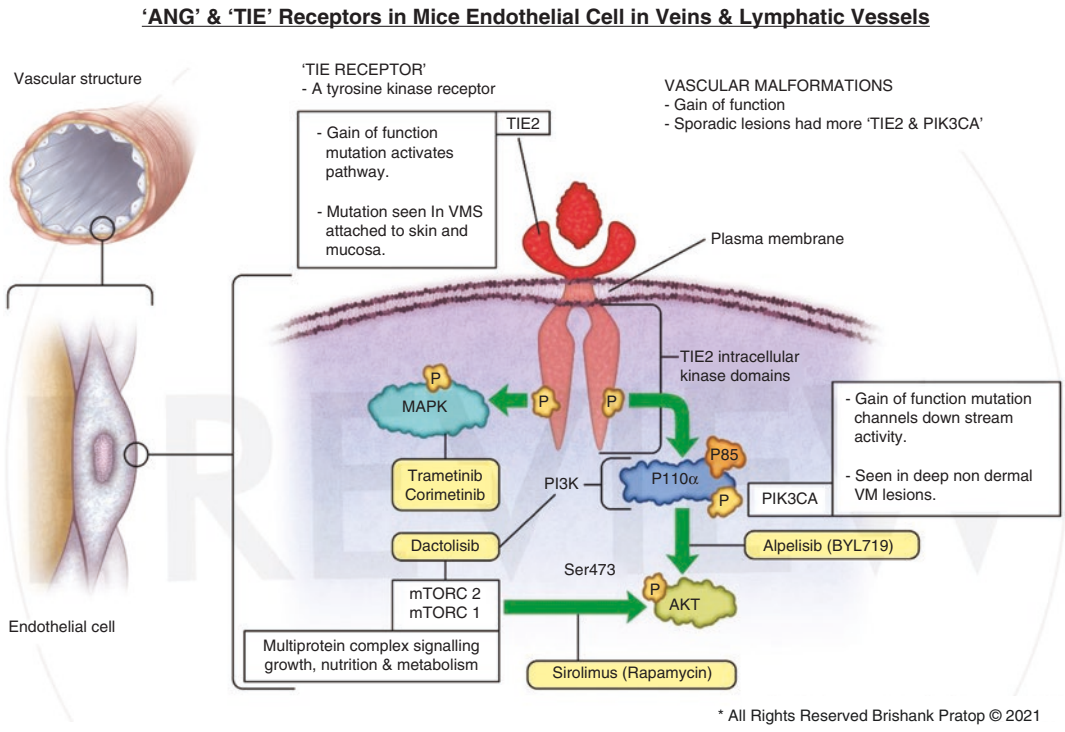

Fig. 2.4 Angiopoietin (ANG) based on two transmembrane tyrosine kinase receptors-TIE1 and TIE 2 (tyrosine kinase with immunoglobulin and epithelial growth factor (EGF) homology domain) homologous receptors have been identified in vascular and lymphatic endothelial cells leading to vascular malformations. This picture illustration represents the identified pathway and the potential therapeutic molecular therapies. Adapted from Kangas J, Nätynki M, Eklund L. Development of Molecular Therapies for Venous Malformations. Basic Clin Pharmacol Toxicol. 2018 Sep;123 Suppl 5:6–19. https://doi.org/10.1111/bcpt.13027. Epub 2018 May 29. PMID: 29668117

2.4 VEGF and PDGF, Growth factors of Raf, Ras in Tyrosine class receptors

Ras and Raf are downstream from the two-peptide amino acid class tyrosine kinase, which is the first step receptor in cancer and vascular cell upregulation.

Upregulation of the receptor or function of the tyrosine kinase (TIE or TK) group of receptors is known to cause cell division and proliferation. Cascade activation of TK to the cell nucleus leads to Growth, virus-directed replication, sepsis, inflammation, oncogenesis, etc.

Vascular endothelial growth factor (VEGF), Platelet-derived endothelial growth factor (PDGF), Epidermal growth factor (EGF), EGFR, PDGF, V EGF, etc. are all tyrosine kinase receptors. There is an extracellular receptor binding site. This sequential protein to protein communication of a phosphorylated cascade is known as RAS–RAF–MEK–ERK/or MAPK/ERK (mitogen-activated protein kinase/extracellular signaled regulated kinase).

RAS is activated by the phosphorylation of the tyrosine kinase class receptor in the cell wall. Activated Ras then activates Raf kinase (both GTPases). Activated Raf downstream phosphorylates MEK the next step of the chain (MEK 1 and 2). Phosphorylated MEK activates the ERK pathway. ERK and MAPK (mitogen-activated protein kinase), well known as RAS–RAF–MEK–ERK cell signaling cascade from the cell surface to the nucleus for growth and proliferation.

2.5 G Protein-related Spectrum

There are multiple G protein receptors related mutations on genes of GNA Q/GNA 11/GNA 14 [30, 31]. This G protein-related spectrum is GNA-vascular anomaly (GNAVA). A few instances of this GNA VA spectrum anomalies are congenital hemangioma, capillary hemangioma, kaposiform hemangioendothelioma. So evidently, these G protein-related receptor mutations can manifest in both tumors and malformations [30].

2.6 PIK3CA-related Overgrowth Spectrum (PROS) [31–36]

Vascular anomalies caused by PIK3CA related mutations are known as PIK3CA-related overgrowth spectrum (PROS). As previously classified independently currently grouped as PROS spectrum. There is a potential targeted therapy for these receptor-related malignant tumors—many PROS-related anomalies present as malformations of a similar kind.

Multiple mutations can give rise to various manifestations in different stages of cell proliferation. Some mutations can give rise to both tumors and malformations. Mutations of a single gene can manifest varying phenotypic characteristics clinically. The mutated gene can express varied manifestations depending on the up-regulation or down-regulation of proliferating, differentiation, and maturation stage affect.

The majority of the known vascular anomalies' mutations are associated with the tyrosine kinase receptor-associated signaling pathway of RAS & PIK3CA [30, 31, 40]. Various somatic mutations affecting the MAP2K1 pathway can present as cutaneous malformations of several types of malignancies not related to vascular tissue but melanomas, lung cancer, and blood-related tumors.

2.7 Mutations and Vascular Lesions: *The Essentials*

Previously focal or multifocal and diffuse vascular lesions were defined with syndrome labeling. However, with the new molecular genetics and identification of somatic mutations, the diagnosis should be based on abnormal genetic pathways. Most of the vascular lesions have a single gene-related Mutation. Nevertheless, the mutation of a single gene may manifest in various forms of vascular anomalies with occasional multiple gene mutations. The identification of genetic mutations is ongoing research with at least close to a hundred publications in the last 25 years.

Mutations can be of broad two types -Germline and somatic (Table 2.1). Alterations in the genetic sequence involving all cells of the gamete organism are called a germline mutation. This type of altered genetic sequence is transmitted to future generations. Most of the vascular lesions are of somatic mutations, which occur after fertilization as variations of the post-zygotic DNA. They are random occurrences affecting the cell's genetic sequence and the derivative, but next generation does not inherit this Mutation type. Understanding these somatic mutations and their pathways may help in control with targeted pharmacotherapy in the future.

Other than that mutation type, alterations in the signaling pathway, the enriched cell type of the Mutation and Signaling pathways are also being understood in vascular anomalies' etiology pathogenesis. More the number of mutated alleles and their frequency of mutant varieties and wild types are also considered.

Gene signaling pathways and Cells enriched by the Mutation Are also being identified for novel therapeutic uses. RAS (phosphatidylinositol-4,5-bisphosphate 3-kinase) PIK3CA cell signaling pathways dependent on the tyrosine kinase receptor are the most common mutations identified in head and neck vascular anomalies.

2.8 Genetic Testing and Advances in the Diagnosis of Vascular Anomalies [3, 4, 26, 30, 31]

Advances in genomics with understanding and utilization of genetic sequencing, testing how provided various molecular basis approaches of pathophysiology in vascular anomalies.

2.8.1 Test Type and Reasons

Ideal genetic testing for vascular anomalies is with a purpose and undertaken with flow chart identification of the Mutation.

Blood, tissue sample of the lesion if obtained, or buccal mucosal scraping can detect Germline mutations. Tissue specimens are fixed in formalin if needed for transport. Simple D -dimer review of lesion activity with the specificities in mind can be performed.

2.9 Biopsy and Immunohistochemistry

2.9.1 GLUT-1 and the Importance of the Vascular Lesion Identification

Glucose transporter protein isoform -1 (GLUT-1) is a standard and ideal marker for hemangiomas.

2.9.2 GLUT 1 Receptor (Fig. 2.5)

Infantile hemangioma (IH) in all evolving stages of the lesion is positive for GLUT 1 (Fig. 2.5). This endothelial stain highlights the cytoplasm inside the vascular lesions' capillary endothelial cells in both the involuting and proliferative stages.

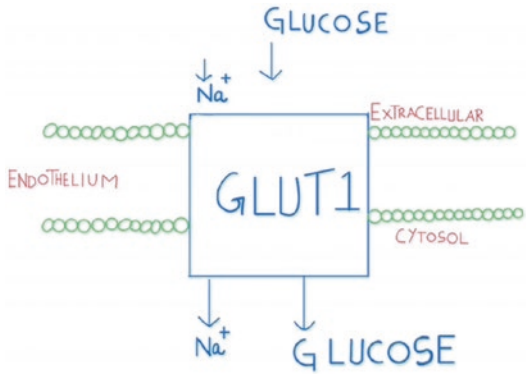

Fig. 2.5 Infantile hemangioma (IH) in all evolving stages of the lesion is positive for GLUT 1. Courtesy Dr Advaith Nair

Congenital Hemangioma (CH)—Rapidly involuting congenital hemangioma (RICH) has endothelial cells mostly GLUT 1 negative. Non-involuting congenital hemangioma (NICH) definitively is Immuno-negative for GLUT 1. Segmental hemangioma is positive for GLUT-1 staining.

2.10 VEGF -3, D2 -40, PROX 1, LYVE -1

These immune stains can differentiate arteries and veins from lymphatics.

Vascular endothelial growth factor receptor -3, Vascular immunostains for D2 -40 (podoplanin), Lymphatic vessels endothelial receptor−1 (LYVE −1 marker PROX 1 (Lymphatic endothelial markers).

2.11 CD31 and CD 34 Antibodies

2.11.1 Factor VIII Related Antigen

Antibodies of CD31 and CD 34 is a pan endothelial cells marker (low affinity for lymphatic endothelial affinity) [30, 31]. CD 31 is specific. These Vascular endothelial Immune peroxidase markers are commonly used along with Factor VIII-related antigen.

2.12 Next-generation Sequencing and Its Promise for Vascular Lesions

2.12.1 DNA Sequencing, What his Next-generation Sequencing?

Deoxyribonucleic acid sequencing, especially human genomic sequencing, has undergone a few generations of development since the early 1970s. Here is a brief review of some fundamentals to understand genetic testing for vascular lesion diagnosis and treatment.

Table 2.2 Table of Sanger and NGS

	Sanger (1977)	Next-Generation Sequencing (1996)
DNA sample	Cell DNA fragments; PCR clones in hotspot areas	DNA libraries; cell-free DNA
Sequencing	Linear read of single fragment	Parallel sequencing of million base pairs simultaneously
Utility	Unknown mutation diagnosis	Detection of unidentified mutations; complex and heterogenous phenotypes
Basic principle of DNA analysis	Chain termination PCR > ddNTPs label>fluorescent signal pick up of fragments	Sheared>tail adapters>DNA library>amplify>sequence> light density read
Cost	Cheap	Varies on the complexity
Sensitivity	Lower	High (lesion heterogeneity and contamination not a factor)
Data	Small storage capacity	Large data with bioinformatic systems

Next-generation sequencing (NGS) techniques are tools for mutation diagnosis. Targeted gene panel sequencing (TS) and Whole-Exome Sequencing and Whole-Genome sequencing are possible for an expressive phenotype's monogenetic disorder.

2.12.2 Sanger Method

The Sanger method took about 13 years for the human DNA genomics study of three million base pairs; NGS does this in a single day (Table 2.2).

Sanger with associates developed dideoxy synthesis with chain-terminating nucleotides at the 3-hydroxyl group. A Detailed nucleotide sequence review in the hundreds in the Sanger method. Sanger method sequences single strands of DNA.

Nevertheless, with the advances of next-generation sequencing (NGS), a whole-exome, genome, transcriptosome, mitochondrial DNA, or Target Gene panel sequencing is performed at a low cost.

2.13 Next-Generation Sequencing (NGS)

Commonly known as "NGS" is also "second generation" DNA sequencing, a significantly faster, low-cost development in the last ten years. This genome, exomes, and transcriptome sequencing will lead to paradigm changes in vascular lesion therapies.

Advances in genomics with understanding and utilization of genetic sequencing, testing how provided various molecular basis approaches of pathophysiology in vascular anomalies.

2.13.1 #1 Copy Number Variations (CNV)

Genome-wide CNV of more than 10,000 base sequence variations are detected done using high-resolution chromosomal microarray (CMA) and Whole-genome sequencing (WGS) methods.

2.13.2 #2 Single Nucleotide Variation (SNV)

This is the most common type of genomic variation affecting a single nucleotide of DNA base, causing short insertion and deletions. This alteration of Nucleotide can be detected using next-generation sequencing (NGS) methods of Whole-exome sequencing (WES) or whole-genome sequencing (WGS). In next Generation sequencing, unlike single-strand sequencing for DNA defects, it involves slicing of the DNA into numerous random strands before amplifying the fragments and then subjecting to Polymerase chain reaction (PCR)to align the exact 5′ and 3′ with attachments of adapters. In PCR, the genetic fragments get amplified to generate clonal clusters. Before that, DNA polymerase will be utilized for complementary; Nucleotide is created

and attached to the strands. This stage utilizes Low-cost fluorescent technology and bioinformatics for Sequencing results.

Unlike vector cloning with the use of restriction enzymes and bacterial cells, the genetic sequencing by PCR aided Sanger and NGS are more accurate and do not use heat and anneal methods.

PCR is utilized for amplification and cloning DNA templates from a biopsy or liquid biopsy sources. The polymerase can rapidly produce a million copies of a single fragment.

NGS is a parallel sequencing of millions of fragmented DNA from a library, while the Sanger method does one fragment in a linear fashion.

The similarities between all the genetic sequencing methods

- Biopsy material for nucleic acid fragment.
- Adapter ligation.
- Multiplex Tag and clone amplification.
- Parallel sequencing.
- Data analysis.

The advances in the future with next impression sequencing technology are with the increase in signal detection, read length, costs, and run time. Exome analysis is by both the parents and the child for variations. Partial genomic hotspot testing or a complete gene panel testing.

The current platforms for next impression sequencing are primarily for research but have great potential for bedside therapeutics. Though the next impression's volume of sequencing is phenomenal for at least one million reads, the infrastructure is still expensive compared to the Sanger method.

2.14 Cell-free DNA (cfDNA) Testing in Prenatal Conditions for Vascular Anomalies

NGS can perform mutational analysis in a prenatal setting of cell-free DNA (cfDNA). Even though this technology has been in use for prenatal testing, tumor-derived cell-free DNA testing is possible with the NGS. The sensitivity of cfDNA may be less compared to actual lesion biopsies. However, it is a viable option in prepartum diagnosis and difficulty with repeated biopsies of highly vascular tissue and anatomically critical areas. cfDNA testing in vascular lesions can be applied to diagnoses, monitoring the evolution, therapeutic guidance, and follow-up. Cf DNA has helped monitor lung cancer therapy targeted at tyrosine kinase inhibitor response for epidermal growth factor activated mutations.

Exome analysis is by both the parents and the child for variations. Partial genomic hotspot testing or complete gene panel testing.

The current platforms for next generation sequencing are primarily for research but have great potential for bedside therapeutics. The sequencing by next generation is phenomenal for at least one million reads, the infrastructure is still expensive compared to the Sanger method (Fig. 2.6 and Table 2.3).

2.15 Therapeutic Hypotheses for the Treatment of Vascular Diseases [39–41, 46–48, 51] (Fig. 2.7)

2.15.1 Targeting the PI3K/AKT/mTOR Pathway

- **mTOR inhibitors, such as Rapamycin in VM, VMCM, MVM, BRBN, and LM; also HHT?**

  Targeting the RAS/BRAF/MAPK/ERK pathway
- Possible inhibitors could be the BRAF inhibitor vemurafenib or the MEK inhibitor trametinib.in CM, CM-AVM1 and 2, PG, NICH, RICH, and verrucous venous malformations.

  Targeting angiogenesis
- Antiangiogenic agents, such as bevacizumab in HHT, IH.

  Targeting TGF-ß pathway
- GVM?

Growth and differentiation of the vascular lesion and its endothelial cell proliferation or

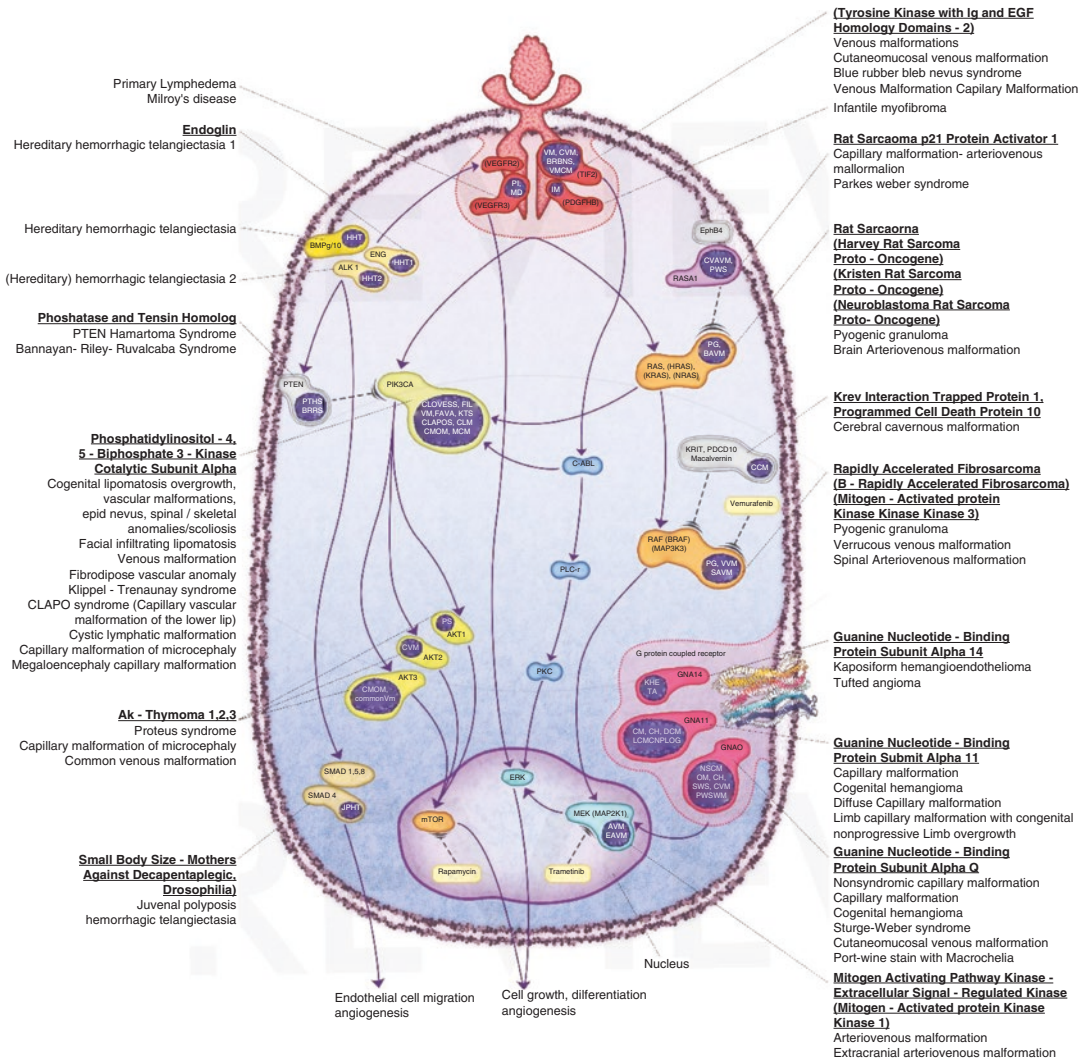

Fig. 2.6 Genetic basis of vascular lesions with Mutation and inheritance—Nair and Chandra [22, 39, 57, 60, 61]. Summary illustration of well-known genetic mutations based on that transmembrane tyrosine kinase MG protein-coupled signaling pathways. The protein mutations are abbreviated with the number's analogs. These vascular malformations caused by the mutations on the syndromic presentations are listed below the receptors which are mutated. The majority of these mutations are based on the well-known Ras-Raf-MEK-ERK and PIK3CA pathways. **Adapted from- International Society for the study of vascular anomalies 2014-classification scheme and associated genetic basis** (Table 2.3); Greene and Goss vascular anomalies: From a clinical histologically genetic framework, Plast Reconstr Surgery 2018 may; 141 (5): 709e–717e; Padia R, Zenner K, Bly R, Bennett J, Bull C, Perkins J. Clinical Application of Molecular Genetics in Lymphatic Malformations. Laryngoscope Investig Otolaryngol 2019, Feb:4(1):170–173

initiated at the cell membrane tyrosine kinase receptor with the RAS- RAF downstream to nucleus MAPK dependent gene transcription. The illustration demonstrates the potential therapy and inhibition of Src-tyrosine kinase family inhibitors; rapidly accelerated fibrosarcoma (RAF) inhibitors; mitogen activating pathway kinase-extracellular signal-regulated kinase (MEK) inhibitors; extracellular signal-regulated kinase is (ERK) inhibitors.

Table 2.3 Study of vascular anomalies 2014-classification scheme and associated genetic basis

Genetic basis of vascular lesion; Inheritance	Vascular tumors	Lymphatic malformations	Vascular malformations; arteriovenous malformation; additional syndromes and anomalies
Follicle-stimulating hormone; stem cell	Infantile hemangioma		
GNAQ GNA 11	Congenital hemangioma		
GNA 14	Kaposiform hemangioendothelioma		
KRAS; NRAS; GNA Q; B RAF	Pyogenic granuloma;		
PDPRB; PLCG; WWT R1-CAMTA 1 gene fusion; PDGF RB	Rare vascular tumors-epithelioid hemangioendothelioma; angiosarcoma; infantile myofibroma		
PIK3CA; VEGFR 3/ FLT-4; FOXC2; SOX 18; CCBE1; GJ C2, CX47;		Primary lymphedema-hereditary, sporadic	
VEGFR 3/FLT-4 dominant/recessive		Nonne–Milroy Syndrome	
FOXC2 dominant		Lymphedema-distichiasis	
SOX18 dominant/ recessive		Hyportrichosis–lymphedema-telangi ectasia	
GATA2		Primary lymphedema with myelodysplasia	
CCBE 1		Primary generalized lymphatic anomaly	
KIF11		Microcephaly with/without chorioretinopathy	
–		Lymphedema or mental retardation syndrome	
PTEN14		Lymphedema–choanal atresia	
PIK3CA somatic			Klippel–Trenaunay syndrome
RASA1/EPHB4 dominant			Parkes Weber syndrome
–			Servelle–Martorell syndrome
GNAQ			Sturge–Weber syndrome
			Capillary malformation & congenital nonprogressive overgrowth
IDH1/IDH2 somatic			Maffucci syndrome
PIK3CA			Megaloencephaly with capillary malformation
STAMBP			Microcephaly with capillary malformation
PIK3CA			CLOVES syndrome
AKT1 somatic			Proteus syndrome
PTEN			Bannayan_Riley_Ruvalcaba syndrome/Cowden syndrome
MAP2K1; RASA1; GDF2; VEGFR2;			Arteriovenous malformation; capillary AVM;

Table 2.3 (continued)

Genetic basis of vascular lesion; Inheritance	Vascular tumors	Lymphatic malformations	Vascular malformations; arteriovenous malformation; additional syndromes and anomalies
ENG (endoglin) ACVRL1/ ALK1(activating A receptor type 2-like 1) Autosomal dominant			Hereditary hemorrhagic telangiectasia (HHT 1 & 2)
SMAD4			Juvenile polyposis
PTEN- autosomal dominant			Hamartoma- tumor syndrome
TIE2 dominant			Cutaneomucosal venous malformation
KRIT1/malcavernin/ PDCD10 dominant			Cerebral cavernous malformation (CCM 1, 2 & 3)
Glomulin dominant			Glomuvenous malformation
TIE2 somatic			Sporadic venous malformation

2.15.2 Sirolimus (Rapamycin)

The literature on Sirolimus and its efficacy is equivocal. The best candidate for this therapeutic advantage is not clear. Strychowsky et al., in the 2018 phase 2 trial using Sirolimus in patients, evaluated the benefits of its empiric use complicated LM. They reported a reduction in cellulitis and incidence of hospitalizations with cellulitis-related complications. The adverse effects of therapy were—Metabolic toxicity (3%), gastrointestinal disturbance (3%), and blood/bone marrow abnormalities (27%). Not all the patients receiving therapy had a genetic test for the PIK3CA mutation confirmed. So, treatment was based on clinical considerations. Other studies have reported anecdotal success with Sirolimus. Sirolimus's current indications for therapy are for pain, lesion enlargement, vesicular ulcerations, bone erosion and expansion, bleeding, airway compression, hematologic abnormalities, and complex symptomatic cases [30–42, 55, 56].

There is significant crosstalk between pathways, which are not well understood. The targeted treatment therapies are focused on this receptor population pathway upregulation or downregulation. One such commonly used and established treatment is organ transplantation with immune suppression with Sirolimus (Rapamycin).

2.15.3 mTOR (Mammalian Target for Rapamycin) [30, 31, 39–45, 50, 52]

mTOR is a downstream enzyme in the PIK3CA pathway. Rapamycin is a chemotherapeutic agent derivative of *Streptomyces hygroscopicus* bacteria, a macrolide used for targeted therapy to block the PIK3CA pathway. Rapamycin (Sirolimus) inhibits cellular proliferation. Sirolimus has varied success in vascular malformations therapy, but clinical outcomes have not been consistent. Authors here present personal experiences in use with syndromic patients has been with adequate caution. Multiple centers have used Sirolimus empirically, and mutational tests before using the therapy have not been consistent.

Sirolimus as a B & T cell suppressor is very well studied in transplant patients, as mentioned with useful utility. Its use in head and neck vascular lesions has shown a qualitative reduction in size and side effects of bleeding and vesicular discharge. Nevertheless, Sirolimus therapy has documented side effects for its use with complicated LM throughout the body. A reduction in cellulitis and hospitalizations with cellulitis-related complications documented with case reports and lesion management publications.

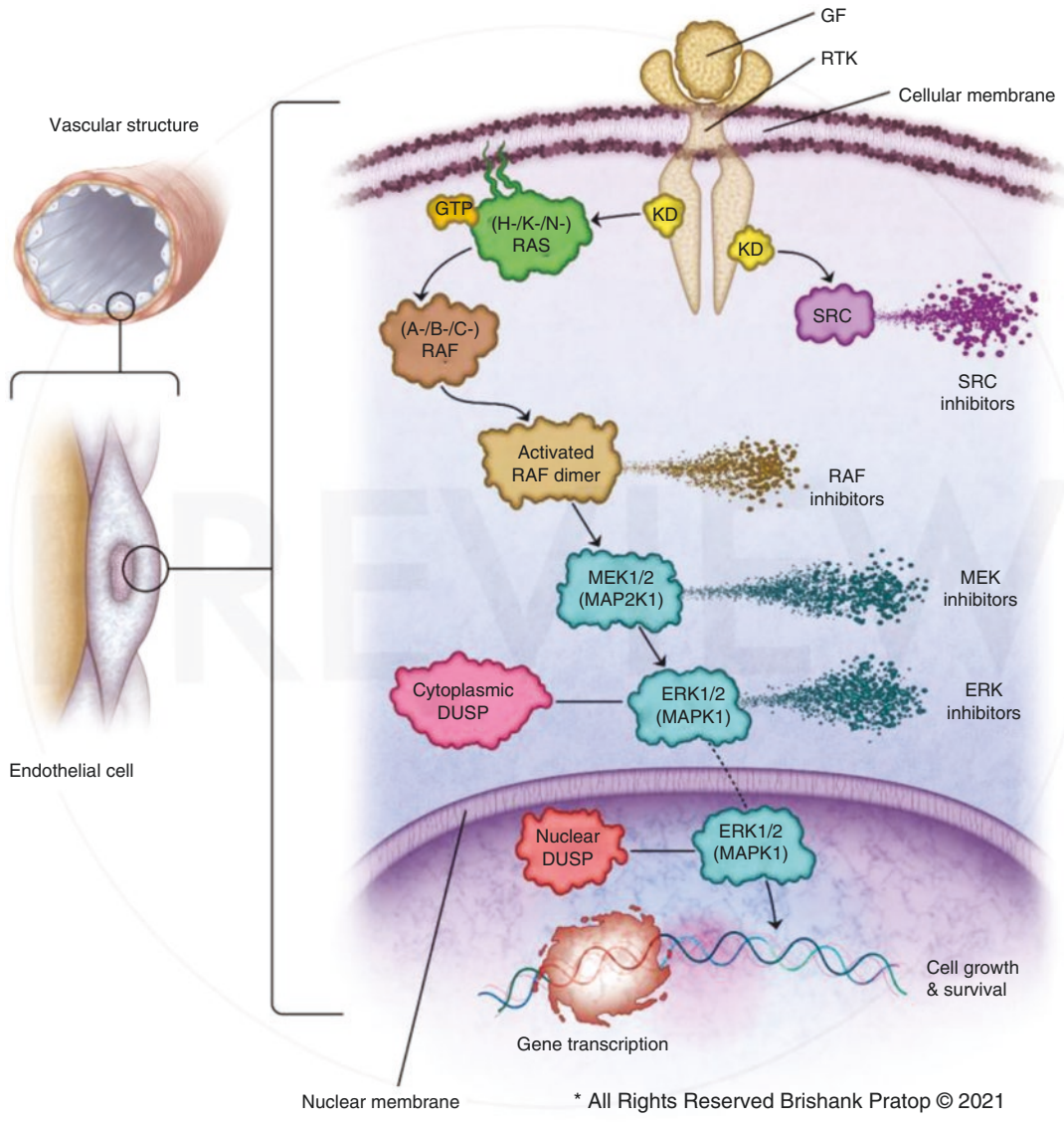

Fig. 2.7 MAPK/ERK pathway (well known as the Ras-Raf-MEK-ERK pathway) potential therapeutic inhibitors. Protein chain in a vascular cell that transmits signal from a cell surface receptor to the DNA in the cell nucleus

Gastrointestinal, general metabolic toxicity, blood dyscrasias, bone marrow suppression (even though one of the treatment indications is dyscrasias of hematological and marrow-derived cell function) were the reported adverse effects of the medications.

The literature on Sirolimus and its efficacy is equivocal. The best candidate for this therapeutic advantage is not clear. Strychowsky et al. [49], in the 2018 phase 2 trial using Sirolimus in patients, evaluated the benefits of its empiric use complicated LM. They reported a reduction in cellulitis and incidence of hospitalizations with cellulitis-related complications. The adverse effects of therapy were—Metabolic toxicity (3%), gastrointestinal disturbance (3%), and blood/bone marrow abnormalities (27%). Not all the patients receiving therapy had a genetic test for the PIK3CA mutation confirmed. So, treatment was based on clinical considerations. Other studies

have reported anecdotal success with Sirolimus. Sirolimus's current indications for therapy are for pain, lesion enlargement, vesicular ulcerations, bone erosion and expansion, bleeding, airway compression, hematologic abnormalities, and complex symptomatic cases [Refs. 2, 34].

2.16 Conclusion

Genetic testing and advances in the understanding of pathogenesis have provided more direct and targeted therapeutics. However, the molecular abnormalities similar to cancer mutations and the phenotypic presentation disparity is intriguing. Nevertheless, mutations provide an objective molecular etiology to educate patients, families, and researchers with HNLMS knowledge for further work with a group of pathologies. Clinical pathways for standardized outcome measures and building data banks for systematic collection in medical trials is needed.

Genetic testing for mutations is a very evolving topic with ongoing research. At the same time, many somatic mutations are not known even after the entire exome sequencing of the parents and the child. The causes for nondetection could be sampling the different hotspots in a different gene or low allele frequency for detection.

Aggressive therapy needs more close follow-up and reporting. HNLM research makes precision-based treatment a possibility. Precision therapy-based treatment decisions on specific molecular and genetic is the future. Biologic factors unique to an individual patient's rare conditions are always with meticulous risk–benefit ratio consideration.

Until now, therapeutic options to treat vascular tumors and malformations have is by classic approaches. To ablate or remove abnormal vessels by laser, sclerotherapy, embolization, and surgery. Detection of a genetic cause, inherited or somatic, has opened up understanding the underlying molecular mechanisms. Most genetic defects directly alter intracellular signaling activities and, subsequently, various downstream actions. Even if all the downstream effects are unknown, the identification of overt signaling opens these diseases to novel ideas to develop treatments.

Lesions caused by constitutively active PI3K/AKT/mTOR pathway (e.g., VMCM, MVM, VM, BRBN, and LM) may benefit from mTOR inhibitors as Rapamycin (see Fig. 2.1). A VM in vivo model is viable by injecting TIE2-L914F mutated human umbilical vein endothelial cells into nude mice. Treatment with Rapamycin prevented VM growth. Additionally, in vitro, Rapamycin significantly reduced mutant TIE2-induced AKT signaling [53–55]. Importantly, in a prospective clinical pilot study, six patients treated with Rapamycin had reduced pain, bleeding, lesional size, and intravascular coagulopathy.

In lesions where the RAS/RAF/MAPK/ERK pathway plays a significant role (e.g., CM, CMAVM1 and 2, PG, NICH, RICH, and verrucous venous malformation), other inhibitors are considered. There are conceivable tests of BRAF (vemurafenib) or MEK inhibitor (trametinib), which are used to treat metastatic BRAF-mutated melanoma (see Fig. 2.1) [30, 31, 57–60]. Because many kinase inhibitors have variable affinities to several intracellular proteins, and multiple cross-talks occur between signaling pathways, numerous studies are needed to characterize the most efficient modalities.

In HHT, receptor mutations lead to decreased BMP signaling, and this may lead in turn to an increase in angiogenic response. Thus, these patients could benefit from antiangiogenic agents, such as bevacizumab. However, diminished ALK activity also leads to increased PTEN phosphorylation and inactivation. There is subsequent PI3K/AKT activation. Rapamycin and other PI3K/AKT inhibitors may thus prove to be efficacious [51].

Other general angiogenesis inhibitors, such as thalidomide or bevacizumab, the anti-VEGF antibody, may also be useful [60–63]. They can inhibit VEGF action, whatever the underlying cause for expression may be. For example, they reduce nosebleeds in patients with HHT. The pathophysiology of GVMs is a little unclear. If earlier data hold, the TGF-ß pathway might serve as a target, in addition to modulation of mTOR.

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