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Emerging Pharmacologic Targets in Cerebral Cavernous Malformation and Potential Strategies to Alter the Natural History of a Difficult Disease
A Review

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IMPORTANCE Cerebral cavernous malformations (CCMs) are vascular lesions of the brain that may lead to hemorrhage, seizures, and neurologic deficits. Most are linked to loss-of-function mutations in 1 of 3 genes, namely *CCM1* (originally called *KRIT1*), *CCM2* (*MGC4607*), or *CCM3* (*PDCD10*), that can either occur as sporadic events or are inherited in an autosomal dominant pattern with incomplete penetrance. Familial forms originate from germline mutations, often have multiple intracranial lesions that grow in size and number over time, and cause an earlier and more severe presentation. Despite active preclinical research on a few pharmacologic agents, clinical translation has been slow. Open surgery and, in some cases, stereotactic radiosurgery remain the only effective treatments, but these options are limited by lesion accessibility and are associated with nonnegligible rates of morbidity and mortality.

OBSERVATIONS We discuss the limits of CCM management and introduce findings from in vitro and in vivo studies that provide insight into CCM pathogenesis and indicate molecular mechanisms as potential therapeutic targets. These studies report dysregulated cellular pathways shared between CCM, cardiovascular diseases, and cancer. They also suggest the potential effectiveness of proper drug repurposing in association with, or as an alternative to, targeted interventions.

CONCLUSIONS AND RELEVANCE We propose methods to exploit specific molecular pathways to design patient-tailored therapeutic approaches in CCM, with the aim to alter its natural progression. In this scenario, the lack of effective pharmacologic options remains a critical barrier that poses an unfulfilled and urgent medical need.

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Cerebral cavernous malformations (CCMs; cavernous angiomas, cavernomas) are low-flow, hemorrhagic vascular lesions of the central nervous system that affect 0.16% to 0.5% of the population.[1] Anatomically, they are composed of abnormal cystic vascular channels lined by a single layer of endothelial cells (ECs) with impaired tight junctions.[2] These vessels are typically arranged in compact masses with no intervening brain parenchyma, so the deriving vascular malformations are angiographically occult. They may undergo acute intracranial hemorrhage (ICH), small subclinical bleeds, or slow diapedesis of red blood cells[3] that produce a characteristic hemosiderin rim on magnetic resonance imaging.[4] In addition to causing stroke from ICH,[5] CCM can also provoke seizures,[6] headaches, and focal neurological deficits (FNDs).[5] About 20% of cases are familial and characterized by the presence of multiple lesions as opposed to sporadic CCM, which has no prevalence within families and typically presents with a single lesion. Radiation-induced cavernous malformation (RICM), a subset of sporadic CCM, can occur in patients previously treated with radiotherapy for brain tumors. Radiation-induced cavernous malformations generally occur after many years, with the time of diagnosis inversely associated with age at radiation treatment. Radiographically and histologically, they are indistinguishable from other sporadic lesions and present similar rates of symptomatic hemorrhage. However, RICM is usually diagnosed at a younger age, and patients are more likely to present with multiple lesions.[7]

Cerebral cavernous malformation has a genetic basis. Its mutational landscape has been first investigated in familial forms, where predisposition to develop cavernomas is inherited through an autosomal dominant pattern with incomplete penetrance. Linkage studies allowed associating the occurrence of CCMs with loss-of-function mutations in 1 of 3 genetic loci: *CCM1* (*KRIT1*) at chromosome 7p,[8] *CCM2* (*MGC4607*) at 7q, and *CCM3* (*PDCD10*) at 3q.[9]

Corresponding mutation rates range between 53% and 65% for *CCM1*, 15% and 19% for *CCM2*, and 10% and 16% for *CCM3*.[10-12] Compared with those in *CCM1* and *CCM2*, mutations in the *CCM3* gene are associated with the most severe phenotype, defined as an earlier onset of ICH and concomitance of multiple meningiomas in some cases.[11] A 2-hit mechanism,[13] whereby a germline mutation in 1 allele of a *CCM* gene is later complemented by a somatic mutation in the other allele, has been described as a trigger in familial CCM.[14] Although CCM is a worldwide disorder, a specific mutation in the *CCM1* gene is prevalent in northern New Mexico. The origin of this founder mutation, also known as the common Hispanic mutation–*CCM1* , has been traced to a Spanish ancestor who settled in New Mexico in the 1600s. The common Hispanic mutation–*CCM1* is responsible for a 15-fold higher population prevalence and is present in virtually all cases of familial CCM in the New Mexico Hispanic population.[15] A founder mutation in the *CCM2* gene has been described in the Ashkenazi Jewish population,[16] while no founder mutations have yet been found in the *CCM3* gene. The association between genotype and phenotype in sporadic forms of CCM is less characterized, although the involvement of the same genes is emerging. Mutations in either the *CCM1*, *CCM2*, or *CCM3* gene have been reported in 57% of sporadic cases with multiple lesions.[12] Again, a 2-hit mechanism has been implicated, in which 2 spontaneous somatic mutations occur sequentially at both alleles of 1 of the known *CCM* genes.[17] Yet other reports suggest a strong association between the presence of cerebral lesions and polymorphisms in the *CCM* genes in sporadic cases.[18] Whether similar genetic alterations take place in RICM is not yet known. The pathophysiology of RICM has been associated with vascular injury[19] and may lay along a spectrum of radiation-induced vasculopathies that evolve from telangiectasia to CCM.

Clinical Presentation, Natural History, and Current Management of CCM

About 60% of CCM lesions present as clinically silent[1] and are discovered incidentally on brain magnetic resonance imaging obtained for other reasons. Sporadic CCM lesions may be associated with a developmental venous anomaly,[20] while familial forms typically present with multiple lesions (even hundreds). In familial patients, modern diagnostic techniques, such as susceptibility-weighted imaging, detect more lesions than were previously identified with T2-weighted gradient echo sequences.[21] These techniques also show that new vascular lesions constantly form de novo with age.[22]

In symptomatic cases, the most common presentation is seizures, followed by ICH and FND in the absence of an acute hemorrhage.[5] The 5-year risk of first-time seizure in asymptomatic CCM is 4% but increases to 6% in patients presenting with ICH or FND.[23] Reported rates of ICH vary widely in the literature. In 2008, the Angioma Alliance Scientific Advisory Board standardized the definition of CCM-associated ICH.[5] A meta-analysis[24] across 7 patient cohorts using this new definition reported an overall 5-year ICH risk of 15.8%.[24] Compared with supratentorial and cerebellar CCMs, lesions located in the brainstem carry the worst prognosis and highest neurologic morbidity, with the combined risk of ICH and FND escalating to more than 50% at 5 years.[24,25] Moreover, the annual risk of ICH in incidentally discovered or previously asymptomatic CCM is significantly higher in familial (4.3% to 6.5%)[26] compared with sporadic cases (0.08% to 0.1%).[1]

Figure 1. Flowchart Summarizing the Clinical Course of Cerebral Cavernous Malformation (CCM) and Medical/Surgical Guidelines

CCM presentation (increasing severity)		Treatment (current guidelines)
Asymptomatic (incidental)	→	Conservative
Symptomatic: first-time seizure	→	Antiepileptics
Symptomatic: refractory epilepsy	→	Surgery
Symptomatic: first ICH (supratentorial)	→	Surgery
Symptomatic: first ICH (brainstem, deep nuclei)	→	Conservative
Symptomatic: second ICH (brainstem, deep nuclei)	→	Surgery/radiation

Depending on the clinical presentation, different strategies are recommended for CCM. For asymptomatic/incidentally discovered lesions, conservative management is the elected choice. Surgery is considered for easily accessible lesions to mitigate the pathological burden. The only medical treatment included in standard management is pharmacologic control of seizures when the condition is not refractory to antiepileptic drugs. However, in this case, evidence suggests early surgery for better seizure control. In all other cases of symptomatic CCM, surgery is the only accepted approach. There is limited evidence for the application of radiation therapy, which is reserved for surgically inaccessible lesions. ICH indicates intracranial hemorrhage.

The clinical approach to CCM depends on presentation, lesion location, and symptom severity (**Figure 1**). In 2017, the Angioma Alliance Scientific Advisory Board published expert guidelines for clinical management of CCM,[27] and we refer the reader to this publication. Briefly, a 3-generation family history should be collected at the time of diagnosis. Genetic testing, including mutation/deletion analysis of *CCM1*, *CCM2,* and *CCM3* genes, is recommended in all cases with multiple lesions without a history of radiation exposure or a positive family history. Further genetic counseling should be offered when a mutation is discovered in the proband. Asymptomatic lesions should be managed conservatively with imaging, although routine imaging follow-up is recommended only in the presence of new or worsening symptoms.[27] Cerebral cavernous malformation–related epilepsy is initially treated with antiepileptic drugs until it becomes refractory. Surgery may be offered for medically intractable epilepsy, supported by evidence for early surgical resection of single lesions to obtain durable seizure control.[6] In general, surgery is reserved for symptomatic lesions owing to its intrinsic risk. Postsurgical morbidity and mortality increase after lesion removal from certain locations; those in eloquent/deep areas and brainstem have the highest risk. Because the overall 5-year probability of a second ICH is as high as 29.5%, surgery is recommended after a first symptomatic bleed for lesions in favorable locations.[28] Advances in surgical navigation, tractography,[29] and awake mapping[30] permit safe resection of lesions in eloquent locations including the brainstem.[31] In contrast, surgery is often delayed in the case of deep lesions until the appearance of a second symptomatic bleed. Stereotactic radiosurgery (SRS) is an alternative option for single lesions in surgically inaccessible areas or in locations with high surgical morbidity, although it is not recommended for asymptomatic lesions or in familial cases.[32]

Failure of Current Strategies

Almost all patients with CCM-related seizures develop epilepsy within 5 years from diagnosis,[23] and up to 40% become refractory to antiepileptic medications,[33] the only pharmacologic treatment currently indicated for CCM. While surgical resection of a single symptomatic lesion results in good seizure control, a favorable outcome is dependent on the duration of seizures. Seizure control after surgery is poor if the duration of epilepsy has been more than 2 years[34] because frequent asymptomatic microbleeds may induce epileptogenic gliosis in a perilesional brain.[35] For all other symptoms, the mainstay treatment options are surgical resection of the lesion and SRS in selected cases, although they are marred by inherent limitations. For example, surgery is often too dangerous or not possible for deep-seated lesions in locations such as the thalamus and brainstem. Moreover, much of the experience on surgical management of CCM comes from highly-specialized centers, which limits generalization to a community setting. Even in experienced hands, the overall risk of major surgical morbidity, such as nonfatal stroke, is around 6%.[28] For deep-seated lesions, such as those in the basal ganglia and thalamus, this risk approaches 18%, with an operative mortality of 2%.[36] For brainstem lesions, surgical resection results in significant postoperative morbidity in 45% of the cases. Of these, up to 12% of patients require tracheostomy and feeding tubes, although they tend to improve over time.[37] Likewise, SRS, when applicable, has a roughly 2-year latency period before the annual risk of hemorrhage is significantly reduced and is limited to small lesions with treatment volumes less than 2 to 3 cm^3 to limit marginal doses.[38] Furthermore, there is still some debate as to whether the observed effects of SRS are indicative of efficacy or a mere reflection of the natural evolution of the disease.[27]

Even in familial cases, for which our understanding of disease progression is more comprehensive, incomplete penetrance and variable presentation within families preclude a reliable risk estimation,[39] hence the necessity for continuous follow-up and/or prompt surgical intervention at the appearance or worsening of symptoms. Nevertheless, our growing knowledge on the natural progression of CCM suggests that there could be a window of opportunity to affect the disease course from time of diagnosis to development of aggressive behavior. In sporadic cases, exploiting this window would control lesion growth and microhemorrhages to limit or abrogate symptomatic ICH and epileptogenesis, which cannot be achieved with current treatment options. Similarly, there is no effective approach to delay lesion growth and de novo formation typical of familial cases, which constantly produce new vascular malformations[22] at a rate that can reach 2.7 per patient per year in the presence of mutations in the *CCM3* gene.[40]

Altered Cellular and Molecular Processes Underlying CCM Lesion Formation

Studies have made progress to link basic and translational science to the emergence of CCM lesions, which reveals novel, putative treatment targets. While a comprehensive overview of CCM-related cellular mechanisms and signaling pathways goes beyond the scope of this review, here we summarize the core events that are critical to elucidate disease pathology and design effective next-generation therapies.

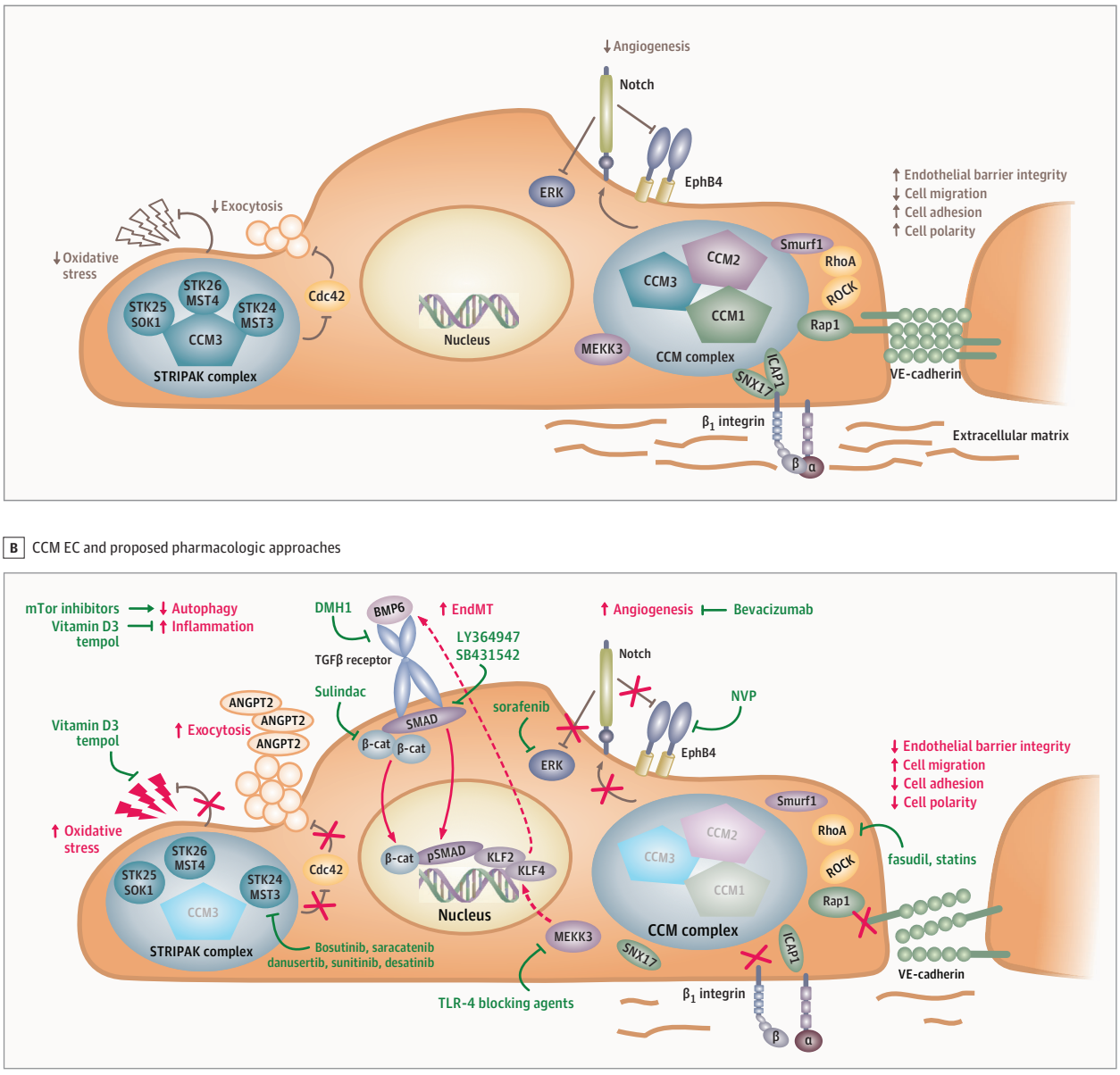
Cerebral cavernous malformations are formed by activated, angiogenic ECs[41] that induce local inflammation and oxidative stress owing to impaired autophagy.[42] At the cellular level, these ECs undergo endothelial-to-mesenchymal transition (EndMT), a process common to other vascular anomalies, such as atherosclerosis and hereditary hemorrhagic telangiectasia,[43] and similar to the epithelial-to-mesenchymal transition (EMT) observed in cancer cells. Several studies confirmed that EndMT[44,45] underlies CCM formation in both familial[46,47] and sporadic[48] cases. Endothelial cells subjected to this transition acquire a stem cell–like and mesenchymal cell–like phenotype characterized by loss of proper polarization, increased migration, and decreased cell-cell and cell-matrix adhesion. Consequences of EndMT are abnormal architecture and leakiness of brain blood vessels, and loss of contact between ECs and nervous cells, which ultimately lead to formation of the typical mulberry-shaped cavernae in the context of an angiogenic and inflammatory microenvironment.

The molecular mechanisms underlying these biologic and cellular processes have been first studied in iCCM1, an inducible, EC-specific *CCM1* knockout transgenic mouse that reproduces the phenotype observed in patients with loss-of-function mutations in the *CCM1* gene, namely, lesions within the central nervous system that are composed of dilated multilumen vascular channels with signs of vascular leakage.[49] Brain ECs derived from iCCM1 mice show loss of cell polarity and disruption of cell-cell contacts owing to altered vascular endothelial (VE)–cadherin distribution along with Notch inhibition,[49] which may induce angiogenesis by releasing a negative control on extracellular-signal-regulated kinase[50] and ephrin receptor B4.[51] These cells show hyperactivated EndMT signaling pathways: they overexpress bone morphogenetic protein 6, which leads to hyperactivation of transforming growth factor β receptor with consequent overexpression of β-catenin, increased small mothers against decapentaplegic phosphorylation, and translocation to the nucleus, followed by upregulation of stem cell, inflammatory, and mesenchymal genes including Kruppel-like factor 4 (*Klf4*)[49] (**Figure 2**). The key role of KLF transcription factors in CCM has been corroborated in an independent mouse model of EC-specific *CCM1* gene loss, in which upregulation of *Klf2* and *Klf4* genes occurs in the early phases of lesion formation,[52] and in mice defective for the *CCM2* gene.[53] Likewise, increased KLF2 and FLF4 protein levels have been observed in both familial and sporadic CCM lesions and are considered a hallmark of CCM-related EndMT (**Table 1**).[47-49,52-54]

In addition to these observations in *CCM1* and *CCM2* models, the peculiar phenotype associated with *CCM3* gene mutations has been specifically examined in mice with inducible EC-specific loss of *CCM3*. In these mice, a distinctive tract is an increased exocytosis and secretion of the proangiogenic factor angiopoietin-2[54] (Figure 2), coupled with decreased EC adhesion and pericyte coverage, which causes disorganized blood vessels with enlarged lumen in the cerebellar and retinal venous plexuses. A similar pattern of vessel disorganization associated with high levels of angiopoietin-2 has also been observed in surgical specimens and lesion-derived ECs from patients with *CCM3* gene mutations[54] (Table 1).

The question remains as to how these molecular pathways and cellular processes derive from the genetic defects identified in

Figure 2. Schematic Representation of Cerebral Cavernous Malformation (CCM) Onset and Proposed Therapeutic Interventions



In normal brain endothelial cells (ECs), the 3 CCM proteins form complete CCM and striatin-interacting phosphatase and kinase (STRIPAK) complexes. Under these conditions, cells are properly polarized and tightly connected to each other by vascular endothelial (VE)–cadherin and to the extracellular matrix by β_1 integrin, so cell adhesion prevails over migration. Blood vessels show a properly organized lumen with a continuous endothelial layer. Angiogenesis is inhibited through activation of a Notch-mediated negative circuit, exocytosis is restricted by inhibition of Cdc42, and oxidative stress is controlled. Loss of either the CCM1, CCM2, or CCM3 protein disrupts these complexes. The consequent loss of VE-cadherin organization is paralleled by hyperactivation of mitogen-activated protein kinase kinase kinase 3 (MEKK3), RhoA/Rho-associated protein kinase (ROCK) and Cdc42, overexpression of Kruppel-like factor 2 (KLF2) and KLF4 with induction of transforming growth factor β (TGFβ) signaling, and inhibition of Notch. Brain ECs undergoing endothelial-to-mesenchymal transition (EndMT) lose their physiological adhesion and polarization properties and acquire a migratory phenotype, thus disrupting the endothelial barrier. Increased oxidative stress, exocytosis, inflammation and angiogenesis, and decreased autophagy emerge as pathogenic events during CCM lesion formation (in red). Based on these biological premises, preclinical studies and ongoing clinical trials advise the repurposing of several targeted drugs for CCM treatment (in green). ANGPT2 indicates angiopoietin-2; BMP, bone morphogenetic protein; DMH1, dorsomorphin homolog 1; EphB4, ephrin receptor B4; ICAP1, integrin cytoplasmic associated protein-1; MST, mammalian sterile 20-like kinase 3; mTor, mammalian target of rapamycin; SMAD, small mothers against decapentaplegic; TLR-4, toll-like receptor 4.

patients with CCM. Quite simply, a loss-of-function mutation in 1 of the *CCM* genes causes defective production of the homonymous protein. So, the lack of either the CCM1, CCM2, or CCM3 protein is the molecular trigger that ultimately facilitates disease onset. The 3 CCM

Table 1. Dysregulated Signaling Pathways and Cellular Processes Identified In Vivo in CCM Animal Models and Ex Vivo in Patient-Derived Specimens (Surgical Samples and/or Lesion-Derived ECs)

Mutated Gene	Study Model	Affected Signaling Pathways and Cellular Processes	Source
Ccm1	Transgenic mice	Disrupted VE-cadherin junctions; activated TGFβ signaling; upregulated Klf4 among other mesenchymal, stem cell, and inflammatory genes; inhibited Notch	Maddaluno et al[49]
Ccm1	Transgenic mice	Upregulated *Klf2* and *Klf4* genes	Zhou et al[52]
Ccm2	Transgenic mice	Upregulated *Klf2* and *Klf4* genes	Whitehead et al[53]
CCM1, *CCM3* Familial and sporadic	Patient-derived specimens	Overexpressed KLF2 and KLF4 proteins	Zhou et al[52]
CCM1, *CCM2*, *CCM3* Familial and sporadic	Patient-derived specimens	Disrupted VE-cadherin junctions; activated TGFβ signaling; overexpressed KFL4 among other mesenchymal, stem cell, and inflammatory protein markers	Bravi et al[48] Cuttano et al[47]
Ccm3	Transgenic mice	Increased secretion of ANGPT2	Jenny Zhou et al[54]
CCM3	Patient-derived specimens	Increased levels of ANGPT2	

Abbreviations: ANGPT2, angiopoietin-2; CCM, cerebral cavernous malformation; EC, endothelial cell; KLF, Kruppel-like factor; TGFβ, transforming growth factor β; VE, vascular endothelial.

Table 2. Interactors of the CCM Proteins Identified by In Vitro Studies, Their Physiological Functions, and Pathogenic Events Mediated by These Interactors Following Disruption of Either the CCM or STRIPAK Complexes

Complex	CCM Protein	Direct Interactor	Physiological Role of the Interactor	Pathological Role of the Interactor in CCM	Source
CCM	CCM1	VE-cadherin	Main constituent of adherens junctions	Disrupted cell-cell contacts, loss of cell polarity and impaired lumen organization	Lampugnani et al[56]
CCM	CCM1	Rap1	Regulation of RhoA/ROCK	Hyperactivated RhoA/ROCK: decreased cell adhesion, disruption of endothelial barrier	Serebriiskii et al[60]
CCM	CCM1	ICAP1 and SNX17	Regulation of β1 integrin	Dysregulated β1 integrin: decreased cell adhesion	Zhang et al[57] Czubayko et al[59]
CCM	CCM2	MEKK3	Several functions in vascular development among which regulation of RhoA/ROCK	Hyperactivated RhoA/ROCK, vascular leakage due to disruption of endothelial barrier, overexpressed KLF2/4	Zhou et al[52] Whitehead et al[53] Fisher et al[61]
CCM	CCM2	Smurf1	Degradation of RhoA	Hyperactivated RhoA/ROCK: decreased cell adhesion	Crose et al.[62]
STRIPAK	CCM3	MST3/STK24	Regulation of Cdc42	Hyperactivated Cdc42: increased exocytosis	Lant et al[63] Song et al[64] Zhang et al[65]
STRIPAK	CCM3	SOK1/STK25 MST4/STK26	Preservation of Golgi integrity and centrosome orientation, protection from oxidative stress	Increased migration, loss of cell polarity, oxidative stress	Ma et al[66] Fidalgo et al[67,68]

Abbreviations: CCM, cerebral cavernous malformation; ICAP1, integrin cytoplasmic associated protein-1; KLF, Kruppel-like factor; MEKK3, mitogen-activated protein kinase kinase kinase 3; MST3, mammalian sterile 20-like kinase 3; ROCK, Rho-associated protein kinase; SNX17, sorting nexin 17; STK, serine/threonine kinase; SOK, suppressor of kinase; STRIPAK, striatin-interacting phosphatase and kinase; TGFβ, transforming growth factor β; VE, vascular endothelial.

proteins do not possess intrinsic enzyme activity and are therefore considered as scaffolds or adaptors.[55] When present at normal levels, they bind to each other in the so-called CCM complex, which also includes VE-cadherin,[56] integrin cytoplasmic associated protein-1,[57,58] sorting nexin 17,[59] and Rap1[60] as direct interactors with CCM1 and mitogen-activated protein kinase kinase kinase 3 (MEKK3)[61] and Smurf1[62] as direct interactors with CCM2. On loss of either the CCM1, CCM2, or CCM3 protein and disruption of the CCM complex, these interactors are no longer regulated. Therefore, they can induce disaggregation of adherens junctions (VE-cadherin),[56] dysregulation of β_1 integrin functions (integrin cytoplasmic associated protein-1 and sorting nexin 17),[57-59] overexpression of KLF2 and KLF4 (MEKK3),[52] and hyperactivation of RhoA signaling via Rho-associated protein kinase (ROCK) (Rap1, Smurf1, and MEKK3)[53,60,62] (Figure 2). Collectively, these altered pathways result in loss of cell polarity and decreased cell adhesion associated with disruption of endothelial barrier integrity and abnormal vascular lumen, all events associated with the onset of EndMT (**Table 2**).[52,53,57,59-68] While the presence of CCM1 and CCM2 proteins is restricted to the CCM complex, the CCM3 protein is also found in the striatin-interacting phosphatase and kinase (STRIPAK) complex, where it binds to germinal center kinase III members: mammalian sterile 20-like kinase 3 also known as serine/threonine kinase 24 (STK24),[69] suppressor of kinase 1 (STK25),[67] and mammalian sterile 20-like kinase 4(STK26)[66] (Figure 2). Loss of the CCM3 protein and consequent disruption of the STRIPAK complex leads to hyperactivation of Cdc42[69] accompanied by induction of cell migration,[67] alteration of cell polarity,[66] impaired ability to contain oxidative stress,[68] and abnormal exocytosis[62-64] (Table 2), which explains the increased secretion of angiopoietin-2 observed in vivo.[54] Although more studies are needed to dissect the consequences of *CCM3* loss-of-function mutations, it seems reasonable that these genetic defects would cause

Table 3. Proposed Drug Repurposing and Targeted Approaches

Study Type and Drug	Targets in CCM	Approved Application	Source
In vitro and in vivo			
Rapamycin analogues	mTor	Antineoplastic drugs	Marchi et al[70]
Sorafenib	ERK	Antiangiogenic drug	Wüstehube et al[50]
NVP	EphB4	None	You et al[51]
DMH1	TGFβ signaling: BMP	None	Maddaluno et al[49]
LY364947 SB431542	TGFβ signaling: SMAD	None	Maddaluno et al[49]
Sulindac	TGFβ signaling: β-catenin	Nonsteroidal antiinflammatory drug	Bravi et al[46]
TLR4-blocking agents	MEKK3-KLF2/4 down-stream of TLR4 signaling	Treatment of sepsis	Tang et al[71]
Fasudil	RhoA	Vasodilator, treatment of cerebral vasospasm	McDonald et al[72]
Simvastatin	RhoA	Statin, treatment of hypercholesterolemia	Whitehead et al[53]
Drug-screening platforms			
Vitamin D3	Potential effect on oxidative stress and/or inflammation	Vitamin supplement	Gibson et al[73]
Tempol	Potential effect on oxidative stress and/or inflammation	Antioxidant and anti-inflammatory drug	Gibson et al[73]
Bosutinib, saracatenib, danusertib, sunitinib, and desatinib	MST3/STK24	Antineoplastic drugs	Olesen et al[74]
Incidental findings and case reports			
Bevacizumab	VEGFA	Antiangiogenic drug	Aguilera et al[75]
Propranolol	Potential effect on angiogenesis	β-Adrenergic blocker, treatment of hypertension, and infantile hemangioma	Zabramski et al[76]
Clinical trials			
Simvastatin	RhoA	Statins, treatment of hypercholesterolemia	Not yet published
Atorvastatin	RhoA	Statins, treatment of hypercholesterolemia	Not yet published

Abbreviations: CCM, cerebral cavernous malformation; DMH1, dorsomorphin homolog 1; EphB4, ephrin receptor B4; ERK, extracellular-signal-regulated kinase; KLF, Kruppel-like factor; MEKK3, mitogen-activated protein kinase kinase kinase 3; MST3, mammalian sterile 20-like kinase 3; mTor, mammalian target of rapamycin; STK, serine/threonine kinase; TGFβ, transforming growth factor β; TLR4, toll-like receptor 4; VEGFA, vascular endothelial growth factor A.

additive effects because the CCM3 protein contributes to both the CCM and STRIPAK complexes. This would explain the extremely severe phenotype observed in patients with loss-of-function mutations of the *CCM3* gene.[11]

New Target Inhibitors and Drug Repurposing Undergoing Preclinical or Clinical Investigation

The biological and molecular bases of CCM suggest that specifically dysregulated pathways will yield therapeutic targets (summarized in **Table 3**).[46,49-51,53,70-76] From a broader perspective, the CCM proteins regulate biological processes whose dysregulation is also observed in cardiovascular diseases and cancer, namely decreased autophagy[70] paralleled by increased angiogenesis,[77] inflammation,[42] and oxidative stress.[78] The prevalence of these processes implies that specific inhibitors could be effective in the symptomatic treatment of CCM. This hypothesis is confirmed by preclinical studies in which defective autophagy was partially restored by pharmacological inhibition of mammalian target of rapamycin.[70] In addition, the hyperactivation of ERK[50] and EphB4 kinase[51] observed in CCM-related angiogenesis was reversed by treatment with the small-molecule inhibitors sorafenib[50] and NVP-BHG712 (NVP),[51] respectively. At the cellular level, EndMT acts as a driving mechanism in CCM lesion onset both in animal models and patients.[46-49] Consistently, pharmacologic inhibition of transforming growth factor β signaling with dorsomorphin homolog 1 (targeting bone morphogenetic protein), LY364947, SB431542 (targeting small mothers against decapentaplegic),[49] or sulindac (targeting β-catenin)[46] proved effective in reverting the CCM phenotype. The MEKK3-KLF2/4 axis was also successfully targeted with inhibitors of the innate immune receptor toll-like receptor 4, which was incidentally identified as upstream inducer of this signaling pathway in mouse models of gram-negative gut infections.[71] RhoA, whose hyperactivation in CCM has been reported in several in vitro and in vivo studies, is another successful target with the kinase inhibitor fasudil[72,79] and the indirect inhibitor simvastatin,[53] which normalized vascular permeability and decreased lesion number in transgenic mice. Some of the previously mentioned drugs are already on the market for other applications and could be repurposed with relative ease, namely the mTor inhibitors, sirolimus and everolimus, and the multikinase inhibitor, sorafenib, approved for cancer treatment; sulindac, a nonsteroidal antiinflammatory drug; toll-like receptor 4 inhibitors, originally developed for sepsis treatment; fasudil, a vasodilator used to treat cerebral vasospasm; and simvastatin, a lipid-lowering medication. In addition to these rationally designed approaches, other candidates for drug repurposing have emerged from screening studies that assessed marketed drugs in the context of CCM. For example, cholecalciferol (vita-

min D_3) and tempol (a free-radical scavenger) emerged from a combined in vitro–in vivo screening of 2100 molecules as efficient in reducing lesion burden in *Ccm2* transgenic mice.[73] Based on their known pharmacologic action, these compounds would be expected to control CCM progression by inhibiting inflammation and oxidative stress. In another study, 14 of 277 tested compounds were confirmed as inhibitors of MST3/STK24 kinase activity, 5 of which are already approved by the US Food and Drug Administration or in phase II/III clinical trials for cancer treatment (namely, bosutinib, saracatenib, danusertib, sunitinib, and desatinib).[74]

While several compounds are being investigated in preclinical studies, only a few pharmacologic agents have reached clinical testing. The potential efficacy of targeting angiogenesis in CCM is supported by 2 case reports: the observation of incidental resolution in a case of CCM on treatment with the anti–vascular endothelial growth factor A antibody, bevacizumab,[75] and lesion regression and reduction of symptomatic ICH in 2 patients with CCM on treatment with propranolol,[76] a β-adrenergic blocker and an antiangiogenic agent used to treat hypertension and infantile hemangioma.[80] Despite these encouraging observations, to our knowledge, there are no ongoing clinical trials to evaluate antiangiogenic drugs in CCM. The focus remains on repurposing statins in an attempt to restore physiologic vascular permeability in brain capillaries. Statins are approved for the treatment of hypercholesterolemia because they are powerful inhibitors of 3-hydroxy-3-methyl-glutarylcoenzyme A reductase, the rate-controlling enzyme in cholesterol synthesis. However, they also impair posttranslational modification of small GTPase proteins, such as RhoA, and have proven efficient in reverting the CCM phenotype in transgenic mice.[53] A first, randomized early phase I trial (NCT01764451) is an imaging investigation conducted by our group in New Mexico to study vascular permeability across the blood-brain barrier with dynamic contrast-enhanced magnetic resonance imaging. The primary outcome will evaluate whether simvastatin improves the blood-brain barrier integrity in patients with familial CCM1. The secondary outcome will correlate permeability data with new lesion formation or growth. A second, phase I/II randomized clinical trial (NCT02603328) is planned to evaluate the long-term effect of atorvastatin on lesion growth in patients with symptomatic (hemorrhagic) CCM. The results of these trials are not yet available.

Conclusions

Despite being a rare disease, CCM is highly prevalent in certain regions, such as New Mexico, and a substantial percentage of patients experience severe symptoms. While clinically silent lesions are left untreated, patients with symptoms, such as seizure, ICH, and/or FND, can consider surgical treatment options, but only in specific cases. Besides antiepileptic drugs, to which up to 40% of patients become refractory, no pharmacologic management of CCM has proven effective enough to be translated to the clinic (Figure 1). A strategy that addresses lesion growth, number, and inherent hemorrhagic potential is needed to alter the disease trajectory in both asymptomatic and symptomatic cases. Indeed, interfering with the natural disease course in CCM would drastically reduce risky surgeries, as well as provide an option to otherwise incurable patients.

We propose to exploit the growing knowledge on the molecular biology that underlies CCM to design patient-tailored therapeutics (Figure 2). Cerebral cavernous malformation proteins interact, either directly or indirectly, with several cellular partners. Cerebral cavernous malformation 1, CCM2, and CCM3 are components of the CCM complex, while CCM3 is peculiarly included into the STRIPAK complex. Loss of CCM proteins is associated with a disruption of such complexes and activates signaling pathways in brain ECs, eventually leading to lesion formation (Table 1 and Table 2). Blocking these dysregulated pathways with targeted inhibitors has proven therapeutic efficacy in animal models and require further investigation. Moreover, several pathologic features of CCM overlap with those observed in cardiovascular diseases and cancer, namely autophagy, angiogenesis, inflammation, and oxidative stress. These commonalities support the feasibility of drug repurposing. In this perspective, 2 pioneering and ongoing clinical trials are evaluating the efficacy of statins to control CCM progression (Table 3).

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