haematologica
Journal of the Ferrata Storti Foundation

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Received: August 16, 2024.
Accepted: October 29, 2024.

Citation: Christina Crossette-Thambiah, Anna M. Randi, and Michael Laffan.
von Willebrand disease and angiodysplasia: a wider view of pathogenesis in pursuit of therapy.
Haematologica. 2024 Nov 7. doi: 10.3324/haematol.2024.285244 [Epub ahead of print]

Von Willebrand Disease and Angiodysplasia: a wider view of pathogenesis in pursuit of therapy

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Disclosures
1. Christina Crossette-Thambiah (CCT)
Speaker fees from Roche and Chugai. Research funding from Astrazenca.
2. Anna M Randi (AM)
Nil
3. Michael Laffan (ML)
Consultation and speaker fees from AstraZeneca, BioMarin, Sobi, LEO Pharma, Takeda, and Pfizer

Author Contributions
CCT and AM wrote the manuscript. ML conceived and wrote the manuscript. All authors read and approved the final version of the paper.

Abstract

Bleeding in the GI tract continues to pose a therapeutic challenge for clinicians in patients with Von Willebrand Disease (VWD). It is associated with significant morbidity and mortality and represents the major unmet need in VWD. Defective angiogenesis in the gut is primarily responsible, resulting in angiodysplastic malformations making bleeding notoriously refractory to standard replacement therapy. A substantial body of evidence now shows that Von Willebrand Factor (VWF) has a role in the regulation of angiogenesis but the mechanisms responsible for the formation of vascular malformations remain incompletely understood. Data from the wider field of vascular malformations may lend insight and point to novel therapeutic approaches. Here we review evidence linking VWF to angiodysplasia, the associated molecular mechanisms and the implications for therapy.

Introduction

Von Willebrand disease is a hereditary bleeding disorder resulting from a deficiency of von Willebrand factor (VWF) function. The clinical bleeding manifestations are largely attributable to the loss of VWF adhesive function, platelet capture and the secondary reduction of FVIII procoagulant activity. Therapeutically the deficiency can be effectively corrected by the use of VWF concentrates, although it is recognised that platelet, endothelial and extravascular VWF are not replaced by this approach. Recent work has identified numerous additional roles for VWF in non-haemostatic processes including immunity, cell proliferation, bone formation and inflammation, but also a role in angiogenesis.(1, 2) Defective angiogenesis in the gut may give rise to vascular malformations (angiodysplasia) that are responsible for a bleeding tendency which is frequently refractory to replacement therapy and represents the principal unsolved therapeutic problem in VWD today. This is a narrative review linking VWF to angiodysplasia, the associated molecular mechanisms and the implications for therapy.

Von Willebrand Disease and Angiodysplasia

The epidemiology of VWD is often confusing. Although 2.5% of the population have a level of VWF function below the lower limit of normal, the point at which this becomes sufficient to cause abnormal bleeding is not agreed. It is usually estimated to be around 30 iu/dL but levels higher than this can nonetheless contribute to a bleeding tendency that may be multifactorial and in some schemes is also classified as VWD.(3) Registries have found the prevalence of gastro-intestinal bleeding (GIB) to be approximately 2.5 times higher in those with VWD compared to non-VWD cohorts.(4) In the general population, angiodysplasia is the most common vascular abnormality of the GI tract in those over 65, with studies showing 11% of this population also have a diagnosis of VWD.(5) Conversely, the reported prevalence of angiodysplasia in VWD is variable, ranging from 2-8% and noted to be higher with increasing age.(6) Angiodysplasia occurs most frequently in those with Type 2A (39%) and Type 3 (14.4%) VWD, the types characterised by loss of high molecular weight multimers (HMWM) of VWF, although it has been documented in all types.(7) Interestingly, a 2024 retrospective review of GI bleeding in VWD found arteriovenous malformation (AVM) to be the most predominant causative lesion, occurring in 35% patients.(8) This sets the problem of VWD-associated angiodysplasia as one not solely of haemostasis, but also one of vascular integrity and angiogenesis whose management may be best considered in parallel with other such vascular malformation syndromes.

The first suggestion that VWF might be responsible for vascular integrity and hence possibly involved in the pathogenesis of angiodysplasia emerged when the nail capillaries of VWD patients were found to show increased fragility, increased tortuosity, and defective contractility to trauma.(9) Later, video capillary microscopy of nailfold vasculature of 100 patients with VWD confirmed the presence of vascular abnormalities with changes in capillary dilatation, extravasates and capillary torquation.(10) The first mechanistic link between VWF and defective angiogenesis was provided by Starke et al. in 2011, who showed that inhibition of VWF expression in human umbilical vein endothelial cells (HUVEC) led to increased proliferation, migration and angiogenesis.(11) This was confirmed in endothelial colony forming cells (ECFC) sourced from VWD patients, although variability between patients has been reported.(12) *In vitro*, full length VWF was able to normalise angiogenesis; *in vivo*, lack of VWF in VWF null mice resulted in increased constitutive vascularity.(11) Xu et al. showed the opposite effect in mice lacking ADAMTS13, in a model of revascularisation after stroke, highlighting the importance of VWF HMWM for regulation of angiogenesis – a finding consistent with the epidemiology in VWD and acquired VW Syndrome (AVWS).(13) Most notably, AVWS with associated GI bleeding has been described in patients with left ventricular assisted devices (LVADs), aortic stenosis (Heyde's Syndrome) and most recently mitral regurgitation; where high shear stress facilitates cleavage of VWF by ADAMTS resulting in loss of HMWM.(14, 15) The AVWS is corrected

and bleeding stops when the cause of the pathological shear is removed.

Evidence that VWF plays an important role in vascular development and angiogenesis is apparent, but the mechanisms by which it regulates angiogenesis and the reason for dependency on HMWM remain unclear. As with non-VWD age- related angiodysplasia, its development only in certain individuals and the predominance in the gut also require explanation.

VWF and pathways regulating angiogenesis.

Like many other biological processes, angiogenesis is controlled by a balance of pro- and anti-angiogenic factors via crosstalk between several signalling pathways, the disruption of which can favour angiodysplasia. Data suggest that some of these effects only become significant when EC are also under the influence of various stressors such as age, muscular compression or inflammation and antigen exposure in tissues where EC express VWF. Such 'second hits' are more likely to be environmental in the context of VWD angiodysplasia, but it is possible that genetic second hits are also involved, as observed in many vascular abnormality syndromes.(16)

As an intracellular, matrix and plasma protein, VWF interacts with many molecules with the potential to regulate angiogenesis. Platelets, growth factors, matrix proteins and intracellular components are all implicated and whilst the contribution of some of these has been delineated, the picture remains incomplete.

1. Angiopoietin-Tie 2 pathway

Prior to release from EC, VWF is stored in organelles called Weibel-Palade Bodies (WPB) which are dependent on VWF for their formation. Numerous other proteins are also stored in WPB allowing VWF to modulate their release. Several of these proteins also bind to VWF and have roles in angiogenesis, most notably Angiopoietin 2 (Angpt2).

In the vasculature, the Angiopoietins (Angpt)- Tie2 system regulates the balance between quiescence and angiogenesis. Angpt1, released by smooth muscle cells and pericytes, promotes vascular homeostasis, stability and quiescence by binding to Tie2, a receptor tyrosine kinase critical for EC function, which signals via the PI3K/AKT/mTor pathway.(17) Angpt2 competes with Angpt1 for binding to Tie2, leading to vascular destabilisation, a precursor to new vessel formation. The effect of Angpt2 is synergistic with the proangiogenic pathway of Vascular Endothelial Growth Factor (VEGF) and its Receptor-2 (VEGFR2), promoting new vessel formation via EC proliferation and migration.(18) Conversely, when VEGF is absent or inhibited, Angpt2 can promote EC death and vessel regression.(17) Angpt2 also potentiates EC inflammatory responses, for example to the proinflammatory cytokine TNFα (19); this may be particularly relevant in the gut, given the high exposure of the gut-vascular barrier to antigens.(20) Interestingly, VWF binds to both Angpt1 and 2 but does not prevent either from interacting with Tie2 (21); similarly Angpt2 does not affect VWF-dependent platelet capture(22); thus the functional relevance of these interactions is still unclear.

In vitro studies showed that VWF-deficient cells release high levels of Angpt2.(11) This was not reflected in plasma, since a study on a large cohort of VWD patients showed normal plasma Angpt2 levels, but interestingly, significant differences in the levels of Angpt1 and VEGF were observed between VWD subtypes.(23) The reason for these findings in unclear; it is possible that released Angpt2 remains bound locally, or that increased release occurs only in specific vascular beds and therefore remains undetectable systemically.

2. Galectin-3

Galectin-3 (Gal-3) may also be implicated in VWF-dependent regulation of angiogenesis. Like

Angpt2, Gal- 3 is a WPB component which binds to VWF intra- and extra-cellularly. It interacts with both VEGFR2 and integrin αvβ3 to promote angiogenesis.(24) Saint-Lu et al noted that in VWF-deficient mice, plasma Gal-3 levels were reduced compared to wild-type and this was corrected following hydrodynamic VWF gene transfer.(25) Contrary to Angpt2, plasma Gal-3 was significantly higher in patients with GIB compared to bleeding at other sites, suggesting unique and distinct roles for these two mediators in VWD-associated angiodysplasia.(23)

3. Integrin αvβ3 and VEGF Receptor2 (VEGFR2)

αvβ3 integrin is the best-characterized endothelial receptor for VWF, expressed on endothelial cells (EC) and smooth muscle cells.(26) It is an obvious candidate for mediating the effect of VWF on angiogenesis because it is known to modulate VEGFR2 and VEGF-mediated increased EC proliferation via the PI3K/AKT/mTor, PLCγ/PKC/RAF/MEK/ERK and RAS/BRAF/MEK1/ERK pathways.(27) *In vitro* studies on VWF-deficient endothelial cells showed that β3 surface expression was decreased due to enhanced internalisation, indicating that VWF binding to αvβ3 stabilises its surface expression. Whether VWF binding to αvβ3 regulates downstream signalling and crosstalk with VEGFR2 signalling remains to be established. VWF can bind multiple angiogenic growth factors, including VEGF-A (see below)(28) and Angpt1-2 (see above). The ability of VWF to bind multiple receptors and ligands may result in VWF-dependent cross linking of cell-surface receptors such as αvβ3, Tie2 and VEGFR2, which would explain why its effects in angiogenesis are dependent on HMWM.(29) A role for VEGFR2 signalling in VWF-dependent angiogenesis is suggested by *in vitro*(11) and *in vivo* data.(13) A model of VWF-dependent crosslinking endothelial cell surface receptors involved in angiogenesis is shown in **Figure 1.**

4. Growth factor binding

Recently, VWF was found to bind a surprising number of growth factors, including pro-angiogenic growth factors VEGF-A, Placental GF (PlGF), Platelet derived GF (PDGF), basic Fibroblast GF (bFGF) and others.(28) The binding was mapped to a short peptide in the A1 domain of VWF, the Heparin Binding Domain (HBD), which overlaps with the site for interaction with platelet GPIb/IX. This is intriguing and raises the question of how VWF can sustain many multiple interactions in such a small region. Confusingly, the VWF HBD appears to have a pro-angiogenic effect in a model of wound healing, by recruiting growth factors to the site of the wound. It is possible that full length VWF and its fragments may have opposite effects. This dual role is not unique to VWF: for example αvβ3 itself can have both pro-angiogenic and anti-angiogenic effect.(26) Whether the role of HBD is dependent on the microenvironment is not known. More studies are required to understand the role of VWF in growth factors signaling and angiogenesis.

5. Other possible mechanisms

Other mechanisms which might be implicated in VWF regulation of angiogenesis include the VWF-LRP4- αvβ3 axis, in which the LDL- receptor related protein (LRP) was identified as a binding partner for VWF on VSMC resulting in VSMC proliferation via p38MAPK (mitogen activated protein kinase) activation.(30) VWF has also been shown to interact with insulin-like growth factor binding protein-7, another WPB component, that modulates angiogenesis via VEGF expression and signaling.(31)

6. VWF and angiogenesis mechanisms: a summary of possible pathways

To summarise, it is not yet possible to provide a complete explanation for the development of angiodysplasia in VWD, but several elements are clear:

•Loss of VWF leads to increased Angpt2 release from EC; increased Angpt2 signaling (depending on

context) promotes angiogenesis by synergising with VEGF signaling; increased Angpt2 likely mimics and/or accentuates inflammaging in the gut.
•VWF binding to αvβ3 on EC surface could modulate VEGFR2 signaling; VEGFR2 activity is increased in VWF-deficient EC.
•The effects of VWF on angiogenesis are at least partly dependent on HMWM
•A fragment of VWF (HBD) interacts with multiple growth factors and is required to recruit growth factors at the site of skin wound healing.
•Platelets store multiple regulators of angiogenesis, both promoters and inhibitors of the process; hence are very likely to play an important role in VWF-dependent angiogenesis which as yet has not been delineated.

Why the gut? Local mechanisms for angiodysplasia

Local factors are likely to explain why the gut is the predominant site for angiodysplasia in patients with and without VWD. Firstly, chronic exposure to high levels of antigen may result in endothelial activation. Although the prevailing dogma is that vascular endothelial cells are quiescent in the adult, recent data indicates that the gut microvasculature is more prone to angiogenesis, possibly because of the oxygen gradient present in the intestinal villi.(32) Secondly, ageing is associated with a loss of pericytes in the murine colon vasculature; this has been attributed to upregulation of Angpt2 production in EC in response to increased macrophage infiltration and TNFα production (inflammaging).(33) The resulting increase in Angpt2-Tie2 signaling leads to loss of pericytes, vascular instability and leakiness. These effects could be accentuated in VWD as described above.

Secondly, vascular instability may be compounded by the repeated muscular compression of the gut, where increased contractility of the muscular propria can cause congestion and failure of the precapillary sphincters.(34) It can also produce a chronic hypoxic state triggering the release of proangiogenic factors such as VEGF. Mechanical and inflammatory factors are both consistent with the increased frequency of angiodysplasia in old age. Emerging data from single cell RNA sequencing of gut endothelium will allow analysis of VWF expression in endothelial subtypes in the gut.(35)

The possible importance of dysregulated angiogenesis at other sites in VWD is largely unexplored. For example, abnormal vasculature may contribute to the high frequency of heavy menstrual bleeding (90%) and slightly increased miscarriage rate in women with VWD.(36) Angiodysplasia in the uterus has been demonstrated in non-pregnant VWD Type 3 pigs with decrease of integrin $\alpha_V\beta_3$ and increase in VEGF expression.(37) Porcine models of VWD Type 1 have revealed significant alterations in VEGF/VEGFR-2 signalling and $\alpha_V\beta_3$, Angpt1, Angpt2 and Tie2 expression during placentation compared to wild-type. (38)

Parallels with other vascular abnormality syndromes

Angiodysplasia is regarded as an acquired disorder, but there are numerous congenital disorders of vessel formation whose genetic basis may help understand angiodysplasia in VWF and help develop therapy. Some also show progression with age. The variable phenotypes suggest interaction with other genetic variants, which would help explain why only some patients with VWD develop angiodysplasia. In some cases, a somatic second hit mutation is required for phenotypic expression. These factors have not been explored in VWD; the mutations involved reveal key signalling pathways and the pathophysiological basis of the associated angiogenic malformations.

1. TGF-β, BMP9-10/ENG/ALK1/SMAD4 pathway

Hereditary haemorrhagic telangiectasia (HHT) is a vascular abnormality syndrome which has parallels with VWF-angiodysplasia. Much like VWD, telangiectasias develop progressively into adulthood in the gastrointestinal mucosa with 25% of patients suffering from GI bleeding after age 50. Variants in *ENG* (Endoglin), *ACVRL1* (ALK1) and rarely *SMAD4* are responsible for HHT and reduce signaling via the transforming growth factor (TGF)-β signaling pathway causing increased EC migration, proliferation and vessel development. There are overlaps between these pathways and those associated with VWF: for example TGF-β has been shown to preserve vascular stabilization via Angpt1/Tie2 and VEGF/VEGFR2 signaling in HUVECs.(39) Furthermore, Angpt2 inhibition was able to alleviate AVM formation in an *in vivo* model of HHT.(40)

2. RAS/RAF/MEK/ERK pathway

Parkes-Weber Syndrome is a rare capillary malformation syndrome observable as cutaneous flat lesions overlying AVMs with bone and soft tissue overgrowth. Transmission is in an autosomal dominant manner, caused by mutation in *RASA1,* which gives rise to prolonged RAS/MAPK/ERK signalling**.** Vascular malformations related to the RAS signalling pathway or 'RASopathies' are varied and generally dissimilar from angiodysplasia. Nonetheless, VEGF and Angpt1 can stimulate the RAS-MAPK pathway in EC, indicating possible shared molecular pathways with VWD vascular abnormalities.(41)

3. PI3K/AKT/mTOR signalling pathway

Naturally occurring *TEK* mutations causing AVM, such as Klippel-Trenaunay Syndrome (KTS), lead to ligand-independent hyperphosphorylation of the receptor and a permanent activation of the PI3K/AKT/mTOR signalling pathway.(16) Interaction between Angpt and Tie2 is central to the activation of the PI3K/AKT/mTOR pathway: Angpt1 binding to Tie2 (encoded by *TEK*) results inPI3K/AKT/mTOR activation. A similar effect may occur in HHT, where reduced signalling via TGFβ /ALK1 pathway leads to loss of its inhibitory action on AKT/mTOR.

8. Therapies and healthcare impact

VWF replacement therapy is effective for most bleeding in patients with VWD but notably less so for GI bleeding and often has little impact when angiodysplasia is present.(42) This experience comes largely from use of plasma derived VWF concentrates in which the multimer composition is degraded to a variable extent.(43) Given the importance of HMW multimers to haemostasis and to vascular integrity, recombinant VWF which contains ultra-large (UL) VWF might be more effective. To date there is a single case report in the literature documenting its successful use in a patient with Systemic Sclerosis and (acquired) Type 2A VWD and GI bleeding unresponsive to plasma derived VWF/FVIII concentrates.(44)

For many years, thalidomide has been the most frequently used and effective anti-angiodysplasia agent. In 2023 a multicentre, double-blind, randomised, placebo-controlled trial demonstrated a significant reduction in bleeding episodes with 100mg or 50mg thalidomide daily compared to placebo.(45) Thalidomide's efficacy has been attributed to suppression of VEGF and possibly TNFβ expression.(46) In the context of VWD angiodysplasia, a further benefit might be derived by its capacity to increase VWF HMWM via inhibition of the degradation of thrombospondin-1 which promotes VWF multimerization.(47)

Bevacizumab, the monoclonal antibody against VEGF, has been efficacious in two cases of GIB associated with AVWS and Heyde's syndrome (48, 49) as well as in a single institutional study of 5 cases of AVWS secondary to LVAD (Left ventricular assist device) insertion.(50) Bevacizumab has been used more widely in HHT and has demonstrated benefit by increasing Hemoglobin and reducing transfusion requirement in the international multicentre INHIBIT-BLEED HHT randomised control trial.(51)

Based on our current knowledge, perhaps the most likely novel therapies that may be of value for VWD patients with angiodysplasia target the VEGFR2 and Angpt/Tie2 pathways, and include antibodies against Angpt-2 alone as well as those capable of neutralising both Angpt2 and VEGF simultaneously, such as the bispecific monoclonal antibody Faricimab which has received approval in retinal vascular diseases. (52) In addition, the anti-angiogenic tyrosine kinase inhibitor Pazopanib has been reported to benefit three patients with VWD angiodysplasia.(53)

Considering the multiple overlapping routes to vascular malformations, parallels with other vascular abnormality syndromes may point to therapeutic alternatives in VWD **(Figure 2).** In HHT, thalidomide's undesirable side effect profile has led to its analogue Pomalidomide being very recently investigated in the PATH-HHT study with encouraging outcomes.(54) The PI3K/AKT/mTOR signaling pathway provided the first genetic targeted therapy for vascular malformations, rapamycin, which has shown efficacy in various vascular malformation syndromes. mTOR is downstream of the activating *TEK* mutations causing KTS and as predicted rapamycin has shown efficacy in KTS.(55) Since this pathway might also be downstream of VWF via modulation of Angpt2-Tie2 signalling, this drug might also be of benefit in VWD. However, more data in VWD-related models is required to confirm this, before its use in patients could be advised. Interestingly, the PI3K/AKT/mTOR pathway has also been shown to be enhanced HHT and novel agents such as Apselisib and Miransertib, targeting PI3K and AKT inhibition respectively, are currently in development.(16)

The emerging role of the RAS/MAP kinase pathway also provides potential for targeted therapy. Trametinib is an oral inhibitor of MEK1 and 2 by blocking Erk phosphorylation. A prospective phase 2 trial TRAMAV investigating Trametenib in refractory AVM is underway.(56)

Overall, the vascular malformation field has delivered numerous novel treatments based on definition of the molecular pathways underlying the disorders; these hold great promise for translation to angiodysplasia and GI bleeding in VWD. Given the complex web of molecular pathways, it also highlights the need to validate the role of these potential targets in the development of VWF-dependent angiogenesis and angiodysplasia before they can safely translate to patients.

Although it is not yet possible to predict whether any of these novel agents will provide the answer to the challenge of intractable GI bleeding from VWD-associated angiodysplasia, the crucial advance has been to understand that the role of VWF is more than simply haemostatic. By broadening our view of this complex molecule's important roles to include regulation of angiogenesis we can utilise understanding from a number of other areas to pursue targeted therapy beyond simple replacement. The frustration of physicians, individual patients enduring prolonged and recurrent inpatient admissions as well as a large economic burden all provide encouragement in pursuit of this goal.(57)

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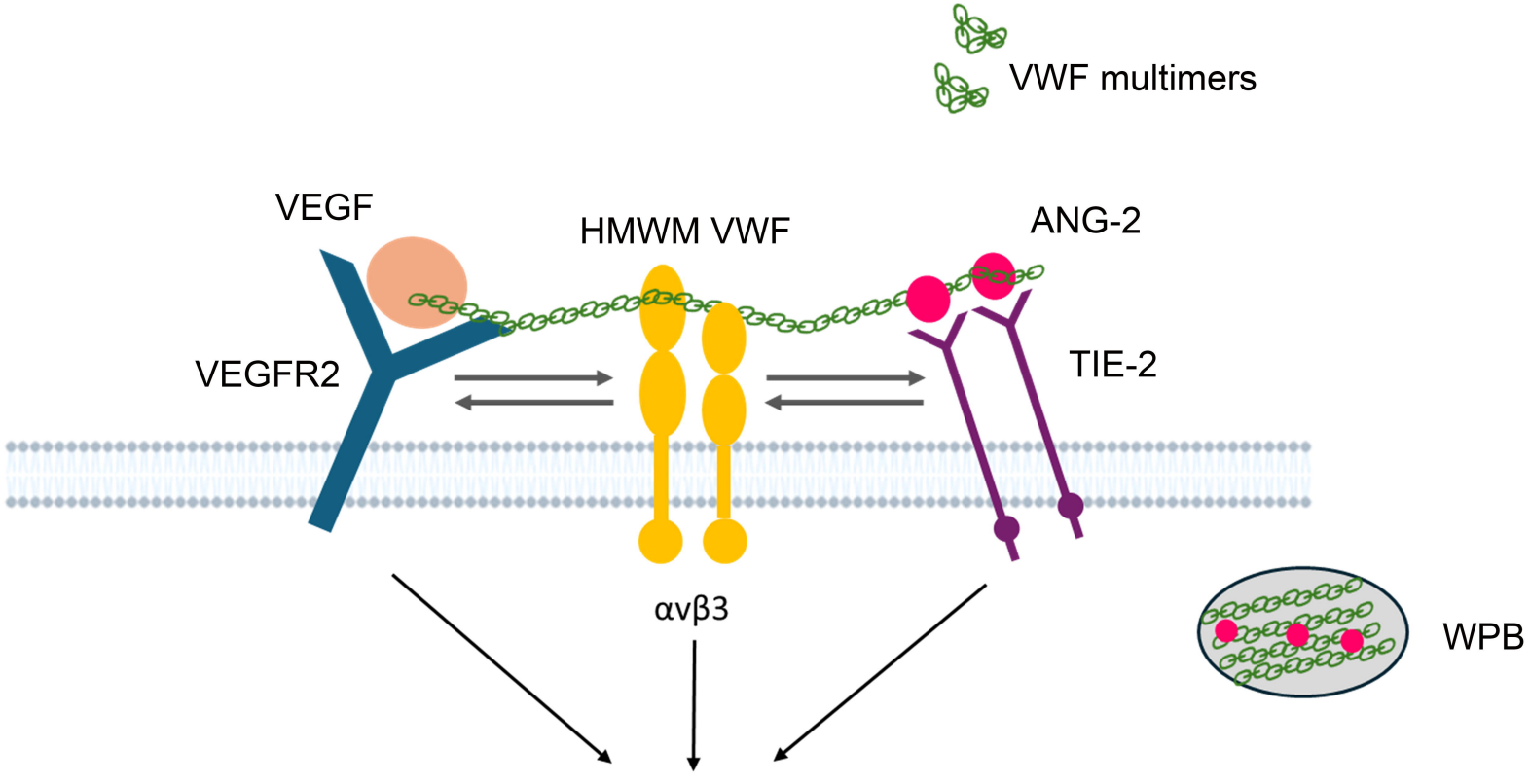
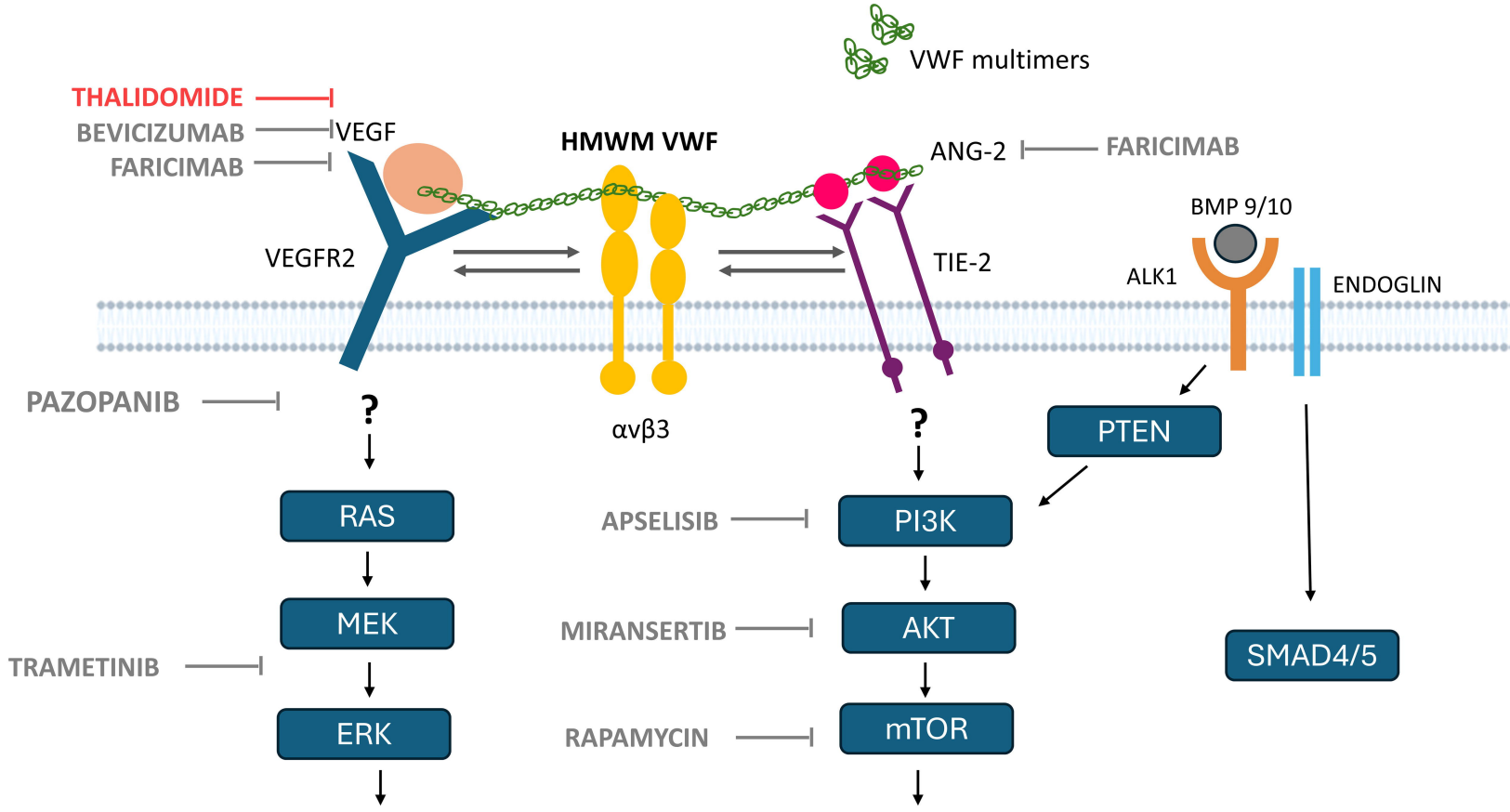
Figure Legends

Figure 1: Model of Von Willebrand Factor (VWF) regulation of Angiogenesis. VWF is likely to modulate angiogenesis via various pathways. VWF is critical to formation of Weibel Palade Bodies (WPB) that store the growth factor Angiopoetin-2 (Angpt2). Loss of VWF leads to increased Angpt2 release from EC (endothelial cells) and subsequent increased Angpt2 signaling. Upon its release, Angpt2 can bind tyrosine kinase receptor Tie2 which promotes angiogenesis by synergising with Vascular Endothelial Grown Factor (VEGF) signaling. VWF can also bind to integrin αvβ3 on endothelial cell surface which modulates Vascular Endothelial Grown Factor (VEGFR2) signaling downstream. However, loss of VWF decreases αvβ3 surface expression which might modify sensitivity to VEGF/VEGFR2 signaling. The ability of VWF to bind multiple receptors and ligands may result in VWF-dependent cross-linking of cell surface receptors αvβ3, Tie2 and VEGFR2, which may explain why its effects in angiogenesis are dependent on HMWM (high molecular weight multimers).

Figure 2: Therapeutic targets in vascular malformation syndromes with possible relevance to Von Willebrand Disease (VWD) Angiodysplasia: There appear to be multiple overlapping routes to vascular malformations which may help to identify overlaps between VWD and other vascular abnormality syndromes. Von Willebrand Factor's (VWF) place in this web of molecular pathways remains unclear but may point to therapeutic alternatives in VWD. Figure 2 shows current therapeutic alternatives which have targets within established VWF pathways (red) and those within pathways of other established vascular malformation syndromes (grey). HMWM : high molecular weight multimers, Angpt2 : Angiopoetin-2, Tie2: tyrosine kinase receptor, VEGF : Vascular Endothelial Grown Factor, VEGFR2 : Vascular Endothelial Grown Factor, PI3K/AKT/mTor: phosphoinositide 3 kinase/serine-threonine protein kinase/mammalian target of rapamycin, RAS/MEK/ERK: Rat sarcoma/ Mitogen-Activated Protein Kinase Kinase 1 (MAPKK1)/ extracellular signal-regulated kinase, BMP: Bone morphogenetic protein, PTEN: phosphatase and tensin homolog, ALK1: Activin receptor-like kinase 1, SMAD: mothers against decapentaplegic homolog.


VWF multimers
VEGF
HMWM VWF
ANG-2
VEGFR2
TIE-2
αvβ3
WPB
EC Proliferation, Migration and Angiogenesis


VWF multimers
THALIDOMIDE
BEVICIZUMAB
FARICIMAB
VEGF
HMWM VWF
ANG-2
FARICIMAB
BMP 9/10
VEGFR2
TIE-2
ALK1
ENDOGLIN
PAZOPANIB
?
αvβ3
?
PTEN
RAS
APSELISIB
PI3K
MEK
MIRANSERTIB
AKT
SMAD4/5
TRAMETINIB
ERK
RAPAMYCIN
mTOR
EC Proliferation, Migration and Angiogenesis