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### Authors

Nguyen, Amy  
Hoang, Van  
Laquer, Vivian  
et al.

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## Angiogenesis in cutaneous disease: Part I

Amy Nguyen, MD,<sup>a</sup> Van Hoang, BS,<sup>b</sup> Vivian Laquer, BS,<sup>c</sup> and Kristen M. Kelly, MD<sup>a,b</sup>  
Irvine, California, and New York, New York

Angiogenesis is an important process in normal physiology and disease pathogenesis. Angiogenesis is controlled in a healthy body by a system of angiogenic growth factors and angiogenesis inhibitors. When angiogenic growth factors are predominantly expressed, blood vessel growth occurs and disease may result. Successful therapies have been developed that target growth factors, their receptors, or the cascade pathways that are activated by growth factor/receptor interactions. There is good evidence that angiogenesis plays an important role in a wide range of cutaneous maladies, and angiogenesis-targeting therapies are playing an increasing role in the management of dermatologic disease. Cutaneous angiogenesis offers an exciting new arena for targeted dermatologic therapeutics. (J Am Acad Dermatol 2009;61:921-42.)

**Learning objectives:** After completing this learning activity, participants should be able to distinguish angiogenic growth factors and inhibitors, recognize angiogenic mediating agents and compare their mechanisms of action, and apply the use of angiogenic mediating agents in clinical and research situations.

**Key words:** angiogenesis; antiangiogenic agents; dermatology.

Angiogenesis is the growth of new blood vessels from preexisting ones. It is a normal process in growth, development, the female reproductive cycle, and wound healing. However, it is also a key element in disease pathogenesis.<sup>1</sup> We will discuss angiogenesis as it relates to cutaneous disease. First, we will review the process of angiogenesis and angiogenesis regulation. This will include a brief description of angiogenic growth factors and inhibitors that have been shown to have a role in cutaneous disease and the key cascade pathways that are activated by growth factor/receptor interactions. We will then describe therapeutic agents developed to inhibit or promote angiogenesis. These introductory sections will provide a basis for our discussion of angiogenesis in cutaneous diseases, which will be included in part II of our review. It is our intent that review and discussion of angiogenesis in cutaneous disease will foster further innovative research on this important topic, resulting

in a better understanding of disease pathogenesis and novel and effective dermatologic therapeutics.

### THE PROCESS OF ANGIOGENESIS IN DISEASE

#### Key points

- **Angiogenesis involves a series of defined steps**
- **Angiogenesis is induced when there is an imbalance of angiogenic growth factors compared to angiogenesis inhibitors**
- **The phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway plays an important role in angiogenesis**

Angiogenesis is composed of several distinct steps (Fig 1).<sup>2,3</sup> Diseased or injured tissues up-regulate the expression of angiogenic growth factors and release these products to adjacent tissues. Growth factors bind to specific receptors located on nearby endothelial cells of preexisting blood vessels, resulting in cell activation. Signals are sent from the cell's surface to the nucleus. Endothelial cells then begin to proliferate and secrete proteases (matrix metalloproteinases [MMPs] which are discussed later) to migrate toward the injured/diseased tissues to degrade the existing vessels' basement membranes. As tracks are formed, endothelial cells differentiate, divide, and migrate. Remodeling occurs in tissues adjacent to the vessels. Endothelial cells eventually anastomose into hollow tubes, produce a new basement membrane, and secrete growth factors to attract supporting cells like pericytes, which stabilize the new vessels.

From the Department of Dermatology,<sup>a</sup> University of California, Irvine, and the University of California Irvine College of Medicine,<sup>b</sup> Irvine, and the Columbia University College of Physicians,<sup>c</sup> New York.

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Correspondence to: Kristen M. Kelly, MD, Department of Dermatology, University of California, Irvine, 1002 Health Sciences Rd, Irvine, CA 92612. E-mail: kmkelly@uci.edu.

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**MATRIX METALLOPROTEINASES****Key points**

- **MMPs are enzymes that digest matrix components and play a role in tissue remodeling**
- **MMPs are thought to be important in tumor growth and metastasis**

MMPs are a family of soluble and membrane-anchored proteinases that collectively degrade components of the extracellular matrix to permit the formation of new blood vessels. MMPs are coded by 24 distinct genes.<sup>4</sup> The enzymes in this family are adapted for digestion of almost every known matrix component.<sup>4</sup> Most MMPs are made only upon demand and in low levels. MMPs have important functions in normal processes, such as development, reproduction, and wound healing, but they are also involved in pathologic angiogenesis, tumor growth, and metastasis.<sup>5</sup> Several MMPs have been linked to angiogenesis, most notably MMP-2, MMP-9, and membrane-type-1 MMP.<sup>6</sup>

**Angiogenesis regulation**

When there is an imbalance of angiogenic growth factors compared to angiogenesis inhibitors, angiogenesis is induced. Table I lists many known angiogenic growth factors and angiogenesis inhibitors. We discuss key angiogenic growth factors and inhibitors involved in cutaneous disease later in this article. We then provide information on other elements with apparent important roles in cutaneous angiogenesis: the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway and hypoxia-inducible factor 1 (HIF-1). Understanding of these key factors and pathways will help provide insight into the role of angiogenesis in cutaneous disease and perhaps most importantly, may provide ideas for targeted therapy.

**ANGIOGENIC GROWTH FACTORS****Vascular endothelial growth factor****Key points**

- **Vascular endothelial growth factor (VEGF) is thought to be the most potent and predominant angiogenesis stimulator**

- **VEGF binds to tyrosine kinase receptors**
- **VEGF effects are mediated through multiple pathways**

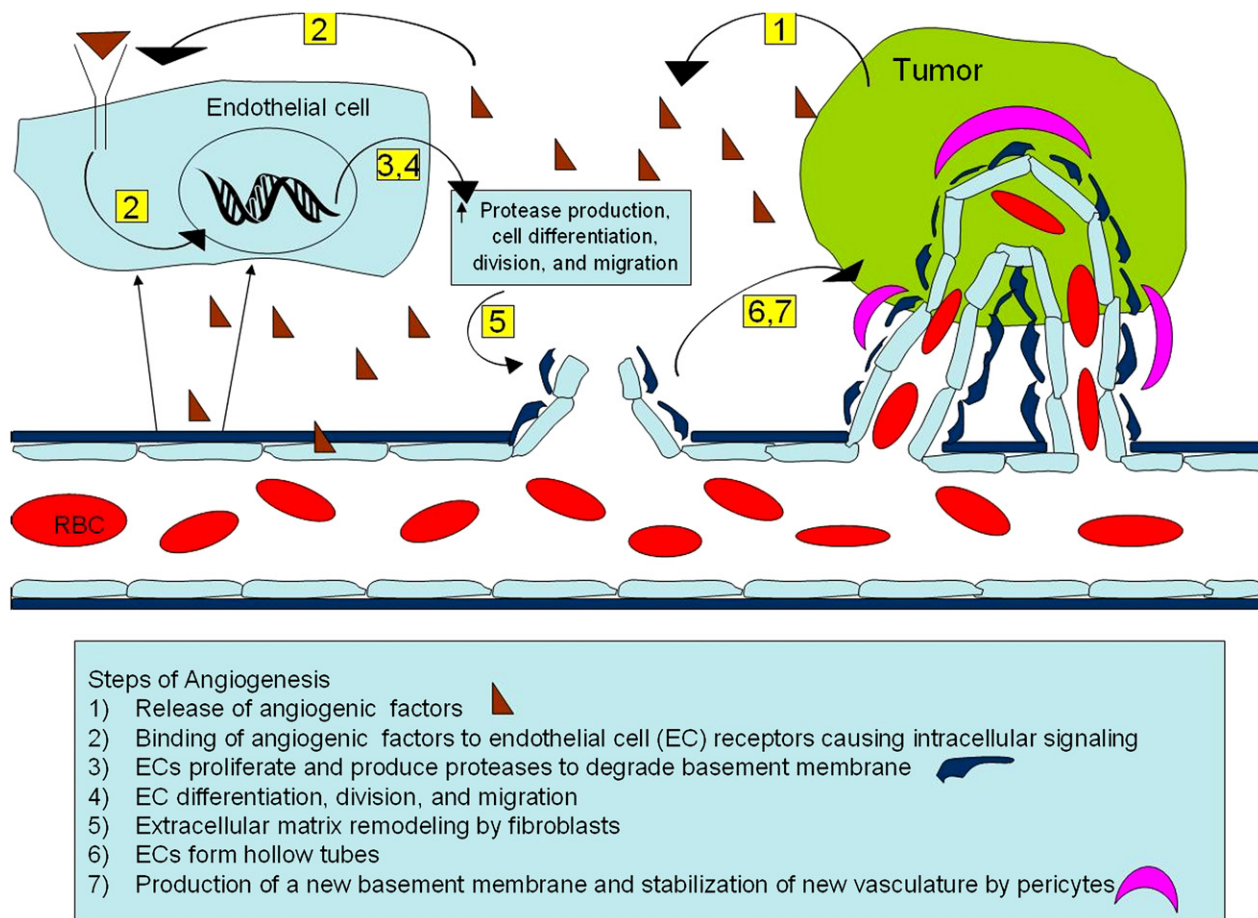
VEGF, also referred to as VEGF-A, is a key factor in both physiologic and pathologic angiogenesis. It is estimated that up to 60% of human cancer cells express VEGF, and many of the angiogenesis inhibitors that are approved by the US Food and Drug Administration (FDA) are aimed at neutralizing VEGF or its receptors. We discuss VEGF first because it is believed to be the most potent and predominant angiogenesis stimulator and it appears to play a key role in angiogenesis related cutaneous disease. VEGF belongs to a family of six structurally related proteins, which include VEGF-B, VEGF-C, VEGF-D, and VEGF-E and placental growth factor.<sup>7</sup> The various VEGFs are closely related; they originate from the same gene and are produced by alternate splicing.<sup>8</sup>

All members of the VEGF family stimulate cellular responses by binding to VEGF receptors (VEGFRs) on cell surfaces. VEGF binds to two known receptors, flt-1 (VEGFR1) and kdr (VEGFR2), which are tyrosine kinase receptors (RTKs).<sup>9-11</sup> The angiogenic effects of VEGF are mediated primarily through VEGFR2.<sup>7</sup> Neuropilin, a binding molecule involved in neuronal guidance, has been shown to enhance the effectiveness of VEGFR2 signaling when coexpressed in the same cell.<sup>12</sup> VEGFR3 is also an RTK, but it binds to VEGF-C and VEGF-D and is implicated in lymphangiogenesis.<sup>7,13</sup> The RTKs stimulate a cascade using secondary messengers to produce factors that stimulate vessel formation.

The primary target of VEGF is the endothelial cell. VEGF effects are mediated through multiple pathways. VEGF's antiapoptotic activity has been linked to the PI3K/Akt pathway and mitogen-activated protein kinase (MAPK).<sup>14,15</sup> VEGF has many activities and functions, including the following: (1) increasing endothelial cell migration, endothelial cell mitoses, and vascular permeability; (2)

**CAPSULE SUMMARY**

- Angiogenesis is a complex process involving angiogenesis growth factors, angiogenesis inhibitors, and the phosphatidylinositol 3-kinase/Akt/mammalian target of the rapamycin (mTOR) pathway.
- Antiangiogenic therapy falls into three main categories: (1) monoclonal antibodies directed against specific angiogenesis growth factors and/or their receptors; (2) small-molecule tyrosine kinase inhibitors; and (3) agents with other mechanisms of action, including the inhibitor of mTOR.
- Antiangiogenic therapy has been used mostly in oncology and ophthalmology.
- The use of antiangiogenic therapy in dermatology is new, but several clinical trials suggest a potential role for these agents to be used in combination with or as an alternative to conventional therapy.



**Fig 1.** Steps in angiogenesis.

promoting chemotaxis for granulocytes and macrophages; and (3) indirectly promoting vasodilatation via nitric oxide release.<sup>7</sup> VEGF has also been shown to regulate blood vessel diameters when binding to VEGFR2.<sup>16</sup> Low concentrations of VEGF promote long and thin vessels, while higher concentrations result in increased vessel diameters.<sup>16</sup>

### Angiopoietin

#### Key points

- **The binding of angiopoietin 1 (Ang1) to Tie2 activates the receptor through autophosphorylation and promotes endothelial cell migration and survival**
- **Ang2 may function as an antagonist to Ang1**

In 1996 and 1997, Ang1 and Ang2 with their corresponding Tie2 receptor were identified as a second family of endothelial specific RTKs that is distinct from the VEGF/VEGFR system.<sup>17-19</sup> There are actually four angiopoietins (Ang1, Ang2, Ang3, and Ang4), but Ang1 and Ang2 are the best characterized. Ang1 is expressed in pericytes, smooth muscle cells, fibroblasts, and some tumor

cells. By contrast, Ang2 is almost exclusively expressed by endothelial cells and is also detectable in Kaposi sarcoma (KS) cells.<sup>20</sup> Both Ang1 and Ang2 bind to the RTK Tie2, which is expressed on the surface of endothelial cells. Binding of Ang1 to Tie2 activates the receptor through autophosphorylation and promotes endothelial cell migration and survival. Unlike VEGF, Ang1 does not directly induce endothelial cell proliferation.<sup>21</sup> Evidence suggests that the Ang1/Tie2 system is important for endothelial cell survival by signaling pathways that inhibit endothelial cell apoptosis and maintaining resting endothelium. By contrast, Ang2 binding to Tie2 does not activate the receptor. Ang2 may function as an antagonist to Ang1.<sup>22</sup> Interestingly, in the absence of VEGF, Ang2 contributes to vascular regression; but in the presence of VEGF, Ang2 may stimulate angiogenesis.<sup>23</sup>

### Basic fibroblast growth factor

#### Key points

- **Basic fibroblast growth factor (bFGF) is produced by keratinocytes and acts as a mitogen for both endothelial cells and keratinocytes**

**Table I.** Angiogenic growth factors and inhibitors\*

Angiogenic growth factors	Angiogenesis inhibitors
Angiogenin	Anastellin
Angiopoietin-1	Angioarrestin
Del-1	Angiostatin
Fibroblast growth factors	Antiangiogenic antithrombin III
Granulocyte colony-stimulating factor	CD59
Hepatocyte growth factor	Chondromodulin
Interleukin-8	Endostatin
Leptin	Heparinases I and III
Midkine	Human chorionic gonadotropin
Placental growth factor	Interferon alfa/beta/gamma
Plasminogen activator inhibitor-1 (low concentrations)	Interferon-inducible protein-10
Platelet-derived endothelial cell growth factor	Interleukin-12
Platelet-derived growth factor	Kringle 5
Pleiotrophin	2-methoxyestradiol
Progranulin	Placental ribonuclease inhibitor
Proliferin	Plasminogen activator inhibitor-1 (high concentrations)
Transforming growth factor-alfa	Proliferin-related protein
Transforming growth factor-beta	Retinoids
Tumor necrosis factor-alfa	Tetrahydrocortisol-S
Vascular endothelial growth factor	Thrombospondin-1
	Tissue inhibitors of matrix metalloproteinases
	Troponin
	Vasculostatin
	Vasostatin

\*Please note that this is only a partial list.

### • bFGF has been linked to the pathogenesis of many cancers, including malignant melanoma

Formerly known as fibroblast growth factor (FGF)-2, bFGF belongs to a family of 22 known FGFs that bind to heparin. These FGFs have been found across different species and have very diverse roles, including angiogenesis, cell proliferation, plasminogen activation, integrin expression, cell migration, embryonic development, and cell differentiation.<sup>24</sup>

bFGF was one of the first angiogenic factors to be isolated.<sup>25</sup> bFGF is the primary FGF expressed in the skin, and it is produced by keratinocytes.<sup>26</sup> In normal skin, bFGF immunoreactivity has been noted to be confined to the upper layers of the epidermis; it was expressed in all epidermal layers in benign and malignant neoplasms.

bFGF acts as an endothelial cell mitogen, inducing tube formation and protease production.<sup>27</sup> It also acts as a mitogen for keratinocytes and may stimulate epithelialization.<sup>28</sup> bFGF has been noted to increase both epithelialization and capillary infiltration in dermal wound healing.<sup>29,30</sup> bFGF is implicated in the pathogenesis of various cancers.<sup>31</sup> Melanoma

cells are well known to secrete bFGF, and the inhibition of bFGF synthesis has been shown to inhibit tumor growth in melanoma cells.<sup>32,33</sup>

### Interleukin-8 Key point

- **Interleukin-8 is a proinflammatory and proangiogenic factor that binds to G protein–coupled receptors, activating the downstream pathway of PI3K/Akt and MAPK to control cell survival, angiogenesis, and migration**

Interleukin-8 (IL-8), also referred to as CXC chemokine ligand-8 (CXCL8), is a proinflammatory chemokine found in monocytes, macrophages, and other nonimmune cells.<sup>34</sup> IL-8 binds with high affinity to two receptors, CXCR1 and CXCR2, but these G protein–coupled rhodopsin-like receptors are not specific and can bind to other ligands in addition to IL-8.<sup>35</sup> Like many other proangiogenic factors, IL-8 is thought to activate downstream pathways involving PI3K/Akt and MAPK, modulating cell survival, angiogenesis, and cell migration.<sup>34</sup> Treatment with ABX-IL8, a neutralizing antibody against IL-8, has been shown to have antiangiogenic effects and promotes tumor cell death in mice.<sup>36</sup>

### **Platelet-derived growth factor**

#### **Key points**

- **Platelet-derived growth factor (PDGF) has been shown to play an analogous role to VEGF in nonmammalian vertebrates and is crucial in early mammalian embryogenesis and organogenesis**
- **PDGF has also been shown to promote dermal wound healing and is strongly linked to fibrotic diseases, such as scleroderma**

PDGFs consist of dimers constructed by combinations of the four isoforms (PDGF-A, -B, -C, and -D). Likewise, its receptor PDGFR has two isoforms ( $\alpha$  and  $\beta$ ). Like VEGF, PDGF activates pathways that involve PI3K, Akt, mTOR, and MAPK.<sup>37</sup> PDGFs belong to the same gene family as VEGF.<sup>38</sup>

PDGF has been shown to play an analogous role to VEGF in nonmammalian vertebrates and is crucial in early mammalian embryogenesis and organogenesis in early development.<sup>39</sup> It is unclear whether PDGF has normal physiologic expression in human adults or whether it only appears in disease.<sup>39</sup> PDGF expression can be induced by several factors under conditions of inflammation and hypoxia, including transforming growth factor- $\beta$  (TGF $\beta$ ), estrogen, IL-1 $\alpha$ , bFGF, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), and lipopolysaccharide.<sup>40</sup> PDGF up-regulates VEGF mRNA expression, and this may be the mechanism for its involvement in vascular diseases.<sup>7</sup> PDGF has also been shown to promote dermal wound healing and it is required by pericytes, which stabilize normal and tumor vasculature.<sup>37,41</sup>

Autocrine PDGF stimulation is seen in some human cancers.<sup>39</sup> PDGF and its receptors have also been strongly linked to fibrotic diseases, including scleroderma, where expression was mainly concentrated around blood vessels.<sup>42,43</sup> Receptors for both PDGF-A and -B are expressed in KS spindle cells, and PDGFRs have been associated with KS, both in the induction of spindle cell growth and in angiogenesis.<sup>37,44</sup>

### **Transforming growth factor- $\beta$**

#### **Key point**

- **TGF $\beta$  normally acts as a tumor suppressor but can promote tumor angiogenesis when aberrantly expressed**

TGF $\beta$  is a pleiotropic cytokine involved in angiogenesis, maintaining epithelial homeostasis, regulation of cell proliferation, differentiation and migration, and immune surveillance.<sup>45,46</sup> The consensus in the available literature is that TGF $\beta$  has differing and contradicting roles in carcinogenesis, whereby it is normally a tumor suppressor but

becomes involved in tumorigenesis when aberrantly expressed. In a study of breast cancer, it was suggested that TGF $\beta$  exerted its influence via tumor angiogenesis rather than regulating apoptosis.<sup>47</sup> TGF $\beta$  may promote tumor angiogenesis by inducing the expression of IL-8 and VEGF.<sup>45</sup>

### **Tumor necrosis factor- $\alpha$**

#### **Key point**

- **TNF $\alpha$  activates B cells and the MAPK pathway to induce upregulation of other angiogenic factors, including IL-8, VEGF, and MMPs**

TNF- $\alpha$  is a 157-amino acid polypeptide isolated from macrophages that have both physiologic and pathologic roles.<sup>48</sup> TNF $\alpha$  has two receptors: TNFR-1 and TNFR-2. Its main receptor TNFR-1 is part of the death receptor family, and TNFR-2 can indirectly mediate cell death via TNFR-1.<sup>49</sup> TNF $\alpha$  is also known to activate the nuclear factor kappa light chain-enhancer of activated B cells and MAPK pathways.<sup>50,51</sup> Although the role of TNF $\alpha$  as a tumor growth promoter or inhibitor is controversial, its involvement in tumor angiogenesis, invasion, and metastasis has been well established and is thought to be related to its ability to induce IL-8, VEGF, and MMPs.<sup>48</sup> TNF $\alpha$  has been noted to be a potent inhibitor of endothelial cell growth in vitro but a proangiogenic factor in vivo, inducing capillary blood vessel formation in rat cornea.<sup>52,53</sup>

## **ANGIOGENESIS INHIBITORS**

### **Angiostatin**

#### **Key point**

- **Angiostatin inhibits endothelial cell proliferation and stimulates endothelial cell apoptosis, possibly through the inhibition of bFGF and VEGF**

Angiostatin was discovered in 1994 and noted to be an endogenous 38-kDa plasminogen fragment that exhibited antimetastatic qualities.<sup>54</sup> It was generated by primary tumors in a murine model and found to specifically inhibit endothelial cell proliferation in vitro.<sup>54</sup> Its antiangiogenic effects result from apoptosis of the vascular endothelial cells and resultant tumor regression.<sup>55</sup> Angiostatin's other potential mechanisms of antiangiogenesis include the inhibition of both bFGF and VEGF, endothelial cell mitotic arrest, cessation of plasmin formation (plasmin participates in tumor invasion), and binding to subunits of adenosine triphosphate synthase on the outer membranes of endothelial cells.<sup>56</sup> Recombinant human angiostatin is now being used clinically for oncologic indications.<sup>57,58</sup>

**Endostatin****Key points**

- **Endostatin inhibits endothelial cell proliferation and angiogenesis by competitively binding to VEGFR2**
- **Endostatin also blocks the effects of bFGF and various MMPs**

Endostatin is a fragment from collagen XVIII that functions as an endogenous inhibitor of endothelial proliferation and angiogenesis.<sup>59</sup> Endostatin has been shown to bind to VEGFR2 and thereby prevent VEGF binding.<sup>60</sup> In addition, endostatin blocks bFGF-induced signal transduction.<sup>61</sup> Taddei et al<sup>62</sup> revealed that endostatin blocks the effects of bFGF and VEGF, resulting in the inhibition of endothelial cell proliferation and migration. Endostatin also has inhibitory effects on MMPs -2, -9, and -13 and integrins located on the surface of endothelial cells.<sup>63,64</sup>

**Interferons alfa and beta****Key point**

- **Interferons alfa and beta are thought to inhibit angiogenesis by down-regulating IL-8, bFGF, and MMP-9**

The interferons (IFNs) are a family of naturally occurring cytokines with antiviral, immunomodulating, and antiproliferative properties. Although the exact mechanism of antiangiogenesis remains unknown, it is postulated that the IFN signaling pathways affect the expression of other factors. IFN $\alpha$  was shown to reduce endothelial cell migration and proliferation and down-regulate VEGF in a murine and in vitro model of hepatocellular carcinoma, as well as a cell line model of melanoma.<sup>65-67</sup> IFN $\alpha$  and - $\beta$  may also down-regulate IL-8, bFGF, and MMP-9.<sup>68</sup> Synthetic forms of both IFN $\alpha$  and - $\beta$  have been made available via recombinant DNA technology using *Escherichia coli*. These drugs have been used to treat a wide range of diseases, including KS and hemangiomas, where the antiangiogenic properties are likely to play a role in the treatment effect.

**Interleukin-12 and interferon gamma****Key point**

- **IL-12 suppresses angiogenesis through the induction of interferon gamma**

IL-12 is a multifunctional cytokine that participates in cell-mediated immune responses and antiangiogenesis. IL-12's antiangiogenic action is carried out by induction of interferon gamma (IFN $\gamma$ ), MMPs, interferon-inducible protein 10 (IP-10), and the angiostatic chemokine MIG.<sup>69</sup> IL-12-induced IFN $\gamma$  leads to decreased endothelial cell adhesion and survival and decreased production of VEGF in

malignant cells.<sup>70,71</sup> IL-12's potent antiangiogenic properties were negated by the administration of IFN $\gamma$ -neutralizing antibodies.<sup>72</sup> This strongly suggests that IL-12's suppression of angiogenesis is dependent on and mediated via the IFN $\gamma$  pathway. Coughlin et al<sup>73</sup> postulate that IL-12 and IFN $\gamma$  can induce neoplastic cells to produce antiangiogenic factors, such as the chemokine IP-10. This observation has promising potential for the development of future cancer therapies.

**Thrombospondin-1****Key point**

- **Thrombospondin-1 inhibits endothelial cell migration and inhibits the activity of VEGF, bFGF, IL-8, and MMP-9**

The thrombospondins (TSPs) are a family of five matricellular glycoproteins that can be found in many different tissues and that have varying functions. TSP-1 and -2 may suppress angiogenesis and tumor growth, whereas TSPs -3, -4, and -5 do not.<sup>74</sup> TSP-1 has been the best characterized, and its role in angiogenesis is known to be complex. TSP-1 inhibits the migration of endothelial cells and induces apoptosis by binding to the endothelial cell membrane CD36 protein.<sup>75</sup> It also binds to and activates TGF $\beta$ , which leads to tumor growth inhibition. TSP-1 inhibits the activity of multiple angiogenic factors, including VEGF, bFGF, IL-8, and MMP-9.<sup>76,77</sup> TSP-1 is overexpressed in early tumor stages, where it plays a limiting factor in tumor growth. Its early overexpression induces hypoxia in an attempt to limit tumor growth, but that same hypoxia subsequently induces VEGF expression. The elevated VEGF counteracts the effect of TSP-1 and stimulates angiogenesis.<sup>78</sup>

**Tissue inhibitors of matrix metalloproteinases****Key point**

- **Tissue inhibitors of matrix metalloproteinases have the critical role of counterbalancing the activity of MMPs, to prevent the uncontrolled destruction of tissue**

It is critical that the proteolytic activity of the MMPs remains controlled so tissue is not destroyed. A group of five tissue inhibitors of metalloproteinases (TIMPs) exist, and each is capable of inhibiting almost every member of the MMP family.<sup>4</sup> The TIMPs are small proteins of about 21,000 Da that contain N- and C-terminal domains. Normally, MMP and TIMP activity is balanced and matrix digestion is carefully regulated. However, there are many diseases in which MMP levels and activity are elevated, resulting in tissue destruction. Tumor invasion and metastasis is an example.<sup>79</sup> The role of TIMPs in angiogenesis is

complex, involves both stimulatory and inhibitory effects, and is not only related to interaction with MMPs.<sup>37</sup> For example, TIMP-2 inhibits the proliferation of bFGF-stimulated endothelial cells. TIMP-3 binds to VEGFR2 and interferes with receptor activation, and TIMP-4 has been shown to have both pro- and antiangiogenic activity. Aside from its angiogenic properties, TIMPs also possess erythroid-potentiating activity and play a role in apoptosis induction and suppression.<sup>4,80</sup>

### Additional elements central to angiogenesis

#### PI3K/Akt/mTOR pathway

##### Key points

- **The PI3K/Akt signaling pathway is central to the regulation of angiogenesis and other cellular processes**
- **Many angiogenic growth factors phosphorylate PI3K resulting in the activation of Akt, which leads to inhibition of endothelial cell apoptosis.**

The PI3K/Akt signaling pathway (Fig 2) is critical for the regulation of several basic cellular functions, including angiogenesis.<sup>81</sup> Akt is activated by many angiogenic growth factors through phosphorylation by PI3K. Akt, also known as protein kinase B, is a serine/threonine kinase that is involved in promoting the survival of a wide range of cell types.<sup>82</sup> Mammalian genomes contain three Akt genes: *Akt1/PKB $\alpha$* , *Akt2/PKB $\beta$* , and *Akt3/PKB $\gamma$* .<sup>83</sup>

Many factors initiate Akt activation, which results in the inhibition of endothelial cell apoptosis and enhanced endothelial cell survival. Angiogenic growth factors, including VEGF and Ang1, are known to activate Akt.<sup>14,84,85</sup> Known inhibitors of endothelial cell apoptosis, including shear stress and insulin, have also been shown to activate Akt.<sup>86,87</sup> Other endothelial cell stimuli, such as insulin-like growth factor-1 (IGF-1), sphingosine-1-phosphate, hepatocyte growth factor, the small proteoglycan decorin, estrogen, reactive oxygen species, and corticosteroids also activate PI3K/Akt signaling.<sup>88-90</sup> mTOR mediates the activation of Akt in several cell types, including endothelial cells. Sirolimus (formerly known as rapamycin), an mTOR inhibitor, blocks the phosphorylation of Akt, resulting in increased endothelial cells apoptosis.<sup>91</sup>

These extracellular signals stimulate PI3K to phosphorylate phosphatidylinositol-4, 5-bis-phosphate to generate phosphatidylinositol-3, 4, 5 triphosphate (PIP3). This leads to membrane localization and activation of phosphatidylinositol-dependent kinase-1 (PDK-1) and Akt.<sup>92,93</sup> Phosphatase and tensin homologue deleted on chromosome 10, a tumor

suppressor phosphatase, dephosphorylates PIP3, thereby reversing the action of PDK1. Activated Akt phosphorylates and inhibits the tuberous sclerosis complex (TSC) composed of TSC1 and TSC2 through the GTPase Rheb that usually inhibits the mTOR protein.<sup>94-96</sup> mTOR forms complexes with other proteins, including regulatory associated protein of mTOR (Raptor), Rictor, and a mammalian ortholog of LST8 (mLST8).<sup>97,98</sup>

Once activated, Akt initiates several processes important to angiogenesis. Akt phosphorylates the endothelial nitric oxide synthase (eNOS). NO synthesis plays an essential role in postnatal neovascularization and endothelial cell migration.<sup>99-104</sup> eNOS knockout animals have an impaired angiogenesis response to stimuli, including ischemia and VEGF.<sup>102</sup> Akt is also involved in VEGF-induced endothelial cell migration required for formation of capillary-like structures.<sup>99</sup> Aberrant activation of the PI3K/Akt pathway has been implicated in several human pathologies.

#### Mammalian target of rapamycin

##### Key points

- **mTOR is a downstream mediator of the PI3K/Akt pathway**
- **This kinase plays a central role in angiogenesis, cell growth, and cell cycle progression, and is the target of several therapeutic agents**

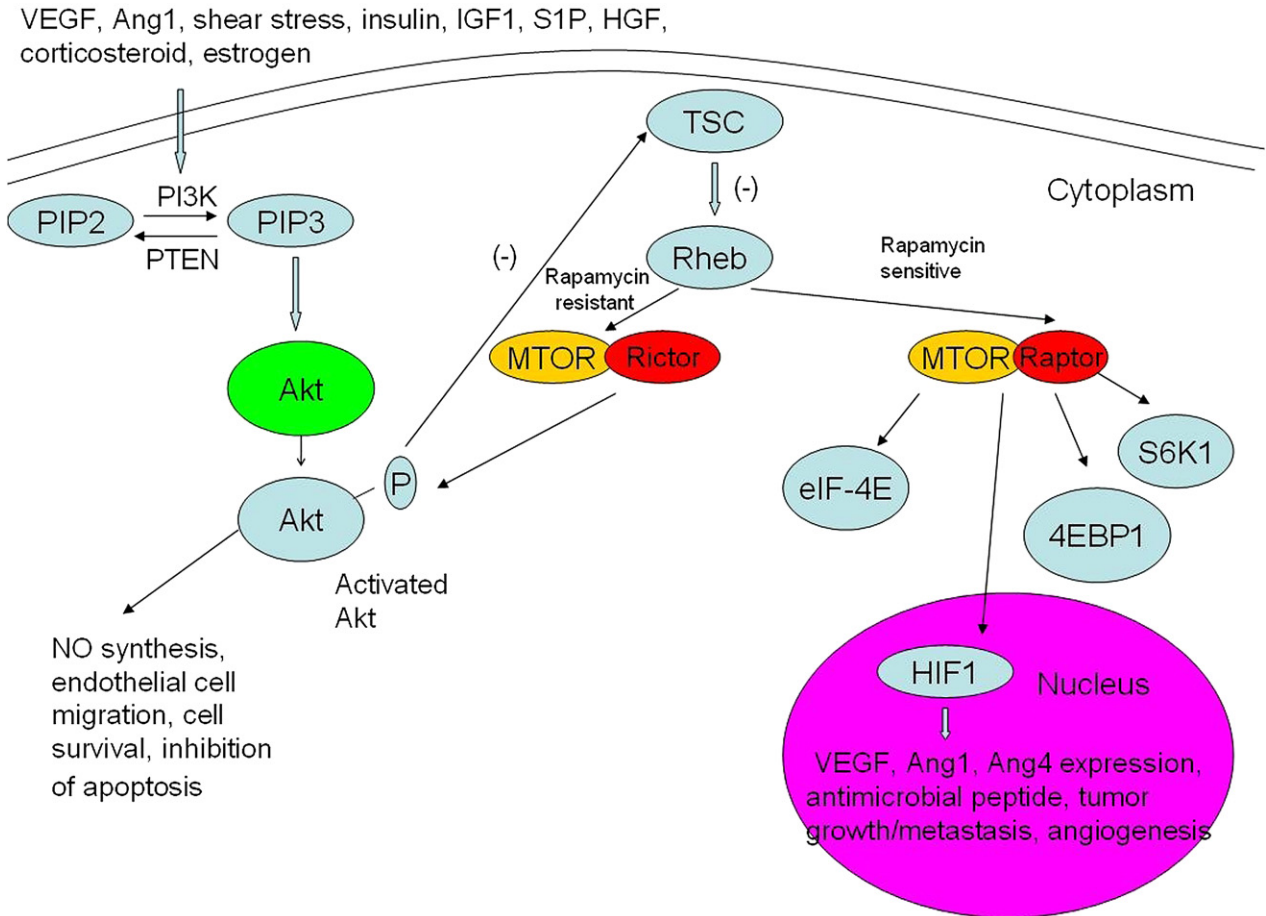
mTOR is a 290-kDa serine-threonine kinase, which is a downstream mediator of the PI3K/Akt pathway. mTOR plays a central role in the control of angiogenesis, cell growth, and cell cycle progression. As noted above, mTOR forms complexes with proteins, including Raptor and Rictor. These complexes have very different functions. Rictor-mTOR complex phosphorylates Akt to fully activate it. Raptor-mTOR complex phosphorylates many factors needed for protein translation including eukaryotic initiation factor 4E, binding protein 1 factor 4E, and protein S6K.<sup>93</sup> This increase in protein translation is a key process in regulation of cell growth. Agents that target mTOR inhibit signals required for angiogenesis, cell cycle progression, cell growth, and proliferation in normal and malignant cells. These agents include sirolimus (rapamycin), temsirolimus, and everolimus. Raptor-mTOR complex is sirolimus-sensitive, and Rictor-mTOR complex is sirolimus resistant.<sup>105-107</sup>

#### Hypoxia-inducible factor

##### Key points

- **HIF-1 mediates the cellular response to hypoxia by up-regulating the expression of many angiogenic factors**





**Fig 2.** The Akt/mammalian target of rapamycin/hypoxia-inducible factor pathway. *4EBP1*, binding protein 1 factor 4E; *Ang1*, angiopoietin-1; *eIF-4E*, eukaryotic initiation factor 4E; *HGF*, hepatocyte growth factor; *HIF1*, hypoxia-inducible factor 1; *IGF-1*, insulin-like growth factor-1; *mTor*, mammalian target of rapamycin; *PI3K*, phosphatidylinositol 3-kinase; *PIP2*, phosphatidylinositol-4, 5-bis-phosphate; *PIP3*, phosphatidylinositol-3,4, 5 triphosphate; *PTEN*, phosphatase and tensin homologue deleted on chromosome 10; *Raptor*, regulatory protein associated with mTor; *Rheb*, a GTPase; *Rictor*, rapamycin-insensitive companion of mTOR; *S1P*, sphingosine-1-phosphate; *S6K1*, a protein; *TSC*, tuberous sclerosis complex composed of TSC1 and TSC2; *VEGF*, vascular endothelial growth factor.

### • The PI3K/Akt pathway and mTOR enhance HIF-1–dependent gene expression

During tumor development, proliferating cells have increased requirements for oxygen and nutrients—above what is generally provided by the normal blood supply. Resultant hypoxia forces the tumor to stay in a steady state of growth in proportion to the oxygen and nutrients available.<sup>108</sup> In order to proliferate, tumors often respond to the hypoxic stress by stimulating cellular metabolism and neovascularization. An important regulator of cellular response to oxygen deprivation is the transcription factor HIF-1. HIF-1 is a heterodimeric protein consisting of alpha (HIF-1 $\alpha$ ) and beta (HIF-1 $\beta$ ) subunits. HIF-1 $\alpha$  expression varies with oxygen tension. Under regular oxygen conditions, HIF-1 $\alpha$  is

continuously expressed but is rapidly destroyed by the ubiquitin–proteasome pathway. Low oxygen tension results in a decrease in the rate of HIF-1 $\alpha$  polyubiquitination and proteolysis, and subsequent accumulation of the HIF-1 $\alpha$  subunit. HIF-1 $\beta$  is a constitutively expressed nuclear protein. The resulting HIF-1 $\alpha$ –HIF-1 $\beta$  heterodimers undergo posttranslational modifications and promote angiogenesis, tumor growth, and metastasis.<sup>109</sup> HIF-1 mediates the angiogenic response to hypoxia by up-regulating the expression of many angiogenic factors. HIF-1 $\alpha$  and -1 $\beta$  form a dimer that binds to the hypoxia-responsive element in the VEGF promoter, resulting in up-regulation of VEGF production.<sup>110</sup> Constitutively expressed HIF-1 $\alpha$  in human endothelial cells was shown to increase expression of Ang2

and Ang4.<sup>111</sup> Recent studies suggest that the PI3K/Akt pathway and its downstream target, mTOR, enhance HIF-1–dependent gene expression. These studies position mTOR as an upstream activator of HIF-1 function in cancer cells and suggest that the antitumor activity of sirolimus is mediated in some parts, through the inhibition of cellular responses to hypoxic stress.<sup>112,113</sup> HIF-1 $\alpha$  is highly expressed in skin epithelium and has also been shown to be involved in macrophage and neutrophil bactericidal pathways.<sup>114</sup> A keratinocyte-specific deletion of HIF-1 $\alpha$  in a mouse model revealed that keratinocyte-HIF1 $\alpha$  offers antibacterial protection by regulating the production of antimicrobial peptides, including cathelicidins.<sup>115</sup> Ultraviolet B light radiation and associated hypoxia induces expression of HIF-1 $\alpha$  along with VEGF in a dose- and time-dependent manner in cultured human keratinocytes.<sup>116</sup>

#### **THERAPEUTIC AGENTS AVAILABLE FOR ANGIOGENESIS INHIBITION**

##### **Key points**

- **Therapies with recognized antiangiogenic properties have been approved by the FDA**
- **These agents have been used mainly in adult oncology and ophthalmology**
- **Use for cutaneous disease is promising, but relatively new and under investigation**

There are anticancer therapies approved for use in adults with recognized antiangiogenic properties. These agents interrupt critical cell signaling pathways involved in tumor angiogenesis and growth. They are divided into two primary categories: (1) monoclonal antibodies directed against specific angiogenesis growth factors and/or their receptors (Table II), and (2) small-molecule tyrosine kinase inhibitors (TKIs; Table III). A third group includes agents with other mechanisms of action, including the inhibitors of mTOR (Table IV). It is worthwhile to note that these agents have been used extensively in oncology and ophthalmology. Use for cutaneous disease is promising, but relatively new and under investigation.

##### **Bevacizumab**

###### **Key point**

- **Bevacizumab is a recombinant antibody that binds to and inhibits the activity of VEGF**

Bevacizumab is a humanized monoclonal antibody against VEGF-A. It binds to VEGF and prevents its interactions with the receptors VEGFR1 and VEGFR2, thereby neutralizing VEGF's endothelial cell mitogenic ability, vascular permeability enhancement, and angiogenic properties.

Bevacizumab comes in a solution and is administered intravenously every 2 to 3 weeks. Reported adverse effects include gastrointestinal perforation, which may be associated with intraabdominal abscess or fistula formation and has, in some cases, resulted in fatality, wound healing impairment or wound dehiscence, and fatal pulmonary hemorrhage in patients with non–small cell lung cancer treated with chemotherapy and bevacizumab. Bevacizumab has been approved by the FDA for the treatment of metastatic colorectal cancer, non–small cell lung cancer, and metastatic human EGFR2–negative breast cancer. Currently, there are several clinical trials investigating the use of bevacizumab in combination with another antiangiogenic agent, or in combination with chemotherapy for the treatment of metastatic malignant melanoma and other diseases of the skin.

##### **Ranibizumab**

###### **Key point**

- **Ranibizumab is an antibody fragment that binds to and inhibits the activity of VEGF and is designed for intraocular use**

Ranibizumab is a bevacizumab-related drug that was developed as an anti-VEGF antibody fragment with the rationale that a smaller molecular mass may have better tissue absorption and fewer inflammatory side effects than a full length antibody.<sup>117</sup> This drug works by binding to and inhibiting VEGF, thereby inhibiting the proliferation and maintenance of blood vessels. Ranibizumab is only available as a solution for intravitreal injection. Major potential risks related to route of administration include serious eye infection, detached retina, increase in eye pressure, bleeding in the eye, or endophthalmitis. Ranibizumab is approved by the FDA for treatment of age-related macular degeneration. There is an ongoing clinical trial studying the effects of VEGF inhibition by local injections of ranibizumab into cutaneous neurofibromas. There is a phase I trial combining the use of pulsed dye laser to induce port wine stain (PWS) blood vessel injury followed by topical application of ranibizumab to prevent blood vessel angiogenesis and recanalization after laser therapy.

##### **Pegaptanib**

###### **Key point:**

- **Pegaptanib is a peptide strand that inhibits the major VEGF isoform and is designed for intraocular use**

Pegaptanib is an anti-VEGF aptamer (short peptide strand) that selectively inhibits VEGF165

**Table II.** US Food and Drug Administration–approved antiangiogenic agents that are monoclonal antibodies directed against specific angiogenesis growth factors and/or their receptors

Drug name, generic (trade name; company)	Mechanism of action	Effect on angiogenesis	FDA approved indications	Some dermatologic indications under clinical investigation
Bevacizumab (Avastin; Genentech)	Monoclonal antibody against VEGF-A	Binds to VEGF and prevents its interactions with the receptors VEGFR1 and VEGFR2	Metastatic colorectal, non–small cell lung, and metastatic HER-2–negative breast cancers	Malignant melanoma, angiosarcoma, and hemangioendotheliomas
Ranibizumab (Lucentis; Genentech)	Monoclonal antibody that binds active forms of VEGF-A	Binds to VEGF and prevents its interactions with the receptors VEGFR1 and VEGFR2	Age-related macular degeneration	Cutaneous neurofibromas and port wine stains
Pegaptanib (Macugen; Pfizer)	Selective VEGF inhibitor that binds extracellular VEGF(165)	Binds to VEGF165 extracellularly and prevents its interactions with the receptors VEGFR1 and VEGFR2	Age-related macular degeneration	No dermatologic indication at present
Cetuximab (Erbix; Imclone/Bristol-Myers Squibb)	Human-murine IgG1 monoclonal antibody to EGFR	Binds to EGFR and prevents tyrosine kinase autophosphorylation, which leads to down regulation of VEGF, IL-8, and bFGF expression; decreases HIF-1 $\alpha$ expression	Metastatic colorectal cancer and squamous cell carcinoma of the head and neck	Cutaneous squamous cell carcinoma
Panitumumab (Vectibix; Amgen)	Fully human IgG2 monoclonal antibody to EGFR	Binds to EGFR and prevents tyrosine kinase autophosphorylation which leads to down regulation of VEGF, IL-8, and bFGF expression	Metastatic colorectal cancer	No dermatologic indication at present
Trastuzumab (Herceptin; Genentech)	Human IgG1 monoclonal antibody to HER-2	Represses angiogenic factors: VEGF, TGF $\alpha$ , Ang-1, PAI-1; induces antiangiogenic factor TSP-1	Adjuvant treatment of HER2 overexpressing breast cancer and metastatic HER2 overexpressing breast cancer	No dermatologic indication at present

*Ang-1*, Angiopoietin-1; *bFGF*, basic fibroblast growth factor; *EGFR*, endothelial growth factor receptor; *HER-2*, human estrogen receptor 2; *HIF-1 $\alpha$* , hypoxia-inducing transcription factor- $\alpha$ ; *IgG*, immunoglobulin G; *IL-8*, interleukin-8; *PAI-1*, plasminogen activator inhibitor-1; *TGF $\alpha$* , transforming growth factor- $\alpha$ ; *TSP-1*, thrombospondin-1; *VEGF-A*, vascular endothelial growth factor-A.

**Table III.** US Food and Drug Administration—approved antiangiogenic agents that act as tyrosine kinase inhibitors

Drug name, generic (trade name; company)	Mechanism of action	Effect on angiogenesis	FDA-approved indications	Some dermatologic indications under clinical investigation
Sunitinib (Sutent; Pfizer)	Inhibitor of multiple RTKs	Targets VEGFR1,2,3	Advanced renal cell and gastrointestinal stromal tumors	Healing of skin ulcers associated with Klippel–Trenaunay syndrome; metastatic melanoma
Sorafenib (Nexavar; Bayer/Onyx)	Inhibitor of multiple RTKs	Inhibits RTKs of multiple proangiogenic factors, such as VEGFR1,2,3 and PDGFR- $\beta$	Advanced renal cell and inoperable hepatocellular cancers	Malignant melanoma
Erlotinib (Tarceva; Genentech/OSI)	Tyrosine kinase inhibitor of EGFR	PI3K inhibition leads to decreased VEGF	Non–small cell lung and pancreatic cancers	Malignant melanoma

EGFR, endothelial growth factor receptor; HIF-1 $\alpha$ , hypoxia-inducible factor-1 alfa; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; RTK, tyrosine kinase receptor; VEGFR, vascular endothelial growth factor receptor.

(bevacizumab and ranibizumab block all VEGF isoforms). Pegaptanib binds VEGF165 extracellularly, preventing receptor connection. VEGF165 is a secreted form of VEGF that stimulates proliferation of endothelial cells.<sup>118</sup> Because VEGF165 is the predominant isoform, inhibition of only VEGF165 is effective at suppressing neovascularization.<sup>119</sup> Pegaptanib is administered via intravitreal injection. Serious adverse events related to intravitreal injection include endophthalmitis, retinal detachment, and traumatic cataracts. Pegaptanib is approved by the FDA for treatment of age-related macular degeneration. There are no reported uses of pegaptanib for dermatologic disease at this time.

### Cetuximab Key points

- **Cetuximab is a recombinant human/mouse antibody that binds to the EGFR, resulting in the down-regulation of VEGF, bFGF, IL-8, and MMP-9**
- **Cetuximab is approved by the FDA for the treatment of metastatic colorectal cancer and squamous cell carcinoma of the head and neck (SCCHN)**

Cetuximab is a chimeric human–murine monoclonal antibody that binds to the EGFR and competitively inhibits binding of epidermal growth factor (EGF) and other ligands, such as TGF. EGFR is highly expressed in many human cancers, and the activation of EGFR induces VEGF expression and therefore angiogenesis.<sup>120</sup> Blockade of EGFR results in inhibition of tumor survival, proliferation, and invasion, tumor cell motility, and angiogenesis.<sup>121</sup> In 1999,

Perrotte et al<sup>122</sup> analyzed the effect of cetuximab on angiogenesis biomarkers in bladder tumors. EGFR blockade with cetuximab down-regulated VEGF, bFGF, and IL-8 expression. This led to tumor cell vessel involution and inhibited tumor growth and metastasis. In 2005, Luwor et al<sup>123</sup> showed decreased levels of HIF-1 after treatment with cetuximab in epidermoid carcinoma cells. This was accompanied by reduced transcriptional expression of VEGF. Zhong et al<sup>124</sup> hypothesized that the mechanism by which cetuximab reduces HIF-1 synthesis is through inhibition of the PI3k/Akt pathway. MMP-9 was also shown to be down-regulated in cetuximab-treated mice as compared to controls, and this may inhibit tumor invasion.<sup>125</sup>

Cetuximab is administered intravenously. Phase I studies showed saturation of cetuximab clearance after administration of 400 mg/m<sup>2</sup> as a loading dose followed by weekly infusions of 250 mg/m<sup>2</sup>.<sup>126</sup> Serious infusion reactions included rapid airway obstruction, hypotension, or cardiac arrest. The most commonly reported adverse event associated with cetuximab treatment is an acneiform neutrophilic folliculitis that occurs in 70% to 80% of patients. The rash appears to correlate with treatment response and may become an important clinical prognostic marker.<sup>127</sup>

Cetuximab is approved by the FDA for the treatment of metastatic colorectal cancer and SCCHN. Patients with recurrent and/or metastatic SCCHN, and especially those with high levels of EGFR, have a very poor prognosis. Several phase II/III studies have shown that cetuximab offers clinical benefit for patients with SCCHN.<sup>128</sup> There is currently a phase II study of cetuximab in squamous cell carcinoma (SCC) of the skin expressing EGFR.

**Table IV.** US Food and Drug Administration—approved antiangiogenic agents with other mechanisms of action

Drug name, generic (trade name; company)	Mechanism of action	Effect on angiogenesis	FDA-approved indications	Some dermatologic indications under clinical investigation where antiangiogenesis is the proposed mechanism of action
Batimastat (British; Biotech)	MMP inhibitor	Inhibits extracellular matrix proteolysis	Vascular stents	No clinical dermatologic uses at present
Sirolimus/Rapamycin (Rapamune; Wyeth-Ayerst)	mTOR inhibitor, immunosuppressant	Inhibits mTOR pathway, decreases VEGF production and response, and inhibits Akt activation	Prophylaxis of organ; rejection and coronary stents	Cutaneous lymphoma, Kaposi sarcoma, angiofibromas, and psoriasis
Temsirolimus (Torisel; Wyeth)	mTOR inhibitor	Inhibits mTOR pathway, down-regulates HIF-1 $\alpha$ , which leads to decreased VEGF, and blocks Akt pathway	Advanced renal cell cancer	Malignant melanoma
Everolimus (XIENCE V; Abbott Afinitor; Novartis)	mTOR inhibitor	Inhibits mTOR pathway, down-regulates HIF-1 $\alpha$ , which leads to decreased VEGF, and blocks Akt pathway	Vascular stents and advanced renal cell cancer	Psoriasis
Bortezomib (Velcade; Millennium)	Proteasome inhibitor	Inhibition of NF- $\kappa$ B, down-regulating VEGF expression; inhibits chemotaxis and capillary formation, and transcription inhibition of VEGF, IL-6, IGF-1, and Ang2	Multiple myeloma and mantle cell lymphoma	Squamous cell carcinoma, malignant melanoma, and cutaneous T-cell lymphoma
Imiquimod (Aldara; Graceway Pharmaceuticals)	Immune modulator	Induces production of angiogenesis inhibitors IFN, IL-10, -12, and -18; up-regulates TIMP and TSP-1, and down-regulates bFGF and MMP-9	Actinic keratosis, superficial BCC, and external genital warts	Kaposi sarcoma, infantile hemangiomas, pyogenic granulomas, hemangioendotheliomas, port wine stains, squamous cell carcinoma, lentigo maligna, and discoid lupus erythematosus
Thalidomide (Thalomid; Celgene)	Immune modulator	Down-regulates expression of bFGF and VEGF; inhibits TNF $\alpha$ , ILs -6 and -12, IGF-1, and NF- $\kappa$ B	Multiple myeloma and erythema nodosum leprosum	Kaposi sarcoma and hemangioendotheliomas

Ang-2, Angiopoietin-2; BCC, basal cell carcinoma; bFGF, basic fibroblast growth factor; HIF-1 $\alpha$ , hypoxia inducible factor- $\alpha$ ; IFN, interferon; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; MMP, matrix metalloproteinase; mTor, mammalian target of rapamycin; NF- $\kappa$ B, nuclear factor kappa B; TIMP, tissue inhibitor of metalloproteinase; TNF $\alpha$ , tumor necrosis factor- $\alpha$ ; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor.

## **Panitumumab**

### **Key point**

- **Panitumumab is a recombinant human antibody that binds to EGFR resulting in the down-regulation of VEGF, IL-8, and bFGF**

Panitumumab is a human monoclonal IgG2 antibody targeting EGFR. Panitumumab is 100% human antibody, whereas cetuximab is a chimeric antibody containing human and murine sequences. It has been hypothesized that the fully human antibody has the potential for better efficacy and may eliminate the risk of an immunogenic reaction.<sup>129</sup> Like cetuximab, panitumumab binds to EGFR and prevents tyrosine kinase autophosphorylation, resulting in the inhibition of cell growth, induction of apoptosis, diminution of proinflammatory cytokines, and down-regulation of vascular growth factors, including VEGF, IL-8, and bFGF.<sup>130</sup> Panitumumab is administered intravenously. The most serious adverse events listed for panitumumab are pulmonary fibrosis, pulmonary embolism, severe dermatologic toxicity, and infusion reactions. The most common reaction is dermatologic toxicity, which occurs in 89% of patients and presents as acneiform dermatitis, pruritus, erythema, and skin exfoliation. Serious skin reactions have in some cases been complicated by infectious sequelae and septic death. Panitumumab is approved by the FDA for the treatment of metastatic colorectal carcinoma. As of this time, there have been no reported uses of panitumumab for dermatologic conditions.

## **Trastuzumab**

### **Key point**

- **Trastuzumab is a monoclonal antibody that selectively binds to human estrogen receptor 2, resulting in the down-regulation of angiogenesis growth factors and up-regulation of TSP-1**

Trastuzumab is a human monoclonal antibody targeted against the human estrogen receptor 2 (HER2). HER2 is a RTK that is overexpressed in 20% to 30% of breast cancers and is associated with increased mortality.<sup>131</sup> Trastuzumab has demonstrated antiangiogenic effects by down-regulating the expression of angiogenic factors VEGF, TGF- $\alpha$ , Ang1, and plasminogen-activator inhibitor-1, while up-regulating the angiogenesis inhibitor TSP-1.<sup>132</sup> Trastuzumab is available as a powder that is reconstituted for intravenous infusion. This drug has been associated with cardiomyopathy presenting as congestive heart failure, serious infusion reactions, and pulmonary toxicity, such as interstitial pneumonitis or acute respiratory distress syndrome. Trastuzumab

has only been shown to be effective in breast cancer, reducing tumor vasculature to a normal state. We found no reports of use of trastuzumab for dermatologic disease.

## **Sunitinib**

### **Key point**

- **Sunitinib is an oral multikinase inhibitor that is currently being evaluated for the treatment of metastatic melanoma**

Sunitinib inhibits the phosphorylation of multiple RTKs, including PDGFR $\alpha$  and - $\beta$  and VEGFR1, 2 and 3. Inhibition of these RTKs results in tumor growth inhibition and regression. It is available in an oral formulation. Reported serious adverse reactions include left ventricular dysfunction, QT prolongation, hemorrhagic events, hypertension, and adrenal insufficiency. Sunitinib is approved by the FDA for the treatment of advanced renal cell carcinoma and gastrointestinal stromal tumors. There is one case report showing that sunitinib improved the healing of skin ulcers associated with Klippel-Trénaunay syndrome.<sup>133</sup> In another case, a patient with metastatic melanoma refused standard chemotherapy and was treated with sunitinib. Disease progression was noted at 6 months.<sup>134</sup> There is an ongoing phase II trial studying the efficacy of sunitinib in the treatment of brain metastases secondary to melanoma.

## **Sorafenib**

### **Key point**

- **Sorafenib is an oral multikinase inhibitor that is being evaluated for the treatment of stage III or IV melanoma**

Sorafenib is also a multikinase inhibitor. It was originally developed as an inhibitor to RAF kinase but was then found to also inhibit multiple intracellular and cell surface kinases (including VEGFR1, 2, 3 and PDGFR- $\beta$ ).<sup>135,136</sup> These kinases are thought to be involved in tumor cell signaling, angiogenesis, and apoptosis. Sorafenib is administered orally; reported serious adverse reactions include cardiac ischemia, hemorrhage, hypertension, and gastrointestinal perforation. The most common adverse reaction was a hand-foot skin reaction and rash. Sorafenib has been approved for the treatment of advanced renal carcinoma and advanced hepatocellular carcinoma. There is currently a phase II study to determine the efficacy of sorafenib in unresectable stage III and stage IV melanoma. In addition, there are ongoing phase III clinical trials studying the use of chemotherapeutic agents (carboplatin and paclitaxel) with or without sorafenib to treat patients with unresectable stage III or stage IV melanoma.

**Erlotinib****Key point**

- **Erlotinib is a tyrosine kinase inhibitor of EGFR which leads to PI3K inhibition and decreased VEGF**

Erlotinib inhibits EGFR tyrosine kinase autophosphorylation and prevents downstream signaling. This leads to inhibition of the PI3K/Akt pathway and the down-regulation of VEGF expression.<sup>137</sup> Erlotinib is administered orally. The most common side effects are acneiform rash and diarrhea. Severe but infrequent adverse reactions include interstitial lung disease—like events (0.7%), myocardial infarction, cerebral vascular accidents, and microangiopathic hemolytic anemia with thrombocytopenia. Erlotinib has been approved by the FDA for the treatment of non-small cell lung cancer and pancreatic cancer. There is an ongoing phase II trial combining erlotinib and bevacizumab for treatment of stage IV melanoma.

**Batimastat****Key point**

- **Batimastat is an MMP inhibitor that requires intraperitoneal or intrapleural administration**

Batimastat is a potent synthetic inhibitor of the major MMPs (-1, -2, -3, -7, and -9) and has been shown to have antiangiogenic and antineoplastic activity. Batimastat binds to the active site of MMPs, thereby inactivating them. In vitro studies showed batimastat to have weak cytostatic activity but no cytotoxic effect, which may improve safety.<sup>138</sup> The use of batimastat is limited by poor oral bioavailability and water solubility, requiring intraperitoneal or intrapleural administration.<sup>139</sup> Side effects include abdominal discomfort, fever, and elevated liver enzymes. Currently, batimastat is only approved by the FDA for use in vascular stents. Clinical development for batimastat has been discontinued because of the difficult routes of administration. An alternative oral formulation was sought, which led to the development of marimastat, prinomastat, metastat, and neovastat. Of these, marimastat and neovastat have been used in clinical trials related to skin disorders.

**Marimastat****Key point**

- **Marimastat is an oral MMP inhibitor that is currently undergoing clinical trials**

Marimastat is the first oral MMP inhibitor. It binds to and inhibits MMPs -1, -2, -3, -7, -9, and -12. The most common associated adverse events were myalgias, arthralgias, and tendonitis.<sup>139</sup> Marimastat is

currently in clinical trials for several indications, including melanoma and vascular malformations, but it has not yet received FDA approval.

**Neovastat****Key point**

- **Neovastat is an MMP inhibitor that has multiple antiangiogenic actions and is currently in clinical trials**

Neovastat is a shark cartilage extract that has been shown to inhibit angiogenesis at multiple levels. Neovastat inhibits MMPs -1, -2, -7, -9, -12, and -13, interferes with VEGFR2 signaling pathways, inhibits endothelial cell proliferation and tubulogenesis, and induces apoptosis in endothelial cells. Common side effects seen in clinical trials were nausea, vomiting, diarrhea, constipation, rash, and acne.<sup>140</sup> Neovastat does not yet have FDA approval, but it is currently being evaluated for use in psoriasis and KS.

**Sirolimus/rapamycin****Key points**

- **Sirolimus is an oral mTOR inhibitor that decreases the production of VEGF, inhibits endothelial response to VEGF and suppresses T cell lymphocyte activation and proliferation**
- **Sirolimus has been used in the treatment of anaplastic large cell lymphoma, KS, angiofibromas, and psoriasis**

Sirolimus, formerly rapamycin, is a macrolide antibiotic with strong antiangiogenic, immunosuppressive, and antiproliferative action. Sirolimus binds to the FK binding protein-12 and forms a complex which then binds to and inhibits mTOR kinase activity. This decreases production of VEGF, inhibits endothelial response to VEGF, and suppresses T cell lymphocyte activation and proliferation.<sup>141-143</sup> Sirolimus has also been shown to inhibit Akt activation by suppressing mTOR complex 2 formation.<sup>106</sup> Sirolimus is administered orally. The use of sirolimus increases susceptibility to infection and development of lymphoma or other malignancy. By inhibiting VEGF and angiogenesis, sirolimus also impairs wound healing, which may be a significant issue following transplant surgery.<sup>144</sup> Sirolimus is approved by the FDA for prophylaxis of organ rejection in renal transplants. There is also a sirolimus-eluting stent that was approved by the FDA in 2003 for use in coronary angioplasty to reduce the rate of restenosis.<sup>145</sup> Sirolimus has been used in treatment of anaplastic large cell lymphoma, KS, angiofibromas, and psoriasis.<sup>146-150</sup> Currently, there is a phase IV study to determine the effect of

sirolimus on prevention of nonmelanoma skin cancers in kidney transplant recipients. There is also an active phase I study using topical sirolimus on basal cell nevus syndrome and a study evaluating use of sirolimus in combination with pulsed dye laser for treatment of port wine stain birthmarks.

### **Temsirolimus**

#### **Key point**

- **Temsirolimus is an mTOR inhibitor designed for both oral and intravenous use**

Temsirolimus is another mTOR inhibitor. Like sirolimus, temsirolimus suppresses the activity of mTOR resulting in reduced levels of HIF-1 and VEGF. It may also suppress the formation of mTORC2 to inhibit Akt activation.<sup>151</sup> Unlike sirolimus, temsirolimus is more water soluble and can therefore be administered both orally and intravenously. Severe adverse reactions associated with temsirolimus include hypersensitivity reaction, hyperglycemia, interstitial lung disease, hyperlipemia, bowel perforation, and renal failure. Temsirolimus is approved by the FDA for the treatment of advanced renal cell carcinoma. There are two separate phase II trials evaluating sorafenib in combination with temsirolimus in patients with metastatic melanoma. A third study is evaluating combination temsirolimus and bevacizumab in stage III or stage IV melanoma.

### **Everolimus**

#### **Key point**

- **Everolimus is an mTOR inhibitor with a better toxicology profile and better oral bioavailability than sirolimus**

Everolimus is a semisynthetic macrolide derived from sirolimus, but with a better toxicology profile, substantially shorter half-life, and better oral bioavailability. Like sirolimus, it binds to the cytoplasmic protein FKBP-12 (FK506-binding protein 12) to form a complex that inhibits mTOR. Everolimus has been shown to decrease the production of HIF-1 $\alpha$ , suppress secretion of VEGF, and inhibit the proliferation of endothelial cells.<sup>152,153</sup> It may also inhibit Akt activation.<sup>151</sup> Reported side effects include headache, polyarthralgia, stomatitis, epistaxis, myelosuppression, and hyperlipidemia. An everolimus drug-eluting coronary stent was approved by the FDA in July 2008. In March 2009, everolimus tablets were also FDA approved for the treatment of advanced renal cell carcinoma after failure of treatment with sunitinib or sorafenib. Serious adverse reactions are noninfectious pneumonitis, infections with opportunistic pathogens, and oral ulcerations. Frigerio et al<sup>154</sup> reported a case of a woman with

treatment resistant, chronic, relapsing psoriasis that was treated with a combination of everolimus 1.5 mg twice daily and cyclosporine (50 mg daily). The patient had significant improvement, but treatment was discontinued because the patient developed leukopenia by the fifth week.

### **Bortezomib**

#### **Key point**

- **Bortezomib, a proteasome inhibitor, blocks angiogenesis by inhibiting nuclear factor kappa B, which leads to decreased VEGF, IL-6, IGF-1, and Ang2**

Bortezomib is a proteasome inhibitor and is the first drug of its class. Proteasomes are enzyme complexes that regulate protein homeostasis in a cell by degrading targeted proteins. Bortezomib impairs the ability of proteasomes to degrade intracellular proteins. This disrupts several cell signaling pathways and leads to cell cycle arrest, apoptosis, and angiogenesis inhibition. Bortezomib blocks angiogenesis by inhibiting nuclear factor kappa B (NF- $\kappa$ B), a protein that is constitutively activated in some cancers. Inhibition of NF- $\kappa$ B down-regulates the transcription of genes involved in neoplastic progression, including VEGF. Roccaro et al<sup>155</sup> showed that bortezomib directly targets angiogenesis by inhibiting chemotaxis and capillary formation. In addition, bortezomib showed the dose-dependent transcription inhibition of VEGF, IL-6, IGF-1, and Ang2. Ang1 expression was also reduced, but only at the highest dose (7.5 nmol/L). Bortezomib is administered intravenously and may cause serious adverse reactions, including peripheral neuropathy, hypotension, congestive heart failure, acute respiratory distress syndrome, reversible posterior leukoencephalopathy syndrome, thrombocytopenia, and neutropenia. Bortezomib is approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. It has been studied in the treatment of SCC and cutaneous T-cell lymphoma,<sup>156</sup> and there are currently three clinical trials using bortezomib and paclitaxel, bortezomib and temozolomide, and bortezomib, paclitaxel, and carboplatin for the treatment of metastatic melanoma.

### **Imiquimod**

#### **Key points**

- **Imiquimod is a topical cream that inhibits angiogenesis by the induction of cytokines that inhibit angiogenesis, up-regulation of endogenous angiogenesis inhibitors, and down-regulation of local proangiogenic factors**



- **Imiquimod has been used off-label to treat angiogenic-dependent dermatologic disease**

Imiquimod (imidazoquinoline) is a synthetic molecule with immunomodulatory, antiviral, and antitumor effects.<sup>157</sup> Imiquimod stimulates the immune system by the induction of cell-mediated immunity through proinflammatory activity of cytokine secretion and activation of macrophages.<sup>158</sup> Antiangiogenic effects are thought to be caused by several mechanisms, including (1) the induction of cytokines that inhibit angiogenesis, such as IFN, IL-10, IL-12, and IL-18<sup>159,160</sup>; (2) the local up-regulation of endogenous angiogenesis inhibitors, including TIMP and TSP-1<sup>161</sup>; and (3) the down-regulation of local proangiogenic factors including bFGF and MMP-9.<sup>161</sup>

Imiquimod is available as a cream for topical administration. The most common side effects are local skin reactions, upper respiratory infections, and headaches. Imiquimod is approved by the FDA for the treatment of actinic keratoses, external genital warts, and superficial basal cell carcinoma. There are currently at least 20 clinical trials involving imiquimod in dermatologic conditions. Recent studies have shown topical imiquimod to be an effective and well tolerated treatment in various angiogenic-dependent dermatologic diseases including SCC, lentigo maligna, hemangiomas, KS, pyogenic granuloma, discoid lupus erythematosus, and in combination with laser treatment for PWS.<sup>162-165</sup>

### Thalidomide

#### Key point

- **Thalidomide exerts its antiangiogenic effects by down-regulating VEGF and bFGF, and may also inhibit TNF- $\alpha$ , IL-6, IL-12, IGF-1, and NF- $\kappa$ B**

Thalidomide has antiangiogenic, antiinflammatory, and immunomodulating effects. It is a potent inhibitor of angiogenesis known to down-regulate the expression of VEGF and bFGF.<sup>166</sup> Thalidomide may also exert antiangiogenic effects by inhibiting TNF- $\alpha$ , IL-6, IL-12, IGF-1, and NF- $\kappa$ B.<sup>167</sup> Thalidomide is administered orally and has been associated with severe birth defects, thromboembolic events, drowsiness/somnolence, peripheral neuropathy, dizziness/orthostatic hypotension, neutropenia, and HIV viral load increase. Thalidomide is first-line therapy for the treatment of erythema nodosum leprosum and is also used for treatment of a wide range of dermatologic diseases.<sup>167</sup> Antiangiogenic mechanisms are likely to play a role in use of this medication for treatment of KS and hemangi endotheliomas.

### Corticosteroids

#### Key point

- **Corticosteroids have been reported to have both angiogenic and antiangiogenic effects**

Corticosteroids are the synthetic form of endogenous adrenal hormones and are a well known class of drugs with antiinflammatory and immunosuppressive properties. Corticosteroids have also been reported to have both angiogenic and antiangiogenic effects. Dexamethasone was found to potentiate the antiangiogenic properties of the chemotherapeutic agent docetaxel in in vitro and in vivo models of prostate cancer by decreasing expression of IL-8, CXCL1, and VEGF.<sup>168</sup> However, in a biosensor model implant study, pretreatment with dexamethasone followed by VEGF appeared to promote angiogenesis and improve successful implantation of the microcapillary sensors, compared to VEGF alone.<sup>169</sup> In a study of an in vitro model of uveal melanoma, triamcinalone acetate was found to have no effect on the expression of VEGF, PDGF, or TSP-1.<sup>170</sup> Corticosteroids can be administered topically, orally, intravenously, or intramuscularly. Common side effects include hypertension, osteoporosis, pathologic bone fractures, peptic ulcers, skin fragility, and insulin resistance. Corticosteroids are used to treat a wide range of disorders in dermatology, endocrinology, rheumatology, allergy, ophthalmology, pulmonology, hematology, oncology, and neurology. From an angiogenesis perspective, corticosteroids are being tested for use in combination with other antiangiogenics to address ophthalmologic disease and are commonly used to slow growth of infantile hemangiomas during the proliferative phase.

### Interferon- $\alpha$ 2b

#### Key points

- **IFN $\alpha$ 2b is a synthetic cytokine that is thought to inhibit angiogenesis by down-regulating angiogenic factors**
- **Use of this drug has been limited because of the risk of spastic diplegia**

IFN $\alpha$ 2a and -2b are synthetic interferons using recombinant DNA from *E coli*. IFN $\alpha$  proteins are naturally occurring molecules with antiviral, antiproliferative, and immunomodulatory activities. As previously mentioned, IFN $\alpha$  is postulated to down-regulate angiogenic factors. IFN $\alpha$ 2b is available as a powder or solution meant to be administered intravenously, intramuscularly, or subcutaneously. The most common side effect is a flu-like syndrome, characterized by fever, chills, tachycardia, malaise, myalgia, and headache. Spastic diplegia has been

reported, especially when this medication is used in children under 1 year of age.<sup>171</sup> IFN $\alpha$ 2b is approved by the FDA for the treatment of condyloma acuminata, chronic hepatitis B and C, hairy cell leukemia, malignant melanoma, AIDS-related KS, and follicular non-Hodgkin lymphoma. This treatment has been used to treat infantile hemangiomas, usually concurrently with corticosteroids or in the event of corticosteroid resistance<sup>172,173</sup>; however, use of this therapy has been limited because of the risk of spastic diplegia. There is an active phase II trial combining chemotherapy with IFN $\alpha$  in cutaneous T-cell lymphoma, phase I trial using IFN $\alpha$  in stage IV tumors, including melanoma, phase I/II trial studying pegylated IFN $\alpha$ 2a in combination with gefitinib in patients with unresectable/metastatic SCC of the skin, phase III trial studying IFN $\alpha$  with or without vaccine therapy for metastatic melanoma, phase III trial studying IFN $\alpha$  following surgery for stage III melanoma, and phase III trial investigating IFN $\alpha$  for hypertrophic scars.

### **ABT-510 (thrombospondin-1 analog)**

#### **Key point**

- **ABT-510 is a synthetic analogue of TSP-1**

ABT-510 is a synthetic analog of the endogenous angiogenesis inhibitor TSP-1. ABT-510 competes with TSP-1 for binding on endothelial cells to produce similar inhibitory effects. ABT-510 is administered subcutaneously. The most common side effects are injection site reaction and fatigue. Severe reactions possibly related to ABT-510 administration include intracranial hemorrhage, transient ischemic attack, and new onset diabetes.<sup>174</sup> This drug is not yet approved by the FDA. A phase I study measured serum markers of angiogenesis in response to ABT-510 treatment of solid malignancies and found a significant decrease in serum bFGF but no significant change of VEGF and IL-8 levels.<sup>174</sup> In contrast, a phase II study of ABT-510 in patients with metastatic melanoma did show decreased level of serum VEGF-A and -C compared to pretreatment levels. This study failed to show clinical efficacy and was terminated early.<sup>175</sup> There is another ongoing phase II study using ABT-510 for the treatment of metastatic melanoma.

### **THERAPEUTIC AGENTS AVAILABLE FOR ANGIOGENESIS PROMOTION**

#### **Recombinant human platelet-derived growth factor**

#### **Key point**

- **Becaplermin is a topically administered recombinant human PDGF used for promoting wound repair**

Becaplermin is currently the only topical recombinant human PDGF (rhPDGF) available for wound management. rhPDGF has similar biologic activity to endogenous PDGF, which includes promoting chemotactic recruitment, promoting proliferation of cells involved in wound repair, and enhancing the formation of granulation tissue.<sup>176</sup>

Adverse events were similar between becaplermin and placebo and were related to the ulcer and diabetes. The most frequently reported adverse events were infection, skin ulceration, and osteomyelitis. rhPDGF has shown negligible systemic absorption<sup>176</sup>; however, in a postmarketing retrospective analysis, an increased rate of mortality secondary to malignancy was observed in adult patients treated with three or more tubes of becaplermin gel. There is an ongoing study to evaluate the risk of malignancy in users of becaplermin. rhPDGF has FDA approval for treatment of lower extremity full-thickness diabetic neuropathic ulcers. It is also being used off label to promote wound healing of flaps, grafts, surgical wounds and ulcerated hemangiomas.<sup>177,178</sup>

### **CONCLUSION**

Angiogenesis involves a series of defined steps. Angiogenesis is induced when there is an imbalance of growth factors compared to inhibitors, and research has elucidated important pathways involved in this biologic process. Therapeutic agents that target angiogenesis have been developed. These drugs have been used mostly in oncology and ophthalmology but hold promise for treatment of cutaneous disease. Further research will determine the role for these agents in dermatology, but it is likely that they will play an important role in future therapies. In part II of this review, we will discuss angiogenesis in specific cutaneous diseases.

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