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REVIEW ARTICLE

INTEGRIN ANTAGONISTS: POTENTIAL THERAPEUTIC AND DIAGNOSTIC IMPLICATIONS

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SUMMARY

The association of different α and β integrin subunits defines a distinct ligand-binding repertoire. Ligands constitute extracellular matrix proteins and/or counterreceptors on adjacent cells, the latter of which result in interactions between cells. Integrins mediate a variety of cell functions, including adhesion, migration, activation and survival. Several integrins have been implicated in regulating leukocyte infiltration into tissues and contributing to the progression of a number of inflammatory and autoimmune diseases. Current evidence supports a general paradigm involving a multistep process for leukocyte migration from the circulation into tissue. One of the pivotal steps within the cascade corresponds to the firm adhesion of the leukocyte to the endothelium. This is most effectively achieved under an inflammatory stimulus in which a number of integrins and their respective ligands are activated and/or upregulated. The role of integrins and extracellular matrix proteins in various physiological and pathological processes (including angiogenesis, thrombosis, apoptosis, cell migration and proliferation), leading to both acute and chronic disease states (i.e., ocular disease, metastasis, unstable angina, myocardial infarction, stroke, osteoporosis, a wide range of inflammatory diseases, vascular remodeling and neurodegenerative disorders), has been recently documented. A key success in this field is evident from the potential role of the platelet αIIb/β3 (also referred to as GPIIb/IIIa) integrin in the prevention, treatment and diagnosis of various thromboembolic disorders. Additionally, there has been progress in the development of leukocyte α4β1 integrin antagonists for various inflammatory indications, and α5β1 integrin antagonists for angiogenesis and vascular-related disorders. Recently, several reports have illustrated the cross talk between integrins and hormonal systems. The rationale for the development of therapeutic and diagnostic candidates based on the key roles of integrins in various diseases will be reviewed.

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INTEGRINS AND SIGNALING

Integrins are a widely expressed family of cell adhesion receptors by which cells attach to extracellular matrices, to each other and to different cells. All integrins are composed of α/β heterodimeric units and are expressed on a wide variety of cells. Most cells express several different integrins. The interaction of integrins with the cytoskeleton and extracellular matrix appears to require the presence of both subunits. The binding of integrins to their ligands is a cation-dependent event. Integrins appear to recognize specific amino acid sequences in their ligands. The most well studied is the Arg-Gly-Asp (RGD) sequence found within a number of matrix proteins, including fibrinogen, vitronectin, fibronectin, thrombospondin, osteopontin, von Willebrand factor and others. However, integrins can bind to ligands via a non-RGD-binding domain, such as the α4β1 integrin receptor, which recognizes and binds to the Leu-Asp-Val (LDV) sequence within CS-1 (connecting segment-1) of fibronectin. There are at least 8 known α subunits and 14 β subunits (1-4). Although the association of the different α and β subunits could in theory result in more than 100 integrins, the actual diversity is much more restricted.

Integrin adhesion receptors contain an extracellular face that engages adhesive ligands and a cytoplasmic face that engages intracellular proteins. Interactions between cell adhesion molecules and extracellular matrix proteins are critical for cell adhesion and for anchorage-dependent signaling reactions in normal and pathological states (5-10). For example, platelet activation induces a conformational change in integrin αIIb/β3, thereby converting it into a high-affinity fibrinogen receptor. Fibrinogen binding triggers a cascade of tyrosine-protein kinases and phosphatases, and recruitment of numerous other signaling molecules into F-actin-rich cytoskele-

ton assemblies in proximity to the cytoplasmic tails of the αIIb and β3 subunits. These dynamics appear to influence platelet function by co-coordinating signals emanating from integrins and G-protein-linked receptors. Studies of integrin mutations confirm that the cytoplasmic tails of αIIb/β3 are involved in integrin signaling, presumably through direct interactions with cytoskeleton and signaling molecules (11).

Blockade of fibrinogen binding to the extracellular face of αIIb/β3 has been shown to be an effective way to prevent platelet-rich arterial thrombi after coronary angioplasty in patients with myocardial infarction and unstable angina (12-14). Once proteins that interact with the cytoplasmic tail of αIIb/β3 are fully identified, it may also be possible to develop selective inhibitors of integrin adhesion or signaling with sites of action inside the cell. This type of intracellular approach to modulating integrin function will perhaps be more difficult compared to direct blockade of the integrin extracellular binding site due to the lack of cellular specificity for the integrin cytoskeleton-coupled intracellular site(s).

Several physiological processes, including cell activation, migration, proliferation and differentiation, require direct contact between cells and the extracellular matrix. Cell–cell and cell–matrix interactions are mediated through several different families of cell adhesion molecules (CAMs), which include selectins, integrins, cadherins and immunoglobulins. The commercial and therapeutic potential of CAMs is on the rise.

Newly discovered CAMs, along with the discovery of new roles for integrins, selectins and immunoglobulins in certain disease states, provide great opportunities for the development of novel therapeutic and diagnostic modalities. The integrin superfamily represents the best opportunity to develop small-molecule antagonists for both therapeutic and diagnostic utility in various key diseases with unmet medical needs.

β1 INTEGRINS

α3β1 Integrin

The α3β1 integrin plays an important role in the development, angiogenesis and pathogenesis of cancer, suggesting potential therapeutic uses for antagonists of this receptor. Recently, an α3β1 integrin binding site was mapped to residues 190-201 (FQGVLQNVRFVF) of the *N*-terminal domain of the secreted protein thrombospondin-1. Data suggested that these residues, which are critical for biological activity, are contained in a structurally well-defined segment of the peptide (15). These data support the role of the NVR motif as a required element of full-length thrombospondin-1 for specific molecular recognition by the α3β1 integrin.

α4β1 Integrin

Leukocytes, with the exception of neutrophils, constitutively express α4β1 integrin (also referred to as VLA-4). Vascular cell adhesion protein 1 (VCAM-1) expressed on endothelial cells and the alternatively spliced type III CS-1 of fibronectin are the two natural ligands of α4β1 integrin. There are at least seven receptors within this subfamily, each with a different ligand specificity. Among the most studied are α4β1 and α5β1 integrins. α4β1 Integrin is predominantly expressed on lymphocytes, monocytes and eosinophils (16), and is a potential target for therapeutics in chronic inflammatory diseases.

Several humanized monoclonal antibodies (MAbs) directed against integrin targets have proven successful in clinical trials and have been approved for use in humans. This has not only resulted in effective therapies for patients, but has also provided important proof-of-concept studies for the development of small-molecule antagonists. Several integrin subclasses are being evaluated for their potential role in pulmonary, dermatological, gastrointestinal and rheumatic diseases. These include the α4 and β2 integrins, as well as an emerging group of targets from the collagen-binding family of integrins. Interfering with integrin signaling pathways represents a future area of great interest.

Potent and selective small-molecule antagonists of α4β1 integrins

The α4β1 integrins are heterodimeric cell-surface molecules that are central to leukocyte–cell and leukocyte–matrix adhesive interactions. The α4β1 receptor is expressed on all leukocytes except neutrophils and interacts with VCAM-1, a member of the immunoglobulin superfamily of CAMs, and with an alternately spliced form of fibronectin containing CS-1. Another member of this subfamily, α4β7 integrin, is also restricted to leukocytes, and binds to mucosal addressin cell adhesion molecule (MAdCAM), the mucosal addressin or homing receptor, which contains Ig-like domains related to VCAM-1 (17). In vivo studies with α4β1 MAbs in several species demonstrate that the interactions between these receptors and their ligands play a key role in immune and inflammatory disorders. To this end, a selective and potent anti-α4β1 MAb and small-molecule antagonists have been designed. These molecules demonstrated in vivo efficacy in several animal models (18, 19).

The leukocyte α4β1 integrin as a potential target for future therapeutics

The accumulation of leukocytes in various organs contributes to the pathogenesis of a number of human autoimmune diseases, such as asthma, rheumatoid arthritis, Crohn's disease, ulcerative colitis, hepatitis C and multiple sclerosis. The inflammatory processes leading to tissue damage and disease are mediated in part by α4 integrins (α4β1 and α4β7) expressed on the leukocyte cell surface. These glycoprotein receptors modulate cell adhesion via interaction with their primary ligands, VCAM-1 and MAdCAM, expressed in the affected tissue. Elevated CAM expression in various organs has been linked with several autoimmune diseases. Monoclonal antibodies specific for α4 integrins or their cognate ligands can moderate inflammation in animal models, suggesting that such inhibitors may be useful for treating human inflammatory diseases.

These leukocyte populations primarily mediate chronic inflammatory disease (e.g., rheumatoid arthritis, asthma, psoriasis and allergy). α4β1 Integrin, however, is not present on circulating unstimulated neutrophils, which constitute a first line of defense against acute infections. Eosinophils selectively accumulate at sites of inflammation in chronic allergic diseases such as bronchial asthma. The role of α4β1 integrin and its regulation by cytokines and other inflammatory mediators during eosinophil adhesion to endothelium and

extracellular matrix proteins and transendothelial migration has been well documented (19-21). The interaction of α4β1 integrin with alternatively spliced fibronectin containing CS-1 has been exploited in the design and targeting of small-molecule receptor blockers that bind to α4β1 integrin. Evaluation of these analogues in animal models of disease indicates that α4β1 integrin blockade has the potential to achieve dramatic in vivo effects in a variety of chronic inflammatory disorders (18-22).

A single inhaled dose of the α4β1 integrin antagonist GW-559090X was investigated for its ability to protect against allergen-induced changes in airways responses and airways inflammation in patients with asthma (23). A randomized, double-blind, three-way crossover study of a single inhaled dose of 3 mg of GW-559090X or placebo in 15 patients with mild intermittent asthma, controlled with short-acting β_2-adrenoceptor agonists only, was conducted. None of the treatments had any effect on allergen-induced changes in airways hyperresponsiveness or exhaled nitric oxide levels. These findings suggest that α4β1 integrin may not play a major role in allergen-induced airways responses and inflammation in asthma.

Paxillin is a signaling adaptor molecule that binds directly to the α4 cytoplasmic tail, and this binding is important for cell migration. Blocking the adhesive functions of α4 integrins has been shown to be an effective therapeutic approach in the treatment of autoimmune diseases, but also carries the risk of defects in development, and hematopoietic and immune surveillance. Interfering with α4 integrin signaling by inhibiting the α4–paxillin interaction decreases α4-mediated cell migration and adhesion to VCAM-1 and MadCAM under shear flow (24). These in vitro effects are accompanied by a selective impairment of leukocyte migration into inflammatory sites when the α4–paxillin interaction is blocked in vivo. Thus, blockade of α4 integrin signaling may offer a novel strategy for interfering with the functions of these receptors in pathological events while sparing important physiological functions.

α5β1 Integrin

α5β1 Integrin and angiogenesis

In contrast to collagen, expression of the extracellular matrix protein fibronectin in provisional vascular matrices precedes permanent collagen expression and provides signals to vascular cells and fibroblasts during blood clotting and wound healing, atherosclerosis and hypertension (25). Fibronectin expression is also upregulated on blood vessels in granulation tissues during wound healing (26). In fact, one isoform of fibronectin, the ED-B splice variant, is preferentially expressed on blood vessels in fetal and tumor tissues, but not on normal quiescent adult blood vessels (27). These observations suggest a possible role for this isoform of fibronectin in angiogenesis. Animals lacking fibronectin die early in development from various defects, including lack of a notochord and somites, as well as an improperly formed vasculature (28). However, a functional role for fibronectin in vasculogenesis or in angiogenesis has never been directly established.

One candidate receptor for some of the biological roles of fibronectin is integrin. Although several integrins bind to fibronectin (2), α5β1 integrin is generally the most selective for fibronectin. It is unclear, however, whether α5β1 integrin plays a direct role in the regulation of vascular development, or angiogenesis in particular. Evidence was recently provided that both fibronectin and its receptor α5β1 integrin directly regulate angiogenesis. Moreover, the interaction of fibronectin and α5β1 integrin is central to the contribution of these two molecules to angiogenesis. Evidence suggests that α5β1 integrin participates in the same pathways of angiogenesis as αvβ3 integrin, pathways that are distinct from those involving αvβ5 integrin. Thus, α5β1 integrin antagonists might be useful tools for inhibiting angiogenesis associated with human tumor growth, e.g., neovascular-related ocular and inflammatory diseases (29, 30).

α5β1 Integrin plays an important role in developmental angiogenesis, but its role in various types of pathological neovascularization has not been completely defined. Upregulation of α5β1 integrin in choroidal neovascularization has been demonstrated (31). Implantation of an osmotic pump delivering approximately 1.8 or 12 mg/kg/day of JSM-6427, a selective α5β1 integrin antagonist, caused significant suppression of choroidal neovascularization, reducing the area of neovascularization by 33-40%. Data from this study suggest that α5β1 integrin plays a role in the development and maintenance of choroidal neovascularization and provides a potential target for therapeutic intervention.

α5β1 Integrin and bacterial invasion

Recent studies have pointed to a key role for α5β1 integrin in the invasion of human hosts by certain bacterial species, leading to antibiotic resistance (32).

β3 INTEGRINS

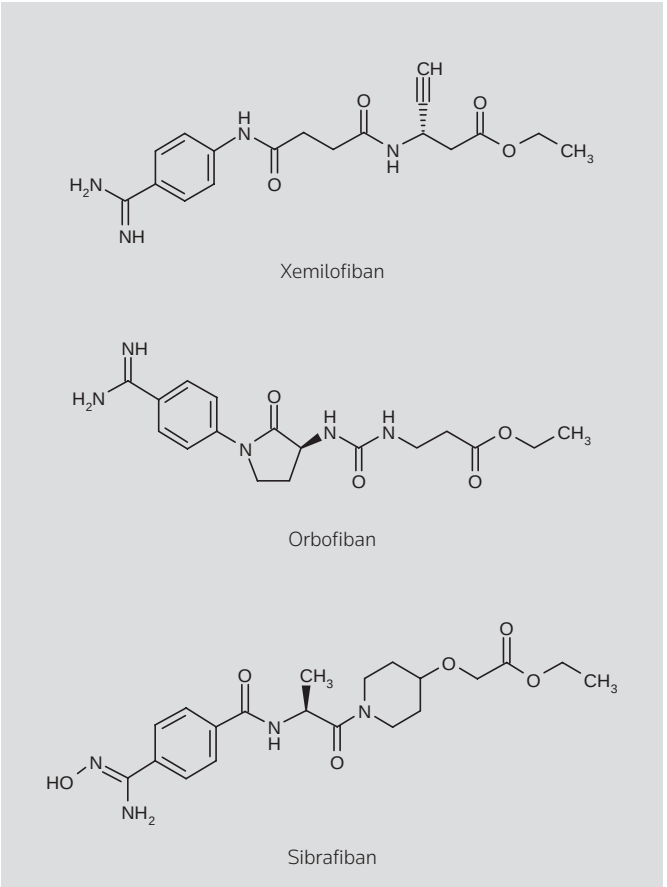
αIIb/β3 Integrin

There is an urgent need for more effective antithrombotic drugs, superior to aspirin or ticlopidine, for the prevention and treatment of various cardiovascular and cerebrovascular thromboembolic disorders. The realization that the platelet αIIb/β3 integrin is the final common pathway for platelet aggregation regardless of the trigger prompted the development of several small-molecule αIIb/β3 receptor antagonists for intravenous (i.v.) and/or oral antithrombotic use. Platelet αIIb/β3 receptor blockade represents a very promising therapeutic and diagnostic strategy for thromboembolic disorders.

Intravenous platelet αIIb/β3 receptor antagonists

A high level of platelet antagonism has been required when i.v. αIIb/β3 antagonists have been employed for acute therapy of coronary artery diseases with heparin and aspirin. Interaction with aspirin and other antiplatelet and anticoagulant drugs leads to shifts in the dose–response curves for both efficacy and unwanted side effects, such as increased bleeding time. As we gain experience with this new class of agents, the benefits and pitfalls associated with their use will become clearer. Antiplatelet therapy has a large role in the prevention and acute treatment of ischemia in patients with acute coronary syndromes. Aspirin, despite being a relatively weak inhibitor of platelet aggregation, decreases the frequency of death or myocardial infarction in all acute ischemic syndromes. αIIb/β3 Integrin antagonists are more potent antiplatelet agents that block the final common pathway of platelet aggregation. For

patients with acute coronary syndromes, compelling data from large-scale trials have established that i.v. αIIb/β3 antagonists decrease the likelihood of coronary events when given before and during angioplasty. In these studies, however, αIIb/β3 inhibition did not prevent later events; after the unstable phase of acute coronary syndromes, cardiac events occurred in treated and placebo groups at similar and significant rates. Accordingly, it was hypothesized that prolonging treatment with oral αIIb/β3 antagonists may provide additional benefit after stabilization.



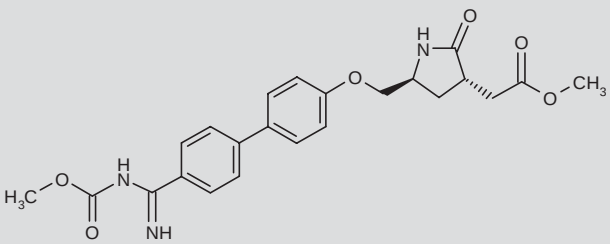
Oral αIIb/β3 antagonists

Clinical experience (efficacy/safety) gained with injectable αIIb/β3 antagonists (i.e., abciximab, eptifibatide, tirofiban) has provided valuable insight into the potential of long-term chronic use of oral αIIb/β3 antagonists. At this point there are still many unanswered questions, and careful studies will be needed to elucidate the safety and efficacy of this route, either alone or in combination with antiplatelet/anticoagulant therapies. More recently, clinical trials of **xemilofiban** (in the Evaluation of Oral Xemilofiban in Controlling Thrombotic Events, or EXCITE, trial) and **orbofiban** (in the Orbofiban in Patients with Unstable Coronary Syndromes, or OPUS-TIMI 16, trial) sponsored by Pfizer, and **sibrafiban** (in the Sibrafiban *versus* Aspirin to Yield Maximum Protection from Ischemic Heart Events Post-acute Coronary Syndromes, or SYMPHONY, trial), sponsored by Hoffmann-La Roche, were withdrawn because of disappointing outcomes (no clinical benefit or increased thrombotic events). This raises serious questions with regard to the potential of oral αIIb/β3 antagonists as compared to the well-documented success of i.v. αIIb/β3 antagonists (33, 34).

Insight into the failure of oral αIIb/β3 antagonists

In contrast to trials of i.v. αIIb/β3 antagonists, the EXCITE trial with xemilofiban including 7,232 patients scheduled for elective percutaneous coronary intervention failed to show a benefit in combined endpoints at 30 days and 6 months (35). The OPUS-TIMI 16 trial with oral orbofiban, which included 10,302 patients who had an acute coronary syndrome within the previous 72 h, showed a trend for lower event rates for the combined endpoints (36). However, this study was prematurely stopped because of excess mortality in the treated group. This raises the question of why are there such conflicting results between i.v. and oral αIIb/β3 antagonists? In the EXCITE and OPUS-TIMI 16 trials, the oral αIIb/β3 antagonist was administered in the background of aspirin treatment, whereas in the SYMPHONY trial low and high doses of oral sibrafiban were compared with aspirin therapy. The SYMPHONY trial and previous dose-ranging studies showed a clear relationship between the dose of sibrafiban and the amount of platelet inhibition and the frequency of bleeding complications. However, during the 90-day treatment period of the SYMPHONY trial, the frequency of the primary endpoint (all-cause mortality, nonfatal myocardial infarction or severe recurrent ischemia) did not differ between sibrafiban and aspirin groups (37). Furthermore, sibrafiban was associated with a higher frequency of complications, particularly increases in minor bleeding events. Despite the less powerful antiplatelet efficacy of aspirin, event rates were similar in the aspirin and sibrafiban groups. This finding suggests that the anti-inflammatory and antiplatelet properties of aspirin might contribute to its benefits. The duration and amount of αIIb/β3 inhibition needed to stabilize plaques and prevent recurrent ischemic cardiac events are not known, nor is it known whether the amount of platelet inhibition required changes over time after the acute coronary syndrome. Use of bedside assays that measure the amount of platelet inhibition may improve the safety and efficacy of αIIb/β3 antagonists once the effective and safe concentrations are known. Additionally, the combination of an αIIb/β3 antagonist and aspirin might be necessary.

In the CAPTURE (C7E3 Anti-Platelet Therapy in Unstable Refractory Angina) and PRISM (Platelet Receptor Inhibition in Ischemic Syndrome Management) trials, i.v. αIIb/β3 antagonists were most effective in patients with elevated serum troponin concentrations (a marker of thrombus formation and a complex lesion), which suggests that patients with high troponin concentrations would perhaps also benefit from lengthy treatment with oral αIIb/β3 antagonists. This hypothesis is supported by results from a subgroup analysis of the FROST (Fibrinogen Receptor Occupancy Study) trial, a placebo-controlled phase II trial with the oral αIIb/β3 antagonist **lefradafiban** in patients with acute coronary syndromes (38). In a subgroup analysis, patients with elevated troponin concentrations on lefradafiban had a greater reduction in cardiac events than did patients with normal troponin concentrations. Subgroup analysis of the SYMPHONY trial, which did not reveal any differences in event rates between



Lefradafiban

groups, did not look at troponin levels. Oral αIIb/β3 antagonists may produce significant benefits when used in addition to aspirin in specified subgroups of patients at high risk of further events, and with individualized dose regimens or improved αIIb/β3 antagonists (optimal pharmacokinetics and pharmacodynamics). The results of the prematurely stopped second SYMPHONY trial in which aspirin was given concomitantly with a low dose of sibrafiban, and the BRAVO (Blockade of the GPIIb/IIIa Receptor to Avoid Vascular Occlusion) trial and other trials will hopefully yield more critical information.

αIIb/β3 Integrin antagonists in the diagnosis of thromboembolic events

Adhesion molecules play a major role in the pathogenesis of many disease states through their ability to mediate cell–matrix interactions. Upon binding to matrix proteins, they alter cellular functions that can be regulated by other pathogenic stimuli acting on the cell. Thus, targeting these receptors should have utility in the diagnosis of diseases the hallmark of which is cellular injury and receptor activation. In order for an agent to be useful as a diagnostic agent it must be specific for the disease, as well as possess adequate contrast. Contrast imaging quality is the result of the combination of the rapid clearance of tissue background and retention at the desired receptor site. The role of the platelet αIIb/β3 integrin receptor and its potential utility as a radiodiagnostic agent in the rapid detection of thromboembolic events have been demonstrated (39). This approach may be useful for the noninvasive diagnosis of various thromboembolic disorders.

αvβ3 Integrin

Although the relationship between integrins and the extracellular matrix is complex and not completely understood, growing evidence points to a role for αvβ3 integrin receptors in growth regulation and anti-apoptosis of tumor cells. A role for integrins in angiogenesis is also supported by the fact that integrins are active in the angiogenic endothelium and dormant on quiescent endothelial cells, and blockade of αvβ3 receptors decreases angiogenesis and tumor regression and triggers endothelial apoptosis. The αv family of integrins associates with various β subunits to form receptors that interact with diverse extracellular matrix proteins, leading to different functional implications (Table I).

αvβ3 Integrin and matrix proteins in vascular remodeling

Vascular remodeling processes play key roles in the pathological mechanisms of atherosclerosis and restenosis. In response to vascular injury, such as by percutaneous transluminal coronary angioplasty (PTCA), matrix proteins such as osteopontin and vitronectin are rapidly upregulated. Osteopontin stimulates smooth muscle cell (SMC) migration via its action on αvβ3 integrin, and thereby contributes to neointima formation and restenosis (40). In addition, the matrix proteins osteopontin and vitronectin induce angiogenesis, which may support neointima formation and arteriosclerosis. Thus, specific matrix proteins acting via selected integrins, particularly αvβ3, may be important targets for selective antagonists aimed at blocking the pathological processes of restenosis (40, 41).

The selective αvβ3 integrin antagonist cRGDfV improved outcomes in a middle cerebral artery occlusion model by preserving the blood–brain barrier, which mechanistically may occur in a VEGF- and VEGF receptor-dependent manner (42).

A series of pyrazole and isoxazole analogues have been developed as antagonists of the αvβ3 receptor. These compounds showed low to subnanomolar potency against αvβ3, as well as good selectivity against αIIb/β3. In HT-29 cells, most analogues also demonstrated significant selectivity against αvβ6. Several compounds showed good pharmacokinetic properties in rats, in addition to antiangiogenic activity in a mouse corneal micropocket model (43).

αvβ3 Integrin antagonists promote tumor regression by inducing apoptosis in angiogenic blood vessels

A single intravascular injection of a cyclic peptide or MAb antagonist of αvβ3 integrin disrupts ongoing angiogenesis in the chick chorioal-

Table I. *αv Integrins, ligands and cellular and tissue distribution.*

αv Integrin	Ligand	Cellular and tissue distribution
αvβ1	Ln, Fn, Opn	Smooth muscle cells, fibroblasts, osteoclasts and tumor cells
αvβ3	Opn, Fg, Vn, Tn, Tsp, Fn, MMP-2	Endothelial cells, smooth muscle cells, osteoclasts, platelets, fibroblasts, tumor cells, epithelial cells and leukocytes
αvβ5	Opn, Fg, Vn, Fn, Tsp	Endothelial cells, smooth muscle cells, osteoclasts, platelets, fibroblasts, tumor cells, epithelial cells and leukocytes
αvβ6	Fn, Fg, Vn, Tn	Epithelial cells and carcinoma cells
αvβ8	Vn	Melanoma, kidney, brain, ovary, uterus and placenta

Ln, laminin; Fn, fibronectin; Opn, osteopontin; Fg, fibrinogen; Vn, vitronectin; Tn, tenascin; Tsp, thrombospondin; MMP-2, matrix metalloproteinase-2.

lantoic membrane (CAM) model (44). This leads to the rapid regression of histologically distinct human tumors transplanted into the CAM. In fact, αvβ3 integrin antagonists also prevent the spontaneous pulmonary metastasis of human melanoma cells (45-51). All human tumors examined in this model were αvβ3-negative, which suggests that these antagonists have no direct effect on the tumor cells. Induction of angiogenesis by a tumor or cytokine promotes entry of vascular cells into the cell cycle and expression of αvβ3 integrin. After angiogenesis is initiated, antagonists of this integrin induce apoptosis of angiogenic vascular cells, leaving preexisting quiescent blood vessels unaffected. These studies are supported by in vitro results. Specifically, cultured human endothelial cells are protected from apoptosis when they are allowed to attach to immobilized anti-αvβ3 MAb LM-609 (46). The adhesion event appears to inhibit the expression of p53 and BAX, while increasing that of Bcl-2. Ligation of αvβ3 is required for the survival and maturation of newly forming blood vessels, an event essential for the proliferation and metastatic properties of human tumors (52, 53). αvβ3 Integrin is preferentially expressed on blood vessels undergoing angiogenesis. Antibody or peptide antagonists of this integrin block angiogenesis in response to human tumors or purified cytokines in several preclinical models. These αvβ3 integrin antagonists promote selective apoptosis of newly sprouting vessels, preventing their maturation. These findings indicate that antibody or peptide antagonists of αvβ3 integrin may have profound therapeutic value in the treatment of diseases associated with angiogenesis (54, 55).

αvβ3 Integrin for targeted drug delivery

In order to selectively block nuclear factor NF-kappa-B (NF-κB)-dependent signal transduction in angiogenic endothelial cells, an αvβ3 integrin-targeted adenovirus encoding dominant negative IκB (dnIκB) as a therapeutic gene was constructed. The RGD-displaying adenovirus mediated the delivery and functional expression of dnIκB via αvβ3, as evidenced by the complete abrogation of TNF-α-induced upregulation of E-selectin, ICAM-1, VCAM-1, IL-6, IL-8, VEGF-A and TIE-2 (56). The approach of targeted delivery of dnIκB into endothelial cells could be applied to diseases such as rheumatoid arthritis and inflammatory bowel disease, where activation of NF-κB activity should be locally restored to basal levels in the endothelium.

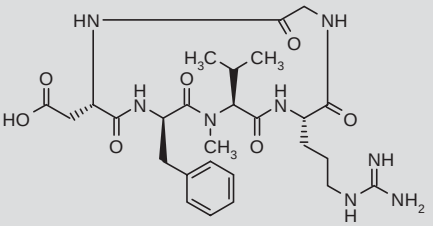
αvβ3 Integrin has been implicated in multiple aspects of tumor progression and metastasis. Many tumors have high levels of expression of αvβ3 that correlate with tumor progression. Therefore, the αvβ3 receptor is an excellent target for drug design and delivery. Reports suggest that a number of high-affinity small-molecule αvβ3 integrin antagonists could be conjugated to paclitaxel for selective delivery to αvβ3-positive metastatic cancer cells (57-60).

αvβ3 Integrin antagonists and chemotherapy/radiotherapy

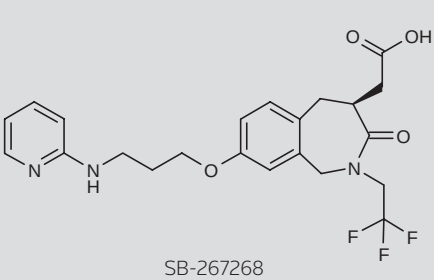
Combination of anti-αvβ3 integrin therapy with other therapeutic approaches such as chemotherapy, radiotherapy and gene therapy has been applied to cancer treatment. Mounting evidence suggests that there is a potentially synergistic effect for combined therapeutic approaches over a single modality. Integrin targeting can also be used for more effective delivery of drugs, genes and radioisotopes, and imaging (i.e., optical, magnetic resonance imaging [MRI], ultrasound, single photon emission computed tomography [SPECT] and positron emission tomography [PET]). αvβ3 Integrin is expressed at low levels on epithelial cells and mature endothelial cells, but is overexpressed on activated endothelial cells of the tumor neovasculature and some tumor cells. The highly restricted expression of αvβ3 integrin during tumor growth, invasion and metastasis presents an interesting molecular target for both early detection and treatment of rapidly growing solid tumors. In the past decade, many radiolabeled linear and cyclic RGD peptide antagonists have been evaluated as αvβ3 integrin-targeted radiotracers. Significant progress has been made on the use of these molecular tracers for imaging tumors of different origin by SPECT or PET in several tumor-bearing animal models. [^{18}F]-Galacto-RGD is under clinical investigation as the first αvβ3 integrin-targeted radiotracer for noninvasive visualization of activated αvβ3 integrin in cancer patients. Radiolabeled multimeric cyclic RGD peptides (dimers and tetramers) are proving to be useful as radiotracers to image tumor αvβ3 integrin expression by SPECT and PET. Some of the fundamental aspects of the development of αvβ3 integrin-targeted radiotracers include the choice of radionuclide and bifunctional chelators, selection of targeting biomolecules, and factors influencing αvβ3 integrin binding affinity and tumor uptake, as well as different approaches for modification of radiotracer pharmacokinetics.

αvβ3 And αvβ5 integrins are important in tumor growth and angiogenesis and have recently been explored as targets for cancer therapy. Radiotherapy also inhibits tumor growth and affects vasculature. We have explored the combination of the integrin antagonist **cilengitide** (EMD-121974) and ionizing radiation. Radiation induces the expression of αvβ3 integrin in endothelial and non-small cell lung cancer models, and cilengitide radiosensitizes tumors in proportion to the levels of target integrin expression (61).

SB-267268, a nonpeptide antagonist of αvβ3 and αvβ5 integrins, attenuates angiogenesis in a murine model of retinopathy of prema-



Cilengitide



SB-267268

turity (ROP) and alters the expression of VEGF and its second receptor (62). Nonpeptidic inhibition of αvβ3 and αvβ5 integrins is effective in ROP and may be a suitable antiangiogenic therapy for other ischemic retinal pathologies.

Antagonists of αvβ3 and αvβ5 integrins inhibit angiogenesis

The enhanced expression of αvβ3 during angiogenesis suggests that it plays a critical role in the angiogenic process. Recent experimental evidence supports this notion. Specifically, antagonists of αvβ3 integrin potently inhibit angiogenesis in a number of animal models. When angiogenesis is induced on CAM with purified cytokines, αvβ3 expression is stimulated 4-fold within 3 days. Topical application of LM-609, an anti-αvβ3 MAb, inhibits this angiogenesis, whereas other anti-integrin antibodies do not. Application of LM-609 or cyclic RGD peptide antagonists, but not other anti-integrin antibodies or control peptides, to tumors implanted on the surface of CAM reduces the growth of blood vessels into the tumor tissue (44). These findings suggest that αvβ3 plays an important biological role in late events of blood vessel formation that are common to embryonic revascularization and angiogenesis. Antagonists of αvβ3 integrin inhibit the growth of new blood vessels into tumors implanted on CAM, without affecting adjacent blood vessels, and also induce tumor regression, with up to 5-fold differences in tumor sizes observed between treated and control tumors. A single intravascular injection of LM-609 halts the growth of tumor xenografts and induces tumor regression, as determined by tumor weight (44-49). Similarly, an injection of a cyclic RGD peptide antagonist of αvβ3, but not an inactive control peptide, induces tumor regression. Histological examination of anti-αvβ3- and control-treated tumors revealed that few, if any, viable tumor cells remained in anti-αvβ3-treated tumors. In fact, the treated tumors contained no viable blood vessels (46).

Antagonists of αvβ3 integrin also inhibit tumor growth in human skin. Exciting results have been obtained from studies of the effect of these antagonists on human angiogenesis in transplanted human neonatal foreskin in SCID mice (46). Tumor growth was either completely suppressed (8 of 12) or significantly inhibited (4 of 12) when compared to mice treated with a control antibody. Furthermore, angiogenesis was significantly inhibited (by at least 75%) in the LM-609-treated animals. Antagonists of αvβ3 integrin also inhibit angiogenesis in various ocular models. Futhermore, αvβ3 integrin antagonists inhibit murine retinal revascularization in an oxygen-induced model of ischemic retinopathy (55).

avb3 Integrin in restenosis

The expression of αvβ3 receptors on cytokine-stimulated blood vessels suggests they may play a role in vascular proliferation and migration events associated with restenosis after angioplasty.

αvβ3 Integrin antagonists versus combination of αvβ3 and αvβ5 integrin antagonists

Given that there are at least two αv integrin pathways for cytokine-mediated angiogenesis (44, 46), combinations of αvβ3 and αvβ5 integrin antagonists might prove to be more effective in certain indications compared to a specific αvβ3 integrin antagonist. However, further work is needed to substantiate this hypothesis.

αvβ3 Integrin in osteoporosis

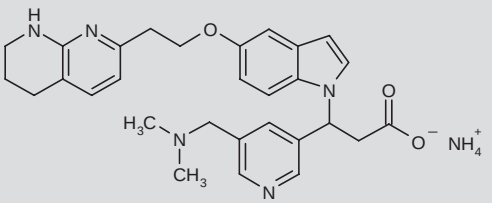
αvβ3 Integrin antagonists have shown that αvβ3 is involved in the bone remodeling process (63, 64). Highly expressed in osteoclasts, this receptor is implicated in the adhesion, activation and migration of osteoclasts on the bone surface, as well as osteoclast polarization. A number of RGD-containing proteins, including osteopontin, bone sialoprotein, vitronectin and fibrinogen, can bind to αvβ3 and regulate bone remodeling. On the other hand, antibodies to αvβ3, RGD peptides and small-molecule αvβ3 integrin antagonists have been shown to be effective in models of bone resorption, providing strong evidence that these inhibitors would be useful agents for the treatment of osteoporosis. RGD analogues have been shown to inhibit the attachment of osteoclasts to bone matrix and to reduce bone resorptive activity in vitro, and αvβ3 integrin appears to play a role in this process. Peptidomimetic antagonists of αvβ3 based on the RGD recognition sequence were synthesized and evaluated in several assay systems. These compounds inhibited the binding of vitronectin to isolated αvβ3, inhibited αvβ3-dependent cell adhesion to vitronectin, and reduced the hypercalcemic response to parathyroid hormone in parathyroidectomized rats. RGD analogues may represent a new approach to modulating osteoclast-mediated bone resorption and may be useful in the treatment of osteoporosis (65, 66).

αvβ3 Integrin ligands

αvβ3 Integrin antagonists as therapeutics

A number of lead αvβ3 integrin antagonists are under preclinical investigation (67-71). However, several key issues are slowing the advance of these promising antagonists. These include pharmacokinetic issues, such as achieving orally active compounds with high oral bioavailability and long half-life, and pharmacodynamic issues, such as measuring ex vivo efficacy in order to predict the optimal dose required for clinical benefit. Additionally, attaining an optimal efficacy/safety ratio (high therapeutic index) for inhibiting pathological angiogenesis with minimal impact on physiological angiogenic processes remains a challenge. A possible solution to the latter problem might be targeted delivery to tumor vasculature (72).

The binding of lead compounds and drugs to human serum albumin (HSA) is a ubiquitous problem in drug discovery since it modulates the availability of the leads and drugs to their intended target, which is linked to biological efficacy. Despite the nanomolar binding affinity of identified lead compounds directed against human αvβ3 and αvβ5 integrins, high HSA binding (96.5-97.3%) is a major limiting factor for these leads. Structure–activity HSA binding data for organic acids recently reported in the literature indicate that the incorporation of polar groups into a given molecule can dramatically decrease the affinity towards HSA. Among the compounds synthesized, 3-[5-[2-(5,6,7,8-tetrahydro-1,8-naphthyridin-2-yl)ethoxy]-1*H*-indol-1-yl]-3-[5-(*N,N*-dimethylaminomethyl)-3-pyridyl]propionic acid (EN: **439844**), was found to be the most promising derivative, with subnanomolar affinity for both αvβ3 and αvβ5, with low HSA protein binding (73).



439844

α4β3 Integrins as diagnostic targets

Imaging of metastatic cancer using technetium-99m-labeled RGD-containing synthetic peptides has been demonstrated. Additionally, detection of tumor angiogenesis in vivo by αvβ3-targeted MRI was shown (74-76).

The αvβ3 integrin is expressed in sprouting endothelial cells in growing tumors, whereas it is absent in quiescent blood vessels. In addition, various tumor cell types express αvβ3 integrin. Due to the selective expression of αvβ3 integrin in tumors, radiolabeled RGD peptides and peptidomimetics are attractive candidates for tumor targeting. The peptidomimetic derivative of RGD and cyclic RGD peptide have high affinity for αvβ3 integrin, and these compounds have tumor-targeting characteristics (77).

A series of radiolabeled cyclic RGD peptide ligands for αvβ3 integrin-targeted tumor angiogenesis are being developed (78). A positron-emitting ^{64}Cu-labeled PEGylated dimeric RGD peptide radiotracer, 64Cu-DOTA-PEG-E[c(RGDyK)]2, has been developed for lung cancer imaging. The PEGylated RGD peptide showed αvβ3 integrin avidity, but PEGylation reduced receptor binding affinity as compared to the unmodified RGD dimer. The radiotracer revealed rapid blood clearance via a predominantly renal route. Minimum nonspecific activity accumulation in normal lung tissue and heart rendered high-quality orthotopic lung cancer tumor images in mice, enabling clear demarcation of both the primary tumor at the upper lobe of the left lung, as well as metastases in the mediastinum, contralateral lung and diaphragm. As a comparison, fluorodeoxyglucose scans of the region were only able to identify the primary tumor, with the metastatic lesions masked by intense cardiac uptake and high lung background. 64Cu-DOTA-PEG-E[c(RGDyK)]2 is an excellent PET tracer for integrin-positive tumor imaging. Further studies to improve the receptor binding affinity of the tracer and subsequently increase the magnitude of tumor uptake without comprising the favorable in vivo kinetics are currently in progress.

α4β7 INTEGRIN

The α4β7 integrin is primarily expressed on mucosal lymphocytes and, apart from interacting with VCAM-1 and CS-1, also binds MadCAM, the expression of which is limited to high endothelial venules of gut mucosal tissues.

Therapeutic implications of α4β7 integrin

Multiple sclerosis (MS) is an autoimmune disease characterized by infiltration of activated T cells into the central nervous system, followed by demyelination and neuronal degeneration. Anti-α4 antibodies have been shown to be effective in a number of animal models of MS.

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by infiltration of activated T cells and monocytes into the synovium. Mechanistic studies in human RA have demonstrated enhanced and activated α4β1 expression on infiltrating leukocytes, and constitutive VCAM-1 expression in the synovial lining. Anti-α4 antibodies are effective in models of adjuvant arthritis and a few small-molecule α4 antagonists have also demonstrated efficacy.

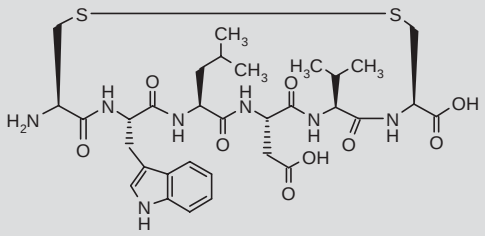
Inflammatory bowel disease (IBD) is thought to be mediated at least in part by α4β7 and interaction with its ligand MadCAM. Antibodies against α4β7 have been shown to reduce inflammation in a number of animal models of IBD. A great deal of effort in the design and development of small-molecule α4 antagonists has centered on identification of the short critical epitopes of VCAM-1 and CS-1 that are responsible for recognition by α4β1 and α4β7.

INTEGRINS IN SICKLE CELL DISEASE

Microvascular complications in sickle cell disease occur as a result of obstruction of small vessels by deoxygenated sickle cells. Cerebrovascular complications are also common and result from obstruction of large blood vessels by thrombosis, with changes in vessels that have some similarity to those found in arteriosclerotic vascular disease. Endothelial damage and activation due to sickle cell–endothelial interactions may contribute to these changes. CAM expression by endothelial cells appears to be stimulated by soluble factors. Antibody inhibition studies support TNF-α and IL-1 produced by sickle leukocytes as the primary soluble factors responsible for the observed expression of CAMs. Both the induction of endothelial CAM expression and subsequent endothelial adherence of sickle erythrocytes may play significant roles in the pathophysiology of sickle-related complications, and reduction in CAM expression may provide a new approach to treatment (79).

Patients with sickle cell disease have elevated circulating levels of cytokines, including TNF-α. TNF-α stimulates expression of adhesion molecules, including VCAM-1, by endothelial cells. Others have demonstrated that VLA-4 (α4β1), a ligand for VCAM-1 or fibronectin, is present on a fraction of sickle reticulocytes. Although red blood cell-mediated vaso-occlusion in the retina and resulting retinopathy are well documented, the effects of red blood cells on the choroidal vasculature are poorly understood. The mechanisms for retention of red blood cells in the retina and choroid appear identical: hypoxia-mediated retention of dense red cells and adherence of red cells in reticulocyte-rich fractions after cytokine stimulation. TNF-α-stimulated retention of red blood cells in the choroid appears to be mediated by VLA-4, presumably on the surface of some reticulocytes. This increased retention was inhibited by **TBC-772**, a VLA-4-neutralizing antibody, or by blocking the CS-1 portion of fibronectin (80).

Abnormal interactions of red blood cells with the vascular endothelium have been implicated in the initiation of vaso-occlusion in sickle cell anemia. Thrombospondin and von Willebrand factor play important roles in mediating red blood cell–endothelium interactions and can bind to the endothelium via αvβ3 receptors. We have



TBC-772

used MAbs directed against αvβ3 and αIIb/β3 integrins to dissect the role of these integrins in red blood cell adhesion. Blockade of αvβ3 may constitute a potential therapeutic approach to prevent red blood cell–endothelium interactions under flow conditions (81).

INTEGRIN–HORMONE CROSS TALK

Evidence that thyroid hormone can act primarily outside the cell nucleus has come from studies of mitochondrial responses to T_3 or T_2 (82, 83), from rapid-onset effects of the hormone at the cell membrane and from actions on cytoplasmic proteins (84-88). The recent description of a plasma membrane receptor for thyroid hormone on αvβ3 integrin (89-91) has provided some insight into effects of the hormone on membrane ion pumps, such as the Na^+/H^+ antiporter (86, 92), and has led to the description of interfaces between the membrane thyroid hormone receptor and nuclear events that underlie important cellular or tissue processes, such as angiogenesis and proliferation of certain tumor cells (93-96).

The potential clinical utility of cellular events that are mediated by the membrane receptor for thyroid hormone may reside in inhibition of such effect(s) in the context of neovascularization or tumor cell growth. We have shown that blocking the membrane receptor for iodothyronines with tetraiodothyroacetic acid (tetrac), a hormone-binding inhibitory analogue that has no agonist activity at the receptor and can arrest the growth of glioma cells and human breast cancer cells in vitro (95). Tetrac is a useful probe to screen for participation of the integrin receptor in the actions of thyroid hormone. The following paragraphs will briefly summarize some of the known effects of thyroid hormone that are mediated by the integrin receptor and then focus on new directions to explore in the area of membrane receptors for the hormone.

αvβ3 Integrin binds thyroid hormone near the RGD recognition site of the protein; the RGD site is involved in the protein–protein interactions linking the integrin to extracellular matrix proteins such as vitronectin, fibronectin and laminin (90). The intact integrin is structurally very plastic (97). Its conformational changes in response to ligand binding may underlie its ability to transduce cell-surface signals into discrete intracellular messages, as well as the ability to expose new surfaces for interactions. The integrin also generates cross talk with other cell-surface receptors. The thyroid hormone signal at the integrin is transduced into mitogen-activated protein kinase (MAPK) activity via phospholipase C and protein kinase C (98). MAPK (ERK1/2) activation is associated with increased Na^+/H^+ antiporter activity locally at the plasma membrane in response to thyroid hormone, and it has been speculated that thyroid hormone effects other ion pumps at the cell surface related to MAPK or PKC activation (92). Also initiated at the cell-surface integrin receptor is the complex process of angiogenesis, monitored in either a standard chick CAM assay or with human endothelial cells in a sprouting assay. This hormone-dependent process requires MAPK activation and elaboration of basic fibroblast growth factor (bFGF), which is the downstream mediator of the effect of thyroid hormone on angiogenesis (93). Tetrac blocks this action of T_4 and T_3, as do RGD peptide and small molecules that mimic RGD peptide. It is possible that desirable neovascularization can be promoted with local application of thyroid hormone analogues, e.g., in wound healing, or that undesirable angiogenesis, such as that which supports tumor growth, can be antagonized in part with tetrac.

Thyroid hormone can also stimulate the proliferation in vitro of certain tumor cell lines (90). Murine glioma cell lines have been shown to proliferate in response to physiological concentrations of T_4 by a mechanism initiated at the integrin receptor that is MAPK-dependent (96). In what may be a clinical corollary, a prospective study of patients with far advanced glioblastoma multiforme in which mild hypothyroidism was induced by propylthiouracil showed an important survival benefit over euthyroid control patients (99). We reported in 2004 that human breast cancer MCF7 cells proliferated in response to T_4 by a mechanism that was inhibited by tetrac (95). A recent retrospective clinical analysis by Cristofanilli et al. (100) showed that hypothyroid women who developed breast cancer did so later in life than matched euthyroid controls and had less aggressive, smaller lesions at the time of diagnosis than controls. Thus, the trophic action of thyroid hormone on in vitro models of both brain tumor and breast cancer appears to have clinical support.

EXPERT OPINION

Efforts focused on leukocyte or endothelial α1 integrins, platelet αIIb/β3, or endothelial/SMC αvβ3 or other αv integrins represent tremendous advances in health and medicine. The platelet αIIb/β3 integrin is leading the way in providing therapeutic and diagnostic applications in various thromboembolic disorders. Examples of currently approved drugs include the antiplatelet αIIb/β3 antagonists abciximab, integrilin and tirofiban, indicated for high-risk patients undergoing PTCA and stenting. ReoPro® (abciximab) is also indicated as an adjunct to PCI for the prevention of cardiac ischemic complications. Aggrastat® (tirofiban) in combination with heparin and aspirin is indicated for the management of patients with unstable angina or non-Q-wave myocardial infarction, including patients who may subsequently undergo PTCA, to decrease the rate of refractory ischemic conditions, new myocardial infarction and death. Eptafibitide (integrilin) is the third inhibitor that has found broad acceptance after abciximab and tirofiban entered the global market. It reduces the risk of acute cardiac ischemic events (death and/or myocardial infarction) in patients with unstable angina or non-Q-wave myocardial infarction both in medically treated patients and those undergoing treatment.

Clinical development of α4β1 integrin antagonists for the treatment of different chronic inflammatory diseases includes natalizumab for the treatment of MS. Not yet approved but in advanced clinical trials is JSM-6427, which inhibits αvβ1 integrin in wet age-related macular degeneration (phase I); cilengitide (anti-αv integrin inhibitor) for glioblastoma (phase III) and head and neck cancer (phase II); and the

anti-αv integrin MAb DI17E6 for colorectal cancer (phase II). Additionally, endothelial and SMC αv integrins represent the next clinical candidates for the treatment of various angiogenesis- and vascular-mediated disorders. More recently, α5β1 integrin has been implicated in the modulation of angiogenesis, similar to αvβ3 integrin. Stimulated endothelial cells depend on αvβ3 function for survival during a critical period of the angiogenic process, as inhibition of the αvβ3–ligand interaction with antibody or peptide antagonists induces vascular cell apoptosis and inhibits angiogenesis. These observations open the door for further analysis of the regulation of cellular function and cell signaling by integrins, as well as for new therapeutic strategies to treat angiogenic diseases.

These strategies have led to the development of αvβ3 and αvβ5 integrin antagonists that promote unscheduled programmed cell death of newly sprouting blood vessels. These antagonists cause regression of preestablished human tumors growing in laboratory animals, and may thus lead to an effective therapeutic approach for solid tumors in humans. These include peptide inhibitors of individual integrins, as well as peptides that inhibit integrins, nonpeptide organic inhibitors, and chimeric or humanized antibody inhibitors of αvβ3 integrin. The first antagonist, a humanized form of the LM-609 antibody (Vitaxin®), has already entered phase II clinical trials and the first of the cyclic peptide antagonists are in initial clinical development.

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DISCLOSURES

The author states no conflicts of interest.

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