Drugs of the Future 2010, 35(1): 16-20

CCC: 0377-8282/2010
DOI: 10.1358/dof.2010.35.1.1450650

MONOGRAPH

VOLOCIXIMAB

Rec INN; USAN

Anti-$\alpha_5\beta_1$ Integrin Monoclonal Antibody
Angiogenesis Inhibitor
Oncolytic

EOS-200-4
M-200

Immunoglobulin G_4, anti-(human $\alpha_5\beta_1$ integrin) (human-mouse clone p200-M heavy chain), disulfide with human-mouse clone p200-M κ-chain, dimer

CAS: 558480-40-3
EN: 330625

SUMMARY

Integrins are dimeric proteins expressed on the surface of most cells in the body. Their primary function is to maintain a dynamic adhesion between the cell and its microenvironment. These transmembrane receptors bind extracellular matrix (ECM) proteins and enable cytoskeletal organization, along with transduction of critical signals into the cells, to promote survival, proliferation, differentiation or migration programs. In addition, there appears to be clear evidence for the importance of integrins in angiogenesis. Their participation in many aspects of vascular biology has been studied. Integrins are the principal adhesion receptors used by endothelial cells to interact with their extracellular microenvironment. Following the wave of research in antiangiogenesis and the development and approval of several such targeted agents, volociximab, an $\alpha_5\beta_1$ integrin inhibitor, has been studied in preclinical and clinical settings. The following monograph provides a brief summary of the available preclinical data and the clinical studies performed so far on volociximab.

PREPARATION

Eos Biotechnology (acquired by PDL in April 2003) initially led the investigation of volociximab to identify a high-affinity antibody to $\alpha_5\beta_1$ integrin (1). The functional domains of $\alpha_5\beta_1$ integrin were cloned and expressed as an Fc fusion protein ($\alpha_5\beta_1$Fc). A BIAcore assay determined that a murine monoclonal antibody (mAb), EOS-100, bound to either $\alpha_5\beta_1$ or $\alpha_5\beta_1$Fc integrin with a dissociation constant (K_d) of 0.2 nM. The variable regions of the murine mAb were then grafted onto the human IgG_4 constant region. This chimeric antibody was designated EOS-200-4 (volociximab) and demonstrated a similar affinity for the $\alpha_5\beta_1$ integrin as EOS-100, with a K_d value of approximately 0.2-0.3 nM (2, 3). Volociximab was stably expressed and purified in Chinese hamster ovary (CHO) cells (2).

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BACKGROUND

Folkman proposed in the 1970s that angiogenesis is required to drive tumor growth beyond a few millimeters and that it is a process by which nascent blood vessels form from existing vasculature to supply new tissue with nutrients. Angiogenesis involves the coordination of vascular cells with fibroblasts, immune cells of blood and tissue origin, and circulating blood components. There is increasing evidence for the importance of integrins in angiogenesis and that they participate in many aspects of vascular biology. Carcinogenesis and metastasis are multistep and complex processes, and can be viewed as a disruption of homeostasis. Also, there is clearly an intricate interplay between altered cell adhesion, survival, proteolysis, migration and lymph/angiogenesis, leading to metastasis. Hence, understanding the adhesive processes of cancer cells is crucial to understanding cancer and its spread, and also to developing targets for therapy.

Integrins are dimeric proteins that contain an α- and a β-subunit. Currently, 18 α- and 8 β-subunits have been identified in vertebrates, and these combine into 24 different dimers. Each dimer has a unique biological function. The specific pairing of the α- and β-subunits determines the receptor diversity, function and versatility in ligand binding, which can induce endothelial cell shape change, motility and growth (3, 4). Among the different integrins, the fibronectin receptor $\alpha_5\beta_1$ appears to be a uniquely proangiogenic integrin (5).

Integrins are expressed on the surface of most cells in the body and were originally referred to as such to denote their function in linking the extracellular matrix (ECM) and the cytoskeleton. Their primary function is to maintain a dynamic adhesion between the cell and its microenvironment. These transmembrane receptors bind ECM proteins and enable cytoskeletal organization, along with transduction of critical signals into the cells to promote survival, proliferation, differentiation or migration programs. Integrins are the principal adhesion receptors used by endothelial cells to interact with their extracellular microenvironment. It has been demonstrated that integrin $\alpha_5\beta_1$ and fibronectin are upregulated in tumor-associated vasculature (3, 6). The fibronectin receptor $\alpha_5\beta_1$ is overexpressed in angiogenic endothelial cells as well.

Vasculogenesis and angiogenesis are the two processes that lead to the formation of new blood vessels during embryogenesis. During these processes growth factors are crucial. For example, vascular endothelial growth factor (VEGF) and its receptors and basic fibroblast growth factor (bFGF) promote not only the initial development of the embryonic vascular network, but also the formation of new blood vessels from pre-existing vessels during development, wound healing and the female reproductive cycle (7-12).

Although growth factors stimulate new blood vessel growth, adhesion to the ECM regulates endothelial cell survival, proliferation and motility during new blood vessel growth. Tumor cells and tumor-associated macrophages are known to secrete matrix metalloproteinases such as MMP-9 and MMP-2 (13). These enzymes degrade the basement membrane, exposing components of the ECM, including fibronectin, vitronectin, laminin, collagen types I and IV, von Willebrand factor, fibrinogen and denatured collagen. During angiogenesis, it is likely that a number of integrins expressed on the surface of activated endothelial cells regulate critical adhesive interactions with a variety of these ECM proteins. Each of these adhesive interactions then regulates distinct biological events of cell migration, proliferation and differentiation. In addition, angiogenesis in different tissues may depend on specific endothelial cell interactions with ECMs that vary considerably in their adhesive protein composition (4, 14).

Studies have demonstrated that blockade of $\alpha_5\beta_1$ ligation inhibits angiogenesis, although the complete mechanism is not yet clear. This may in part be possible through the inhibition of signaling and the induction of the cell death program through caspases. It has been shown that integrin $\alpha_5\beta_1$ antagonists induce a proapoptotic pathway in proliferating endothelial cells that results from activation of initiator caspases (15). Studies seem to indicate that key unligated integrins can also induce cell death when cells are still attached to the ECM through integrin $\alpha_v\beta_3$ or other integrins.

The α_v integrins are also thought to participate in angiogenesis by providing survival signals to activated endothelial cells (7). Unligated integrin $\alpha_5\beta_1$ has been shown to inhibit endothelial cell survival and angiogenesis by induction of apoptosis in ECM-adherent cells by a pathway termed integrin-mediated death (IMD). This pathway may involve activation of protein kinase A (PKA), and subsequently, caspase-8 (17, 18). Interaction between the integrin $\alpha_5\beta_1$ and its ligand fibronectin following growth factor stimulation is important in vascular development (5). Antagonists that specifically block the binding of fibronectin to $\alpha_5\beta_1$ integrin inhibit endothelial cell survival and proliferation in vitro and in vivo, even when other integrins are present that can bind to the ECM (19). Furthermore, inhibitors of $\alpha_5\beta_1$ integrin and fibronectin block angiogenesis in experimental animal models (17). Endothelial cell expression of the $\alpha_5\beta_1$ integrin and the ligand fibronectin are both upregulated during tumor angiogenesis (6, 20-22), but not on unstimulated, quiescent blood vessels. It has been shown that VEGF fails to upregulate $\alpha_5\beta_1$ expression. Thus, the functional roles of integrin $\alpha_5\beta_1$ and fibronectin in angiogenesis appear to be the direct consequence of their growth factor-induced expression.

In addition to its role in angiogenesis, there is evidence of integrin crosstalk with other receptors as well. Integrin $\alpha_5\beta_1$ is an integrin that is upregulated in proliferating endothelial cells. Interaction between fibronectin and integrin $\alpha_5\beta_1$ promotes cell survival through Bcl-2, migration through RhoA and proliferation through ERK-, Akt- and FAK-dependent mechanisms (17, 18).

Several $\alpha_5\beta_1$ antagonists, such as SJ-749, SJ-755 and the peptide Ac-PHSCN-NH2 (ATN-161), have been developed as anticancer agents (6, 23-28). Volociximab is a high-affinity IgG_4 chimeric (82% human, 18% murine) mAb that specifically binds to $\alpha_5\beta_1$ integrin. The activity of the mAb appears to be independent of growth factor stimulus, suggesting that $\alpha_5\beta_1$ signaling occurs downstream of growth factor signaling, and is possibly a final common pathway for the development of neovasculature (6).

PRECLINICAL PHARMACOLOGY

Kim et al. studied expression patterns of fibronectin and its receptor $\alpha_5\beta_1$ during angiogenesis on the vasculature in human normal and tumor tissues and in response to growth factor stimulation in animal models of angiogenesis. Evidence was provided that both fibronectin and its receptor integrin $\alpha_5\beta_1$ directly regulate angiogenesis. There was evidence that the function-blocking $\alpha_5\beta_1$ antibody selectively blocked angiogenesis induced by the growth factor, indicating that integrin $\alpha_5\beta_1$ has a functional role in the angiogenic response of human blood vessels to growth factors. They further studied the role of integrin $\alpha_5\beta_1$ in tumor angiogenesis and growth, demonstrating that targeting vascular cell integrin $\alpha_5\beta_1$ can lead to inhibition of tumor growth and angiogenesis. The study reported that antibody antagonists of the central cell-binding domain of fibronectin, as well as three classes of integrin $\alpha_5\beta_1$ antagonists (antibody, peptide and a novel nonpeptide antagonist), are potent inhibitors of tumor growth and tumor-induced angiogenesis (6).

In several other preclinical models, selective antagonists targeted to $\alpha_5\beta_1$ integrin have also been shown to inhibit tumor growth. The antibody has no potentially adverse effects on the resting endothelium, suggesting that while the $\alpha_5\beta_1$ interaction with fibronectin is a critical event for proliferating endothelium, other receptor–ligand interactions are critical in maintaining the resting state of vessels. It was observed by Ramakrishnan et al. that the inhibitory potential of volociximab appeared to be greater than that of anti-VEGF under various conditions in vitro, due to its ability to inhibit multiple growth factor pathways and induce apoptosis in proliferating endothelial cells (17, 27, 29).

Volociximab was more effective than HuMV833 (an anti-VEGF mAb) in inhibiting the proliferation of human umbilical vein endothelial cells (HUVEC) in response to VEGF. No additive effects were observed after the coadministration of volociximab with HuMV833 (30). These preliminary studies seemed to indicate a mechanism of action independent of growth factor stimulus and causing apoptosis of actively proliferating, but not resting, endothelial cells, as mentioned above. In addition to the direct effects of volociximab on endothelial cells, there may be an indirect antiangiogenic effect in macrophages. Volociximab significantly and specifically inhibited the release of proangiogenic cytokines, including IL-8 and the growth-related alpha protein GRO-α, by differentiated macrophages. Other cytokines, such as the proinflammatory factors IL-6 and TNF-α, were unaffected by volociximab (30).

In a simian model of choroidal neovascularization (CNV), intravitreal and i.v. injection of volociximab significantly inhibited laser-induced neovascularization at the back of the eye. Hence, volociximab can also potentially be used for disorders such as macular degeneration (2, 3).

SAFETY

In preclinical models (monkey and rabbit models of CNV) administration of volociximab appeared to have no detrimental effect (2, 29).

Most of the clinical studies reported below appear to demonstrate a tolerable side-effect profile for the drug. The most commonly observed adverse events include fatigue, anorexia, fever, arthralgias and myalgias, gastrointestinal symptoms (nausea and vomiting), headache, edema and hypertension. In the most recently published phase I clinical trial by Ricart et al., no patients were reported to have any grade III toxicities. Grade II toxicities included fatigue, nausea, vomiting, edema and arthralgias. Neither hematological toxicity nor infectious complications were observed. One of the 223 infusions was interrupted due to an infusion reaction, and subsequent infusions were administered uneventfully with the use of premedication. There was no documented dose-limiting toxicity (DLT). No injection-site reactions were reported. Two patients developed anti-volociximab antibodies, but these were not associated with any adverse events (31).

In a combination phase I trial (volociximab in combination with carboplatin and paclitaxel) for non-small cell lung cancer (NSCLC), of a total of 22 patients, grade III bowel obstruction was observed in 1 patient and was considered a DLT in 1 of the cohorts. Again, more than half of the patients reported constipation, asthenia, nausea, arthralgia and paresthesia. Seven patients reported at least one serious adverse event (all grade 3), including deep vein thrombosis, peripheral arterial occlusion, proteinuria, pneumonitis, small intestinal obstruction, pleural effusion, hypoxia, dehydration and orthostatic hypotension. It is possible that some of the adverse events can be attributed to carboplatin and paclitaxel used in combination with volociximab (32).

CLINICAL STUDIES

A phase I pharmacokinetic and biological correlative study was conducted by Ricart et al. based on the data for antiangiogenic activity in preclinical models of angiogenesis. Patients with advanced solid malignancies were treated with escalating doses of volociximab administered i.v. over 60 min. Blood samples were assayed to determine plasma pharmacokinetic parameters, detect human anti-chimeric antibody formation and determine the saturation of $\alpha_5\beta_1$ sites on peripheral blood monocytes. Twenty-one patients received a total of 223 infusions of volociximab at doses ranging from 0.5 to 15 mg/kg. It was observed that the saturation of free $\alpha_5\beta_1$ integrin sites on monocytes by volociximab was dose-dependent. The investigators concluded that the dose reductions from the highest dose determined in this study may result in increased clearance of volociximab, as well as incomplete saturation of all $\alpha_5\beta_1$ integrin target sites. They determined that the highest dose that can be administered to patients was 15 mg/kg. Antitumor activity was seen in a patient with metastatic renal cell carcinoma refractory to sunitinib, who had a minor response. Five other patients who had malignancies with documented progressive disease before study entry experienced durable stable disease that lasted for at least 4 months. One of these patients who had metastatic melanoma with hepatic metastases had stable disease for 14 months. At the dose range examined in this study, volociximab showed nonlinear pharmacokinetic parameters consistent with a target saturation effect (31).

Volociximab is also being studied in various tumor types as combination therapy. A phase I trial of volociximab in combination with carboplatin and paclitaxel in patients with advanced NSCLC was reported last year (32). The highest dose of volociximab tested was 30 mg/kg every 3 weeks in combination with carboplatin and paclitaxel, and appeared to be tolerated well by patients. A total of 22 patients were treated and the drug appeared to show a good clinical response in several patients. A partial response was seen in 6 patients and stable disease was seen in 12 of 18 patients.

A phase II study comparing pegylated liposomal doxorubicin with and without volociximab in recurrent ovarian or primary peritoneal cancer (refractory to platinum/taxane-based chemotherapy [maximum two prior lines]) did not show evidence of benefit for the addition of volociximab. The investigators attempted to compare the efficacy and safety of pegylated liposomal doxorubicin with volociximab versus pegylated liposomal doxorubicin alone (33). Reports of the phase II component are awaited.

Other studies include volociximab in combination with gemcitabine for pancreatic cancer and in combination with erlotinib for stage IIIB or IV NSCLC. Volociximab in combination with gemcitabine for pancreatic cancer was well tolerated up to 15 mg/kg/week in a phase II trial. Overall response for volociximab with gemcitabine for pancreatic cancer included a confirmed partial response in 1 patient and stable disease in 10 of 20 patients. Median time to progression was 3.4 months (34).

A multicenter, open-label phase II study in metastatic renal cell carcinoma patients enrolled a total of 40 patients who received volociximab 10 mg/kg i.v. every 2 weeks until disease progression. Twenty-one patients (52.5%) had prior antiangiogenic therapy. Stable disease was observed in 80% of the patients, with a duration of response ranging from 2 to 22 months. There was one confirmed partial response. The time to progression for 35% of the patients was between 5.8 and 22 months, with a median of 4 months. Overall survival at 6 and 22 months was 79% and 68%, respectively (35).

In a multicenter phase II study of volociximab in patients with relapsed metastatic melanoma, weekly volociximab demonstrated insufficient clinical activity to proceed to the second stage of the trial (36).

A trial of volociximab in combination with liposomal doxorubicin in relapsed advanced epithelial ovarian and primary peritoneal carcinoma has also been initiated, with reports of the phase II component awaited (ClinicalTrials.gov Identifier NCT00635193).

FUTURE DIRECTIONS

In the 1970s, Folkman explored the theory that angiogenesis could support tumor growth and therefore be a target for cancer therapy. This theory was then fostered by Folkman for over two decades. Ultimately, it was vindicated in animal tumor models via modern molec-

ular and cell biology techniques. Subsequently, with the successful clinical trials of a humanized mAb that neutralizes VEGF, the theory of targeted antiangiogenic treatment for cancer has taken root. We also have a better understanding of the cellular pathways of vasculogenesis and neovascularization, although our knowledge is far from complete. Bevacizumab (Avastin®) is an mAb directed against VEGF that was launched by Genentech in the U.S. in 2004 for the treatment of colorectal cancer and is now approved for use in several different cancer types. A number of other antiangiogenic agents have been approved, with varying results, some being more promising than others. Angiogenesis clearly represents a vital pathway for targeting cancer. The pursuit to identify a targeted agent with minimal off-target activities and excellent clinical activity continues.

Following the wave of research, volociximab has been studied in preclinical and clinical settings. The mAb appears to have demonstrated some promising early results. It is important to note, however, that the widespread use of antiangiogenic agents has been partially humbling. Although clinical activity is seen, these agents have so far proven to be "disease stabilizers" at best. There is evidence of tumor shrinkage for several months, followed by "escape of the cancer". This resistance to therapy is different from the traditional concept of drug resistance and probably involves the activation and/or upregulation of alternative proangiogenic signaling pathways within the tumor. In this context, the development of additional and newer targets of angiogenesis is important. Most tumors use multiple pathways and growth factors for angiogenesis. It appears that VEGF is most commonly associated with the hypoxic core of the tumor, as it is transcriptionally regulated by hypoxia, whereas certain other factors, such as bFGF, may be associated with the growing edge of the tumor (37-39). Also, the development of combinations to shut down different pathways, either as a horizontal or a vertical blockade, is an active area of research, and volociximab may be a candidate considering its favorable toxicity profile. However, much clinical research must be performed before volociximab becomes a viable option.

SOURCES

Biogen Idec (US); developed in collaboration with Facet Biotech Corp.; licensed to Ophthotech Corp. for wet age-related macular degeneration.

DISCLOSURES

The author states no conflicts of interest.

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