Introduction

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Glioblastoma (GBM) is the most common malignant brain tumor and is associated with a poor prognosis (median survival is approximately 15 to 20 months in protocol-eligible patients with newly diagnosed GBM). Approximately 15,000 persons in the United States are diagnosed with GBM each year. Despite extensive research efforts, there have been few therapeutic advancements and consequently only incremental improvement in patient survival. The new standard of care for newly diagnosed GBM, introduced in 2005, is temozolomide given concurrently with radiotherapy and for 6 months post-radiotherapy. This management strategy has resulted in some improvement, but the prognosis for the majority of patients with GBM nonetheless remains discouraging. The juncture has nearly been reached where basic research in GBM is producing exciting new data that are defining the molecular basis for tumor development and suggesting biomarkers that are both predictive and prognostic. This new information is increasingly being used to design drugs and drug trials that target specific steps in the aberrant molecular biology of this aggressive and ultimately fatal cancer.

Integrins are a family of cell surface receptor proteins that are involved in many critical pathways that contribute to the malignant phenotype of GBM. Integrins therefore represent potential anti-GBM targets for specific anti-integrin inhibitors. Accordingly, anti-integrins and integrin inhibitors are currently in advanced trials as novel agents to treat GBM.

This monograph provides an overview of GBM pathophysiology, reviews the interplay between cancer cells and their environment, and highlights the involvement of integrins in this relationship. Additionally, specific anti-integrin agents in development are described along with current clinical trials suggesting integrin inhibitors are valid and novel anti-GBM therapeutic agents. Constraints associated with targeted therapies are considered, and the future outlook summarized as pursuit of targeted therapies for GBM continues.

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CME/CNE Pretest

Understanding the Clinical Applicability of
Integrin Inhibition in the Treatment of Glioblastoma

CME/CNE Instructions

Answer each pretest question by entering it in the space provided on the registration form on page 18. Responses to the pretest will not affect CME credit for this activity and will only be used to assess the efficacy of the activity.

1. **A 60-year-old patient with normal neurologic function and newly diagnosed GBM managed with an aggressive multimodal treatment approach is likely to have a median survival of:**

   A. 9 to 12 months
   B. 12 to 15 months
   C. 15 to 20 months
   D 20 to 24 months

2. **Which of the following is true about integrins?**

   A. One integrin can bind many ligands, but each ligand can only bind to 1 integrin.
   B. Integrins activate the NF-κB pathway.
   C. Integrins are involved with angiogenesis but not vasculogenesis.
   D. Overexpression of integrins in cancer is limited to the tumor cells themselves.

3. **MGMT promoter methylation:**

   A. Is important because patients without promoter methylation respond better to TMZ therapy.
   B. Precludes patients from enrolling in cilengitide clinical trials.
   C. Is associated with better survival outcomes in newly diagnosed GBM and appears predictive of response to TMZ and perhaps cilengitide.
   D. Was associated with subtype in the TCGA GBM classification studies.

4. **Preclinical studies have shown that cilengitide:**

   A. Has no effect on apoptosis.
   B. Induces cell adhesion.
   C. Is anti-angiogenic.
   D. Inhibits apoptosis.

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Overview

Integrins play a crucial role in tumor signaling, survival, migration, and angiogenesis, making them potent targets for cancer therapy, particularly for gliomas and glioblastomas. Several integrin inhibitors are being studied and have been shown to be effective with minimal side effects. This monograph will provide an overview of integrin biology and the role of anti-integrins in the treatment of glioblastomas.

Target Audience

The intended audience for this activity is oncologists, hematologists/oncologists, oncology nurses, and other health care professionals involved in the treatment of patients with cancer.

Unlabeled and Investigational Usage

The audience is advised that this continuing medical education activity may contain references to unlabeled uses of FDA-approved products or to products not approved by the FDA for use in the United States. The faculty members have been made aware of their obligation to disclose such usage. All activity participants will be informed if any speakers/authors intend to discuss either non-FDA approved or investigational use of products/devices.

Understanding the Clinical Applicability of Integrin Inhibition in the Treatment of Glioblastoma

Marc C. Chamberlain, MD; Timothy Cloughsey, MD; David A. Reardon, MD; Patrick Y. Wen, MD

Glioblastoma (GBM) is the most common primary malignant brain tumor, with an incidence rate in the United States of 3.19 per 100,000 person-years.[1] GBM is also the most lethal, with an overall US 1-year survival rate from 1995 through 2007 of only 34.6%, decreasing to less than 5% of patients surviving for 5 years after diagnosis. Protocol-eligible patients (ie, patients younger than 70 years of age, independent in activities of daily living, and with no medical comorbidities) managed with an aggressive multimodal treatment approach have a median survival of approximately 15 to 20 months,[2,3] compared with 12 months if they receive radiation therapy only, and 3 months with no treatment.[4]

Part of the challenging nature of GBMs is related to their heterogeneous and invasive phenotype, characterized by areas of microvascular proliferation, brain invasion, and necrosis. Understanding the complex mechanisms that define GBM as among the most vascular of human cancers and finding agents to interfere with these processes have been a major focus of GBM research.

Primary GBMs (accounting for >90% of all adult GBMs) develop de novo and usually occur in patients aged 50 years and older. Their distinguishing characteristics include epidermal growth factor receptor (*EGFR*) amplification. Of the 40% to 50% of primary GBMs with this amplification, approximately one-half have the EGFRvIII variant, a constitutively active EGFR which has become a therapeutic vaccine target. Secondary GBMs are much less common and result from the progression of de-differentiating low-grade gliomas into GBMs, typically in younger patients.[2] Approximately two-thirds of secondary tumors have p53 suppressor gene mutations and overexpression of platelet-derived growth factor receptor (*PDGFR*), among others. These 2 subtypes respond similarly to conventional temozolomide (TMZ)-based therapy. However, with their genetic and transcription pattern differences, this similarity in response to treatment may not be upheld with targeted therapy. Mutations in the gene encoding isocitrate dehydrogenase (*IDH1*) have also been observed in patients with GBM.[5] Isocitrate dehydrogenase catalyzes the oxidative

carboxylation of isocitrate to α-ketoglutarate, leading to the production of nicotinamide adenine dinucleotide phosphate (NADPH). The IDH1 protein is believed to play a substantial role in cellular control of oxidative damage through generation of NADPH.[6,7] A genomic analysis of human GBMs indicated that patients harboring this mutation tended to be younger, with a mean age of 33 years compared to 53 years for patients who have wild type *IDH1*. Also, this mutation was predominantly present in patients with secondary GBM. Furthermore, patients with *IDH1* mutations had significantly improved prognosis compared to patients wild type *IDH1*.[5]

GBMs are distinguished from lower grade gliomas by the presence of endothelial proliferation and necrosis. These cancers are highly invasive locally, rarely metastasize, and are histologically heterogeneous both with respect to neoplastic and stromal tissues. GBM arises as a stepwise accumulation of both genetic and epigenetic aberrations with concomitantly and commonly deregulated growth factor signaling. The complexity of this process is exemplified by the presence of at least 5 growth factor receptor families expressed on the GBM cell. These growth factor receptor families include vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), PDGFR, EGFR, and insulin-like growth factor receptor (IGFR), each comprising numerous members and each with specific activating ligands.[8] Molecular hallmarks of GBMs include *EGFR* amplification and activating mutations. In addition, some reports have theorized that deletion of the gene encoding the nuclear factor of the κ-light polypeptide gene enhancer in B-cells inhibitor-α (*NFKBIA*) may promote tumorigenesis in GBMs that are devoid of *EGFR* alterations.[9]

Standard GBM Treatment

One of the most important management considerations for patients with GBM is to provide supportive care. A major aspect of caring for patients with GBM is providing medical management with antiepileptic drugs for tumor-related seizures, corticosteroids for peritumoral edema, anticoagulation therapy for venous

thromboembolism, and appropriate therapy for symptoms of fatigue, cognitive dysfunction, and depression.[2]

Newly diagnosed disease

For newly diagnosed GBM, surgery and radiotherapy comprised the basic management approach for years, yet provided only modest survival benefit.[8] Initial efforts to identify a beneficial chemotherapeutic drug for newly diagnosed GBM were largely unsuccessful. FDA-approved chemotherapy was added to standard care[8] practice guidelines for newly diagnosed GBMs after it was reported in 2005 that daily TMZ, an oral alkylating agent, administered concomitantly with radiotherapy for up to 49 days followed by 6 monthly 5-day courses of TMZ, produced a modest but significant 37% reduction in risk of death during the median 28-month follow-up interval (HR 0.63; 95% CI, 0.52 to 0.75; $P < .001$).[10] Progression-free survival (PFS) at 6 months was 53.9% in the TMZ group, compared with 36.4% in the radiotherapy-only group. Two- and 5-year overall survival (OS) was 27.2% and 9.8% in TMZ-treated patients, compared with 10.9% and 1.9% in patients receiving radiotherapy only.[11]

Recurrent disease

GBM is accompanied by a nearly 100% recurrence rate despite aggressive upfront treatment. Surgery in selected patients who might benefit provides only a limited survival advantage, and the potential benefits of re-radiotherapy for recurrent disease are controversial.[2] Although TMZ therapy was reported to improve 6-month PFS in patients with recurrent anaplastic gliomas (35% response rate; 46% 6-month PFS),[12,13] the response rate in patients with recurrent GBMs was very limited (5.4% response rate; 21% 6-month PFS).[14] Alternative dosing schedules of TMZ (metronomic daily TMZ or dose-dense TMZ) have been utilized for recurrent GBM previously treated with upfront TMZ and may offer therapeutic benefit in a subset of patients with recurrent GBM.[15,16]

Inhibition of angiogenesis has been at the forefront of GBM therapeutic research for several years. Early trials investigating thalidomide and other less potent angiogenic inhibitors (AI) have largely been negative in recurrent GBM. However, studies of newer AIs (ie, bevacizumab) have produced improvements in radiographic response rates and 6-month PFS in patients with recurrent GBM that resulted in the FDA approval of bevacizumab as a salvage therapy in recurrent GBM in 2009 and its addition to practice guidelines.[17] In 1 study where patients received bevacizumab as a single agent or combined with irinotecan, the primary endpoint of proportions of patients achieving 6-month PFS were 42.6% and 50.3%, respectively,[18] and 29% in patients receiving bevacizumab alone in another study.[19] Median overall survival was 31 to 37 weeks in both studies. In addition to bevacizumab, there are at least 9 compounds with anti-angiogenic properties in phase 2 or phase 3 clinical trials.[20]

Bevacizumab is a humanized monoclonal antibody that targets VEGF, the first angiogenic mediator described in GBM pathogenesis. VEGF is abundantly expressed in glioma cells and is upregulated in these cells by EGFR.[21] This strategy exemplifies the increasing interest in targeting a discrete molecule, with the intuitive rationale that inhibiting a factor that is critically involved in tumor angiogenesis should diminish the blood supply of the tumor and therefore have at least a cytostatic anti-tumor effect. Paradoxically, preclinical data are emerging that suggest that bevacizumab treatment may lead to enhanced tumor cell invasion in response to the treatment-elicited hypoxic tumor microenvironment. Using a xenograft human GBM model, bevacizumab treatment induced a reduction in large- and medium-sized blood vessels. However, it also resulted in a switch to a more invasive phenotype as evidenced by a 68% ($P < .001$) increase in cells invading normal brain, which were infiltrating at distances much farther from the tumor core compared with controls.[22] These preclinical data suggesting increasing invasiveness led to clinical analyses of patterns of recurrence after bevacizumab therapy. In a retrospective review of 55 consecutive patients with recurrent malignant gliomas who received bevacizumab and chemotherapy, a significant increase in the volume of infiltrative tumor relative to enhancing tumor in bevacizumab responders was observed.[23] In another study, at a median follow-up of 7 months, 79 of 162 patients with high-grade gliomas taking bevacizumab developed diffuse invasive recurrence (DIR).[21] The hazard risk of DIR increased with time and was similar in those with newly diagnosed and recurrent disease. The pattern of relapse did not affect overall survival ($P = .253$) and the duration of bevacizumab therapy increased the interval to recurrence ($P < .0001$) and improved overall survival ($P < .0001$). The interval to progression was similar in the DIR and local recurrence groups (6.5 vs. 6.3 months, $P = .296$).[24] Other clinical studies with bevacizumab and recurrent GBM, on the other hand, did not convincingly demonstrate an increase in GBM invasion.[25-27] Therefore,

> Glioblastoma is accompanied by a nearly 100% recurrence rate despite aggressive upfront treatment.

Table 1. Integrins in Tumor Cells

Tumor Type	Integrins Expressed
Breast	$\alpha_6\beta_4/\alpha_v\beta_3$
Cervical	$\alpha_v\beta_3/\alpha_v\beta_6$
Colon	$\alpha_v\beta_6$
Glioblastoma	$\alpha_v\beta_3/\alpha_v\beta_5$
Melanoma	$\alpha_v\beta_3/\alpha_5\beta_1$
Non-small Cell Lung Carcinoma	$\alpha_5\beta_1$
Ovarian	$\alpha_4\beta_1/\alpha_v\beta_3$
Pancreatic	$\alpha_v\beta_3$
Prostate	$\alpha_v\beta_3$

Source: Desgrosellier JS, et al. *Nat Rev Cancer.* 2010;10:9-22.

the relationship of bevacizumab therapy to invasive recurrence is controversial and additional studies are required to further discern this relationship.

GBM Phenotype

As experience with targeted therapies in clinical trials for GBM increases in concert with knowledge of the often unique molecular pathology of these tumors, the basis for and influences on the tumor phenotype has become an important research area. Malignant cells can assume

Figure 1. Cancer Phenotypes

Cancer cells are associated with a variety of phenotypes.
Source: Hanahan D, et al. *Cell.* 2000;100:57-70.

one or more of several phenotypes (Figure 1).[28] GBMs are highly vascularized, characterized by aggressive invasion of the surrounding brain tissue with extensive microvascular proliferation. However, a cellular hierarchy exists that contributes to their heterogeneity.[8,29-31] Invasiveness is accomplished by migration that is mediated by cell-cell interactions as well as by adhesion to and degradation of the extracellular matrix (ECM). For a tumor to grow beyond 1 to 2 mm, it must induce angiogenesis, or the sprouting and proliferating of endothelial cells from extant vessels in its periphery.[32] In normal adults, formation of new blood vessels is rare, occurring primarily in wound healing and endometrial re-epithelialization. The pathological angiogenesis that is essential for tumor growth differs from normal angiogenesis. GBM-induced vessels are structurally abnormal, lack a blood-brain barrier, exhibit significant tortuosity, and are generally described as "leaky."[33]

The process whereby new vessels sprout from existing vessels requires a cascade of events involving numerous key molecular players. Upregulation of VEGF-A, PDGF, and basic FGF-2 is associated with the increase in proliferative activity of endothelial cells, is involved with their survival, and marks the transition from low-grade to high-grade gliomas.[20,34,35]

Tumors can enhance their vascularity by means other than angiogenesis.[36] Tumors can co-opt host vessels, or they can induce vasculogenesis by stimulating colonization by circulating bone marrow-derived cells. Angiogenesis is now understood to be an integral aspect of tumor progression.

Malignant phenotypes can be upregulated or downregulated in vitro by alterations in the quantity and activity of the numerous signal pathway factors involved in their expression. The therapeutic potential of targeting factors associated with angiogenesis, invasiveness, and survival continues to be investigated. As complex interrelationships that control GBM pathophysiology at the molecular level unravel, the role of integrins in these processes has become a focus of attention.

Integrins

Integrins are a family of transmembrane receptor proteins. They are heterodimers, each composed of one α and one β subunit bound together noncovalently. There are at least 24 known heterodimers formed by 18 α and 8 β subunits. Natural integrin ligands include important components of the ECM, such as vitronectin, fibronectin, laminin, FGF, matrix metalloproteinase (MMP)-2, thrombospondin, osteopontin, collagen, fibrin, and fibrinogen.[37,38] Binding of integrins can be redundant; that is, many integrins can bind the same ligand, allowing substitution of functions. Integrins can also be promiscuous, with a single integrin binding multiple ligands.
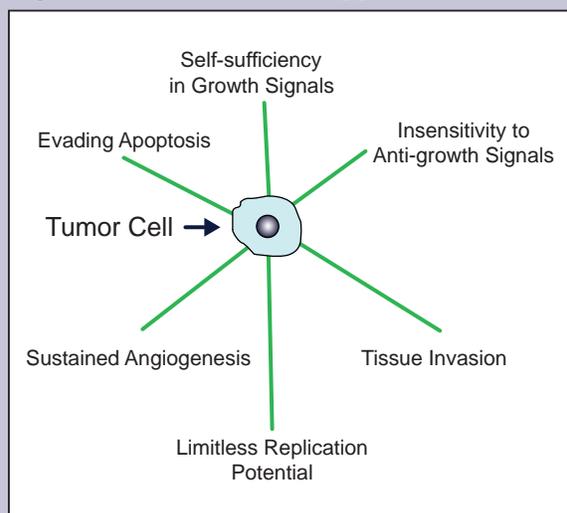
The structure of integrins includes, for each subunit, a large extracellular domain responsible for ligand binding, a single membrane-spanning domain, and a short intracytoplasmic tail. Many integrins bind their ligands in the short arginine-glyci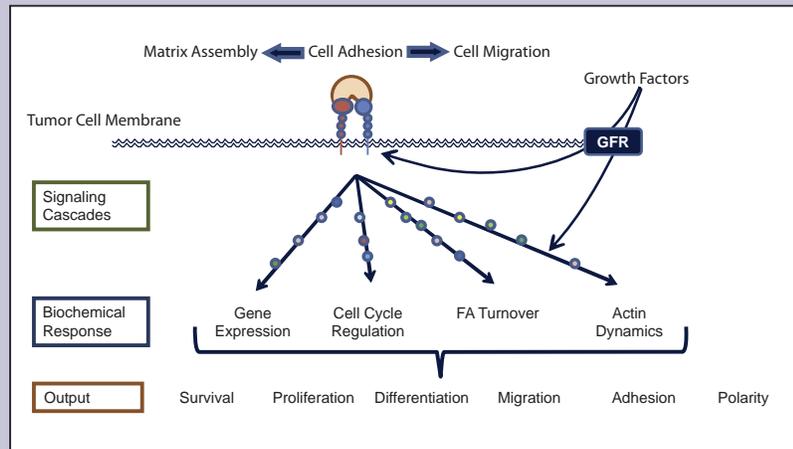ne-aspartate (RGD) amino acid sequence that comprises the cell attachment site for many proteins.[39] Ligand binding induces integrins to form focal adhesion complexes in the cell membrane. Focal adhesion complexes are composed of clusters of integrins with signaling and adaptor proteins (Figure 2).[40,41] These complexes recruit focal adhesion kinases, which activate intracellular downstream signaling pathways, including the nuclear factor (NF)-κB, phosphoinositide-3 kinase, Src, and Ras-MAP kinase cascades. These pathways regulate functions involved in proliferation, motility, cytoskeleton organization, and survival. Given the role of integrins in regulating these pathways, it follows that alterations to integrin activity facilitates the development of a malignant phenotype.

Integrins fulfill signaling functions that are essential for cell migration, proliferation, survival, and for adhesion to the ECM.[40] These activities provide the basis for their critical role in angiogenesis, and integrins have been referred to as providing a "functional hub" for this process.[42] Recruitment of pericytes to newly created blood vessels involves binding of integrins on endothelial cells to vascular adhesion molecules on the pericytes.[43] Cell migration is mediated by remodeling the intracellular actin cytoskeleton in the leading edge of the cell, with coordinated extracellular cell-matrix adhesions providing the traction necessary for cell movement.[44] "Cross-talk" occurs between integrins and cytokines and growth factors on host cells. These cells include myeloid and other cell types known to be associated with vasculogenesis, and this cross-talk stimulates their infiltration into the tumor microenvironment.[36,42-48]

Integrins are expressed in all cell types that are involved in angiogenesis, including many tumors (Table 1). Some integrins are limited to certain cell types or tissues, while others are more ubiquitous. Five integrins ($\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$, $\alpha_V\beta_5$) that are expressed by epithelial cells are usually also expressed in solid tumors originating from epithelial cells, although often at altered levels.[43] Three integrins ($\alpha_5\beta_1$, $\alpha_V\beta_3$, $\alpha_V\beta_6$) that may be undetectable in normal epithelia are overexpressed in some tumors. Expression of these 3 integrins along with $\alpha_6\beta_4$ and $\alpha_V\beta_5$ is associated with disease progression in some tumors. Increased integrin expression in cancers is not limited to the tumor cells. In GBMs, for example, integrin $\alpha_V\beta_3$ is highly overexpressed not only on the advancing margins of the tumor, but in proliferating vascular endothelial cells as well. These integrins, therefore, have a possible role in tumor invasion.[49] The $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins are known to be critically associated with angiogenesis in many tumor types.[50]

Two recent advancements in understanding the role of integrins in GBM biology underscore the importance of these factors. Brain tumors are now known to contain a subpopulation of pluripotent stem cells (sometimes referred to as glioma stem or initiating cells) that are responsible for tumor growth and many of its malignant phenotypes (Figure 1).[51,52] These cells have more therapeutic resistance than differentiated tumor cells.[53] Contributing to the heterogeneity of the tumor, these stem cells are lodged in a perivascular niche they help maintain by secreting proangiogenic factors thereby creating a microenvironment that promotes their self-renewal.[54]

This understanding of the dependency of tumor survival on stem cells may partially explain why many therapies that do well in preclinical studies are shown to be ineffective when used in human patients.[53] Recently, integrins were shown to be associated with cancer stem cell function. For example, integrins α_6 and β_1 are elevated on GBM stem cells, and disrupting function reduced tumor formation and increased survival in a mouse model.[55]

Figure 2. Integrins and the Regulation of Multiple Significant Functions

Integrins are involved in the regulation of a variety of cellular and physiological processes.
KEY: FA — focal adhesion; GFR — growth factor receptor
Source: Legate KR, et al. *Genes Dev.* 2009;23:397-418.

Table 2. Anti-integrin Agents Under Development for Cancer Therapy

Agent	Integrin Target	Therapeutic Target	CR Phase
Peptidomimetics (RGD-based)			
Cilengitide	$\alpha_V\beta_3/\alpha_V\beta_5$	Glioblastoma, squamous cell carcinoma of the head and neck, non-small cell lung cancer, other	3
ATN 161	$\alpha_5\beta_1$	Glioblastoma	2
HYD1	β_1	Multiple myeloma	PC
Antibodies			
Intetumumab (CNTO95)	α_V	Hormone refractory prostate cancer, melanoma, solid tumors	2
Volociximab	$\alpha_5\beta_1$	Renal cell carcinoma, melanoma, pancreatic	2
Etaracizumab	$\alpha_V\beta_3$	Prostate	2
Natalizumab	α_4	Phase 1 / 2 terminated; marketed for MS	X
DI 17E6	$\alpha_V\beta_3$	Colorectal cancer, prostate	2
PF-04605412	$\alpha_5\beta_1$	Solid tumors	1
IMGN388	α_V	Solid tumors	1
264RAD	$\alpha_V\beta_6$	Cancer	PC
Small Organic Molecules			
E7820	α_2	Colorectal cancer, lymphoma	2
MK0429	$\alpha_V\beta_3$	Hormone refractory prostate cancer	1
GLPG 0187	5 integrin receptors	Bone metastases in metastatic bone cancer	1
Celastrol	β_1 integrins	Prostate, pancreas	PC

KEY: CR — clinical research; PC — preclinical
Source: Reardon DA, et al. *Future Oncol.* 2011;7:339-354.

Another recent advancement is the further elucidation of the association between integrins and the tumor response to hypoxia. Areas of hypoxia in tumors are refractory to radiotherapy and are foci of malignant phenotypes including angiogenesis and proliferation.[56] As part of their heterogeneity, GBMs have areas of hypoxia and necrosis in the invasive periphery of the tumors.[57] The tumor cells respond to oxygen deprivation by the activating hypoxia-inducible factor (HIF), which is a key regulator of angiogenesis. HIF upregulates genes involved in glycolytic energy metabolism and that are also associated with tumor invasiveness, angiogenesis, and cell survival.[58,59] The gene regulating VEGF is among those that are upregulated. In addition, it was recently shown that molecular signaling induced by integrin ligation is one of the pathways through which HIF-1α activity is increased.[56]

Anti-integrin Therapy

The involvement of integrins in several aspects of the survival and progression of different cancers provides a rational basis for developing integrin-targeting therapeutic agents. Integrins are expressed on both endothelial and tumor cells. Therefore, potential effects of anti-integrin strategies in cancer therapeutics are 3-fold: 1) anti-angiogenesis, 2) anti-invasion, and 3) anti-tumor.[60] In addition, integrin inhibitors have been shown to augment the effect of radiation, and may augment that of other therapies including cytotoxins, cell signaling inhibitors, immunotherapies, vascular targeting agents, and anti-angiogenics.[38]

Because of their diverse activities in many biochemical processes that are related to a variety of pathologies in addition to cancer, anti-integrin compounds have been under

development for several indications, and 4 are currently FDA-approved for non-cancer indications.[61] Drug design often focuses on developing compounds or biologics against specific integrin subtypes or extracellular domains that are known to have increased expression in the disease of interest. Three types of integrin inhibitors are being evaluated in pre-clinical or clinical cancer trials (Table 2):

- Peptidomimetics
- Antibodies
- Small organic molecules

The safety of selective integrin monoclonal antibodies was confirmed in phase 1 studies, which included failing to elicit "class-associated" toxicities. Significantly, dose-escalation studies were unable to define a maximum tolerated dose.[62-64] Several phase 2 studies are ongoing.

Peptidomimetics that are primarily RGD-based competitively block ligand binding to integrin. Cyclized peptides, such as the pentapeptide cilengitide, have been shown to have 10-fold to 1,000-fold increased activity compared with their linear counterparts, such as ATN 161.[65]

Finally, small organic molecules are of interest. Because they lack peptide bonds, these compounds are orally active, unlike the antibodies and peptidomimetics being investigated that are administered intravenously. These compounds have higher stability as well.

Cilengitide – an RGD-based Integrin Antagonist

Cilengitide is a cyclicized RGD pentapeptide that selectively and potently inhibits the activation of $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins. Binding occurs on the extracellular domain, thus the activity of cilengitide does not depend on accessing the interior of the cell.

Cilengitide is administered by intravenous infusion, and the pharmacokinetic profile is characterized by reaching maximum plasma concentrations within 1 hour of starting administration; that is, peak concentrations are achieved by the end of the infusion interval. The elimination half-life is approximately 3 to 5 hours.

Preclinical Research

Cilengitide has been studied in several cancer models, and preclinical studies demonstrated that it blocks angiogenesis and has direct anti-tumor and anti-invasive effects. For example, basic studies in an in vitro cell adhesion model showed that cilengitide blocked cell adhesion mediated by integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$. In addition, cilengitide reduced cell adhesion and inhibited proliferation in an in vitro stem cell model for vasculogenesis.[66] Using cilengitide concentrations similar to those in the adhesion model, FGF- and VEGF-dependent angiogenesis

was inhibited in a chick chorioallantoic membrane model. Growth of cells from melanoma, lung, and pancreatic tumors was completely inhibited by cilengitide.

Several studies demonstrated a potentiation or synergy when cilengitide is combined with radiation and chemotherapy. In one study of endothelial and cancer cells in vitro, radiation increased the expression of $\alpha_V\beta_3$ in all cell lines, with the sensitizing effect of cilengitide proportional to the levels of target expression.[67] A key study with combined cilengitide and radiotherapy in an orthotopic glioma xenograft model identified a time dependency for maximum synergistic effect.[68] The administration of cilengitide within a window of 4 to 8 hours of the administration of radiation therapy was critical to providing the effect. This has been translated to the clinical research protocols by specifying that cilengitide administration should begin 4 hours before radiotherapy. Interestingly, in vivo studies utilizing mouse models have demonstrated that low (nanomolar) concentrations of RGD-mimetic $\alpha_V\beta_3$ and $\alpha_V\beta_5$ inhibitors can paradoxically stimulate tumor growth and tumor angiogenesis. Low concentrations of these inhibitors promoted VEGF-mediated angiogenesis by altering $\alpha_V\beta_3$ integrin and VEGF receptor-2 trafficking, consequently promoting endothelial cell migration to VEGF. The proangiogenic effects of low concentrations of RGD-mimetic integrin inhibitors could compromise their efficacy as anticancer agents and have major implications for the use of RGD-mimetic compounds in humans.[69] These results imply that the role of integrins in tumorigenesis and angiogenesis is complex, and the concentration of integrin inhibitors used is a critical consideration.

Overall, studies in a variety of in vivo and in vitro models demonstrated that cilengitide:

- Blocks binding of α_V integrins to ECM[70-72]
- Is anti-angiogenic[70,73-75]
- Blocks endothelial cell proliferation[66,70,73]
- Blocks adhesion, migration, and differentiation[66,67,71]
- Induces apoptosis[67,71,76]
- Displays additive activity in combination with a wide variety of classical and molecular targeted therapeutics[77,78]

Cilengitide Clinical Trials

Cilengitide is currently the lead prototype in the RGD peptidomimetic category of anti-integrin therapies, and is under investigation for several cancers, including GBM. The primary treatment regimen being evaluated comprises 1-hour infusions administered twice weekly,

Table 3. Summary of Clinical Trials with Cilengitide in Malignant Glioma

	# Pts	Dose, mg 2x/week	MGMT Status	Co Tx or Comparator*	C	O	P1	P2	P3
Newly Diagnosed									
1. Stupp 2010	52	500	M/U	RTX/TMZ	X			X	
2. Nabors (NABTT) 2009	112	500/2,000	M/U	RTX/TMZ	X			X	
3. CORE	240	2,000†	U	RTX/TMZ		X		X	
4. CECIL	108	2,000	U	*Cetuximab**		X		X	
5. CENTRIC	504	2,000	M	RTX/TMZ‡		X			X
Recurrent									
1. Nabors (NABTT) 2007	51	120-2,400/m²	M/U	None	X		X		
2. MacDonald 2008	33	120-2,400/m²	M/U	None	X		X		
3. Reardon 2008	81	500/2,000	M/U	None	X			X	
4. Gilbert (NABTC) 2007	30	500/2,000	M/U	None	X			X	
5. ABTC	52	2,000	M/U	Cediranib		X	X		

* Co Tx — cotreatment, or if a multi-arm comparator study, comparator is in bold/italics*
† 5x/week during radiotherapy;
‡ Cilengitide with RTX/TMZ vs. RTX/TMZ alone

Sources: Nabors LB, et al. *J Clin Oncol.* 2009;27:S15 (abstract 2001); National Cancer Institute. http://www.cancer.gov/clinicaltrials/search/view?cdrid=654715&version=healthprofessional. Accessed March 12, 2011; Reardon DA, et al. *Future Oncol.* 2011;7:339-354.

KEY: ABTC — Adult Brain Tumor Consortium, C — study completed, M — methylated, NABTT — New Approaches to Brain Tumor Therapy, O — study ongoing, RTX — radiotherapy, TMZ — temozolomide, U — unmethylated

although some variations are being explored. Doses approaching 5 grams have been safely administered following this schedule. Several studies have been completed, 3 are ongoing, and more studies are planned (Table 3).

Phase 1 studies

Cilengitide has been evaluated in 3 phase 1 studies relevant to GBM. The first study enrolled 37 patients, was open to patients with any cancer, and allowed dose escalation to 1,600 mg/m².[79] Pharmacokinetics was dose-independent and time-invariant. There were minimal adverse effects and no indication of dose-limiting toxicity. This led to 2 additional phase 1 studies in patients with recurrent brain tumors:

NABTT study

The New Approaches to Brain Tumor Therapy (NABTT) Consortium directed a phase 1 study in 51 adults with recurrent malignant glioma. Doses ranged from 120 mg/m² to 2,400 mg/m². There was no indication of dose-limiting toxicity (DLT) and the maximally tolerated dose (MTD) was not reached.[80] The treatment was generally well-tolerated and there were no hemorrhages. There were a total of 14 events with a toxicity level of grade 3 or 4 and a relationship of possibly to definitely related to cilengitide. Ten of these 14 events occurred in < 2% of patients. The more common events, which occurred in 4% of patients, were hyperglycemia and hyponatremia, and these events are typically associated with the underlying disease and/or concurrent medications such as corticosteroids. There was some evidence of efficacy, with several patients having durable responses that were not dose-dependent.

Pediatric study

A companion trial also evaluated doses of 120 mg/m² to 2,400 mg/m² in 33 patients ≤ 21 years of age with refractory CNS tumors.[81] Treatment was well-tolerated. Although 3 of 13 patients at 2,400 mg/m² experienced intratumoral hemorrhages, 2 were asymptomatic. Their relationship to treatment was not clear. However, as a precaution from the results of this study, the recommended phase 2 dose was set at 1,800 mg/m².