Passive Antibody-Mediated Immunotherapy for the Treatment of Malignant Gliomas

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KEYWORDS

- Monoclonal antibody
- Epidermal growth factor receptor variant III
- Epidermal growth factor receptor
- Tenascin-C • Bevacizumab • Glioblastoma multiforme

Malignant gliomas are the most common primary intracranial tumors and among the most lethal of solid and hematopoietic cancers.[1] Their ruthless malignant progression invades and destroys normal brain tissue, resists traditional therapies, and causes death for sure. Infiltration throughout the brain is a prominent feature of high-grade gliomas and is the principal basis for refractoriness to local therapies, including surgery.[1] Although in most cases, recurrent tumor is first noted radiographically near or within several centimeters of the resection cavity, the tumor has already infiltrated widely by that time.[2] Invasion of tumor cells within normal brain structures makes normal brain function vulnerable to therapies, which do not discriminate between neoplastic and normal cells. Potential for such discrimination arises, however, from differences between tumor and normal cells, in the protein-carbohydrate complexes on their surface.[3] Immunotherapeutic approaches selectively target tumor cells by exploiting these differences in cell surface molecules. This strategy has the dual benefit of increasing efficacy against the tumor cells and decreasing toxicity to nonneoplastic cells. Although many immunotherapeutic approaches are being investigated, this article focuses on monoclonal antibodies (mAbs), or serotherapies, that have progressed to clinical trials against malignant gliomas.

MONOCLONAL ANTIBODIES

mAbs, made in large numbers outside the body, are considered agents of passive immunotherapy, which does not require the patient's immune system to take an active role in fighting the cancer.[4,5] The first mAbs were produced by hybridoma cells formed by fusing a mouse myeloma cell with a mouse B cell making a specific antibody.[6] Because the antibodies, which are all identical, are made from a single (mono) hybridoma clone, they are called monoclonal antibodies. mAbs can be used in treating malignant gliomas either alone or attached to a cytotoxic or radioactive agent.[7] Repeated treatments with murine mAbs frequently trigger allergic reactions and human antimouse antibody responses in patients. This is prevented by humanizing the mAbs by linking the murine antibody variable region to human IgG constant regions.[8,9] Human antibodies should have even less immunogenicity.[10] Yang and colleagues[11] reported generation of mAb E7.6.3, a human anti-EGF (epidermal

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Neurosurg Clin N Am 21 (2010) 67–76
doi:10.1016/j.nec.2009.08.010
1042-3680/09/$ – see front matter

growth factor) receptor IgG2 mAb from humanized transgenic mice.

The antineoplastic activity of mAbs could have multiple mechanisms: (1) opsonization of cancer cells and subsequent activation of immune effector mechanisms,[12] (2) disruption of cellular signaling,[13,14] and (3) inducing antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cellular cytotoxicity, whereby an effector cell of the immune system lyses a target cell by releasing granzymes, porforins, tumor necrosis factor α, and interferon-γ.[15–17] Bleeker and colleagues[18] showed that anti-EGF receptor antibodies cause cell death by compromising EGF-induced signaling and inducing ADCC. Several EGF receptor mAbs have shown promise in vitro and in murine xenograft models.[19–26]

TARGET DETERMINATION OF PASSIVE ANTIBODY IMMUNOTHERAPY

Effective mAb therapy requires that the targeted tumor-specific antigen has stable cell surface expression (ie, low turnover time) of at least 1×10^5 molecules per tumor cell.[27] Glioblastoma multiformes (GBMs) overexpress many different antigens, including EGF receptor, melanoma associated antigen, Her2/neu, tyrosinase, Trp-1, Trp-2, gp100, IL-13Rα2, survivin,[28] and EphA2.[29] Treatment targeting these antigens is potentially toxic as they are also expressed on cells of normal tissue. Targeting a novel epitope unique to cancer cells would be preferable. EGF receptor variant III (EGFRvIII), the most common genetic alteration of the EGF receptor, is such a protein.[30–34]

An alternative mAb strategy targets a ligand/receptor pair essential for tumor proliferation or maintenance. An example includes vascular endothelial growth factor (VEGF) and its receptor, which stimulate the extensive vascularization of high-grade gliomas. One final method is the targeting of tenascin-C, an extracellular matrix protein, which drives tumor cell invasion of normal tissue.

ANTI-EGFR THERAPY

The EGF receptor is overexpressed, mutated, or both in many solid tumors, notably high-grade gliomas, where it is overexpressed in 40% to 50% of cases.[35] Cetuximab is a human:mouse chimeric mAb that binds with high specificity to the extracellular domain of EGF receptor and prevents receptor dimerization and signaling.[36] Crystal structural studies showed that binding of the cetuximab antigen-binding fragment (Fab) to domain III of the receptor prevents growth factor binding. Ferguson[37] showed that the heavy-chain (V_H) region of the antibody sterically prevents domain I of the EGF receptor from adopting the conformation required for the dimerization essential for preventing potential ligand-independent modes of EGF receptor activation. In preclinical studies, cetuximab inhibits growth and increases apoptosis in GBM cell lines. There are conflicting data regarding whether EGF receptor amplification imparts cetuximab sensitivity.[38,39]

These preclinical studies have led to multiple human clinical trials (**Table 1**). Seventeen patients with pathologically confirmed GBM underwent standard postoperative radiation and temozolomide treatment followed by weekly infusions of cetuximab in a phase 1/2 trial, ''Radiochemotherapy with Temozolomide and Cetuximab in patients with primary GBM (GERT trial)''.[40] Median follow-up was 13 months in 17 patients, of whom 7 received gross total resection, 7 had subtotal resection, and 3 had biopsy only. This study concluded that the combination of radiation, temozolomide, and cetuximab is safe and well tolerated. At 6 months, 81% of patients were free of tumor progression, and at 12 months, 87% of patients were still alive. Methylated methyl guanine methyl transferase (MGMT) was not associated with longer overall or progression-free survival, and analysis of EGF receptor status is ongoing.[40]

Single-agent cetuximab has been tried in patients with recurrent high-grade glioma after surgery, radiotherapy, and chemotherapy.[41] Patients were stratified into 2 treatment arms according to the amplification status of the EGF receptor gene as determined by fluorescence in situ hybridization. A total of 55 patients underwent treatment with cetuximab (28 with and 27 without an increased EGF receptor copy number). The EGF receptor mAb was generally well tolerated, the median duration of progression-free survival was 1.9 months, and the median duration of overall survival was 5.0 months. The rates of 6-month progression-free survival and overall survival were 10% and 40%, respectively. Although progression-free survival lasted less than 5 months in most (n = 49) patients, 5 patients survived without tumor progression for at least 9 months (range, 9.5 to >16.5). No significant correlation was found between response, duration of survival, and EGF receptor copy number.[41]

Cetuximab has also been tried in a phase 2 trial that combined cetuximab, bevacizumab, and irinotecan for patients with primary GBMs following tumor progression after radiation therapy and temozolomide treatment.[42] The mean duration of overall survival was 29 weeks, and the mean time to tumor progression was 24 weeks. Thirty

Table 1
Summary of clinical trials using anti-EGF receptor mAbs

Treatment	Patients Evaluated	Number of Patients	Results	Secondary Result
Cetuximab (phase 1/2)	Newly diagnosed GBM after surgical treatment and standard postoperative radiation and temozolomide	17 (7 gross total resection, 7 subtotal resection, 3 biopsy only)	OS = 87% at 12 months PFS = 81% at 6 months	MGMT not associated with longer survival. EGF status analysis ongoing
Cetuximab (phase 2)	Recurrent GBM	55 (28 with increased EGF copy number, 27 without)	PFS = 1.9 months OS = 5 months 6 month PFS = 10% 6 month OS = 40%	No correlation between response and EGF receptor copy number
Cetuximab with bevacizumab and irinotecan (phase 2)	Recurrent GBM	43	OS = 29 weeks 6 month PFS = 30.2% Mean TTP = 24 wk	6-month PFS for responders was 72.7% when compared to 23.8% for nonresponders
Nimotuzumab (phase 1/2)	Newly diagnosed GBM or anaplastic astrocytoma after surgical treatment and standard postoperative radiation and temozolomide	28 (16 GBM, 12 anaplastic astrocytoma)	Response rate was 37.9% Median OS, 22.17 months	
^{125}I-mAb 425 (phase 2)	Newly diagnosed patients with GBM or astrocytoma with anaplastic foci after surgery, postoperative radiation $\pm$ chemotherapy	180	OS GBM = 13.4 months OS astrocytoma with anaplastic foci = 50.9 months	

Abbreviations: MGMT, methyl guanine methyl transferase; OS, overall survival; PFS, progression-free survival; TTP, time to progression.

percent of patients were free of tumor progression at 6 months.

Nimotuzumab (h-R3) is a humanized mAb that targets the extracellular domain of the EGF receptor. It has both antiangiogenic and proapoptotic effects. A phase 1/2 trial using nimotuzumab and radiation in 28 individuals with newly diagnosed high-grade gliomas (16 with GBM and 12 with anaplastic astrocytoma) found an objective response rate (defined in this study as either a complete or partial response) of 7.9%. Median duration of overall survival was 22.17 months with a median follow-up of 29 months.[43]

EGF receptor mAbs attached to cytotoxic agents have also been evaluated in clinical trials. mAb 425, a murine mAb raised against human A431 carcinoma cells (which have very high levels of EGF receptor on the cell surface), conjugated with iodine 125,[44] has been used to treat high-grade gliomas in a phase 2 study [45] of 180 patients with either GBM or astrocytomas with anaplastic foci (AAF). When radiolabeled mAb 425 was administered after surgery and radiation therapy, with or without chemotherapy, the median durations of overall survival of patients with GBM and AAF were 13.4 and 50.9 months, respectively.

ANTI-EGFRVIII THERAPY

EGFRvIII, the most common genetic variation of the EGF receptor, is expressed only in cancer tissue and is thus an ideal immunotherapeutic target.[46] This variant is missing 801 coding bases, a deletion which includes exons 2 to 7 of the wt receptor.[47] The deletion of amino acids 6 to 273 from the extracellular domain of the wild-type protein is accompanied by the encoding of a novel glycine residue at the fusion junction (**Fig. 1**). EGFRvIII was initially discovered in GBM, where it is expressed in approximately 40% of cases. Among the EGFRvIII mAbs generated against this antigen, mAb 806 is the most tumor-specific.[48,49] Treatment with mAb 806 significantly decreased tumor volume and prolonged the survival of mice with xenografts from EGFRvIII expressing U87 glioma cells.[50]

Y10 is an IgG2a murine mAb. Although Y10 is generated towards an artificially created murine homolog of human EGFRvIII,[51] it recognizes human and murine equivalents of this tumor-specific antigen. Y10 inhibits DNA synthesis and cellular proliferation in vitro and induces autonomous complement-mediated and antibody-dependent cell-mediated cytotoxicity. Intratumoral injection of Y10 in an intracranial B16 melanoma expressing EGFRvIII increased the median duration of survival by 286%. A human chimeric antibody based on Y10 has been developed for clinical use. It induces lysis of human EGFRvIII-expressing malignant glioma cells autonomously and in the presence of activated human macrophages.[52]

One drawback of EGFRvIII serotherapy is that mAbs binding EGFRvIII are rapidly internalized at ambient temperatures.[48,53–55] This disadvantage has been mitigated by modified labeling methods, which has resulted in EGFRvIII mAbs successfully targeting radioisotopes in the radioimmunotherapy of tumors in rodent models.[53,54,56] Despite this preclinical work, mAbs targeting EGFRvIII have not been tested clinically. Clinical trials evaluating a peptide vaccine targeting this unique tumor antigen[57] are underway.

ANTIANGIOGENESIS THERAPIES

Microvascular proliferation is a histopathologic hallmark of GBMs and an independent prognostic factor for adult gliomas.[58,59] Increasing evidence suggests that tumor angiogenesis is not an adaptive response to tumor-induced hypoxia, but

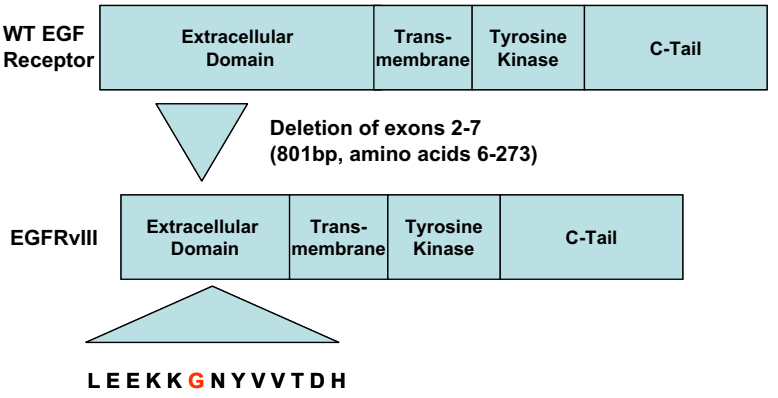

Fig. 1. EGFRvIII. An in-frame deletion of exons 2 to 7 of the EGF receptor permits fusion of exon 1 and exon 8, which generates a novel glycine.

rather a result of decisive genetic mutations that activate an angiogenic transcriptional program which is further modified by the regional tumor oxygen status. Antiangiogenic therapies are being developed in hopes that disrupting the tumor vasculature will lead to regression of the tumor. The most successful therapy from this approach is serotherapy against VEGF and its cognate receptor.[60–62]

Bevacizumab is a humanized mAb that binds to circulating VEGF-A (the most common isoform in GBMs). Treatment with bevacizumab in combination with irinotecan (CPT-11), a topoisomerase-1 inhibitor, resulted in increased duration of survival in patients with metastatic colorectal cancer and has subsequently received approval for treatment of other solid tumors, including lung and breast cancers.[63,64] Bevacizumab and irinotecan were first used for patients with malignant glioma when a medical oncologist, Dr Stark-Vance, who had success treating patients with colorectal cancer, applied this regimen to 21 patients with recurrent GBM and saw a response rate of 43% (1 patient with complete response and 8 patients with partial response using MacDonald's criteria).[65] Prompted by these exciting results, Vredenburgh and colleagues[60,61] treated 32 patients with recurrent malignant gliomas in a prospective trial with bevacizumab and irinotecan and saw dramatic rates of radiographic response (61% in GBM and 67% in anaplastic glioma) and a near doubling of the rate of 6-month progression-free survival of 30% for GBM and 56% for anaplastic gliomas, compared with historical controls of 15% and 31%, respectively. Long-term follow-up of these patients revealed that they lived no longer than historical controls.[66] Norden and colleagues[67] published similar results from Dana Farber Cancer Institute after using bevacizumab and irinotecan treatment for 33 patients with recurrent GBM and 21 patients with recurrent anaplastic glioma. This study showed that patients who failed treatment with bevacizumab and irinotecan were unlikely to respond favorably to subsequent cytotoxic chemotherapy or other novel therapeutics. They were likely to have very rapid disease progression and clinical deterioration.

The 2 earlier studies did not evaluate the incremental benefit of adding irinotecan to bevacizumab. Fine[68] treated 79 patients with recurrent GBM in a phase 2 study with bevacizumab alone. The response rate was 60%, and the rate of 6-month progression-free survival was 30% (similar to the other 2 studies), suggesting that irinotecan treatment is not necessary. Moreover, the toxicity of bevacizumab in the Fine study was less than that of the other 2 studies. Preliminary results of a Genentech study of patients with recurrent GBM randomized between bevacizumab alone or bevacizumab in combination with irinotecan[65] showed a response rate and progression-free survival rate of 28% and 42.6% in patients receiving bevacizumab monotherapy versus 37.8% and 50.3% in patients receiving the combination therapy; this suggests that irinotecan adds benefit to bevacizumab treatment. The median duration of overall survival for patients receiving bevacizumab alone was 9.2 months, and for the combination group, it was 8.7 months. Neither of these numbers is significantly different from those of historical controls.

Although the superior rates of radiographic response and 6-month progression-free survival from the bevacizumab trials are enticing, many clinicians question bevacizumab's benefit, given the lack of improvement in overall survival. Although the magnetic resonance imaging (MRI) shows decreased volume of contrast enhancement (**Fig. 2**), bevacizumab may simply be normalizing tumor blood vessels to decrease leakage of gadolinium and may have no real antitumor affect. Laboratory studies and randomized trials are seeking to resolve this issue. Meanwhile, patients with newly diagnosed GBM are being treated with bevacizumab immediately after surgery and radiation in ongoing clinical trials.

SEROTHERAPY AGAINST EXTRACELLULAR MATRIX PROTEINS

Tenascin-C is an extracellular matrix protein expressed in more than 90% of gliomas but not in the normal brain; its presence increases with increasing tumor grade.[69–72] Although the function of tenascin-C is debated, the protein seems to be important for cellular processes including adhesion, migration, and proliferation. Several anti-tenascin-C antibodies have been generated.[73–75] The tenascin-C mAb 81C6, a murine IgG2b that targets an isoform of tenascin-C expressed in malignant gliomas,[73,76] has been used in most clinical studies. This antibody does not cross-react with normal brain and has been used in conjugation with ^{131}I radioisotope.

^{131}I-labeled mAb 81C6 injected into the tumor resection cavity to deliver a target dose of 44 Gy was evaluated in 10 human GBM trials. Newly diagnosed GBM patients treated with surgery, postoperative radiotherapy, temozolomide, and ^{131}I-labeled mAb 81C6 in the phase 2 trial had a median duration of overall survival of 91 weeks.[77–79] A further analysis after 231 weeks of median follow-up revealed an average time to

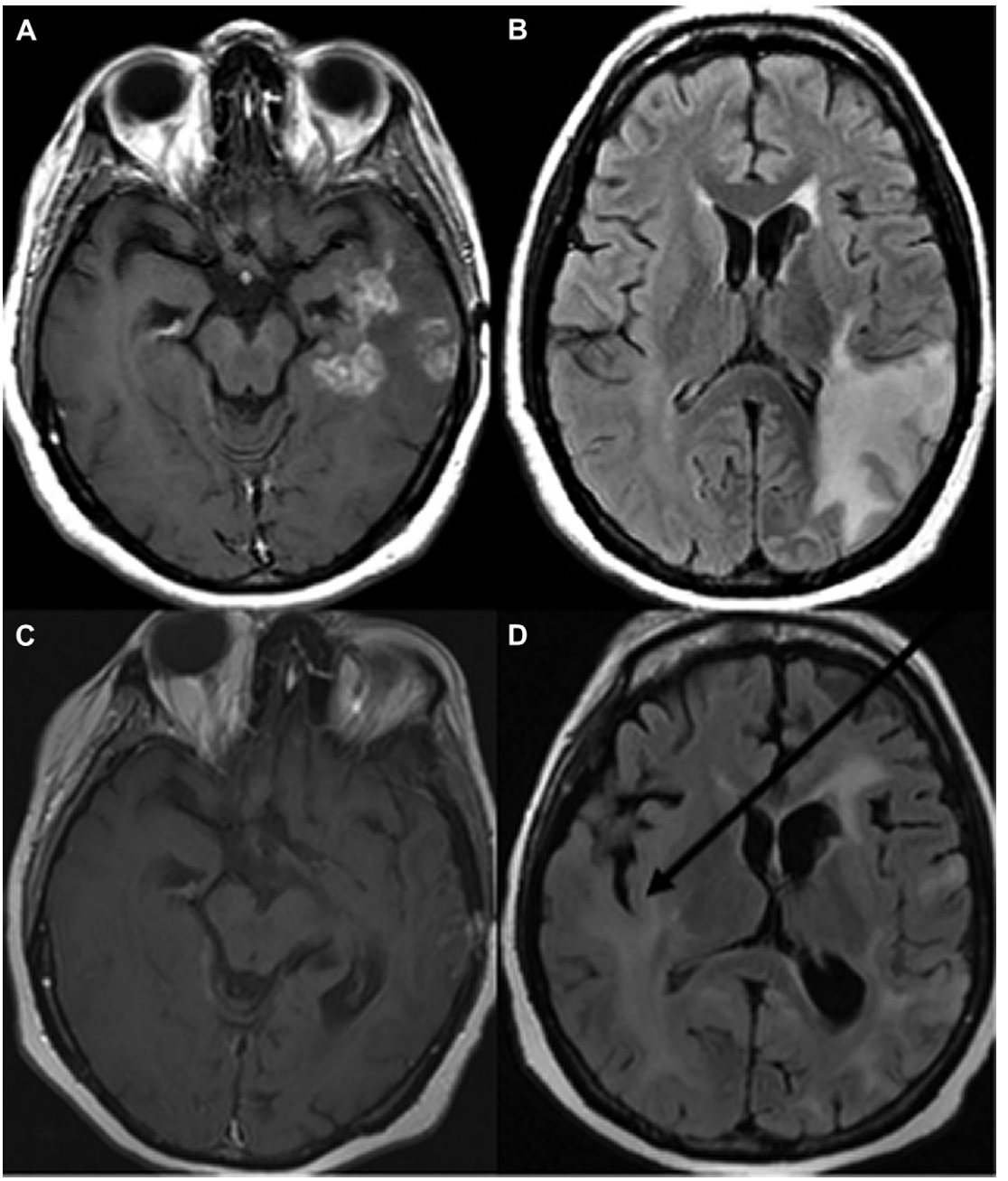

Fig. 2. MRI images from a patient with recurrent GBM before and after bevacizumab treatment showing significant radiographic response. Pretreatment axial T1 with contrast (*A*) and axial FLAIR (fast fluid attenuated inversion recovery) (*B*). Posttreatment axial T1 with contrast (*C*) and axial FLAIR (*D*). *Arrow* points to interval white matter change that may indicate new tumor.

progression of 77.3 weeks (17.8 months) and an overall survival duration of 102.1 weeks ($n = 14$). These data are the basis of a randomized phase 3 trial, which is currently recruiting patients to either the control treatment arm of surgery and postoperative radiochemotherapy or the treatment arm of surgery, postoperative radiochemotherapy, and ^{131}I-conjugated 81C6 mAb.

DISCUSSION

mAbs are widely accepted as standard-of-care agents for several solid and hematopoietic

cancers. They afford high target specificity, relatively low toxicity, and efficacy based on target inhibition and immune enhancement. The authors optimistically await completion of randomized trials evaluating mAbs such as bevacizumab, cetuximab, and ^{131}I-labeled mAb 81C6 for malignant gliomas and of preclinical studies of newer mAbs against other targets.

There are many ways to improve mAb therapy. Clinical testing and use of newer agents will require the humanization of mouse mAbs to reduce the risk of infusion and allergic reactions. This involves cloning the immunoglobulin light and heavy chains from an established hybridoma and directed amino acid substitution. Alternatively, antibodies could be created in mice that are genetically engineered to produce fully human antibodies. Alteration of either the mAb's Fc protein or its glycosylation may enhance ADCC, which should improve tumor lysis. Even single-residue substitutions and/or minor changes in glycosylation may enhance binding of the Fc portion of the antibody to the Fc receptor gamma. This improves opsonization of target-expressing tumor cells for cell killing by ADCC.

The need for brain tumor therapeutic agents to traverse the blood-brain barrier has prompted development and testing of smaller antibody fragments, such as diabodies, minibodies, Fab fragments, single-chain Fv domains, and single-chain antibodies derived from camels and llamas.[80–82] These antibody fragments range in size from 15 to 60 kD; they become distributed throughout the tumor more quickly and homogeneously when compared to full mAbs but have more limited tumor retention, serum half-life, and ADCC functionality. Tumor retention, however, can be improved with multivalent constructs.[4,83,84]

Many investigators believe that the initiation and maintenance of malignant gliomas, and particularly their early and aggressive recurrence, depend on limited numbers of highly tumorigenic stem cells.[85–87] mAbs directed against epitopes unique to brain tumor stem cells theoretically hold special promise. CD133 has been identified as 1 stem cell marker for malignant gliomas.[88–90] However, CD133 is also shared by neural/hematopoietic stem cells, and therapy directed indiscriminately against this stem cell marker may cause significant side effects. Therapy directed against CD33, the marker shared by acute myeloid leukemia cancer stem cells and hematopoietic stem cells, causes myelosuppression and neutropenia.[91] To avoid such toxicity, identification of markers highly specific for tumor progenitor cells will likely be required. Screening of glioma cells for expression of such cell surface markers is being pursued. Identification of a tumor-specific marker unique to brain tumor stem cells would permit development of mAbs that would selectively target the cells of a tumor that are critical to its self-renewing proliferation and resistance to therapy. Success would validate the stem cell hypothesis of cancer, the mAb strategy of therapy, and the hope for a cure of malignant brain tumors.

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