During the last decade, we have witnessed several key advances in the field of neuro-oncology. First, there were conceptual advances in the molecular and cell biology of malignant gliomas including the discovery in 2004 of brain tumor stem cells. Second, the Cancer Genome Atlas project has been extremely useful in the discovery of new molecular markers, including mutations in the IDH1 gene, and has led to a new classification of gliomas based on the differentiation status and mesenchymal transformation. In addition, use of the 1p/19q marker and O6-methylguanine-DNA methyltransferase methylation status have been identified as guides for patient selection for therapies and represent the first steps toward personalized medicine for treating gliomas. Finally, progress has been made in treatment strategies including the establishment of temozolomide as the criterion standard for treating gliomas, the adoption of bevacizumab in the clinical setting, and developments in experimental biological therapies including cancer vaccines and oncolytic adenoviruses.


In the past 10 years there have been a number of scientific discoveries that have dramatically influenced the diagnosis and treatment of malignant gliomas. These discoveries have given crucial information about the cellular origin of gliomas and how we can use molecular defects in these tumors to predict their prognosis and generate more accurate and effective therapeutic regimens.

CELL BIOLOGY OF GLIOMAS: BRAIN TUMOR STEM CELLS

In 2004, 2 groups working independently identified and isolated a relatively small population of cells from human glioma samples that displayed several characteristics of neural stem cells. These newly identified cells, referred to by several authors as brain tumor stem cells, are thought to be the tumor-initiating cells that are responsible for the resistance of gliomas to chemotherapy and radiotherapy and thus responsible for tumor recurrence after treatment. Like neural stem cells, these brain tumor stem cells can form spheroids when cultured in a rich-growth factor medium and display the capacity for self-renewal. In addition, when cultured in differentiation media, brain tumor stem cells express the differentiation markers normally exhibited by glial cells, oligodendrocytes, and neurons. CD133, a transmembrane glycoprotein with unknown function, has been proposed as a biochemical marker of cancer stem cells in brain tumors and other neoplasms. Importantly, brain tumor stem cells express proteins such as Sox2, Nanog, and Oct4 that play a key role in the maintenance of self-renewal in neural stem cells. Investigators have shown that tumors formed in mice with human brain tumor stem cells are more histologically similar to primary glioblastomas than tumors generated with human glioma cell lines.

The existence of brain tumor stem cells and their role in tumorigenesis are not ac-
de novo and secondary or progressive. De novo gliomas, the one in which they are divided into primary or have been proposed, the most widely accepted being, per-

During the last decade, several classifications of gliomas have been proposed, the most widely accepted being, perhaps, the one in which they are divided into primary or de novo and secondary or progressive. De novo gliomas are more common; they are observed in individuals older than 50 years, appear in a matter of weeks or months and, at the molecular level, do not harbor mutations in the p53 gene but do have amplification of the epidermal growth factor receptor (EGFR) gene. Secondary glioblastomas are diagnosed in younger patients and, in some cases, there is evidence of progression from benign astrocytomas. At the molecular level, these secondary glioblastomas harbor mutations in the p53 gene.

Recently, Phillips et al proposed another classification of gliomas. These authors identified the molecular signature of 3 subsets of gliomas that were termed proneural, proliferative, and mesenchymal. In the proneural tumors, chromosome gains and losses are not evident, the phosphatase and tensin homologue (PTEN) gene is intact, and EGFR is not amplified; however, the notch signaling pathway is activated. In the proliferative and mesenchymal subtypes, there is a gain of chromosome 7 and loss of chromosome 10, EGFR is normal or amplified, and PTEN is mutant. The main difference between the 2 groups is the presence of a vascular phenotype in the mesenchymal one. This classification has important prognostic implications because tumors with proneural characteristics are generally less aggressive than those in the other subgroups. More recently, based in the Cancer Genome Atlas, a new gene expression–based molecular classification of glioblastoma multiforme has been described (Table 1).

**THE CANCER GENOME ATLAS: PRELIMINARY RESULTS IN GliOMAS**

The Cancer Genome Atlas, as defined by the National Cancer Institute, is a comprehensive and coordinated effort to accelerate the understanding of the genetics of cancer using innovative genome analysis technologies. In an initial article from the Cancer Genome Atlas, researchers used sequencing whole-genome–amplified genomic DNA samples from gliomas and healthy brain tissue samples to examine the expression and DNA methylation in 206 glioblastoma multiforme tumors. The analyses showed the roles of ERBB2, NF1, and TP53, uncovered frequent mutations of the phosphatidylinositol-3-OH kinase regulatory subunit gene PIK3R1, and provided a network view of the pathways altered in the development of glioblastoma. Although some of these data were expected, the whole-genome approach allowed the group to identify frequent mutations of NF1 in sporadic gliomas and thereby demonstrate that NF1 is a tumor suppressor gene in gliomas. Interestingly, NF1 was previously identified as a tumor suppressor gene in animal models. The mutations of the PIK3R1 gene were also a new finding and, taken together with the inactivation of PTEN (the main negative regulator of the pathway), demonstrate the relevance of this pathway in gliomas. The methylation analyses showed abnormal regulation of O6-methylguanine-DNA methyltransferase (MGMT), a finding that was expected based on previous studies, but also established a link between methylation of MGMT and mismatch repair deficiencies in a hypermutator phenotype in brain tumors. Overall, the Cancer Genome Atlas project has confirmed previous solid information about tumor suppressor and oncogene activation pathways and has proven its power as a discovery tool.

**MUTATIONS OF THE ISOCITRATE DEHYDROGENASE GENES IN GliOMAS**

Perhaps the most significant discovery of the Cancer Genome Atlas is the finding of mutations of isocitrate dehydrogenase genes. Interestingly, more than half of the IDH1 mutations affect R172, which is analogous to R172 of IDH2. These mutations are relevant for the homeostasis of the cell because they change the enzymatic activity of the encoded isocitrate dehydrogenase. Mutations in IDH, compared with

### Table 1. Subtypes of Glioblastomas

<table>
<thead>
<tr>
<th>Subtypes</th>
<th>Differential Genetic Alterations</th>
<th>Molecular Markers</th>
<th>Cellular Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proneural</td>
<td>IDH1 (point mutation), PDGFRα, p53 (mutation, LOH)</td>
<td>Oligodendrocytic development (PDGFRα), proneural development (SOX)</td>
<td>Oligodendrocytic</td>
</tr>
<tr>
<td>Neural</td>
<td>Several genetic abnormalities (no significant differences with other subtypes)</td>
<td>Oligodendrocytic, astrocytic, and neural</td>
<td>Oligodendrocytic</td>
</tr>
<tr>
<td>Classical</td>
<td>EGFR (amplification), EGFRvIII, wt p53, CDKN2A (deletion p16/p14arf) or aberrant Rb/CDK4</td>
<td>Neural and stem cells (notch, Sonic hedgehog)</td>
<td>Astrocytic</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>NF1 (mutation, low expression), PTEN (mutation)</td>
<td>Mesenchymal (YKL40, MET), astrocytic (CD44), Schwann cell (S100A), tumor necrosis</td>
<td>Undefined</td>
</tr>
</tbody>
</table>

*Data collected from results reported in Verhaak et al, 2010*
no mutations, are associated with younger age and better prognosis in adults with gliomas; thus, definition of a new subset of glioblastomas—those with IDH mutation—may be warranted. Interestingly, astrocytomas and oligodendroglial tumors often carry IDH mutations but not other mutations or amplifications that are frequently found in the early stages of the progression of gliomas. This finding, in addition to the presence of IDH1 mutations in tumors with features of astrocytes and oligodendrocytes (such as oligoastrocytomas and anaplastic oligoastrocytomas), suggests that IDH mutations occur in stem cells that are precursors of the astrocyte and oligodendrocyte lineages (Figure 1). Although IDH1 mutations constitute an early event in the progression of malignant gliomas, they are absent in pilocytic astrocytomas, suggesting that pilocytic astrocytomas are histologically different from gliomas classified as grade II and III according to the World Health Organization grading system. This hypothesis is further supported by the tandem duplication of chromosome 7q34 leading to a fusion between KIAA1549 and BRAF that has recently been observed in most pilocytic astrocytomas studied but is not present in tumors with IDH1 mutations.14-16

Figure 1. Schematic illustration of the cellular origin of gliomas: IDH genes as genetic markers. The original cell causing the tumor is still under discussion. However, both mutations in neural stem cells and astrocytes result in generation of gliomas in transgenic mouse models. Mutations in the IDH1 and IDH2 genes are observed in astrocytomas and oligodendroglial tumors, suggesting that, in some cases, the mutation has occurred in a progenitor cell before the differentiation into both lineages has been initiated. IDH1 mutations also identified a potential subset of secondary glioblastomas with long survival times. In addition, lack of mutations of IDH genes and presence of the BRAF translocation in pilocytic astrocytomas of children suggest that these tumors could have a distinct genetic signature, different from that of low-grade astrocytomas in the adult population.

PERSONALIZED MEDICINE

It is difficult to discuss personalized medicine for patients with gliomas given the paucity of effective treatment options; nevertheless, a few molecular markers offer a hint of what the future may harbor for these patients. Although many aspects of the molecular biology of gliomas have been known for years, only recently have molecular defects in these tumors served as a guide for their diagnosis and/or treatment (Table 2). Perhaps the first examples of therapeutically relevant molecular markers in gliomas are chromosomes 1p and 19q.19 Codeletion of 1p and 19q is found in gliomas with oligodendroglial differentiation (in fact, this type of differentiation should be suspected in gliomas whose genome encompasses the codeletion) or anaplastic oligoastrocytomas and it is associated with a partial response to temozolomide and better prognosis.19 Another molecular marker with therapeutic relevance is methylation of the MGMT promoter. To examine the relevance of MGMT in the response of malignant gliomas to temozolomide, investigators tested the relationship between MGMT DNA-repair gene silencing in tumors and patient prognosis (Figure 2). The data were obtained during a randomized trial comparing the prognosis of patients undergoing radiotherapy alone with the prognosis of patients undergoing radiotherapy combined with concomitant and adjuvant chemotherapy with temozolomide. Intriguingly, the MGMT promoter was found to be methylated in almost half of the cases.17 Among patients in whose tumors the MGMT promoter was methylated, survival was longer in those treated with temozolomide and radiotherapy than in those who received radiotherapy alone. This difference in survival was not significant in the absence of MGMT promoter methylation. Thus, according to this study, only patients with glioblastoma containing a methylated MGMT promoter benefit from temozolomide.

ADVANCES IN CURRENT THERAPY FOR GLIOMAS

After more than 20 years of clinical trials for malignant gliomas, we are now witnessing the success of some therapeutic strategies. Actually, in the last 10 years, the US Food and Drug Administration approved the only 2 new drugs for the standard treatment of gliomas, temozolomide and bevacizumab.

Table 2. Basis for Personalized Medicine in Neuro-oncology

<table>
<thead>
<tr>
<th>Molecular Marker</th>
<th>Tumor Type</th>
<th>Clinical relevance</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGMT methylation</td>
<td>Malignant gliomas</td>
<td>Sensitivity to temozolomide</td>
<td>Hegi et al, 200515</td>
</tr>
<tr>
<td>IDH1 mutation</td>
<td>Astrocytoma grade II-IV (secondary glioblastoma)</td>
<td>Better prognosis</td>
<td></td>
</tr>
<tr>
<td>1p/19q Co-deletion</td>
<td>Oligodendrocytoma</td>
<td>Prolonged survival/chemosensitivity</td>
<td>van den Bent et al, 200318</td>
</tr>
<tr>
<td>BRAF mutation + wild type IDH1</td>
<td>Juvenile pilocytic astrocytoma</td>
<td>Distinguish pilocytic astrocytomas (WHO grade I) from diffuse astrocytomas (WHO grade II)</td>
<td>Jones et al, 200815</td>
</tr>
</tbody>
</table>

Abbreviation: WHO, World Health Organization.
The criterion standard chemotherapy for gliomas is temozolomide. Temozolomide is taken orally and has a low frequency of serious adverse events, which enormously facilitates its use around the world. Survival is superior by several weeks in patients treated with temozolomide and radiotherapy compared with patients treated with radiotherapy only.20 Bevacizumab (Avastin) was the first US Food and Drug Administration–approved biological therapy designed to inhibit the formation of new blood vessels in tumors. Bevacizumab is a monoclonal antibody that binds to and inhibits vascular endothelial growth factor.21 The basis for bevacizumab’s accelerated approval in 2009 for treating recurrent glioblastoma was uncontrolled phase II trials with a total of 215 patients, a single-arm phase II trial of bevacizumab with irinotecan added at progression, and a randomized phase II trial of the same regimen or first-line treatment with the combination of bevacizumab and irinotecan.22 Despite its promise, bevacizumab has not dramatically improved survival in patients with glioma. However, bevacizumab does improve progression-free survival and eliminates the need for steroids to treat edema. Therefore, bevacizumab offers some therapeutic benefits to patients with glioma and can improve their quality of life.23 Not all gliomas are sensitive to anti–vascular endothelial growth factor therapy.24 In addition, some of the tumors that are initially sensitive to the therapy recur with an aggressive phenotype (Figure 3). In fact, magnetic resonance imaging of patients with glioblastomas treated with bevacizumab showed the development of multifocal recurrence and strongly indicated the presence of an infiltrative/invasive pattern.25 In this regard, Bergers and Hanahan26 recently proposed several hypothetical mechanisms that might underlie the evasive resistance of tumor cells to antiangiogenic therapy. These models include an increased capability of the tumor cells to develop an invasive phenotype without promoting angiogenesis. In fact, there is strong evidence that malignant glioma cells adapt to pathological conditions (such as necrosis) or to therapies that challenge angiogenesis by migrating more aggressively into healthy tissue.27 Collectively, these observations indicate that the clinical success of antiangiogenic therapy might depend on its combination with simultaneous therapy aimed at preventing multifocal recurrence by inhibiting tumor infiltration. Preclinical and clinical data have established the effectiveness of antiangiogenic therapies for human malignant gliomas. However, more studies need to be undertaken, with a special focus on identifying the mechanisms of the resistant phenotype and, ultimately, testing combined therapies.

**EXPERIMENTAL BIOLOGICAL THERAPIES**

There is no question that the immune system should be able to eradicate any cancer if these cells are an immune

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**Figure 2.** Silencing of MGMT promoter correlates with sensitivity to temozolomide (TMZ). Patients whose gliomas encompass a methylated, and thus silenced, MGMT promoter are more responsive to TMZ than patients whose tumors express MGMT. Temozolomide adds a methyl group to the 06 of the guanine in the DNA of a glioma (A). If MGMT is not expressed owing to epigenetic modifications of its promoter, TMZ-mediated damage of DNA would be followed by growth arrest or cell death (B). However, in patients whose tumors display normal MGMT expression, this enzyme will remove the methylating groups from the DNA, rendering cancer cells resistant to TMZ (C).

**Figure 3.** Early success and long-term failure of bevacizumab. This series of magnetic resonance images shows a glioblastoma multiforme in the parieto-occipital region of the left hemisphere. A, Note the typical enhancement ring after injection of gadolinium. B, Approximately 6 months later and after treatment with bevacizumab, the enhanced tumor is dramatically diminished. C, Three years after diagnosis, the tumor recurred, displaying a more diffuse, infiltrative pattern. This pattern of recurrence is also observed in animal models in which glioma xenografts are treated with antivascular endothelial growth factor therapy.
target. For that reason, it is not surprising that several groups are developing a therapeutic approach for gliomas in the form of cancer vaccines. Notably, investigators have attempted to develop strategies against pure cancer antigens. A vaccine has been developed to target the most common mutant form of the epidermal growth factor in gliomas. This mutant, termed variant III or Delta-EGFR (epidermal growth factor receptor), occurs in a subset of glioblastoma multiforme tumors (but not all) and is not found in healthy cells. In a phase II study, one group of patients received an initial set of 3 vaccinations at 2-week intervals, standard temozolomide plus radiotherapy, and monthly vaccines thereafter; the other group of patients received only temozolomide plus radiotherapy. The median time to tumor progression was significantly longer for the vaccinated patients than for patients receiving the same treatment except for the immunization. A subsequent trial was completed in which the vaccine was given only to patients who were screened in advance for the mutant receptor before admission into the clinical trial. Median survival for these patients was impressive, reaching almost 2.5 years. Another recently developed biological approach for gliomas in the form of cancer vaccines. No-30 tensive success was achieved, reaching almost 2.5 years. A subsequent trial was completed in which the vaccine was given only to patients who were screened in advance for the mutant receptor before admission into the clinical trial. Median survival for these patients was impressive, reaching almost 2.5 years. Another recently developed biological approach that is now being used in the clinical setting is oncolytic viruses in combination with chemotherapy. Currently, an oncolytic adenovirus, Delta-24-RGD, is being tested in a phase I clinical trial for patients with malignant glioma at the University of Texas MD Anderson Cancer Center. Importantly, both herpes simplex viruses and oncolytic adenoviruses show synergy when combined with chemotherapy, particularly temozolomide. Concomitant treatment with temozolomide and herpes simplex viruses increases the virus titer. Oncolytic adenoviruses express a protein that silences the MGMT promoter and thus enhances the chemosensitivity of glioma cells to temozolomide. Oncolytic viruses in combination with chemotherapy should be adopted for clinical use during the next 5 years.

CONCLUSIONS

Ten years ago, Levin33 wrote an overview of Neuro-oncology for the Archives of Neurology. He quoted Dickens’ “Tale of Two Cities” to illustrate his position: “It was the best of times, it was the worst of times.” His reflection is still valid but today we are more optimistic. Our knowledge about brain tumors has met crucial milestones toward a more specific treatment and management of patients with this disease. In the next 10 years, patients with gliomas should have, for the first time, therapeutic strategies that will cure their tumors or make them controllable chronic disease.

Author Contributions: Study concept and design: Fueyo, Gomez-Manzano, and Yung. Drafting of the manuscript: Fueyo and Gomez-Manzano. Critical revision of the manuscript for important intellectual content: Fueyo, Gomez-Manzano, and Yung. Obtained funding: Fueyo. Study supervision: Yung.

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**Announcement**

**Trial Registration Required.** As a member of the International Committee of Medical Journal Editors (ICMJE), *Archives of Neurology* will require, as a condition of consideration for publication, registration of all trials in a public trials registry (such as http://ClinicalTrials.gov). Trials must be registered at or before the onset of patient enrollment. The trial registration number should be supplied at the time of submission.

For details about this new policy, and for information on how the ICMJE defines a clinical trial, see the editorials by DeAngelis et al in the September 8, 2004 (2004;292:1363-1364) and June 15, 2005 (2005;293:2927-2929) issues of *JAMA*. Also see the Instructions to Authors on our Web site: www.archneurol.com.