

# Understanding the Origins of Gliomas and Developing Novel Therapies: Cerebrospinal Fluid and Subventricular Zone Interplay

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Glioblastoma multiforme (GBM), the most common malignant primary brain tumor in adults, carries a poor prognosis, with median survival generally less than 1 year. Although initial therapy often eradicates the bulk of the tumor, disease recurrence, usually within 2 cm of the original tumor, is almost inevitable. This may be due to a failure of current therapies to eradicate viable chemotherapy- and radiotherapy-resistant neoplastic progenitor cells, which may then repopulate tumors. An increasing body of preclinical data suggests that these cells may correspond to stem cells derived from the subventricular zone (SVZ), which migrate to tumor sites and contribute to glioma growth and recurrence. Therapeutic targeting of SVZ stem cell populations via cerebrospinal fluid (CSF)-directed therapy may provide a means for limiting tumor recurrence. This approach has proved successful in the treatment of medulloblastoma, another brain tumor thought to be derived from stem cells. We discuss the rationale and design considerations for a clinical trial to evaluate the efficacy of CSF-directed therapy for preventing GBM recurrence.

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**G**lioblastoma multiforme (GBM) is the most common type of primary malignant brain tumor in adults. Despite current treatment strategies, the median survival for patients with GBM is about 1 year from the time of diagnosis, and more than 90% of patients die within 2 years.<sup>1-3</sup> Standard therapy consists of surgical resection followed by radiotherapy and adjuvant chemotherapy.<sup>4,5</sup> Although initial therapy often eradicates the majority of the tumor, recurrence within 2 cm of the original site occurs in more than 90% of patients.<sup>6,7</sup> This may be due, in part, to a failure of current therapies to eradicate viable chemotherapy-

and radiotherapy-resistant neoplastic progenitor cells that can repopulate the tumor.

An extensive body of in vitro data suggests that the origin and primary location of these putative tumorigenic cells is the subventricular zone (SVZ). Clinical observations that malignant cells are often present in the cerebrospinal fluid (CSF) both at the time of GBM diagnosis and at the time of tumor recurrence, and that tumor proximity to the SVZ affects the timing and topology of tumor recurrence, suggest that therapy-resistant progenitor cells may find shelter in the CSF or in periventricular regions adjacent to the CSF, where systemic chemotherapy penetration is poor.<sup>8,9</sup> This review discusses data suggesting that CSF and SVZ malignant cell progenitor populations may represent a reservoir of cells that can repopulate tumor beds and thus contribute to GBM recurrence. Specifically, we hypothesize that: (1) stem cell-like neural or glial precursor cells contribute to glioma growth and recurrence; (2) these putative tumorigenic cells derive from the SVZ, a known site of self-renewing undifferentiated cells in the adult brain; and (3) tumorigenic cells migrate from the SVZ to parenchymal brain sites in a non-random manner analogous to neuronal and glial migration that occurs during neurogenesis (Figure 1). We further propose that if these hypotheses prove sufficiently robust, CSF-directed therapy may provide an effective means for preventing GBM recurrence. Practical considerations related to designing a clinical trial that would

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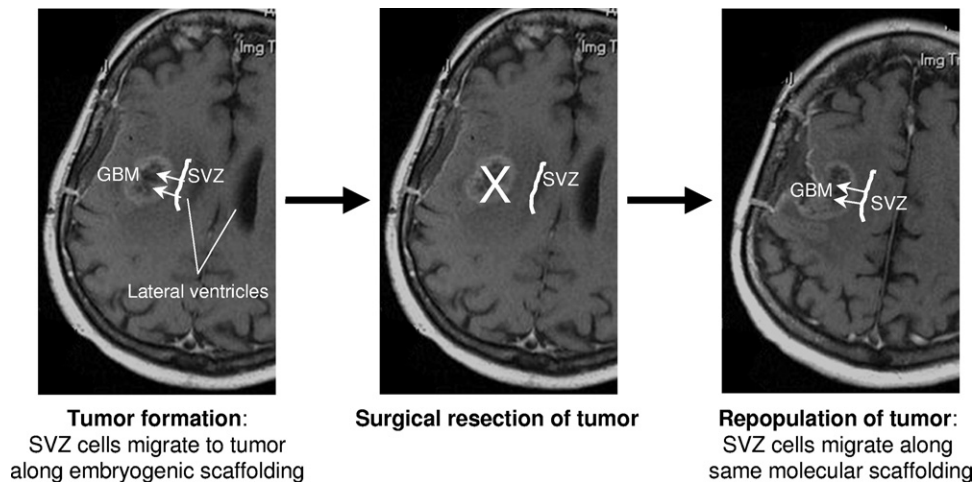
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**Figure 1.** Schematic diagram of proposed stem cell hypothesis of glioblastoma multiforme (GBM) tumor formation and repopulation following surgical resection. SVZ, subventricular zone.

evaluate the efficacy of CSF-directed therapy for preventing tumor recurrence are then discussed.

### THE SVZ, STEM CELLS, AND GLIOMA FORMATION

As early as 1944, anatomical and histological observations led Globus and Kuhlenbeck to suggest that central nervous system (CNS) neoplasms could be traced to “embryonal cell nests” within the subependymal zone, a cell layer surrounding the lateral ventricles.<sup>10</sup> Periventricular regions were further implicated in tumor formation in early animal models that induced brain tumors by in utero exposure to ethyl-nitrosourea (ENU). Tumors induced by ENU were found to almost always occur in areas adjacent to the ventricles.<sup>11</sup> Positional differences in tumor susceptibility also were identified in another important animal model that used intracerebral implantation of carcinogen pellets to induce brain tumors in rats.<sup>12</sup> These studies demonstrated a higher incidence of tumor induction when the carcinogen was implanted in positions adjacent to the ventricular space (77%) than when pellets were situated near the meninges (11%).<sup>12</sup>

The periventricular subregion implicated in these animal models of glioma formation is the SVZ. The SVZ is a mitotically active cell layer that retains the ability to produce neurons and glia throughout the life of the animal, functioning as a source of stem-like cells in adults.<sup>13-15</sup> In the normal brain, multipotent cells that originate in the SVZ are thought to respond to injury and growth factors. This is supported by observations that 6-hydroxydopamine lesions and infusion of transforming growth factor  $\alpha$  stimulate massive proliferation, directed migration, and neuronal differentiation of SVZ progenitor cells in rats.<sup>16</sup> In vivo infusion of epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and cytokines also

has been shown to induce proliferation and migration of SVZ cells away from the lateral ventricle walls into adjacent parenchyma.<sup>17,18</sup>

The cancer stem cell hypothesis suggests that a fraction of tumor cells with stem cell properties may be involved in the initiation and maintenance of tumors.<sup>19,20</sup> Stem-like cells have been isolated from other areas of the adult brain such as the hippocampus and cerebral cortex, in various species including humans.<sup>21</sup> However, the SVZ stem cell populations lie most proximal to the cerebral ventricles, which, as described above, have been strongly implicated in the development of gliomas. Glioma stem cells and normal neural stem cells share many of the same signaling pathways.<sup>22</sup>

### ANIMAL MODELS OF GLIOMA FORMATION

A growing body of preclinical evidence suggests that stem-like cells of the SVZ are involved in glioma formation. In animal models, these cells appear to correspond to glial precursor cells that express glial fibrillary acidic protein (GFAP) and nestin. In their landmark study in mice, Holland et al oncogenically transformed glial precursor cells using nestin or GFAP promoters and found that nestin positive glial precursors were easily transformed into tumorigenic cells.<sup>23</sup> These observations support the hypothesis that glioma formation may be stimulated and/or maintained by transformation events in progenitor cells. Alternatively, it has been suggested that normal cells from the SVZ may be recruited to migrate into tumor sites by signals from other tumor cells, and subsequently either fuel or combat tumor growth. This hypothesis is supported by observations that normal nestin<sup>+</sup>/GFAP<sup>+</sup> progenitor cells migrate from the SVZ to surround and infiltrate gliomas that have been initiated by transplanted tumor cells in rats and mice.<sup>24,25</sup> Interestingly, Glass et al found that the appearance of nestin<sup>+</sup>/GFAP<sup>+</sup> cells in

transplanted tumors was actually associated with suppressed tumor growth as well as improved survival.<sup>25</sup>

The rodent ENU model for glioma formation also has provided some important insights into the temporal dynamics of glioma-like tumor formation. In ENU-induced tumors, nestin-expressing cells are among the first cells to appear, and clusters of nestin<sup>+</sup> cells are detected long before tumors are detectable by histology or magnetic resonance imaging (MRI).<sup>26</sup> A tumor transition stage marked by the appearance of a second type of cell, osteopontin-expressing GFAP<sup>+</sup> cells, also has been identified. Once osteopontin<sup>+</sup> cells appear, tumors become evident on MRI.<sup>26</sup> Furthermore, there is a large time window (~40 days) between the appearance of nestin<sup>+</sup> cells and the advent of osteopontin-expressing cells, suggesting that there may be early pre-tumor markers that could allow for early detection and potentially prevention.<sup>26</sup>

Stem cells of the SVZ are thought to exist within a supportive niche that maintains and controls critical stem cell properties through contact-mediated and paracrine signaling between stem cells and the niche microenvironment.<sup>27</sup> Soluble factors secreted by vascular endothelial cells have been found to promote self-renewal and inhibit differentiation of neural stem cells, suggesting a vascular niche.<sup>28</sup> It has been suggested that glioma tumor stem cells also may rely on interactions with a vascular niche to maintain their stem-like properties and their ability to drive tumor growth.<sup>29,30</sup> Consistent with this notion, many studies have found that proangiogenic growth factors such as bFGF,<sup>31-34</sup> EGF,<sup>32-35</sup> and platelet-derived growth factor (PDGF)<sup>31</sup> permit maintenance and expansion of tumor stem cells in culture. In vivo effects of PDGF and EGF on tumor generation also have been observed. For example, intracerebral infusion of PDGF alone is sufficient to cause proliferation of SVZ stem cells and the generation of large glioma-like hyperplasias.<sup>36</sup>

Insights into microenvironmental properties responsible for maintaining stem cell viability and proliferation may prove invaluable for developing novel therapies that target cancer stem cells that are notoriously resistant to most chemotherapeutic agents and radiation.<sup>30,37-39</sup>

## HUMAN GLIOMAS AND STEM-LIKE CELLS

Glioma progression and neuronal differentiation may involve activation of molecular developmental pathways. In patients with GBM, the stem-like cells relevant to tumor formation may correspond to neural precursor cells that express the surface protein CD133. Populations of CD133<sup>+</sup> cells have been isolated from resected human glioblastomas,<sup>32</sup> and transplantation of isolated human CD133<sup>+</sup> GBM cells into mouse brains can initiate and sustain the growth of glioma-like tumors.<sup>33</sup> More recently, CD133 has been localized to

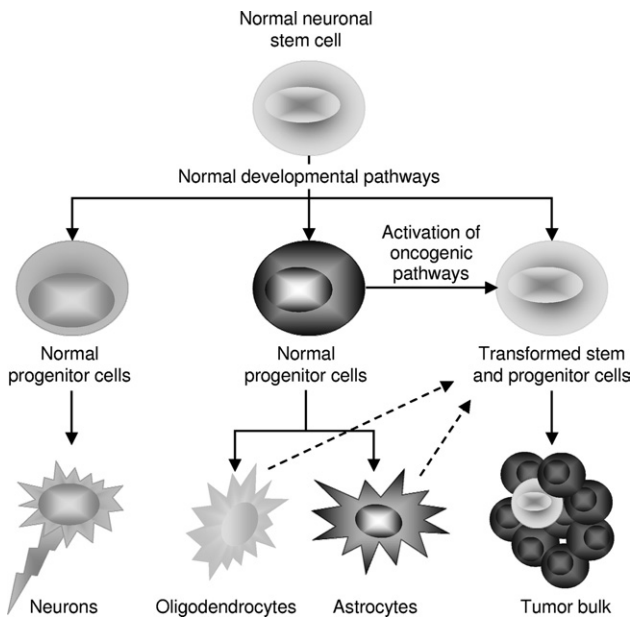
ependymal cells of the SVZ in adult mouse and human brains.<sup>40,41</sup> However, unlike GFAP-expressing adult neural stem cells, adult CD133<sup>+</sup> ependymal cells have generally been regarded as postmitotic.<sup>41</sup> Despite this, Coskun et al demonstrated that CD133<sup>+</sup> ependymal cells continuously produce new neurons and suggested that they may represent an additional, perhaps more quiescent stem cell population in the SVZ of the mammalian forebrain.<sup>40</sup>

Gene expression profiling studies in humans also have provided evidence that undifferentiated stem-like cells contribute to some types of gliomas. According to microarray expression profiles, there are three distinct subtypes of high-grade gliomas: proneural, mesenchymal, and proliferative.<sup>42-45</sup> Proliferative and mesenchymal tumors express markers of neural stem cell and/or transit-amplifying cells, whereas tumors of the proneural subclass express markers of neuroblasts or immature neurons.<sup>45</sup> Observations from this study also suggest that glioma progression and neuronal differentiation involve differential expression of the same molecular developmental pathways. Comparisons of primary tumors with recurrent tumors indicate that a substantial proportion of proneural and proliferative primary tumors shift to a mesenchymal expression profile upon recurrence<sup>45</sup> in a manner that parallels activation patterns observed in neural stem cell lines following treatment with brain-derived neurotrophic factor (BDNF). Specifically, BDNF-stimulated neural or glial differentiation tends to occur with Notch pathway activation and Akt pathway down-regulation. Likewise, the notch pathway is activated in proneural tumors, and the Akt pathway is activated in mesenchymal and proliferative tumors.<sup>45,46</sup>

The shift from proneural expression profiles to mesenchymal or proliferative expression that occurs with tumor recurrence may be viewed as a de-differentiation of tumor cells (Figure 2).<sup>45,47</sup> Alternatively, the shift to an undifferentiated expression profile may reflect a migration of stem cell-like populations into the tumor upon recurrence.<sup>45</sup> Therefore, therapeutic targeting of stem cell populations has the potential to play an important role in limiting tumor recurrence. One approach to targeting this cell population may be via CSF-directed chemotherapy, an approach that has proved successful in the treatment of medulloblastoma, another type of brain tumor believed to be derived from stem cells.

## LESSONS LEARNED FROM MEDULLOBLASTOMA THERAPY

Medulloblastoma is the most common malignant brain tumor in children. Although debate exists over the cellular origin of medulloblastomas, the leading hypothesis implicates precursor cells of the medullary velum of the fourth ventricle.<sup>48</sup> The medullary velum is



**Figure 2.** Stem cells that putatively form malignant gliomas originate from the transformation of neural stem cells or progenitor cells. Oligodendrocytes and astrocytes may follow the same process.<sup>47</sup>

thought to be a source for neural precursor cells during the development of the cerebellum, and thus the relationship between this periventricular area and medulloblastoma may be analogous to the hypothesized relationship between the SVZ and GBM.

Treatment of medulloblastoma has conventionally included surgery, systemic chemotherapy, and radiotherapy. However, because of the high incidence of neurocognitive and other side effects, more recent treatment strategies strive to avoid radiotherapy. Strategies omitting radiotherapy have included high-dose chemotherapy with autologous stem cell transplantation (ASCT) accompanied by intraventricular chemotherapy during initial treatment. The incorporation of intraventricular chemotherapy into the initial treatment has yielded promising results. In the German HIT-SKK'92 trial (age <3 years, N = 43), the 10-year progression-free survival (PFS) rate was 82% for patients who had no postoperative residual tumor and 50% for patients with residual tumor.<sup>49</sup> This was a very favorable outcome compared with the 29% to 41% 5-year PFS rates observed in previous studies that did not include CSF-directed chemotherapy.<sup>50</sup> Previous trials also had observed high rates of disease progression throughout the neuraxis, which seem to have been prevented using the HIT-SKK'92 trial's combination of high-dose systemic and intraventricular chemotherapy.

However, the prognosis remains poor for patients with recurrent medulloblastomas, and CSF-directed therapy appears to provide little benefit. The German HIT-REZ'97 study for relapsed medulloblastoma compared ASCT with high-dose systemic and CSF-directed

chemotherapy to oral chemotherapy and documentation controls.<sup>51</sup> Patients in the high-dose systemic chemotherapy arm had a median PFS duration of 11.6 months and a median overall survival of 21.1 months.<sup>51</sup> Given the poor prognosis at relapse and the encouraging results seen with CSF-directed chemotherapy at initial treatment, it has been proposed that CSF-directed chemotherapy should be included as part of initial treatment in all children with newly diagnosed primary embryonic brain tumors. Perhaps similar benefits may be observed by incorporating CSF-directed therapy into the initial treatment of adult patients with newly diagnosed GBM.

## THE POTENTIAL OF CSF-DIRECTED THERAPY IN ADULT PRIMARY BRAIN TUMORS

There have been no clinical trials investigating the potential of CSF-directed chemotherapy in preventing tumor recurrence in adults with primary brain tumors. If such a clinical trial is to be developed, several questions must be addressed. First, the patient population to be investigated must be identified. This might include all patients with newly diagnosed GBM, only patients with radiologic or cytologic evidence of CSF spread, or perhaps only patients with tumors that are adjacent to the SVZ at presentation. An MRI study has shown that GBMs that were both in contact with the SVZ and infiltrated the cortex were most likely to be multifocal at diagnosis, and recurred at greater distances from the initial lesion.<sup>9</sup> These observations suggest that tumors that are in intimate contact with the SVZ may be most related to SVZ stem cells, and that patients with this type of tumor may potentially derive the most benefit from CSF-directed therapy.

Once a patient population has been identified, an appropriate CSF-directed treatment regimen must be selected. In general, CSF-directed therapies include intra-CSF chemotherapy, systemic chemotherapy with agents that produce high drug concentrations within the CSF (eg, high-dose methotrexate), neuraxis irradiation, or combinations of these approaches. Cancer stem cells are known to be resistant to radiation and to multiple chemotherapeutic agents.<sup>39,52</sup> GBM-isolated CD133<sup>+</sup> stem cells have demonstrated resistance to many agents, including temozolomide, carboplatin, paclitaxel, etoposide, and carmustine<sup>37,38</sup> but appear to be relatively sensitive to certain cytotoxic (eg, cytarabine, cyclophosphamide) and anti-angiogenic (eg, bevacizumab) agents.<sup>30,52-54</sup> Because stem cells may be relatively sensitive to cytarabine and because a sustained-release formulation of cytarabine for intrathecal use is available, this might be a good choice of agent. When considering treatment with CSF-directed chemotherapy combined with irradiation, issues such as the relative resistance of stem cells to radiotherapy<sup>37,55</sup> and the poten-

tial for increased neurotoxicity risk associated with combining these two therapies<sup>56</sup> should be considered.

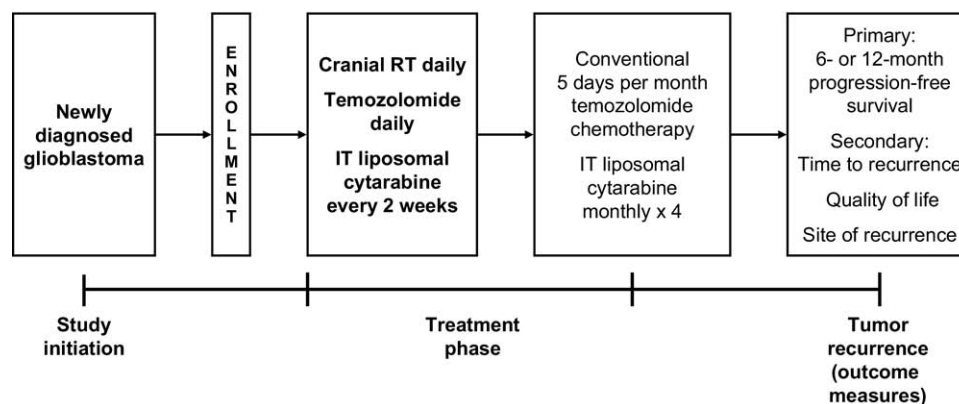
The ideal dosage schedule and route of drug administration also must be addressed in trial designs. We propose that using low but sustained concentrations may be more appropriate than administering drugs at higher, more rapidly depleted concentrations. Pertaining to the route of administration, CSF-directed therapy may be administered via repeated lumbar punctures or by an intraventricular (eg, Ommaya) reservoir. The placement of an Ommaya reservoir may be preferable to repeated lumbar punctures, in terms of comfort to the patient. In addition, the ventricular placement of an Ommaya reservoir allows for higher intraventricular drug concentrations<sup>57</sup> and thus potentially greater exposure of the SVZ to drug.

The size and design of any trial should recognize the novel nature of the GBM stem cell hypothesis upon which it is based. Outcome measures should include traditional end points such as median survival, time to progression, or PFS. However, the evaluation of other outcome measures, such as cytologic progression, radiologic progression, site of tumor recurrence, and novel surrogate markers (eg, biochemical or proteomic CSF markers, stem cell assays, quality of life assessments) also may prove to be useful indicators of therapeutic efficacy, particularly in a randomized trial where sample size is a potential barrier to detecting significant changes in survival. For example, detecting a moderate improvement in overall survival would require hundreds of patients ( $N = 550$  for change from 14.6 to 19.5 months median survival; hazard ratio [HR], 0.75; 85% power;  $\alpha = 0.05$ ). Looking for a similar improvement in PFS would also require 300–500 patients (eg,  $N = 472$ ; 6.9 *v* 9.2 months median PFS; HR, 0.75; 85% power;  $\alpha = 0.05$ ). A randomized phase II trial would also require a large sample size. Reducing the required sample size to less than 200 patients may require setting unrealistic goals (eg,  $N = 158$ ; 6.9 *v* 10.7 months median PFS; HR, 0.65; 80% power;  $\alpha = 0.10$ ).

A phase II, two-stage single-arm trial using a minimax or optimal design (Figure 3) might be preferable to a randomized phase II or III trial.<sup>58</sup> A minimax design minimizes sample size by requiring fewer patients if the trial is carried through both stages to completion. On the other hand, an optimal design minimizes the expected sample size given an “unfavorable” response rate, by requiring fewer patients in stage 1 of the trial.<sup>58</sup> According to these models, if an end point of 6-month PFS ( $p_0 = 0.5$  and  $p_1 = 0.75$ ) is selected, then a maximum of 32 patients (21 in stage I) would be required for a minimax design ( $\alpha = 0.05$ ; 90% power;  $\langle r_1/n_1 \rangle$ : 12/21;  $\langle r/n \rangle$ : 20/32; probability of early termination: 81%), and only 41 patients (13 in stage I) would be needed for an optimal two-stage design ( $\alpha = 0.05$ ; 90% power;  $\langle r_1/n_1 \rangle$ : 7/13;  $\langle r/n \rangle$ : 25/41; probability of early termination: 71%; see Table 1 for alternate scenarios). Although the lack of randomization or masked outcome assessment in these types of single-arm trials can potentially introduce substantial bias, and the small sample size makes identification of important patient subgroups difficult or impossible, this trial design may be the most feasible and may provide preliminary evidence of efficacy, justifying a larger randomized trial.

## CONCLUSIONS

The frequent recurrence of GBMs close to their original site of occurrence may be due to a failure of current therapies to eradicate viable tumor progenitor cells that can repopulate the tumor. Cells that contribute to GBM growth and recurrence may be stem/progenitor cells that migrate from the SVZ to the cortical site where the growing tumor ultimately becomes symptomatic, and is then recognized clinically and radiographically. These progenitor/stem cells migrate to the same location at recurrence as they did initially because the migration is not random. Rather, cells migrate along the cellular “scaffolding” originally established to facilitate precursor cell migration during em-



**Figure 3.** Proposed design for a clinical trial evaluating the use of intrathecal (IT) chemotherapy in the initial treatment of newly diagnosed patients with glioblastoma multiforme. RT, radiation therapy; IT, intrathecal therapy.

**Table 1. Phase II Minimax and Optimal Designs**

$\alpha$	Power	Minimax Design Rejection Rules				Optimal Design Rejection Rules			
		$< r_1/n_1$	$< r/n$	EN	PET	$\leq r_1/n_1$	$\leq r/n$	EN	PET
For PFS at 6 months with $p_0 = 0.5$ and $p_1 = 0.75$									
0.05	90%	12/21	20/32	23	81%	7/13	25/41	21	71%
0.10	90%	13/22	15/25	22	86%	6/12	17/28	18	61%
0.05	80%	7/14	15/23	18	60%	6/11	16/25	15	73%
0.10	80%	9/15	11/18	15	85%	5/9	13/22	12	75%
For PFS at 6 months with $p_0 = 0.5$ and $p_1 = 0.65$									
0.05	90%	28/57	54/93	75	50%	22/43	57/99	64	62%
0.10	90%	19/40	41/72	58	44%	18/35	47/84	53	63%
0.05	80%	39/66	40/68	66	95%	15/28	48/83	44	71%
0.10	80%	10/22	29/50	38	42%	12/23	34/60	36	66%

Abbreviations: EN, estimated number; PET, probability of early termination; PFS, progression-free survival.

bryogenesis. In animal models, these progenitor cells correspond to glial precursor cells that express GFAP and nestin. In resected human GBMs, the stem-like cells relevant to tumor formation appear to correspond to neural precursor cells that express the surface protein CD133. When transplanted into mouse brains, these CD133<sup>+</sup> cells can initiate and sustain the growth of glioma-like tumors.

Therapeutic targeting of stem cell populations has the potential to play an important role in limiting tumor recurrence. One approach to targeting this cell population may be via CSF-directed chemotherapy, an approach that has proved successful in the treatment of medulloblastoma. Medulloblastomas are thought to develop from stem cells arising from the ventricular lining, in a manner that may be analogous to the proposed relationship between glioblastoma and stem cells of the SVZ. The inclusion of CSF-directed chemotherapy in the initial treatment of children with medulloblastoma has substantially improved PFS rates. Incorporation of CSF-directed therapy into the initial treatment of patients with newly diagnosed GBM may provide similar benefits and merits investigation.

## REFERENCES

- Buckner JC. Factors influencing survival in high-grade gliomas. *Semin Oncol.* 2003;30 Suppl 19:10-4.
- Curran WJ Jr, Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst.* 1993; 85:704-10.
- DeAngelis LM. Brain tumors. *N Engl J Med.* 2001;344: 114-23.
- Mirimanoff RO, Gorlia T, Mason W, Van den Bent MJ, Kortmann RD, Fisher B, et al. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. *J Clin Oncol.* 2006;24:2563-9.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352:987-96.
- Hochberg FH, Pruitt A. Assumptions in the radiotherapy of glioblastoma. *Neurology.* 1980;30:907-11.
- Wallner KE, Galicich JH, Krol G, Arbit E, Malkin MG. Patterns of failure following treatment for glioblastoma multiforme and anaplastic astrocytoma. *Int J Radiat Oncol Biol Phys.* 1989;16:1405-9.
- Hsu S, Cole BF, Recht L, Glantz LK, Mills P, Leks A, et al. Frequency and prognostic significance of a positive cerebrospinal fluid (CSF) cytology in patients with gliomas [abstract]. *Neurology.* 1997;48:A35.
- Lim DA, Cha S, Mayo MC, Chen MH, Keles E, Vandenberg S, et al. Relationship of glioblastoma multiforme to neural stem cell regions predicts invasive and multifocal tumor phenotype. *Neurooncology.* 2007;9:424-9.
- Globus JH, Kuhlenbeck H. The subependymal cell plate (matrix) and its relationship to brain tumors of the ependymal type. *J Neuropathol Exp Neurol.* 1944;3:1-35.
- Ivankovic S, Druckrey H. Transplacental induction of malignant tumors of the nervous system. I. Ethyl-nitrosourea (ENU) in BD IX rats. *Z Krebsforsch.* 1968;71:320-60.
- Hopewell JW, Wright EA. The importance of implantation site in cerebral carcinogenesis in rats. *Cancer Res.* 1969;29:1927-31.
- Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. *Science.* 1994;264:1145-8.
- Luskin MB. Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron.* 1993;11:173-89.
- Thomas LB, Gates MA, Steindler DA. Young neurons from the adult subependymal zone proliferate and migrate along an astrocyte, extracellular matrix-rich pathway. *Glia.* 1996; 17:1-14.
- Fallon J, Reid S, Kinyamu R, Opole I, Opole R, Baratta J, et al. In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. *Proc Natl Acad Sci U S A.* 2000;97:14686-91.

17. Calza L, Giuliani A, Fernandez M, Pironi S, D'Intino G, Aloe L, et al. Neural stem cells and cholinergic neurons: regulation by immunolesion and treatment with mitogens, retinoic acid, and nerve growth factor. *Proc Natl Acad Sci U S A*. 2003;100:7325-30.
18. Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D. In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci*. 1996;16:2649-58.
19. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nat Rev Cancer*. 2003;3:895-902.
20. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature*. 2001;414:105-11.
21. Taupin P, Gage FH. Adult neurogenesis and neural stem cells of the central nervous system in mammals. *J Neurosci Res*. 2002;69:745-9.
22. Dietrich J, Imitola J, Kesari S. Mechanisms of disease: the role of stem cells in the biology and treatment of gliomas. *Nat Clin Pract Oncol*. 2008;5:393-404.
23. Holland EC, Hively WP, DePinho RA, Varmus HE. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes Dev*. 1998;12:3675-85.
24. Dunsch C, Zhou Q, Weimar JD, Frankel B, Robertson JH, Pourmottabed T. Up-regulation of neurogenesis generating glial progenitors that infiltrate rat intracranial glioma. *J Neurooncol*. 2005;71:245-55.
25. Glass R, Synowitz M, Kronenberg G, Walzlein JH, Markovic DS, Wang LP, et al. Glioblastoma-induced attraction of endogenous neural precursor cells is associated with improved survival. *J Neurosci*. 2005;25:2637-46.
26. Jang T, Savarese T, Low HP, Kim S, Vogel H, Lapointe D, et al. Osteopontin expression in intratumoral astrocytes marks tumor progression in gliomas induced by prenatal exposure to N-ethyl-N-nitrosourea. *Am J Pathol*. 2006;168:1676-85.
27. Scadden DT. The stem-cell niche as an entity of action. *Nature*. 2006;441:1075-9.
28. Shen Q, Goderie SK, Jin L, Karanth N, Sun Y, Abramova N, et al. Endothelial cells stimulate self-renewal and expand neurogenesis of neural stem cells. *Science*. 2004;304:1338-40.
29. Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B, et al. A perivascular niche for brain tumor stem cells. *Cancer Cell*. 2007;11:69-82.
30. Folkins C, Man S, Xu P, Shaked Y, Hicklin DJ, Kerbel RS. Anticancer therapies combining antiangiogenic and tumor cell cytotoxic effects reduce the tumor stem-like cell fraction in glioma xenograft tumors. *Cancer Res*. 2007;67:3560-4.
31. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. *Proc Natl Acad Sci U S A*. 2004;101:781-6.
32. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. *Cancer Res*. 2003;63:5821-8.
33. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. *Nature*. 2004;432:396-401.
34. Yuan X, Curtin J, Xiong Y, Liu G, Waschmann-Hogiu S, Farkas DL, et al. Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene*. 2004;23:9392-400.
35. Savarese TM, Jang T, Low HP, Salmons R, Litofsky NS, Matusevic Z, et al. Isolation of immortalized, INK4a/ARF-deficient cells from the subventricular zone after in utero N-ethyl-N-nitrosourea exposure. *J Neurosurg*. 2005;102:98-108.
36. Jackson EL, Garcia-Verdugo JM, Gil-Perotin S, Roy M, Quinones-Hinojosa A, VandenBerg S, et al. PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling. *Neuron*. 2006;51:187-99.
37. Kang MK, Kang SK. Tumorigenesis of chemotherapeutic drug-resistant cancer stem-like cells in brain glioma. *Stem Cells Dev*. 2007;16:837-47.
38. Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, et al. Analysis of gene expression and chemoresistance of CD133<sup>+</sup> cancer stem cells in glioblastoma. *Mol Cancer*. 2006;5:67-78.
39. Trumpp A, Wiestler OD. Mechanisms of disease: cancer stem cells-targeting the evil twin. *Nat Clin Pract Oncol*. 2008;5:337-47.
40. Coskun V, Wu H, Bianchi B, Tsao S, Kim K, Zhao J, et al. CD133<sup>+</sup> neural stem cells in the ependyma of mammalian postnatal forebrain. *Proc Natl Acad Sci U S A*. 2008;105:1026-31.
41. Pfenninger CV, Roschupkina T, Hertwig F, Kottwitz D, Englund E, Bengzon J, et al. CD133 is not present on neurogenic astrocytes in the adult subventricular zone, but on embryonic neural stem cells, ependymal cells, and glioblastoma cells. *Cancer Res*. 2007;67:5727-36.
42. Burton EC, Lamborn KR, Feuerstein BG, Prados M, Scott J, Forsyth P, et al. Genetic aberrations defined by comparative genomic hybridization distinguish long-term from typical survivors of glioblastoma. *Cancer Res*. 2002;62:6205-10.
43. Mohapatra G, Bollen AW, Kim DH, Lamborn K, Moore DH, Prados MD, et al. Genetic analysis of glioblastoma multiforme provides evidence for subgroups within the grade. *Genes Chromosomes Cancer*. 1998;21:195-206.
44. Nigro JM, Misra A, Zhang L, Smirnov I, Colman H, Griffin C, et al. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. *Cancer Res*. 2005;65:1678-86.
45. Phillips HS, Kharbanda S, Chen R, Forrester WF, Soriano RH, Wu TD, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell*. 2006;9:157-73.
46. Kanamori M, Kawaguchi T, Nigro JM, Feuerstein BG, Berger MS, Miele L, et al. Contribution of Notch signaling activation to human glioblastoma multiforme. *J Neurosurg*. 2007;106:417-27.
47. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359:492-507.
48. Katsetos CD, Del Valle L, Legido A, de Chadarevian JP, Perentes E, Mork SJ. On the neuronal/neuroblastic nature of medulloblastomas: a tribute to Pio del Rio Hortega and Moises Polak. *Acta Neuropathol*. 2003;105:1-13.

49. Rutkowski S, Bode U, Deinlein F, Ottensmeier H, Warmuth-Metz M, Soerensen N, et al. Treatment of early childhood medulloblastoma by postoperative chemotherapy alone. *N Engl J Med.* 2005;352:978-86.
50. Rutkowski S. Current treatment approaches to early childhood medulloblastoma. *Expert Rev Neurother.* 2006;6:1211-21.
51. Bode U, Simon A, Hasan C, Zimmermann M, Fleischhack G. The role of HD chemotherapy (CT) in the treatment of therapy-resistant CNS PNETs. HIT-REZ-97 results. *Hematol Rep.* 2006;2:1-15.
52. Rich JN. Cancer stem cells in radiation resistance. *Cancer Res.* 2007;67:8980-4.
53. Dietrich J, Han R, Yang Y, Mayer-Pröschel M, Noble M. CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. *J Biol.* 2006;5:22-36.
54. Doetsch F, García-Verdugo JM, Alvarez-Buylla A. Regeneration of a germinal layer in the adult mammalian brain. *Proc Natl Acad Sci U S A.* 1999;96:11619-24.
55. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature.* 2006;444:756-60.
56. Omuro AM, DeAngelis LM, Yahalom J, Abrey LE. Chemoradiotherapy for primary CNS lymphoma: an intent-to-treat analysis with complete follow-up. *Neurology.* 2005;64:69-74.
57. Shapiro WR, Young DF, Mehta BM. Methotrexate: distribution in cerebrospinal fluid after intravenous, ventricular and lumbar injections. *N Engl J Med.* 1975;293:161-6.
58. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials.* 1989;10:1-10.