

Immunosuppression in Patients with High-Grade Gliomas Treated with Radiation and Temozolomide

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Abstract

Purpose: Patients with high-grade gliomas (HGG) routinely receive radiation, temozolomide, and glucocorticoids. As each of these is immunosuppressive, we conducted a prospective, multicenter study to follow CD4 counts over time and determine whether low CD4 counts were associated with adverse outcomes.

Experimental Design: Patients with newly diagnosed HGG had CD4 counts drawn before initiating standard therapy and monthly thereafter for 1 year. Information on hospitalizations, infections, glucocorticoid use, survival, and cause of death were also collected.

Results: Ninety-six evaluable patients were accrued [85% glioblastoma, median age of 57, median Karnofsky performance status (KPS) = 90]. The median CD4 count before radiation and temozolomide treatment was 664 cells/mm³. The CD4 count nadir occurred 2 months after initiating therapy when 73% of patients had CD4 counts less than 300 cells/mm³ and 40% had less than 200 cells/mm³. CD4 counts remained low throughout the year of follow-up. Patients with CD4 counts less than 200 cells/mm³ at 2 months had shorter survival than those with higher counts (median: 13.1 vs. 19.7 months, $P = 0.002$). Median survival was related to CD4 toxicity grades (I = 23.8 months, II = 19.7 months, III-IV = 13.1 months, $P = 0.009$). The adjusted HR for death attributable to 2-month CD4 count below 200 was 1.66 ($P = 0.03$). Eighty-eight percent of deaths resulted from disease progression, whereas only 2.5% were due to infection.

Conclusions: Severe reductions in CD4 counts in patients with newly diagnosed HGG treated with radiation and temozolomide treatment are common, treatment-related, long-lasting, and associated with early death from tumor progression. *Clin Cancer Res*; 17(16); 5473-80. ©2011 AACR.

Introduction

Most patients with high-grade gliomas (HGG) receive glucocorticoids, radiation, and chemotherapy as part of their therapeutic regimen. Each of these treatment modalities is toxic to lymphocytes and their combined use can result in substantial immunosuppression and opportunistic infections. Pneumocystis jiroveci pneumonia (PJP), formerly known as Pneumocystis carinii pneumonia (PCP), occurs in severely immunosuppressed patients. This infection is caused by a unicellular fungus

which exists in the respiratory tract of healthy mammals and humans throughout the world. It is a major cause of life-threatening illness in patients with AIDS and solid-organ transplant recipients treated with potent immunosuppressive agents (1, 2). PJP also occurs in patients with lymphoma or leukemia following chemotherapy (3-10).

PJP has also been noted with increasing frequency in patients with primary brain tumors (11). CD4 counts were found to be severely reduced in individual patients with HGGs and PJP at the Johns Hopkins Hospital. These observations prompted a study measuring CD4 counts before and during radiation in patients with newly diagnosed HGGs treated only with glucocorticoids and radiation (12). Chemotherapy was not administered, as this study was conducted before temozolomide was known to be efficacious in this patient population. We found that CD4 counts began in the normal range but fell rapidly. After 6 weeks of radiation, 47% of patients had CD4 counts less than 300 cells/mm³ and 26% of patients had CD4 counts less than 200 cells/mm³.

In 2005, temozolomide was shown to have a substantial survival benefit in patients with glioblastoma when

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Translational Relevance

Patients with high-grade gliomas (HGG) routinely receive radiation, temozolomide, and glucocorticoids. Each of these treatment modalities has been associated with lymphopenia, immunosuppression, and opportunistic infections. CD4 counts were followed for 1 year in 96 patients with newly diagnosed HGGs to quantify the degree and duration of treatment-related reductions in CD4 counts and to determine whether low CD4 counts were associated with adverse outcomes. After 2 months of treatment, 40% of patients had CD4 counts less than 200 cells/mm³. These patients continued to have low CD4 counts for the full year of follow-up and died earlier from tumor progression. These findings raise concerns that severe treatment-related lymphopenia may compromise survival in patients with HGGs and perhaps other cancers as well. Further studies of the consequences of treatment-related lymphopenia and immunosuppression could be important to the design and conduct of translational research and improving patient outcomes.

administered daily during radiation and for 5 days each month for 6 months thereafter (13). Given the paucity of other efficacious therapies, this regimen quickly became standard therapy for this disease. Prophylactic antibiotics are recommended, as earlier experience in combining temozolomide and radiation in patients with HGGs was associated with PJP and a high mortality rate (14). As reductions in CD4 counts are observed following radiation and glucocorticoid administration and temozolomide is known to be toxic to CD4 lymphocytes (15), we hypothesized that adding temozolomide to the treatment of all patients with HGGs could result in further immunosuppression and infectious complications. In addition, information on treatment-related immunosuppression is needed to guide immunologically based clinical trials in patients with brain tumors. The study we report here is a federally funded, prospective, multicenter investigation which followed patients with HGGs receiving standard radiation, temozolomide, and glucocorticoids to determine the frequency and severity of immunosuppression, as monitored by CD4 counts, and the association between low CD4 counts and infections or other adverse outcomes.

Materials and Methods

This study was conducted by the New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium and funded by the NIH through the National Cancer Institute [NCI; NIH: CA-62475 New Approaches to Brain Tumor Therapy CNS Consortium (principal investigator: S.A. Grossman)]. Participating institutions included the Johns Hopkins University, Wake Forest University,

and the H. Lee Moffitt Cancer Center, Tampa, FL. This study was reviewed and approved by the NCI and the Institutional Review Board of each participating institution.

This study was designed to prospectively monitor CD4 counts in patients with newly diagnosed HGG before, during, and after the administration of standard radiation, temozolomide, glucocorticoids, and PJP prophylaxis. The specific objectives of the study were to determine: (i) the frequency and severity of CD4 count reductions and (ii) the relationship between reduced CD4 counts and significant infections or other adverse outcomes.

Eligible patients were at least 18 years of age with newly diagnosed, histologically confirmed anaplastic astrocytoma (WHO grade III), anaplastic oligodendroglioma (WHO grade III), or glioblastoma multiforme (GBM; WHO grade IV) who were to receive standard radiation and temozolomide treatment. Eligible patients were permitted to have had prior surgery with or without placement of Gliadel wafers (polifeprosan 20 with carmustine implant; Eisai, Inc.) and glucocorticoids as required for control of peritumoral edema. Patients were required to have a Karnofsky performance status (KPS) of 60% or above, a negative HIV serology, and written informed consent. Prior radiation or systemic chemotherapy for their HGGs rendered patients ineligible for this study.

This study was designed to have 85% power to detect reductions in CD4 counts to less than 300 cells/mm³ in 30% of 100 HGG patients receiving radiation and temozolomide treatment versus 5% of 25 low-grade glioma patients observed without antineoplastic therapies using a 2-sided significance level of 0.05. CD4 counts and complete blood counts with differential and platelet count were monitored monthly. Data were also collected monthly on radiation and chemotherapy treatments, documented infections, hospitalizations, and dexamethasone and antibiotic use. All episodes of pneumonia or inpatient hospitalization prompted a record review to determine whether *Pneumocystis jiroveci* or other opportunistic infections were present. The above information was collected for 1 year. All patients were followed for survival at least every 2 months.

The original study design included the accrual of 25 patients with newly diagnosed low-grade gliomas where observation without glucocorticoids or antineoplastic therapies was planned. This subcohort was added to ensure that gliomas were not inherently associated with low CD4 counts. However, early results from the patients with HGGs showed that pretreatment CD4 counts were within the normal range despite weeks of peri- and post-operative glucocorticoids prior to these patients being enrolled. As these observations answered this question, accrual to the low-grade glioma cohort glioma was quickly discontinued and results from this cohort are not included in this report.

The statistical design was based on preliminary observations in HGGs treated with only radiation and glucocorti-

coids where a reduction in CD4 counts was evident at 6 weeks.⁽¹²⁾ Therefore, the primary outcome measure was chosen to be the proportion of subjects with a CD4 count less than 300 cells/mm³ at 2 months. We also hypothesized that low CD4 counts would be associated with an increased incidence of infection. Furthermore, we intended to explore whether there was an association between low CD4 counts and infection or survival. NCI Common Terminology Criteria for Adverse Events (CTCAE) 3.0 toxicity classifications were used to define the severity of CD4 count reductions and used for the relevant analyses. These criteria define grade III–IV toxicity as CD4 counts were less than 200 cells/mm³. In the event that a CD4 measurement at 2 months was missing, the 3- or 1-month values were used.

Patient baseline characteristics were summarized using descriptive statistics. The proportion of patients with low CD4 counts at each specific time point was assumed to follow an independent binomial distribution. Overall survival time was calculated from the time of histologic diagnosis until death from any cause. Survival time was censored if the subject was alive at the time of last follow-up. Survival probability was estimated using the Kaplan–Meier method (16).

Univariate analysis was used to assess an association between the known prognostic factors of patients at baseline and overall survival. Important patient characteristics associated with survival were identified in the univariate analysis using a value of $P \leq 0.05$. These characteristics were selected as covariates to construct the multivariate proportional hazards regression model. (17) KPS, an established prognostic factor in HGGs, was added as a covariate to this model. The proportional hazards regression model was used to estimate the HR for death attributable to prognostic factors. All P values are reported as 2 sided, and all analyses were

conducted using the SAS software (version 9.1, SAS Institute).

Results

Study population

A total of 103 subjects were accrued to this protocol between August 2004 and July 2008. Seven patients who did not receive temozolomide were excluded from analysis. Data from the remaining 96 subjects who received standard radiation and temozolomide treatment are the focus of this report. Fifty percent of the subjects were female, 95% were Caucasian, and 80% had a debulking surgical procedure (Table 1). The histologic diagnosis was glioblastoma in 85%, anaplastic astrocytoma in 12%, anaplastic oligodendroglioma in 2%, and an otherwise unspecified malignant glioma in 1%. The median age of the subjects was 57 years (range: 28–85) and the median KPS was 90. Eighty-one percent of the subjects were prescribed anticonvulsants and 82% were taking glucocorticoids when they began radiation and temozolomide treatment. The average elapsed time between surgery and the initiation of radiation and temozolomide treatment was 3.9 weeks (range: 2–12.6). In addition to the standard radiation and temozolomide treatment, 24% of patients received experimental agents. These were noncytotoxic agents unlikely to affect lymphocyte counts. They included talampanel, an oral glutamate receptor blocker (14 subjects); cilengitide, an integrin antagonist (5 subjects); poly-ICLC, an immunostimulant (3 subjects); and BSI, a PARP inhibitor (1 subject). Talampanel and cilengitide were initiated on the first day of radiation and temozolomide treatment and continued until tumor progression or toxicity, whereas BSI and poly-ICLC were added in the tenth week of therapy. As this study was designed before the overall importance of O⁶-methylguanine-DNA methyltransferase (MGMT) was

Table 1. Baseline patient characteristics

	All patients (N = 96)	Patients with CD4 counts ≥200 at 2 mo (N = 58)	Patients with CD4 counts <200 at 2 mo (N = 38)	P ^a
Age, median (range)	57.4 (28–85)	56.6 (28–85)	60.9 (33–78)	0.07
Age, ≥ 55 years %	61 (64)	35 (60)	26 (66)	0.42
Sex, percent male	48 (50)	30 (52)	18 (47)	0.68
KPS of 80–100, n (%)	90 (94)	53 (91)	37 (97)	0.24
Biopsy only, n (%)	19 (20)	9 (16)	10 (26)	0.19
Weeks from diagnosis to treatment, median (range)	3.9 (2–12.6)	4.2 (2–12.6)	3.7 (2.3–7.6)	0.08
Baseline CD4 count, median (range)	664 (90–2,010)	933 (145–2,010)	401 (90–1,309)	0.0001
Baseline lymphocyte count, median (range)	1,418 (331–4,736)	1,645 (672–4,736)	1,044 (331–2,790)	0.0001
Baseline steroid use, n (%)	79 (82)	44 (76)	35 (92)	0.04
Histology, glioblastoma n (%)	81 (84)	46 (79)	35 (92)	0.09

^aComparing subjects with CD4 counts above and below 200 at 2 months.

appreciated, the MGMT status of patients accrued to this multi-institutional study was not evaluated.

This protocol was a noninterventional study to follow CD4 counts and outcomes with standard radiation and temozolomide treatment. As a result, it did not specify the duration of post-radiation temozolomide treatment which is commonly prescribed for 6 months or longer. The median time that patients on this multi-institutional study received temozolomide was 5.8 months (SD = 3 months). Only 4 patients (4%) continued on temozolomide for 11 months or more. Eighty-five of the 96 patients (89%) did not have second-line chemotherapy during the 12 months they were followed on this study. Of the 11 patients who received salvage chemotherapy during the follow-up period, only 2 were treated with bevacizumab. Others received BCNU, CCNU, Gliadel wafers, imatinib, IL-13, bortezomib, tamoxifen, and/or a dendritic cell vaccine.

CD4 counts over time

CD4 counts were reliably obtained from patients on this study. No deaths occurred during the first 2 months of therapy and all patients had a baseline CD4 count and 1 or more follow-up CD4 counts during the first 3 months after beginning radiation and temozolomide treatment.

Serial CD4 count results are provided in Figure 1. The median CD4 count in these patients before initiating radiation and temozolomide treatment was 664 cells/mm³ (range: 90–2,010 cells/mm³). Two months after initiating radiation and temozolomide treatment, the median CD4 count was 255 cells/mm³ (range: 8–1,580 cells/mm³) and subsequent CD4 counts remained persistently low during the 12 months of observation (Figs. 1 and 2). The lowest CD4 counts were observed 2 months after beginning antineoplastic therapy when 70 patients (73%; 95% CI: 63%–82%) had CD4 counts below 300 cells/mm³, and 38 (40%; 95% CI: 30%–50%) had CD4 counts below 200 cells/mm³. Although there was a wide

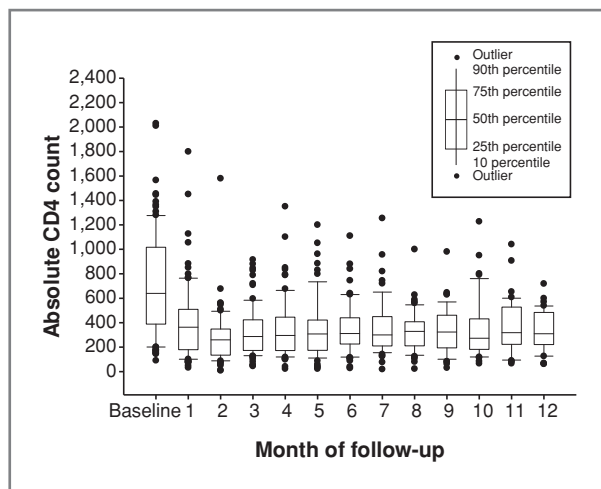


Figure 1. CD4 count trend over time.

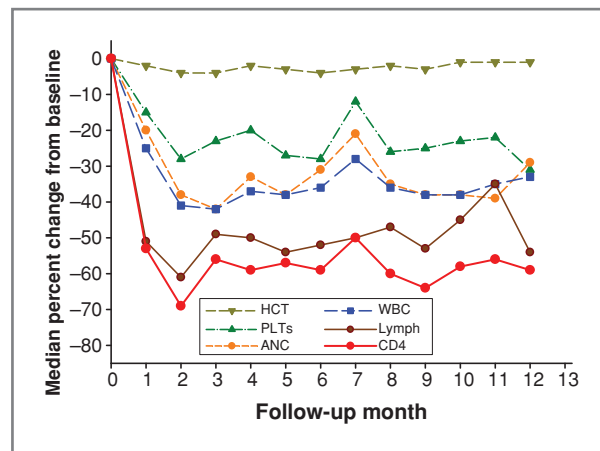


Figure 2. Percent decline in hematologic values over time. HCT, hematocrit; PLT, platelet counts; WBC, white blood cell; ANC, absolute neutrophil counts.

range of CD4 counts noted at baseline, this variability was greatly reduced at 2 months (Fig. 1).

Age, sex, KPS, extent of surgery, and time from surgery to treatment were not associated with a low posttreatment CD4 count (Table 1). Subjects who developed grade III–IV CD4 count depression had significantly lower CD4 counts (458 vs. 887 cells/mm³, $P = 0.0001$) and total lymphocyte counts (1,044 vs. 1,645 cells/mm³, $P = 0.001$) before beginning radiation and temozolomide treatment than those who did not. In addition, they were more likely to be on glucocorticoids (92% vs. 76%, $P = 0.04$) before beginning radiation and temozolomide treatment, have a diagnosis of glioblastoma (92% vs. 79%, $P = 0.09$), and have had a biopsy rather than resection (26% vs. 16%, $P = 0.19$).

Two months after initiating radiation and temozolomide treatment, the average absolute reduction in CD4 counts was 476 cells/mm³. The median percentage reduction in CD4 counts at 2 months was 69% in all patients. Patients with baseline CD4 counts of more than 500 cells/mm³ fell by 72%, whereas those with baseline CD4 counts of less than 500 cells/mm³ fell by 49%. The reduction in CD4 counts paralleled changes in total lymphocyte counts but was not related to changes in the white blood cell counts, neutrophil counts, platelet counts, or hematocrit (Fig. 2). The percentage of subjects who developed CD4 counts of less than 200 cells/mm³ was similar in those receiving radiation and temozolomide treatment alone or with the addition of talampanel, cilengitide, BSI, or poly-ICLC (42% vs. 31%, $P = 0.4$).

Infection

A total of 40 subjects (41%) had a documented infection during the 12-month study period. Infections were seen in 17 individuals (46%) with CD4 counts that fell below 200 cells/mm³ and in 23 (40%) with CD4 counts that remained above 200 cells/mm³ ($P = 0.54$). Seven subjects developed pneumonia and 1 of these was diagnosed as PJP despite

recommended PJP prophylaxis. Only 2 of the 81 deaths (2.5%) that occurred in the study population were due to infection, and these occurred in subjects who survived for 11 and 25 months. The median survival time of individuals with infection was 16.8 months (95% CI: 15.2–20.7 months) and of those without infection was 17.8 months (95% CI: 13.2–23.5 months). Also, time-to-first infection was assessed as a time-dependent covariate in a univariate and a multivariate proportional hazards model. After adjusting for baseline age, surgical procedure, histology, KPS, and CD4 counts (greater than or less than 200 cells/mm³), the adjusted HR for death attributed to infection was 1.16 (95% CI: 0.73–1.83; *P* = 0.5, Table 2).

Hospitalizations

Fifty-three of the 96 subjects (55%) were hospitalized during the year of observation. Hospitalizations were more frequent in subjects with CD4 counts less than 200 cells/mm³ at 2 months than in those with higher CD4 counts (73% vs. 43%, *P* = 0.003). Among those who required hospitalization, the median number of hospitalizations was 2 (range: 1–6) for all CD4 counts, 2 for those with CD4 counts less than 200 at 2 months, and 1 for those with higher CD4 counts (*P* = 0.8). The reasons for hospitalization were highly variable and more than 50 different admission diagnoses were recorded. Examples included additional surgery, thromboembolic disease, change in mental status or seizures, nausea/vomiting, peripheral edema, fatigue, weakness, pain management, glucose control, constipation, drug allergy, gastrointestinal bleeding, hematoma, meningitis, pneumonia, and spontaneous pneumothorax.

Survival

At the time of analysis, 81 of the 96 subjects had died. The overall median survival time was 17.3 months (95% CI: 14.6–19.7 months). The unadjusted and adjusted HRs with respect to the factors associated with overall survival are listed in Table 2. As shown in Table 2, an analysis of the unadjusted HR for death identified previously known prognostic factors for patients with HGGs: histology (GBM vs. other HGGs, *P* = 0.003), extent of surgery (biopsy vs. craniotomy, *P* = 0.003), and age (≥ 55 vs. < 55 , *P* = 0.02). Baseline CD4 counts (< 500 vs. ≥ 500 , *P* = 0.002) and CD4 counts at 2 months (< 200 vs. ≥ 200 , *P* = 0.003) were identified as related to survival. The median survival time in patients with CD4 counts lesser than 200 cells/mm³ at 2 months was 13.1 months (95% CI: 9.1–16.4) compared with 19.7 months (95% CI: 15.9–24.0) in subjects with higher CD4 counts (*P* = 0.002, log-rank test). The relevant Kaplan–Meier survival curves are presented in Figure 3 (HR = 1.99, 95% CI: 1.27–3.1).

A proportional hazards model was constructed to estimate the risk of death attributed to low CD4 count at 2 months while accounting for known baseline clinical prognostic factors (Table 2). Covariates entered into this model included those identified in the univariate analysis (histology, surgical procedure, age and baseline, and 2-month CD4 counts) as well as KPS and infection. The adjusted HRs for death for 3 known prognostic factors were confirmed in this model: histology (GBM vs. other) = 3.39 (*P* = 0.006), surgical procedure (biopsy vs. craniotomy) = 2.3 (*P* = 0.004), and KPS (60–70 vs. 80–100) = 3.18 (*P* = 0.02). The adjusted HR for death attributable to baseline CD4 count (dichotomized at 500) was 1.29

Table 2. Associations between patient characteristics and survival

Characteristic	HR (95% CI)	<i>P</i>
Univariable association		
Histology: GBM vs. other	3.28 (1.50–7.17)	0.003
Biopsy vs. craniotomy	2.22 (1.31–3.78)	0.003
CD4 count at baseline: < 500 vs. ≥ 500 ^a	2.07 (1.32–3.24)	0.002
CD4 count at 2 mo: < 200 vs. 200 ^a	1.99 (1.27–3.11)	0.003
KPS: 60–70 vs. 80–100	1.95 (0.78–4.87)	0.15
Age: ≥ 55 vs. < 55	1.73 (1.08–2.77)	0.02
Anticonvulsant use: yes vs. no	1.47 (0.85–2.54)	0.17
Steroids use: yes vs. no	1.09 (0.60–1.98)	0.78
Infection ^b	1.04 (0.07–1.61)	0.86
Multivariable association		
Histology: GBM vs. other	3.39 (1.43–8.02)	0.006
KPS: 60–70 vs. 80–100	3.18 (1.18–8.56)	0.022
Biopsy vs. craniotomy	2.30 (1.31–4.04)	0.004
CD4 count at 2 mo: < 200 vs. 200 ^a	1.66 (1.05–2.64)	0.03
Age: ≥ 55 vs. < 55	1.37 (0.83–2.28)	0.22
Infection ^b	1.16 (0.73–1.83)	0.53

^aCD4 count at baseline is dichotomized at 500 (normal vs. abnormal). CD4 count at 2 months is dichotomized at 200.

^bTime-dependent covariate (time-to-first infection).

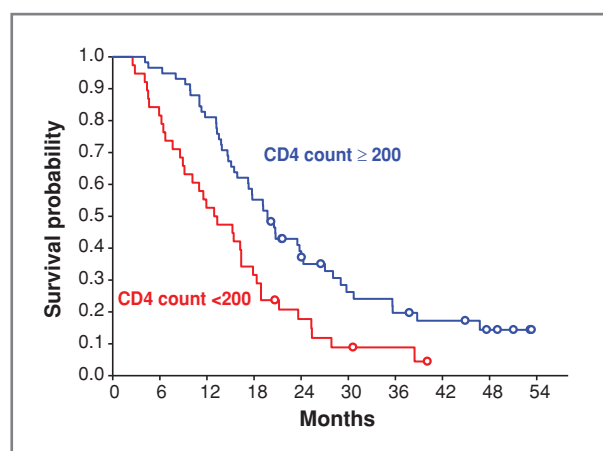


Figure 3. Overall survival by CD4 count at 2 months. Survival in patients with CD4 counts less than 200 cells/mm³ at 2 months was substantially different from those with higher CD4 counts as measured by median survival (13.1 months; 95% CI: 9.1–16.4) vs. 19.7 months; 95% CI: 15.9–24.0), log-rank test ($P = 0.002$), or the adjusted HR for death (1.66; $P = 0.03$). Kaplan–Meier estimates of overall survival according to absolute CD4 counts less than 200 or 200 or above.

($P = 0.38$). The corresponding HR attributable to CD4 count at 2 months (dichotomized at 200) was 1.66 ($P = 0.03$) after 2 months of therapy. Further analysis revealed that survival was related to the CTC toxicity grades for CD4 counts at 2 months. The median survival of patients who developed grade I ($n = 9$), grade II ($n = 49$), and grade III–IV ($n = 38$) reductions in CD4 counts at 2 months were 23.8 months, 19.7 months, and 13.1 months, respectively ($P = 0.009$). A common HR for death with respect to the severity of the toxicity grade (per unit increase) was 1.7 (95% CI: 1.13–2.54, $P = 0.01$) after adjusting for age, surgical procedure, histology, and KPS.

Discussion

This is the first study to follow serial CD4 counts in patients with newly diagnosed HGGs treated with radiation and temozolomide treatment and to correlate the findings with clinical outcomes. Despite weeks of peri- and post-operative glucocorticoids, CD4 counts in these patients were usually within the normal range before radiation and temozolomide treatment were started. This suggests that the disease itself is not associated with marked immunosuppression. These findings are consistent with a previous report of patients with newly diagnosed glioblastoma treated with radiation, glucocorticoids, but no chemotherapy (12). In these patients, CD4 counts began in the normal range, but after 6 weeks of radiation, 47% had CD4 counts less than 300 cells/mm³ and 26% had less than 200 cells/mm³. In the study reported in this article where temozolomide was added to radiation and glucocorticoids, 73% of patients had less than 300 cells/mm³ and 40% had less than 200 CD4 cells/mm³ 2 months after initiating therapy. Patients with the lowest CD4 counts at 2 months

were more likely to have begun radiation and temozolomide treatment with lower total lymphocyte and CD4 counts. In most subjects, this grade III–IV toxicity persisted for the full year of follow-up. Subjects with CD4 counts below 200 cells/mm³ 2 months after beginning radiation and temozolomide treatment had significantly shorter survival than others after adjusting for known prognostic factors. Remarkably, the cause of death in these study subjects was early tumor progression and was unrelated to opportunistic infection.

The significant reduction in CD4 counts observed in this study is consistent with available information on the toxicities of the administered agents. Glucocorticoids are potent lympholytic agents and their use has been associated with PJP and other opportunistic infections (18–21). Lymphocytes are also highly sensitive to the effects of radiation. Although radiation for HGGs is delivered only to the affected region of the brain, modeling studies suggest that there is substantial exposure to circulating lymphocytes, as blood flow to the brain is high and the radiation beam is on for approximately 30 minutes during the 6 weeks of therapy (22). Finally, temozolomide administered to patients with melanoma as a single agent, without glucocorticoids or radiation, produces profound lymphopenia and a striking reduction in CD4 counts that lasts for months after temozolomide is discontinued (15). Thus, it is plausible that the combined use of glucocorticoids, radiation, and temozolomide in patients with HGGs results in significant and long-lasting reductions in CD4 and total lymphocyte counts that can lead to clinically significant immunologic impairment.

Our findings that low CD4 counts at 2 months are independently associated with shorter survival are of special concern. Our initial hypothesis was that the addition of temozolomide would result in more highly immunosuppressed patients who would be at risk to develop severe opportunistic infections that might shorten their lives. However, we found that with appropriate PJP prophylaxis serious infections were not a significant cause of death. Instead, patients died early from progressive growth of their HGGs. One potential explanation for this finding is that highly immunocompromised patients were poor prognosis patients with the largest postoperative tumor burden who required higher doses of glucocorticoids. Although this would be consistent with data in Table 1 showing that patients with lower CD4 counts at 2 months were older (60.9 vs. 56.6 years) and more likely to have been biopsied than resected (26% vs. 16%), these differences are not statistically significant. As noted in Table 1, patients with lower CD4 counts were more likely to be on glucocorticoids when beginning radiation and temozolomide treatment (92% vs. 79%, $P = 0.04$). The extent to which CD4 count depletion is related to glucocorticoid dose is difficult to determine, as steroid doses were changed frequently but recorded only every 2 months. However, the multivariable analysis which adjusts for significant prognostic variables shows that

the CD4 count at 2 months was independently associated with survival (Table 2).

An alternative explanation is that the severe immunosuppression noted in 40% of patients treated with this regimen compromises survival. It has long been thought that the immune system plays a significant role in controlling the growth of cancers. This is the basis for a large body of experimental work on tumor vaccines and other immunologic approaches designed to stimulate the immune system to "fight" the cancer. If stimulating the immune system can improve cancer outcomes, it is possible that immunosuppression could compromise the ability of the body to assist in tumor control. In fact, severe immunosuppression has been related to poor outcomes in patients with cancer. More rapid disease progression and shorter survival have been reported in patients with hematologic and solid tumor malignancies and a diminished immune response reflected by reduced total lymphocyte counts (23–26), lack of intratumoral lymphocytes at diagnosis (27–29), delayed or incomplete lymphocyte recovery following chemotherapy (30, 31), or autologous bone marrow transplantation (32–35). Although the correlation of immune status and clinical outcome is well described, the precise biological mechanisms remain poorly defined.

The observations reported in this article suggest that the immune status may play a significant role in survival in patients with HGGs. Additional studies are required to confirm these findings. Our trial, which opened in 2004 as the first to explore the effects of radiation and temozolomide treatment on CD4 counts and patient outcome, did not include detailed immunologic assessments and measurements of lymphocyte subtypes over time. However, now that the depth and duration of treatment-related

immunosuppression and its potential relationship to survival have been described, future studies should investigate the immunologic consequences of therapy in depth. This is a complex and controversial research topic. Our observations suggest that severe immunosuppression is associated with poor outcomes, whereas other investigators have recently reported that temozolomide treatment-related lymphopenia might enhance the results of immunotherapy in patients with glioblastoma (36).

In addition, further studies should also explore if the association that we describe between treatment-induced immunosuppression and poor survival is also present in other systemic malignancies where patients are intensively treated with radiation and chemotherapy. A better understanding of the general consequences of treatment-related lymphopenia and immunosuppression are critical to improving the design and conduct of translational research and improving the outcomes of patients with malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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