Serum IgE, Tumor Epidermal Growth Factor Receptor Expression, and Inherited Polymorphisms Associated with Glioma Survival

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Abstract

In population-based glioma patients, we examined survival in relation to potentially pertinent constitutive polymorphisms, serologic factors, and tumor genetic and protein alterations in epidermal growth factor receptor (EGFR), MDM2, and TP53. Subjects were newly diagnosed adults residing in the San Francisco Bay Surveillance Epidemiology and End Results Area during 1991 to 1994 and 1997 to 1999 with central neuropathology review (n = 873). Subjects provided blood for serologic studies of IgE and IgG to four herpes viruses and constitutive specimens for genotyping 22 polymorphisms in 13 genes (n = 471). We obtained 595 of 697 astrocytic tumors for marker studies. We determined treatments, vital status, and other factors using registry, interview, medical record, and active follow-up data. Cox regressions for survival were adjusted for age, gender, ethnicity, study series, resection versus biopsy only, radiation, and chemotherapy. Using a stringent P < 0.001, glioma survival was associated with ERCC1 C8092A [hazard ratio (HR), 0.72; 95% confidence limits (95% CL), 0.60-0.86; P = 0.0004] and GSTT1 deletion (HR, 1.64; 95% CL, 1.25-2.16; P = 0.0004); glioblastoma patients with elevated IgE had 9 months longer survival than those with normal or borderline IgE levels (HR, 0.62; 95% CL, 0.47-0.82; P = 0.0007), and EGFR expression in anaplastic astrocytoma was associated with nearly 3-fold poorer survival (HR, 2.97; 95% CL, 1.70-5.19; P = 0.0001). Based on our and others' findings, we recommend further studies to (a) understand relationships of elevated IgE levels and other immunologic factors with improved glioblastoma survival potentially relevant to immunologic therapies and (b) determine which inherited ERCC1 variants or other variants in the 19q13.3 region influence survival. We also suggest that tumor EGFR expression be incorporated into clinical evaluation of anaplastic astrocytoma patients. (Cancer Res 2006; 66(8): 4531-41)

Introduction

About 14,000 people are diagnosed with and >10,000 die from glioma each year in the United States (1). Primary brain and central nervous system (CNS) tumors rank first among cancer types for the average years of life lost with an average of 20.1 years (compared, e.g., with 6.1 years for prostate cancer and 11.8 years for lung cancer; ref. 2). Survival from glioblastoma, the most common form of glioma in adults, is very poor; <3% of those ages ≥65 years and only 30% for those ages <45 years at diagnosis survive 2 years (1). Histologic type and grade, age, extent of resection, tumor location, radiation therapy, some chemotherapy protocols, Karnofsky performance status, and other functionality measures have been consistently and convincingly linked to glioma survival (3, 4). Investigators are currently trying to identify and understand tumor markers or patient characteristics that might influence survival or response to treatment (reviewed in ref. 5). Most studies have relied on patients in clinical trials (who generally have better survival than the overall population of glioma patients) or comparisons of long-term versus short-term survivors from selected clinical series. Patients with long-term survival, although uncommon for glioblastoma, could provide important clues to identify key pathways for developing future therapies.

Constitutive genetic influences on glioma prognosis and survival. It is being increasingly showed that common gene polymorphisms influence response to cancer therapies or otherwise influence prognosis and survival (recently reviewed in refs. 6, 7). Variants in cell cycle, DNA repair, detoxification, or immune response genes might alter function and the response of tumor cells to therapeutic agents or provide variation in host defenses against the tumor. Glioma survival has been associated with polymorphisms in *EGF, GSTP1* and *GSTM1, HLA A*32* and *B*55,* and *GLTSCR1 S397S* and *ERCC2 D711D* (8–11).

Immune and serologic factors. Immunotherapies are being intensely studied in attempts to improve brain tumor survival (12–15). That some brain tumors can depress the immune system has been known for some time (16, 17). The humoral and innate arms of the immune system have not been well studied in brain tumor survival. Our previous studies showed significant inverse associations of glioma case-control status with serum IgE levels as well as IgG levels to the varicella-zoster virus (VZV; refs. 18, 19), suggesting a potential protective effect on glioma development. If a causal relationship exists between IgE or IgG on glioma formation or growth, then elevated serum levels may also be associated with longer survival times. Therefore, we are now examining whether these serologic factors relate to glioma survival.

Studies of tumor markers in relation to survival. Although combined loss of 1p and 19q in oligodendroglial tumors are well-established favorable prognostic indicators (recently reviewed in ref. 20), there are no equally well validated prognostic indicators for astrocytic tumors. Amplification/overexpression of epidermal growth factor receptor (EGFR) is more common in older versus younger anaplastic astrocytoma patients (21). EGFR amplification/overexpression may also contribute to resistance to therapeutic

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modalities (22). In glioblastoma, EGFR overexpression may be associated with poor survival in younger adults (23–25). Recent studies examining expression profiles alone (26, 27) or in conjunction with comparative genomic hybridization (28) have identified candidate markers that represent promising leads for possible validation in larger studies.

In this article, we examine survival among population-based glioma cases with uniform neuropathology review in relation to polymorphisms in a variety of metabolic, DNA repair, and immune function genes; to total IgE and positivity of IgG to four herpes viruses; and to tumor genetic and expression alterations in TP53, EGFR, and MDM2.

Materials and Methods

The University of California San Francisco (UCSF) Committee for Human Research and individual hospital institutional review boards approved pertinent methods for this study.

Subjects. Glioma eligibility criteria, ascertainment, interview, medical record abstraction, and mortality follow-up methods for the glioma cases have been previously described (29). Briefly, any adult (age >20 years) newly diagnosed with glioma [International Classification of Disease for Oncology, morphology codes 9380-9481 between August 1991 and April 1994 (series 1) and May 1997 and August 1999 (series 2)] who resided in six San Francisco Bay Area counties at the time of diagnosis was eligible to participate. Potentially eligible cases were identified using a rapid case ascertainment program available through a Surveillance Epidemiology and End Results (SEER) participating registry, the Northern California Cancer Center (NCCC). Median time from diagnosis to recruitment was 98 days. The study group included consenting patients or their proxies who were interviewed about a variety of factors, who gave written consent to obtain and review pathology specimens and records, and whose review confirmed an eligible diagnosis. Blood and/or buccal specimens also were obtained from willing cases.

Neuropathology review. Pathology records and specimens were obtained from diagnosing hospitals, and a neuropathologist reviewed all tumors; Richard Davis reviewed cases diagnosed between 1991 and 1994, and Kenneth Aldape reviewed cases diagnosed between 1997 and 1999. Tumors were classified according to the WHO criteria described by Kleihues et al. (30), using a coding form developed for this study. Glioblastoma, anaplastic astrocytoma, and astrocytoma correspond to WHO grades 4, 3, and 2, respectively.

Determination of vital status. We determined vital status through linkage with NCCC-SEER in July 2004. We followed those not identified as deceased with a letter and follow-up phone calls. For those contacted and determined to be alive (n = 152), date of returned postcard or the last phone contact was their last known date alive. For six cases that we could not locate, we used the date of last contact as determined by NCCC-SEER. To summarize, patient survival time was either date of death or date of last contact from date of histologic diagnosis. For patients not known to be dead, the patient was censored as of the date of last contact.

Determination of treatment information. We previously described in detail methods for classifying treatments (29). To summarize, we used three sources of data, NCCC-SEER, medical record abstraction, and clinical trials database from UCSF, to code treatments, as follows: surgery (resection versus biopsy only), radiation treatment (given versus not given), and chemotherapy (given or not given).

Genotyping constitutive polymorphisms. We include polymorphisms that were measured on most of the subjects for whom blood or buccal specimens had been obtained. Twenty-two polymorphisms in 12 genes were considered. References are given for genotyping methods for polymorphisms we have published, and Table 1 shows the primers and conditions for polymorphisms that we have not previously published. Note that we use the National Center for Biotechnology Information standard abbreviations for gene names[5] and the IUPAC-IUB standard initials for amino acids (31). The genes and polymorphisms in alphabetical order are *CCR5* delta 32 deletion (32); *ERCC1* C8092A; *ERCC2* K751Q and R156R (33); *GSTM1* deletion; *GSTP1* I105V and A114V; *GSTT1* deletion (34); *IL4R* I75V, E400A, C431R, S503P, Q576R, S752A; *IL4* C34T; *MDR1* C3435T (35); *MEH* H113Y and R139H; *MGMT* L84F (21) and I143V; and *XRCC1* H280R and R399G. To summarize methods for constitutive DNA isolation and genotyping, DNA was isolated from heparinized whole blood using Qiagen column purification. For subjects who provided buccal specimens, buccal swabs were inserted into a 1.5-mL tube with 300 to 600 μL of 50 mmol/L NaOH and vortexed. The brush was then removed from the tube, making sure all liquid was reserved. The tube was boiled in a water bath at 95°C for 5 minutes. The tube was centrifuged at 14,000 rpm for 1 minute, then the liquid was transferred into a freezing vial, and the amount of liquid was measured. The sample was neutralized by adding a 1:10 volume (10% final concentration) of 1 mol/L Tris-EDTA (pH 8). DNA concentration was measured using Hoescht-33258 fluorimetry. Up to 10 μL was used in a 50-μL PCR reaction. DNA was stored at −80°C. Restriction enzymes for genotyping were purchased from NEN Life Sciences (Boston, MA); PCR was carried out on an ABI 9600 thermocycler. Each reaction included the following: 1 μL of forward primer, 1 μL of reverse primer, 5 μL of deoxynucleotide triphosphates, 5 μL of 10× buffer solution (50 mmol/L KCl at pH 8.3); 37 μL of distilled water, 0.25 μL Taq, and 1 μL of DNA to give a final reaction volume of ~50 μL. All PCR reactions were at 94°C for 30 seconds, at the various annealing temperatures specific for each gene sequence (Table 1) for another 30 seconds, then at 72°C for a final 30 seconds; run on 3% to 4% agarose gel; and digested overnight or for 4 hours at 37°C. Quality control measures include blinded analyses, replicates of 10% of samples, and positive controls (blood-derived DNA from all known genotypes), and negative controls for contamination (no DNA) were run routinely with patient samples.

Serologic markers. We assessed IgG seropositivity for herpes simplex virus (HSV), Epstein Barr Virus (EBV), Varicella Zoster Virus (VZV), cytomegalovirus (CMV), and total IgE (normal, borderline, and elevated), as previously described (18, 19). IgE assays were available only for series 2 subjects.

Tumor markers. We examine mortality in relation to six tumor markers assessed in glioblastoma and anaplastic astrocytoma. We did not have funding for assessment of markers in nonastrocytic gliomas, and there were too few astrocytoma grade 2 for separate consideration in this article. The markers studied are *TP53* mutation (present in exons 5-8 versus absent), *EGFR* and *MDM2* gene copy numbers (≤3 considered not amplified versus >3 considered amplified), and expression of *TP53, EGFR,* and *MDM2* proteins as determined by immunohistochemistry (for *TP53* and *MDM2,* we assessed staining as 0, none; 1, <5%; 2, 5-30%; or 3, >30% nuclei staining; for EGFR, we assessed membrane/cytoplasmic staining as 0, no staining; 1, weak/focal; and 2, strong diffuse). We recently published details of the laboratory methods for assessing these markers (21).

Statistical methods. We estimated median months survival from time of histologic diagnosis and 95% confidence limits (95% CL) overall and by genotypes, serologic results, and tumor marker status with life table (Kaplan-Meier) methods using the SAS PROC LIFETEST (36). We estimated hazard ratios (HR) for the days of survival after histologic diagnosis with the various individual markers using Cox proportional hazards regression models estimated with SAS PROC PHREG (37), initially adjusting for age, gender, ethnicity (White, non-White), and series (1 or 2). A gene-dose model was used for polymorphisms (*GSTM1* or *GSTT1*: 0 = not null, 1 = null; for single nucleotide polymorphisms, 0, 1, and 2 represent the number of variants), and IgE levels were coded 0 = normal, 1 = borderline, 2 = elevated. Because there were six polymorphisms measured in interleukin-4 receptor (*IL4R*), we also computed a variable that gave the total number of variants (0-10 observed) in *IL4R* for each subject. The most likely haplotypes carried by each person were estimated using a Bayesian method implemented in PHASE 2.0.[6] *IL4R* haplotypes were coded 0 = more common variant in the

[5] National Center for Biotechnology Information Entrez Gene (http://www.ncbi.nlm.nih.gov), 2005.

[6] Stephens, M. Software for haplotype estimation (http://www.stat.washington.edu/stephens/software.html), 2003.

Table 1. NCBI gene symbols, rs numbers, primers, and conditions for selected polymorphisms genotyped for participants in the San Francisco Bay Area Adult Glioma Study, 1991-2000

Symbol	rs no.	Enzyme	Primer Sequence		Annealing temperature (°C)	Cycles
			Forward	Reverse		
*IL4 C34T**	rs2070874	*Bsm*A1	GATTTGCAGTGACAATGTGAG	TCCTATGCTGAAACTTTGTAG	57	30
IL4R I75V (I50V)-N	rs1805010	*Bsm*FI	GCCTACAGGTGACCAGCCTA	AGCCCACAGGTCCAGTGTAT	60	40
IL4R E400A	rs1805011	*Aci*I	TGCGATGTGTGGAGTTGTTT	AAAGCCCCCATTCTCCTCT	60	40
IL4R C431R	rs1805012	*Tsp*45I	CAGAGAGCCTGTTCCTGGAC	TGCAAGTCAGGTTGTCTGGA	60	40
IL4R S503P	rs1805015	*Hin*fI	CTTACCGCAGCTTCAGCGAC	CACAGTGGTTGGCTCAGAGA	60	40
IL4R Q576R	rs1801275	*Pvu*I or *Bsi*I	TCGGCCCCACCAGTGGCGATC	CCAGTCCAAGGTGAACAAGGGG	67	40
IL4R S752A	rs1805016	AVA II	GGCAGTGGCATTGTCTACTC	GGATGGAAGGATGATGAGGA	63	40
MEH H113Y	rs1051740	ASP 1	GCTGCTTCCACTATGGCTTC	TCAATCTTAGTCTTGAAGTGACGGT	60	34
MEH R139H	rs2234922	RSA	GGGGTACCAGAGCCTGACCGT	AACACCGGGCCCACCCTTGGC	68	38
MGMT I143V †	rs2308321		TGCCCCCCTGTCTTCCA	GCTGCTGCAGACCACTCT	—	—
XRCC1 H280R	rs25489	RSA I	CCCCAGTGGTGCTAACCTAA	ACACCCTGAAGGATCTTCCC	60	40
XRCC1 R399G	rs25487	*Msp*I	CCAAGTACAGCCAGGTCCTA	AGTCTGACTCCCCTCCGGAT	58	32

NOTE: In addition, we previously published genotyping methods for *CCR5* delta 32 deletion (32), *ERCC1 C8092A, ERCC2 K751Q* and *R156R* (33), *GSTM1* and *GSTT1* deletions, *GSTP1 I105V* and *A114V* (34), *MDR1 C3435T* (35), and *MGMT L84F* (21).

**IL-4 C34T* PCR product diluted 1:50 and 1 μL used for nested PCR with primers 5′-AAGTGACTGACAATCTGGTGA-3′ and 5′-GATTTGCAGTGA-CAATGTGAG-3′ (*Bsm*A1 digestion at 55°C).

†*MGMT* L143V genotype was determined using an allelic discrimination 5′-nuclease assay (Taqman) on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA), in 96-well format. Taqman primers and probes were designed using the Primer Oligo Design Software v2.0 (Applied Biosystems). Probes used for detection of *MGMT-L143V* SNP were vic-CCCATCCTCATCCCGT-NFQ and fam-CCCATCCTCGTCCCGT-NFQ.

single nucleotide polymorphism (SNP) and 1 = less common variant in the SNP with SNPs ordered according to numerical position (i.e., I75V, E400A, C431R, S503P, Q576R, and A752S); for example, haplotype 111110 would be 75V, 400A, 431R, 503P, 576R, and A752. Six of 25 estimated haplotypes were sufficiently common for additional analyses (011110, 000010, 000000, 111110, 100111, and 100000). Cox regressions included left truncation (the days between blood draw or buccal collection and the date of diagnosis) for individual polymorphisms, haplotypes, and serologic factors because the constitutive specimen collection took place after diagnosis. Because constitutive polymorphisms and serologic factors possibly could influence survival regardless of histologic type, models were run for all glioma, and separately for glioblastoma, and anaplastic astrocytoma; the all-glioma models were stratified by four histologic groups: glioblastoma, anaplastic astrocytoma, astrocytoma, and other. We selected polymorphisms or serologic factors for more in-depth analyses that yielded $P < 0.10$ for the above models. More in-depth analyses included adjustment for surgery (resection or biopsy only), radiation, and chemotherapy (given, not given) in addition to age, gender, ethnicity, and series (see ref. 29 for more details on these factors). Each of six IL4R haplotypes was included in a separate survival model (adjusting for age, gender, series, surgery, chemotherapy, and radiation therapy); each haplotype was coded as present if the subject carried the haplotype (either homozygous or heterozygous) and absent if the subject did not carry the haplotype. Some multivariate models included several polymorphisms in addition to the above adjustment factors. Because tumor markers vary by histologic type and were measured only in astrocytic tumors, separate models for glioblastoma and anaplastic astrocytoma were run.

Tabled results provide nominal *P*s without correction for multiple comparisons. However, to minimize conclusions based on false positives due to the large number of comparisons, we emphasize findings with $P < 0.001$ [this is roughly equivalent to the Bonferroni correction for $P = 0.05 / 33$ (22 polymorphisms + 5 serologic variables + 6 tumor markers)].

To compare our results to the only other study (11) that reported several of the same polymorphisms studied here, we compared survival distributions with the log-rank test among a subgroup of anaplastic oligodendroglioma, anaplastic astrocytoma, or anaplastic oligoastrocytoma patients by their combined genotypes of *GSTM1* deletion, *GSTP I105V* and *A114V IIAA* versus those who did not fall in this group.

Results

The study group consisted of 873 glioma subjects: 519 with glioblastoma, 105 with anaplastic astrocytoma, and the remaining with other histologic types (Table 2). The relatively low overall genotyping rate of 54% (471 of 873; Table 2) is due to 241 subjects from the first series (1991-1994) having died before obtaining funding for blood collection; we obtained blood from 186 of 231 (81%) of the remaining subjects in that period. We obtained blood or buccal specimens from 71% (283 of 401) of participants recruited from 1997 to 1999. We obtained tumors from 89% (595 of 667) of patients with astrocytic tumors (glioblastoma, anaplastic astrocytoma, or astrocytoma grade 2). Although survival is substantially longer for subjects with genotyping data, subjects with tumor marker data had very similar survival to all subjects with comparable tumor types (Table 2).

Constitutive polymorphisms. The following polymorphisms were not associated with glioma, glioblastoma, or anaplastic astrocytoma survival with $P > 0.10$: *ERCC2 K751Q* and *R156R, GSTM1* deletion, *GSTP1 I105V* and *A114V, IL4 C34T, MEH H113Y, MGMT I143V,* and *XRCC1 H280R* (data not shown). For all glioma, using the stringent criteria of $P < 0.001$, *ERCC1 C8092A* was associated with improved survival, and *GSTT1* deletion was associated with poorer survival (Table 3). *CCR5* delta 32 deletion, *IL4R_C431R, IL4R_E400A* (Table 3), and the number of *IL4R* variants (HR, 0.94; 95% CL, 0.90-0.99; $P = 0.03$) were associated with better survival using a nominal $P < 0.05$. Multivariate analyses, including various combinations of polymorphisms with $P < 0.05$, and the other usual adjustment factors showed that *ERCC1 C8092A*

Table 2. Characteristics of glioma subjects, San Francisco Bay Area Adult Glioma Study, 1991-2000

	All glioma*	Glioblastoma	Anaplastic astrocytoma
All case subjects			
No.	873	519	105
Mean age (SE)	55.4 (0.6)	61.6 (0.6)	51.1 (1.5)
% Male	56.4	56.3	52.4
% White	82.8	84.0	76.2
Median mos survival (95% CL)	11.0 (10.0-12.5)	7.1 (6.5-7.8)	13.0 (9.9-19.5)
Subjects with any genotyping or serologic data			
No.	471	230	57
Mean age (SE)	50.8 (0.7)	57.6 (0.8)	47.4 (1.8)
% Male	59.2	63.0	50.9
% White	83.0	83.5	80.7
Median mos survival (95% CL)	19.7 (17.7-22.7)	12.4 (10.8-13.7)	20.7 (18.5-41.6)
All cases with astrocytic tumors			
No.	667		
Mean age (SE)	58.8 (0.6)		
% Male	55.6		
% White	82.3		
Median mos survival (95% CL)	8.0 (7.3-9.0)		
Astrocytic subjects with any tumor marker data			
No.	595	452	88
Mean age (SE)	58.7 (0.6)	61.8 (0.6)	51.4 (1.7)
% Male	58.0	57.7	58.0
% White	84.2	85.4	78.4
Median mos survival (95% CL)	8.6 (7.7-9.6)	7.2 (6.7-8.0)	14.6 (10.6-20.6)

NOTE: Median months survival and 95% CLs estimated with Kaplan-Meier methods.
Abbreviations: SE, Standard Error; 95% CL, 95% Confidence Limit.
*Numbers of subjects with other histologies were astrocytoma grade 2 (n = 43), anaplastic oligodendroglioma (n = 26), anaplastic oligoastrocytoma (n = 16), oligodendroglioma (n = 67), oligoastrocytoma (n = 40), ependymoma (n = 8), juvenile pilocytic astrocytoma (n = 15), medulloblastoma (n = 10), and others (n = 24).

(HR, 0.69; 95% CL, 0.58-0.84; P = 0.0001) and *GSTT1* deletion (HR, 1.66; 95% CL, 1.26-2.20; P = 0.0003) remained associated with glioma survival using the stringent criteria, whereas *XRCC1 H280R* gave an HR of 1.61 (95% CL, 1.11-2.32; P = 0.01), and the number of variants in *IL4R* yielded an HR of 0.95 (95% CL, 0.89-1.00; P = 0.07). Haplotype analysis of the six *IL4R* polymorphisms indicated persons with the haplotype with the rarer variant in all positions except A752S (i.e., haplotype 111110) had better survival than those who did not have this haplotype but not at the stringent significance level (HR, 0.64; 95% CL, 0.47-0.87; P = 0.004).

Although no polymorphisms were associated with glioblastoma survival using the stringent criteria of P < 0.001, *CCR5* delta 32, *ERCC1 C8092A,* and *GSTT1* deletion were associated with P < 0.05 (Table 3), as was the number of variants in *IL4R* (HR, 0.93; 95% CL, 0.88-0.99; P = 0.04) and the above mentioned haplotype (HR, 0.66; 95% CL, 0.46-0.95; P = 0.03). In a multivariate model using the same polymorphisms used in the model for all glioma above and the other usual adjustment factors, we obtained the following results: *ERCC1 C8092A* (HR, 0.72; 95% CL, 0.58-0.89; P = 0.003), *XRCC1 H280R* (HR, 1.69; 95% CL, 1.03-2.79; P = 0.04), *GSTT1* deletion (HR, 1.59; 95% CL, 1.11-2.28; P = 0.01), and for the number of variants in *IL4R* (HR, 0.95; 95% CL, 0.88-1.01; P = 0.11). For anaplastic astrocytoma, no polymorphisms met the stringent criteria, but *MEH R139H, MGMT L84F,* and *IL4R S503P* and *Q576R* were associated with survival with nominal P < 0.05 (Table 3), as was the number of *IL4R* variants (HR, 0.85; 95% CL, 0.72-0.99; P = 0.04).

For comparison with results by Okcu et al. (11), 81 patients diagnosed with either anaplastic oligodendroglioma (N = 19), anaplastic astrocytoma (N = 53), or anaplastic oligoastrocytoma (N = 9) had genotyping data for *GSTM1, GSTP I105V,* and *A114V*. Median survival for those with *GSTM1* deletion and *GSTP 105/114 IIAA* was 20.0 months (95% CL, 15.5-48.5; n = 18) versus 36.6 months (95% CL, 10.6-95.6; n = 63) for those with other combinations (log-rank comparison, P = 0.14).

Serologic factors. Positivity of IgGs to VZV and EBV was not associated with glioma survival (P > 0.10; data not shown). Elevated IgE levels were associated with improved glioblastoma survival (P = 0.0007) using stringent criteria (Fig. 1), and positivity for IgG to HSV was associated with poorer anaplastic astrocytoma survival (P = 0.03). Also note that overall, IgE levels were positively correlated with the numbers of IL4R variants (correlation = 0.13; P = 0.05). The mean numbers of days between glioblastoma diagnosis and blood collection did not materially differ between people with normal, borderline, and elevated IgE levels (mean ± SE: 119 ± 9, 129 ± 16, and 129 ± 13; Table 4).

The three markers most highly associated with survival from analyses of individual markers (*ERCC1 C8092A* genotype, *GSTT1* deletion status, and IgE) were not correlated, and we did not find noteworthy differences in HRs from models that included these markers individually or in combinations of two to three of these markers (data not shown).

Table 3. Cox regressions of survival by selected genotypes using gene dose, 0-2 variants, San Francisco Bay Area Adult Glioma Study, 1991-2000

Genes	All glioma				Glioblastoma				Anaplastic astrocytoma			
	*n**	*n* died†	Median mos survival‡	*P*, HR (95% CI)§	*n**	*n* died†	Median mos survival‡	*P*, HR (95% CI)§	*n**	*n* died†	Median mos survival‡	*P*, HR (95% CI)§
CCR5 delta32	457	330			222	220			56	40		
Homozygous not delta32	392	288	19.5		193	193	12.2		51	36	20.6	
Heterozygous	62	40	30.6	0.04	29	27	14.9	0.02	5	4	30.7	0.56
Homozygous delta32	3	2	150.9	0.72 (0.52-0.98)	—	—	—	0.62 (0.41-0.93)	—	—	—	0.66 (0.16-2.68)
ERCC1A C8092A	450	325			220	218			56	40		
CC	238	175	18.1		115	114	11.1		27	23	20.1	
AC	176	123	20.1	0.0004	84	83	13.5	0.004	27	16	38.1	0.14
AA	36	27	23.4	0.72 (0.60-.86)	21	21	17.4	0.73 (0.59-0.91)	2	1	—	0.57 (0.27-1.21)
GSTT1	446	322			215	213			55	40		
Not deleted	356	250	20.5	0.0004	175	173	13.2	0.02	46	32	22.9	0.23
Deleted	90	72	17.2	1.64 (1.25-2.16)	40	40	10.2	1.54 (1.08-2.20)	9	8	14.7	1.74 (0.71-4.31)
IL4R_I75V	466	337			229	226			57	41		
II	140	102	20.4		66	66	10.7		16	13	25.3	
IV	235	174	18.1	0.09	127	125	12.7	0.10	23	15	20.7	0.55
VV	91	61	24.6	0.87 (0.74-1.02)	36	35	15.8	0.84 (0.68-1.03)	18	13	30.5	0.87 (0.55-1.37)
IL4R_E400A	466	337			229	226			57	41		
EE	347	251	18.5		166	165	12.3		45	32	20.0	
EA	104	76	21.7	0.03	54	53	12.8	0.13	10	8	46.8	0.11
AA	15	10	30.1	0.78 (0.63-0.97)	9	8	10.6	0.82 (0.63-1.06)	2	1	—	0.55 (0.27-1.15)
IL4R_C431R	465	337			229	226			57	41		
CC	366	266	18.2		178	177	11.7		46	33	20.0	
CR	92	67	24.5	0.04	48	46	13.2	0.12	10	8	46.8	0.21
RR	7	4	43.7	0.76 (0.59-0.98)	3	3	19.0	0.78 (0.57-1.07)	1	0	—	0.60 (0.27-1.34)
IL4R_S503P	464	336			228	225			57	41		
SS	319	229	18.5		152	151	12.4		38	28	19.7	
SP	131	99	19.9	0.10	69	68	12.4	0.14	17	13	38.1	0.03
PP	14	8	85.2	0.84 (0.68-1.04)	7	6	19.0	0.82 (0.64-1.07)	2	0	—	0.46 (0.24-0.91)
IL4R_Q576R	462	335			227	224			57	41		
QQ	277	198	18.5		131	130	11.3		34	26	19.7	
QR	157	116	19.9	0.08	80	79	13.2	0.06	19	13	38.1	0.03
RR	28	21	25.5	0.85 (0.70-1.02)	16	15	17.9	0.80 (0.63-1.01)	4	2	—	0.54 (0.31-0.93)
IL4R_A752S	459	334			226	223			56	41		
SS	410	294	19.9		197	194	12.5		47	36	20.1	
SA	47	39	17.4	0.93	28	28	12.8	0.79	9	5	36.2	0.10
AA	2	1	—	1.02 (0.73-1.41)	1	1	7.3	0.95 (0.63-1.43)	—	—	—	0.43 (0.16-1.16)
MEHR139H	388	276			185	183			47	33		
HH	273	198	19.9		128	127	12.9		33	27	19.1	
HR	104	70	27.0	0.12	54	53	14.1	0.77	12	5	—	0.002
RR	11	8	30.7	0.82 (0.64-1.05)	3	3	9.7	0.95 (0.70-1.30)	2	1	—	0.14 (0.04-0.49)
MGMT L84F	453	329			223	220			56	41		
LL	344	249	19.8		177	175	12.4		39	27	30.7	
LF	101	76	19.2	0.79	46	45	12.6	0.59	13	11	19.1	0.03
FF	8	4	—	1.03 (0.82-1.31)	—	—	—	0.91 (0.65-1.27)	4	3	17.9	1.77 (1.05-2.96)
XRCC1 H280R	463	335			227	224			57	41		
HH	411	298	20.0		203	200	12.7		49	37	20.7	
HR	50	37	15.2	0.11	24	24	11.0	0.10	8	4	—	0.82
RR	2	0	—	1.32 (0.94-1.87)	—	—	—	1.47 (0.93-2.33)	—	—	—	0.88 (0.29-2.67)

Abbreviations: 95% CI, 95% confidence interval; HR, Hazard Ratio.

*Number of cases.

†Number of deceased.

‡Kaplan-Meier estimates of median months survival.

§HR adjusted for age, gender, ethnicity (White/non-White), series, radiation (given/not given), surgery (resection/biopsy only), chemotherapy (given/not given); the models for all glioma include stratification by four diagnosis groups (glioblastoma, anaplastic astrocytoma, astrocytoma, and other); models also account for time between histologic diagnosis and constitutive specimen collection (left truncation).

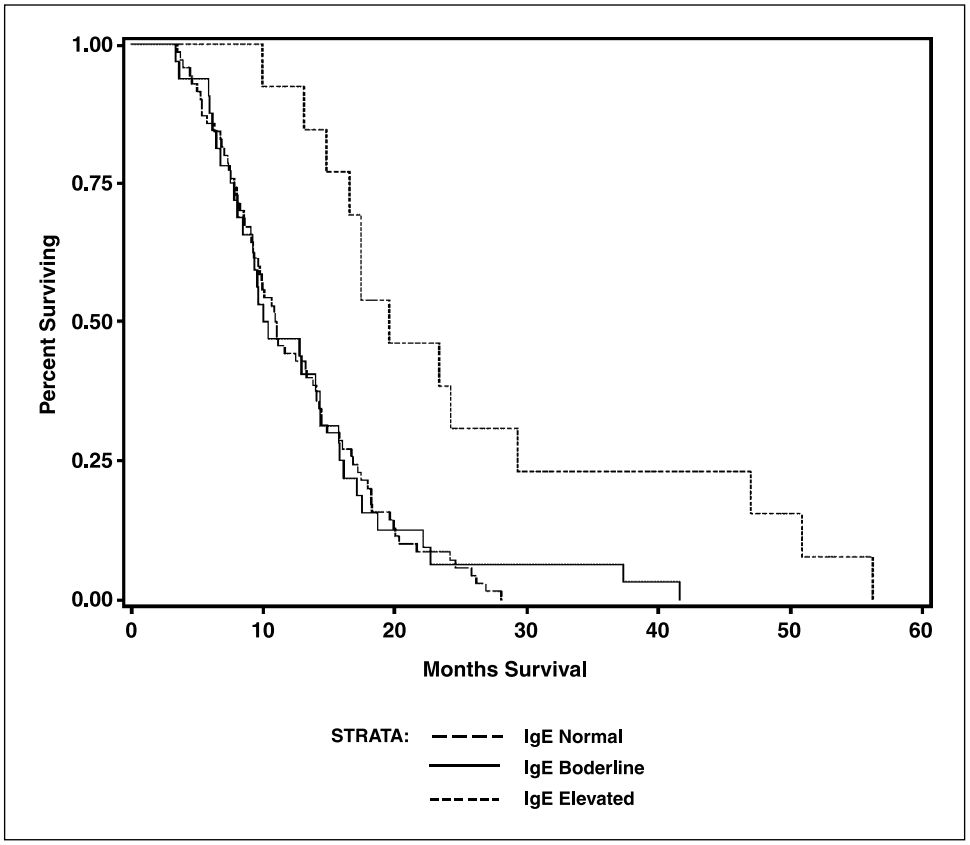

Figure 1. Percent survival distributions for people with glioblastoma who had normal (---, n = 70), borderline (—, n = 32), and elevated (---, n = 13) serum IgE levels.

Tumor markers. EGFR expression (P = 0.008) and MDM2 amplification (P = 0.04) were associated with better glioblastoma survival, whereas TP53 expression was associated with poorer survival (P = 0.05), although these associations did not meet the stringent statistical significance criteria. In a multivariate model including these three tumor markers and other adjustment factors for 403 glioblastoma patients, we found HR of 0.75 (95% CL, 0.61-0.92; P = 0.005) for EGFR expression, HR of 1.1 (95% CL, 0.98-1.19; P = 0.10) for MDM2 expression (coded 0-3), and HR of 1.11 (95% CL, 1.00-1.22; P = 0.03) for TP53 expression (coded 0-3; Table 5).

For anaplastic astrocytoma, tumor EGFR expression was associated with three times worse survival (Table 5; Fig. 2; P = 0.0001). EGFR amplification also was associated with about 2-fold worse survival (P = 0.04) as was MDM2 expression (P = 0.003; Table 5), but neither met the stringent significance criteria.

Discussion

Each of the three classes of biomarkers evaluated here proved to be associated with variations in patient survival. To limit the number of possible false-positive associations, we used $P < 0.001$ for statistical significance criterion to account for the numbers of markers and comparisons tested.

Serologic IgE measurements. We observed the typical mortality profile for the deadly glioblastoma histology with nearly 65% of our study subjects deceased within 12 months of diagnosis. However, none of the 13 patients with elevated IgE succumbed during this same time. The very longest survivors (e.g., >40 months) were persons with high IgE, and they lived on average 9 months longer than those with normal or borderline levels. This suggests that those with higher IgE levels might have either better antitumor defenses or less aggressive tumors with weaker anti-immunologic effects. Given that half as many glioblastoma patients have elevated IgEs as normal population controls (11% versus 23%, respectively; ref. 18), it may be that high IgE levels are correlated with an effective antitumor response. The exact nature and specificity of this response warrants further investigation. In particular, the relationship to atopic allergy should be studied, as it may be relevant to glioma immunotherapy. In addition, correlating IgE levels with degree of tumor burden or extent of disease may yield insights into the relationship between secreted tumor specific factors and host immune responses.

This highly significant association of increased serum IgE with survival is the most novel of the present findings and raises the question whether IgE itself may have antitumor activity via its direct activity on glioma cells or other resident cells within the CNS in proximity to the tumor. Supporting the plausibility of this hypothesis is the fact that the inducible low-affinity IgE receptor CD23 can be expressed by astrocytes *in vitro* (38) and *in vivo* (39). In astrocytes, cell surface stimulation of CD23 expression induced production of nitric oxide and IL1β (39), the latter up-regulates Fas and Fas ligand expression (40) and induces apoptosis (41). In addition, the *CD23* gene is localized to chromosome 19p13.3 (42), a region with copy number gains and losses in gliomas (43–45). Although CD23 expression in glioma cells and tumors has not been examined, these genetic observations would predict that common cytogenetic subgroups of glioma could express varying amounts of IgE receptor and possibly different capacities for IgE-mediated signaling in patients with very high IgE levels. Modulating CD23 signaling was previously proposed to have therapeutic applications

(46), and further efforts should be made to assess the actions of IgE within both malignant and normal astrocytes.

Because a primary limitation of this study is that constitutive DNA samples were unavailable for those people with the poorest survival owing to population registry ascertainment, results may not be generalizable to all glioblastoma patients. However, the delay from diagnosis to blood draw did not materially differ for glioblastoma patients in the three IgE groups, and the median survival of 12 months for all glioblastoma patients with blood specimens is typical of that seen in clinical trials.

Constitutive polymorphisms. Improved survival among *ERCC1* C8092A variant carriers is of interest for several reasons. First, the very low *P* suggests that the association is unlikely to be due to chance. Second, the ERCC1 protein is involved in annealing DNA single-strand breaks and resolving DNA interstrand cross-links (47) and may affect sensitivity to cancer therapies (48, 49). In our study group, 82% of glioblastoma and 89% of anaplastic astrocytoma patients had radiation therapy, and 21% of glioblastoma and 34% of anaplastic astrocytoma patients had chemotherapy (29). Both increased expression of ERCC1 (50, 51) and heritable variants of the *ERCC1 C8092A* polymorphism (52) may be inversely related to non–small cell lung cancer survival time. *ERCC1 8092A* is also associated with greater gastrointestinal toxicity from platinum-based therapy (53). *ERCC1 N118N* was found to be significantly associated with clinical response to 5-fluorouracil plus oxalplatin in metastatic colorectal cancer (54). *ERCC1* also is involved in chromosomal repair in cells damaged by ionizing radiation (55, 56). Interestingly, radiation exposure and EGFR signaling both induce *ERCC1* expression (57), suggesting a need to explore links between these markers. Third, although *C8092A* is in the untranslated region of *ERCC1*, it is a nonsynonymous polymorphism in *ASE-1* (a gene that is the antisense of *ERCC1*; ref. 58). This overlap is conserved in the mouse and even in the yeast ERCC1 homologue RAD10, suggesting an important biological function. Yamamoto et al. (59) indicate that the mouse PAF49 may be the homologue of human ASE-1, and that the protein plays an important role in rRNA transcription. Fourth, *ASE-1* and *ERCC1* are located close to putative glioma tumor suppressor genes *GLTSCR1* and *GLTSCR2* in 19q13.3 (24). Yang et al. (8) showed better survival for oligodendroglioma patients with the *GLTSCR1* TT versus CT or CC genotypes ($P = 0.02$); they also reported that the ASE-1 polymorphism was not significantly associated with oligodendroglioma survival but did not provide the HR. Because our results may be due to linkage of the *ERCC1 C8092A* polymorphism with other genes or variants in this region, further work is warranted to identify which polymorphisms in the 19q13.3 region may be causally related to glioma prognosis.

The results for IL4R polymorphisms are noteworthy, although they did not meet stringent statistical significance criteria of $P < 0.001$. IL4Rα chain influences IgE production through interactions with IL4 and IL13, promotes differentiation of Th2 cells, and can inhibit IL4-mediated cell proliferation and IL5 up-regulation by T cells.[5] Two variants (S503P and Q576R) associated with increased asthma risk have been shown to be associated with decreased glioblastoma risk (60). In our study, several *IL4R* variants singly or combined (as variant counts or as a haplotype) were associated with increased survival; the two *IL4R* variants *S503P* and *Q576R*, when combined on the same allele are part of the haplotype associated with improved survival in the present study. This haplotype was the only common *IL4R* haplotype also to be associated with type 1 diabetes (61), high IL4R activity (62), and lower risk to asthma/allergy (63).

Only one other glioma survival study genotyped patients for some of the same polymorphisms reported here. Okcu et al. (11) examined GST polymorphisms in relation to glioma survival among 278 White adults ages 21 to 64 years with overall median survival of

Table 4. Cox regressions of survival by selected serologic factors, San Francisco Bay Area Adult Glioma Study, 1991-2000

	All glioma				Glioblastoma				Anaplastic astrocytoma			
	*n**	*n* died †	Median mos survival ‡	*P*, HR (95%CI)§	*n**	*n* died †	Median mos survival ‡	*P*, HR (95% CI)§	*n**	*n* died †	Median mos survival ‡	*P*, HR (95%CI)§
IgE (series 2 only)	228	160			115	115			25	17		
Normal	131	90	16.8		70	70	10.9		16	10	21.5	
Borderline	71	50	20.6	0.01	32	32	10.2	0.0007	7	5	36.6	0.70
Elevated	26	20	24.9	0.75 (0.60-0.94)	13	13	19.5	0.62 (0.47-0.82)	2	2	22.3	0.81 (.29-2.31)
IgG for CMV	361	255			170	169			39	29		
Negative	153	99	28.0	0.39	63	63	14.4	0.09	18	13	20.7	0.36
Positive	208	156	18.4	1.13 (0.86-1.48)	107	106	10.6	1.34 (0.96-1.88)	21	16	41.6	0.63 (0.24-1.70)
IgG for HSV	355	250			167	166			38	28		
Negative	98	58	58.3	0.03	33	33	15.0	0.06	12	8	95.6	0.03
Positive	257	192	18.2	1.42 (1.03-1.95)	134	133	12.1	1.49 (0.99-2.26)	26	20	19.8	4.31 (1.16-16.10)

Abbreviation: 95% CI, 95% confidence interval; HR, Hazard Ratio.

*Number of cases.

†Number of deceased.

‡Kaplan-Meier estimates of median months survival.

§HR adjusted for age, gender, ethnicity (White/non-White), series, radiation (given/not given), surgery (resection/biopsy only), chemotherapy (given/not given); all glioma models include stratification by four diagnosis groups (glioblastoma, anaplastic astrocytoma, astrocytoma, and other); models also account for time between histologic diagnosis and constitutive specimen collection (left truncation). IgE coded as 0-2.

21.2 months, similar to the 19.7 months observed for all glioma subjects here with genotyping data. They found no difference in median months survival by *GSTT1* variant (21.6 for null and 21.4 for not null), but the HR of 1.2 (95% CL, 0.73-1.8) adjusted for age, diagnosis group, chemotherapy, radiation therapy, and total resection versus subtotal resection or biopsy was consistent with our findings (HR, 1.6; 95% CL, 1.2-2.2), and the confidence intervals overlap. In 78 patients with anaplastic tumors, those who were *GSTM1* null and homozygous *GSTP1 I105V* and *A114V IIAA* had nonsignificantly (P = 0.06) longer survival compared with those with other combinations of these genes (11), whereas we found the converse with P = 0.14. Because differences between the studies are compatible with chance, additional studies and well-conducted meta-analyses will be necessary to confirm or refute potential associations.

Tumor markers. It is very interesting that EGFR expression was associated with somewhat better survival among glioblastoma patients but much poorer survival among anaplastic astrocytoma patients. For anaplastic astrocytoma patients with EGFR expression, survival was only 9.9 months, nearly as poor as that of glioblastoma patients. This finding supports our and others' previous suggestion that anaplastic astrocytomas that overexpress EGFR might represent tumors that are more similar to glioblastoma (21). Incomplete tumor sampling is one possible explanation of why some anaplastic astrocytomas were found to overexpress EGFR. As we previously suggested, EGFR expression might be incorporated into clinical evaluation of anaplastic astrocytoma, and, if positive, might suggest greater scrutiny of the tumor for evidence of glioblastoma features. Because our criteria for glioblastoma included microvascular proliferation or necrosis, tumors with these features would not have been classified as anaplastic astrocytoma.

EGFR overexpression was more strongly associated than *EGFR* amplification with survival among both glioblastoma and

Table 5. Cox regressions of glioma survival by tumor EGFR, MDM2, and TP53 genetic and expression alterations, San Francisco Bay Area Adult Glioma Study, 1991-2000

	Glioblastoma				Anaplastic astrocytoma			
	*n**	*n* died †	Median mos survival ‡	*P*, HR (95% CI)§	*n**	*n* died †	Median mos survival ‡	*P*, HR (95% CI) §
EGFR amplification	400	399			70	55		
No	251	250	6.8	0.46	56	41	20.3	0.04
Yes	149	149	7.9	0.93 (0.75-1.14)	14	14	4.5	2.31 (1.04-5.12)
EGFR expression	409	408			82	67		
Negative	216	216	6.9	0.008	55	42	20.6	0.0001
Positive	193	192	7.8	0.76 (0.63-0.93)	27	25	9.9	2.97 (1.70-5.19)
MDM2 amplification	400	398			70	55		
No	362	360	7.2	0.04	69	54	16.3	NC
Yes	38	38	7.2	0.70 (0.49-0.98)	1	1	10.6	
MDM2 expression‖	413	412			81	65		
No stain	122	122	7.0		47	37	19.4	
<5%	117	116	8.0		21	16	19.1	
5-30%	101	101	6.6	0.14	12	11	4.0	0.003
>30%	73	73	7.4	1.07 (0.98-1.18)	1	1	30.1	1.81 (1.22-2.70)
MDM2 Expression‖								
Negative	122	122	7.0	0.28	47	37	19.4	0.14
Positive	291	290	7.3	1.13 (0.91-1.40)	34	28	11.3	1.53 (0.87-2.67)
TP53 expression‖	435	433			85	67		
No stain	72	72	7.2		23	21	11.0	
<5%	123	122	8.0		15	12	12.6	
5-30%	117	117	7.3	0.05	19	15	19.1	0.47
>30%	123	122	6.8	1.10 (1.00-1.21)	28	19	20.3	0.93 (0.75-1.15)
TP53 expression‖								
Negative	72	72	7.2	0.38	23	21	11.0	0.64
Positive	363	361	7.3	1.12 (0.87-1.45)	62	46	19.5	0.87 (0.50-1.54)
TP53 mutation	407	405			76	58		
No mutation	345	343	7.2	0.54	49	41	11.3	0.43
Mutation	62	62	8.4	1.09 (0.83-1.44)	27	17	36.6	0.78 (0.42-1.46)

Abbreviations: NC, not calculated; 95% CI, Confidence Interval; HR, Hazard Ratio

*Number of cases.

†Number of deceased.

‡Kaplan-Meier estimates of median months survival.

§HR adjusted for age, gender, ethnicity (White/non-White), series, radiation (given/not given), surgery (resection/biopsy only), chemotherapy (given/not given).

‖HR results for tumor immunohistochemistry marker coded ordinally: 0, no stain; 1, <5%; 2, 5-30%; and 3, >30%;

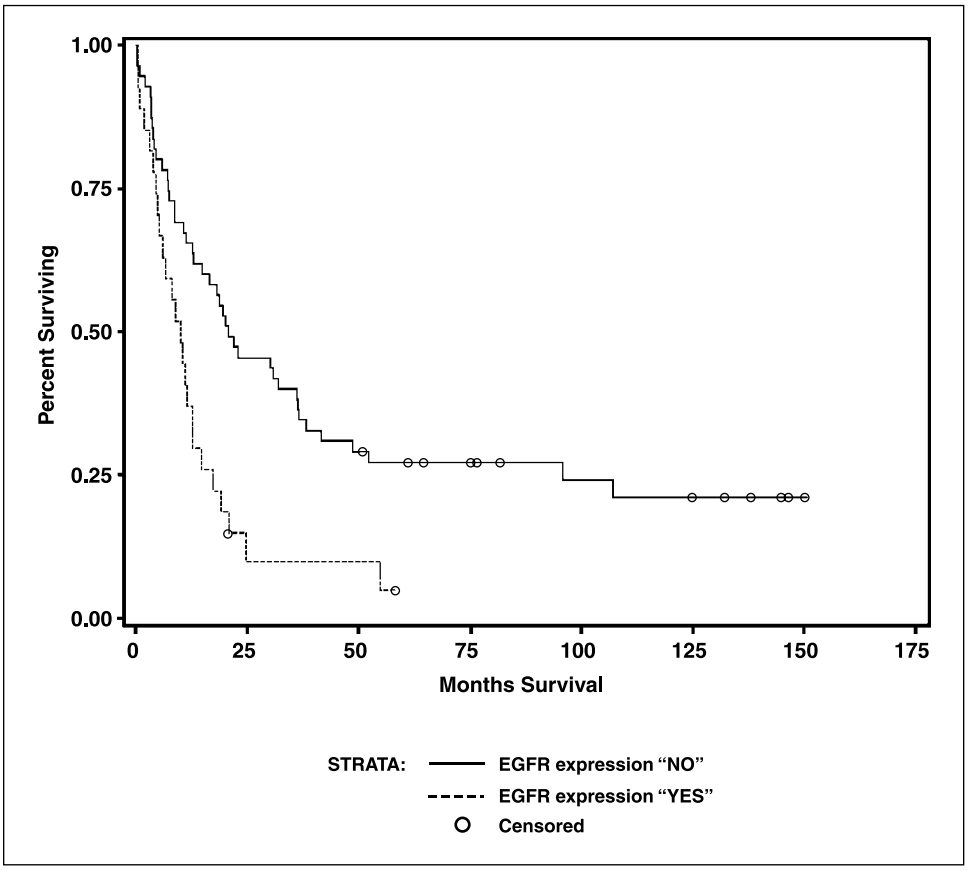

Figure 2. Percent survival distributions for people with anaplastic astrocytoma whose tumors did (---, $n = 27$) and did not (—, $n = 55$) express EGFR.

anaplastic astrocytoma patients. Other studies also have reported that *EGFR* amplification does not correlate with glioblastoma survival (27, 64). About a third of tumors overexpressing EGFR do not contain gene amplification. The factors responsible for overexpression in the absence of gene amplification are not known, although recent studies have shown that a constitutive polymorphism in the regulatory portion of the *EGFR* gene is associated with increased expression and cancer risk (65, 66).

Others and we (23–25) previously reported age-dependent associations of EGFR expression with glioblastoma survival with overexpression associated with longer survival in older patients and with shorter survival in younger patients. In the present study, EGFR overexpression was associated with better glioblastoma survival irrespective of age group. The previous reports were from patients from clinical trials and thus may represent a somewhat different patient group than a population-based series. Taken together, the results indicate that (*a*) among glioblastoma patients, the predictive value of EGFR expression may depend on the patient population and (*b*) among anaplastic astrocytoma patients, EGFR expression is a marker predictive of clinical behavior similar to glioblastoma.

Also noteworthy is the very poor median survival (4 months) among anaplastic astrocytoma patients with 5% to 30% of cells staining for MDM2.

In the only other comparable population-based series, Ohgaki et al. (64) reported a significant difference in glioblastoma survival for those whose tumors had TP53 mutation (median months = 8.2) versus those without (median months = 7.2; $P = 0.02$) but did not adjust for age or other factors. We found very similar median glioblastoma survival times by *TP53* tumor mutation status to their report, but after adjusting for age and other pertinent factors, we found a slightly positive nonsignificant HR for *TP53* tumor mutation and glioblastoma survival.

Conclusions

Based on these findings and those of others, we recommend that (*a*) EGFR expression be included in clinical evaluation of anaplastic astrocytoma patients; (*b*) confirming and understanding the relationship of elevated IgE levels, and possibly other immunologic factors, with improved glioblastoma survival should be a high priority in glioblastoma research; and (*c*) further work to understand the relationship of inherited *ERCC1* or other variants in the 19q13.3 region is warranted. Our results also support the need for larger studies of the role of inherited variation in glioma survival.

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