

# Impact of tumor location and pathological discordance on survival of children with midline high-grade gliomas treated on Children's Cancer Group high-grade glioma study CCG-945

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**Abstract** Children with high-grade glioma (HGG) have a poor prognosis compared to those with low-grade glioma (LGG). Adjuvant chemotherapy may be beneficial, but its optimal use remains undetermined. Histology and extent of resection are important prognostic factors. We tested the hypothesis that patients with midline HGG treated on Children's Cancer Group Study (CCG) CCG-945 have a worse prognosis compared to the entire group. Of 172 children eligible for analysis, 60 had midline tumors primarily localized to the thalamus, hypothalamus and basal ganglia. Time-to-progression and death were determined from the date of initial diagnosis, and survival curves were calculated. Univariate analyses were undertaken for extent of resection, chemotherapy regimen, anatomic location, histology, proliferation index, MGMT status and p53 over-expression. For the entire

midline tumor group, 5-year PFS and OS were  $18.3 \pm 4.8$  and  $25 \pm 5.4$  %, respectively. Many patients only had a biopsy (43.3 %). The sub-groups with near/total resection and hypothalamic location appeared to have better PFS and OS. However, the effect of tumor histology on OS was significant for children with discordant diagnoses on central pathology review of LGG compared to HGG. Proliferative index (MIB-1 > 36 %), MGMT and p53 over-expression correlated with poor outcomes. Children treated on CCG-945 with midline HGG have a worse prognosis when compared to the entire group. The midline location may directly influence the extent of resection. Central pathology review and entry of patients on clinical trials continue to be priorities to improve outcomes for children with HGG.

**Keywords** Childhood malignant gliomas · Anaplastic astrocytoma · Glioblastoma multiforme · Chemotherapy · Midline tumors · Thalamus · Hypothalamus · Basal ganglia

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## Introduction

The Children's Cancer Group (CCG) randomized clinical trial (RCT) CCG-943 compared irradiation followed by

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nitrosourea-based chemotherapy to irradiation alone. This was the first phase III trial demonstrating that adjuvant chemotherapy improved survival for children with HGG, with a 5-year event-free survival (EFS) and overall survival (OS) of 33 and 36 %, respectively [1].

Subsequently, the CCG-945 study compared a novel regimen known as “8-drugs-in-1-day” (“8-in-1”), given both pre- and post-irradiation, to the chemotherapy-containing arm in CCG-943. Although neither treatment arm was superior, important observations were made regarding central neuropathology review, tumor histology and the extent of resection (EOR) [2].

Although the original CCG-945 report assigned a poor prognosis to “midline” HGG patients, this group had the highest rate of pathological discordance and merited further study. Furthermore, the rarity and dismal outcome of HGG in the hypothalamic/optic chiasm region are new and important observations.

## Materials and methods

### Patient population

From 1985–1990, 172 eligible children with newly-diagnosed HGG outside of the brainstem or spinal cord were randomized to receive following surgery either eight cycles of the control regimen consisting of lomustine, vincristine and prednisone (Regimen A) following involved-field irradiation (54 Gy in 1.8 Gy fractions) with concomitant weekly vincristine  $\times 8$ , or the experimental regimen known as “8-in-1” (Regimen B) which included lomustine, vincristine, hydroxyurea, procarbazine, cisplatin, cytaraboside, dacarbazine and methylprednisolone. In Regimen B, two cycles of postoperative chemotherapy were administered prior to involved-field irradiation (54 Gy in 1.8 Gy

fractions), the latter without weekly vincristine. Young patients (<2–3 years, depending upon enrollment year) were non-randomized to Regimen B (NB) to avoid or delay irradiation. Specimens were centrally reviewed by a panel of five neuropathologists, as were institutional radiology and surgical reports. EOR was defined as: biopsy only (<10 %), partial resection (10–50 %), subtotal (STR, 50–90 %), and near-total/total resection (NTR, >90 %). A pathologic diagnosis was termed discordant when central review determined the tumor to be other than HGG [2]. Surviving patients were followed up to 2004.

This report focused on 60 patients with “midline” HGG. In addition to progression-free survival (PFS) and OS, outcomes were determined according to EOR, chemotherapy regimen, tumor location and discordant diagnosis.

### Tumor biology

Study neuropathologists were blinded to patient status, EOR, tumor location or histology. The proliferation index (MIB-1 score) of formalin-fixed paraffin embedded (FFPE) sections used the percentage of Ki-67 antigen immunoreactivity in approx. 2,000 cells from 5 to 10 high power fields (hpf) [3]. O<sup>6</sup>-methylguanine-DNA methyltransferase (MGMT) expression was determined using immunohistochemistry (IHC) (mouse anti-MGMT antibody, Zymed) [4] with labeling score determined semi-quantitatively in 5–10 hpf: no/little expression (0/1), scattered positive cells (2), most/nearly all cells (3/4). A score of 3 or 4 was classified as “over-expression” whereas 0, 1 or 2 were considered “no over-expression” comparable to normal brain. p53 protein expression was detected by IHC using an antibody that recognized wild-type and/or mutant p53 (DO-7, Dako) [5], and assessed: little/no p53 expression (0/1), 25–50 % (2), 50–75 % (3) or >75 % of tumor cells (4).

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Statistical considerations

The Kaplan–Meier method calculated time to recurrence and death from the date of diagnosis [6]. Follow-up stopped at death or the end of the study date for survival and at recurrence or the end of study (whichever happened first). Patients were censored for any other events when determining time to recurrence. The log rank test was used to compare time-to-event distributions. Data management and analyses were performed using SAS (v. 9.1.3).

Results

Patient characteristics

Of 172 enrolled patients, 60 with primarily midline tumor were eligible (Table 1). Eleven (18.3 %) are long-term survivors. There were 26 males (43.3 %) with 47 (78 %) Caucasians. The mean age at diagnosis was 7.61 years. Thirty-nine (65 %) were under 10 years with 21 (35 %) <5 years. Twenty-three patients received Regimen A (38.3 %); 1/23 was non-randomized (NA), whereas 37 received Regimen B (61.7 %), 16/37 being non-randomized. Tumors were primarily localized to the thalamus (37, 61.7 %), hypothalamus (10, 16.7 %), basal ganglia (6, 10.0 %), or mid-brain (4, 6.7 %). NTR was performed on only five patients (8.3 %); the remainder underwent a biopsy (26, 43.3 %), partial (15, 25.0 %) or subtotal (14, 23.3 %) resections. Institutional diagnoses were predominantly anaplastic astrocytoma (AA) in 39 patients (65 %) and glioblastoma (GBM) in 14 (23.3 %). Central review reclassified 19 patients as LGG, 26 patients (43.3 %) had AA (22, 36.7 %) or other anaplastic gliomas (4, 6.7 %) and 12 with GBM (20.0 %), representing 63.3 % of the cohort (Online resource Table 1).

Extent of resection

For the midline tumor group, the 3- and 5-year PFS was  $23.3 \pm 5.3$  and  $18.3 \pm 4.8$  %, respectively (Fig. 1). According to EOR the following 3- and 5-year PFS were: biopsy,  $19.2 \pm 7.1$  %,  $7.7 \pm 4.3$  %; partial resection,  $33.3 \pm 11.1$  %,  $33.3 \pm 11.1$  %; STR,  $14.3 \pm 7.6$  %,  $14.3 \pm 7.6$  %; NTR,  $40.0 \pm 17.9$  %,  $40.0 \pm 17.9$  % (Fig. 2a). NTR appeared to have better PFS but these results were not significant (NS,  $p = 0.26$ ). The 3- and 5-year OS were  $26.7 \pm 5.5$  and  $25.0 \pm 5.4$  %, respectively (Fig. 1). Analysis of OS demonstrated the following 3- and 5-year survivals: biopsy,  $23.1 \pm 7.6$  %,  $19.2 \pm 7.1$  %; partial resection,  $33.3 \pm 11.1$  %,  $33.3 \pm 11.1$  %; STR,  $21.4 \pm 9.5$  %,  $21.4 \pm 9.5$  %; NTR,  $40.0 \pm 17.9$  %,  $40.0 \pm 17.9$  % (Fig. 2b). Similar to PFS, NTR appeared to

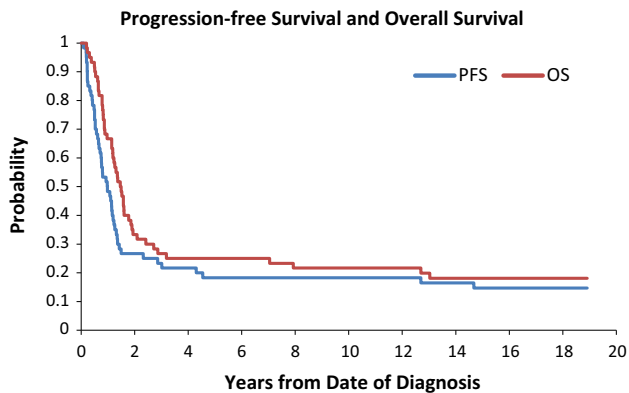
**Table 1** Summary table of patient demographics, management, CCG-945 study assignment and primary tumor location

	Number	Percent
<i>Sex</i>		
F	34	56.7
M	26	43.3
Total	60	100.0
<i>Age at diagnosis (years)</i>		
0–4	20	33.0
5–9	19	31.7
10–14	16	26.7
15+	5	8.3
Total	60	100.0
<i>Vital status</i>		
Alive	11	18.3
Deceased	49	81.7
Total	60	100.0
<i>Race</i>		
Asian	3	5.0
African–American	6	10.0
Hispanic	3	5.0
Mixed	1	1.7
Caucasian	47	78.3
Total	60	100.0
<i>Off therapy—reason</i>		
Death	2	3.3
Completed protocol	18	30.0
Other off-therapy	1	1.7
Withdrawn by parent	3	5.0
Withdrawn by physician	2	3.3
Relapse off protocol	3	5.0
Relapse on protocol	30	50.0
Toxicity off treatment	1	1.7
Total	60	100.0
<i>Resection</i>		
Biopsy	26	43.3
Partial	15	25.0
Subtotal	14	23.3
Near-total/total	5	8.3
Total	60	100.0
<i>Treatment</i>		
A	22	36.7
A (non-randomized)	1	1.7
B	21	35.0
B (non-randomized)	16	26.7
Total	60	100.0
<i>Primary tumor location</i>		
Basal ganglia	6	10.0
Hypothalamus	10	16.7
Midbrain	4	6.7
Other (Chiasm 2; Pineal region 1)	3	5.0
Thalamus	37	61.7
Total	60	100.0

have improved survival but the results were not significant ( $p = 0.55$ ).

#### Chemotherapy regimen

There were no statistical differences between chemotherapy regimens ( $p = 0.48$ ) with 3- and 5-year PFS for Regimen A/NA ( $26.1 \pm 8.5 \%$ ,  $26.1 \pm 8.5 \%$ ) and Regimen B/NB ( $21.6 \pm 6.4 \%$ ,  $13.5 \pm 5.1 \%$ ), respectively



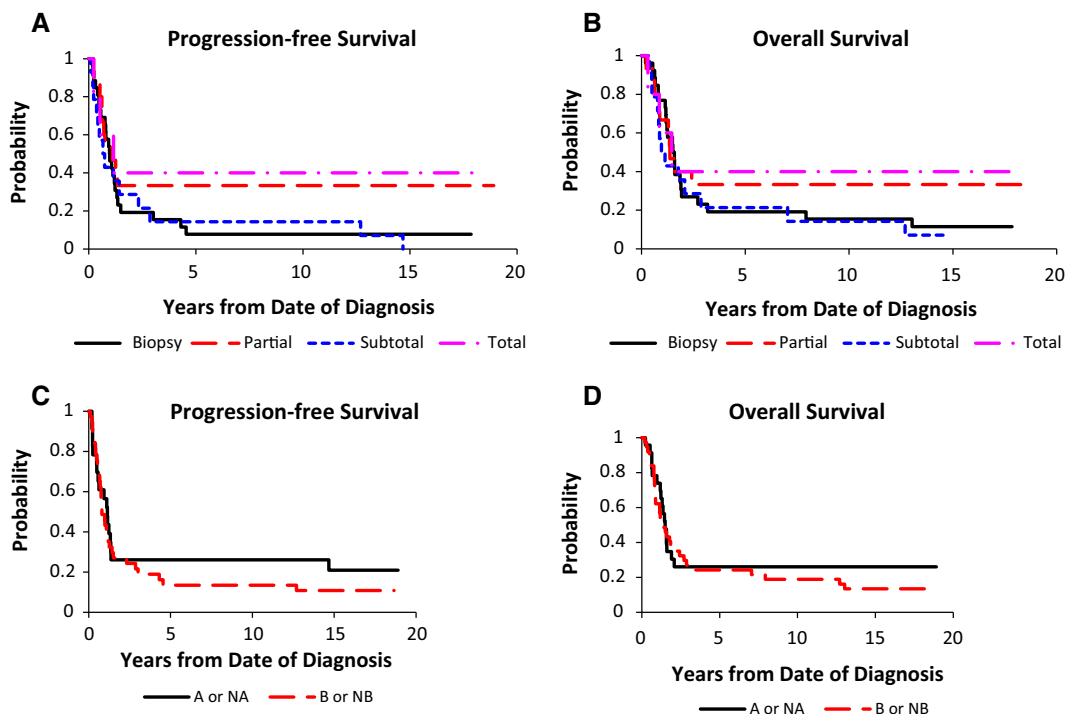
**Fig. 1** Progression free survival (PFS) and overall survival (OS) for the midline HGG cohort. The *x*-axis is time (months) and the *y*-axis represents the proportion of surviving patients

(Fig. 2c). Similarly there was no significant difference in OS between regimens ( $p = 0.49$ ) with 3- and 5-year OS for Regimen A/NA ( $26.1 \pm 8.5 \%$ ,  $26.1 \pm 8.5 \%$ ) and Regimen B/NB ( $27.0 \pm 7.0 \%$ ,  $24.3 \pm 6.7 \%$ ), respectively (Fig. 2d).

#### Anatomic tumor location

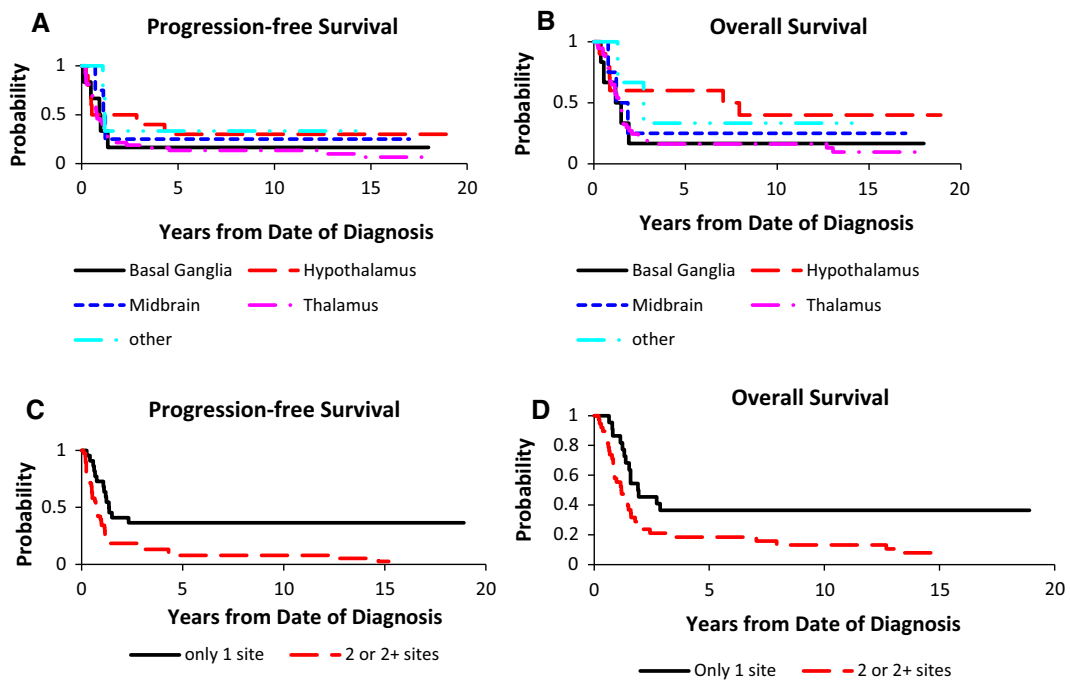
PFS revealed 3- and 5-year PFS for thalamus ( $18.9 \pm 6.0 \%$ ,  $13.5 \%$ ), hypothalamus ( $30 \pm 13.9 \%$ ,  $30 \pm 12.5 \%$ ), basal ganglia ( $16.7 \pm 10.8 \%$ ,  $16.7 \pm 10.8 \%$ ) and midbrain ( $25.0 \pm 15.3 \%$ ,  $25.0 \pm 15.3 \%$ ) (Fig. 3a). Survival analysis revealed 3- and 5-year OS for thalamus ( $18.9 \pm 6.0 \%$ ,  $16.2 \pm 5.6 \%$ ), hypothalamus ( $60 \pm 14.3 \%$ ,  $60 \pm 14.3 \%$ ), basal ganglia ( $16.7 \pm 10.8 \%$ ,  $16.7 \pm 10.8 \%$ ) and midbrain ( $25.0 \pm 15.3 \%$ ,  $25.0 \pm 15.3 \%$ ) (Fig. 3c). There were no significant differences for PFS ( $p = 0.59$ ) or OS ( $p = 0.39$ ) among primary tumor locations. However, the patient group with hypothalamic tumors appeared to have better survival outcomes ( $p = 0.28$ ).

For patients whose tumors were localized primarily to one site versus more than one site, PFS was significantly different ( $p = 0.0013$ ) with 3- and 5-year PFS  $36.4 \pm 9.7$  and  $36.4 \pm 9.7 \%$  for one site versus  $15.8 \pm 5.5$  and  $7.9 \pm 3.8 \%$  for more than one site, respectively (Fig. 3b). OS was also significantly different ( $p = 0.0084$ ) with 3-

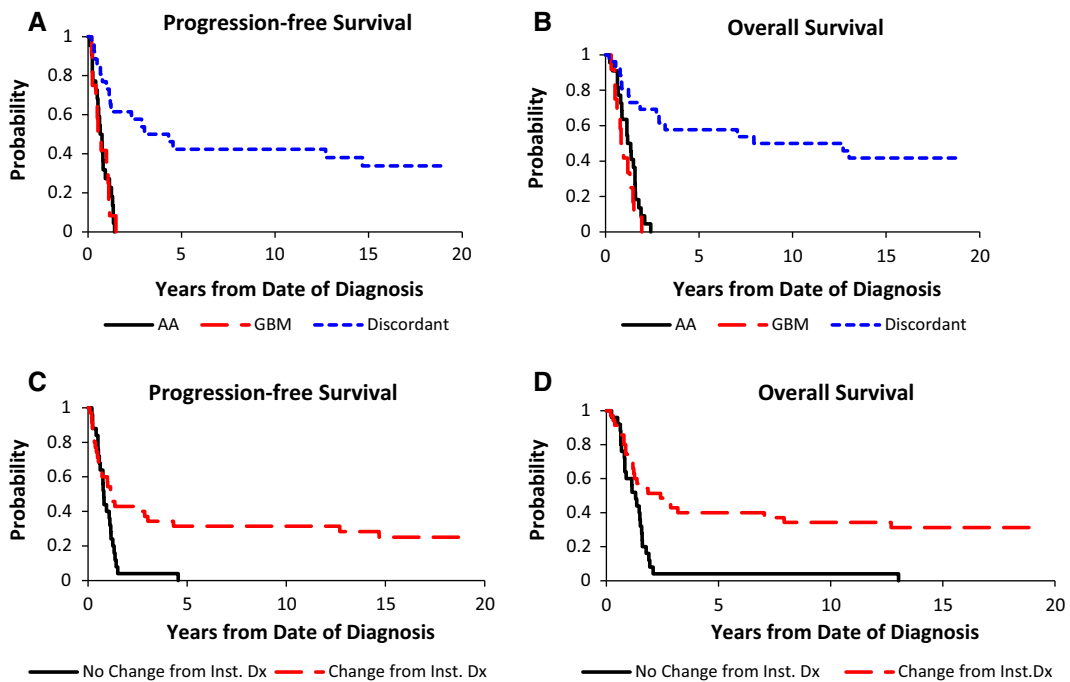


**Fig. 2** Extent of surgical resection and survival. **a** PFS ( $p = 0.26$ ) and **b** OS ( $p = 0.55$ ). Chemotherapy regimen and survival. **c** PFS ( $p = 0.48$ ) and **d** OS ( $p = 0.49$ ). *Solid line* Regimen A or non-

randomized to Regimen A (prednisone, CCNU and vincristine); *Dotted line* Regimen B or non-randomized to Regimen B



**Fig. 3** Anatomic site and survival curves. Primary tumor site and **a** PFS ( $p = 0.59$ ) or **c** OS ( $p = 0.39$ ). Number of involved sites and **b** PFS ( $p = 0.0013$ ) or **d** OS ( $p = 0.0084$ )



**Fig. 4** Tumor histology and survival curves for **a** PFS or **b** OS. *Solid line* anaplastic astrocytoma (AA, WHO III); *dashed line* glioblastoma multiforme (GBM, WHO IV); *dotted line* diagnoses discordant for

pediatric HGG. **c, d** Comparing changed diagnoses following central review to those patients with an unchanged diagnosis ( $p < 0.01$ ). Diagnosis changed, *dotted line*; diagnosis unchanged, *solid line*

and 5-year OS  $36.4 \pm 9.7$  and  $36.4 \pm 9.7$  % for one site versus  $21.1 \pm 6.2$  and  $18.4$  % for more than one site, respectively (Fig. 3d).

### Histology

Central review showed 1- and 3-year PFS of  $27.3 \pm 8.8$  and  $0$  % for AA and  $33.3 \pm 12.2$  and  $0$  % for GBM, respectively. For those with a discordant diagnosis resulting in reclassification as low grade glioma (LGG), 1-, 3- and 5-year PFS were  $73.1 \pm 8.5$ ,  $53.8 \pm 9.4$  and  $42.3 \pm 9.3$  % (Fig. 4a). One- and 3-year OS were  $58.3$  and  $0$  % for all HGG whose diagnoses remained unchanged. For AA, 1- and 3-year OS were  $63.6 \pm 9.9$  and  $0$  %, and  $41.7 \pm 13.0$  and  $0$  % for GBM (Fig. 4b). For patients revised to a LGG, 1-, 3- and 5-year OS were  $80.8 \pm 7.6$ ,  $61.5 \pm 9.3$  and  $57.7 \pm 9.4$  % (Fig. 4b). There were significant differences for PFS ( $p < 0.0001$ ) and OS ( $p < 0.0001$ ) when comparing HGG (AA, GBM) to a discordant diagnosis.

Patients were also classified into two groups: “No Change” and “Change” from Institutional Diagnosis. One-, 3- and 5-year PFS were  $40.0 \pm 9.3$ ,  $40.0 \pm 2.8$ , and  $0$  % for no change and  $54.3 \pm 8.2$ ,  $37.1 \pm 7.9$ , and  $31.4 \pm 7.5$  % for changed diagnosis; these findings were significant ( $p = 0.0078$ , Fig. 4c), as were 1-, 3- and 5-year OS of  $60.0 \pm 9.5$ ,  $4 \pm 2.8$  and  $4 \pm 2.8$  % for no change and  $71.4 \pm 7.5$ ,  $42.9 \pm 8.1$ , and  $40.0 \pm 8.0$  % for changed diagnosis ( $p = 0.0009$ , Fig. 4d).

### Tumor biology

Analysis was also performed on patient subsets: (i) MIB-1 score ( $n = 32$ ), (ii) MGMT expression score ( $n = 23$ ) and (iii) p53 expression score ( $n = 27$ ). The MIB-1 score separated into three groups: group 1 (“0”:  $<18$  %;  $n = 21$ ), group 2 (“1”:  $18$ – $36$  %;  $n = 6$ ) and group 3 (“2”:  $>36$  %;  $n = 5$ ) [3]. Three- and 5-year PFS were  $28.6 \pm 9.1$  and  $23.8 \pm 8.5$  % (group 1),  $16.7 \pm 10.8$  and  $16.7 \pm 10.8$  % (group 2) and  $0$  and  $0$  % (group 3), respectively (Online resource Fig. 1A). Similarly, 3- and 5-year OS were  $33.3 \pm 9.6$  and  $33.3 \pm 9.6$  % (group 1),  $16.7 \pm 10.8$  and  $16.7 \pm 10.8$  % (group 2) and  $0$  and  $0$  % (group 3), respectively (Online resource Fig. 1B). There were significant differences among the MIB-1 score groups for PFS ( $p = 0.03$ ) and OOS ( $p = 0.01$ ).

MGMT scores were grouped as “0: no over-expression” (score = 0, 1 or 2;  $n = 19$ ) or “1: over-expression” (score = 3 or 4;  $n = 4$ ) [4]. Three- and 5-year PFS were  $26.3 \pm 9.2$  and  $21.1 \pm 8.4$  % (group 1) and  $0$  and  $0$  % (group 2), respectively (Online resource Fig. 2A). Similarly, 3- and 5-year OS were  $26.3 \pm 9.2$  and  $26.3 \pm 9.2$  % (group 1) and  $0$  and  $0$  % (group 2), respectively (Online

resource Fig. 2B). Differences between MGMT score groups were not significant for PFS ( $p = 0.19$ ) or OS ( $p = 0.13$ ).

Finally, the p53 score was grouped as “0: normal” (score = 0 or 1;  $n = 17$ ) or “1: over-expression” (score = 2, 3 or 4;  $n = 10$ ) [5]. Three- and 5-year PFS were  $29.4 \pm 10.1$  and  $23.5 \pm 9.2$  % (group 1) and  $10 \pm 6.7$  and  $10 \pm 6.7$  % (group 2), respectively (Online resource Fig. 3A). Similarly, 3- and 5-year OS were  $35.3 \pm 10.7$  and  $35.3 \pm 10.7$  % (group 1) and  $10 \pm 6.7$  and  $10 \pm 6.7$  % (group 2), respectively (Online resource Fig. 3B). Differences between p53 score groups were not significant for PFS ( $p = 0.27$ ) or OS ( $p = 0.099$ ). Although MGMT and/or p53 over-expression correlated with poor PFS and OS, these results were not statistically significant (NS), unlike those observed for MIB-1 scores  $>36$  %.

### Discussion

In the current analysis of 60 children with midline HGG, the 5-year PFS and OS of  $18.3$  and  $25.0$  % were reduced when compared to the overall group of 172 patients with 5-year EFS and OS of  $33$  and  $36$  % [2]. EOR correlated with both PFS and OS in the midline group but did not reach significance; only 5 patients ( $8.3$  %) underwent extensive resections. Posterior fossa (PF) and cerebral hemispheric locations were more amenable to aggressive resections [2]. CCG-945 patients were stratified according to the EOR combining operative reports with postoperative imaging (CT) [7]. A radical resection ( $>90$  %) was performed in only  $37$  %, but  $49$  % with tumors in the cerebral hemispheres and  $45$  % in the PF compared with  $8$  % of midline tumors underwent NTR ( $p < 0.0001$ ). Only radical resection was associated with significantly improved PFS [7]. A single institution retrospective study of 39 children with pre- and post-operative MRI studies reported similar outcomes with a median OS of 122.2 months in patients with GTR compared with 14.1 in those with residual disease [8]. HIT-GBM-C reported a  $63$  % 5-year OS for patients with complete resection [9]. Although the EOR on CCG-945 represented the spectrum of neurosurgery offered in the late 1980s, this was prior to the wider availability and adoption of MRI scanning, stereotactic navigation, advanced neurophysiologic intraoperative monitoring and dedicated pediatric neurosurgeons, so it is likely that more aggressive neurosurgical management will have a positive impact on pediatric HGG in midline locations.

In this midline HGG cohort, neither chemotherapy regimen was superior, similar to the overall group [2]. Concurrent temozolomide during involved-field irradiation followed by adjuvant temozolomide has become the



standard of care for adult GBM [10]. However, a recent COG trial for newly-diagnosed HGG using a similar treatment strategy (ACNS0126) did not improve outcomes compared to CCG-945 ( $p = 0.98$ ) [11]. MGMT over-expression was adversely associated with survival [12]. A follow-up Phase I/II COG study using chemo-irradiation and an adjuvant chemotherapy regimen combining temozolomide with BCNU also did not improve outcomes [13]. Bevacizumab-containing regimens are under evaluation for both children with newly diagnosed and recurrent HGG [14].

When compared to the overall group, the midline location had poorer survival; however, tumors localized to the hypothalamus (10 of 60; 16.7 %) displayed a non-significant trend towards improved OS, whereas HGG originating in the thalamus (37 of 60; 61.7 %) or basal ganglia (6 of 60; 10 %) had worse outcomes (NS). Tumors that involved more than one contiguous site had significantly worse outcomes. The most likely explanation for the improved outcome for hypothalamic tumors is that 7 of 10 (70 %) were LGG after central review. Conversely, hypothalamic or optic chiasm patients with centrally reviewed HGG had no survivors; this observation has not been previously reported.

Central pathology review for children with HGG entered on treatment protocols is essential. Similar to the overall group, centrally reviewed AA histology correlated with improved PFS and OS when compared with GBM. Discordant diagnoses were evident in 21 of 60 patients (35 %), including revision of 19 of 39 AA and 1 of 14 GBM diagnoses to LGG. Of the other HGG, four AA were reclassified as GBM and five GBM were changed to AA. Patients with centrally reviewed midline LGG had significantly better outcomes on univariate analysis when compared with midline HGG. 70 of 250 patients entered on CCG-945 based upon institutional pathology were revised to LGG upon central review; 5-year PFS and OS were 63 and 79 % versus 19 and 22 %, respectively, in patients with confirmed HGG [15]. The most recent WHO guidelines post-dated our central re-review in 2003; a few additional HGG may have been reassigned to a LGG diagnosis [16]. Expedited central pathology review is therefore highly recommended prior to assignment of treatment on prospective HGG clinical trials [17, 18].

Pollack et al. retrospectively studied the proliferative indices of 98 eligible HGG from the overall CCG-945 cohort. The MIB-1 score was associated with 5-year EFS independent of tumor histology. MIB-1 labeling was a categorical variable and indices between 18 and 36 % had intermediate outcomes when compared to <18 or >36 % with better and worse prognoses, respectively [3]. In the midline HGG group, MIB-1 labeling >36 % had significantly worse PFS and OS when compared to patients with

<36 % labeling; survival curves for tumors with indices from 18 to 36 % could not be distinguished from those with <18 % MIB-1 labeling.

Esteller et al. correlated MGMT inactivation with the response of adult HGG patients to alkylating agents [19]. Hegi et al. [20] retrospectively investigated adult GBM patients treated on an intergroup phase III RCT [10]. MGMT gene silencing significantly correlated with response to temozolomide and was an independent prognostic factor. Pollack et al. [4] studied 109 specimens from the entire CCG-945 group; all received alkylator therapy. Five-year PFS was 42.1 % in the 97 patients with low levels of MGMT expression versus 8.3 % in the 13 patients with MGMT over-expression ( $p < 0.02$ ). Lee et al. assessed MGMT promoter methylation in a small cohort of 19 pediatric GBM and discovered low methylation rates (6–16 %) depending upon the laboratory assay used [21]. Buttarelli et al. found 7/24 pediatric HGG (30 %) had MGMT promoter methylation, whereas 19/28 (68 %) had MGMT protein over-expression [22]. However, using a methylation-specific polymerase chain reaction (PCR) assay, Srivastava et al. found that 10/20 pediatric GBM had MGMT promoter methylation [23].

MGMT expression was also an independent prognostic factor in this study. In the midline HGG cohort, the patients with MGMT over-expression had very poor outcomes; however, statistical significance could not be determined due to small patient numbers. Current clinical trials are prospectively assessing MGMT gene silencing and/or protein expression and future studies will stratify patients or assign treatments based on MGMT status. However, it may be necessary to assess both MGMT promoter methylation and protein expression in future pediatric HGG studies.

TP53 mutational status was assessed in 77 eligible tumor samples from the CCG-945 cohort; a higher frequency of TP53 mutations was found in 11.8 % of tumors from children under 3 years compared with 40 % in older children [24]. p53 expression ( $n = 115$ ) as well as TP53 mutational status ( $n = 121$ ) was then studied in a larger group of tumors from the initial CCG-945 cohort. p53 over-expression was significantly associated with adverse outcomes, independent of histology and other prognostic factors [5]. In the midline HGG cohort, only a few samples could be assessed for TP53 mutations; data was insufficient to generate statistical significance. However, in this group, 10/27 patients over-expressed p53 and had adverse PFS and OS when compared with patients without p53 over-expression. These results support assessing p53 expression status in pediatric HGG.

Other markers of pediatric HGG are being discovered using advanced cytogenetic approaches [25]. Comparing pediatric GBM to either normal controls or adult GBM,

two subsets of pediatric GBM have been identified, one associated with *Ras/Akt* pathway activation having a poor prognosis [26–28]. Insights of the genomic alterations of adult GBM from The Cancer Genome Atlas Research Network [29] and other consortia [30] are being applied to pediatric GBM, leading to identification of IDH1 mutations in adolescent HGG [31], and driver mutations in histone variant H3.3 and chromatin remodeling genes in pediatric and young adult GBM [32–34]. These findings will improve tumor classification and identify targets and pathways for novel therapeutic approaches for pediatric HGG.

In summary, children treated on CCG-945 with midline HGG fare poorly when compared to the entire group. Although EOR is limited by midline anatomic location, improvements in imaging, intraoperative monitoring and surgical adjuncts and techniques may contribute to improved outcomes. Our study reinforces the importance of central pathology review. Since optimal adjuvant chemotherapy in pediatric HGG is undetermined, patient enrolment on clinical trials remains a priority to improve outcomes.

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