Clinical Outcomes and Prognostic Features of Diffuse Hemispheric Glioma, H3 G34-Mutant: An International Multi-institutional Study

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ABSTRACT

Purpose: Knowledge of prognostic factors and long-term survival in patients with diffuse hemispheric glioma, H3 G34–mutant (DHG, H3 G34), remains limited in this tumor with a poor prognosis.

Experimental Design: This retrospective, multi-institutional study investigated prognostic variables for patients with DHG, H3 G34, and their association with progression-free survival (PFS) and overall survival (OS). Uni- and multivariable Cox proportional hazard models were applied with multiple imputed datasets.

Results: A total of 153 patients (142 G34R, 9 G34V, 2 via DNA methylation) were included. The median age at diagnosis was 17 years (range, 2–45). Initial gross/near total resection (GTR/NTR) was achieved in 43% of patients. Radiation was given in 91% (85% focal irradiation), and initial chemotherapy was given in 87% [70% temozolomide-based (TMZ), 25% TMZ/lomustine, 5% non-TMZ]. Median OS was 24 months [interquartile range (IQR), 22–28] with a median PFS of 14 months (IQR, 12–19). Twelve patients (8%) were found to be long-term survivors (≥5 years). Exploratory multivariable analysis showed that adjuvant radiotherapy [HR, 0.076; 95% confidence interval (CI), 0.033–0.17] and achieving GTR/NTR compared with < NTR (HR, 0.51; 95% CI, 0.33–0.78) were associated with improved PFS. Multivariable analysis showed improved OS with increasing age at diagnosis (HR, 0.70; 95% CI, 0.57–0.87), initial radiotherapy (HR, 0.38; 95% CI, 0.15–0.96), and initial GTR/NTR compared with < NTR (HR, 0.60; 95% CI, 0.37–0.97).

Conclusions: This cohort highlights prognostic factors for patients with DHG, H3 G34, and describes relapse patterns and therapy approaches. Clinical trials and prospective registries are needed to improve outcomes.

Introduction

Diffuse hemispheric glioma, H3 G34-mutant (DHG, H3 G34), is an aggressive brain tumor that predominantly affects the adolescent and young adult population, accounting for <1% of all gliomas in this age group (1). Outcome is poor, with a median survival generally between 17 and 22 months (2, 3). These tumors are characterized by a histone mutation in the *H3-3A* gene at amino acid

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Translational Relevance

Previous studies on diffuse hemispheric glioma, H3 G34-mutant (DHG, H3 G34), have largely focused on molecular features in tumors affecting adolescents and young adults, with limited data on patient characteristics and outcomes. Key gaps remain in understanding prognostic factors, treatment responses, and long-term survival. We present a comprehensive multi-institutional clinical cohort to address these gaps. Our findings indicate that older age at diagnosis, upfront gross/near total resection, and initial radiation are associated with improved prognosis. Tumor biology may be influenced by patient factors, including age and sex. Additionally, we show that 8% of patients diagnosed with DHG, H3 G34, survive beyond 5 years, further highlighting disease heterogeneity. Finally, post hoc analysis shows that adding adjuvant lomustine to temozolomide improves progression-free survival in both adult and pediatric patients and overall survival in pediatric patients, supporting the exploration of additional systemic therapies for this aggressive brain tumor.

codon 35, known as codon 34 in histone legacy amino acid numbering, in which glycine is replaced by either arginine (G34R) or valine (G34V; refs. 4, 5). This mutation is a key defining feature of DHG, H3 G34, a tumor first described in 2012 and now a distinct entity in the current World Health Organization classification of central nervous system (CNS) tumors, fifth edition (6–11). Although DHG, H3 G34 is conventionally defined as a glioma, this type of tumor originates from interneuron progenitor cells (9, 12, 13).

Efforts to summarize the current literature about clinical characteristics and patient outcomes for DHG, H3 G34 have demonstrated numerous knowledge gaps (3, 14). Notable limitations in the literature include inconsistent prognostic variables of DHG, H3 G34; a lack of understanding of the best available treatment paradigms; and poor insight into long-term survivors. Most studies attempting to address these questions have been constrained by small sample sizes, which limit their strength to inform care (2, 15–19).

Positive prognostic factors related to modifiable treatment-related factors have been very limited, mainly showing the importance of gross/near total resection (GTR/NTR; refs. 3, 20). Other reported positive prognostic factors have included female sex, age ≥18 years, and *MGMT* promoter methylation, especially with focal chromosome losses at the *MGMT* locus (3, 20). Diagnostic imaging features have been assessed in limited capacity with regard to their impact on prognosis (20). Numerous molecular studies have characterized coalterations in DHG, H3 G34, revealing patterns of tumor behavior. These include loss-of-function mutations in *TP53* and *ATRX*, found in the majority of patients and, more recently, *PDGFRA* overexpression in most tumors, whereas mutations in this receptor tyrosine kinase are present in more than 50% of patients at diagnosis and exceed 80% at relapse or recurrence (2, 7, 9, 20). Additionally, *CCND2* or *CDK6* amplifications have been identified in subsets of tumors although their prognostic significance remains inconsistent (21). Finally, minimal data exist for patients surviving more than 5 years after diagnosis (2, 20).

We collaborated internationally to develop a multicenter study to describe current treatment paradigms of initial multimodality therapy, understand variables associated with progression-free survival (PFS) and overall survival (OS), including clinical, radiographic, and molecular factors, describe relapse patterns and treatment, and describe a cohort of long-term survivors (≥5 years from diagnosis).

Materials and Methods

Patient selection

The study design was an international, multi-institutional, retrospective study of patients diagnosed with a G34-mutant brain

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tumor. Eligible for inclusion were any patients diagnosed with a G34-mutant brain tumor after January 1995 via sequencing or DNA methylation profiling. Each patient was required to have at least one outcome measure, including time to progression, OS, or follow-up duration with known vital status. Patients who remained alive at the last follow-up required at least 6 months of follow-up from diagnosis.

Data collection

Patient data were collected via convenience sampling from collaborating institutions by way of the site investigator. Patient demographic, molecular, radiographic, and treatment characteristics, as well as outcome measures, were collected using a standardized data collection form with variable definitions (Supplementary Table S1). Demographic variables included age and sex, whereas clinical variables included initial tumor histology, tumor mutation status, radiographic characteristics from the MRI reports, disease extent and location, initial and relapse treatment strategies, disease relapse occurrence, and survival details. Initial treatments were defined as those given before disease relapse. Initial tumor histology was classified as either high-grade glioma (HGG) or embryonal. G34 tumor mutation subtype and molecular coalterations were collected at the time of initial tissue biopsy or resection, including *TP53*, *ATRX*, *PDGFRA*, *EGFR*, and *MGMT* promoter methylation.

Statistical analysis

Descriptive statistics were reported as counts and percentages for categorical variables and medians with interquartile ranges (IQR) for non-normally distributed continuous variables. Patient characteristics were compared between extent of surgical resection groups (GTR/NTR vs. < NTR vs. Biopsy) and initial chemotherapy type used before relapse [temozolomide (TMZ) vs. TMZ/lomustine (CCNU)] and mortality outcome. χ^2 tests were used to compare categorical variables between groups, and the Wilcoxon rank-sum test was used for nonparametric continuous variables. Multiple imputation was performed using the fully conditional specification method, assuming the data were missing at random (22). Variables included in the imputation are shown in Supplementary Table S2. The imputation was repeated 30 times, equivalent to the percentage of patients containing any missing data, and was followed by the application of Rubin's rule to combine parameter estimates and standard errors (23). To establish the validity of the imputed data, we compared observed values of complete cases with imputed values. Imputation was not performed on any outcome events or variables with greater than 20% missing data. PFS and OS from the time of diagnosis to first progression, death, or the date of last follow-up were analyzed using Kaplan–Meier plots and log-rank tests to compare the survival distribution between groups. Kaplan–Meier survival estimates were also generated at 12, 24, and 36 months with 95% confidence intervals (CI). Univariable analysis using Cox proportional hazard models was performed on a priori selected variables for PFS and OS. The proportional hazards assumption was tested using the Kolmogorov-type supremum test and exploring time by covariate interactions. An exploratory Cox proportional hazards multivariable model was examined using clinically relevant variables and those with $P < 0.1$ on univariable analysis for PFS and OS. A stratified Cox model for OS was used to relax the proportional hazards assumption by stratifying the baseline hazard function for sex (24). A sensitivity analysis was performed to adjust for potential intracluster correlation within countries using a marginal Cox model (25). Statistical analyses were performed using SAS STAT 15.1 version 9.4 (RRID:SCR_008567). A two-sided P value of <0.05 was the threshold for statistical significance unless otherwise specified.

Ethics approval

This study was approved by the IWK Health Centre Research Ethics Board (File No. 1028579). All institutions obtained approval as required by their local research ethics boards. Given the retrospective nature of the study, a waiver of consent was used for deidentified clinical data.

Results

Demographic characteristics

A total of 153 patients diagnosed with an H3 G34–mutant brain tumor were included in our study from 31 participating institutions and nine different countries (Supplementary Fig. S1). Twelve institutions contributed pediatric patients only (<18 years old), six institutions contributed adult patients only (≥18 years old), and 13 institutions contributed both pediatric and adult patients. Our cohort had a median age at diagnosis of 17 years (range, 2–45); 92 patients (60%) were younger than 18 years old. Ninety-three of the included patients (61%) were male (**Table 1**; **Fig. 1**).

Radiographic and molecular characteristics

The disease location at diagnosis was available in 128 patients. Tumors most commonly involved more than one lobe in 33%, either with a solitary mass in 18% ($n = 23$) or as multifocal disease in 15% ($n = 19$), followed by tumors localized to the frontal lobe in 31% ($n = 40$; **Fig. 1**). With respect to imaging characteristics on MRI, moderate or diffuse contrast enhancement was present in 63% ($n = 64$), whereas 37% ($n = 37$) had minimal or no enhancement (**Table 1**). Diffusion restriction was present in 86% ($n = 72$). Tumor margins were infiltrative in 71% ($n = 57$) and well defined in 29% ($n = 23$). Peritumoral edema was either not present or had minimal halo in 56% ($n = 47$). Of 120 patients with known disease extent at diagnosis, one patient had distant metastatic disease.

Based on histopathologic interpretation, 139 patients (94%) were initially diagnosed with an HGG, whereas nine patients (6%) were diagnosed with embryonal tumors (six primitive neuroectodermal tumors and three embryonal tumors, not otherwise specified). Further molecular investigation demonstrated that among the 153 included patients, 142 (94%) were classified as G34R and nine (6%) were classified as G34V. Patients with the G34V mutation are described in more detail within Supplementary Table S3. The two remaining patients (1%) were diagnosed through DNA methylation–based CNS tumor classification only, and sequencing was not available. With respect to coalterations, 71% (62/87) had *MGMT* promoter methylation, 93% (123/132) had *TP53* mutations, 75% (74/99) had *ATRX* mutations, 59% (51/87) had *PDGFRA* single-nucleotide variants (SNV), and 38% (26/68) had *PDGFRA* amplification. For the 64 patients with both *PDGFRA* amplification and mutation information submitted, 15 (23%) had both amplification and SNV present. Five of seventy-seven (6.3%) patients had EGFR SNV, whereas another 16.9% (13/77) had EGFR amplification without overlap.

Initial treatment

With respect to the extent of surgery, GTR/NTR was achieved in 63 patients (43%), whereas 58 patients (39%) had less than NTR

(subtotal or partial resection), and 26 patients (18%) had biopsy only (**Fig. 2A**). Two of the 26 patients who had biopsy alone underwent a second surgery after diagnosis to help minimize residual tumor. Eleven patients underwent surgical resection alone without chemotherapy or radiation as initial treatment. Two of these patients elected not to pursue radiotherapy, whereas three others progressed rapidly before radiation. The remaining patients did not have information about the rationale for radiation and chemotherapy omission. Of these 11 patients (one GTR/NTR, eight < NTR, and two biopsy only), only one was progression-free at 6 months, whereas the remaining 10 patients progressed quickly within an average of 3 months (0.25–6 months) after surgery.

Initial radiotherapy was delivered to 139 patients, for whom the radiation type was known for 124 patients. A total of 112 patients received focal irradiation, five patients received craniospinal irradiation (CSI), and seven had other delivery techniques (five whole brain, one whole ventricular, and one unknown). The median dose of focal irradiation was 59.4 Gy (IQR, 59.4–60 Gy). Fourteen patients received no initial radiotherapy. Of the 139 patients who received radiotherapy, 112 patients had this as adjuvant therapy after surgical resection, and 22 patients had it as part of their definitive therapy after biopsy alone. Of the five patients who received CSI, four were identified as having embryonal tumors, and the reason for choosing the remaining patient is unknown, as their tumor was considered an HGG without dissemination at diagnosis.

For the 151 patients with available initial chemotherapy data, 132 patients received chemotherapy, defined as the use of any initial systemic anticancer therapy, whereas 19 patients did not receive chemotherapy before progression/relapse. The type of chemotherapy regimen was available for 130 of the 132 patients (98%). Ninety-one patients (70%) were treated with TMZ-based therapy without CCNU, 32 (25%) were treated with TMZ with the addition of CCNU, and seven patients (5%) had non-TMZ–based systemic therapy (Supplementary Table S4). Patients who received TMZ with CCNU chemotherapy were more often <18 years old at diagnosis compared with those who received TMZ alone (84% vs. 50%, $P = 0.0006$). Most patients who received chemotherapy also received concurrent chemoradiation in 87 of 108 (81%) with data available.

Table 1. Demographic and clinical characteristics.

Variable	*N*	*n* (%)
Demographics		
Age at diagnosis (years)	153	
Median (range)		17 (2–45)
Sex	153	
Male		93 (60.8)
Female		60 (39.2)
Imaging characteristics		
Contrast enhancement	101	
None/minimal		37 (36.6)
Moderate		48 (47.5)
Diffuse		16 (15.8)
Diffusion restriction	84	
Yes		72 (85.7)
No		12 (14.3)
MRI tumor margins	80	
Well-defined		23 (28.7)
Infiltrative		57 (71.3)
Peritumoral edema	84	
None/minimal		47 (55.9)
Moderate		25 (29.8)
Significant		12 (14.3)
Tumor characteristics		
Primary tumor histology	148	
High-grade glioma		139 (93.9)
Embryonal		9 (6.1)
Mutation subtype	153	
G34R		142 (92.8)
G34V		9 (5.9)
DNA methylation only		2 (1.3)
MGMT promoter hypermethylation, present	87	62 (71.3)
TP53 mutation, present	132	123 (93.2)
ATRX mutation, present	99	74 (74.7)
PDGFRA mutation, present	87	51 (58.6)
EGFR mutation, present	79	5 (6.3)

Relapse treatment and characteristics

A total of 151 patients were evaluable for progression, of which 129 patients (85%) had progressed or relapsed, whereas 22 patients (15%) remained alive without disease progression. Of the 129 patients who relapsed, six were missing the date of relapse; therefore, they were excluded from PFS models. Relapse patterns were documented in 95 patients with 56 (59%) having local recurrence within the prior radiation field, four (4%) recurring locally but without initial radiation, 15 (16%) having distant relapse outside of the radiation field, and 20 (21%) having combined patterns of disease relapse. There was no reported systemic metastatic disease at relapse in our cohort.

Data about disease relapse treatment were available for 106 of 129 (82%) patients, including 25 patients who did not receive any further cancer-directed treatment. The median number of lines of salvage therapy for patients with disease progression was one (IQR, 1–2). Seventy-seven of the 81 patients who received relapse treatment had complete details surrounding the modalities used (**Fig. 2B**). Three patients underwent surgical resection alone, five received radiation only, and 32 received only chemotherapy. For those receiving multimodal relapse therapy, three had surgery and radiation, 13 had surgery and chemotherapy, 14 had radiation and chemotherapy, whereas seven received surgical resection, radiation, and chemotherapy. Details of relapse therapy were incomplete for four patients. One received chemotherapy without surgery with unknown radiotherapy status, two underwent surgery with unknown chemotherapy and radiotherapy status, and one had surgery with radiotherapy with unknown chemotherapy status.

For the 29 patients who had surgical resection at relapse, nine had GTR/NTR, six had subtotal resection, and three had partial resection, with the remaining undergoing an unknown extent of surgical resection. Of the 30 patients treated with radiation after relapse, 28 had focal radiation, one had CSI, and one had delivery of an unknown type. Twenty-six of the 30 patients received reirradiation to the prior radiation field. Reirradiation doses were available for 24 of 26 patients, with a median dose of 34 Gy (IQR, 30–42.5 Gy). Two patients had whole brain radiotherapy at 30 Gy, and one had 18 Gy via CyberKnife. For the 67 patients who received chemotherapy at relapse, 22 received TMZ-based chemotherapy, three received TMZ/CCNU, 39 received non-TMZ systemic therapy, and three additional patients received chemotherapy of an unknown type. The most frequently used non-TMZ therapy at

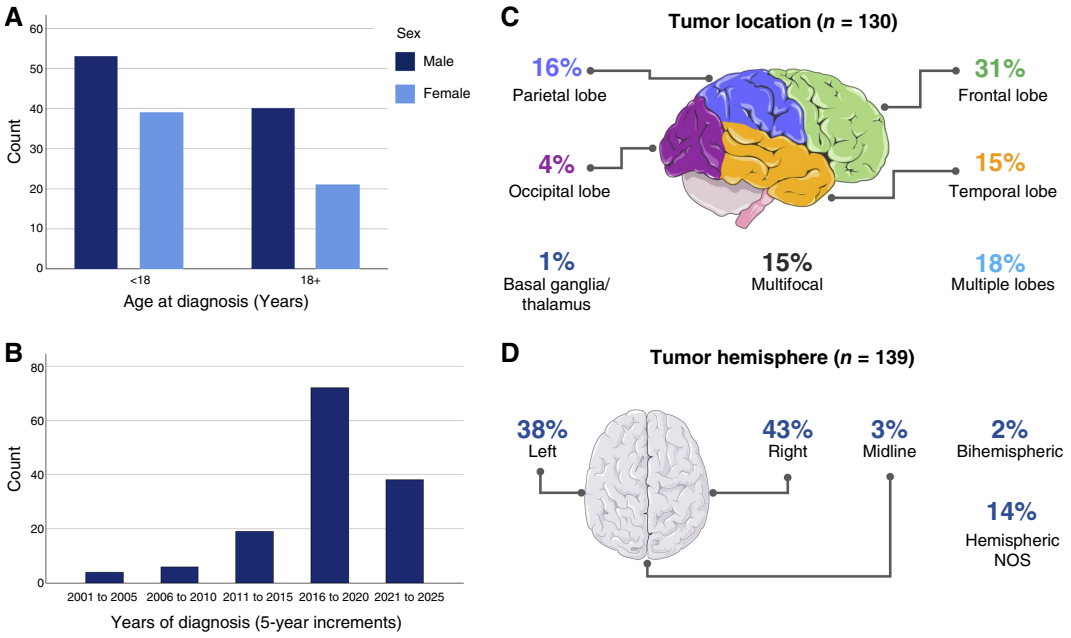

Figure 1.

Patient and tumor characteristics at disease diagnosis. **A,** Patient age and sex. **B,** Year of diagnosis. **C,** Anatomic location of tumor. **D,** Hemisphere involvement. NOS, not otherwise specified. [Figures 1C and 1D were adapted from Servier Medical Art (Servier; smart.servier.com), licensed under Creative Commons Attribution 4.0 International License (CC BY 4.0)].

relapse was bevacizumab, used in 23 patients, either alone or in conjunction with other agent(s).

Patient outcomes and prognostic factors

At a median follow-up of 20 months (IQR, 13–32), 111 (73%) patients died of disease, 31 (20%) were alive with disease, and 11 (7%) were alive without radiologic evidence of disease. Nine of these 11 patients without radiologic disease at the last follow-up had upfront GTR/NTR, whereas two patients had a short tandem repeat followed by radiation. The median PFS was 14 months (IQR, 12–19), and in those who relapsed, the median time from disease progression to death was 12 months (IQR, 6–21). The median OS for patients diagnosed with DHG, H3 G34 was 24 months (IQR, 22–28), with 12-, 24-, and 36-month OS rates of 81.3% (95% CI, 75.3–87.8), 49.2% (42.1–59), and 30.5% (23.3–39.9), respectively (**Fig. 3A**).

In univariable survival modeling, the Kaplan–Meier estimate of the OS function showed crossing survival functions (non-proportional hazards) for patient sex (**Fig. 3E**). In the first 18 months after diagnosis, females showed no significant difference in mortality compared with males (HR, 0.77; 95% CI, 0.43–1.36, $P = 0.36$). After 18 months, female patients showed a statistically significant increased risk of mortality (HR, 1.68; 95% CI, 1.065–3.088, $P = 0.031$). The selection of the time cut point to explore time by gender interaction was post hoc and data-driven. Based on these results, patient characteristics of females and males were compared, demonstrating that 92% of females (33/36) had *MGMT* promoter methylation compared with 57% (29/51) of males ($P = 0.0004$). This was the only variable indicating any statistically significant difference between sexes based on available covariates. Similarly, sex demonstrated a non-proportional hazard of PFS, with survival functions crossing multiple times, although the Wald χ^2 test was not statistically significant and was therefore not included in multivariable analysis.

Results generated from the univariable Cox analysis (**Table 2**) show that longer PFS was associated with greater extent of initial surgical resection (GTR/NTR vs. <NTR; HR, 0.52; 95% CI, 0.34–0.78), use of chemotherapy before disease relapse (HR, 0.53; 95% CI, 0.30–0.93), and inclusion of radiation as part of initial treatment (HR, 0.091; 95% CI, 0.047–0.18), whereas involvement of deep brain structures was associated with shorter PFS (HR, 1.71; 95% CI, 1.03–2.85). Exploratory multivariable analysis (**Table 2**; **Fig. 4**) showed longer PFS to be associated with the use of initial radiation (HR, 0.076; 95% CI, 0.033–0.17) and greater extent of initial surgical resection (GTR/NTR vs. < NTR; HR, 0.51; 95% CI, 0.33–0.78).

Univariable analysis revealed that longer OS was associated with older age at disease diagnosis by continuous 6-month increments (HR, 0.75; 95% CI, 0.61–0.89), a greater degree of initial surgical resection (GTR/NTR vs. < NTR; HR, 0.54; 95% CI, 0.34–0.84), use of initial radiation (HR, 0.19; 95% CI, 0.10–0.37), and use of chemotherapy prior to disease relapse (HR, 0.41; 95% CI, 0.23–0.69). A sex-stratified multivariable analysis shows greater OS to be associated with increasing age at diagnosis by continuous 6-month increments (HR, 0.70; 95% CI, 0.57–0.87), initial radiation (HR, 0.38; 95% CI, 0.15–0.96), and a greater degree of initial surgical resection (GTR/NTR vs. < NTR; HR, 0.60; 95% CI, 0.37–0.97).

Post hoc exploratory analysis

Additional post hoc exploratory survival analyses were completed for clinically relevant patient characteristics. Patients who received initial TMZ/CCNU ($n = 30$) versus TMZ alone ($n = 88$) as part of initial therapy after radiation had improved PFS (HR, 0.57; 95% CI, 0.35–0.93; $P = 0.02$). When looking at both adult and

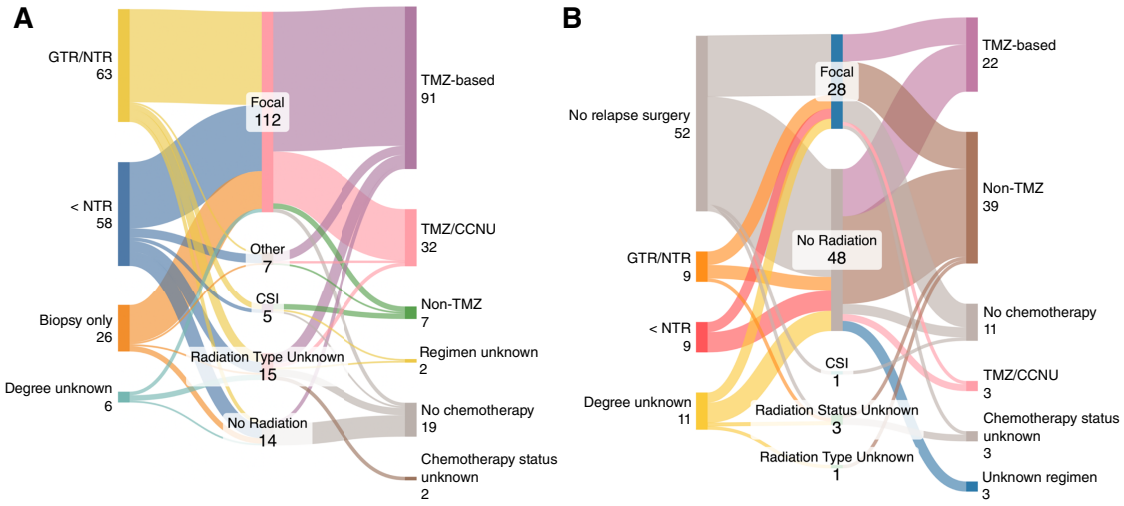

Figure 2.

Regimen details. **A,** Treatment before disease relapse. **B,** Treatment at disease relapse. (Left) Extent of surgical resection, (middle) radiation modality used, and (right) chemotherapy regimen used.

pediatric patients, there was no significant OS difference between TMZ/CCNU ($n = 32$) versus TMZ alone ($n = 91$; HR, 0.65; 95% CI, 0.39–1.11; $P = 0.11$). However, in the assessment of the subgroup of patients <18 years old, OS was improved with TMZ/CCNU ($n = 27$) versus TMZ alone ($n = 41$; HR, 0.48; 95% CI, 0.26–0.87; $P = 0.015$; Supplementary Fig. S2B). In our cohort, *MGMT* promoter methylation was not associated with improved PFS ($P = 0.24$) or OS ($P = 0.62$), whereas *PDGFRA* mutation was associated with improved OS ($P = 0.032$; Supplementary Fig. S2G). Additional analysis revealed that those with well-defined margins on MRI at disease diagnosis demonstrated longer PFS (HR, 0.48; 95% CI, 0.27–0.83; $P = 0.0093$), although OS was not different (HR, 0.57; 95% CI, 0.32–1.03; $P = 0.061$). Furthermore, patients with well-defined tumor margins compared with infiltrative margins on MRI were more likely to achieve GTR/NTR (81.8% vs. 21.4%, $P < 0.0001$). With respect to those with G34V mutations ($n = 9$), survival was not different compared with those with the G34R mutation ($P = 0.61$; **Fig. 3F**).

We subsequently looked at post-relapse survival, although missing post-relapse data points were not random and were more likely to be missing in those who died. Post hoc univariable assessment showed that patients who had surgery at relapse had a longer median time to death, 16 months (95% CI, 11–22) versus 7 months (95% CI, 5–8) for those without surgery ($P = 0.0049$). Similarly, those who had chemotherapy after relapse had a longer median time to death at 6 months (95% CI, 4–8) versus 10 months (95% CI, 8–16) in favor of chemotherapy ($P = 0.0001$). Radiation after progression did not seem to affect the time from relapse to death for those receiving radiation [median, 9.0 months (95% CI, 7–17) versus 8 months (95% CI, 7–10), $P = 0.9166$].

Sensitivity analysis

Results remained consistent for univariable analysis when country was incorporated into the models as a cluster variable, except for mutation subtype in the PFS outcome model. With only eight (5.6%) patients having the G34V mutation subtype and seven located in the United States, the data were underpowered.

Long-term survivors ≥5 years

Our cohort identified 12 (8%) patients who lived with DHG, H3 G34 beyond 5 years after diagnosis (Supplementary Table S5). The median length of follow-up for these patients was 72 months (range, 67–81). At the last follow-up, seven (58%) had died of disease, two (17%) were alive with disease, and three (25%) remained alive without radiologic evidence of disease. Eight (67%) were <18 years, and nine (75%) were male. All long-term survivors had the G34R mutation subtype with initially localized disease and received initial chemotherapy and irradiation. Eleven of the 12 patients had complete initial treatment details, and 10 received initial surgical resection, nine (82%) had GTR/NTR, one had < NTR, and one patient had biopsy only. For the patients treated with irradiation, 10 received focal radiation, one had CSI, and all had received initial TMZ-based therapy (four with CCNU and seven without CCNU). With respect to tumor biology for long-term survivors, *MGMT* promoter methylation was present in 88% (7/8), 78% (7/9) had *ATRX* loss, 91% (10/11) had *TP53* alteration, 83% (5/6) had *PDGFRA* mutation, and 14% (1/7) had *MYC-N* amplification.

Discussion

Our cohort demonstrates that patients with DHG, H3 G34 are diagnosed at an average age of 17 years and have a median OS of 24 months (IQR, 22–28). Although this OS is longer than reported in a recent systematic review, it is consistent with the findings described in a retrospective cohort by Le Rhun and colleagues and a literature analysis by Williams and colleagues, each focusing on DHG, H3 G34 (3, 20, 26). Our cohort shows longer OS relative to other HGG types, surpassing the median of 15 months for IDH wild-type (WT) glioblastoma and 11 months for diffuse midline glioma, H3 K27M–mutant (H3 K27M-mt DMG; refs. 27, 28). The median age in our study matches that of Williams and colleagues, confirming a predominantly pediatric population that extends into young adulthood, but is younger than the median age of 22 years

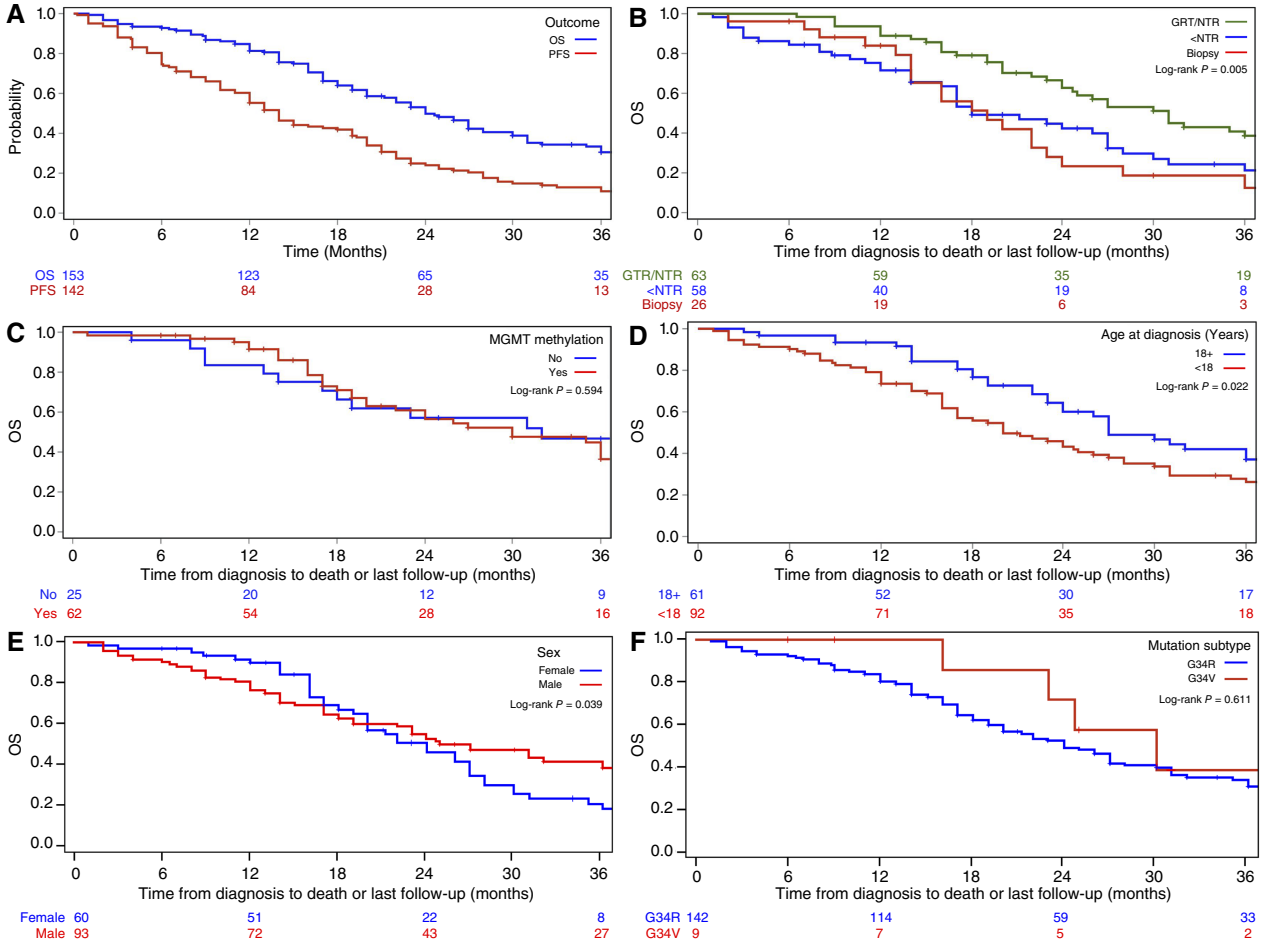

Figure 3.

Kaplan–Meier survival plots. **A,** Patient cohort demonstrating PFS and OS. **B,** Degree of initial surgical resection: <NTR vs. GTR/NTR vs. biopsy only. **C,** *MGMT* promoter methylation: present vs. absent. **D,** Age at disease diagnosis: <18 years old vs. ≥18 years old. **E,** Patient sex: female vs. male. **F,** Mutation subtype: G34R vs. G34V.

reported by Le Rhun and colleagues, which was predominantly a cohort of patients >18 years old (20, 26). Nearly 8% of our patients survived longer than 5 years, closely reflecting the 7% reported in Le Rhun and colleagues (20). In a cohort of adolescent and young adult patients with gliomas, DHG, H3 G34 accounted for <1% of all gliomas and was about eight times less common than H3 K27M-mt DMG and three times less common than diffuse pediatric-type HGG, H3 WT, and IDH WT (1). Median survival of DHG, H3 G34 was slightly longer than for these other pediatric-type diffuse HGG subtypes, consistent with the outcomes observed in our cohort (1).

OS seems significantly improved in patients diagnosed at an older age. In addition, patient sex may influence outcomes. Although prior studies have suggested female sex as a favorable prognostic factor, our cohort revealed a notable interaction between sex and OS in which after 15 to 18 months, females exhibited a higher risk of mortality compared with males (20). Exploratory analysis showed that females were more likely to have *MGMT* promoter methylation ($P = 0.0004$). These findings suggest that similar to other cancers, the prognostic roles of age and sex may reflect underlying epigenetic differences or the developmental timing of the tumor progenitor cell (29). Future tumor biology studies could help clarify how age and sex influence disease behavior and outcomes.

We confirmed that improved PFS and OS were associated with more extensive initial surgical resection, which is consistent with previous studies (3, 20, 26, 30, 31). Additionally, exploratory analysis revealed that patients who presented with well-defined tumor margins on MRI were more likely to receive initial GTR/NTR ($P < 0.0001$) and experience a longer period of disease control ($P = 0.0093$). Ill-defined tumor margins make it impractical to grossly distinguish tumor from surrounding healthy brain parenchyma, limiting the ability to achieve complete resection and increasing the likelihood of tumor recurrence (32, 33). Given our findings, clinical trials involving DHG, H3 G34 should prospectively assess outcomes of patients stratified based on the extent of initial resection, as this is a consistent finding across several studies. Additionally, initial radiotherapy was also associated with improved PFS and OS. However, assessing the true impact of radiation is challenging, as only a small subset of patients did not

Table 2. Univariable and multivariable Cox regression for PFS and OS.

	Univariable			
	PFS		OS	
Variable	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**
Age				
6-month increase in age (continuous)	1 (0.85–1.18)	0.98	0.75 (0.61–0.89)	**0.0027**
Age				
≥18 years old (ref) vs. <18 years old	0.94 (0.65–1.35)	0.73	1.57 (1.06–2.34)	**0.022**
Sex				
Female vs. male (ref)	1.22 (0.84–1.75)	0.31[a]		
Sex[a]				
Female vs. male (ref) ≤18 months			0.77 (0.43–1.36)	0.36
Female vs. male (ref) >18 months			1.68 (1.07–3.09)	**0.031**
Primary tumor histology				
HGG (ref) vs. embryonal	0.95 (0.46–1.97)	0.89	0.88 (0.39–2.01)	0.77
Mutation subtype				
G34V (ref) vs. G34R	0.72 (0.35–1.48)	0.38	1.27 (0.52–3.11)	0.61
Deep structure involvement				
No (ref) vs. Yes	1.71 (1.03–2.85)	**0.039**	1.44 (0.85–2.45)	0.17
Degree upfront surgery				
<NTR (ref) vs. GTR/NTR	0.52 (0.34–0.78)	**0.0016**	0.54 (0.34–0.84)	**0.0058**
<NTR (ref) vs. biopsy	1.53 (0.91–2.59)	0.1095	1.01 (0.56–1.79)	0.98
Initial radiation				
No (ref) vs. yes	0.091 (0.047–0.18)	<**0.0001**	**0.19 (0.10–0.37)**	<**0.0001**
Initial chemotherapy				
No (ref) vs. yes	0.53 (0.30–0.93)	**0.028**	0.41 (0.23–0.69)	**0.0012**
	Multivariable			
	PFS *N* = 142		**OS *N*= 153**	
Age	—	—		
6-month increase in age (continuous)			0.70 (0.57–0.87)	**0.0011**
Degree upfront surgery				
<NTR (ref) vs. GTR/NTR	0.51 (0.33–0.78)	**0.0017**	0.60 (0.37–0.97)	**0.039**
<NTR (ref) vs. biopsy	1.68 (0.95–2.97)	0.076	1.34 (0.72–2.49)	0.361
Initial radiation				
No (ref) vs. yes	0.076 (0.033–0.17)	<**0.0001**	0.38 (0.15–0.96)	**0.042**
Deep structure involved				
Yes (ref) vs. no	0.88 (0.50–1.57)	0.67	1.24 (0.62–2.48)	0.53
Initial chemotherapy				
No (ref) vs. yes	0.96 (0.49–1.99)	0.91	0.54 (0.24–1.18)	0.12

[a]Sex for OS and PFS outcomes showed multiple crossings of hazards.
Bold values indicate statistically significant results.

receive radiation before relapse and often experienced rapid disease progression.

In our cohort, patients received variable treatment plans, supporting the fact that there is no standard of care for these devastating tumors. Despite this, patients typically receive initial maximal safe resection, focal radiotherapy, and chemotherapy consisting of TMZ with or without CCNU (Research Square rs.3.rs-5375436/v1; 3, 20, 31, 34). A quarter of our cohort was treated with TMZ in combination with CCNU. These patients were more likely to be <18 years ($P = 0.0006$) and were likely following the pediatric treatment as per the Children's Oncology Group ACNS0423 study (31). Based on this dichotomization of chemotherapy data, we found improved PFS for those receiving adjuvant TMZ and CCNU combination compared with TMZ alone. The OS data demonstrated an improvement only in those <18 years old in favor of the TMZ and CCNU combination. This analysis was limited, as it was completed via post hoc univariable analysis. In addition, the number of patients ≥18 years of age receiving TMZ and CCNU was limited. Taken together, our data and previously published data support the continued use of adjuvant radiation plus alkylator chemotherapy (i.e., TMZ for adults and TMZ with or without CCNU for pediatric patients with DHG, H3 G34) outside of clinical trials, as suggested by the current National Comprehensive Cancer Network pediatric and adult CNS cancer guidelines (35–38).

The median time from progression to death was 12 months (IQR, 6–21), and close to 40% of patients will have distant or combined patterns of disease relapse, suggesting that imaging of both the brain and spine should be performed at diagnosis and during surveillance. DHG, H3 G34 are known to further acquire mutations at relapse,

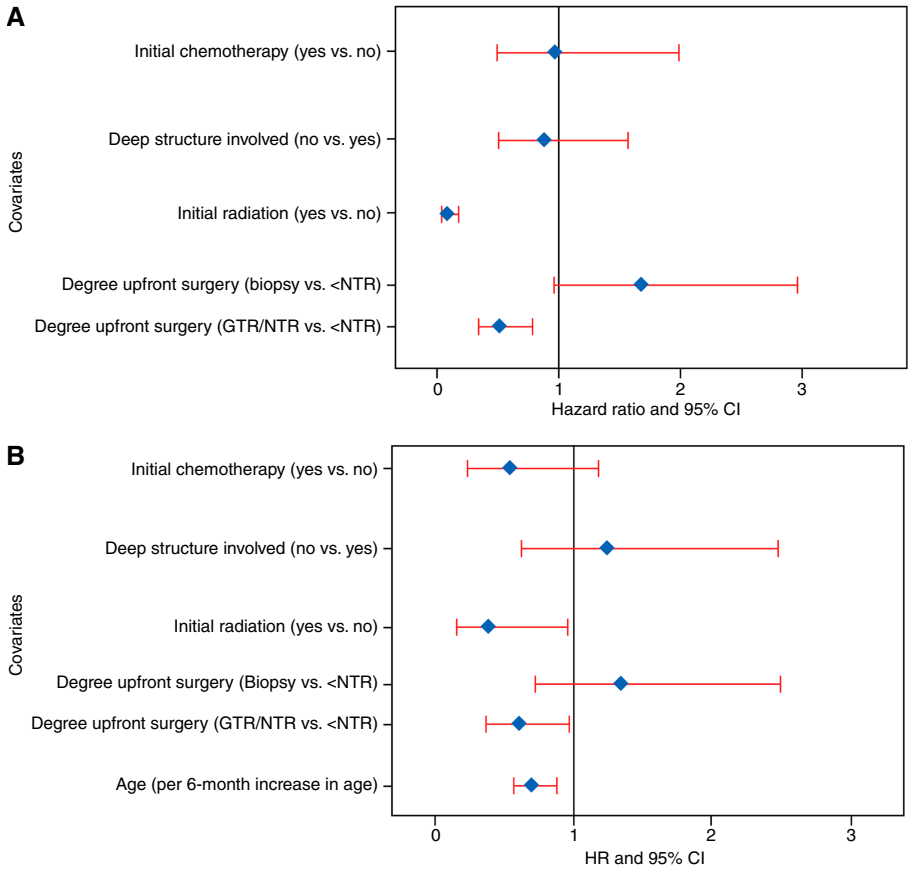

Figure 4.
Forest plots based on multivariable analysis. **A,** PFS. **B,** OS.

with *PDGFRA* mutation rates at relapse increasing from ~50% to more than 80%. Some have described posttreatment hypermutation, suggesting biological degeneration and helping explain the acceleration of disease processes at relapse (9, 39). These findings suggest that repeat biopsies or the use of ctDNA in cerebrospinal fluid at disease progression may help guide ongoing treatment decisions.

Following disease relapse, more than 75% of patients received some form of salvage therapy, including various combinations of surgery, radiation, and chemotherapy, although most were treated with chemotherapy alone. Among the 30 patients who received radiation at relapse, 26 underwent reirradiation within overlapping fields from initial treatment. Recognizing the limitations of incomplete data, both post-relapse surgery and chemotherapy were associated with longer survival, whereas post-relapse irradiation was not. We hypothesize that radiation resistance may be occurring due to biological evolution and possibly related to the high prevalence of *TP53* mutations previously associated with radiation resistance (40).

Our cohort confirms the overall poor prognosis of DHG, H3 G34, with most patients experiencing rapid disease progression and dying within a short time after diagnosis. Nonetheless, a small subset of patients survived longer than 5 years after diagnosis. All long-term survivors had the G34R subtype, presented with localized disease at diagnosis, and received both initial radiation and chemotherapy; the majority also underwent initial surgical resection, achieving GTR or NTR. However, only a minority of these long-term survivors remained disease-free at last follow-up, with more than half ultimately dying from their disease despite surviving beyond 5 years. These findings highlight the devastating trajectory of this tumor but suggest that DHG, H3 G34 exhibits a biological spectrum of aggressiveness that may share some parallel patterns observed in IDH-mutant gliomas (41, 42).

Investigation of molecular biomarkers that stratify clinical behavior continues to be an area needing further understanding, especially about how it might help us halt disease progression. Our cohort contained 6% of patients with the G34V mutation subtype, which is consistent with other published literature (20, 26). We did not detect a survival difference between subtypes, unlike a recent review, although both analyses are impaired by small numbers (26). *MGMT* promoter methylation was common in our cohort. *MGMT* promoter methylation status has been confirmed as an independent prognostic factor in patients with primary adult glioblastoma, regardless of adjuvant therapy, as well as a predictive factor for survival benefit with TMZ (43–45). However, *MGMT* promoter methylation does not represent an independent prognostic biomarker in patients with pediatric HGG, and its role in predicting benefit from TMZ in this population remains unclear (17, 46). In our cohort, *MGMT* promoter methylation was not associated with a statistically significant improvement in overall OS, contrasting with prior reports. However, this finding is consistent with the observations of Williams and colleagues (7, 20, 26).

TP53 and *ATRX* mutations were present in the tissue of most patients in our cohort, consistent with previous work suggesting that these mutations are an essential and unifying feature of DHG, H3 G34 (4, 7). Our cohort displayed *ATRX* mutations in 75% of

patients, which is about 10% lower than previously described, warranting additional exploration, as this may be due to testing variation across sites and interpretation (9, 11). Nearly 60% of patients demonstrated *PDGFRA* mutation at diagnosis. When compared with the current literature, there are conflicting reports about the frequency of finding *PDGFRA* mutations in DHG, H3 G34 (9, 11, 19). Despite this, *PDGFRA* is considered to be a critical oncogenic driver in the development of DHG, H3 G34 through downstream signaling pathways (9). In our cohort, the *PDGFRA* mutation seemed to be a positive prognostic factor for OS, in contrast to prior studies suggesting an association with poor survival (19, 47). This observation should be interpreted cautiously, as molecular data for *PDGFRA* were incomplete for a considerable subset of patients. Our high frequency of *PDGFRA* amplification (38%) is unusual, further urging the need for a central registry with tissue specimen requirements (9). Future work should also explore *PTEN* and *CDKN2A/B* mutations as well as broader biological underpinnings, as these have been found to be more common in DHG, H3 G34 when compared with H3 K27M-mutated gliomas (11). Our molecular data for this study came from institutional reporting in which a significant proportion of data were missing or absent. Therefore, our molecular analyses are less robust than some previous reports, and molecular variables were not included in our multivariable analyses.

Limitations of our study include its data sourcing from multiple centers, its retrospective nature using convenience sampling, and the lack of central review for imaging and molecular data relying on individual center reporting. Imaging patterns at diagnosis and patterns of relapse, including the presence or absence of leptomeningeal disease, could not be verified centrally and remain important avenues for future investigation. Missing variables about molecular correlates were a challenge and are important both at diagnosis and at progression. These items highlight the continued need for wide international efforts, as well as the creation of a prospective international patient registry. Given disparities in molecular coalteration frequency and missing data, extensive tissue analysis continues to be necessary to correlate better with modifiable treatment and clinical factors.

The extent of resection (GTR/NTR) and radiotherapy are identified as the most important treatment factors that correlate consistently with improved PFS and OS. Our data suggest a potential benefit in adding CCNU to TMZ, especially for patients <18 years old. However, the true benefit of dual alkylators in this population should be further investigated. Older age also demonstrated improved OS, and our findings about sex and well-defined imaging margins suggest that biological correlates should be further explored to help explain these findings. The development of clinical trials, an international registry, and a biobank should be pursued for this rare tumor to help improve understanding and refine treatment algorithms.

Data Availability

Data supporting the findings of this study will be made available upon reasonable request to the corresponding author and subject to local data protection laws.

Authors' Disclosures

J. Bennett reports personal fees from Servier Canada, Alexion Canada, and Rhythm Pharmaceuticals outside the submitted work. S. Perreault reports grants from Bayer, Novartis, and SpringWorks Therapeutics and personal fees from Alexion, Ipsen, and DayOne outside the submitted work. M. Zápotocký reports personal fees and nonfinancial support from AstraZeneca and nonfinancial support from Ipsen outside the submitted work. J.R. Hansford reports other support from Servier Pharma, Alexion Pharma, EcoR1, and Medison Pharma outside the submitted work. S. Cheng reports other support from Ipsen and Alexion outside the submitted work. N. André reports grants and other support from Bristol Myers Squibb and AstraZeneca; other support from Pierre Fabre, Norgine, Novartis, and ITM; nonfinancial support from Merck; personal fees from Ipsen; and personal fees and other support from Alexion outside the submitted work. M.J. Lim-Fat reports other support from Ipsen outside the submitted work. S. Lapointe reports personal fees from Bayer, Alexion, Novocure, Servier, and GSK outside the submitted work. U. Sener reports personal fees from Merck & Co and Alexion Pharmaceuticals outside the submitted work. C. Hawkins reports personal fees from Servier Canada during the conduct of the study. B.S. Imber reports grants and personal fees from GT Medical Technologies and grants from AstraZeneca and Novartis outside the submitted work. M.A. Karajannis reports grants from the NIH/NCI during the conduct of the study and personal fees from PeerView, Aptitude, Physicians' Education Resource, and Medscape outside the submitted work. D.T.W. Jones reports other support from Heidelberg Epignostix GmbH outside the submitted work and patents for Method for classification of cancer and Method for characterization of cancer pending and licensed to Heidelberg Epignostix GmbH. K. Ligon reports other support from Servier (consultant) and Travera (consultant; equity). C. Erker reports other support from Ipsen and Alexion outside the submitted work. No disclosures were reported by the other authors.

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Note

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