Prognostic impact of distinct genetic entities in pediatric diffuse glioma WHO-grade II—Report from the German/Swiss SIOP-LGG 2004 cohort

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Abstract
Reports on pediatric low-grade diffuse glioma WHO-grade II (DG2) suggest an impaired survival rate, but lack conclusive results for genetically defined DG2-entities. We analyzed the natural history, treatment and prognosis of DG2 and investigated which genetically defined sub-entities proved unfavorable for survival. Within the prospectively registered, population-based German/Swiss SIOP-LGG 2004 cohort 100 patients (age 0.8-17.8 years, 4% neurofibromatosis [NF1]) were diagnosed with a
INTRODUCTION

Low-grade gliomas (LGG) are a heterogeneous group of slow-growing glial or glioneuronal brain tumors usually assigned to WHO-grades I or II, which may arise in all regions of the CNS. Circumscribed pilocytic astrocytomas (PA; WHO-grade I) represent the best characterized and largest subgroup in children and adolescents with a share of 70% to 80% in most pediatric series. According to the 2016 WHO-classification of tumors of the central nervous system diffuse gliomas of WHO-grade I (DG2) include diffuse astrocytomas WHO-grade I (DA) and oligodendrogliomas WHO-grade II (ODG). Mixed gliomas (oligoastrocytoma WHO-grade II [OA]) were considered to mostly represent either DA or ODG as defined by genetic features, in particular in adult patients. Although the infiltrative histologic pattern is identical to the adult counterpart, pediatric DG2 differ in terms of clinical behavior, genetics and prognosis from their adult counterparts. Diffuse low-grade gliomas in adults usually harbor IDH-gene mutations. While astrocytomas of adults are characterized by concomitant mutations of ATRX and TP53, ODG are defined by codeletion of chromosome arms 1p/19q with preserved ATRX-expression. Conversely, pediatric DG2 are usually IDH1/2-wild-type. While no constant mutations of IDH1/2 or ATRX were found in DA, pediatric ODG typically lack 1p/19q-codeletion. Whole genome analyses documented that pediatric LGG may harbor several different alterations of BRAF-, FGFR1-, MYB- or MYBL1-genes which are usually mutually exclusive. Moreover, pediatric DG2 comprise just 6% to 10% in LGG-cohorts (<18 years), and even recent series of molecularly investigated tumors report only up to 36 cases preventing general conclusions about the prognostic significance of their molecular genetic profile. While long-term overall survival (OS) is generally excellent in pediatric LGG, early series indicated shorter OS for diffuse astrocytoma as compared to PA and even in the more recent reports of Stokland et al and Ater et al OS remained reduced for DG2. Following biopsy (41%) or variable extent of resection (59%), 65 patients received no adjuvant treatment. Radiologic progression or severe neurologic symptoms prompted chemotherapy (n = 18) or radiotherapy (n = 17). Multiple lines of salvage treatment were necessary for 19/35 patients. Five years event-free survival dropped to 0.44, while 5 years overall survival was 0.90 (median observation time 8.3 years). Extensive genetic profiling of 65/100 DG2 identified Histone3-K27M-mutation in 4, IDH1-mutation in 11, BRAF-V600-mutation in 12, KIAA1549-BRAF-fusions in 6 patients, while the remaining 32 tumor tissues did not show alterations of these genes. Progression to malignant glioma occurred in 12 cases of all genetically defined subgroups within a range of 0.5 to 10.8 years, except for tumors carrying KIAA1549-BRAF-fusions. Histone3-K27M-mutant tumors proved uniformly fatal within 0.6 to 2.4 years. The current LGG treatment strategy seems appropriate for all DG2-entities, with the exemption of Histone3-K27M-mutant tumors that require a HGG-related treatment strategy. Our data confirm the importance to genetically define pediatric low-grade diffuse gliomas for proper treatment decisions and risk assessment.

What’s new?

Pediatric low-grade diffuse gliomas histologically resemble their adult counterparts, but they differ greatly in terms of genetics, clinical behavior, and prognosis. Here, the authors investigated genetic mutations in 65 pediatric grade 2 diffuse gliomas (DG2), looking for a correlation with long-term outcome. All 4 tumors carrying the K27M mutation in the Histone3 gene were fatal. Conversely, none of the 6 tumors carrying a particular duplication of the BRAF gene, called the KIAA1549-BRAF fusion, progressed to malignant glioma. The authors characterized the tumor genetics with respect to prognosis, age at onset, and response to treatment.
not treated within a comprehensive treatment strategy.\textsuperscript{14,15,17,23,24} Thus, no general conclusion could be drawn so far.

Therefore, we investigated the natural history, response to treatment and long-term outcome of 100 pediatric patients with centrally confirmed DG2 who were included in the prospectively registered, population-based German/Swiss cohort of the SIOP-LGG 2004 study. We focused on the frequency of genetically defined DG2-entities and their prognostic impact.

2 | PATIENTS AND METHODS

2.1 | Eligibility

The prospective, multinational/multicenter SIOP-LGG 2004 study registered patients with LGG of all CNS-sites from 2004 to 2012. Inclusion criteria comprised age <18 years, histologic diagnosis of LGG according to the effective WHO-classification (2000, 2007), without prior nonsurgical therapy.\textsuperscript{19} Central review for radiology and neuropathology recommended; central neuropathological review was mandatory for DG2 included in this series.

Informed consent was obtained from patients, parents and/or guardians. The Institutional Review Board approved SIOP-LGG 2004 study observed the Declaration of Helsinki in its revised version (Edinburgh, Scotland, 2000), the WHO and European Community rules of "Good Clinical Practice" (effective January 17, 1997). The SIOP-LGG 2004 study was registered at ClinicalTrials.gov PRS NCT00276640, EudraCT number 2005-005377-29.

2.2 | Treatment strategy

All patients with DG2 followed the study strategy: at diagnosis, best safe resection of the primary tumor was recommended. Patients with complete resection were to be observed, as well as patients following incomplete resection, biopsy or radiological diagnosis if no threatening neurological symptoms were present. Severe initial symptoms or clinical/radiological progression during observation were an indication for the start of nonsurgical/adjuvant treatment, if resection remained infeasible. Children <8 years and all children with neurofibromatosis (NF1) were allowed to receive primary chemotherapy. Older children ≥8 years without NF1 were allowed to receive either primary RT or chemotherapy.

Chemotherapy was scheduled for 18 months with a 24-week induction (7 courses at 3-4 weeks intervals) and 60 weeks consolidation (10 courses every 6 weeks). Details for standard (vincristine/carboplatin) and intensified (additional etoposide) induction and consolidation were reported by Gnekow et al.\textsuperscript{19} Focal radiotherapy (RT) was scheduled with a total dose of 54 Gy (1.8 Gy/fraction). Brachytherapy with 125-Iodine-seeds was applied for suitable tumors.\textsuperscript{25} Treatment for progression after primary RT or chemotherapy was not standardized, but included all modalities after discussion in local and reference tumor boards.

For radiological response assessment, contrast-enhanced MRI was performed at defined intervals at week 24, 54 and 85 after the start of nonsurgical therapy. Complete, partial and objective responses (regression) as well as stable disease (SD) were considered positive responses.\textsuperscript{19,26}

2.3 | Materials and neuropathological evaluation

Formalin-fixed paraffin-embedded (FFPE) tissue specimens of DG2 were retrieved from the archive of the German DGN Brain Tumor Reference Center, Bonn, Germany.

All DG2-tumors were re-reviewed by two neuropathologists (M. G., T. P.) and classified according to the WHO-classification of tumors of the CNS\textsuperscript{2} using standard histological and immunohistochemical methods. Nondiffuse low-grade astrocytic tumors such as PA and glioneuronal tumors were not included in our study.

Immunohistochemical analysis (IHC) was performed on a Ventana Benchmark XT Immunostainer (Roche Ventana, Darmstadt, Germany) with antibodies against glial fibrillary acidic protein (GFAP; Dako, Glostrup, Denmark), synaptophysin, chromogranin, neurofilament protein, CD34 (all from Dako), microtubule-associated protein2 (Map2; Sigma, St. Louis, MO), p53-protein (DO-7, Dako), Olig-2 (R&D Systems, Abingdon, United Kingdom), alpha-thalassemia/mental retardation syndrome X-linked-protein (ATRX; Sigma), Ki67 (MIB-1; Dako), mutant BRAF-V600E (clone VE1, Roche), IDH-R132H (clone H09, Dianova) and H3-K27M (rabbit polyclonal, Millipore).

2.3.1 | DNA- and RNA-extraction

Hematoxylin-eosin (H&E) stained sections of each case were reviewed before selection for DNA-extraction. All samples selected contained at least 80% of vital tumor. DNA from FFPE- tumor-sections was purified using the QiAamp DNA Mini Tissue Kit (Qiagen GmbH, Düsseldorf, Germany) according to the manufacturer’s instructions after proteinase-K digestion. Total RNA was isolated from 5 μm-thick FFPE-tissues with RNeasy FFPE-kit (Qiagen). DNA-quantity and DNA-quality were determined by Qubit (Thermo Fisher) fluorometric measurement. RNA-quantity and RNA-quality were determined using NanoDrop 2000 instrument (Thermo Fisher).

2.3.2 | Pyrosequencing analysis of mutational hotspots of IDH1, IDH2, H3F3A, TERT and FGFR1

For pyrosequencing analysis, single-stranded DNA-templates were immobilized on streptavidin-coated Sepharose high performance beads (GE Healthcare, Uppsala, Sweden) using the PSQ Vacuum Prep Tool and Vacuum Prep Worktable (Biotage, Uppsala, Sweden), then incubated at 80°C for 2 minutes and allowed to anneal to 0.4 mM sequencing primer at room temperature. Pyrosequencing was performed using PyroGold Reagents (Biotage) on the Pyromark Q24 instrument (Biotage), according to manufacturer’s instructions.
2.3.3 | MLPA and FISH

MLPA analyses for the determination of chromosome 1p/19q copy-number status, the SALSA MLPA P088 Oligodendroglioma 1p-19q probemix (MRC Holland, Amsterdam, The Netherlands) assay was used in accordance with manufacturer’s instruction. For the detection of rearrangements/copy number alterations of BRAF, MYB, MYBL1, FGRFR1 and CDKN2A, the SALSA MLPA P370 probemix (MRC Holland) was used. Briefly, 100 ng of tumor-DNA was heat-denatured for 5 minutes and cooled down to 25°C. Hybridization of the sample to probemix was performed for 16 hours at 60°C. After ligation, polymerase chain reaction (PCR) was carried out in a total volume of 50 μL containing 10 μL of the ligation mix in a thermocycler (Biometra, Göttingen, Germany). A LIZ-labeled internal size standard was added to the tumor samples. Fragments were separated and quantified on an ABI3730 capillary sequencer after denaturation (Applied Bioscience, Darmstadt, Germany) and afterward analyzed with the GeneMapper software (Applied Bioscience). After normalization of the assay against normal cerebellar tissue (FFPE material), a difference of minus threefold standard deviation from the mean was considered as significant loss. MYB-FISH1 analysis was performed with commercial probes (Cytocell) as published previously.23

2.3.4 | RNA-analysis for KIAA1549-BRAF and other recurrent fusions by Nanostring assay

The information of probes for detection of fusion genes is given in Table S1. A total of 100 ng RNA was added to the nCounter Elements TagSet in hybridization buffer and incubated at 67°C for 20 hours. Samples were then processed on the nCounter Preparation Station and cartridges were scanned on the nCounter Digital Analyzer. Raw NanoString counts were subjected to normalization using counts obtained for positive control probe sets. The normalized data was then subjected to background noise subtraction. A statistical outlier detection method was used to detect the presence of fusion/duplication events.

2.3.5 | Molecular inversion probe assay

To identify copy-number gains and losses, we used a molecular inversion probe (MIP) array including approximately 330 000 inversion probes (Thermo Fisher, Santa Clara, CA). The MIP assay was performed according to the protocols of the manufacturer. The raw MIP data file was analyzed using the Nexus Copy Number 8.0 Discovery Edition software (Biodiscovery, El Segundo, CA). SNPFAST2 segmentation algorithm was used to make copy-number and loss of heterozygosity (LOH) calls.

2.3.6 | Epigenetic classification

A total of 250 ng of tumor-derived DNA was bisulfite-converted and hybridized to Illumina Epic methylation bead chips. Epigenetic classification was performed as published by Capper et al.27

2.4 | Statistics

Median and range are given for continuous variables; absolute and relative frequencies for categorical variables. Comparisons of two unpaired samples regarding a categorical/continuous variable were performed using Fisher’s exact test/Mann-Whitney U test.

Survival curves were estimated by the Kaplan-Meier method and compared between independent groups using log-rank test. OS was calculated from date of diagnosis until death (of any cause), but from start of therapy in the chemotherapy and RT subgroups. Event-free survival (EFS) was calculated from date of diagnosis until event, defined as relapse after complete resection, clinical or radiological progression, start of nonsurgical therapy or death. OS and EFS were calculated from the date of surgery to evaluate the variable resection. PFS was calculated from start of nonsurgical therapy until event, defined as relapse after complete remission, clinical or radiological progression or death.

Multivariable Cox regression with forward stepwise selection (inclusion criterion: score test \( P \leq .05\); exclusion criterion: likelihood ratio test \( P \geq .10\) ) was used to analyze the prognostic values of molecular biomarkers and clinical features on OS and EFS. Variables included age at diagnosis (\( \leq 1 \) year, \( 1 \) to \( <5 \) years, \( 5 \) to \( <11 \) years, \( 11 \) to \( <16 \) years, \( 16 \) years), sex, NF1-status, localization, tumor diameter and contrast enhancement on diagnostic MRI, extent of resection, histology, genetic subgroup (IDH1-mutant, KIAA1549-BRAF-fused, BRAF-V600E-mutant, wild-type for these genes). Patients aged \( <1 \) year at diagnosis (\( n = 2 \)) or with Histone3-K27M-mutation (\( n = 4 \)) were excluded from multivariable analysis. Extent of resection was included as time-dependent variable for OS and EFS coming into effect at the date of surgery. Results are reported as hazard ratios (HR) with 95% confidence interval (CI) and \( P \)-value of likelihood-ratio test for selected variables.

Analyses were exploratory, and \( P \) values were considered as descriptive measures to detect and study meaningful effects. In particular, no significance level was fixed.

3 | RESULTS

Diffuse glioma grade 2 (DG2) was diagnosed in 114 patients (8.6%) among 1320 histologically verified LGG from the German/Swiss SIOP-LGG 2004 cohort (\( n = 1645 \)). Fourteen patients without central neuropathological review of tumor tissue were excluded (Figure 1). Data for the remaining 100 patients are summarized in Table 1. Median age at diagnosis was 9.5 years; NF1 was diagnosed clinically in four patients.

The majority of tumors were localized in the cerebral hemispheres (42%); thalamic localization was predominant for tumors in the supratentorial midline (21/27). DG2 occurred at similar frequencies in the caudal brainstem, cerebellum and spinal cord. No tumor was disseminated at diagnosis, but progression with dissemination occurred in two patients.

The diagnostic MRI showed a largest tumor diameter of 0.8 to 10.7 cm (median 3.3 cm) in 80 patients, while irregular shape
precluded measurements in 20 patients. Margins were not sharply delineated in 38/81, moderately sharp in 26/81 and sharp in 17/81 tumors (no information 19/100). Mostly inhomogeneous contrast-enhancement of light, moderate or high intensity according to standards of the German pediatric brain-tumor-network radiologic reference-center\textsuperscript{26,28} was detectable in 33/92 patients, it was absent in 59/92 (no information 8/100). Cysts were described in 18/81 and perifocal edema in 16/83 cases (no information 19/100 and 17/100 cases, respectively). Radiologic review of diagnostic MRI was available for 76/100 patients and for 92/100 during follow-up.

At the time of diagnosis 92 patients were symptomatic with increased intracranial pressure (33/92), seizures (34/92), cranial-nerve palsies (5/92), long-tract paresis (11/92), pain/paresthesia (4/92), ataxia (3/92) or visual impairment (2/92). Furthermore, DG2 was an incidental finding in eight patients.

Although the majority of patients had an initial tumor volume reduction, 41 had primary biopsy only; 36 patients underwent several subsequent resections and biopsies. There were no surgery-associated deaths.

During the study period (median follow-up 8.3 years) 65 patients did not receive adjuvant treatment, while 18 received primary chemotherapy (13 standard vincristine/carboplatin, 3 intensified induction, 2 temozolomide) and 17 primary RT (Figure 1). Multiple lines of salvage treatment were necessary for 19/35 patients. In summary, 26 patients received RT as primary or salvage treatment (photons n = 18, protons n = 3, 125-iodine-seed n = 5). There was one chemotherapy-associated toxic death; 13 patients died.

Initial neuropathological diagnosis for the DG2 (n = 100) according to the WHO 2007-classification was diffuse astrocytoma (DA) in 89% of cases (Figure S1), oligoastrocytoma (OA) in 7% and oligodendroglioma (OGD) in 4%. Molecular/genetic assessment could be performed in 65/100 (57/89 DA, 5/7 OA, 3/4 OGD), while no further/insufficient amounts of FFPE-material was available for this purpose in 35 cases (Figure 2); these patients were excluded from further inter-group comparisons. IDH\textsuperscript{-}mutations were found in 11, B\textsuperscript{RAF}-V600\textsuperscript{-}mutations in 12, KIAA1549-B\textsuperscript{RAF}-fusions in 6 and Histone3-K27M-mutations in 4 DG2. These alterations were mutually exclusive. One IDH1-mutated tumor showed the genetic features of an adult-type oligodendroglioma with 1p/19q-codeletion and retained ATRX-protein. Thirty-two tumors were negative for these investigated genetic alterations. Extended analyses could be performed in 20/32 cases wild-type for these genes and where sufficient tumor material was available, but revealed no additional genetic alteration. In particular, methylation-based classification did not result in a confident score for any of the defined methylation tumor subgroups according to Capper et al.,\textsuperscript{27} MYB-FISH, molecular inversion probe (MIP) and Nanostring assays for rare fusions were negative. The association between genetic parameters and clinical features are depicted in Table 2.

Median follow-up for the 65 patients with genetically classified tumors was 8.5 years. Median age at diagnosis was 9.6 years and highest for patients with IDH1-mutated tumors (13.2 years). The four histological DG2 with Histone3-K27M-mutation (6%) were localized in the midline (thalamus, 2 cases; caudal brainstem, 2 cases), with a median tumor diameter of 3.6 cm. After initial biopsy, adjuvant...
| Table 1 | Epidemiologic data, DG2-cohort |
|---------|--------------------------------|
|         | All (n = 100) | Chemotherapy (n = 18) | Radiotherapy (n = 17) |
| Gender  |               |                       |                       |
| Female  | 46            | 10 (56%)              | 9 (53%)               |
| Male    | 54            | 8 (44%)               | 8 (47%)               |
| Neurofibromatosis NF I | 4 | 2 (11%) | 0 |
| Median age at diagnosis (years; range) | 9.5 (0.8-17.8) | 4.2 (1.2-16.0) | 10.9 (1.4-16.3) |
| Age group |               |                       |                       |
| <1 year | 2             | 0                      | 0                      |
| ≥1 to <5 years | 24 | 9 (50%) | 2 (12%) |
| ≥5 to <11 years | 34 | 6 (33%) | 7 (41%) |
| ≥11 to <16 years | 32 | 2 (11%) | 7 (41%) |
| ≥16 years | 8             | 1 (6%)                 | 1 (6%)                 |
| Age group |               |                       |                       |
| ≤1 year | 2             | 0                      | 0                      |
| >1 to <8 years | 40 | 13 (72%) | 5 (29%) |
| ≥8 years | 58            | 5 (28%)                | 12 (71%)               |
| Dissemination |         |                       |                       |
| Primary | 0             | 0                      | 0                      |
| Secondary | 2            | 1 (6%)                 | 1 (6%)                 |
| Tumor localization |       |                       |                       |
| Cerebral hemispheres | 42 | 4 (22%) | 3 (18%) |
| Supratentorial midline | 27 | 6 (33%) | 7 (41%) |
| Mono-/bi-thalamic | 15/6 | 2/3 | 1/1 |
| Visual pathways | 1 | 0 | 1 |
| Cerebellum | 10 | 0 | 1 (5.5%) |
| Caudal brainstem | 11 | 5 (28%) | 3 (18%) |
| Spinal cord | 10 | 3 (17%) | 3 (18%) |
| Tumor diameter at initial MRI (cm; median, range) | 3.3 (0.8-10.7) | 4.9 (1.9-10.7) | 3.4 (0.8-5.7) |
| No data | 20            | 2 (11%)                | 3 (18%)                |
| Extent of resection |       |                       |                       |
| Complete | 26            | 1 (6%)                 | 0                      |
| Subtotal | 10            | 0                      | 1 (6%)                 |
| Partial | 23            | 3 (17%)                | 4 (23%)                |
| Biopsy (stereotactic/endooscopic) | 41 (29/2) | 14 (77%) (10/0) | 12 (71%) (10/0) |
| Patients with re-interventions (median number of surgeries; range) | 36 (1; 1-3) | 10 (2; 1-3) | 7 (1; 1-3) |
| Histology |       |                       |                       |
| Diffuse astrocytoma | 89 (4 NF1) | 15 (83%) | 16 (94%) |
| Oligoastrocytoma | 7 | 2 (11%) | 1 (6%) |
| Oligodendroglioma | 4 | 1 (6%) | 0 |
| Malignant transformation (AA III/GBM IV/radiologic) | 12 (4/5/3) | 3 (17%) (1/2/0) | 3 (18%) (0/0/3) |
| Median observation time (years; range) | 8.3 (0.04-25.4) | 8.0 (0.04-14.9) | 9.0 (0.6-25.4) |
| No data | 20            | 2 (11%)                | 3 (18%)                |
| Last patient status |       |                       |                       |
| Alive |               |                       |                       |
| Complete remission | 33 | 1 (5.5%) | 4 (23.5%) |
| Stable disease | 47 | 11 (61%) | 9 (53%) |
treatment was started in three patients (two chemotherapy, one RT) due to severe neurologic symptoms (n = 2) or tumor progression (n = 1) 2 weeks to 4.4 months after initial diagnosis. One patient was initially observed, re-operated upon progression after 6 months; the tumor showed anaplasia in this sample. All four patients progressed and died despite salvage treatments after 0.6 to 2.4 years (median 1.6 years).

All IDH1-mutated tumors (17%) arose in the cerebral hemispheres; median tumor diameter was 4.9 cm. After partial resection or biopsy, five tumors progressed after 3.2 months to 9.7 years. In two tumors pathological examination showed progression to anaplastic astrocytoma in the second resection 0.9 and 1.9 years after initial diagnosis. After high-grade glioma (HGG) treatment, one patient was alive (follow-up 5.0 years), the other died. One patient was irradiated following further progression, one had multiple surgeries and the fifth received a sequence of standard chemotherapy-resection-salvage therapy. At 6.2 years median follow-up 10 patients were alive without (n = 3) or with stable (n = 7) disease.

The tumors with BRAF-V600-mutation (18%) were localized in the supratentorial midline (6/12), cerebral hemispheres (5/12) and cerebellum (1/12). Five patients progressed 1 month to 7.7 years after diagnosis. After initial resection in one patient and second resection in another, both achieved complete remission. One patient was irradiated and one received primary and salvage chemotherapy followed by second surgery with progression to anaplastic astrocytoma. This patient died. At 7.5 years median follow-up 10 patients were alive without (n = 6), with stable (n = 3) or progressive (n = 1) tumor.

Patients with tumors showing KIAA1549-BRAF-fusions (9%) had a median age at diagnosis of 4.8 years. The tumors occurred in the cerebellum (3/6), cerebral hemispheres (2/6) and caudal brainstem (1/6). Progression was observed in three patients following 7 months to

| TABLE 1 (Continued) | All (n = 100)a | Chemotherapy (n = 18)b | Radiotherapy (n = 17)b |
|----------------------|---------------|-----------------------|-----------------------|
| Progression         | 3             | 1 (5.5%)              | 0                     |
| Dead                | 13            | 5 (28%)               | 4 (23.5%)             |
| Not known/no data   | 4             | 0                     | 0                     |
| Median age at start of therapy (years; range) | —              | 4.3 (1.4-18.2)b       | 13.0 (4.9-17.8)       |

aNo percentages given, since cohort size is n = 100.
bPercentages relate to treatment group.
 cicluded if diagnosis <18 years of age/start of treatment at 18 years of age.
c Including one tumor of the lateral ventricle.
dMeasurement of tumor diameters in the three standard planes.

Three radiologic diagnosis of high-grade glioma (1 gliomatosis cerebri/1 diffuse intrinsic pontine glioma/1 new focal contrast enhancement). Abbreviations: AA III, (anaplastic) astrocytoma WHO-grade III; GBM IV, glioblastoma multiforme WHO-grade IV.
| Gender                  | Molecular-genetic group (n = 65) | Histone3-K27M (n = 4)a | IDH-mutant (n = 11) | BRAF-V600E (n = 12) | KIAA1549-BRAF-fusion (n = 6) | Wild-type (n = 32) |
|-------------------------|----------------------------------|------------------------|---------------------|---------------------|-------------------------------|------------------|
| Female                  | 27 (42%)                         | 2 (50%)                | 7 (64%)             | 3 (25%)             | 3 (50%)                       | 12 (37.5%)       |
| Male                    | 38 (58%)                         | 2 (50%)                | 4 (36%)             | 9 (75%)             | 3 (50%)                       | 20 (62.5%)       |
| Neurofibromatosis NF I  | 1 (1.5%)                         | 0                      | 0                   | 0                   | 0                             | 1 (3.1%)         |
| Median age at diagnosis (years; range) | 9.6 (0.8-17.8)             | 8.0 (1.5-13.4)         | 13.2 (9.8-17.5)     | 11.3 (0.9-13.2)     | 4.8 (2.8-17.8)                | 6.8 (0.8-15.6)   |
| Age group               |                                  |                        |                     |                     |                               |                  |
| <1 year                 | 2 (3%)                           | 0                      | 0                   | 1 (8.3%)            | 1 (3.1%)                      |
| ≥1 to <5 years          | 17 (26%)                         | 1 (25%)                | 0                   | 2 (16.7%)           | 3 (50%)                       | 11 (34.4%)       |
| ≥5 to <11 years         | 20 (31%)                         | 2 (50%)                | 1 (9%)              | 2 (16.7%)           | 2 (33.3%)                     | 13 (40.6%)       |
| ≥11 to <16 years        | 20 (31%)                         | 1 (25%)                | 6 (55%)             | 6 (50%)             | 0                             | 7 (21.9%)        |
| ≥16 years               | 6 (9%)                           | 0                      | 4 (36%)             | 1 (8.3%)            | 1 (16.7%)                     | 0                |
| Age group               |                                  |                        |                     |                     |                               |                  |
| ≤1 year                 | 2 (3%)                           | 0                      | 0                   | 1 (8%)              | 0                             | 1 (3%)           |
| >1 to <8 years          | 26 (40%)                         | 2 (50%)                | 0                   | 2 (17%)             | 5 (83%)                       | 17 (53%)         |
| ≥8 years                | 37 (57%)                         | 2 (50%)                | 11 (100%)           | 9 (75%)             | 1 (17%)                       | 14 (44%)         |
| Dissemination           |                                  |                        |                     |                     |                               |                  |
| Primary                 | 0                                | 0                      | 0                   | 0                   | 0                             | 0                |
| Secondary               | 2 (3.1%)                         | 1 (25%)                | 0                   | 0                   | 0                             | 1 (3.1%)         |
| Localization            |                                  |                        |                     |                     |                               |                  |
| Cerebral hemispheres    | 32 (49%)                         | 0                      | 11 (100%)           | 5 (41.7%)           | 2 (33.3%)                     | 14 (43.7%)       |
| Supratentorial midline  | 11 (17%)                         | 2 (50%)                | 0                   | 6 (50%)             | 0                             | 3 (9.4%)         |
| Mono-/bi-thalamic       | 7/2                              | 1/1                    | 0                   | 3/1                 | 0                             | 3/0              |
| Visual pathways         | 0                                | 0                      | 0                   | 0                   | 0                             | 0                |
| Cerebellum              | 9 (14%)                          | 0                      | 0                   | 1 (8.3%)            | 3 (50%)                       | 5 (15.6%)        |
| Caudal brainstem        | 6 (9%)                           | 2 (50%)                | 0                   | 0                   | 1 (16.7%)                     | 3 (9.4%)         |
| Spinal cord             | 7 (11%)                          | —                      | 0                   | 0                   | 0                             | 7 (21.9%)        |
| Tumor diameter at initial MRI (cm; median, range) | 3.3 (1-10.7)                  | 3.6 (1.9-7.0)          | 4.9 (2.0-6.7)       | 2.2 (1.5-3.8)          | 1.5 (1.4-4.0)     | 3.8 (1.3-10.7)   |
| No data                 | 11 (17%)                         | 0                      | 4 (36%)             | 3 (25%)             | 2 (33.3%)                     | 2 (6.2%)         |
| Extent of resection     |                                  |                        |                     |                     |                               |                  |
| Complete                | 25 (%)                           | 0                      | 2 (%)               | 6 (50%)             | 5 (83.3%)                     | 12 (37.5%)       |
| Subtotal                | 5 (%)                            | 0                      | 1 (%)               | 0                   | 0                             | 4 (12.5%)        |
| Partial                 | 21 (%)                           | 0                      | 5 (%)               | 4 (33.3%)           | 1 (16.7%)                     | 11 (34.4%)       |
| Biopsy                  | 14 (%)                           | 4 (100%)               | 3 (%)               | 2 (16.7%)           | 0                             | 5 (15.6%)        |
| Patients with re-resection (median number; range) | 24 (1; 1-2)                   | 1 (1; −)              | 6 (2; 1-2)          | 2 (1; −)             | 1 (2; −)                      | 14 (1; 1-2)      |
| Histology               |                                  |                        |                     |                     |                               |                  |
| Diffuse astrocytoma     | 57 (87.7%)                       | 4 (100%)               | 10 (91%)            | 9 (75%)             | 6 (100%)                      | 28 (87.5%)       |
| Oligoastrocytoma        | 5 (7.7%)                         | 0                      | 1 (9%)              | 1 (8.3%)            | 0                             | 3 (9.4%)         |
| Oligodendroglioma       | 3 (4.6%)                         | 0                      | 0                   | 2 (16.7%)           | 0                             | 1 (3.1%)         |
| MIB1-labeling-index     |                                  |                        |                     |                     |                               |                  |
| <1%                     | 8 (12%)                          | 1 (25%)                | 0                   | 2 (16.7%)           | 2 (33.3%)                     | 3 (9.4%)         |
| 1 to <5%                | 53 (82%)                         | 3 (75%)                | 10 (91%)            | 10 (83.3%)          | 4 (66.6%)                     | 26 (81.2%)       |
| ≥5%                     | 4 (6%)                           | 0                      | 1 (9%)              | 0                   | 0                             | 3 (9.4%)         |
3.9 years after initial diagnosis. First and second resections were complete in two patients and regrowth stabilized spontaneously in one patient. No patient received adjuvant treatment. At 10.0 years median follow-up all patients are alive without (n = 5) or with stable (n = 1) disease.

The remaining tumors without evidence of these major genetic alterations (49%) occurred along the CNS, affecting also the spinal cord. Half of them were completely/subtotally resected. For 23 patients a *wait and see* approach was used after initial surgery (n = 15) or after multiple resections (n = 8), revealing progression to anaplastic astrocytoma in one patient who later died. One patient with a stable spinal primary developed chondrosarcoma of the skull-base. Six patients started chemotherapy and three received RT; treatment started at diagnosis (5/9) or following tumor progression (4/9). Subsequent salvage treatments were applied in 6/9 patients including second or multiple resections. One treatment-associated death occurred 2 weeks after initial diagnosis and 4 days after the start of chemotherapy from infection-triggered multi-organ failure. Progression to glioblastoma multiforme was diagnosed in 1/9 patient. One other patient had the radiologic diagnosis of gliomatosis cerebri after RT and salvage-chemotherapy. At 8.6 years follow-up 29 patients are alive without (n = 14), with stable (n = 12) or progressive (n = 2) tumor.

In summary, malignant transformation was documented histologically in nine patients with DG II (anaplastic astrocytoma WHO-grade III, n = 4; glioblastoma multiforme WHO-grade IV, n = 5), while three cases fulfilled radiologic criteria of malignancy (1 DIPG, 1 gliomatosis cerebri, 1 rapid progression with sudden, increasing contrast enhancement).

Survival data including the results of univariable analyses are detailed in Table 3 and illustrated in Figure 3A-D.

OS for the entire cohort of 100 patients was 0.90 (±0.03) at 5 years and 0.85 (±0.04) at 10 years. It was shorter for midline tumors, for tumors following biopsy only, and for those with a larger initial diameter. Five-years OS was 0.72 (±0.11) for the chemotherapy and 0.82 (±0.09) for the RT group.

OS for the 65 patients with genetically classified tumors was 0.88 (±0.04) at 5 years and 0.83 (±0.06) at 10 years. While 5-years OS was zero for the Histon3-K27M-mutated patients, it was 0.92-1.00 for the other four genetically defined subtypes.

Five-years EFS for the entire cohort was 0.44 (±0.05). It was longer for tumors of the cerebral hemispheres and cerebellum, after

| TABLE 2  (Continued)                                                                 |
|---------------------------------------------------------------|
| Molecular-genetic group (n = 65) | Histone3-K27M (n = 4) | IDH-mutant (n = 11) | BRAF-V600E (n = 12) | KIAA1549-BRAF-fusion (n = 6) | Wild-type (n = 32) |
|--------------------------------|---------------------|---------------------|-------------------|-----------------------------|-------------------|
| p53-accumulation               |                     |                     |                   |                             |                   |
| Positive                       | 15 (23%)            | 1 (25%)             | 8 (73%)           | 2 (16.7%)                   | 4 (12.5%)         |
| No data                        | 8 (12%)             | 0                   | 0                 | 2 (16.7%)                   | 5 (15.6%)         |
| Malignant transformation (MT)  | 8 (12%)             | 1 (25%)             | 2 (18%)           | 1 (8.3%)                    | 4* (12.5%)        |
| Median interval to MT (years, range) | 1.6 (0.5-10.8)        | 1.4 (0.9-1.9)       | 1.2               | 1.3 (0.08-7.7)              | 1.0 (0.6-3.9)      |
| Median interval to first event (years, range) | 0.8 (0.04-11.2)          | 0.15 (0.04-0.4)    | 1.3 (0.3-9.7)      | 1.3 (0.08-7.7)              | 0.8 (0.04-11.2)    |
| Adjuvant treatment             |                     |                     |                   |                             |                   |
| No adjuvant therapies          | 49 (75.4%)          | 1 (25%)             | 9 (82%)           | 10 (83.3%)                  | 23 (71.9%)        |
| Primary chemotherapy           | 10 (15.4%)          | 2 (50%)             | 1 (9%)            | 1 (8.3%)                    | 6 (18.9%)         |
| Primary radiotherapy           | 6 (9.2%)            | 1 (25%)             | 1 (9%)            | 1 (8.3%)                    | 3 (9.4%)          |
| Last patient status            |                     |                     |                   |                             |                   |
| Alive                          |                     |                     |                   |                             |                   |
| Complete remission             | 28                  | 0                   | 3                 | 6                           | 5                 |
| Stable disease                 | 23                  | 0                   | 7                 | 3                           | 1                 |
| Progression                    | 3                   | 0                   | 0                 | 1                           | 0                 |
| Dead                           | 9 (14.1%)           | 4 (100%)            | 1 (10%)           | 1 (8.3%)                    | 0                 |
| Not known/no data              | 2                   | 0                   | 0                 | 1                           | 3 (9.4%)          |
| Median observation time (years, range) | 8.5 (0.04-14.9)        | 1.6 (0.6-2.4)       | 6.2 (1.6-10.5)     | 7.5 (2.1-13.2)              | 10 (1.8-13.4)      |

*a*Only midline tumors were analyzed for Histone gene mutations.

*b*Including one tumor of the lateral ventricle (wild-type).

*c*Measurement of tumor diameters in the three standard planes.

*d*Further treatment followed high-grade glioma protocols.

*e*Including one patient with the radiologic diagnosis of gliomatosis cerebri.

*f*Event according to protocol definition: relapse after complete remission, clinical or radiological progression, start of nonsurgical therapy or death of any cause.
| TABLE 3 | Univariable analysis of overall, event-free and progression-free survival |
|-----------------|-----------------------------|-----------------------------|-----------------|------------------|
|               | Overall survival            | Event-free survival         |                 |
|               | n                           | 5 years %, (±SE)            | 10 years %, (±SE) | P*              | 5 years %, (±SE) | 10 years %, (±SE) | P*          |
| All DG2       | 100                         | 89.6 (±3.1)                | 84.6 (±4.1)     | —               | 43.9 (±5.1)     | 34.7 (±6.1)     | —           |
| Gender        |                             |                             |                 |                  |                 |                  |             |
| Female        | 46                          | 88.9 (±4.7)                | 85.2 (±5.8)     | 35.2 (±7.3)     | 35.2 (±7.3)     | —               | —           |
| Male          | 54                          | 90.3 (±4.1)                | 84.0 (±5.9)     | 51.7 (±7.0)     | 37.5 (±8.2)     | —               | —           |
| Age at diagnosis |                             |                             |                 |                  |                 |                  |             |
| <1 year       | 2                           | 0 events                   | 0 events        | 2 events        | 2 events        | —               | —           |
| ≥1 to <5 years| 24                          | 87.5 (±6.8)                | 87.5 (±6.8)     | 45.5 (±10.2)    | 39.8 (±10.4)    | —               | —           |
| ≥5 to <11 years| 34                          | 85.0 (±6.2)                | 85.0 (±6.2)     | 40.6 (±8.5)     | 40.6 (±8.5)     | —               | —           |
| ≥11 to <16 years| 32                          | 93.8 (±4.3)                | 81.5 (±9.3)     | 49.3 (±9.5)     | 16.2 (±12.9)    | —               | —           |
| ≥16 years     | 8                           | 100                        | 50.0 (±35.4)    | 29.2 (±22.6)    | 29.2 (±22.6)    | —               | —           |
| Age at diagnosisb |                             |                             |                 |                  |                 |                  |             |
| <1 year       | 2                           | 0 events                   | 0 events        | 2 events        | 2 events        | —               | —           |
| ≥1 to <8 years| 40                          | 87.4 (±5.3)                | 87.4 (±5.3)     | 44.6 (±7.9)     | 41.4 (±8.0)     | —               | —           |
| ≥8 years      | 58                          | 90.8 (±3.9)                | 78.7 (±7.7)     | 44.3 (±7.0)     | 24.0 (±11.1)    | —               | —           |
| Localization  |                             |                             |                 |                  |                 |                  |             |
| Cerebral hemispheresc | 42                          | 97.4 (±2.6)                | 91.3 (±6.4)     | 56.1 (±8.2)     | 29.9 (±13.8)    | —               | —           |
| Supratentorial midline | 27                          | 77.8 (±8.0)                | 77.8 (±8.0)     | 36.7 (±9.3)     | 31.4 (±9.4)     | —               | —           |
| Cerebellum    | 10                          | 100                        | 100             | 54.9 (±17.2)    | 54.9 (±17.2)    | —               | —           |
| Caudal brainstem | 11                          | 81.8 (±11.6)               | 60.6 (±15.7)    | 9.1 (±8.7)      | 9.1 (±8.7)      | —               | —           |
| Spinal cord   | 10                          | 90.0 (±9.5)                | 90.0 (±9.5)     | 40.0 (±15.5)    | 40.0 (±15.5)    | —               | —           |
| Extent of resection |                             |                             |                 | 0.028           | 0.027           | —               | —           |
| Complete      | 26                          | 90.9 (±8.7)                | 90.9 (±8.7)     | 75.4 (±9.8)     | 67.0 (±11.7)    | —               | —           |
| Subtotal      | 10                          | 100                        | 100             | 76.2 (±14.8)    | 76.2 (±14.8)    | —               | —           |
| Partial       | 23                          | 92.9 (±6.9)                | 92.9 (±6.9)     | 55.9 (±10.5)    | 39.1 (±12.6)    | —               | —           |
| Biopsy        | 41                          | 75.0 (±6.8)                | 72.5 (±7.1)     | 18.9 (±6.4)     | 0.0             | —               | —           |
| Histology     |                             |                             |                 |                  | 0.437           | 0.013           |             |
| Diffuse astrocytoma | 89                          | 88.3 (±3.5)                | 82.8 (±4.5)     | 46.2 (±5.5)     | 38.0 (±6.5)     | —               | —           |
| Oligoastrocytoma | 7                           | 100 (0 events)             | 100 (0 events)  | 14.3 (±13.2) (6 events) | 14.3 (±13.2) (6 events) | —               | —           |
| Oligodendrogloma | 4                           | 100 (0 events)             | 100 (0 events)  | 50.0 (±25.0) (2 events) | 0.0 (4 events) | —               | —           |
| Tumor diameter at diagnosisd |                           |                             |                 | 0.048           | 0.13            | —               | —           |
| ≤3 cm         | 39                          | 97.2 (±2.7)                | 90.3 (±7.2)     | 53.0 (±8.4)     | 42.5 (±9.6)     | —               | —           |
| >3 cm         | 41                          | 80.5 (±6.2)                | 76.5 (±7.1)     | 28.9 (±7.1)     | 25.3 (±7.1)     | —               | —           |
| Contrast enhancemente |                             |                             |                 | 0.639           | 0.11            | —               | —           |
| No enhancement | 59                          | 91.3 (±3.7)                | 85.7 (±5.3)     | 46.8 (±6.8)     | 41.3 (±7.0)     | —               | —           |
| Enhancement present | 33                          | 87.1 (±6.0)                | 81.6 (±7.7)     | 31.0 (±8.3)     | 20.7 (±10.1)    | —               | —           |
| Molecular subgroups |                         |                             |                 | 0.003           | 0.003           | —               | —           |
| Histone3-K27M-mutation | 4                           | 0.0                        | 0.0             | 0.0             | 0.0             | —               | —           |
| IDH1-mutation | 11                          | 100                        | 75.0 (±21.7)    | 63.6 (±14.5)    | 50.9 (±16.3)    | —               | —           |
| BRAF-V600E-mutation | 12                          | 91.7 (±8.0)                | 91.7 (±8.0)     | 75.0 (±12.5)    | 75.0 (±12.5)    | —               | —           |
| KIAA1549-BRAF-fusion | 6                           | 100                        | 100             | 66.7 (±19.2)    | 44.2 (±22.2)    | —               | —           |
| Wild-type     | 32                          | 93.5 (±4.4)                | 87.7 (±7.0)     | 55.9 (±8.8)     | 48.5 (±9.1)     | —               | —           |
| All molecularly subtyped | 65                          | 88.8 (±4.0)                | 82.9 (±5.5)     | 58.2 (±6.2)     | 50.5 (±6.4)     | —               | —           |
| Treatment groups |                             |                             |                 |                  |                 |                  |             |
| Chemotherapy  | 18                          | 72.2 (±10.6)               | 72.2 (±10.6)    | —               | 33.3 (±11.1)    | 14.8 (±8.9)     | —           |
| Histone3-mutated excluded | 16                          | 81.3 (±9.8)                | 81.3 (±9.8)     | —               | 37.5 (±12.1)    | 16.7 (±9.9)     | —           |
TABLE 3  (Continued)

|                          | Overall survival  |                      | Progression-free survival |
|--------------------------|-------------------|----------------------|---------------------------|
|                          | 5 years           | 10 years             | 5 years                   | 10 years |
| Radiotherapy             | 17                | 82.4 (±9.2)          | 76.0 (±10.5)              | —         | 69.5 (±11.5)          | 69.5 (±11.5) | — |
| Histone3-mutated         | 16                | 87.5 (±8.3)          | 80.8 (±10.0)              | —         | 73.9 (±11.3)          | 73.9 (±11.3) | — |

*P*-value log-rank-test.

Infants (age at diagnosis ≤ 1 years) excluded from analysis due to small group size.

Including 1 tumor of the lateral ventricle (wild-type).

$n = 20$ no data.

$n = 8$ no data.

Multivariable analysis confirmed extent of resection for OS and age, contrast enhancement at diagnosis, histology, localization and extent of resection for EFS as independent prognostic factors (Table 4).

Five-years PFS after first adjuvant treatment was 0.33 (±0.11) after chemotherapy and 0.70 (±0.12) after RT; excluding patients with Histone3-K27M-mutated tumors 5-years PFS was 0.38 (±0.12) for 16 patients receiving chemotherapy and 0.74 (±0.11) for 16 patients receiving RT.

FIGURE 3  Overall, event-free and progression-free survival. A, OS and EFS for the entire group. B, OS and PFS for the chemotherapy and radiotherapy groups. C, OS for molecular genetic subgroup. D, EFS for molecular genetic subgroup

(A) Overall survival (%)

(B) Progression-free survival (%)

(C) Event-free survival (%)

(D) Overall survival (%)
**TABLE 4**  Multivariable analysis of overall and event-free survival

| Predictors for overall survival | HR | 95% CI | P*  |
|--------------------------------|----|--------|-----|
| **Gender**                     |    |        |     |
| Female vs male (ref.)          |    |        | N/S .556 |
| **Age at diagnosis**           |    |        |     |
| ≥1 to <8 years vs ≥8 years (ref.) |    |        | N/S .692 |
| **Age at diagnosis**           |    |        |     |
| ≥1 to <5 years vs ≥16 years (ref.) |    |        | N/S .705 |
| ≥5 to <11 years vs ≥16 years (ref.) |    |        |     |
| ≥11 to <16 years vs ≥16 years (ref.) |    |        |     |
| **Localization**               |    |        |     |
| Supratentorial midline vs cerebral hemispheres (ref.) |    |        | N/S .851 |
| Cerebellum vs cerebral hemispheres (ref.) |    |        |     |
| Caudal brainstem vs cerebral hemispheres (ref.) |    |        |     |
| Spinal cord vs cerebral hemispheres (ref.) |    |        |     |
| **Extent of resection**        |    |        |     |
| Biopsy vs resected (complete, subtotal, partial) (ref.) | 6.00 | 1.25-28.91 | .027|
| **Histology**                  |    |        |     |
| Oligoastrocytoma vs diffuse astrocytoma (ref.) |    |        | N/S .711 |
| Oligodendroglioma vs diffuse astrocytoma (ref.) |    |        |     |
| **Tumor diameter at diagnosis** |    |        |     |
| >3 cm vs ≤3 cm (ref.)          |    |        | N/S .127 |
| **Contrast enhancement**       |    |        |     |
| Enhancement present vs no enhancement (ref.) |    |        | N/S .526 |
| **Molecular subgroup**         |    |        |     |
| IDH-mutation vs wild-type (ref.) |    |        | N/S .795 |
| BRAF-V600E-mutation vs wild-type (ref.) |    |        |     |
| KIAA1549-BRAF-fusion vs wild-type (ref.) |    |        |     |

| Predictors for event-free survival | HR | 95% CI | P*  |
|------------------------------------|----|--------|-----|
| **Gender**                         |    |        |     |
| Female vs male (ref.)              |    |        | N/S .622 |
| **Age at diagnosis**               |    |        |     |
| ≥1 to <8 years vs ≥8 years (ref.)  | 2.58 | 1.24-5.35 | .012|
| **Age at diagnosis**               |    |        |     |
| ≥1 to <5 years vs ≥16 years (ref.) |    |        | N/S .237 |
| ≥5 to <11 years vs ≥16 years (ref.) |    |        |     |
| ≥11 to <16 years vs ≥16 years (ref.) |    |        |     |
| **Localization**                   |    |        |     |
| Supratentorial midline vs cerebral hemispheres (ref.) | 0.67 | 0.31-1.54 | .010|
| Cerebellum vs cerebral hemispheres (ref.) | 2.80 | 0.78-10.06 |     |
| Caudal brainstem vs cerebral hemispheres (ref.) | 5.22 | 1.73-15.75 |     |
| Spinal cord vs cerebral hemispheres (ref.) | 2.58 | 0.71-9.35 |     |
| **Extent of resection**            |    |        |     |
| Subtotal vs complete (ref.)        | 2.00 | 0.32-12.35 | <.001|
| Partial vs complete (ref.)         | 5.38 | 1.68-17.27 |     |
| Biopsy vs complete (ref.)          | 43.36 | 10.87-172.95 |     |
DISCUSSION

4.1 Clinical and epidemiologic aspects of pediatric diffuse glioma WHO-grade II

This large cohort of 100 prospectively registered, centrally confirmed pediatric diffuse gliomas of WHO-grade II (DG2) achieved good OS following the SIOP-LGG 2004 protocol. Patients’ demographics including age at diagnosis, gender distribution and primary tumor site are similar to previous reports. Pediatric patients with DG2 tend to be older than population-based cohorts with LGG of all histologies, highlighted by an age peak at 10 to 14 years. Although 40% of our patients were older than 10 years, registration of adolescents ≥16 years did not start before 2007 following amended eligibility criteria of the protocol. DG2 is a rare diagnosis among CNS-tumors of patients affected by NF1, which was seen in only 4% of our patients. In accordance with the reports of Fouladi et al and Fisher et al, most DG2 in our cohort were hemispheric tumors, while other series did not relate histology to site. Correspondingly, seizures were among the most frequently reported presenting symptoms comparable to the observation in young adults. Although 40% of our patients were older than 10 years, registration of adolescents ≥16 years did not start before 2007 following amended eligibility criteria of the protocol. DG2 is a rare diagnosis among CNS-tumors of patients affected by NF1, which was seen in only 4% of our patients. In accordance with the reports of Fouladi et al and Fisher et al, most DG2 in our cohort were hemispheric tumors, while other series did not relate histology to site.

Contrast enhancement

Enhancement present vs no enhancement (ref.)

2.28

1.02-5.10

N/S: .828

Molecular subgroup

IDH-mutation vs wild-type (ref.)

—

—

N/S: .828

BRAF-V600E-mutation vs wild-type (ref.)

—

—

N/S: .828

KIAA1549-BRAF-fusion vs wild-type (ref.)

—

—

N/S: .828

4.2 Treatment results for histologically defined diffuse glioma WHO-grade II

All DG2 were treated according to the SIOP-LGG 2004 therapy algorithm. After diagnosis, almost two thirds of patients were managed with surgery and observation alone, corroborating results of our previous cohort. Despite intermittent progression in 25/65 patients, 85% achieved complete remission or stable disease after re-resections or by spontaneous tumor stabilization. Nevertheless, malignant transformation was observed during follow-up in six patients; an additional six developed malignant features among the 35 patients receiving adjuvant therapy. This was detected after a follow-up of 0.5 to 10.9 years (median 1.9 years), 5/12 patients had received RT 1.9 to 10.9 years prior to malignant transformation not excluding the evolution of radiation-induced secondary neoplasms.

Adjuvant treatment was started in 35 patients for radiologic tumor progression and/or severe/progressive neurologic symptoms. Reflecting protocol-stratification median age at start of treatment was higher in the RT group. Within both therapy-groups more than three quarters of DG2 were located in midline structures and 94% had only been partially resected or biopsied. During the past two decades front-line RT for
pediatric LGG has been replaced by chemotherapy for younger patients. Still, almost half of the DG2 treatment group was irradiated primarily achieving 70% PFS in the long term, well comparable to previous reports. Three quarters were irradiated including salvage approaches. Several reports on chemotherapy for LGG included WHO-grade II tumors and explorative data suggested impaired survival for DG2. Two randomized chemotherapy trials allowed analysis of the impact of DG2 histology upon outcome. While 5-years EFS was 34% ± 10% for diffuse astrocytomas compared to 49% ± 6% for PA, and 5-years OS reached 79% ± 8% vs 88% ± 4%, histology was not prognostic at multivariate analysis in the COG trial. After vincristine/carboplatin in the international SIOP-LGG 2004 trial, patients with WHO-grade II glioma had a 5-years OS of 80.0%, and DG2 conferred a statistically noticeable adverse influence on survival compared to PA in multivariable analysis (HR 5.56 [95% CI: 2.52-12.23]), while PFS was not impaired. These results implied an unfavorable response to salvage treatment for progressive tumors beyond first-line therapy, “linking progression to survival,” and suggested the existence of prognostically relevant molecular genetic subtypes.

### Treatment results differentiated for the molecular genetic subgroups

We identified five genetically and clinically distinct major subtypes among 65 patients with sufficient tissue for molecular testing. This cohort did not show demographical differences to the cohort without residual material and therefore can be considered representative for DG2 (Table S3).

### Histone3-K27M-mutation

The most striking finding was the presence of Histone3-K27M-mutation in four midline tumors (6%). Histone3-mutations are a hallmark of malignant diffuse midline glioma WHO-grade IV according to the WHO-classification, but have also been described in low-grade circumscribed and diffuse, mostly thalamic and brainstem tumors preceding malignant evolution and death. In the retrospective analysis of 289 pediatric low-grade glioma by Yang et al., they constituted 6% and were allocated to a “high-risk group”. Co-occurrence with other genetic alterations was described for various low grade histologies, but was not seen in tumors that were uniformly progressive within few months and all patients died. We advocate to test all diffuse midline gliomas for this mutation to identify and exclude Histone3-K27M-mutated midline diffuse gliomas in future pediatric LGG protocols, even if they lack signs of anaplasia.

### IDH-mutation

Seventeen percent of the DG2 carried an IDH-mutation which was described at various frequencies (0%-42%) in previous, often smaller pediatric series. Although IDH-mutations are a hallmark for DG2 in adults, their presence in pediatric DG2 has been rarely observed. Only one of our cases with IDH1-mutation had chromosome 1p/19q-codeletion and retained ATRX. Furthermore, 8/10 tumors showed nuclear accumulation of p53, reported for 5/12 in the series of Johnson et al. Although only two of our patients needed adjuvant treatment, early malignant transformation was seen in another two. Thus, treatment according to the pediatric LGG-strategy seems appropriate for IDH-mutated DG2, but we support the suggestion of increased clinical surveillance for this subgroup.

### BRAF-V600-mutation

BRAF-V600-mutation was found in 18% of the tumors of our cohort, and was reported at variable frequencies up to 25% for pediatric DG2. Although the presence of this mutation was rated as unfavorable with more frequent non-response to adjuvant treatment and shorter PFS in the basket group of LGG, no specific data are available for DG2. The majority of our patients were just observed after surgery and only one patient each received primary RT or chemotherapy. The latter, however, progressed despite salvage treatment and exhibited malignant histology upon subsequent resection. BRAF-mutations had been detected in 44% of pediatric LGG that later progressed to HGG, but only in 6% that did not transform in the population-based report of Mistry et al. We agree to recommend radical resection in amenable locations for this DG2 subgroup, while the consideration to recommend targeted therapy for stable tumors to prevent transformation awaits further confirmation.

### KIAA1549-BRAF-fusion

While FGFR1-mutations and MYB-rearrangements had been common in other LGG-series, we did not identify such alterations in our cohort having excluded non-diffuse or glioneuronal tumor entities of WHO-grade I like angiocentric glioma or dysembryoplastic neuroepithelial tumor. Instead, we identified a small group of histological DG2 with KIAA1549-BRAF-fusions (9%). Although KIAA1549-BRAF-fusions were described in ganglioglioma and unspecified LGG by Ramkisson et al., the frequency appears low in diffuse glioma. These tumors of infra- and supratentorial location occurred in younger patients, were amenable to surgery and mostly completely resected at first or second intervention. Indeed, some of them may represent areas of PA lacking specific histological features of PA and showing diffuse growth patterns. This has been described in the margin of bona fide PA.
assays we did not find distinct genetic events in half of all tumors, while smaller series detected at least one alteration in most DG2.\textsuperscript{2,10,15}

The increasing number of genetic alterations identified for each histologic variant calls for an increasingly differentiated diagnostic program.\textsuperscript{9,15} Yet, even the extensive characterization of LGG in the series of Yang could not detect molecular changes in 27% of tumors (78/289).\textsuperscript{23} In that paper recurrent TERT-mutations were associated with worse prognosis in older children. In our series, TERT-promotor mutations were absent indicating different tumor cohorts. Corroborating their findings of an "intermediate" biologic behavior for this subgroup,\textsuperscript{23} the majority of our wild-type tumors were managed by surgery and "wait and see" strategy, although almost one-third needed adjuvant treatment and 6/9 patients received multimodal therapy including multiple surgeries. Although 28/32 patients were alive at last follow-up, four tumors had evolved to high-grade, including one with radiographic signs of gliomatosis cerebri and three patients had died.

While the unfavorable course for Histone3-K27M-mutated tumors is indisputable, no differences were found with respect to EFS after diagnosis or to OS for the other genetic DG2-entities. Reflecting treatment stratification, primary chemotherapy was rather applied in younger patients and associated with multiple lines of treatment for 77% of patients. Nevertheless, the favorable outcome of our treatment groups with a 5-years OS of 72% and 82% after primary chemotherapy and RT, respectively, underscores the appropriateness of the study strategy for pediatric DG2.

Malignant transformation was seen, however, in all groups, except for the KIAA1549-BRAF-fusion positive tumors. Thus, re-biopsy of recurrent or progressing lesions is warranted even in early progression. Malignant transformation of pediatric LGG has been shown for BRAF-V600E-mutated tumors and was linked to the concurrent presence of CDKN2A-deletion.\textsuperscript{6,23,48,49} Although the underlying molecular alteration was already traced in the initial biopsy in the report of Mistry et al.,\textsuperscript{49} and identical genetic alterations were found in primary and recurrent tumors in the series of Bergthold et al.,\textsuperscript{47} its later evolution cannot be ruled out. An unfavorable outcome of tumors with CDKN2A-deletion was linked to specific tumor sites.\textsuperscript{23} However, none of our tumors had concomitant CDKN2A-loss. IDH-mutations are the hallmark of adult diffuse glioma, but are present in secondary glioblastoma, as well.\textsuperscript{6,8} The propensity for malignant transformation has to be accepted for IDH-mutated tumors often in association with ATRX-mutation or p53-aberration.\textsuperscript{8,50,51} Despite p53-accumulation in 8/11 patients, concurrent IDH- and ATRX- or p53-mutations were not found. The molecular clues for malignant evolution have still to be identified for those tumors termed wild-type in our report. None of our patients had received targeted treatment. Yet, identification of characteristic genetic alterations does not only enhance diagnostic accuracy, but will offer additional treatment options in the presence of druggable driver mutations in future protocols.\textsuperscript{7,52}

## 5 | CONCLUSIONS

Pediatric DG2 represents a group of genetically distinct entities and their biological features and clinical behavior are different from their adult counterpart. Even IDH-mutated tumors in older children and adolescents seem to represent a more indolent tumor group compared to IDH-mutated gliomas in adult patients. However, except for DG2 with KIAA1549-BRAF-fusions, tumors of all genetic subtypes may progress to high-grade glioma. Although Histone3-K27M-mutated midline gliomas can show histologies lacking signs of anaplasia, all patients with Histone3-K27M-mutated DG2 had a fatal outcome. Therefore, DG2 should be genetically classified upfront employing appropriate methods for diagnosis and forecasting outcome. In addition, genetic classification will be a prerequisite for targeted therapy within future protocols.

We found no significant differences with respect to outcome as compared to WHO-grade I tumors. Thus, the current LGG treatment strategy seems appropriate for all DG2-entities, with the exception of Histone3-K27M-mutated tumors that require a HGG-related treatment. More than half of all patients were safely followed by observation, while multimodal adjuvant treatment controlled progressive tumors in most cases.

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## CONFLICT OF INTEREST

No author stated a conflict of interest.

## DATA ACCESSIBILITY

The datasets of the current study are available from the corresponding authors on reasonable request.

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## REFERENCES

1. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803-820.
2. Louis DN, Ohgaki H, Wiestler OD, et al. WHO classification of tumours of the central nervous system. 4th ed. Lyon: IARC Press; 2016.
3. Qaddoumi I, Sultan I, Gajjar A. Outcome and prognostic features in pediatric gliomas: a review of 6212 cases from the Surveillance, Epidemiology, and End Results database. Cancer. 2009;115:5761-5770.
4. Stokland T, Liu JF, Ironside JW, et al. A multivariate analysis of factors determining tumor progression in childhood low-grade glioma: a population-based cohort study (CCLG CNS9702). Neuro Oncol. 2010;12:1257-1268.

5. Gnekow AK, Falkenstein F, von Hornstein S, et al. Long-term follow-up of the multicenter, multidisciplinary treatment study HIT-LGG-1996 for low-grade glioma in children and adolescents of the German Speaking Society of Pediatric Oncology and Hematology. Neuro Oncol. 2012;14:1265-1284.

6. Ryall S, Tabori U, Hawkins C. A comprehensive review of paediatric low-grade diffuse glioma: pathology, molecular genetics and treatment. Brain Tumor Pathol. 2017;34:51-61.

7. Jones DTW, Kieran MW, Bouffet E, et al. Pediatric low-grade gliomas: next biologically driven steps. Neuro Oncol. 2018;20:160-173.

8. Jones DT, Mulholland SA, Pearson DM, et al. Adult grade II diffuse astrocytomas are genetically distinct from and more aggressive than their paediatric counterparts. Acta Neuropathol. 2011;121:753-761.

9. Ellison DW, Hawkins C, Jones DTW, et al. cIMPACT-NOW update 4: diffuse gliomas characterized by MYB, MYBL1, or FGFR1 alterations or BRAF(V600E) mutation. Acta Neuropathol. 2019;137:683-687.

10. Zhang J, Wu G, Miller CP, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet. 2013;45:602-612.

11. Ater JL, Zhou T, Holmes E, et al. Randomized study of two chemotherapy regimens for treatment of low-grade glioma in young children: a report from the Children's Oncology Group. J Clin Oncol. 2012;30:2641-2647.

12. Fisher PG, Tihan T, Goldhwaite PT, et al. Outcome analysis of childhood low-grade astrocytomas. Pediatr Blood Cancer. 2008;51:245-250.

13. Wisoff JH, Sanford RA, Heier LA, et al. Primary neurosurgery for pediatric low-grade gliomas: a prospective multi-institutional study from the Children's Oncology Group. Neurosurgery. 2011;68:1548-1554. discussion 54–5.

14. Lassaletta A, Zapotocky M, Mistry M, et al. Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas. J Clin Oncol. 2017;35:2934-2941.

15. Qaddoumi I, Orisme W, Wen J, et al. Genetic alterations in uncommon low-grade neuroepithelial tumors: BRAF, FGFR1, and MYB mutations occur at high frequency and align with morphology. Acta Neuropathol. 2016;131:833-845.

16. Cruz GR, Dias Oliveira J, Moraes L, et al. Analysis of KIAA1549-BRAF fusion gene expression and IDH1/IDH2 mutations in low grade pediatric astrocytomas. J Neurooncol. 2014;117:235-242.

17. Ramkissoon LA, Horowitz PM, Craig JM, et al. Genomic analysis of diffuse pediatric low-grade gliomas identifies recurrent oncogenic truncating rearrangements in the transcription factor MYBL1. Proc Natl Acad Sci USA. 2013;110:8188-8193.

18. Fouladi M, Hunt DL, Pollack IF, et al. Outcome of children with centrally reviewed low-grade gliomas treated with chemotherapy or without radiotherapy on Children's Cancer Group high-grade glioma study CCG-945. Cancer. 2003;98:1243-1252.

19. Gnekow AK, Walker DA, Kandels D, et al. A European randomised controlled trial of the addition of etoposide to standard vincristine and carboplatin induction as part of an 18-month treatment programme for childhood (4-16 years) low grade glioma—a final report. Eur J Cancer. 2017;81:206-225.

20. Buckner J, Giannini C, Eckel-Passow J, et al. Management of diffuse low-grade gliomas in adults—use of molecular diagnostics. Nat Rev Neurol. 2017;13:340-351.

21. Reuss DE, Sahm F, Schrimpf D, et al. ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an “integrated” diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. Acta Neuropathol. 2015;129:133-146.

22. Di Carlo DT, Duffau H, Cagnazzo F, Benedetto N, Morganti R, Perrini P. IDH wild-type WHO grade II diffuse low-grade gliomas. A heterogeneous family with different outcomes. Systematic review and meta-analysis. Neurosurg Rev. 2018. https://doi.org/10.1007/s10143-018-0996-3 [Epub ahead of print].

23. Yang RR, Albadala A, Wang WW, et al. Pediatric low-grade gliomas can be molecularly stratified for risk. Acta Neuropathol. 2018;136:641-655.

24. Bandopadhyay P, Bergthold G, London WB, et al. Long-term outcome of 4,040 children diagnosed with pediatric low-grade glioma: an analysis of the Surveillance Epidemiology and End Results (SEER) database. Pediatr Blood Cancer. 2014;61:1173-1179.

25. Ruge MJ, Simon T, Suchorska B, et al. Stereotactic brachytherapy with iodine-125 seeds for the treatment of inoperable low-grade gliomas in children: long-term outcome. J Clin Oncol. 2011;29:4151-4159.

26. Gnekow AK, Kandels D, van Tilburg C, et al. SIOP-E-BTG and GPOH Guidelines for Diagnosis and Treatment of Children and Adolescents with Low Grade Glioma. Klin Padiatr. 2019;231:107-135.

27. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555:469-474.

28. Stock A, Mynarek M, Pietsch T, et al. Imaging characteristics of wingless pathway subgroup medulloblastomas: results from the German HIT/SIOP-trial cohort. AJNR Am J Neuroradiol. 2019;40:1811-1817.

29. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. Neuro Oncol. 2018;20:iv1-iv86.

30. Hernois Driever P, von Hornstein S, Pietsch T, et al. Natural history and management of low-grade glioma in NF-1 children. J Neurooncol. 2010;100:199-207.

31. D’Angelo F, Cecarelli M, Tala GL, et al. The molecular landscape of glioma in patients with neurofibromatosis 1. Nat Med. 2019;25:176-187.

32. Ater JL, Xia C, Mazewski CM, et al. Nonrandomized comparison of neurofibromatosis type 1 and non-neurofibromatosis type 1 children who received carboplatin and vincristine for progressive low-grade glioma: a report from the Children’s Oncology Group. Cancer. 2016;122:1928-1936.

33. Margol AS, Yeo KK, Xia C, et al. A comparative analysis of clinicopathological features and survival among early adolescents/young adults and children with low-grade glioma: a report from the Children's Oncology Group. J Neurooncol. 2018;140:575-582.

34. Fan X, Li Y, Shan X, et al. Seizures at presentation are correlated with better survival outcomes in adult diffuse glioma: a systematic review and meta-analysis. Seizure. 2018;59:16-23.

35. Goel NJ, Abdullah KG, Lang SS. Outcomes and prognostic factors in pediatric oligodendroglioma: a population-based study. Pediatr Neurosurg. 2018;53:24-35.

36. Zhou H, Vallieres M, Bai HX, et al. MRI features predict survival and molecular markers in diffuse lower-grade gliomas. Neuro Oncol. 2017;19:862-870.

37. Bion S, Warmuth-Metz M, Schneckenburger M, Mirow C, Gnekow AK. Contrast enhancement of low grade gliomas (LGG) during follow-up. Neuro Oncol. 2011;13:415-418.

38. Mishra KK, Puri DR, Missett BT, et al. The role of up-front radiation therapy for incompletely resected pediatric WHO grade II low-grade gliomas. Neuro Oncol. 2006;8:166-174.

39. Lassaletta A, Scheinemann K, Zelcer SM, et al. Phase II weekly vincristine for chemotherapy-naive children with progressive low-grade glioma: a Canadian Pediatric Brain Tumor Consortium Study. J Clin Oncol. 2016;34:3537-3543.

40. Massimino M, Spreafico F, Riva D, et al. A lower-dose, lower-toxicity cisplatin-etoposide regimen for childhood progressive low-grade glioma. J Neurooncol. 2010;100:65-71.
41. Pratt D, Natarajan SK, Banda A, et al. Circumscribed/non-diffuse histology confers a better prognosis in H3K27M-mutant gliomas. Acta Neuropathol. 2018;135:299-301.
42. Ryall S, Krishnatry R, Arnoldo A, et al. Targeted detection of genetic alterations reveal the prognostic impact of H3K27M and MAPK pathway aberrations in paediatric thalamic glioma. Acta Neuropathol Commun. 2016;4:93.
43. Ishibashi K, Inoue T, Fukushima H, et al. Pediatric thalamic glioma with H3F3A K27M mutation, which was detected before and after malignant transformation: a case report. Child’s Nervous Syst. 2016;32:2433-2438.
44. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360:765-773.
45. Johnson A, Severson E, Gay L, et al. Comprehensive genomic profiling of 282 pediatric low- and high-grade gliomas reveals genomic drivers, tumor mutational burden, and hypermutation signatures. Oncologist. 2017;22:1478-1490.
46. Korshunov A, Meyer J, Capper D, et al. Combined molecular analysis of BRAF and IDH1 distinguishes pilocytic astrocytoma from diffuse astrocytoma. Acta Neuropathol. 2009;118:401-405.
47. Bergthold G, Bandopadhayay P, Hoshida Y, et al. Expression profiles of 151 pediatric low-grade gliomas reveal molecular differences associated with location and histological subtype. Neuro Oncol. 2015;17:1486-1496.
48. Frazao L, do Carmo Martins M, Nunes VM, et al. BRAF V600E mutation and 9p21: CDKN2A/B and MTAP co-deletions—markers in the clinical stratification of pediatric gliomas. BMC Cancer. 2018;18:1259.
49. Mistry M, Zhukova N, Merico D, et al. BRAF mutation and CDKN2A deletion define a clinically distinct subgroup of childhood high-grade glioma. J Clin Oncol. 2015;33:1015-1022.
50. Korshunov A, Ryzhova M, Hovestadt V, et al. Integrated analysis of pediatric glioblastoma reveals a subset of biologically favorable tumors with associated molecular prognostic markers. Acta Neuropathol. 2015;129:669-678.
51. Sturm D, Pfister SM, Jones DTW. Pediatric gliomas: current concepts on diagnosis, biology, and clinical management. J Clin Oncol. 2017;35:2370-2377.
52. Packer RJ, Pfister S, Bouffet E, et al. Pediatric low-grade gliomas: implications of the biologic era. Neuro Oncol. 2017;19:750-761.

SUPPORTING INFORMATION
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