CHAPTER 15

Clinical, radiological, histological, and genetic characteristics of long-term survivors of diffuse intrinsic pontine glioma: A collaborative report from the International and SIOPE DIPG Registries

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ABSTRACT

INTRODUCTION Diffuse intrinsic pontine glioma (DIPG) is a pediatric malignant brainstem tumor with median survival of <1 year. The International and European Society for Paediatric Oncology DIPG Registries collaborated to assess clinical, radiological, and histo-molecular characteristics of long-term survivors (LTS) of DIPG. **METHODS** Data were abstracted from registry databases, including patients from North America, Australia, Germany, Austria, Switzerland, the Netherlands, Italy, France, United Kingdom, and Croatia. **RESULTS** Among 1,130 patients with radiographically confirmed DIPG, 122 (11%) were excluded. Of 1,008 remaining, 101 (10%) were LTS (overall survival ≥2 years). Median survival was 11 months (range 0-167 months), and 1-, 2-, 3-, 4- and 5-year OS were 42.3%, 9.6%, 4.3%, 3.2%, and 2.2%, respectively. Median age was similar between LTS (7.2 years) and short-term survivors (STS; 6.8 years). LTS more commonly presented at age <3 or >10 years ($p < 0.0001$) and with longer symptom duration ($p < 0.0001$). Cranial nerve (CN) palsy was more common in STS ($p = 0.008$), as was ring enhancement ($p = 0.007$), necrosis ($p = 0.009$), larger cranio-caudal (CC) tumor dimension ($p = 0.04$), and extra-pontine extension ($p = 0.04$) on diagnostic magnetic resonance imaging. LTS more commonly received chemotherapy at diagnosis ($p = 0.005$). Histological grade was not significantly different between the groups, but in multivariate analysis, LTS and STS were more likely to harbor *HIST1H3B* ($p = 0.002$) and *H3F3A* ($p = 0.04$) mutations, respectively. **DISCUSSION** We report a number of clinical, radiological, and genetic factors that correlate with survival for children with DIPG. These findings are important for risk stratification in future clinical trials and demonstrate the prognostic value of molecular data gained by performing diagnostic biopsy.

INTRODUCTION

Diffuse intrinsic pontine glioma (DIPG) is a highly malignant brainstem tumor of middle childhood. Despite therapy, median survival is <1 year [1]. Long-term survival in DIPG, historically defined as overall survival (OS) >2 years, has anecdotally been reported in <10% of patients [1]. Clinical and imaging characteristics previously associated with longer survival include younger age, longer symptom latency, and lack of ring enhancement on diagnostic magnetic resonance imaging (MRI) [1,2]. Up to 90% of DIPGs harbor a pathognomonic histone point mutation in *H3F3A* (65% of cases) or *HIST1H3B* (25% of cases); the latter appears to confer longer survival. Ten percent of patients have a histone 3 wild-type tumor [3].

Involved-field radiation therapy (RT) remains standard of care but confers only a 3 to 4-month survival advantage. Benefit from neoadjuvant [4] or adjuvant [2,5] chemotherapy has not been consistently confirmed in prospective trials.

The rarity and inconsistent classification of DIPG, an imaging-based diagnosis, have long hampered cross-cohort comparisons. The primary aim of this multi-national collaborative effort between the International DIPG Registry (IDIPGR) and European Society for Paediatric Oncology DIPG Registry (SIOPE-DIPGR) [6,7] was to define clinical, radiological, histological, and molecular factors associated with short and long-term survival in the largest cohort of centrally-reviewed DIPGs to date.

METHODS

Study population

The study was approved by the institutional review board at Cincinnati Children's Hospital Medical Center and included 1,130 patients with centrally radiographically-confirmed DIPG diagnosed from 1990–2015. Patients from the IDIPGR (n = 409) were age 0–27 years from the United States, Canada, and Australia. Those from the SIOPE-DIPGR (n = 721) were age 0–21 years from the Netherlands, Germany, Austria, Switzerland, Italy, France, United Kingdom, and Croatia. Patients were referred to the registries as previously described [6,7]. All patients with radiographically-confirmed DIPG, regardless of age or symptom duration, were eligible. Exclusion criteria are listed in Figure 1. Patients with neurofibromatosis type 1 were excluded from the IDIPGR but not the SIOPE-DIPGR.

Clinical variables

Clinical data were abstracted from registry databases (JB, BC, SVZ, NC) using standardized Case Report Forms (CRFs). Cerebellar signs included dysmetria, ataxia, dysarthria, or

nystagmus (without associated CN palsies). Pyramidal tract signs included mono-, hemi-, or quadriparesis, hyperreflexia, or positive Babinski sign. Since OS (time from diagnosis to death or last follow-up) is regarded as the most reliable outcome variable for DIPG, progression-free survival was not reported. Long-term survivors (LTS) and short-term survivors (STS) were those with OS ≥24 or <24 months, respectively. Very long-term survivors (VLTS) were those with OS ≥ 60 months.

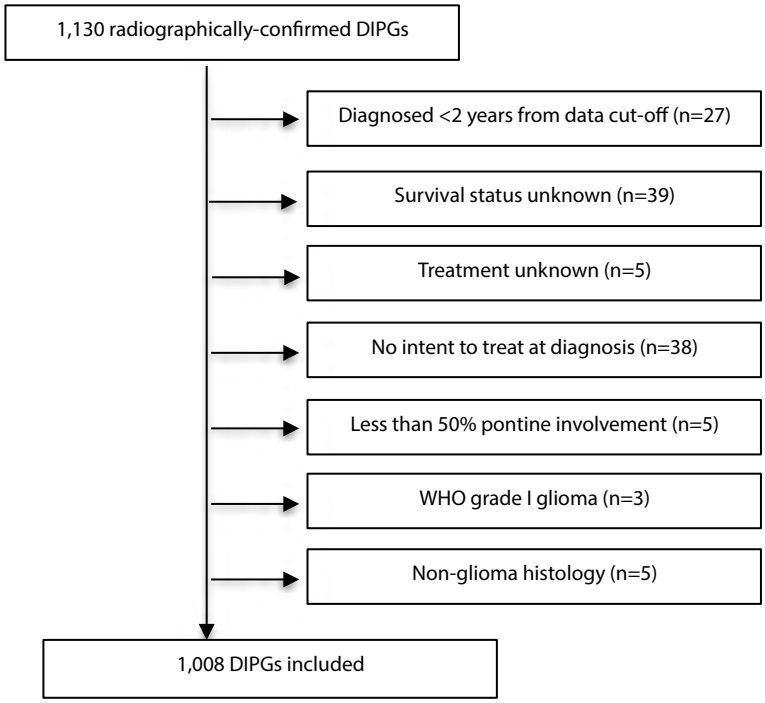


FIGURE 1 | Flow chart of patients excluded from this study.
WHO = World Health Organization

Radiological variables

Anonymized diagnostic MRIs were centrally reviewed by one of six neuro-radiologists (MW, BB, ES, RC, JL, BJ). MRI findings were classified as "typical" or "unlikely DIPG, other diagnosis suspected"; the latter were excluded. Typical DIPGs arose from and diffusely involved ≥50% of the pons. Exclusionary features included focally exophytic morphology, marked diffusion restriction, or secondary brainstem involvement by a tumor centered elsewhere in the brain or spine. Diagnostic imaging from all LTS and 10% of STS were cross-validated by a neuro-radiologist from the other registry.

Histopathological and molecular variables

Histology was defined according to the 2007 WHO grading system [8]. Forty-three IDIPGR tumor specimens were centrally reviewed (CF, CH). Databases were queried for the most common genomic alterations reported in DIPG. Molecular methods varied by institution. Histone mutations were assessed by Sanger sequencing, whole exome sequencing, whole genome sequencing, polymerase chain reaction, or immunohistochemistry (IHC) to detect H3 K27M-mutant protein or H3 K27 tri-methylation (H3 K27me3). K27M mutation in *H3F3A* (H3.3 K27M) or *HIST1H3B* (H3.1 K27M) was considered mutually exclusive, even if both were not evaluated.

Statistical analyses

Continuous and categorical patient characteristics were summarized by median (range) and frequency (%), respectively. Univariate differences between categorical and continuous variables were assessed by Fisher exact and Wilcoxon rank sum tests, respectively. Multivariate logistic regression was performed on variables with <15% missing data and univariate *p*-value of <0.1, with the exception of transverse tumor dimension (excluded due to high correlation with CC dimension). For each subgroup analysis, a multivariate logistical regression model, including statistically significant variables from the primary multivariate analyses, was used to determine subgroup significance and adjusted for confounding factors. The Kaplan-Meier method was used to estimate survival as a continuous variable. Statistical significance was defined as p-value <0.05. Statistical evaluation was done using R (Vienna, Austria, Version 3.1.3).

RESULTS

Survival

A total of 1,008 patients met inclusion criteria, including 374 and 634 from the IDIPGR and SIOPE-DIPGR, respectively. Median survival was 11 months (range 0–167 months), and 1-, 2-, 3-, 4- and 5-year OS were 42.3%, 9.6%, 4.3%, 3.2%, and 2.2%, respectively. Clinical, treatment, histological, molecular, and outcome data of 101 LTS (10%) and 16 VLTS (1.6%) are shown in Figure 2 and Supplementary Figure 1, respectively. KM survival analyses for age, symptom duration, chemotherapy, histology, and molecular status are shown in Figure 3.

ID	Age	Sex	CN Palsy	Cerebellar	Pyramidal	Symptom Duration (weeks)	Chemo	RT	Re-RT	Chemo Type	Tissue	WHO Grade	H3 Status	Status at LFU	OS (months)
DIPG 149	73		No	No	Yes	< 6	Yes	Yes	No	Other					24
DIPG 164	50						Yes	Yes	No	Other					24
DIPG 336	189		Yes	No	Yes	6-12	Yes	Yes	No						24
DIPG 354	125		Yes	No	Yes	< 6	Yes	Yes	No						24
FR 299	94		Yes	Yes	No	< 6	No	Yes	No				H3.3		24
GER 368	81		No	No		< 6	Yes	Yes	No	HDAC		IV			24
GER 399	188		Yes	No	No	12-24	Yes	Yes	No						24
GOSH 30	49		Yes	No	Yes	< 6	Yes	Yes	No						24
NETH 162	204		Yes	Yes	Yes	6-12	Yes	Yes	No	EGFR		IV	H3.3		24
DIPG 215	71						Yes	Yes	No	Unkn					24
DIPG 155	145		No	No	No	6-12	Yes	Yes	No			II			24
DIPG 22	93		No	No	Yes	< 6	Yes	Yes	No	Bev		IV	H3.1		24
DIPG 83	97		Yes	No	Yes	< 6	Yes	Yes	No	Other					25
DIPG 160	54			Yes	Yes		Yes	Yes	No	Unkn					25
FR 333	70		No	Yes	No	< 6	Yes	Yes	Yes	EGFR		IV	H3.1		25
GER 370	93		Yes	No	Yes	< 6	Yes	Yes	No	HDAC					25
IT 19	44		Yes	Yes	No	< 6	Yes	Yes	Yes	EGFR					25
IT 79	241		Yes	Yes	Yes	6-12	Yes	Yes	Yes	EGFR					25
NETH 111	97		No	No	Yes	< 6	No	Yes	No						25
DIPG 40	78					< 6	Yes	Yes	No	EGFR					25
DIPG 371	77		Yes	No	No	< 6	Yes	Yes	No	Other					26
FR 250	93		Yes	Yes	Yes	< 6	Yes	Yes	No	EGFR					26
FR 258	75		No	No	Yes	< 6	Yes	Yes	No	EGFR			H3		26
FR 270	91		Yes	No	Yes	6-12	No	Yes	No						26
FR 337	39		Yes	Yes	Yes		Yes	Yes	No	EGFR		III	H3.1		26
GER 383	42		Yes	Yes	No	< 6	Yes	Yes	No	HDAC					26
DIPG 247	188		No	Yes	Yes	6-12		Yes	No						26
FR 350	79		Yes	No	Yes		Yes	Yes	Yes	mTOR		II	H3.3		27
GER 372	77					< 6	Yes	Yes	No						27
DIPG 35	145		Yes	No	Yes	< 6	Yes	Yes	No	Bev		III			27
DIPG 96	78		Yes	No	Yes			Yes	Yes						28
DIPG 526	98		Yes	Yes	No	6-12	Yes	Yes	No	Bev		II	H3.3		28
FR 366	170		Yes	No	Yes		Yes	Yes	Yes	EGFR			WT		28
GER 375	142		No	No	No	>24	Yes	Yes	No			IV	WT		28
GER 390	59		No	Yes	Yes	< 6	Yes	Yes	No						28
IT 10	64		Yes	No	Yes		Yes	Yes	No			III			28
GER 393	138		Yes	No	No	6-12	Yes	Yes	No						29
IT 11	48		Yes	Yes	Yes	< 6	Yes	Yes	No						29
GER 369	106		Yes	Yes		< 6	Yes	Yes	No	HDAC		II			30
GER 376	136		No	No	Yes	< 6	Yes	Yes	No	HDAC					30
GER 401	54		Yes	No	Yes	< 6	Yes	Yes	No						30
IT 20	207		Yes	No	No	< 6	Yes	Yes	Yes	EGFR		IV			30
IT 81	104		Yes	No	No	12-24	Yes	Yes	Yes	EGFR					30
NETH 141	183		Yes	Yes	Yes	12-24	Yes	Yes	No			IV			30
DIPG 157	56		Yes	Yes	Yes	12-24	Yes	Yes	No	EGFR					31
DIPG 79	33					6-12	Yes	Yes	No						32
GER 373	78		Yes	Yes	Yes	< 6	No	Yes	No						32
NETH 160	147		Yes	Yes	Yes	< 6	No	Yes	No						32
DIPG 107	54						Yes	Yes	No			II			32
DIPG 31	34		No	No	No	12-24		Yes	No			IV	H3.3		32
DIPG 486	92		Yes	No	Yes	6-12	Yes	Yes	Yes	mTOR					33
GER 388	214		No	Yes	No	12-24	Yes	Yes	No			IV	H3.3		33
DIPG 114	27					6-12	Yes	Yes	No						33
DIPG 16	264		No	No	Yes	6-12	Yes	Yes	No	Bev		II			34

Age
- < 3 years
- 3-10 years
- >10 years

Sex
- Female
- Male

CN/Cerebellar/ Pyramidal
- Yes
- No

Symptom Duration
- < 6 weeks
- 6-12 weeks
- 12-24 weeks
- >24 weeks

RT, Chemo, Re-RT
- Yes
- No

Chemo Type
- Cytotoxic
- Targeted
- Both

Tissue
- Biopsy
- Autopsy
- Both

WHO Grade
- II
- III
- IV

Histone Status
- H3.3
- H3.1
- Wild-Type

Status as LFU
- Alive
- Deceased

Survival
- ≥2 years
- ≥3 years
- ≥4 years
- ≥5 years

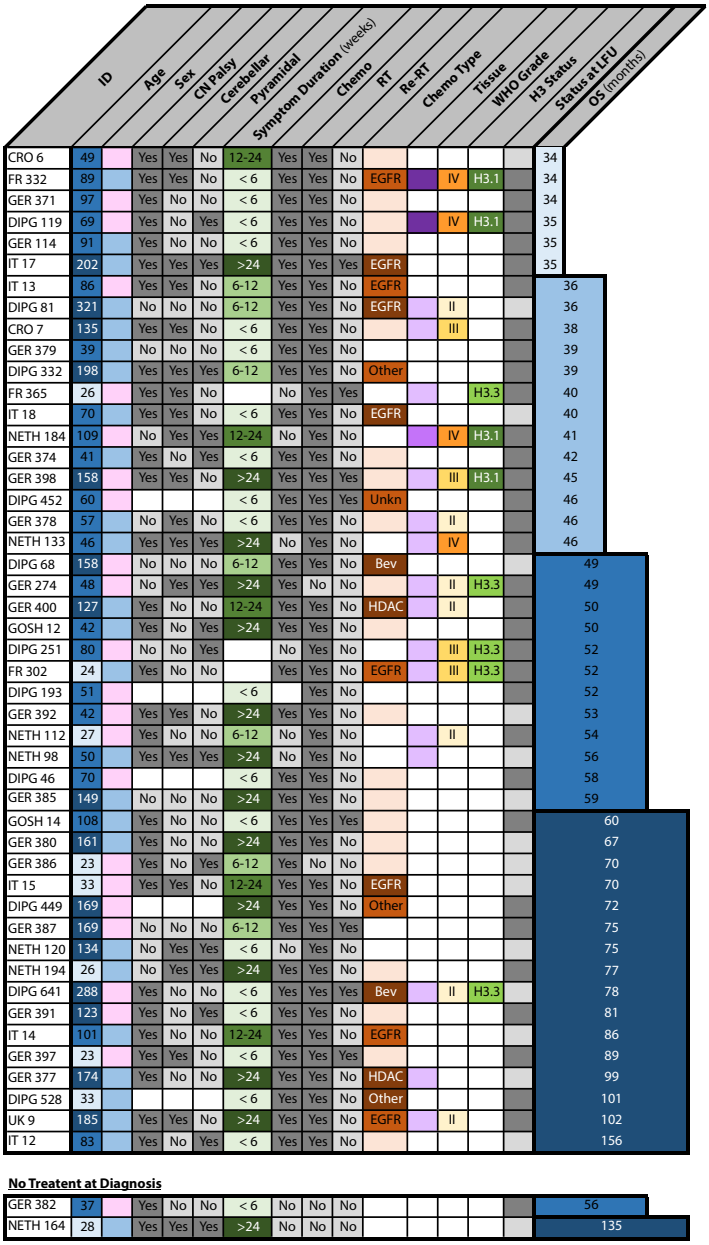
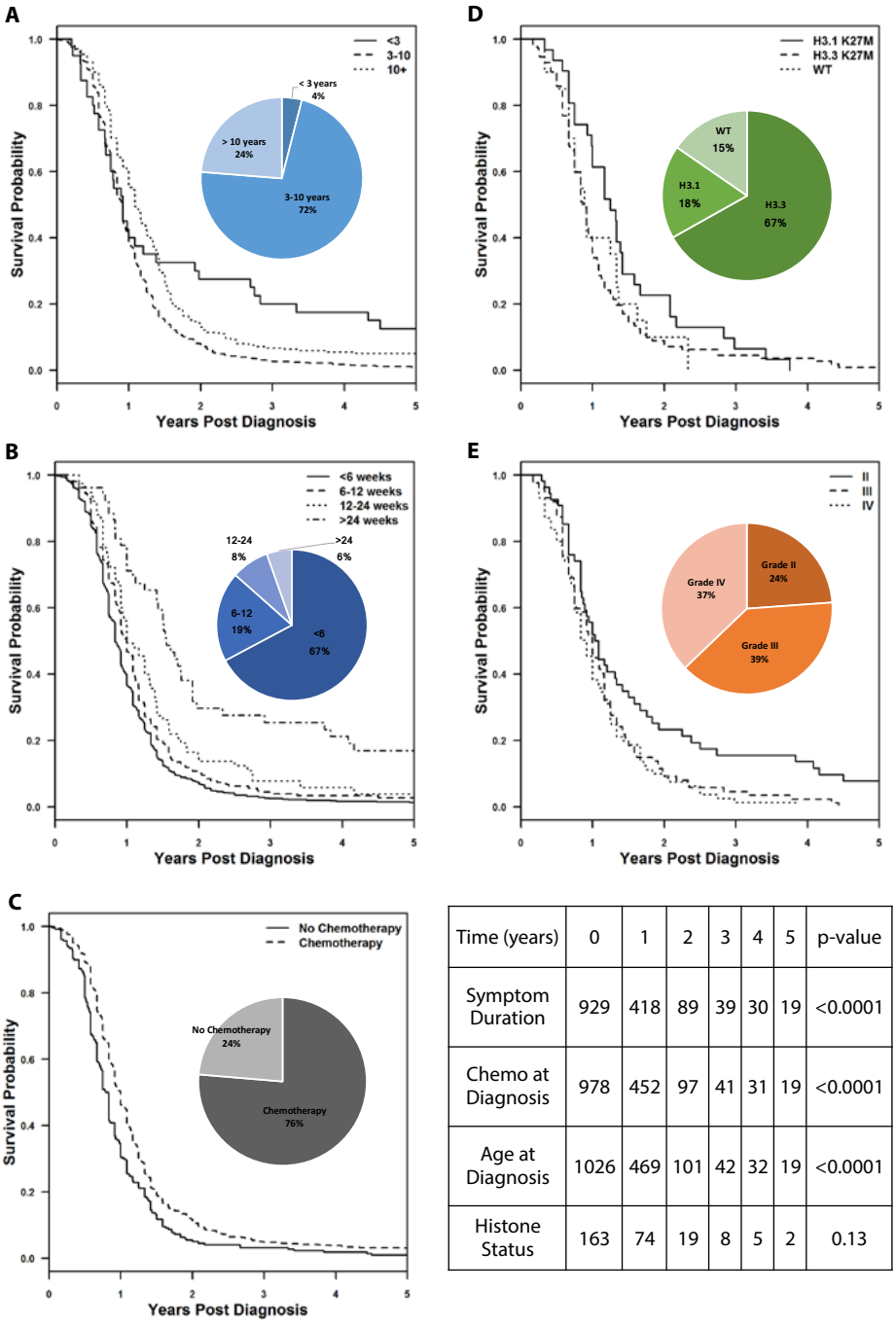


FIGURE 2 | Clinical, histological, and molecular characteristics of long-term survivors of DIPG.

CN = cranial nerve, RT = radiation therapy, WHO = World Health Organization, LFU = last follow up, OS = overall survival, HDAC = histone deacetylase inhibitor, EGFR = epidermal growth factor receptor, Unkn = Unknown, Bev = bevacizumab



Time (years)	0	1	2	3	4	5	p-value
Symptom Duration	929	418	89	39	30	19	<0.0001
Chemo at Diagnosis	978	452	97	41	31	19	<0.0001
Age at Diagnosis	1026	469	101	42	32	19	<0.0001
Histone Status	163	74	19	8	5	2	0.13

FIGURE 3 | Kaplan Meier curves representing overall survival based on **(A)** patient age (years), **(B)** symptom duration (months), **(C)** chemotherapy at diagnosis, **(D)** histone status, or **(E)** WHO grade.

Clinical presentation

Median age was 6.8 years (range 0–26.8 years); 4% were age <3 years at diagnosis. Of those with data available, 755/917 (82%), 468/915 (51%), and 567/920 (62%) presented with at least one CN palsy, pyramidal tract, or cerebellar sign, respectively. On univariate analysis (Table 1), LTS were more likely to be <3 or >10 years old (p<0.0001) and have longer symptom duration at diagnosis (p<0.0001), while STS were more likely to present with ≥1 CN palsy (p = 0.008). Multivariate analyses (Table 2) confirmed the association of age (p = 0.02) and symptom duration (p<0.0001) with long-term survival but failed to associate CN palsy with short-term survival.

TABLE 1 | Results of univariate analyses comparing clinical, radiological, and histological characteristics of long- and short-terms survivors of DIPG.

Clinical Variables		**LTS (n = 101)**	**STS (n = 907)**	**p-value**
Registry	International	33 (9%)	341 (91%)	0.39
	SIOPE	68 (11%)	566 (89%)	
Gender	Male	51 (50%)	420 (46%)	0.46
	Female	50 (50%)	485 (54%)	
Age (years)	Median	7.2 (1.9–26.8)	6.8 (0–26.5)	0.61
	<3	11 (11%)	29 (3%)	**<0.0001**
	3-10	57 (56%)	668 (74%)	
	>10	33 (33%)	205(23%)	
Symptom Duration (weeks)	<6	45 (51%)	564 (69%)	**<0.0001**
	6-12	19 (21%)	156 (19%)	
	12-24	11 (12%)	62 (8%)	
	>24	14 (16%)	35 (4%)	
Symptoms at Diagnosis	Cranial Nerve Palsy			
	Yes	63 (73%)	692 (83%)	**0.008**
	No	25 (27%)	137 (17%)	
	Pyramidal Tract Sign			
	Yes	39 (44%)	429 (52%)	0.5
	No	50 (56%)	397 (48%)	
	Cerebellar Sign			
	Yes	46 (53%)	521 (63%)	0.08
	No	41 (47%)	312 (37%)	
CSF Diversion	Yes	22 (22%)	196 (22%)	1
	No	79 (78%)	709 (78%)	
Chemotherapy at Diagnosis	Yes	85 (88%)	644 (75%)	**0.005**
	No	12 (12%)	214 (25%)	
Tumor Size (mm)	AP	36 (18–57)	36 (14–70)	0.98

TABLE 1 | Results of univariate analyses comparing clinical, radiological, and histological characteristics of long- and short-terms survivors of DIPG. *(Continued)*

Radiological Variables				
Tumor Size (mm)	Transverse	43 (15–76)	45 (17–81)	0.08
	CC	40 (20–88)	43 (16–107)	**0.04**
Pons Size (mm)	AP	36 (21–50)	35 (20–58)	0.12
	Trans	49 (31–62)	48 (22–78)	0.62
Extra-Pontine Extension	Yes	78 (86%)	739 (92%)	**0.04**
	No	13 (14%)	60 (8%)	
Hemorrhage	Yes	11 (14%)	136 (19%)	0.35
	No	68 (86%)	588 (81%)	
Necrosis	Yes	20 (26%)	306 (42%)	**0.009**
	No	56 (74%)	424 (58%)	
Hydrocephalus	Yes	14 (18%)	136 (18%)	1
	No	65 (82%)	632 (82%)	
Tumor Margin	Ill-defined	64 (75%)	605 (82%)	0.14
	Well-defined	21 (25%)	132 (18%)	
Ring Enhancement	Yes	19 (23%)	281 (38%)	**0.007**
	No	63 (77%)	457 (62%)	
Histological Variables				
Biopsy	Yes	38 (38%)	249 (28%)	**0.03**
	No	61 (62%)	652 (72%)	
Autopsy	Yes	11 (18%)	65 (10%)	**0.04**
	No	49 (82%)	597 (90%)	
WHO Grade	II	12 (41%)	40 (21%)	0.08
	III	9 (31%)	76 (40%)	
	IV	8 (28%)	73 (39%)	

SIOPE = European Society for Paediatric Oncology, CSF = cerebrospinal fluid, AP = anterior-posterior, CC = cranio-caudal, WHO = World Health Organization.

Therapy

Thirty-eight patients (3%) who did not receive therapy at diagnosis were excluded. Clinical characteristics of treated versus untreated patients are shown in Supplementary Figure 2A. Untreated patients were more often <3 years old at diagnosis. Seven underwent biopsy (1 diffuse astrocytoma [DA], 2 anaplastic astrocytoma [AA], and 4 glioblastoma multiforme [GBM]), and four underwent autopsy (1 AA, 2 GBM, 1 primitive neuroectodermal tumor [PNET]). At progression, one received chemotherapy; none received RT. Median OS for untreated patients was 1 month (range 0–135 months). Two were LTS (both infants), including one who is alive 135 months from diagnosis (Supplementary Fig. 2B).

TABLE 2 | Results of multivariate Cox proportional analysis of clinical, radiological, and biological variables predicting survival.

Clinical Variables		**Odds Ratio (95% CI)**	**p-value**
Age (years)	<3	1	**0.02**
	3-10	0.35	
	>10	0.79	
Symptom Duration (weeks)	<6	0.18	**<0.0001**
	6-12	0.26	
	12-24	0.43	
	>24	1	
Cranial Nerve Palsy	Yes	0.57	0.08
	No	1	
Chemotherapy at Diagnosis	Yes	3	**0.01**
	No	1	
Radiological Variables			
Tumor Dimension (mm)	AP	-	0.58
	Trans	0.99	
	CC	-	
Extra-Pontine Extension	Yes	0.95	0.91
	No	1	
Molecular Variables			
H3F3A Mutation	Yes	1	**0.04**
	No	1.14	
HIST1H3B Mutation	Yes	1	**0.002**
	No	0.78	
ACVR1 Mutation	Yes	1	0.09
	No	0.75	
TP53 Mutation	Yes	1	0.36
	No	1.09	

Necrosis, enhancement, and WHO grade were excluded from multivariate analysis since >15% of data for these variables were missing. Multivariate analysis of genomic data adjusted for age, symptom duration, and use of chemotherapy at diagnosis.

Status of both RT and chemotherapy was known for 968 patients of whom 721 (74%) received both, 231 (24%) RT alone, and 16 (2%) chemotherapy alone. In uni- and multivariate analyses, LTS were significantly more likely to have received chemotherapy at diagnosis ($p = 0.005$ and $p = 0.01$, respectively). Chemotherapy type was known for 702 patients (70%); 350 (50%), 193 (27%), and 159 (23%) received cytotoxic only, targeted only, or both, respectively. On univariate analysis, there was no survival difference based on type of targeted therapy (Table 1). However, multivariate logistical

regression adjusted for age and symptom duration demonstrated greater odds of long-term survival with use of an epidermal growth factor receptor (EGFR) inhibitor (OR 2.32, p = 0.03) or bevacizumab (OR 2.67, p = 0.03), an anti-vascular endothelial growth factor (VEGF) antibody, at diagnosis (Table 2).

Imaging

Table 1 summarizes diagnostic imaging characteristics. STS demonstrated larger CC tumor dimension (p = 0.04), extra-pontine extension (p = 0.04), tumor necrosis (p = 0.009), and ring enhancement (p = 0.007). Hemorrhage, hydrocephalus, and tumor margins were not statistically different between LTS and STS. Metastatic disease was present in 18 STS (2%) and no LTS at diagnosis.

Histology and molecular

More SIOPE-DIPGR patients (245/634; 39%) underwent biopsy than IDIPGR patients (54/372; 14%), while more IDIPGR patients (61/376; 16%) underwent autopsy (16/363; 4% SIOPE-DIPGR) (Supplementary Table 1). LTS from both registries more often underwent biopsy (p = 0.04; Table 1). Histology and WHO grade were available for 288 biopsies and 76 autopsies. WHO grade (II-IV) did not influence survival. Biopsy specimens included GBM (n = 80), AA (n = 76), anaplastic oligodendroglioma (n = 10), DA (n = 37), fibrillary astrocytoma (n = 4), oligodendroglioma (n = 2), low-grade astrocytoma (n = 8), or unknown (n = 71). Histology of autopsy tissue included GBM (n = 48), AA (n = 12), DA (n = 3), and unknown (n = 13).

Genomic data were available for 181 patients (18%) (Supplemental Table 2; available upon request), including 21 LTS (Fig. 4). Patients with H3.1 K27M had longer median OS (15 months), and H3.1 K27M was strongly associated with long-term survival in multivariate analysis (p = 0.002; Table 2). In contrast, H3.3 K27M was associated with short-term survival STS (p = 0.04, median survival 10.4 months). Patients with H3 wildtype tumors (n = 26) had a median OS of 10.5 months. WHO grade was not associated with histone mutation status (p = 0.18). Mutations in *TP53* or *ACVR1* were not associated with survival. Interestingly, of those age >10 years at diagnosis, who as a group demonstrated higher likelihood of long-term survival, 38/50 (78%) harbored H3.3 K27M, 9 (18%) were H3 wild-type, and only 3 (6%) had H3.1 K27M.

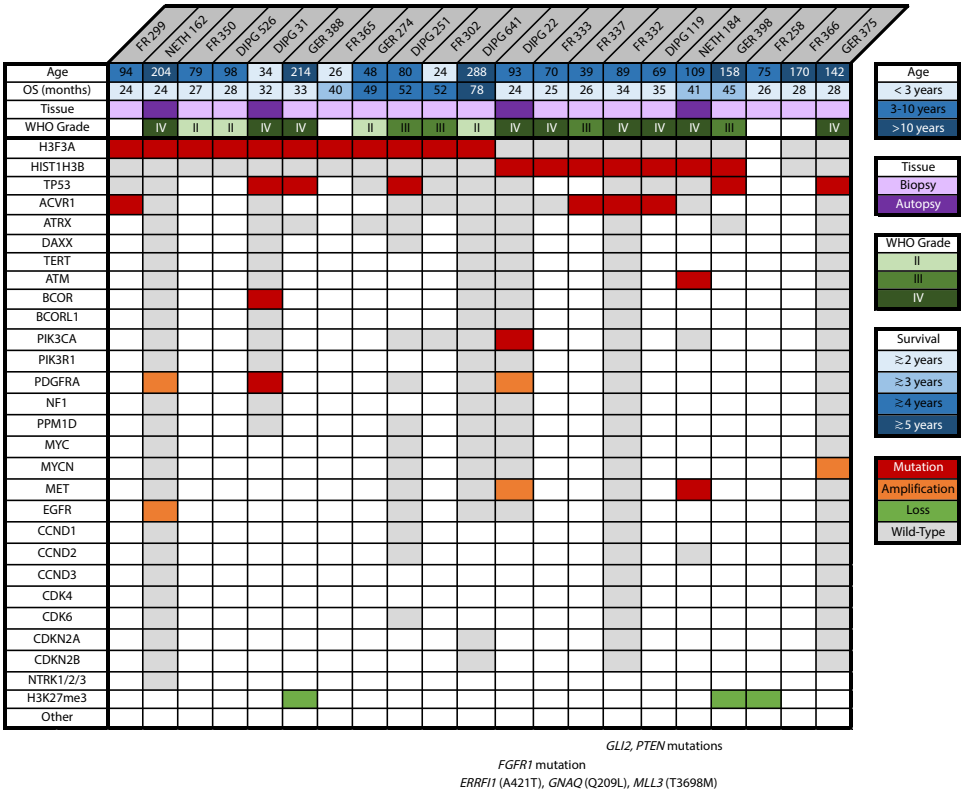


FIGURE 4 | Genomic aberrations in long-term survivors of DIPG.

WHO = World Health Organization

DISCUSSION

This study confirms the relevance of some previously reported survival-associated factors in patients with DIPG and offers unique insight into 101 LTS (including 16 VLTS). Median survival for all 1,008 patients was 11 months [1,5]. Median survival of LTS and VLTS was 33 (range 24–156) and 78 (range 60–156) months, respectively. Two-year OS of 9.7% in this study was consistent with large retrospective studies [2,5] that reported 9.2% and 9% 2-year OS in 153 and 316 DIPG patients, respectively.

Previously, 43 VLTS had been reported in the literature [1,9–14]. In Supplementary Figure 1, we compare characteristics of 22 previously published VLTS to our 16 VLTS, eight of whom (0.02% of the total cohort) are still alive with median follow up of 6.5 years (range 5–13 years). Five-year OS of 2.3% in this study is comparable to 2.6% reported by Jackson *et al*. [1] in 191 DIPG patients; however, two of their five VLTS would have been excluded from our study for atypical MRI features. Freeman *et al*. reported

nine VLTS (6.9%) among 130 DIPGs treated with hyper-fractionated RT (POG-8495) [11], though only four (3%) would have met inclusion criteria on our study.

In this study, age <3 or >10 years, longer symptom latency, lack of CN palsy, and chemotherapy at diagnosis were predictors of long-term survival. Of 41 patients age <3 years at diagnosis, 36 received upfront RT ± chemotherapy; five received chemotherapy alone. Although median OS for younger children (11 months) was the same as the entire cohort, a greater proportion were LTS or VLTS. Other studies have reported similar findings [1,2,5,15]. Broniscer *et al*. described 10 patients age <3 years with radiographically-confirmed DIPG, who received RT ± chemotherapy (n = 8) or chemotherapy only (n = 2) at diagnosis (n = 6) or progression (n = 4). Five (50%) were LTS, including one treated without RT. Wagner *et al*. similarly reported higher median survival (13.6 versus 10 months) in 13 children with DIPG age <4 years at diagnosis; only eight (61%) received RT [5]. Limited molecular data on five children with DIPG age <3 years in this study precluded making conclusions about biologic differences in this age group. We postulate that unique mechanisms, such as potently oncogenic NTRK fusions described in infantile midline HGG and DIPG [16], may underlie their observed survival advantage.

In our study, patients age >10 years at diagnosis had longer median OS (13 months) and were more likely to be LTS. Bailey *et al*. similarly reported five LTS (all >9 years) among 43 radiographically-confirmed DIPGs [17]. By contrast, Veldhuijzen van Zanten *et al*. reported no difference in OS between patients age 9–18 years versus younger [15]. Although pathogenic mechanisms, such as low-grade histology or IDH mutation may influence survival in older patients, 78% of patients >10 years old in our study harbored the poor prognostic H3.3 K27M mutation.

Consistent with prior reports [1,2], symptoms for >24 weeks at diagnosis was strongly associated with longer survival in uni- and multivariate analyses. CN palsy at diagnosis predicted shorter survival on univariate but not multivariate analysis. Previous studies reporting association of CN palsy with shorter survival included all brainstem tumors, not just DIPG, and/or diagnosis based on CT scan, making comparison difficult [18].

Neoadjuvant or adjuvant chemotherapy at diagnosis correlated with long-term survival in both uni- and multivariate analyses. This finding differs from the long-standing view that chemotherapy provides no survival benefit for DIPG, a principle largely based on small, non-randomized clinical trials. Effective cross-comparison of therapeutic studies for DIPG has been hindered by wide variation in inclusion criteria, as demonstrated in meta-analyses by Hargrave *et al*. and Jansen *et al*. in which only six of 29 DIPG-specific therapeutic trials between 1984 and 2012 had comparable eligibility [19,20]. In a randomized study, Wagner *et al*. reported better median OS in DIPG patients treated

with adjuvant chemotherapy after RT (11.3 months) compared to those receiving RT alone (9.5 months) (p = 0.03) [5]. Similarly, others have reported superior median OS in DIPGs treated with adjuvant or neoadjuvant chemotherapy and RT [4].

Multivariate logistical regression demonstrated higher odds of long-term survival with use of EGFR inhibitors (e.g. gefitinib, erlotinib, nimotuzumab, rindopepimut, or cetuximab) (p = 0.03) or bevacizumab (p = 0.03) at diagnosis. The EGFR pathway has been a much studied therapeutic target in DIPG [21]. The phase II study of gefitinib with RT in newly-diagnosed DIPGs noted 2-year OS of 19.6% with PFS >36 months in 3 patients [22]. In Geoerger *et al.*'s biopsy-mandated phase I study of erlotinib with RT in newly-diagnosed DIPG, median OS was 12 months and EGFR over-expression trended towards longer PFS (10.1 vs 6.3 months; p = 0.058) but not OS [23]. Despite only modest activity of nimotuzumab in progressive DIPG patients, two lived for 663 and 481 days from the start of therapy [24].

Despite efficacy in adult GBM, bevacizumab has shown little activity in pediatric trials for newly-diagnosed [25] or progressive DIPG [26] (median PFS 2.3 months). However, in a phase I trial of vandetanib, a selective VEGFR2 and EGFR inhibitor, in patients with newly-diagnosed DIPG, Broniscer *et al*. reported 2-year OS of 21.4%, and higher levels of plasma VEGF were associated with longer PFS (p = 0.02) [28]. Modestly improved survival in some patients receiving EGFR or VEGF-directed therapies may correspond to tumor-specific pathways. Numbers were too few to assess patient outcomes based on genomically-matched targeted therapy in our study, but our findings support prospective assessment of biopsy tissue to define potential therapeutic targets, as recently undertaken in two multi-institutional/multi-national trials (NCT01182350, NCT02233049).

Children who did not receive treatment at diagnosis experienced early death with a median survival of 1 month (Supplementary Fig. 2A). Consistent with prior literature [5], these patients were more often age <3 years at diagnosis (26% versus 4% of treated patients). Interestingly, two young children (ages 28 and 37 months) who did not receive therapy were ultimately LTS (Supplementary Fig. 2B).

Based on the radiographic definition of DIPG by Barkovich *et al*. [29], patients with <50% pontine involvement (n = 5) were excluded. Similar to a prior report [5], these five patients had better median OS (20 months), two among them were LTS. Greater CC tumor dimension and extra-pontine extension were associated with shorter survival; the former contrasts with a report by Young-Poussaint *et al*., in which larger tumor at diagnosis was associated with longer survival [30].

As previously described [30], tumor necrosis (p = 0.005) and ring enhancement (p = 0.005) were associated with short-term survival in univariate analysis. Jansen *et al.* developed a validated multi-parametric prediction model [2] in which age <3 years, longer symptom duration, and use of chemotherapy were predictors of longer survival, while ring enhancement predicted shorter survival (p = 0.001). Unfortunately, in the current study, tumor necrosis and ring enhancement could not be assessed in multivariate analysis due to missing data.

The biological landscape of DIPG has been intensely studied since 2012, when first-in-human histone mutations were described [14]. Here, we report the largest patient cohort with known histone mutation status and clinical and radiological correlation to date. Our findings confirm the independent association of H3.1 K27M and H3.3 K27M with long- and short-term survival, respectively [3,14]. In our study, median OS did not significantly differ between histone wildtype and mutant DIPGs, which contrasts with Khuong-Quang *et al.*'s report of dramatically longer median OS (4.59 years) for patients with histone-WT tumors [14].

On univariate analysis, WHO grade was not statistically different between LTS and STS (Table 1), but on KM analysis, WHO grade II was associated with longer survival (Fig. 3E). In the most recent WHO classification for CNS tumors [32], K27M-mutant midline gliomas have been reclassified as WHO grade IV regardless of histology making this point less relevant. Tumors classified as PNET (now called "embryonal tumor not otherwise specified") may represent true embryonal mimics of DIPG or may result from sampling error in the context of intratumoral heterogeneity. Three such patients were identified (Supplementary Table 3) and excluded from the primary analysis. Embryonal pontine tumors often demonstrate sharper margination and eccentric location, while others have radiological characteristics indistinguishable from DIPG [33], like those in our study. Young age is the most consistent distinguishing clinical factor between an embryonal pontine tumors and DIPG [33]. One of three embryonal patients in our study was 27 months old at diagnosis.

A limitation of this study is use of disease-specific registry data that is susceptible to enrollment bias on the part of participating institutions, which tend to be large academic centers, and patients or families who self-refer. Variation in standards of care for patients with DIPG (e.g. enrollment on clinical trials) may have also influenced findings. The anonymity of registry data makes some overlap of registry patients with those previously reported possible, biasing our findings toward similarity with published literature since they are not completely independent cohorts. The primary strength of this study is the requirement for central review of diagnostic imaging and cross validation of findings by highly-experienced pediatric neuro-radiologists and use of

standardized CRFs. This study represents the largest, most comprehensively-annotated cohort of radiographically-confirmed DIPGs reported, offering the most accurate rates of long- and very-long term survival for this rare tumor. Identification of robust survival-associated factors in this study is vital for development of prognostic sub-groups and emphasizes patient subsets from whom the most could be learned from analyzing pre-treatment biopsy tissue. Understanding biological differences that confer survival advantage in DIPG paves the road toward development of sub-group-specific therapies that may improve outcome in this devastating disease.

SUPPLEMENTARY DATA

Study	ID	Age	Sex	CN Palsy	Cerebellar	Pyramidal	Symptom Duration (weeks)	Chemo	RT	Re-RT	Chemo Type	Tissue	WHO Grade	H3 Status	Status at LFU	OS (months)
IDIPG/SIOPE DIPG Registries	GOSH 14	108		Yes	No	No	< 6	Yes	Yes	Yes						60
	GER 380	161		Yes	No	No	>24	Yes	Yes	No						67
	GER 386	23		Yes	No	Yes	6-12	Yes	No	No						70
	IT 15	33		Yes	Yes	No	12-24	Yes	Yes	No	EGFR					70
	DIPG 449	169					>24	Yes	Yes	No	Other					72
	GER 387	169		No	No	No	6-12	Yes	Yes	Yes						75
	NETH 120	134		No	Yes	Yes	< 6	No	Yes	No						75
	NETH 194	26		No	Yes	Yes	>24	Yes	Yes	No						77
	DIPG 641	288		Yes	No	No	< 6	Yes	Yes	Yes	Bev		II	H3.3		78
	GER 391	123		Yes	No	Yes	< 6	Yes	Yes	No						81
	IT 14	101		Yes	No	No	12-24	Yes	Yes	No	EGFR					86
	GER 397	23		Yes	Yes	No	< 6	Yes	Yes	Yes						89
	GER 377	174		Yes	No	No	>24	Yes	Yes	No	HDAC					99
	DIPG 528	33					< 6	Yes	Yes	No	Other					101
	UK 9	185		Yes	Yes	No	>24	Yes	Yes	No	EGFR		II			102
	IT 12	83		Yes	No	Yes	< 6	Yes	Yes	No						156
Ref. 1	SJCRH 5	197		Yes		Yes	< 6	Yes	Yes		EGFR					64
	SJCRH 3	88		Yes	Yes	Yes	>24	Yes	Yes		EGFR					94
	SJCRH 4	101		Yes	Yes	Yes	< 6	Yes	Yes		Other					117
	SJCRH 1	13		Yes	Yes	Yes	>24	Yes	Yes				II			120
	SJCRH 2	30		Yes	Yes	Yes	>24	Yes	Yes							158
Ref. 11	POG 9	78		Yes	Yes	No	>24	No	Yes							64
	POG 6	144		No	No	No	< 6	No	Yes				III			78
	POG 8	86		Yes	Yes	Yes	6-12	No	Yes							86
	POG 2	96		Yes	Yes	Yes	6-12	No	Yes				II			89
	POG 4	66		Yes	Yes	Yes	>24	No	Yes				II			91
	POG 7	86		Yes	No	No	6-12	No	Yes							92
	POG 5	180		Yes	No	No	6-12	No	Yes				III			96
	POG 3	144		Yes	Yes	Yes	< 6	No	Yes							99
	POG 1	132		No	Yes	No	>24	No	Yes				II			109
Ref. 14	Sick Kids 1	20		Included atypical radiological or clinical features if HGG histology									IV	WT		75+
	Sick Kids 2	180											III	WT		190+
	Sick Kids 3	30											III	WT		158+
	Sick Kids 4	36											IV	WT		120+
Ref. 13	Finland 1	156		"typical" clinical findings				Yes	Yes	No	Other		II/III			60+
Ref. 9	NCI 1	31						Yes	Yes	No	Other					60+
Ref .10	Toronto 1	4		Yes	No	No	< 6	Yes	No	No						183
	Toronto 2	42		Yes	No	No	< 6	Yes	Yes	No						233

Age
< 3 years
3-10 years
>10 years

Sex
Female
Male

CN/Cerebellar/ Pyramidal
Yes
No

Symptom Duration
< 6 weeks
6-12 weeks
12-24 weeks
>24 weeks

RT, Chemo, Re-RT
Yes
No

Chemo Type
Cytotoxic
Targeted
Both

Tissue
Biopsy
Autopsy

WHO Grade
II
III
IV

Status as LFU
Alive
Deceased

Histone Status
H3.3
H3 WT

SUPPLEMENTARY FIGURE 1 | Very long-term survivors of DIPG in the current study compared to those described in the literature. Yellow highlight indicates atypical radiological features that would have been excluded in the current study.

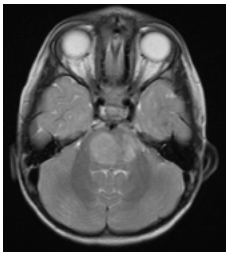
CN = cranial nerve, RT = radiation therapy, WHO = World Health Organization, LFU = last follow up, OS = overall survival, HDAC = histone deacetylase inhibitor, EGFR = epidermal growth factor receptor, Unkn = Unknown, Bev = Bevacizumab

A

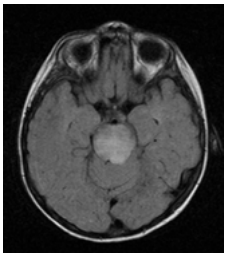
Clinical Variables		Untreated (n=38)	Treated (n=1,008)
LTS	Yes	2 (5%)	101 (10%)
	No	36 (95%)	907 (90%)
Age (years)	Median	6.3 (0-15.4)	6.8 (0-26.8)
	<3	10 (26%)	40 (4%)
	≥3	28 (74%)	963 (96%)
Symptom Duration (weeks)	<6	26 (68%)	609 (67%)
	6-12	8 (21%)	175 (19%)
	12-24	1 (4%)	73 (8%)
	>24	3 (8%)	49 (6%)
Symptoms at Diagnosis: Cranial Nerve Palsy	Yes	26 (79%)	755 (82%)
	No	7 (21%)	162 (18%)
Pyramidal Tract Sign	Yes	17 (52%)	429 (52%)
	No	16 (48%)	397 (48%)
Cerebellar Sign	Yes	20 (62%)	521 (63%)
	No	12 (38%)	312 (37%)
Median OS (range)		1 month (0-135)	11 months (0-167)

B

GER 382



NETH 164



ID	GER 382	NETH 164
Age	37	28
Gender		
CN Palsy	Yes	Yes
Cerebellar	No	Yes
Pyramidal	No	Yes
Symptom Duration	< 6	>24
Chemo	No	No
RT	No	No
Re-RT	No	No
Status at LFU		
OS (months)	56	135

SUPPLEMENTARY FIGURE 2 (A) Comparison of characteristics of patients who received therapy or did not receive therapy at diagnosis. **(B)** MRI images and clinical characteristics of two DIPG long-term survivors who did not receive therapy.

SUPPLEMENTARY TABLE 1 | Number of biopsies and autopsies performed by country or region.

SIOPE-DIPGR	Biopsy n, %	Autopsy n, %
France	109/113, 96%	2/115, 2%
Germany/Switzerland/Austria	81/278, 29%	4/16, 25%
The Netherlands	29/114, 25%	10/113, 9%
Italy	17/79, 22%	0/71, 0%
Croatia	2/7, 29%	0/5, 0%
United Kingdom	7/43, 16%	0/43, 0%
IDIPGR		
United States/Canada/Australia	54/372, 15%	61/376, 16%

SUPPLEMENTARY TABLE 3 | Clinical, radiological, and molecular characteristics of patients with PNET.

Patient ID	Age (months)	Symptom Duration	Symptoms	Treatment at Diagnosis	OS (months)	Source of Tissue	Molecular
DIPG-0051	27	Unknown	Unknown	RT+vorinostat	6	Biopsy	WT H3.3
DIPG-0165	53	<6 weeks	CN, pyramidal	RT+vorinostat	7	Biopsy	WT PDGFRA and EGFR
DIPG-0236	62	<6 weeks	Unknown	RT	5	Autopsy	Mutant TP53 and NF1 Amplified MYC-N WT H3.3, H3.1, ACVR1, PDGFRA, EGFR, ATRX, DAXX, PIK3CA, MET, CDKN2A/B, CCND1/2, CDK6, PPM1D

RT=radiation therapy, CN=cranial nerve, OS=overall survival, WT=wild-type

REFERENCES

[1] Jackson S, Patay Z, Howarth R, et al. Clinico-radiologic characteristics of long-term survivors of diffuse intrinsic pontine glioma. J Neurooncol. 2013;114:339–44.

[2] Jansen MH, Veldhuijzen van Zanten SE, Sanchez Aliaga E, et al. Survival prediction model of children with diffuse intrinsic pontine glioma based on clinical and radiological criteria. Neuro Oncol. 2015;17(1):160-6.

[3] Castel D, Philippe C, Calmon R, et al. Histone H3F3A and HIST1H3B K27M mutations define two subgroups of diffuse intrinsic pontine gliomas with different prognosis and phenotypes. Acta Neuropathol. 2015;130:815–27.

[4] Gokce-Samar Z, Beuriat PA, Faure-Conter C, et al. Pre-radiation chemotherapy improves survival in pediatric diffuse intrinsic pontine gliomas. Childs Nerv Syst. 2016;32:1415–23.

[5] Wagner S, Warmuth-Metz M, Emser A, et al. Treatment options in childhood pontine gliomas. J Neurooncol. 2006;79:281–7.

[6] Baugh J, Bartels U, Leach J, et al. The international diffuse intrinsic pontine glioma registry: an infrastructure to accelerate collaborative research for an orphan disease. J Neurooncol. 2017;132:323–31.

[7] Veldhuijzen van Zanten SEM, Baugh J, Chaney B, et al. Development of the SIOPE DIPG network, registry and imaging repository: a collaborative effort to optimize research into a rare and lethal disease. J Neurooncol. 2017;32:255–66.

[8] Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 2007;114:97–109.

[9] Warren K, Bent R, Wolters PL, et al. A phase 2 study of pegylated interferon α-2b (PEG-Intron(®)) in children with diffuse intrinsic pontine glioma. Cancer. 2012;118:3607–13.

[10] Hargrave D, Chuang N, Bouffet E. Conventional MRI cannot predict survival in childhood diffuse intrinsic pontine glioma. J Neurooncol. 2008;86:313–9.

[11] Freeman CR, Bourgouin PM, Sanford RA, et al. Long term survivors of childhood brain stem gliomas treated with hyperfractionated radiotherapy. Clinical characteristics and treatment related toxicities. Cancer. 1996;77:555–62.

[12] Allen J, Siffert J, Donahue B, et al. A phase I/II study of carboplatin combined with hyperfractionated radiotherapy for brainstem gliomas. Cancer. 1999;86:1064–9.

[13] Porkholm M, Valanne L, Lönnqvist T, et al. Radiation therapy and concurrent topotecan followed by maintenance triple anti-angiogenic therapy with thalidomide, etoposide, and celecoxib for pediatric diffuse intrinsic pontine glioma. Pediatr Blood Cancer. 2014;61:1603–9.

[14] Khuong-Quang D-A, Buczkowicz P, Rakopoulos P, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. Acta Neuropathol. 2012;124:439–47.

[15] Veldhuijzen van Zanten SEM, Jansen MHA, Sanchez Aliaga E, et al. A twenty-year review of diagnosing and treating children with diffuse intrinsic pontine glioma in The Netherlands. Expert Rev Anticancer Ther. 2015;15:157–64.

[16] Wu G, Diaz AK, Paugh BS, et al. The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma. Nat Genet. 46:444–50.

[17] Bailey S, Howman A, Wheatley K, et al. Diffuse intrinsic pontine glioma treated with prolonged temozolomide and radiotherapy--results of a United Kingdom phase II trial (CNS 2007 04). Eur J Cancer. 2013;49:3856–62.

[18] Fisher PG, Breiter SN, Carson BS, et al. A clinicopathologic reappraisal of brain stem tumor classification. Identification of pilocystic astrocytoma and fibrillary astrocytoma as distinct entities. Cancer. 2000;89:1569–76.

[19] Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. Lancet Oncol. 2006;7:241–8.

[20] Jansen MHA, van Vuurden DG, Vandertop WP, et al. Diffuse intrinsic pontine gliomas: a systematic update on clinical trials and biology. Cancer Treat Rev. 2012;38:27–35.

[21] Gilbertson RJ, Hill DA, Hernan R, et al. ERBB1 is amplified and overexpressed in high-grade diffusely infiltrative pediatric brain stem glioma. Clin Cancer Res. 2003;9:3620–4.

[22] Pollack IF, Stewart CF, Kocak M, et al. A phase II study of gefitinib and irradiation in children with newly diagnosed brainstem gliomas: a report from the Pediatric Brain Tumor Consortium. Neuro-Oncol. 2011;13:290–7.

[23] Geoerger B, Hargrave D, Thomas F, et al. Innovative Therapies for Children with Cancer pediatric phase I study of erlotinib in brainstem glioma and relapsing/refractory brain tumors. Neuro-Oncol. 2011;13:109–18.

[24] Bartels U, Wolff J, Gore L, et al. Phase 2 study of safety and efficacy of nimotuzumab in pediatric patients with progressive diffuse intrinsic pontine glioma. Neuro-Oncol. 2014; 16:1554-9.

[25] Hummel TR, Salloum R, Drissi R, et al. A pilot study of bevacizumab-based therapy in patients with newly diagnosed high-grade gliomas and diffuse intrinsic pontine gliomas. J Neurooncol. 2016;127:53–61.

[26] Gururangan S, Fangusaro J, Poussaint TY, et al. Efficacy of bevacizumab plus irinotecan in children with

recurrent low-grade gliomas--a Pediatric Brain Tumor Consortium study. Neuro-Oncol. 2014;16:310–7.

[27] Aguilera DG, Mazewski C, Hayes L, et al. Prolonged survival after treatment of diffuse intrinsic pontine glioma with radiation, temozolamide, and bevacizumab: report of 2 cases. J Pediatr Hematol Oncol. 2013;35:e42–6.

[28] Broniscer A, Baker JN, Tagen M, et al. Phase I study of vandetanib during and after radiotherapy in children with diffuse intrinsic pontine glioma. J Clin Oncol. 2010;28:4762–8.

[29] Barkovich AJ, Krischer J, Kun LE, et al. Brain stem gliomas: a classification system based on magnetic resonance imaging. Pediatr Neurosurg. 1990;16:73–83.

[30] Poussaint TY, Kocak M, Vajapeyam S, et al. MRI as a central component of clinical trials analysis in brainstem glioma: a report from the Pediatric Brain Tumor Consortium (PBTC). Neuro-Oncol. 2011;13:417–27.

[31] Sethi R, Allen J, Donahue B, et al. Prospective neuraxis MRI surveillance reveals a high risk of leptomeningeal dissemination in diffuse intrinsic pontine glioma. J Neurooncol. 2011;102:121–7.

[32] David N Louis MD, Ohgaki H. WHO classification of tumours of the central nervous system. World Health Organization, 2007.

[33] Sufit A, Donson AM, Birks DK, et al. Diffuse intrinsic pontine tumors: a study of primitive neuroectodermal tumors versus the more common diffuse intrinsic pontine gliomas. Journal of Neurosurgery: Pediatrics. 2012;10:81–8.