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Brainstem biopsy in pediatric diffuse intrinsic pontine glioma in the era of precision medicine: the INFORM study experience

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Abstract ***Purpose:*** Diffuse intrinsic pontine glioma (DIPG) is a highly aggressive paediatric brain tumour with fatal outcome. The Individualised Therapy For Relapsed Malignancies In Childhood (INFORM) registry study offers comprehensive molecular profiling of high-risk tumours to identify target alterations for potential precision therapy. We analysed molecular characteristics and clinical data after brainstem biopsy of all enrolled newly diagnosed DIPGs.
Patients and methods: From −February 2015 to February 2018, 21 subsequent primary DIPG cases were enrolled in the nation-wide multicentre INFORM registry study after brainstem biopsy. Whole-genome, whole-exome sequencing and DNA methylation analysis were performed, and RNA-sequencing was added in case of sufficient material. Clinical data were obtained from standardised questionnaires and the INFORM clinical data bank.
Results: Tumour material obtained from brainstem biopsy was sufficient for DNA analysis in all cases and RNA analysis in 16 of 21 cases. In 16 of 21 cases (76%), potential targetable alterations were identified including highly relevant *MET* and *NTRK1* fusions as well as an *EZH2* alteration not previously described in DIPG. In 5 of 21 cases, molecular information was used for initiation of targeted treatment. The majority of patients (19/21) presented with neurological deficits at diagnosis. Newly arising or worsening of neurological deficits post-biopsy occurred in nine patients. Symptoms were reversible or improved notably in eight cases.
Conclusion: In this multicentre study setting, brainstem biopsy of DIPG was feasible and yielded sufficient material for comprehensive molecular profiling. Relevant molecular targets were identified impacting clinical management in a substantial subset. Death or severe bleeding occurred in none of the cases. One of 20 patients experienced unilateral paraesthesia possibly related to biopsy.

1. Introduction

The Individualised Therapy For Relapsed Malignancies In Childhood (INFORM) registry, a multicentre, multinational study, started recruiting patients in Germany in February 2015 [1,2] and has prospectively enrolled more than 400 high-risk or relapsed paediatric cancer cases to date, offering a platform for comprehensive molecular profiling of the tumours to identify alterations as targets for potential precision therapy approaches. The registry is a collaborative effort of several trial groups of the German Society of Paediatric

Oncology and Hematology including the high-grade glioma study group, Hirntumor (HIT)—high-grade glioma (HGG). Owing to the very unfavourable prognosis with a median survival of less than one year [3,4], enrolment of patients with diffuse intrinsic pontine glioma (DIPG) in the registry is allowed already at primary diagnosis.

Current standard of care (SOC) treatment for DIPG patients includes radiotherapy (https://www.cancer.gov/types/brain/hp/child-glioma-treatment-pdq#section/_45). Concomitant oral temozolomide (e.g. EudraCT, number 2007-000128-42) or other regimens are frequently applied [4,5]. Unresectability, dismal prognosis and typical neuroradiologic features have been the main reasons not to consider tissue sampling in these children in the past [6,7], resulting in relatively lack of biological information on tumour-driving events and potential treatment targets in comparison to other paediatric brain tumours.

Recent large single-centre series report safety and feasibility of tissue sampling on paediatric brainstem tumours in about 95–100% of cases [8–11]. In addition, a recent study by Gupta *et al* [12]. (DIPG-BATS (Biology and Treatment Study)) demonstrates that morbidity and mortality rates are comparably low in a multicentre study using a standardised surgical procedure and upfront instruction of the neurosurgeons.

First molecular data from newly diagnosed DIPG and autopsy material demonstrate the prototypic midline glioma alterations including histone 3.3 or 3.1 K27M mutation [13–15], *ACVR1* and *TP53* mutations, as well as RAS-MAPK pathway alterations involving *PDGFRA*, *MET* and *NTRK* [16–19].

The aims of the present study were as follows: (1) to evaluate feasibility for applying comprehensive molecular profiling on brainstem biopsies including whole-exome sequencing (WES), low-coverage whole-genome-sequencing (lc-WGS), DNA methylation analysis, RNA sequencing and microarray gene expression profiling in the context of the population-based paediatric precision oncology registry study INFORM, (2) to identify targetable alterations and (3) to perform a clinical risk-benefit assessment on 21 subsequently newly diagnosed DIPG patients in a multicentre study setting.

2. Materials and methods

Within the first three years after the start of the INFORM registry (February 2015–February 2018), a total of 411 high-risk or relapsed paediatric cancer patients were prospectively enrolled. Of these, 21 subsequent patients with primary diagnosis of DIPG from 10 paediatric oncology centres across Germany were included in the present study. Written informed consent was obtained at the local centre, covering sequencing analysis of tumour and germline as well as scientific evaluation of molecular and clinical results. The study protocol was reviewed and approved by the Institutional Review Board of the University Hospital of Heidelberg (Ethics committee, University Hospital of Heidelberg).

For technical comparison of sequencing data, 29 non-DIPG high-grade glioma patients from 19 German paediatric oncology centres enrolled in the INFORM registry were added.

Brain biopsies on DIPG were performed in 12 German neurosurgery centres as part of the institutional SOC management as two patients were sent after surgery to a neighbouring oncology centre. Because the INFORM registry is a molecular profiling platform not including the sample taking, no neurosurgical guidelines were provided. Furthermore, enrolment in the INFORM registry is only possible after obtaining of tumour samples. Therefore, no study-specific or specialised training regarding the execution of biopsy was given to the neurosurgeons in the participating centres upfront.

The extracted tumour material was snap-frozen in accordance with local protocols and sent to the University of Heidelberg, Department of Neuropathology, for further processing according to the INFORM pipeline [1]. Nucleic acid extraction for tumour and germline material as well as comprehensive molecular profiling using next-generation sequencing (NGS) methods including WES, lc-WGS, DNA methylation analysis and—when possible—RNA sequencing and microarray gene expression profiling was performed as described previously [2].

Bioinformatic processing was conducted as per protocols established for the INFORM registry and previously outlined [2].

Gene expression profiling from Affymetrix GeneChip U133 Plus 2.0 array was visualised and analysed using the online ‘R2’ platform (http://r2.amc.nl). Expression levels were compared with publicly available data sets available within the ‘R2’ platform.

Detailed clinical information on preoperative conditions, the operative procedure as well as postoperative complications were assessed retrospectively by standardised questionnaires which were completed by the responsible treating paediatric oncology centre (see Supplementary Table 1).

3. Results

3.1. Demographics and preoperative clinical condition of the study cohort

Twenty-one patients with newly diagnosed DIPG were enrolled after biopsy into the prospective paediatric precision oncology INFORM registry (2015–2018) and could be included in this study. Clinical characteristics of the patient cohort are outlined in Table 1. All except two patients presented with neurological deficits at

Table 1
Clinical characteristics of the patient cohort.

Clinical characteristics	Total n = 21 INFORM DIPG patients
Sex, no. (%)	
Female	13 (61.9)
Male	8 (38.1)
Age, no. (%)	
≤ 4 yr	4 (19.0)
> 4 yr	17 (81.0)
Age, mean (range)	7.5 (3–15)
Karnofsky–Lansky, no. (%)	
≤ 70%	10 (47.6)
≥ 80%	11 (52.4)
Steroids perioperative, no. (%)	
Yes	19 (90.5)
No	0
Missing data	2 (9.5)
Level of resection, no. (%)	
Biopsy (R4)	19 (90.5)
Partial resection (R3)	1 (4.8)
Missing data	1 (4.8)
Preoperative condition, no. (%)	
Neurological deficits	19 (90.5)
Ataxia	16 (76.2)
Cranial nerve palsy	17 (81.0)
Long tract signs	9 (42.9)

INFORM, Individualised Therapy For Relapsed Malignancies In Childhood; DIPG, diffuse intrinsic pontine glioma.

diagnosis, with cranial nerve palsy affecting 81%, 76% suffering from ataxia and long tract signs being described in 43%. The further two patients showed unspecific signs of increased intracranial pressure at diagnosis.

3.2. Biopsy-obtained material is suitable for comprehensive molecular profiling

Tissue sampling was performed as per the institutional SOC procedures of the participating centres. A mean of eight tissue samples (range 1–24, data not available for two cases) were obtained by biopsy with variable number of cores. Median time before freezing of the tumour samples was 1hr 14min, ranging from 0 min to 15 h. Tumour cell content assessed by histopathological evaluation was relatively high (range 40–90%), and material was suitable for molecular analysis in all cases. Because of limited amount of tumour tissue or limited RNA quality and quantity, RNA analysis was missing or incomplete in nine cases (Fig. 1).

The quality of sequencing results were comparable with a control group of 29 non-DIP HGGs analysed within INFORM (Supplement Table 2).

3.3. Comprehensive analysis reveals actionable alterations and novel targets with implications for clinical management

Histopathological evaluation by local pathology as well as central review (Brain Tumour Reference Centre of the German Society of Neuropathology and Neuroanatomy [DGNN]) confirmed the diagnosis of a diffuse midline glioma World Health Organisation (WHO) grade III–IV in 18 of 21 (86%) cases. Two tumours were classified as WHO grade II lesions at the local neuropathological review, but as WHO grade III–IV lesions on reference assessment. For one tumour, the local diagnosis was diffuse midline glioma WHO grade IV H3-K27M mutated, but anaplastic astrocytoma WHO grade III Isocitrate dehydrogenase (IDH)-mutated according to the reference assessment. Exome sequencing confirmed IDH1 mutation in this case and wildtype status for H3.

An in-house–developed classifier scoring algorithm based on genome-wide DNA methylation patterns was applied [20,21]. With this tool, tumour samples were allocated to previously established methylation subgroups, with 19 of 21 (90%) of cases falling into the methylation class ‘diffuse midline glioma H3 K27M mutant’ [21,22]. The tumour harbouring an *IDH1*-mutation was assigned to the respective methylation class ‘IDH glioma, subclass high-grade astrocytoma’. The methylation profile of one further tumour was most similar to the methylation class ‘glioblastoma, IDH wildtype, subclass MYCN’, in line with the finding of a *MYC* as well as a *MYCN* amplification in this case and absence of a H3 mutation.

The K27M mutation in H3.3 or H3.1 encoding genes, characteristic of DIPG [16–18], was detected in 71% (15/21) and 14% (3/21) of cases, respectively. One case most closely allocated to the ‘diffuse midline glioma H3 K27M mutant’ methylation subgroup was wildtype for these genes. Interestingly, this case harboured a hotspot *EZH2* mutation known to be activating, but yet undescribed in DIPG [23–25]. Further typical alterations for DIPG, such as *TP53*, *ATRX*, *ACVR1* and *PPM1D* mutations, were identified in 67%, 19%, 14% and 14%, respectively (Fig. 2, Supplement Table 3).

Germline alterations indicative of underlying hereditary cancer predisposition syndrome could not be detected.

Detected alterations (e.g. genetic alterations such as gene amplifications/deletions, point mutations, fusions as well as gene expression changes) were assessed for matching drugs. A previously described prioritisation algorithm established for the INFORM registry and based on the type of alteration and its entity-specific relevance [2] was applied to the reported alterations, assigning each to a priority score on a 7-step scale ranging from ‘very high’ (1/7) to ‘very low’ (7/7). No actionable targets were identified in 5 of 21 (24%) of cases. Forty-eight percent (10/21) harboured at least one alteration with a priority of ‘moderate’ (3/7) to ‘very high’ (1/7).

The most frequent targetable alteration was *PDGFRA* amplification present in 4/21 cases (with *PDGFRA* amplification as well as fusion in one case). Two cases harboured oncogenic fusions involving *MET*

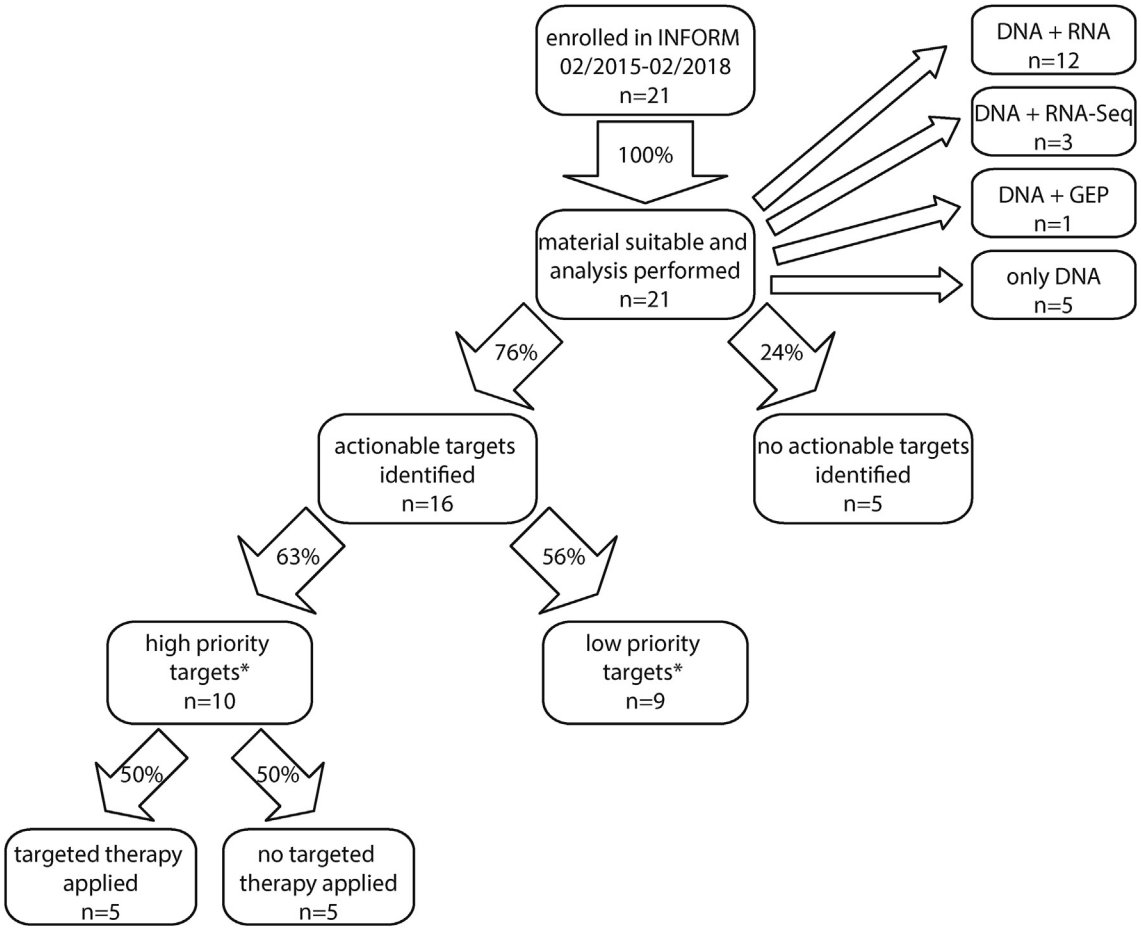

Fig. 1. Flow chart illustrating percentages for suitability of material, identification of actionable targets as well as implication on therapy. * high priority ≥ moderate (≥3/7), low priority < moderate (<3/7). Seven cases harboured alterations with high priority only; for six cases, only low-priority targets were identified, and three cases had high as well as low-priority targets. INFORM, Individualised Therapy For Relapsed Malignancies In Childhood.

and *NTRK1*, which have been described in paediatric high-grade glioma [11,16,26,27] and constitute promising drug targets. An overview of alterations is given in Fig. 2 (for details see Supplement Table 3).

Identification of actionable targets had implications on the further treatment in 5 of 21 (24%) cases at the time of follow up based on the treating physician's choice. Two patients with tumours harbouring genetic *PDGFRA* alterations were treated with receptor tyrosine kinase inhibitors targeting PDGFRA (dasatinib and sorafenib, respectively) in addition to the SOC treatment with temozolomide and irradiation. Based on a *PIK3CA* mutation, SOC treatment was complemented by the mammalian target of rapamycin inhibitor everolimus for one patient. Targeted therapy plus irradiation without temozolomide backbone was initiated in two patients—one treated with cetuximab and trametinib based on the detection of a *KRAS* amplification and one patient with a tumour harbouring a *MET* fusion as well as a *PDGFRA* amplification who received crizotinib and dasatinib. None of the patients treated with targeted therapy were enrolled in an early phase clinical trial because of the lack of matching open phase I/II trials in Germany.

3.4. Adverse events

Biopsy and sampling procedure were allowed according to institutional Standard Operating Procedure (SOPs). Owing to the multicentric nature of the INFORM registry, different biopsy techniques were applied. Details on the execution of brainstem biopsies are listed in Table 2.

Perioperative clinical information was available for 20 of 21 patients. Neurological deficits potentially related to surgical intervention (either newly arisen or worsening of pre-existing deficits) were described in 9 of 20 cases. Symptoms included cranial nerve palsy, hemiparesis, movement difficulties and somnolence. Symptoms were transient in seven cases with complete resolution. One patient showed hydrocephalus after surgery requiring placement of a ventriculoperitoneal shunt which led to resolution of symptoms. Permanent left-sided hemihyposthesia arose after surgical intervention in one patient.

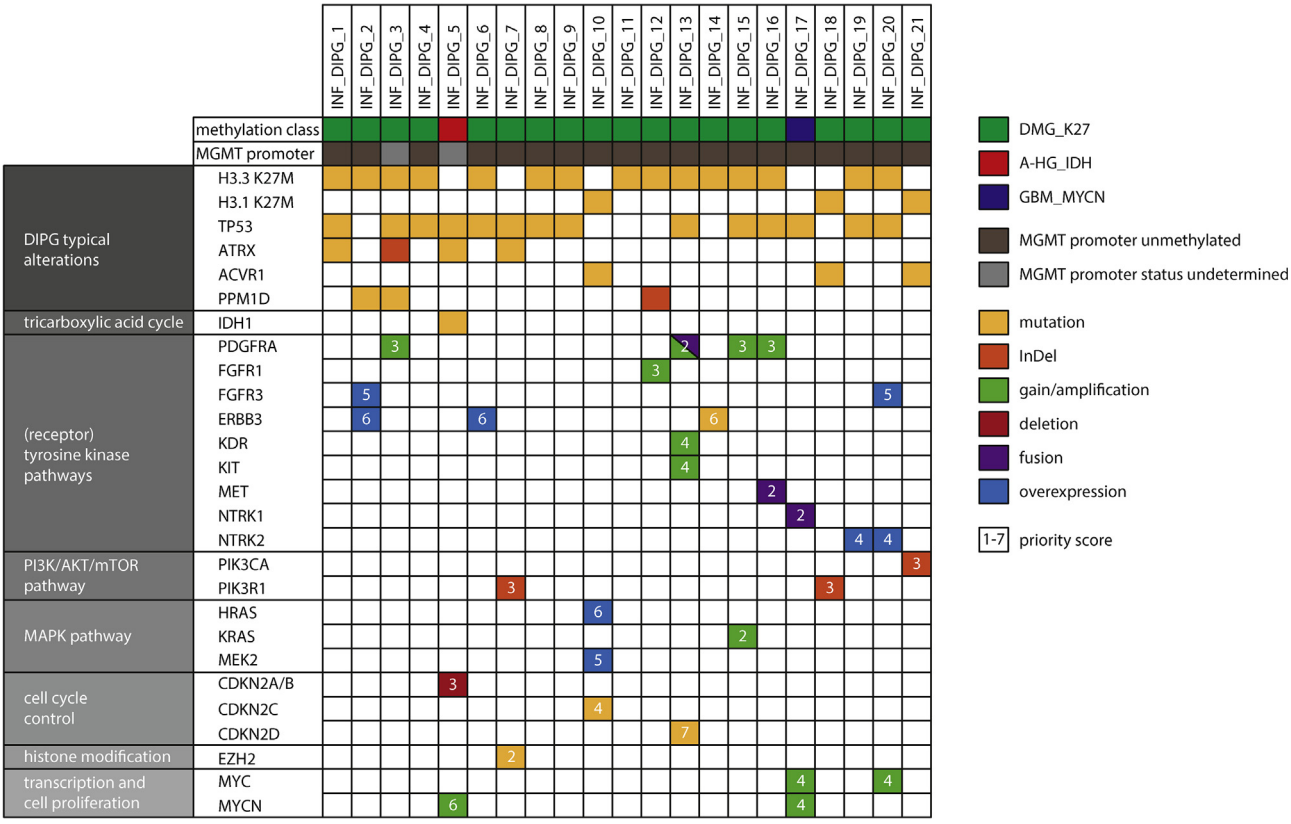

Fig. 2. Overview of subgroup affiliation based on genome-wide DNA methylation analysis, typical variants as well as potential targetable molecular alterations priority scores: very high (1), driving mutation/rearrangement in directly targetable gene which is a confirmed driver in specific entity; high (2), likely activating genetic alteration in directly targetable gene which is a confirmed driver in any entity or genetic alteration confirmed to activate an actionable pathway in specific entity; moderate (3), genetic alteration in presumed driver or presumed pathway activation (genetic) in specific entity or confirmed pathway activation known in other entity; intermediate (4), genetic alteration in presumed driver gene or presumed pathway activation (genetic) known in another entity or overexpression of directly targetable driver gene in specific entity or genetic alteration in a gene known to induce susceptibility to inhibition of another gene; borderline (5), genetic alteration in possible driver gene in any entity or overexpression of confirmed driver gene in another entity; low (6), possible pathway activation (genetic) or pathway activation (expression) in any entity; very low (7), circumstantial evidence. DIPG, diffuse intrinsic pontine glioma.

Table 2
Details on execution of brainstem biopsies.

Biopsy technique and approach	Total n = 21 INFORM DIPG patients
Biopsy technique, no. (%)	
Frame-based stereotactic biopsy	12 (57)
Navigated needle biopsy (frameless)	3 (14)
Open biopsy (craniotomy)	5 (24)
Endoscopic	0
Robotic	1 (5)
Approach, no. (% of minimal invasive biopsies)	
Frontal	5/16 (31)
Suboccipital/transcerebellar	11/16 (49)
Localisation of biopsy, no. (%)	
In the middle of the tumour mass	5/17 (29)
At the margin of the tumour mass	7/17 (41)
Multiple sides	5/17 (29)

INFORM, Individualised Therapy For Relapsed Malignancies In Childhood; DIPG, diffuse intrinsic pontine glioma.

Postoperative imaging was performed in 17 of 21 cases—in three cases directly after surgery, within 48 h in eight cases and beyond 48 h in five cases (data not available for one case). No changes compared with the preoperative status were observed in 13 cases; one patient had an asymptomatic bleeding, and another had a leakage of cerebrospinal fluid (no information available for two cases). Relevant secondary haemorrhage or cases of death due to brainstem biopsy were not observed. None of the patients had a prolonged postoperative stay in the intensive care unit exceeding more than seven days.

4. Discussion

The INFORM study provides a population-based precision oncology approach for relapsed or high-risk

newly diagnosed paediatric cancers [1,2]. Because DIPG is a highly aggressive, non-resectable brain tumour with a uniformly fatal disease course, INFORM allows inclusion of newly diagnosed DIPG cases.

We could demonstrate that comprehensive molecular characterisation from limited tumour material is feasible in a 'real-world' setting—both regarding amount and quality of the obtained material as well as the turnaround time of sample processing, NGS and bioinformatics processing steps. About 3–4 weeks (median 22 days) were required from arrival of tumour tissue until the preliminary target analysis report was available via the INFORM documentation platform. A short turnaround time is crucial because most DIPG patients suffer from aggressive clinical courses with limited time span for postsurgical clinical interventions.

Analysis of tumour material obtained from biopsy of DIPG allows for detailed molecular profiling with regards to the uniqueness of each individual tumour harbouring typical mutations as well as a specific combination of additional molecular characteristics. Potentially targetable alterations are of particular interest in this context—underlined by the fact that in this study, we identified high priority targets in half of the cases. Promising targets are, in particular, fusion products involving the *MET* and *NTRK1* gene, respectively. *MET* fusions were previously described as being oncogenic and potential drug targets [27]. A clinical phase I trial on the NTRK inhibitor larotrectinib in paediatric patients with TRK fusion–positive solid tumours could demonstrate clinically relevant antitumour activity already in phase I in a tumour-agnostic trial design [28]. Unexpected findings not described in DIPG before include detection of an *EZH2* mutation, which might constitute a promising drug target. EZH2 is the functional enzymatic component of the polycomb repressive complex 2, responsible for trimethylation on histone 3 K27 (H3K27me3) and subsequent transcriptional silencing [29]. With the previously described local hypermethylation effect of H3K27M in mind [30–32], the described *EZH2* mutation might hypothetically mimic the H3K27M controlling the transcription of specific loci, for example, of tumour suppressor genes. The EZH2 inhibitor tazemetostat showed antitumour activity in adult patients with selected advanced solid tumours [33], and a paediatric phase I study is ongoing.

Several early phase clinical trials for paediatric DIPG patients are recruiting. These include targeted agents such as receptor tyrosine kinase inhibitors (e.g. ClinicalTrials.gov Identifiers NCT01644773,), inhibitors of the cyclin-dependent kinases (e.g. NCT02644460), immune-modulating drugs such as PD-1 inhibitors (e.g. NCT02359565) [34] as well as, for example, Histone deacetylase (HDAC) inhibitors (e.g. NCT02717455,). The results of these studies and their potential impact on clinical outcomes are eagerly awaited. However, as per the molecular target pattern identified in this study, all-comer studies including any DIPG without defining the presence of a matching molecular target will unlikely exhibit efficacy signals. In contrast, the Biological Medicine for Diffuse Intrinsic Pontine Glioma Eradication (BIOMEDE) trial assigns patients to different molecular strata based on the expression of specific targets (epidermal growth factor receptor and phosphatase and tensin homologue deleted on chromosome 10) (NCT02233049). It remains to be shown, however, if immunohistochemistry based expression data of targets are predictors of treatment response to matching drugs in DIPG.

In the era of precision oncology approaches, it is important to assess not only the gain of molecular information for potential clinical management but also associated adverse events in particular if tissue sampling in high-risk anatomic regions is required such as the brainstem in DIPG. In our cohort of 21 newly diagnosed DIPG patients treated at 10 German paediatric oncology centres and operated on in 12 German neurosurgery centres, postinterventional mild neurological deficits were observed in 45% (9/20) of cases. It is of note that despite the fact that 12 neurosurgical teams with a wide range of different biopsy approaches (Table 2) were involved, the overall outcome of this 'real-world' series compares similar to previous single-institutional reports with 0 of 20 deaths or severe bleedings in our cohort [8–10]. However, permanent mild morbidity was observed in 1 of 20 cases (5%) which appears higher compared to less than 1% in large single-centre studies conducted in experienced centres [8–10]. A prospective multicentre study (DIPG-BATS) including 50 patients recently published by Gupta *et al.* described 20 adverse events postbiopsy, one patient with persistent neurological deficits but also no biopsy-related deaths [12]. These numbers are in the same range compared with our results.

To conclude, within our INFORM paediatric precision oncology study, we demonstrated that material obtained from DIPG biopsy in a multicentre approach was suitable for molecular analysis in all cases and state-of-the-art comprehensive molecular profiling was performed within a meaningful timeframe. Relevant targets with potential impact on further treatment were identified in the majority of cases. Tumour biopsy in DIPG in the context of a paediatric precision oncology program was not associated with mortality, or relevant permanent morbidity. In the context of the dismal outcome of DIPG patients with current standard treatment protocols, we believe that these results

support biopsy and comprehensive molecular analysis of DIPGs in the context of prospective multicentre study protocols. Our data will support future trial designs of interventional molecular matching studies in DIPG.

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Conflict of interest statement

None declared.

Authorship statement

E.P., A.E.D., M.B.-J., B.C.W., S.S., D.T.W.J. and O.W. performed data analysis and interpretation. E.P., G.P.B., M.B.-J., B.C.W., K.W.P., C.M.v.T., P.F., C.H.-M. and D.E.R. established workflows and performed data processing. E.P., A.E.D., H.B. and O.W. contributed to design and interpretation of the analyses. H.W., K.W.P., C.M.v.T., T.M., M.J., M.C.F., P.H.D., U.W.T., M.U.S., M.M., K.B., T.S., M.D., M.K., S.K., M.E., A.O.vB., T.P., K.K. and C.M.K. provided data, patient materials and reviewed the manuscript. E.P., A.eD., H.B. and O.W. prepared the manuscript and figures. R.W., P.L., A.E., S.M.P., D.T.W.J., H.B. and O.W. contributed to project management and provided leadership.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2019.03.019.

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