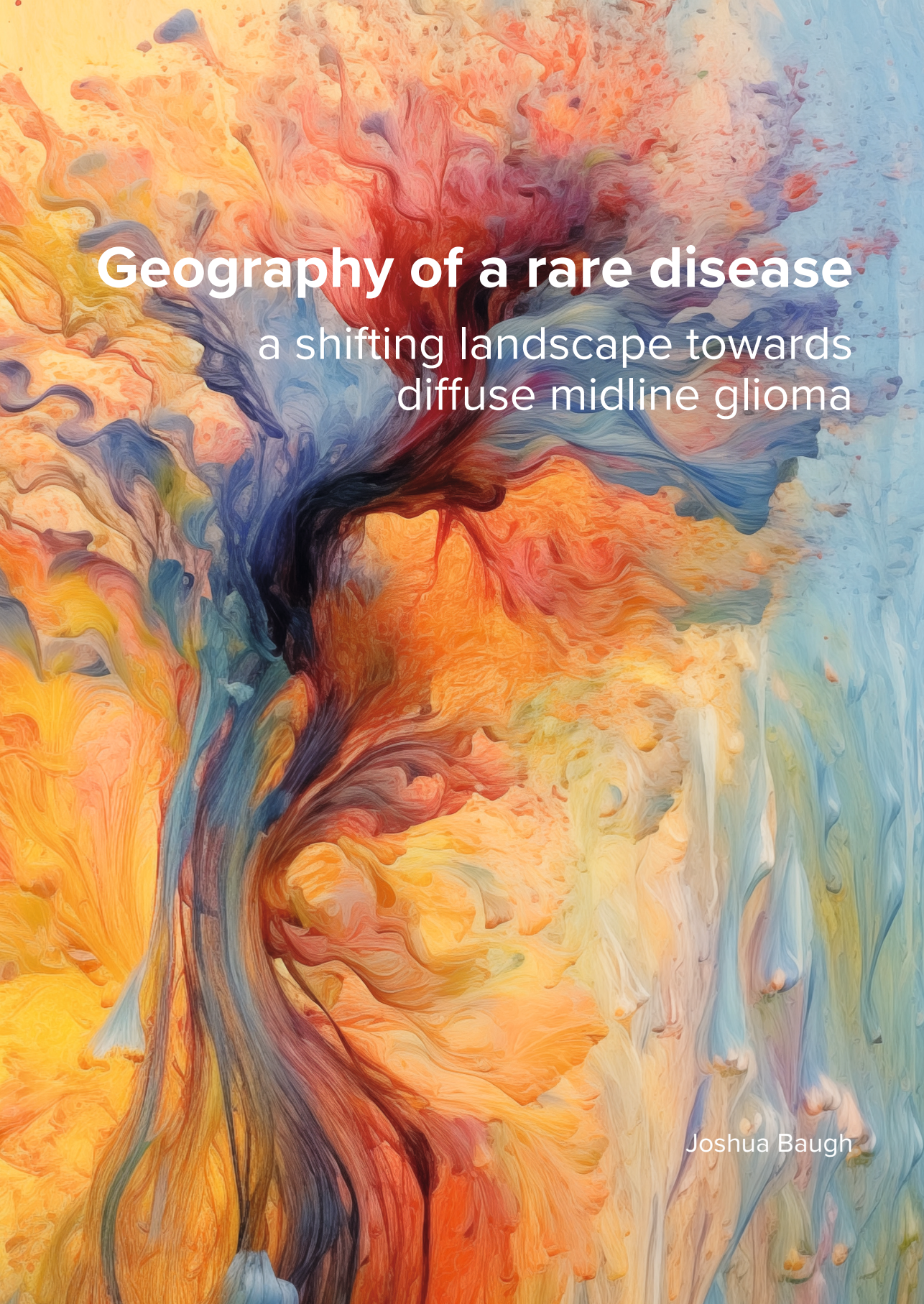

# Geography of a rare disease

## a shifting landscape towards diffuse midline glioma

Joshua Baugh

# GEOGRAPHY OF A RARE DISEASE: A SHIFTING LANDSCAPE TOWARDS DIFFUSE MIDLINE GLIOMA (DMG)

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# GEOGRAPHY OF A RARE DISEASE: A SHIFTING LANDSCAPE TOWARDS DIFFUSE MIDLINE GLIOMA (DMG)

## GEOGRAFIE VAN EEN ZELDZAME ZIEKTE: EEN VERSCHUIVEND LANDSCHAP RICHTING DIFFUUS MIDLIJN GLIOOM (DMG)

(met een samenvatting in het Nederlands)

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
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ingevolge het besluit van het College voor Promoties
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“In examining disease, we gain wisdom about anatomy and physiology and biology. In examining the person with disease, we gain wisdom about life.”

Oliver Sacks

# CONTENTS

# 1

# General Introduction and Thesis Outline

## INTRODUCTION TO DIFFUSE INTRISNIC PONTINE GLIOMA (DIPG)

Diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain tumor related death in children with a median overall survival of less than 1 year.[1,2] The diagnosis is accompanied with significant co-morbidities due to tumor location. At presentation most children have abnormal gait and coordination difficulties, long track signs and cranial nerve palsies.[3] Symptoms tend to resolve during the six weeks of frontline radiation and in the short months after completion but return quickly at progression, which occurs at a median of 6 months after diagnosis. [4]

Brainstem gliomas account for 10-15% percent of all pediatric central nervous system tumors and DIPG represents 75% of all brainstem gliomas.[5,6] Pediatric high-grade gliomas located in the brain stem account for the largest proportion (37.9%) of deaths in children ages 0-14 (Figure 1).[5] The incidence rate is estimated at 0.32 - 0.48 per million in the USA or 100-150 DIPG patients per year.[7] A retrospective analysis in the Netherlands found a similar incidence of 0.54 cases per million or 5-8 patients each year.[8]

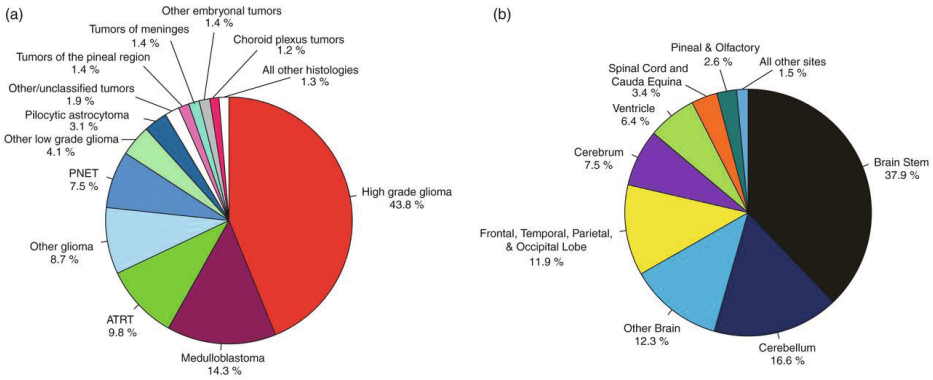


**Fig 1.** Distribution of deaths due to primary CNS tumors in children aged 0–14 from 2007–2011 as published in Ostrom et al. Neuro-Oncology 2015.[5]

Diagnosis of a DIPG tumor is based on MRI, which typically shows a large diffuse tumor centered in the pons, encompassing >50% of the pons and often encasing the basilary artery. DIPGs are classically hypointense on T1 and hyperintense on T2-weighted MRI sequences.[9] DIPG usually concerns high grade gliomas (WHO grade III-IV) but can also include WHO grade II diffuse gliomas, when located in the pons. [10] WHO tumor grade alone is not prognostic of survival.[11] A biopsy is indicated in the case of atypical appearance on MRI or in the context of a clinical trial.[1] The procedure can be safely performed in a specialized center by an experienced neurosurgeon but requires a delicate and invasive surgery, not without risk.[12]

Treatment is difficult because of the delicate location within the pons from which the majority of cranial nerves originate. This makes radical surgery impossible.[13] In addition, several anti-cancer drugs appear to be inactive in DIPG, either due to tumor drug resistance and/or poor drug distribution in the tumor due to unfavorable pharmacokinetics over a largely intact blood brain barrier.[14,15] Radiotherapy can significantly reduce the tumor and often improves clinical signs, but these effects are almost always temporary.[16] For these reasons, the prognosis of DIPG has remained unchanged in the past decades with a median overall survival rate of eleven months and a two-year overall survival rate of less than 10 percent.[2]



## BARRIERS TO CLINICAL TRANSLATION

Significant barriers to the development of new therapies include limited patient numbers, and tumor specimens that inhibit the throughput and power of DIPG research. This is in part due to rarity of the disease but also a lack of centralized data and tumor resources.

Since the digitalization of information there has been an explosion of data welcoming the so-called era of "Big Data".[17] The field of health care has been no exception, yet health care data proves difficult to mine for research purposes. Most clinical data is unstructured and exist behind firewalls with restricted access due to the sensitivity of health information and privacy regulations overseeing the use and sharing of personal health information.

In the wake of GDPR (the EU General Data Protection Regulation) in 2018, further governance over data became a mandate in Europe, inherently complicating global data sharing activities.[18] Health data which are available, exist mostly in electronic health records (EHR) systems employed by hospitals. These data information systems are examples of unstructured and semi-structured data, designed with clinical care in mind, not research.

For epidemiological level cancer data, researchers rely on the reporting of hospital-based cancer registries (HBCRs). These registries serve an important role in evaluating outcomes and the quality of a care delivered in the hospital's cancer program.[19] If these data are standardized and abstracted by trained cancer registrars, they can provide useful information on the delivery of services (e.g. radiation, chemotherapy) and the treatment outcomes, and toxicities, which can then be feedback into a broader population-based cancer registry (PBCR).[20] HBCRs and PBCRs function symbiotically in this way.[19]

PBCRs do not rely on a single data source and are able to provide population level data and if coverage is high, complete incidence and mortality data. PBCRs allow for comparison across populations over time and improve diversity in sampling (eliminate biases) to allow extrapolation of findings between countries. This enable research at scale.[21] In the United States the CBTRUS is the authoritative source for epidemiological data on pediatric brain tumors.

In the CBTRUS however, DIPG and DMG tumors are not reported specifically, rather they are grouped broadly into "high-grade glioma of the brain stem".[5] This lack of specificity renders large population-based cancer registries (PBCRs), like CBTRUS, ineffective in addressing clinical research questions specific to DIPG. A rare disease registry that focuses specifically on DIPG is therefore needed to collate clinical, imaging and biological material for research.

## A SHIFTING DIPG/DMG LANDSCAPE

Further hindering drug development is an incomplete understanding of tumor biology and imperfect diagnostic classification driven by the dearth of available tumor tissue. Following the development of autopsy and biopsy procurement protocols these tissues rapidly became available for research purposes.[22,23] The availability of biological material coupled with rapid advancement in molecular biology techniques has led DIPG to be reclassified based on molecular genomics.[24] With the hallmark discovery of histone 3 (H3) mutations in 2012 occurring in 80% of DIPG, a lysine to methionine substitution at amino acid 27 (H3K27M), the key oncogenic driver of DIPG was uncovered, revolutionized our biological understanding of the disease pathogenisis.[25,26] Importantly for purposes of this thesis, variants in histone mutations now define biological subgroups with distinct clinical features and prognosis (Figure 2).[27,28]

Mutations in the mutually exclusive H3 gene variations of HIST1H3B/C (H3.1K27M) confer a survival advantage over H3F3A (H3.3K27M) tumors and represent approximately 30% of cases. H3.3 mutant DMGs represent 60% of cases and constitute the most aggressive and radiation treatment resistant genotype, with an overall survival (OS) of 9 months. These tumors most commonly occur in the pons but also the thalamus, with a peak incidence in children of 7.5 years of age. H3.1 mutations are more common in younger children and portend a longer OS of 15 months and are found exclusively in the pons.[29,30,31] Approximately 10% of tumors are histone 3 wild-type but exhibit EZHIP overexpression resulting in equal loss of H3K27M trimethylation and a similarly poor survival to H3.3K27M mutated tumors.[32]

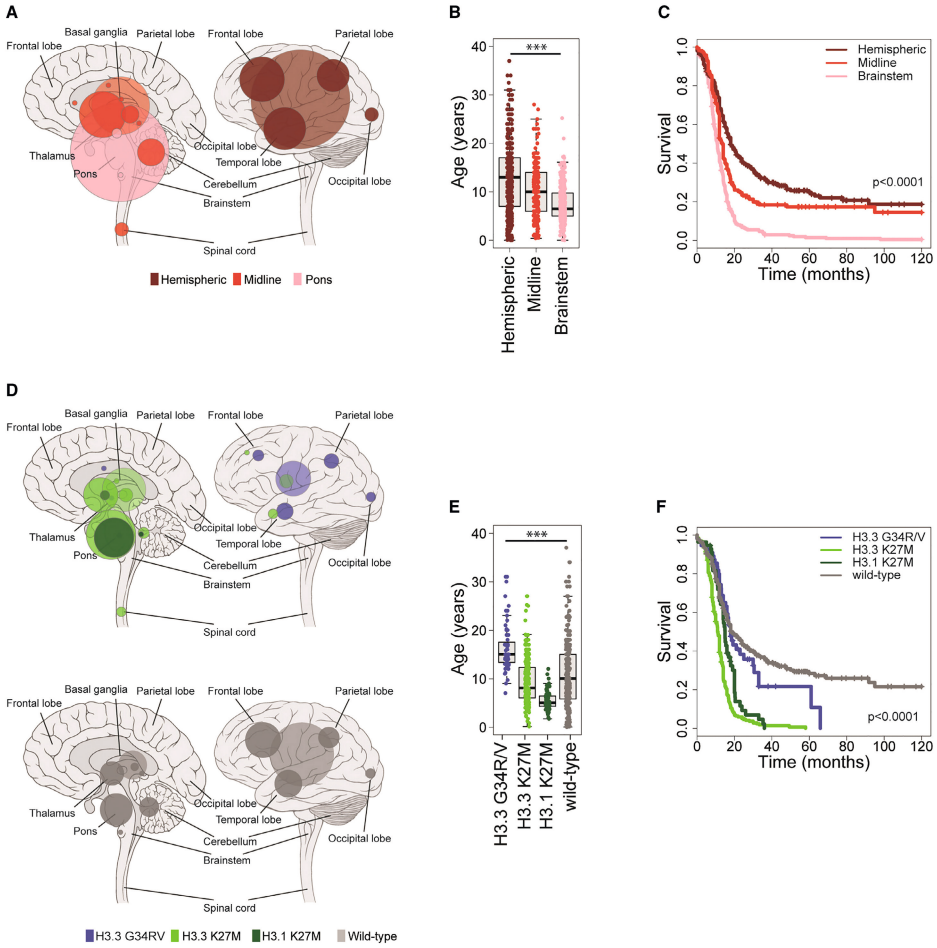


**Fig 2.** Clinicopathological and Molecular Subgroups of pHGG/DIPGs, as published in: Mackay A. et al. *Cancer Cell* 2017.[29]

Both H3K27M (oncohistones) and the oncohistone mimic EZHIP lead to broad epigenetic dysregulation by inactivating the polycomb repressive complex 2 (PRC2) and inhibiting H3 global trimethylation (H3K27me3).[33] Importantly these driver mutations occur during narrow windows of childhood development associated with waves of brain myelination and in distinct midline structures to drive tumorigenesis. DMGs are thought to arise in cancer stem cell-like oligodendrocyte precursor cells (OPC), however spatiotemporal differences in terms of age and anatomical location between H3.3 and H3.1 mutations point to distinct cells of origin. However, the influence of precise developmental conditions such as different histone variants, anatomical locations and ages are still under investigation.[34]

The complex biological underpinnings of DMG are just starting to be understood. Molecular differences within each subgroup of DMG are well described however the functional role of the many concurrent mutations aside from the oncohistones are not well characterized.[35] It is clear there is significant inter- and intra-tumoral heterogeneity in diffuse midline gliomas which only increases the need for pooling of samples and data resources. [36,37]

In 2016, based on several biopsy studies the diagnosis of DIPG was redefined as diffuse midline glioma (DMG), H3K27M-mutant.[24] All tumors with histone 3 alterations are considered WHO Grade IV by definition.[11] The disease however remains characteristic of its original phenotype and universally fatal. Until clinical research efforts can catch up and translate these exciting biological findings in the clinic this poor survival will likely remain.

Our understanding of these tumors is evolving and with that comes a constant adjustment of classification systems and debates about inclusion, exclusion, and response criteria for use in clinical trials. It is better to be inclusive of both the genotypic and phenotypic definitions of DMG and DIPG respectively, to capture the entire spectrum of this universally fatal disease, especially as biopsy of this disease is still not routinely performed in all clinical practices.

## THE SEARCH FOR A CURE

Exciting drug development has followed the discovery of oncohistones, aimed at targeting DMG's epigenic vulnerabilities. Panobinostat, a potent histone deacetylase (HDAC) inhibitor showed strong preclinical evidence of restoring H3K27-methylation and normalizing gene expression.[38] Despite promising preclinical evidence however, it did not translate to clinical effect as all patients still succumb to their disease, as with all other experimental agents used to date.

The feverish pace of biological discoveries following the discovery of the histone modifications in 2012, as the driver mutations underlying the pathogenesis of DIPG, led to a great deal of optimism that the field of genomics and precision medicine would lead to a cure. The promise of precision medicine however has thus far failed to materialize.[39] Recent biological discoveries dispel the idea that brain tumors are "monogenetic and monoclonal" and necessitate a holistic view of the cancer.[40]

Robust data information systems and infrastructure will be vital to improving our understanding of the disease complexity and inform the development of a synergistic multipronged treatment approach. To date, there are too few patients with centralized clinical data and imaging to enable clinical and translational investigations capable

of elucidating the high-level associations between treatment and survival in DIPG.[30] A rare disease registry specific to DIPG offers a mechanism to centralize, standardize and collate clinical, imaging and biological data to enable a better understanding of disease patterns and the prognostic factors underlying survival.[41]

## THESIS AIMS AND OUTLINE



This thesis aims to describe the clinical and translational landscape of DIPG, and the research challenges addressed in my PhD project. I aim to 1) describe the development and establishment of a centralized data collection in the SIOPE and International DIPG/DMG Registries, providing a research infrastructure mechanism capable of "assessing the landscape" of this rare disease. 2) The challenges arising with the diagnostic evolution of DIPG to DMG, a transition from a clinicoradiographic (phenotype) based diagnosis to a molecular based (genotype) classification and the clinical implications of implementation by specialty and in terms of access to advanced diagnostics along socioeconomic lines. 3) And finally, using the SIOPE DIPG Registry as a high-level epidemiological tool to "survey the land", studies on clinical, radiologic, pathologic, and molecular characteristics, and exceptional (long-term) and treatment-related survival patterns.

Chapter 1: General introduction to DIPG, barriers to developing therapies, the need for centralized data collection and changes that came along with the transition towards molecular diagnostics and evolution to DMG.

Chapter 2: We describe the establishment of the International DIPG Registry, an infrastructure to accelerate collaborative research for an orphan disease. Outlining the organizational structure, recruitment, policies, and procedures, along with the project's status at the time.

Chapter 3: The establishment and development of the SIOPE DIPG network, registry and imaging repository: a parallel registry enabling collaborative research efforts. In addition, we provide a status update on data collection efforts and an initial report on survival trends.

Chapter 4: A study documenting the transition to the widespread utilization of molecular diagnostics in pediatric high-grade glioma in the wake of the 2016 WHO CNS tumor classification. We performed a survey studying the impact among practitioners in pediatric neuro-oncology to understand the differences in implementation along socioeconomic lines.

Chapter 5: Following further changes to the 2021 WHO CNS Tumor Classification, we investigated differences between pediatric neuro-oncologists and neuropathologists in their perception of molecularly defined subtypes for pediatric high-grade gliomas.

Chapter 6: We perform the first large-scale collaborative Registry study comparing clinical, radiologic, and molecular characteristics between short-term survivors and long-term survivors of DIPG, to better elucidate prognostic factors.

Chapter 7: We examine the survival benefit of additional therapies beyond standard of care frontline radiation. This study suggests what survival benefits may be gained by which general therapeutic approach, as a first step to quantifying survival differences observed in a historical cohort. Our landmark methodology is an innovative approach to deal with immortal time bias and better estimate survival outcomes in DIPG.

Chapter 8: General discussion and future directions

Chapter 9: English summary/Nederlandse samenvatting

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# 2

# The international diffuse intrinsic pontine glioma registry: an infrastructure to accelerate collaborative research for an orphan disease

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## ABSTRACT

Diffuse intrinsic pontine glioma (DIPG), a rare, often fatal childhood brain tumor, remains a major therapeutic challenge. In 2012, investigators, funded by the DIPG Collaborative (a philanthropic partnership among 29 private foundations), launched the International DIPG Registry (IDIPGR) to advance understanding of DIPG. Comprised of comprehensive deidentified but linked clinical, imaging, histopathological, and genomic repositories, the IDIPGR uses standardized case report forms for uniform data collection; serial imaging and histopathology are centrally reviewed by IDIPGR neuro-radiologists and neuro-pathologists, respectively. Tissue and genomic data, and cell cultures derived from autopsies coordinated by the IDIPGR are available to investigators for studies approved by the Scientific Advisory Committee. From April 2012 to December 2016, 670 patients diagnosed with DIPG have been enrolled from 55 participating institutions in the US, Canada, Australia and New Zealand. The radiology repository contains 3558 studies from 448 patients. The pathology repository contains tissue on 81 patients with another 98 samples available for submission. Fresh DIPG tissue from seven autopsies has been sent to investigators to develop primary cell cultures. The bioinformatics repository contains next-generation sequencing data on 66 tumors. Nine projects using data/tissue from the IDIPGR by 13 principle investigators from around the world are now underway. The IDIPGR, a successful alliance among philanthropic agencies and investigators, has developed and maintained a highly collaborative, hypothesis-driven research infrastructure for interdisciplinary and translational projects in DIPG to improve diagnosis, response assessment, treatment and outcome for patients.

## BACKGROUND

Diffuse intrinsic pontine glioma (DIPG) is an aggressive childhood brainstem tumor with a dismal prognosis.[1, 2] The diagnosis of DIPG has, hitherto, been based on imaging and clinical findings. The reluctance to conduct brainstem biopsies, a paradigm which has recently been challenged, [3–6] long hindered understanding of this fatal disease. Radiation therapy prolongs survival by only 2–3 months and remains the standard of care,[7] while chemotherapy has proven ineffective [8].

In 2012, next-generation sequencing studies using autopsy and some biopsy tissue elucidated the genomic landscape of DIPG [9, 10]. Despite these discoveries, DIPG research remains challenging, due to the tumor's relative rarity, the limited power of single-institution or small-scale studies, presence of inter- and intra-tumoral heterogeneity, and a lack of understanding of mechanisms of therapy resistance [11, 12].



In 2011, physicians, scientists, and patient advocacy groups met at the first International DIPG Symposium, and advocated for the establishment of a focused international effort to develop uniform criteria for diagnosis, classification, disease assessment, and to study DIPG biology and therapeutic strategies through the development of in vitro and in vivo models. In 2012, with financial support from the DIPG Collaborative, a philanthropic partnership which now includes 29 private foundations, and international investigators banded together to establish the International DIPG Registry (IDIPGR) and a parallel European SIOPE Registry. The IDIPGR continues to expand and maintains a highly-collaborative, hypothesis-driven research infrastructure to support a wide spectrum of interdisciplinary and translational projects in DIPG. Here, we report the logistical challenges, pitfalls, and successes of developing this registry, which we hope will serve as a model for other orphan disease registries.

## METHODS

### Structure of the DIPG registry

The IDIPGR consists of the Operations Center (OC), a Steering Committee (SC), Scientific Advisory Committee (SAC), Research Ethics Panel, Quality Assurance Group, and collaborating institutions. An organizational chart is provided in Fig. 1.

Cincinnati Children's Hospital Medical Center (CCHMC) is the Operations Center and repository for all clinical and neuroimaging data and pathology specimens from collaborating institutions. Tissue from Canada is stored in a separate biobank at The Hospital for Sick Children.

The SC serves as the governing board, providing oversight of the IDIPGR. SC consists of experts in the field of DIPG, and one patient/family representative. Non-voting members include two registry staff members: the IDIPGR project co-ordinator and the regulatory and ethics officer. The SAC consists of senior basic, translational and clinical experts in DIPG research, including two external reviewers, and is responsible for evaluating and prioritizing submitted research proposals. The Registry's policies and detailed organizational information are outlined in the DIPG Registry and Repository constitution. The SC meets semi-annually by teleconference or in-person. Biannually, an in-person meeting of the SC and SAC is conducted.

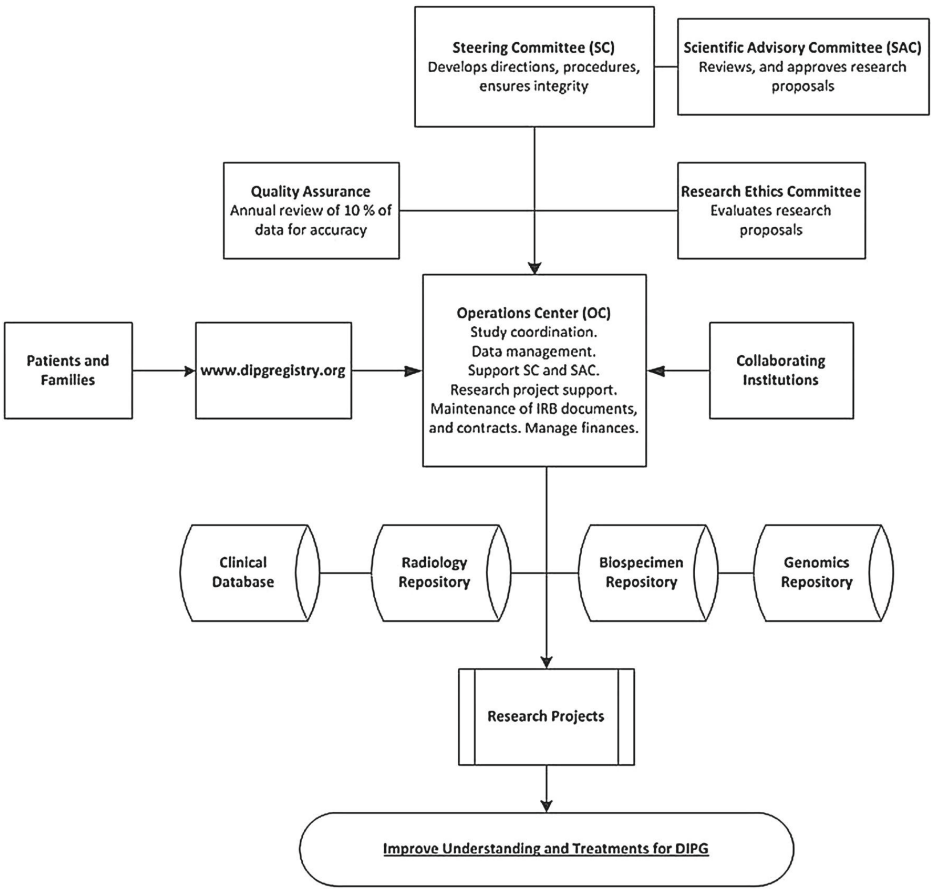


**Fig. 1** Organizational of the DIPG registry and repository

## Registry website (http://www.dipgregistry.org)

A website, http://www.dipgregistry.org, representing the International and SIOPE DIPG Registries, serves as a direct link between families/medical professionals and registry personnel, providing a list of registry-affiliated oncologists around the world, clinical trials and research updates, investigator profiles and educational information about DIPG, palliative care and autopsy. The website also facilitates consultations or self-referrals to the registries. Information on the website is updated monthly by the registry coordinators and the Principle Investigator to reflect the most up-to-date information available.



## Recruitment and data collection

There are two principal mechanisms for identification and recruitment of participants (a) self-referral by patients and their families via the DIPG Registry website or (b) procurement of deceased patient records from participating institutions, after Institutional Review Board (IRB) approval or non-humansubjects determination. All patients, regardless of age, with an institutional diagnosis of DIPG are eligible for enrolment in the International DIPG Registry.

*Self-referral*

Prospective patients and their families may self-refer by contacting the IDIPGR office directly at http://www.dipgregistry.org or by phone. Physicians and medical staff may also provide prospective patients with the IDIPGR brochure. Once self-referral is made, registry staff contact the patient or parent/guardian (for minor patients) to obtain consent for registry participation. When possible, written assent to participate is also obtained from patients ≥11 years old. Once written consent has been obtained, registry staff work directly with the treating medical team to collect information, imaging, and tissue samples, if available.

Parents/legal guardians of deceased patients may also self-refer to the IDIPGR and grant registry personnel access to the decedent's medical information by signing a HIPAA release form.

*Institutional referral*

Each collaborating institution is responsible for providing source documentation for registry personnel to abstract data from medical records of their DIPG patients. The IDIPGR coordinator works with a designee from each collaborating institution to obtain source documentation, including medical records, radiographic imaging on CD-ROM, available pathological material. Data are abstracted from the medical record by the

IDIPGR coordinator, who is solely responsible for completing case report forms (CRF) and entering into the Registry database. If release of individually identifiable medical records of deceased patients is not permissible from a collaborating institution, the CRFs may be completed on site using a data abstraction guide developed to ensure uniform interpretations and collection of variables/data points. Radiographic images submitted on CD-ROM are de-identified, uploaded, and stored in the research picture archiving and communication systems (PACS) system housed at CCHMC. All paraffin blocks/slides or frozen tissue for central pathology review and/or future research are de-identified and sent to CCHMC, or The Hospital for Sick Children (HSC) for Canadian sites.

Collaborating institutions can inform prospective, living patients about the IDIPGR, either verbally or by providing IRB-approved brochures. Interested patients or families may then self-refer to the IDIPGR for enrolment. For international sites, local staff may obtain informed consent per institutional and country policies using site-specific consent forms approved by their ethics committee based on the IRB-approved consent template provided by the IDIPGR.

*Data inclusion moratorium*

Investigators at each institution may elect to place a 1-year moratorium on inclusion of their data (clinical, imaging, pathology/tissue) as part of any research or publications from the IDIPGR. The moratorium begins when the first patient records are accessed. The collaborating institution may request an extension of the moratorium if needed for projected publication of institutional data donated to the registry.

## Regulatory strategies

*Institutions in the United States*

The IRB approval for the IDIPGR is maintained at the CCHMC operations center. According to HIPAA regulation 45 CFR 164.512, the request for and release of decedent personal health information (PHI) for research purposes is permitted and HIPAA requirements are fulfilled as part of a decedent PHI request form (available on request). All PHI received by the IDIPGR is coded. The IDIPGR research personnel function as the honest brokers ensuring that no identifying information is released to researchers. Many collaborating institutions have consulted with their IRB and acted in accordance with institutional policy. Some of the submissions to IRBs have included the decedent request form and brief explanation of use of PHI through completion of their IRB application. Most IRBs have granted a non-human subjects research determination and grant HIPAA waivers. Since the informed consent is obtained by Registry staff, institutions should not need to obtain full IRB approval.

*International institutions*

For international sites, local staff may obtain informed consent of living participants according to institutional and country policies using a site-specific consent form approved by their Research Ethics Board. Privacy laws do not typically permit release of PHI, requiring most international sites to submit data on CRFs and maintain source documents, including consent forms, on-site.

## Protocols and procedures



*Clinical database*

Demographic, clinical, treatment, and outcome data are abstracted from existing clinical records, pathology and imaging reports by the two IDIPGR coordinators using standardized case report forms (CRFs). Clinical CRFs have been developed in conjunction with investigators from the SIOPE DIPG Registry for collection of identical data that would enable facile collaboration. Data are coded and stored in Oncore, a clinical trials management software system. Abstracted data elements include: demographics, diagnosis, date of diagnosis, imaging, signs and symptoms and physical exam at diagnosis, treatment, response evaluations, central pathology review characteristics, central imaging review characteristics, and molecular profile. All source documentation is maintained at the operations center in patient binders for access for future studies and quality assurance. Annually, members of the oncology quality assurance team at CCHMC review 10% of all patient data for completion and accuracy and provide formal reports regarding their findings.

*Imaging repository*

All available imaging on each enrolled subject is submitted to the central imaging repository at the OC on CD, and loaded onto a dedicated, research-only, picture archiving and communication system (PACS). Data are reviewed prior to placement in the research PACS to ensure all patient identifiers are removed from images, and a study ID generated, linkable to the subject identity only by DIPG Registry staff.

MR imaging is reviewed by the study primary neuroradiologists (BVJ, JLL) at diagnosis, post-radiation, best response to each therapy, and at the time of progression with each therapeutic intervention. An international central neuroimaging review panel is available as needed to define/cross validate evaluation parameters. All cases are reviewed by both primary neuroradiologists and consensus opinions utilized in cases in which there is disagreement. The primary goal of central review is to confirm the imaging diagnosis of DIPG, provide measurements of tumor extent, and basic descriptive assessments of imaging appearance. Each case is evaluated and classified

as: (1) typical DIPG imaging appearance, (2) some atypical features, but likely DIPG, and (3) unlikely DIPG, other diagnosis suspected. A tumor is considered a typical DIPG if it arises from the pons, exhibits a diffuse pattern of involvement, and involves ≥50% of the pons at diagnosis. Each case classified as unlikely DIPG by consensus opinion of both neuroradiologists will be designated as such in the IDIPGR database and excluded from analyses. Imaging features suggestive of an alternative diagnosis may include: tumor not arising from the pons (medulla or midbrain origin), a primarily focal exophytic morphology, very sharply defined margins, or marked diffusion restriction of the majority of the lesion. Cases in which there is secondary brainstem involvement by a tumor centered in the thalami, cerebral hemispheres, or cerebellar hemispheres are excluded. Only tumors that appear to originate in the brainstem are included in the registry. After consensus review, MR imaging data will be entered into the registry database for use by approved research studies.

*Biospecimen repository*

If biopsy or autopsy materials are available, submission is requested at the time of enrolment. The Division of Pathology at CCHMC archives and digitizes all pathology cases using Biomaterial Tracking and Management Research (BTM). De-identified pathology images and reports are centrally reviewed by the study primary neuropathologists at CCHMC (CF) or HSC (CH) in Canada for Canadian patients. Frozen specimens originating from referring institutions in Canada are sent to HSC for long-term storage. Frozen specimens originating from all other institutions around the world are stored at CCHMC.

*Genomics repository*

Molecular data, including genome-wide DNA copy number, karyotyping, expression profiling (mRNA and miRNA), methylation analysis, and DNA or RNA sequencing is collated into an International DIPG Bioinformatics Repository. Both original raw data and processed files are requested, and can be uploaded along with annotation files to a secure ftp site. For autopsy tissue that has been donated to the IDIPGR, if molecular/genomics testing have not been conducted or are not available, next generation sequencing consisting of whole genome sequencing, RNA sequencing, paid for through IDIPGR funds are being conducted by core facilities or commercial vendors and deidentified, raw data are then deposited in the genomics repository. Investigators who have donated tissue to the IDIPGR can receive raw NGS data generated from specimens submitted to the IDIPGR.

Researchers are invited to contribute any relevant data in addition to that which may be found in the published literature or databases. Investigators known to have unpublished data are approached to contribute pre-publication. Data may be held in this context in a

non-public (password-controlled) area. Data generated from biospecimens contributed to the Registry are also incorporated into the repository in a prospective manner. Sample identifiers are linked to those in the IDIPGR to allow correlation of molecular and clinico-pathological variables. The IDIPGR staff maintain the link. The repository is held at the genomic data facility in the Bioinformatics Division at CCHMC. By combining these data, we intend to generate a comprehensive, accessible database of the molecular profiles of DIPG for the academic community.

## RESULTS



### Current status

The IDIPGR has enrolled 670 patients, from 55 collaborating institutions in the United States, Canada, and Australia, and New Zealand with an additional 500 patients committed from 25 other sites in these countries, which are at various points in their approval and data submission processes. Data have been abstracted on all enrolled patients. A summary of available clinical, radiographic, genomics data and biospecimens are summarized in Table 1. Currently, 81 tumor specimens are housed in the pathology biorepository with approximately 98 more specimens committed for submission on enrolled patients. Next Generation Sequencing data from tumor and germline are currently available on 66 patients.

### Registry research studies

Nine studies, from various investigators in the US, Canada and Europe have been approved by the SAC utilizing registry resources. Several of these studies have external funding, including funding from the DIPG Collaborative. The studies are in various stages of conduct and analysis and include:

1. Joint International and SIOP-E DIPG Registry long-term survivor project. To describe the clinical, radiographic, pathological and biologic characteristics of long-term survivors with DIPG and correlate key variables with outcome.
2. An epidemiological study to determine incidence patterns of DIPG in North America. Our Canadian collaborators have presented the Canadian epidemiology data and we plan to expand this study to examine incidence of DIPG in other countries.
3. External validation of the Survival Prediction Model for Diffuse intrinsic pontine glioma. A survival prediction model, developed within a cohort of European DIPG patients is being validated using the International DIPG Registry cohort.
4. DIPG: Contemporary Survival Endpoints. A study examining reported survival endpoints in order to better define progression and aid the development of objective measures for robust clinical trials.

5. Establishment of in vitro and in vivo Models. Fresh tissues from autopsy are being shared to establish in vitro and in vivo models for drug screening.
6. Comprehensive Molecular-Based Cross-Species Comparison of DIPG Biology examines overlapping genetic alterations between mouse and human DIPG, allowing for identification of novel subtype-specific oncogenic pathways.
7. Imaging Phenotype and Survival in DIPG. This proposal seeks to identify specific imaging features at baseline that significantly correlate with overall survival and assess multi-reader agreement and concordance with imaging features of the DIPG registry.
8. DIPG as a complication of Medulloblastoma Therapy. This proposal seeks to study the incidence of brainstem glioma as a complication of therapy for medulloblastoma.
9. Radiogenomic Evaluation in Diffuse Intrinsic Pontine Glioma.

## Consultations, second opinions and education via (http://www.dipgregistry.org)

Since the website launched in April 2012, over 218,000 people have visited the site from 183 countries. Dipgregistry.org is now an established centralized resource for patients, families and physicians around the world in search of up-to-date information regarding DIPG including DIPG education, currently open clinical trials, latest literature and research developments, access to an international network of oncologists for consultations. To date, 283 consultations have been provided to patients, families and physicians around the world. These consultations are provided by DIPG Registry participating investigators from around the world depending on the origin of the consult requests. Since the IDIPGR is housed at CCHMC, initial review of consult requests is conducted by Registry Coordinators, IDIPGR PI (MF) and triaged accordingly. Consultation by the IDIPGR PI, review of imaging by IDIPGR neuroradiologists (JL and BVJ) is provided free of charge to patient.

**Table 1** Patient characteristics and available imaging, pathology and molecular data

| Category | Subcategory | December 2016 n |
|---|---|---|
| Total enrollment | | 670 |
| Collaborating institutions | | 55 |
| Sex | Female:male | 351:319 |
| Age at diagnosis (years) | Mean age (range) | 7.4 (<0.1 to 26.8 years) |
| Ethnicity | African American | 43 |
| | Asian | 15 |
| | Caucasian | 278 |
| | Other | 11 |
| | Unknown | 323 |
| Neuroimaging | | 541 (3558 studies) |
| | Diagnostic | 448 |
| | MRI | 3182 studies |
| | CT | 376 studies |
| | Central review | 438 |
| | Not DIPG | 29 |
| | Typical DIPG | 300 |
| | Some atypical features | 109 |
| Biospecimens | | 81 (460 specimens) |
| | Central review | 54 |
| | Autopsies coordinated | 24 |
| | Fresh tissue shared for in vitro and in vivo modeling | 7 |
| | Frozen | 57 |
| | FFPE | 19 |
| | Slides only | 8 |
| Molecular data | | 66 |
| | Whole exome | 21 |
| | Whole genome | 29 |
| | Targeted only | 16 |

### Tissue procurement efforts

Recently, greater acceptance of the safety of DIPG biopsy, [3–5, 13] development of autopsy-based protocols, [14–16] and next generation sequencing (NGS) efforts have advanced our understanding of the molecular basis of DIPG, identifying aberrations such as the highly recurrent histone mutations (H3F3A or HIST1H3B/C/I) [9, 10, 17] and ACVR1 [18–20]. The IDIPGR repository contains tissue specimens on 81 enrolled patients and NGS data on 66 enrolled patients. Registry funding supports conduct of comprehensive whole genome sequencing, RNA sequencing and 850 K methylation array on all available specimens. Spurred on by the explosion of knowledge about this disease through tissue donations, many patients and families have contacted the IDIPGR to assist in organizing autopsies at participating institutions to donate tissue for research to the Registry. Registry staff have organized 24 autopsy donations to the Registry. Tumor specimens from seven patients have been sent to registry investigators to establish patient-derived cell culture and xenograft models for drug screening and other studies. To date, one cell culture has been successfully established.

### Funding strategies

One of the major obstacles to establishing and maintaining registries for rare cancers is the lack of sustained funding opportunities to support such efforts. The IDIPGR has been generously supported by the DIPG Collaborative from 2012 to 2018 for a total of $1.4 million. The DIPG Collaborative also fully funds SIOPE DIPG Registry. The DIPG Collaborative and IDIPGR formed in parallel in 2012, when the need for collaborative research and funding became evident. The DIPG Collaborative is comprised of 29 foundations supporting DIPG research world-wide. The growth of the DIPG Collaborative has been vital to sustaining the International DIPG Registry and this relationship remains an integral part of maintaining and improving research for this orphan disease.

## DISCUSSION

In a rare orphan disease like DIPG, scientific progress and development of effective therapies have often been impeded by the lack of large scale, well-annotated, clinico-radiologic and biologic data available about the disease. The IDIPGR provides the infrastructure for acquisition of biological specimens, imaging, and correlative clinical and genomics data to facilitate basic and translational research studies in this rare disease. The increased availability and centralization of data and specimens from DIPG patients, and the effective collaboration among clinical, translational and basic researchers as well as philanthropic foundations represent a welcome paradigm shift in DIPG research in which data and tumor specimens are no longer rate-limiting resources.

The highly collaborative, international, hypothesis-driven and hypothesis-generating research infrastructure of the IDIPGR can support a wide spectrum of interdisciplinary and translational research that will be critical to improving diagnosis, classification, response assessment and treatment options for this vulnerable population. Centralized, standardized and linked pathologic, clinical, genomics and radiological data enable investigators to develop new approaches to DIPG diagnosis, classification and response assessment that will inform the development and conduct of future clinical trials.

Tissue acquisition from autopsies on the DIPG Registry has led to critical collaborations among basic science and translational investigators to develop primary cell cultures and xenografts to support assay development and high throughput screening of novel agents for the treatment of DIPG. Keys to these discovery efforts are the ability to integrate analysis of relevant genes and pathways and assess potential biomarkers. Comprehensive genomics and functional proteomics efforts are already on the way through international collaborations by scientists utilizing the IDIPGR infrastructure and biorepository. Promising drugs can then be tested in the animal models and cell lines developed from registry tissue and provide the rationale for scientifically-sound clinical trials to improve outcome. Dipgregistry.org provides a platform to disseminate results and aid recruitment of patients for future studies.



## Data requests and research proposals

Data and samples from the registry are available to researchers affiliated with the registry and to external researchers world-wide. Research proposals (application available on the DIPG registry website) from participating investigators are evaluated by the SAC for scientific merit, prioritization, feasibility and appropriate use of resources before approval. If approved, de-identified clinical, radiographic, pathologic, genomic data and biological specimens may be released to investigators. IDIPGR statistician and bioinformaticians perform and provide detailed analyses to the investigators for manuscript preparation.

## Future directions

The ready availability of the IDIPGR resources to external investigators has promoted robust, hypothesis-driven international and interdisciplinary collaborative research on all aspects of DIPG. Areas of focus for the IDIPGR are: to prospectively enrol patients diagnosed with DIPG, expand participation to other regions around the world, develop supplemental web-based educational materials for families and medical teams to improve awareness and treatment of DIPG. Ultimately, the IDIPGR's extensive and robust infrastructure for collaborative research may serve as a platform to develop and conduct innovative, multi-institutional trials to improve the outcome for patients with DIPG.

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# 3

# Development of the SIOPE DIPG network, registry and imaging repository: a collaborative effort to optimize research into a rare and lethal disease

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## ABSTRACT

Diffuse intrinsic pontine glioma (DIPG) is a rare and deadly childhood malignancy. After 40 years of mostly single-center, often non-randomized trials with variable patient inclusions, there has been no improvement in survival. It is therefore time for international collaboration in DIPG research, to provide new hope for children, parents and medical professionals fighting DIPG. In a first step towards collaboration, in 2011, a network of biologists and clinicians working in the field of DIPG was established within the European Society for Paediatric Oncology (SIOPE) Brain Tumour Group: the SIOPE DIPG Network. By bringing together biomedical professionals and parents as patient representatives, several collaborative DIPG-related projects have been realized. With help from experts in the fields of information technology, and legal advisors, an international, web-based comprehensive database was developed, The SIOPE DIPG Registry and Imaging Repository, to centrally collect data of DIPG patients. As for April 2016, clinical data as well as MR-scans of 694 patients have been entered into the SIOPE DIPG Registry/Imaging Repository. The median progression free survival is 6.0 months (95% Confidence Interval (CI) 5.6–6.4 months) and the median overall survival is 11.0 months (95% CI 10.5–11.5 months). At two and five years post-diagnosis, 10 and 2% of patients are alive, respectively. The establishment of the SIOPE DIPG Network and SIOPE DIPG Registry means a paradigm shift towards collaborative research into DIPG. This is seen as an essential first step towards understanding the disease, improving care and (ultimately) cure for children with DIPG.

## INTRODUCTION

Diffuse intrinsic pontine glioma (DIPG) is a pediatric brain cancer for which there is no curative treatment yet. Despite multiple clinical trials studying (combinations of) cytotoxic chemotherapy, including novel agents, the median overall survival of 9 months has not improved over the past decades [1, 2]. Although major advances have been accomplished in knowledge on the biological background of the disease by discovery of a high prevalence of specific mutations in genes encoding for histone 3.1 and 3.3, ACVR1 and P53 [3–9], much is yet to be learned on the mechanisms that contribute to treatment resistance. Research on the DIPG patient population, however, is hampered because integrative, large scale clinical, radiological and biological data are lacking.



There are several factors that contribute to the scarcity of data. First, DIPG is an orphan disease with a yearly incidence of 2.32 per 1,000,000 residents aged 0–20 years [10]. Second, DIPGs are diagnosed clinically, based on typical MR-imaging findings [11], in combination with a classic triad of neurological symptoms [12]. Biopsy procedures to obtain tumor material have long been considered dangerous and not contributing to the diagnosis, treatment approach or survival outcome [13]. Fortunately, recent years have seen an emergence of studies that include biopsies, however, the discovery of new mutations have caused an on-going debate about the actual definition of the disease itself [9, 14]. This is exemplified by the recently published new WHO classification of central nervous system tumors, that has reclassified DIPG to the category of WHO grade IV diffuse midline gliomas with histone mutations [15]. Inconsistent definition of DIPG has hampered in- and exclusion or response criteria for clinical trials, which resulted in a great variety of mostly incomparable clinical trials, many of which are single-center, single-arm studies with only few patients enrolled [10].

Collaboration and data sharing are promising strategies for tackling rare diseases, by facilitating uniform and hypothesis-driven research [16]. To overcome the current lack of data and improve the integration, speed, quality, and coherence of research, we aimed to (1) create a DIPG research-infrastructure consortium, and (2) initiate collaborative collection of comprehensive data on DIPG patients. This paper describes the methodology of the set-up of an international research network infrastructure, the SIOPE DIPG Network and SIOPE DIPG Registry, including legal and IT aspects, as well as preliminary patient inclusion data.

## MATERIALS AND METHODS

### *The establishment of a research-infrastructure consortium*

In January 2011, in a DIPG meeting organized by the Semmy Foundation in Amsterdam, the SIOPE DIPG Network was established as a sub-committee of the high-grade glioma (HGG) working group of International Society of Paediatric Oncology Europe (SIOPE). The SIOPE DIPG Network is a collaboration of pediatric oncologists, neurologists, neurosurgeons, radiotherapists, radiologists, pathologists, molecular biologists, psychologists and others motivated to carry out excellent clinical and biological research in the field of DIPG. Initially started as a European network, it has extended to colleagues from all over the world, with participants from Russia, Turkey and Mexico.

The SIOPE DIPG Network is comprised of (i) an executive committee, (ii) a group of scientific advisors, (iii) National Coordinators (NCs) and (iv) members. The Executive Committee (i) manages and controls the DIPG Network, and abides by and enforces the mission and the core values of the Network. Scientific Advisors (ii) are individuals with expertise in areas such as: biostatistics and biometry, medical ethics and health policy, basic science research, translational research, (neuro)psychology, neuroimaging, or other areas not mentioned. Scientific Advisors are consulted to advise the Executive Committee in matters of development and implementation of research protocols including ideas for innovative studies that could be executed using the Network. NCs (iii) are those DIPG Network members that coordinate collaboration between the SIOPE DIPG Network and biologists and clinicians in their countries. NCs identify and select hospitals and scientific experts in their countries, that are involved in the treatment of DIPG patients and that potentially may join the DIPG Network. DIPG Network members (iv) participate in research projects initiated by the DIPG Network following the principles of Good Clinical Practice. Potential members need to be approved by the Executive Committee before subscription to the DIPG Network. Network members are free to decide on whether they wish to participate in a research project on a case-by-case basis and at their sole discretion.

The mission of the SIOPE DIPG Network is to serve as a research-infrastructure for the design and execution of high quality, international multicenter laboratory and clinical studies, intended to enhance the understanding of DIPG and to improve outcome of patients suffering from DIPG. The mission, aims, core values and structure of the SIOPE DIPG Network are described in the SIOPE DIPG Network Bylaws (see Legal aspects).

### *Collaborative collection of comprehensive data*

The establishment of a DIPG registry was set as first project of the Network, with the purpose to include clinical, biological and centrally reviewed radiology data of patients

with DIPG, both in- and outside clinical trials. The SIOPE DIPG Registry is composed of an online web application and database for clinical data, and an Imaging Repository for radiological data (Fig. 1).

In parallel, an International DIPG Registry was initiated and developed, which includes patient data from the USA, Canada, Australia and New Zealand. To allow for the inclusion of uniform data, standardized electronic Case Report Forms (e-CRFs; Fig. 2) were developed by the SIOPE DIPG Network, in coordination with colleagues from the International DIPG Registry. The online e-CRFs collect data on demographics, medical history and physical exam at time of diagnosis together with the results from radiological and pathological review by the local hospital, treatment data (including radiotherapy, chemotherapy, surgery and supportive care such as steroids), data on clinical and radiological follow up, and last known status of the patient (see Data Entry Manual; Supplementary material 1).



In parallel to the clinical data, anonymized MRI-scans scans are uploaded via a secure FTP server or sent on CDs. De-identification/ pseudonymization, according to the country's law, is performed either in the referring center, during upload or at the time of receipt. When fully anonymized, these images are uploaded into the SIOPE DIPG Imaging Repository (Fig. 1). Expert neuroradiologists are brought together in a central neuroradiology panel. This panel has access to view assigned images from the Imaging Repository for blinded central review of submitted cases.

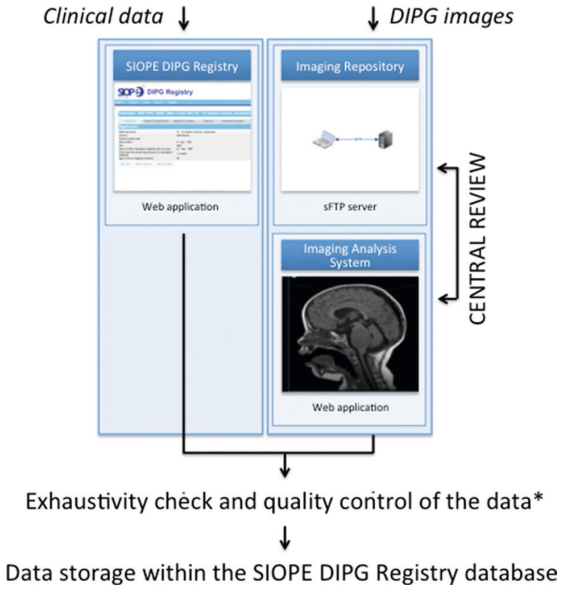


**Fig. 1** Organizational chart of the SIOPE DIPG registry and imaging repository. For details on the quality control process please see Supplementary Fig. 1

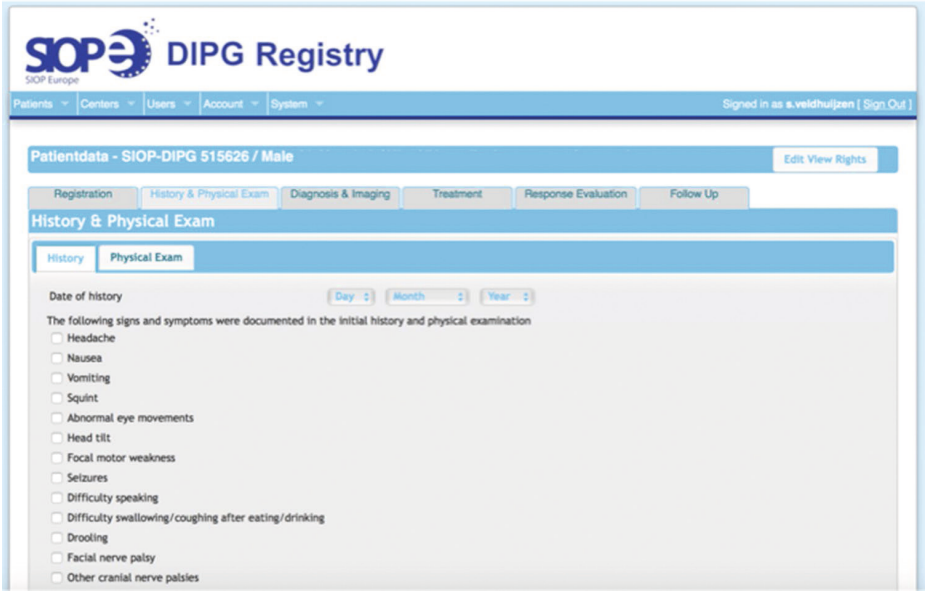


**Fig. 2** Screenshot of the SIOPE DIPG Registry showing the electronic case report forms (e-CRFs). The open tab represents the e-CRF for history and physical exam

### *Eligibility criteria*

The criteria for patient inclusion in the SIOPE DIPG Registry are: (i) patients with DIPG, or with focal Pontine Glioma (fPG), defined as a T1-weighted hypointense and T2-weighted hyperintense tumor with at least 50% involvement of the pons (DIPG) or less than 50% involvement of the pons (fPG) on T2, and as confirmed by expert neuroradiologists via the central radiology review procedure described above (ii) age at diagnosis between 0 and 21 years, and (iii) written informed consent in case of prospective registration. Furthermore, in order to enable validation of the diagnosis following the current guideline, a minimum of diagnostic criteria is required i.e. clinical and radiological data (MRI scans) to be shared in the registry and, if available, pathology data.

### *Ethical considerations*

The SIOPE DIPG Registry is conducted according to the principles of the Declaration of Helsinki. No personal identifiers, besides date of birth, are included in the e-CRFs. If in a certain country this is not allowed, age at diagnosis is submitted instead. All patients are assigned a unique SIOPE DIPG Registry number. Per member site, a separate list, kept under a special password, connects the DIPG Registry number with the personal identifiers. Access to this list is restricted to a local coordinator at each site.

In most participating countries informed consent is not mandatory for retrospective registration of (mostly deceased) patients. If required a consent form is sent to parents and signed. Prospective registration of living patients requires an informed consent procedure. National coordinators are responsible for the translation of the standardized informed consent to the language of their country. Translated forms will be centrally collected and available to local hospitals upon request. In this procedure, a SIOPE Network member informs parents (and patients), after which he/she provides the Patient Information Form (Supplementary material 2) and requests for informed consent. Parents or patients may reject participation at all times.

### *Data collection*

Each country represented in the SIOPE DIPG Network is committed to delivering data to the SIOPE DIPG Registry and Imaging Repository. After subscription to the Network, the approved Network member receives a username and password to enter data into the Registry. Data collection covers both retrospective and prospective registration. Retrospective data will be collected from local hospitals, national registries and clinical trials. For prospective registration, Network members are encouraged to inform their patients about the existence of the SIOPE DIPG Registry followed by the informed consent procedure. In case of decline, the e-CRFs will be left blank, but a unique Registry number is created, which will only be used for epidemiologic studies. To describe data retrieval, as well as responsibility and ownership of the data, uniform international agreements for collaborative research purposes were created (see Legal aspects).



### *Exhaustivity check and quality control of the data*

To ensure the reliability, validity, and completeness of the data [17], an appropriate program of Quality Control was implemented (Supplementary Fig. 1). Quality Control of data is an integral part of the project and takes place at all stages: before, during and after data entry.

### *Data storage and safety*

Based on the e-CRF's, an optimized relational database was constructed. The database along with the web application is hosted on a dedicated server where the web application is the single point of contact with the database. All end-user connections use the secure HTTP (HTTPS) protocol to ensure protection of the privacy and integrity of the exchanged data. The server is placed within a Virtual Private LAN protected by a dedicated firewall ring. For server maintenance purposes direct access to the server is only possible through a restricted virtual private network (VPN) connection. The DIPG Registry is built on a generic framework in which presentation, logic and data layer

are separated. The framework was designed with several active protection features to prevent unsolicited use of the application such as user/role/session validation, the use of antiforgery tokens and brute force protection. To ensure data safety, database input controls and extensive audit trailing are used. Every action within the system and the database is logged. The server, application and database are monitored 24h/7days and backups are made and stored daily on a different server in order to provide a disaster recovery scenario. The SIOPE DIPG Registry framework herewith provides a stable, secure and generic basis in any of its products. A penetration test (black box approach) was performed to validate the effectiveness of the (visible) security implemented on the SIOPE DIPG Registry and Imaging Repository. This test will be repeated on a regular basis.

### *Legal aspects*

The daily and financial management, and hosting of the SIOPE DIPG Registry is carried out by the Dutch Childhood Oncology Group (DCOG), a National Paediatric Haematology-Oncology Society (NaPHOS) member of SIOPE. DCOG is mandated by the Executive Committee of the DIPG Network to act as a legal entity on its behalf in matters concerning the DIPG Registry, by a letter of mandate.

The construction of a collaborative research infrastructure, with geographical differences in health care structures and legislation faces considerable challenges. Experts in the field of sensitive data transfer and access rights have been consulted to certify issues concerning data anonymization, -collection, and -safety. To meet multinational standards, two legal documents have been drafted, abiding to EU law and taking into account SIOPE DIPG Network members' national laws. The first contains the SIOPE DIPG Network Bylaws (Supplementary material 3), that describe the mission, aims, core values and structure of the SIOPE DIPG Network as well as terms and conditions for submitting, reviewing and approving proposals for research projects using data from the SIOPE DIPG Registry. Furthermore, the Bylaws provide a Scientific Advisory Agreement for consultation of experts outside the SIOPE DIPG Network, such as specialised neuroradiologist for central radiology review. Second is the SIOPE DIPG Registry and Imaging Repository Regulatory Document (Supplementary material 4), describing the terms and conditions for management, maintenance of and access to the DIPG Registry and Imaging Repository.

### *Use of data*

For strategic decisions concerning novel collaborative clinical and biological research projects in the field of DIPG, NCs meet or consult several times a year. In this way the SIOPE DIPG Network itself is responsible for the optimal use of obtained data. Data from the SIOPE DIPG Registry and Imaging Repository are available to researchers

for collaborative, interdisciplinary, and translational studies. For use of the data from the Registry, the researcher must be a member of the SIOPE DIPG Network. The availability of data to the researcher is conditional to obtained approval from the Executive Committee, after submission of a project proposal, and permits and licenses required by the researcher's national law. The Executive Committee may set additional conditions to a specific project and stipulates the general terms and conditions with regard to receipt and use of data. Subsequently, only requested, relevant data are selected from the DIPG Registry and made available to the researcher. The researcher owns results of a research project, including the intellectual property rights thereto. Publication of results generated with data from the SIOPE DIPG Registry requires to comply with rules concerning authorship, as defined by the International Committee of Medical Journal Editors (ICMJE). Each year, the Executive Committee sends a report to the members of the SIOPE DIPG Network on the number of approved, performed and rejected projects.



## RESULTS

### *International collaboration in DIPG research*

Since its inception in 2011, the SIOPE DIPG Network has expanded each year. Currently, 27 countries (Austria, Belgium, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Lithuania, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland, The Netherlands, United Kingdom, Turkey, Russia, and Mexico; Supplementary Fig. 2) have committed to the SIOPE DIPG Network and Registry. There is also a close collaboration with the International DIPG Registry, which represents the collaborative efforts of physicians and researchers from North America, Canada, Australia and New Zealand (Supplementary Fig. 2). To coordinate similar data collection, there are frequent telephone conferences and annual working visits between the SIOPE DIPG Network chair, the SIOPE DIPG Registry coordinator and International DIPG Registry team stationed at the Clinical Management and Research Support Core (CMRSC) at Cincinnati Children's Hospital Medical Center. Both DIPG registries are financially supported by The DIPG Collaborative, a collection of more than 20 parent foundations with the common interest of promoting and funding research into DIPG.

### *SIOPE DIPG registry and imaging repository*

Currently, as a prerequisite to start prospective patient inclusion in the SIOPE DIPG Registry, members of the SIOPE DIPG Network are in the process of Medical Ethical Committee and IRB review, with some countries already including patient data upon approval. As of April 2016, six countries have submitted retrospective data of 694 patients to the SIOPE DIPG Registry and Imaging Repository. Data were retrieved from

three national registries, two local hospitals, and one clinical trial. Figure 3 shows the age distribution of patients included in the SIOPE DIPG Registry, with a median age of 7 years (standard deviation (SD) ± 3.5). Table 1 shows the patient characteristics, clinical, radiological and biological disease characteristics, and treatment details of the total cohort. For 94 patients, tumor material was available for genetic analysis. Results are shown in Table 2. The median progression free survival, defined as time from diagnosis to clinical signs of disease progression (i.e., increase of symptoms or new symptoms) and/or radiological tumor progression on MRI, was 6.0 months (95% Confidence Interval (CI) 5.6–6.4 months). The median OS, defined as time from diagnosis to death, was 11.0 months (95% CI 10.5–11.5 months). PFS and OS are both plotted in Fig. 4a. Figure 4b, c show the PFS and OS stratified by mutational status. Figure 4d, finally, shows the distribution of time from progression to death (median 4 months). Ten percent of patients were alive at 2 years post diagnosis. At 5 years post diagnosis only two percent were alive. No disease-free survival was observed.

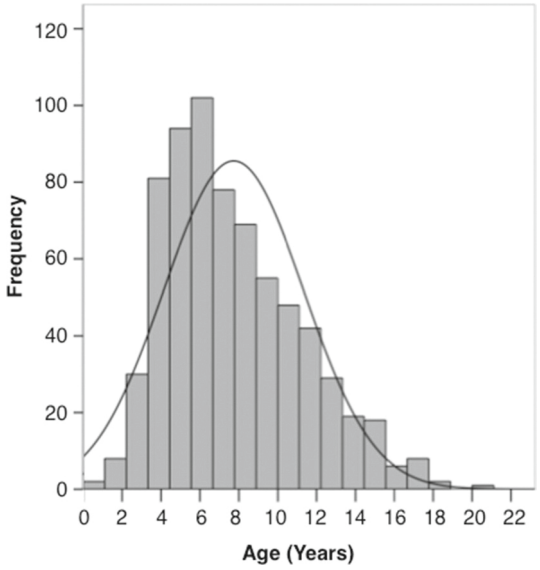


**Figure 3.** Histogram showing the age distribution of the total cohort

## DISCUSSION

A first step is made to improve the infrastructure of research into DIPG. This was done by (1) the establishment of the SIOPE DIPG Network, and (2) the development and initiation of the SIOPE DIPG Registry and Imaging Repository. This initiative, enabling collaborative research, is seen as major first step towards improving care and (ultimately) cure for children with DIPG.

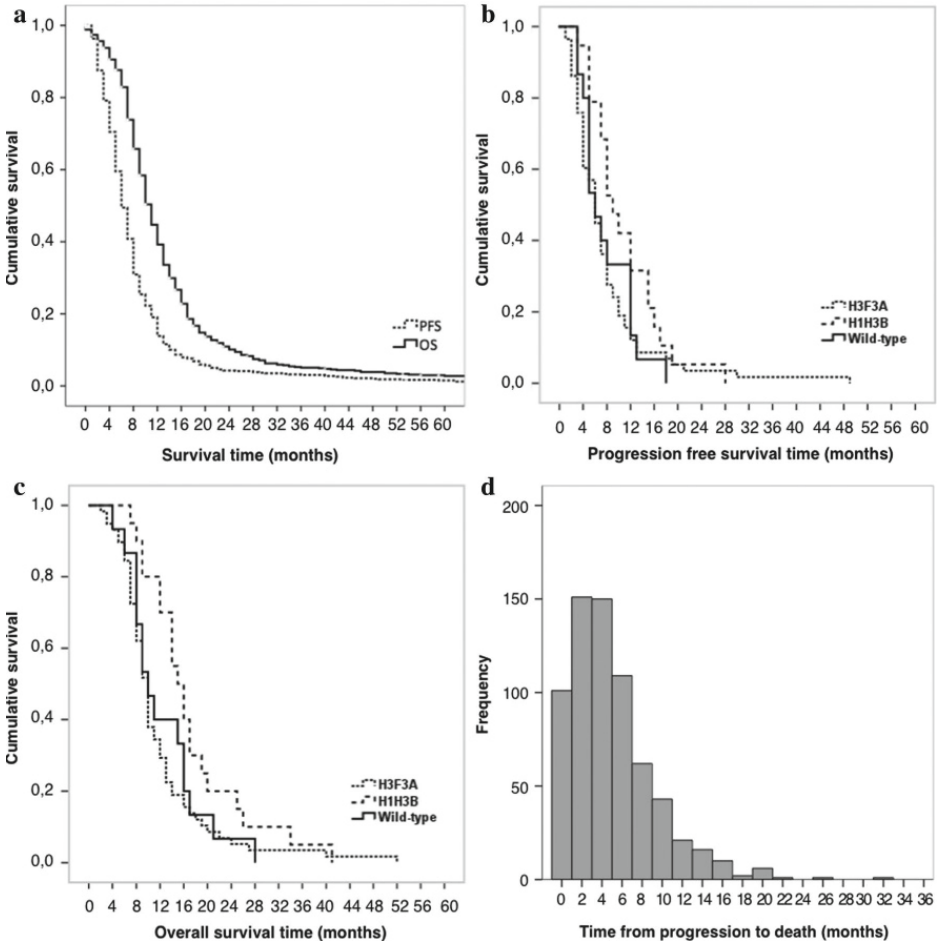


**Fig. 4** Survival data. a Kaplan Meier estimates of progression free survival (PFS; n = 684) and overall survival (OS; n = 691). b Kaplan Meier estimates of progression free survival (PFS) stratified by mutational status (H3F3A n = 59, H1H3B n = 20, wild-type n = 15). c Kaplan Meier estimates of overall survival (OS) stratified by mutational status (H3F3A n = 59, H1H3B n = 20, wild-type n = 15). d Histogram showing the distribution of time from progression to death

Collaboration is pursued to overcome the factors hampering research into DIPG. This paper is the first to publish pooled patient data of almost 700 DIPG patients collected from national registries, local hospitals and clinical trials. To date, published patient data are largely from phase I/II trials, which cover only a small percentage of the actual population diagnosed with DIPG. This possibly results in publication/selection bias. Future registration of all DIPG patients, both in- and outside trials, will give the opportunity to analyze 'real-life' DIPG patient data resulting in better description of incidence, characteristics and survival of DIPG patients. Also, it will generate a representative reference cohort, which may be used as historical control in any future

study. With the SIOPE DIPG Network and SIOPE DIPG Registry/Imaging Repository, an infrastructure has been created that allows for research transparency, international collaboration and the elimination of duplication of research efforts. Already two international studies were published by the SIOPE DIPG Network, concerning palliative care and end-of-life decisions [18] and steroid use [19] in DIPG patients. The first large-scale international study including all patients registered in the SIOPE DIPG Registry and International Registry, with an estimated total of >1000 DIPG cases, is currently being conducted. This study will evaluate the characteristics of long-term surviving patients in comparison to the total group of patients.

The preliminary patient data of the 694 patients currently included in the SIOPE DIPG Registry, shows an equal gender distribution, rapid onset of symptoms pre-diagnosis (86% <12 weeks of which 66% within 6 weeks), a clinical presentation including cranial nerve palsy in the majority (85%) of patients, and two-third of patients showing gadolinium contrast enhancement on the diagnostic MRI, of which 57% (39% of the total cohort) showed partial ring-like enhancement suggestive for necrosis. At time of diagnosis, only 1% of the diagnostic MRIs showed metastasis in the brain, and 2% in the spine. Eighteen percent of patients present with hydrocephalus. Biopsy was performed in one-third of the patients, showing a range of WHO grades. From the 94 patients in whom histone mutational status was determined, two-third harbored a H3F3A mutation, versus 21% of patients harboring a H1H3B mutation, and 16 % were classified as wild-type. This distribution, as well as the observed difference in survival in favor of the H1H3B mutational subgroup, is in line with international literature [4, 6, 9, 20]. Almost all patients received radiotherapy, 9% received re-irradiation, and a sticking 72% received chemotherapy, which is contradictory since there is no chemotherapeutic strategy yet, that has shown to be effective [1, 2]. Autopsy was performed in only 4% of patients. Currently, the majority of patients included in the SIOPE DIPG Registry are patients with a radiologically confirmed and centrally reviewed T1-weighted hypointense and T2-weighted hyperintense tumor with at least 50% involvement of the pons (DIPG) [11]. The recent WHO re-classification, however, may imply that the inclusion criteria for the SIOPE DIPG Registry need to be adjusted to also include patients with non-pontine diffuse midline gliomas in the future.

Dependent on the extent to which biopsies and autopsies will be (re-)introduced for DIPG, data on biological characteristics will gradually increment in the Registry, which will increase the knowledge on DIPG etiology, pathogenesis, possible drug targets and the mechanisms that contribute to the observed resistance to treatment. Furthermore, big-data analysis of aggregated clinical, radiological and especially biological data facilitates the discovery of patterns that indicate patient subgroups, which enables consensus formation on classification, in-/exclusion and response criteria, and improves the quality and comparability of future trials. Moreover, joining forces within an international research-infrastructure will stimulate the initiation of,

and active accrual in, international multicenter trials, with sufficient power to address the many unanswered research questions. This, together with the recent evolution of ideas concerning therapeutic strategies [21–24], should facilitate the identification and selection of novel tolerable and effective therapies.

Data collection in the Registry will have some (initial) limitations. Due to the former lack of local hospital- and national registrations, lack of specific ICD-codesFootnote1, and due to a presumed limited documentation of clinical, radiological and pathological data, retrospective data collection will very likely be incomplete. Based on data from the Dutch retrospective study [10], and included parties in the SIOPE DIPG Network (with a total number of about 600 million residents aged 0–19 years; April 2016) it is estimated that over 350 children are eligible for prospective registration in the SIOPE DIPG Registry each year. It is expected that annually about 200 patients (60%) will be registered in the first years, and that this number will increase when the SIOPE DIPG Network expands, resulting in higher data completeness per country over time.



Recent publications in DIPG literature have shown that coupling genetic data to clinical data will become increasingly important to understand and/or predict the clinical behavior of the disease [9, 14]. Therefore, as for now, data of the most common genetic aberrations are entered in the Registry via a 'Biopsy/Autopsy e-CRF'. A next step of the SIOPE DIPG Registry is to establish a (virtual) biobank of DIPG material, linked with the DIPG Genomics Repository at Progenetix (dipg.progenetix.org), a cancer genome database [25]. Ideally, the increased availability of DIPG tumor tissue will lead to generally available, representative, and possibly even patient subgroup-specific cell cultures and xenograft models, which enable thorough basic research and high-throughput screening of candidate therapies. Other future perspectives are to include questionnaires for Quality of Life research since research on this important subject is largely lacking, especially data on end-stage disease symptoms and the associated specific needs for palliative and end-of-life care [18]. The collection of conventional MR-imaging data in the Imaging Repository, will in the future be expanded to multimodality MR-imaging and other advanced imaging techniques such as PET. The data also might be useful for educational purposes (e-learning) in an aim to improve diagnostics of these tumors.

To conclude, with the collaborative efforts of professionals treating children with DIPG, patient/parent organizations, legal advisors, experts in the field of information technology and imaging experts, an international research-infrastructure was successfully set up, which led to the development and initiation of the SIOPE DIPG Registry. With already 694 patients registered, this Registry stimulates collaborative preclinical and clinical research efforts. The first study using data from both the SIOPE and International Registry is already in its final stages. The existence of the International DIPG Registry, surveying similar data as the SIOPE DIPG Registry,

allows for external cross-validation of data, generating robust data on the DIPG patient population. Big data analysis of the Registry's data will potentially lead to the discovery of patterns that pave the way to the identification of effective therapies towards a cure for patients suffering from DIPG.

The methodology used for the SIOPE DIPG Registry will, most likely, be easily translatable to other pediatric cancer registries, as almost all of these are orphan diseases that could benefit from international registration and collaboration in research.

**Table 1** Demographics, disease characteristics and treatment data of the total cohort (n = 694)

| Category | Variable | n | Valid (%) |
|---|---|---|---|
| Total | | 694 | |
| Country | Germany | 312/694 | 45 |
| | Netherlands | 132/694 | 19 |
| | France | 118/694 | 17 |
| | Italy | 79/694 | 11 |
| | United Kingdom | 45/694 | 7 |
| | Croatia | 8/694 | 1 |
| Gender | Female | 359/694 | 52 |
| | Male | 335/694 | 48 |
| Age | (mean, SD) | 7.7 | ±3.5 |
| Symptom duration | <6 weeks | 413/627 | 66 |
| | 6–12 weeks | 127/627 | 20 |
| | 13–24 weeks | 47/627 | 8 |
| | >24 weeks | 40/627 | 6 |
| Cranial nerve palsy | Yes | 484/568 | 85 |
| | No | 84/568 | 15 |
| Pyramidal signs | Yes | 270/562 | 48 |
| | No | 292/562 | 52 |
| Cerebellar signs | Yes | 338/562 | 60 |
| | No | 224/562 | 40 |
| T1-weighted | Hypo-intense | 422/439 | 96 |
| | Iso-intense | 16/439 | 4 |
| | Hyper-intense | 1/439 | 0 |
| T2-weighted | Hypo-intense | 5/465 | 1 |
| | Iso-intense | 2/465 | 0 |

**Table 1** Demographics, disease characteristics and treatment data of the total cohort (n = 694 *(continued)*

| Category | Variable | n | Valid (%) |
|---|---|---|---|
| | Hyper-intense | 458/465 | 99 |
| Pontine involvement | <50% | 3/550 | 0 |
| | >50% | 547/550 | 100 |
| Tumor size | Anterior-posterior Ø in mm (mean, SD) | 36 | ±7 |
| | Transverse Ø in mm (mean, SD) | 43 | ±8 |
| | Cranial-caudal Ø in mm (mean, SD) | 42 | ±9 |
| Enhancement | Yes | 336/516 | 65 |
| | No | 180/516 | 35 |
| Ring-enhancement | Yes | 191/491 | 39 |
| | No | 300/491 | 61 |
| Margin | Ill-defined | 363/481 | 76 |
| | Well-defined | 118/481 | 24 |
| Extension | Yes | 493/549 | 90 |
| | No | 56/549 | 10 |
| Metastasis brain | Yes | 7/547 | 1 |
| | No | 540/547 | 99 |
| Metastasis spine | Yes | 8/420 | 2 |
| | No | 412/420 | 98 |
| Hemorrhage | Yes | 60/458 | 13 |
| | No | 398/458 | 87 |
| Necrosis | Yes | 191/473 | 40 |
| | No | 282/473 | 60 |
| Hydrocephalus | Yes | 89/505 | 18 |
| | No | 416/505 | 82 |
| Radiation | Yes | 650/691 | 94 |
| | No | 41/691 | 6 |
| Chemotherapy at diagnosis | Yes | 498/689 | 72 |
| | *Oral | 252/495 | 51 |
| | *IV | 230/495 | 46 |
| | *Both | 13/495 | 3 |
| | *Cytotoxic | 323/495 | 65 |

**Table 1** Demographics, disease characteristics and treatment data of the total cohort (n = 694 *(continued)*

| Category | Variable | n | Valid (%) |
|---|---|---|---|
| | *Targeted | 129/495 | 26 |
| | *Both | 43/495 | 9 |
| | *EGFR | 111/495 | 22 |
| | *mTOR / PI3K | 15/495 | 3 |
| | *EGFR/mTOR | 1/495 | 0 |
| | *HDAC inhibitor | 37/495 | 8 |
| | *Other | 331/495 | 67 |
| | No | 191/689 | 28 |
| Chemotherapy at progressive disease | Yes | 370/684 | 54 |
| | No | 314/684 | 46 |
| Re-irradiation | Yes | 61/694 | 9 |
| | No | 633/694 | 91 |
| Hydrocephalus treatment | Yes | 158/694 | 23 |
| | No | 536/694 | 77 |
| Biopsy | Yes | 260/694 | 37 |
| | *WHO Grade IV | 91/260 | 35 |
| | *Glioblastoma multiforme | 76/91 | 84 |
| | *DIPG^ | 15/91 | 16 |
| | *WHO Grade III | 71/260 | 27 |
| | *Anaplastic astrocytoma | 61/71 | 86 |
| | *Anaplastic oligoastrocytoma | 8/71 | 11 |
| | *Anaplastic oligodendroglioma | 2/71 | 3 |
| | *WHO Grade II | 38/260 | 15 |
| | *Diffuse astrocytoma | 20/38 | 53 |
| | *Low-grade astrocytoma n.o.s | 11/38 | 29 |
| | *Fibrillary astrocytoma | 4/38 | 10 |
| | *Oligoastrocytoma | 2/38 | 5 |
| | *Oligodendroglioma | 1/38 | 3 |

**Table 1** Demographics, disease characteristics and treatment data of the total cohort (n = 694 *(continued)*

| Category | Variable | n | Valid (%) |
|---|---|---|---|
| | *WHO Grade unknown | 60/260 | 23 |
| | No | 434/694 | 63 |
| Autopsy | Yes | 16/380 | 4 |
| | *WHO Grade IV | 12/16 | 75 |
| | *Glioblastoma multiforme | 12/12 | 100 |
| | *WHO Grade II-IV | 1/16 | 6 |
| | *Astrocytoma | 1/1 | 100 |
| | *WHO Grade unknown | 3/16 | 19 |
| | No | 364/380 | 96 |

**Table 2** Genetic characteristics of patients with available tumor material (n = 94)

| Category | Variable | n | Valid % |
|---|---|---|---|
| Total | | 94 | |
| Material type | Biopsy | 86/94 | 92 |
| | Autopsy | 8/94 | 8 |
| Histone mutations | H3F3A | 59/94 | 63 |
| | H1H3B | 20/94 | 21 |
| | H1H3C | 0/16 | – |
| | H1H3I | 0/16 | – |
| | Wild-type | 15/94 | 16 |
| Additional mutations | ACVR1 | 9/45 | 17 |
| | Wild-type | 45/54 | 83 |
| | TP53 | 18/29 | 62 |
| | Wild-type | 11/29 | 38 |
| | ATM | 3/16 | 19 |
| | Wild-type | 13/16 | 81 |
| | PIK3CA | 5/30 | 17 |
| | Wild-type | 25/30 | 83 |
| | PIK3R1 | 3/15 | 20 |
| | Wild-type | 12/15 | 80 |
| | MET | 1/15 | 7 |
| | Wild-type | 14/15 | 93 |

# 4

# Transitioning to Molecular Diagnostics in Pediatric High Grade Glioma: Experiences with the 2016 WHO Classification of CNS Tumors

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## ABSTRACT

**Background:** Pediatric neuro-oncology was profoundly changed in the wake of the 2016 revision of the WHO Classification of Tumors of the Central Nervous System. Practitioners were challenged to quickly adapt to a system of tumor classification redefined by molecular diagnostics.

**Methods:** We designed a 22 question survey studying the impact of the revised WHO classification on pediatric high-grade glioma. The survey collected basic demographics, general attitudes, issues encountered, and opinions on pediatric subtypes. Participant answers were analyzed along socioeconomic lines utilizing the human development index (HDI) of the United Nations and membership in the group of seven (G7) world economic forum.

**Results:** 465 participants from 53 countries were included, 187 pediatric neurooncologists (40%), 160 neuropathologists (34%), and 118 other experts (26%). When asked about pediatric high grade glioma entities, participants from very high development countries preferred treating a patient based on genetic findings. Participants from high and medium development countries indicated using traditional histology and tumor location as mainstays for therapeutic decisions. Non-G7 countries tended to regard the introduction of molecularly characterized tumor entities as a problem for daily routine due to lack of resources.

**Conclusions:** Our findings demonstrate an overall greater reliance and favorability to molecular diagnostics among very high development countries. A disparity in resources and access to molecular diagnostics has left some centers unable to classify pediatric high-grade glioma per the WHO classification. The forthcoming edition should strain to abate disparities in molecular diagnostic availability and work towards universal adaptation.

## INTRODUCTION

With the revised 4th edition of the WHO Classification of Tumors of the Central Nervous System, published in 2016, the field of neuro-oncology entered the molecular era. The diagnostic approach and classification of diffuse glioma, ependymoma, and medulloblastoma, among other tumor types, underwent major changes. A rapid shift in neuropathology laboratories and neuro-oncology clinics around the world was required to implement molecular advancements and a revised tumor typing system. The intention of the revision was to increase precision and add objectivity in the identification of defined diagnostic entities that can aid the treatment of patients, and more accurately predict prognosis [1]. The 5th edition of the WHO classification with more molecularly defined brain tumor subgroups is in the final stages of development [2], but key questions regarding implementation have not yet been answered. We sought to address if the implementation of molecularly defined entities into practice is adequately and equally perceived to be of added clinical benefit and supported by neurooncological professionals worldwide.



Within pediatric oncology, there is a broad disparity in financing and access to cancer care worldwide [3]. At current levels of care, models estimate that between 2020 and 2050, 9.3 million children will die from cancer in low- and middle-income countries. This represents 84% of pediatric cancer deaths worldwide [4]. Access to diagnostics is a well-documented problem in low- and middle-income countries [5]. Underdiagnosis and late diagnosis being key contributors to disparities in pediatric cancer outcomes [6]. The standard set by the WHO Classifications of Tumors of the CNS plays a pivotal role in how and if pediatric high-grade gliomas are diagnosed worldwide. Our survey on pediatric high-grade glioma (pedHGG) serves as a model disease to suggest an increasing diagnostic gap dependent on national socioeconomic environments. Knowledge on the influence of national socioeconomic environments may help increase applicability and usability of current and future pediatric CNS tumor classification.

## METHODS

The survey was designed and pretested by the European Society for Paediatric Oncology High Grade Glioma Working Group (SIOPE HGG WG). An online version of the survey was built using SurveyMonkey® (San Mateo, Ca, USA). Addressees of this survey study were primarily neuropathologists, pediatric neurooncologists, neurosurgeons, radiation oncologists, neuroradiologists and other professionals in the field of neuro-oncology. These professionals were actively approached worldwide by email on behalf of the SIOPE HGG WG between March 22 and May 8, 2019. Members of the neuro-oncology community were contacted using contacts from a prior international survey within the International Society of Neuropathology (ISN) [7], listservs from the SIOPE

Brain Tumour Group, the German Society of Pediatric Oncology and Hematology (GPOH), the German Neurooncology Working Group (NOA), the German Society of Neuropathology and Neuroanatomy (DGNN), and other international collaborators in the field of pediatric neuro-oncology. Multiple replies from the same IP and/or email address were excluded.

The survey consisted of twenty-two questions, twelve "Yes" or "No" questions, eight multiple choice questions, and two demographic questions. Within each thematic section we identified one key question. Respondents who failed to answer four out of six predefined key questions (including questions 1, 3, 10, 14, 16 and 17) were excluded. All key questions were dichotomous, "Yes or No". Key questions covered subjects including *(i)* awareness of the revised 2016 WHO classification, *(ii)* awareness of the newly introduced entity diffuse midline glioma (DMG), H3K27M mutant, *(iii)* opinions on the upcoming 5$^{th}$ WHO classification regarding introducing infantile glioma, *(iv)* introducing pediatric subtypes for anaplastic astrocytoma and glioblastoma, *(v)* introducing anaplastic pilocytic astrocytoma grade III and, *(vi)* removing gliomatosis cerebri (Appendix A).

The 2018 United Nations (UN) Human Development Index (HDI) was selected for the socioeconomic analysis. A country's HDI represents the mean of three key dimensions of human development: life expectancy, education, and standard of living. The ranking system classifies countries with a HDI >.80 as very high human development, ≥.70 to <.80 high, <.70 to ≥.56 medium, and <.56 as low human development respectively [8]. For comparison purposes, we coupled our HDI analysis with membership in the G7 (group of the seven world leading economies) [9]. The analysis was performed using IBM SPSS Statistics version 25 (Armonk, NY, USA). Data were analyzed using Pearson's Chi-square and Fisher's Exact Test. Full results available in Appendix B and C. Research involving human subjects according to the World Medical Association Declaration of Helsinki did not apply. Thus, the present study did not require an IRB review. Independent professionals, no patients, were asked for voluntary participation investigating their experience and opinion. No personal identifying data were collected and participation did not involve any advantage, disadvantage or any potential harm.

## RESULTS

### *Demographics*

The questionnaire was completed by 482 participants. 17 respondents (4%) did not meet inclusion criteria. Participants represented a broad spectrum of specialties; 187 pediatric neurooncologists (40%), 160 neuropathologists (34%), and 118 (26%) other experts. These experts included 45 neuroradiologists (10%), 29 radiation oncologists

(6%), 20 neurosurgeons (4%), 8 adult neurooncologists (2%), 7 scientists (2%), and 9 not specified (2%). 394 participants (87%) were from very high HDI countries, 44 (10%) high HDI and 15 (3%) medium HDI countries. A total of 53 countries were represented. No low development (HDI <.56) countries participated. 261 (57%) of participants were from G7 countries (Table 1). Within the HDI groups, select countries represented a large portion (>10%) of participation. These include among the very high HDI group Germany (n=115/29%) and the USA (n=43/11%), within the high HDI group Brazil (n=18/40%) and China (n=13/24%), and within the medium HDI group India (n=6/40%) and Egypt (n=3/20%), (Table 2).

**Table 1.** Demographics of participants utilizing the United Nations Human Development Index (HDI) and membership in the group of seven (G7) world economic forum.

| | **Survey Participants No. (%) N=454** | **Participant's Country No. (%) N=53** | **Reference List (United Nations) No. (%) N=189** |
|---|---|---|---|
| **Human Development Index (HDI)** | | | |
| Very High | 394 (87%) | 37 (70%) | 59 (31%) |
| High | 45 (10%) | 9 (17%) | 53 (23%) |
| Medium | 15 (3%) | 7 (13%) | 39 (21%) |
| Low | 0 | 0 | 38 (20%) |
| **Economic Tier** | | | |
| G7 | 261 (57%) | 7 (13%) | 7 (4%) |
| Non-G7 | 193 (43%) | 46 (87%) | 182 (96%) |

**Table 2.** Representation by country. Number of participants and % within HDI group.Medium*, High**, Very High HDI countries*** [8]

| **Country** | **Participants** | **Country** | **Participants** |
|---|---|---|---|
| Argentina*** | 4 (1) | Latvia*** | 1 (.3) |
| Australia*** | 9 (2.3) | Lebanon** | 1 (2.2) |
| Austria*** | 6 (1.5) | Lithuania*** | 2 (.5) |
| Belgium*** | 6 (1.5) | Luxembourg*** | 1 (.3) |
| Brazil** | 18 (40) | Malta*** | 1 (.3) |
| Canada*** | 16 (4.1) | Mexico** | 5 (11) |
| Chile*** | 3 (.8) | Morocco* | 1 (6.7) |
| China** | 13 (24) | Netherlands*** | 10 (2.5) |
| Colombia** | 3 (6.7) | New Zealand*** | 2 (.5) |
| Croatia*** | 2 (.5) | Norway*** | 7 (1.8) |

**Table 2.** Representation by country. Number of participants and % within HDI group.Medium*, High**, Very High HDI countries*** [8] *(continued)*

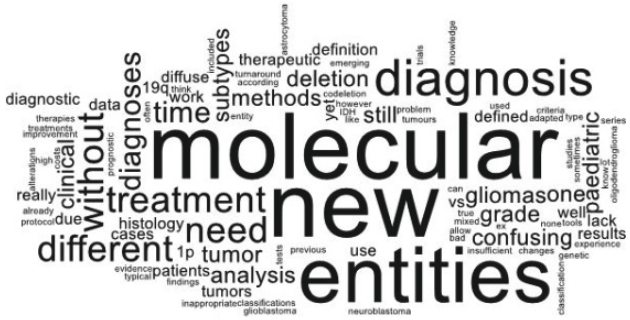
| Country | Participants | Country | Participants |
|---|---|---|---|
| Czech Rep.*** | 6 (1.5) | Pakistan* | 1 (6.7) |
| Denmark*** | 6 (1.5) | Poland*** | 2 (.5) |
| Egypt* | 3 (20) | Portugal*** | 3 (.8) |
| El Salvador* | 1 (6.7) | Russia*** | 9 (2.3) |
| Finland*** | 4 (1) | Slovakia*** | 2 (.5) |
| France*** | 20 (5.1) | Slovenia*** | 4 (1) |
| Germany*** | 115 (29) | South Africa** | 2 (13) |
| Greece*** | 4 (1) | South Korea*** | 2 (.5) |
| Honduras* | 1 (6.7) | Spain*** | 8 (2) |
| Hong Kong *** | 2 (.5) | Sweden*** | 10 (2.5) |
| Hungary*** | 5 (1.3) | Switzerland*** | 10 (2.5) |
| India* | 6 (40) | Thailand** | 2 (4.4) |
| Iran** | 2 (4.4) | Turkey** | 1 (2.2) |
| Israel*** | 1 (.3) | UK*** | 24 (6.1) |
| Italy*** | 23 (5.8) | Uruguay*** | 1 (.3) |
| Japan*** | 20 (5.1) | USA*** | 43 (11) |
| Jordan** | 2 (4.4) | | |

## *Overall Experiences with the Revised 4th Edition*

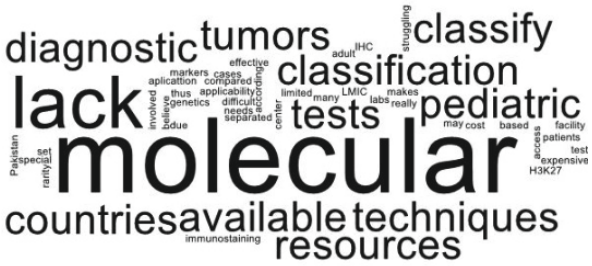
Participants were asked to report if the implementation of the revised WHO classification had caused problems and voluntarily provided details. 57% reported experiencing issues with the revision, representing 52/53 of the participating countries. 24% elaborated on their experiences in the free text portion of the survey. Feedback is visualized in Figure 1.

Very high HDI participants shared such experiences as; “some molecular tests are not readily available or validated for clinical practice”, “lack of consensus for treatment of new entities”, “new subtypes are not well known in all cooperating specialties”, “emerging new data which show new results, very often without a real influence on survival”, “a lot of the new WHO chapters do not describe pediatric gliomas well”, “treatment protocols not yet adapted to the new classification” and “changes in criteria for diagnoses create a lot of confusion in series with retrospective evaluation of patients”.

Participants from high/medium HDI countries shared experiencing including; "because of rarity compared to adult cases, it is not cost-effective to set up tests (IHC, molecular) for pediatric tumors", "lack of applicability due to lack of access to special techniques", "we do not have the facility to do molecular markers and or H3K27 immunostaining" and "resources for the classification according to the WHO 2016 are not available in many of the diagnostic labs in countries with limited resources, which makes it difficult to classify the tumors".




*Very High HDI*




*High/Medium HDI*

**Figure 1.** Overall experiences with the 2016 WHO Classification of CNS Tumors. Feedback from survey participants visualized using word clouds. Very High development countries (on the left) versus High/Medium development (on the right).

## *Diffuse Midline Glioma (DMG) and Diffuse Intrinsic Pontine Glioma (DIPG)*

Participants from very high HDI countries more often used the diagnosis of DMG, H3K27M mutant, than high/medium HDI country participants, i.e., 93% vs 65% respectively (question 4, p <.001). Those from G7 countries also reported using DMG, H3K27M mutant, more often (p <.001). When asked about the use of DIPG at diagnosis, 59% of very high HDI respondents in comparison to 38% of high/medium

HDI respondents, reported using both DIPG and DMG depending on context (question 6b, p .02). G7 country participants were also more likely to use both terms (p .01). Regarding treatment of H3K27 wildtype diffuse astrocytoma, WHO grade II, of the pons that fulfills radiological criteria of DIPG, high/medium HDI respondents answered like other H3K27M high grade gliomas (question 8c, p .004). Non-G7 country participants demonstrated the same preference (p.01). On the contrary, very high HDI respondents preferred personalized treatment, depending on genetic findings (53% vs 36% respectively: question 8d, p .01).

### *Infants*

On the introduction of infantile (high grade) glioma as a new tumor entity in the 5$^{th}$ WHO classification, 54% of very high HDI participants were in support, in comparison to 35% from high/medium HDI countries (question 11a, p .02). Regarding classification of infantile glioma, high/medium HDI participants selected WHO grade III/IV (question 12c, p .05), and very high HDI participants selected "depending on genetic findings" (question 12d, p .01).

### *Pediatric Subtypes*

Concerning routine analysis of IDH status for (pediatric) anaplastic astrocytoma and glioblastoma, high/medium HDI participants were not in support, because of a low percentage of IDH mutant pediatric HGG (question 13a, p <.001). In contrast, very high HDI participants supported "obligatory" testing for all cases (question 13b, p <.001). G7 country participants also felt routine analysis of IDH status should be obligatory (p. 04). In the matter of introducing new pediatric subtypes for anaplastic astrocytoma and glioblastoma, 90% of very high HDI participants were in favor, in comparison to only 74% of the high/medium HDI group, selecting "genetic findings suggest it" (question 15b, p .003).

### *Gliomatosis Cerebri*

Among those that support defining gliomatosis cerebri, very high HDI participants more often selected introducing a specific phenotype of an underlying glioma (question 18a, p. 02). While high/medium HDI participants selected introducing a specific tumor subtype (question 18b, p .01, Table 3).

**Table 3.** Survey results by Human Development Index (HDI). Only significant results displayed. Full length survey available in Appendix A and results in Appendix B.

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q4_Do you use the diagnosis of diffuse midline glioma, H3K27M mutant? | Yes | 367 (93) | 39 (65) | **<.001** |
| | No | 24 (6) | 21 (35) | |
| | No information given | 3 (1) | 0 | |
| Q6_please specify why or when you still use the term DIPG. (multiple answers possible) | Not answered | 267 (68) | 23 (38) | |
| | b. I use both terms depending on the respective context | Yes 133 (59)<br>No 94 (41) | Yes 14 (38)<br>No 23 (62) | **.02** |
| Q8_How would you treat a child (3 years and older) with a diffuse astrocytoma WHO grade II of the pons, H3K27 Wildtype, which fulfills clinical, radiological criteria of DIPG? | Not answered | 23 (6) | 1 (2) | |
| | c. Like other high grade gliomas, H3K27M | Yes 47 (13)<br>No 324 (87) | Yes 16 (27)<br>No 43 (73) | **.004** |
| | d. Individually, depending on other genetic findings including methylation | Yes 196 (53)<br>No 175 (47) | Yes 21 (36)<br>No 38 (64) | **.01** |
| Q11_Please specify why you think there is a need to introduce a new tumour entity of "infantile glioma" for histologically diagnosed high grade gliomas in infants younger than 3 years? (multiple answers possible) | Not answered | 152 (39) | 14 (23) | |
| | a. Prognosis is usually better | Yes 131 (54)<br>No 111 (46) | Yes 16 (35)<br>No 30 (65) | **.02** |
| Q12_If you think that there is indeed a need for a new tumour entity of "infantile glioma" would you classify this new entity as; | Not answered | 106 (27) | 10 (17) | |
| | c. WHO grade III/ IV (depending on histological grade like it is now) | Yes 66 (23)<br>No 222 (77) | Yes 18 (36)<br>No 32 (64) | **.05** |
| | d. Individually depending on genetic findings including methylation signature | Yes 154 (54)<br>No 134 (46) | Yes 16 (32)<br>No 34 (68) | **.01** |

**Table 3.** Survey results by Human Development Index (HDI). Only significant results displayed. Full length survey available in Appendix A and results in Appendix B. *(continued)*

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q13_What do you think about routine analysis of IDH status in paediatric anaplastic astrocytoma and glioblastoma? | Not answered | 2 (.5) | 0 | |
| | a. Not adequate because of low percentage (<10%) of IDH mutant paediatric HGG | Yes 46 (12)<br>No 346 (88) | Yes 26 (43)<br>No 34 (57) | **<.001** |
| | b. Obligatory for all cases | Yes 176 (45)<br>No 216 (55) | Yes 12 (20)<br>No 48 (80) | **<.001** |
| Q15_Please specify why you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytoma and glioblastoma in children (3 years and older) and adolescents/young adults? | Not answered | 120 (30) | 14 (23) | |
| | b. Genetic findings including methylation suggest specific paediatric subtypes of anaplastic astrocytoma/ glioblastomas | Yes 246 (90)<br>No 28 (10) | Yes 34 (74)<br>No 12 (26) | **.003** |
| Q18_Please specify why you think there is still a need for diagnosis of gliomatosis cerebri with typical neuroradiological features of diffuse growth pattern involving two and more cerebral lobes?" | Not answered | 159 (40) | 18 (30) | |
| | a. Diagnosis in the renaming of a specific phenotype of an underlying glioma. | Yes 134 (57)<br>No 101 (43) | Yes 16 (38)<br>No 26 (62) | **.02** |
| | b. Diagnosis in the renaming of a specific tumour subtype of its own for an underlying glioma histology | Yes 44 (19)<br>No 191 (81) | Yes 16 (38)<br>No 26 (62) | **.01** |

## DISCUSSION

Perceptions among the neuro-oncology community of the 2016 WHO Classification for CNS Tumors are not well documented, and the new 5th edition will soon be published. Our study provides input from nearly 500 neurooncological experts, representing 53 countries and eight disciplines. Results of this survey are the first to document international differences in acceptance and implementation along socioeconomic lines of the 2016 revised 4th edition, where molecular diagnostics were introduced for the first time as basis for new tumor entities and sub-entities. The process of adoption and adaptation has not been the same in countries with a highly developed national health system, as it has been in countries with much fewer financial and medical resources. Our findings demonstrated an overall greater reliance and favorability among very high HDI country participants to genetic testing. Participants in our study from very high HDI countries were significantly more likely to treat a patient individually based on genetic findings. This applied to how they would treat an infantile glioma and H3K27 wildtype DIPG for example, whereas high and medium development countries chose using conventional grading systems based on histology and tumor location. Furthermore, when asked about the use of routine IDH1 analysis for pediatric anaplastic astrocytoma and glioblastoma, only very high HDI countries considered this obligatory. The same divide was evident in the use of molecular diagnosis of diffuse midline glioma, H3K27M mutant. Significantly more very high HDI country participants reported using the DMG diagnosis and differentiating between DMG and the radiological diagnosis of DIPG depending on the context.



Socioeconomic differences and resulting attitudes as suggested in our study were largely a distillation of whether participants have access to molecular diagnostic tools. Our survey documents participants from lesser developed and some high and even very high development countries find access to molecular test to be a barrier. A 2016 survey by the International Society of Neuropathology (ISN) underlined the issues surrounding access to molecular diagnostics. They found 25% of participating countries and 79/314 neuropathology centers declared not to have access to molecular diagnostics for brain tumors. Furthermore, 12% of the neuropathologists surveyed claimed to be unfamiliar with molecular techniques [7]. Disparities in diagnostic usage stem from a lack of availability, accessibility, or acceptability [10]. In the context of molecular diagnostics for CNS tumors, evident in our survey is that they are in fact available and accepted, however not internationally accessible.

The ambition of the World Health Organization, with 194 Member States, is “to achieve better health for everyone, everywhere, united in a shared commitment” [11]. How WHO sponsored working groups, such as cIMPACT-NOW (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy), which try to adapt and explain identified issues with the current 2016 WHO classification, [12] can recommend

solutions to abate issues of access to molecular tests remains to be seen. It has been acknowledged there would be a transition period during the adaption of a molecular based system [1], however our survey results provide a glimpse into the current state of affairs. The development of an "integrated system" approach that uses both phenotype and genotype for CNS tumors is meant in part as a stop gap during the transition to a more genotype- based system, yet some diagnoses already required genotyping [2]. Our survey results demonstrate the mechanisms to introduce a genetic layer of neuropathological diagnoses have not been sufficient so far to bridge the resource gap in a large part of the world. As a result, many centers in lower income settings cannot adequately diagnose pediatric high grade glioma patients *per* the WHO 2016 criteria.

To make the WHO classification of CNS tumors more inclusive, alternative recommendations can be made for limited molecular evaluations by widely accessible tests such as FISH analysis or immunohistochemical surrogates, correlated with histology and complementary imaging. Guidelines to limit molecular testing in the setting of resource constraints and limited access to diagnostics are needed and would also be helpful for more general tumor types lacking effective targeted therapies. Should the WHO classification always consider if there is a clinical impact for each genotype? If not, how can phenotypic tumor typing still be useful and integrated moving forward? Participants in our survey mention a clinical disconnect between the WHO diagnostic requirements for genotyping and implications for therapy. Why is that the case and how can it be remedied?

A WHO CNS tumor classification that predominantly incorporates clinically significant phenotypes would enable centers without access to advanced molecular diagnostics to participate in the global neuro-oncology community more actively. Expanding cancer networks and population-based cancer registries to include more low-and middle-income countries, will increase access to diagnostic services, treatments, and foster research [4]. In pediatric HGG, rare disease registries that also function as networks, such as the SIOPE DIPG/DMG Registry and the International DIPG/DMG Registry, provide promising avenues to increase inclusion of countries beyond very highly developed countries. These organizations provide an infrastructure and international network of neuro-oncology expertise with the goal of enabling interdisciplinary and translational projects specifically for DIPG/DMG [13,14]. In collaboration with organizations like the WHO, pediatric cancer registries/networks can aid the rapid deployment of neuropathological expertise, molecular diagnostics, and treatments for high grade gliomas into high, middle and low-income countries. Bold research, financing, and implementation agendas are needed to bridge disparities in pediatric neuro-oncology cancer care and control worldwide [3]. Suggestive from our survey, the WHO Classification of CNS Tumors can play a role in perpetuating or eliminating disparities within the neuro-oncology community.

Although our study included voices from several underrepresented and less developed countries it should be acknowledged that participation from these countries remains a limitation. We had 13% representation from high/medium development countries, comprising 16 countries, and no representatives from low-income countries. Furthermore, sometimes only one or two participants answered for each high/medium HDI countries, thus, subgroup analyses of for example views between clinical neurooncologists or neuropathologists could not be made. Nevertheless, our limited socioeconomic and geographic diversity is reflective of the disparities within pediatric cancer care worldwide, as outlined above [3]. Despite these limitations, our study raises the most geographically and socioeconomically diverse set of voices to date from the pediatric neuro-oncology community.

## CONCLUSIONS

The 2016 revision of WHO classification drastically changed the practice of pediatric neuro-oncology. Around the world, practitioners were challenged to quickly adapt to a system of tumor classification redefined using molecular diagnostics. Our survey for the first time documents how disparities in access to molecular diagnostics can shape the implementation of the WHO 2016 tumor classification, and how perspectives towards diagnosis and treatment can differ in resource constrained settings during the molecular era. The forthcoming 5th edition should strain to abate disparities in molecular diagnosis between rich and poor countries and define a “minimum needed” molecular panel for each histotype.



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## SUPPLEMENTARY DATA

### Appendix A. Full Length Survey Copy

### Pediatric HGG and YOUR experience with the revised WHO classification

1. Are you aware of the revision of the WHO Classification of Tumours of the Central Nervous System that occurred in 2016?

*If you are a neuropathologist who needs to work with the revised classification please don't feel offended and continue :) ...*

- No
- Yes

2. Do you use the revised WHO Classification in your daily practice?

- No
- Yes

3. Are you aware of the newly introduced tumour entity "diffuse midline glioma, H3K27M mutant (WHO grade IV)"?

- No
- Yes

4. Do you use the diagnosis of diffuse midline glioma, H3K27M mutant?

- No
- Yes

5. Do you still prefer DIPG ("diffuse intrinsic pontine glioma") as neuroradiological/clinical diagnosis instead of diffuse midline glioma, H3K27M mutant, when located within the pons?

- No
- Yes

6. **If you answered YES to the previous question** (*"Do you still prefer DIPG as neuroradiological/clinical diagnosis instead of diffuse midline glioma ...?"*), please specify why or when you still use the term DIPG
(several answers are possible):

- DIPG is a well defined and established diagnosis/diagnostic term
- I use both terms depending on the respective context
- Patients can better understand DIPG as diagnosis than diffuse midline glioma, H3K27M mutant

- Diffuse midline glioma, H3K27M mutant, does not cover all DIPG
- Any other answer?_____

7. Do you believe there is an entity of DIPG, **H3K27 WILDTYPE**, WHO IV?
- No
- Yes

8. How would you treat a child (3 years and older) with a diffuse astrocytoma WHO grade II of the pons, **H3K27 WILDTYPE**, which fulfils clinical/neuroradiological criteria of DIPG?
- Like a low grade glioma
- Like a diffuse midline glioma, H3K27M mutant
- If using different protocols for diffuse midline gliomas, H3K27M mutant, and other high grade gliomas: Like other high grade
- gliomas
- Individually, depending on other genetic findings including methylation

9. Do you think there is a need to introduce a new tumour entity of **"Diffuse midline glioma of the pons, H3K27 WILDTYPE (WHO grade IV)**" with typical neuroradiological features of a DIPG?
- No
- Yes

10. Do you think there is a need to introduce a new tumour entity of "infantile glioma" for histologically diagnosed high grade gliomas in infants younger than 3 years?
- No
- Yes

11. **If you answered YES to the previous question** (*"Do you think there is a need to introduce a new tumour entity of "infantile glioma" ..."*), please specify why (several answers are possible):
- Prognosis is usually significantly better
- Genetic findings including methylation suggest a tumour entity of its own
- Therapy is usually different from high grade gliomas of older children and adults
- Any other reason? _____

12. If you think that there is indeed a need for a new tumour entity of "infantile glioma" would you classify this new entity as
- WHO grade I
- WHO grade II
- WHO grade III/IV (depending on histological grade like it is now)
- Individually depending on genetic findings including methylation signature
- Without a defined WHO grade

13. What do you think about routine analysis of IDH status in paediatric anaplastic astrocytomas and
glioblastomas?

- Not adequate because of low percentage (<10%) of IDH mutant paediatric HGG
- Obligatory for all cases
- Only if sufficient tumor material is available
- I don´t know
- Any other comment? _____

14. Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytomas and
glioblastomas in children (3 years and older) and adolescents/young adults?

- No
- Yes
-

15. **If you answered YES to the previous question** (*"Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytomas and glioblastomas ..."*), please specify why (several answers
are possible):

- Prognosis is usually better than in older adults
- Genetic findings including methylation suggest specific paediatric subtypes of anaplastic astrocytomas/glioblastomas
- Any other reason? _____

16. Do you think there is a need to introduce a new tumour entity of "Anaplastic pilocytic astrocytoma (WHO
grade III)" or "Anaplastic astrocytoma with piloid features (WHO grade III)", respectively, for pilocytic
astrocytomas with anaplastic features?

- No
- Yes

17. Do you think there is still a need for diagnosis of gliomatosis cerebri with typical neuroradiologcal features
of diffuse growth pattern involving two and more cerebral lobes ?

- No
- Yes

18. **If you answered YES to the previous question** (*"Do you think there is still a need for diagnosis of gliomatosis cerebri ..."*), please specify (several answers are possible):

- Diagnosis in the meaning of a SPECIFIC PHENOTYPE of an underlying glioma, but not as a tumour subtype or entity of its own

- Diagnosis in the meaning of a SPECIFIC TUMOUR SUBTYPE of its own for an underlying glioma histology
- Diagnosis in the meaning of a TUMOUR ENTITY of its own independently of an underlying glioma histology
- Any other suggestions? _____

19. In summary, has the implementation of the revised WHO Classification caused any problems?
- No
- Yes

20. **If you answered YES to the previous question** (*"In summary, has the implementation of the revised WHO Classification caused any problems?"*), please specify your relevant issues (several answers are
possible):
- Introduction of new tumour entities
- Abolishment of tumour entities
- Renaming of tumour entities
- Insufficient diagnostic definitions of tumour entities
- Diagnostic definitions are less relevant for pediatric than for adult neurooncology
- Diagnostic definitions are sometimes hard to explain to patients/parents
- Any other problems? _____

21. What is your field of expertise?
- Paediatric Oncologist/Paediatric Neurooncologist
- Neuropathologist
- Neurosurgeon
- Radiotherapist
- Radiologist/Neuroradiologist
- Scientist/Biologist/Physician Scientist
- Any other field of expertise? _____
-

22. In which country are you working?
and_____ YOUR experience

**Appendix B.** Full Survey Results by HDI. Participant No. (% within HDI)

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q1_Are you aware of the revision of the WHO Classification of Tumors of the Central Nervous System that occurred in 2016? | Yes | 375 (95) | 58 (97) | 1.0 |
| | No | 19 (5) | 2(3) | |
| Q2_Do you use the revised WHO Classification in your daily practice? | Yes | 366 (93) | 55 (92) | .70 |
| | No | 24 (6) | 4 (7) | |
| | No information given | 4 (1) | 1 (2) | |
| Q3_Are you aware of the newly introduced tumour entity "diffuse midline glioma, H3K27M mutant (WHO grade IV)"? | Yes | 380 (96) | 56 (93) | .43 |
| | No | 12 (3) | 4 (7) | |
| | No information given | 2 (1) | 0 | |
| Q4_Do you use the diagnosis of diffuse midline glioma, H3K27M mutant? | Yes | 367 (93) | 39 (65) | **<.001** |
| | No | 24 (6) | 21 (35) | |
| | No information given | 3 (1) | 0 | |
| Q5_Do you still prefer DIPG as radiological/clinical diagnosis instead of diffuse midline glioma, H3K27M mutant, when located within the pons? | Yes | 180 (46) | 32 (53) | .50 |
| | No | 212 (54) | 28 (47) | |
| | No information given | 2 (.5) | 0 | |
| Q6_If you answered YES to the previous question, please specify why or when you still use the term DIPG. (multiple answers possible) | Not answered (% total HDI) | 267 (68) | 23 (38) | |
| | a. DIPG is a well defined and established diagnosis/ diagnostic term | Yes 104 (46) No 123 (54) | Yes 14 (38) No 23 (62) | .40 |
| | b. I use both terms depending on the respective context | Yes 133 (59) No 94 (41) | Yes 14 (38) No 23 (62) | **.02** |
| | c. Patients can better understand DIPG as diagnosis than diffuse midline glioma, H3K27M mutant | Yes 74 (33) No 153 (67) | Yes 7 (19) No 30 (81) | .09 |
| | d. Diffuse midline glioma, H3K27M mutant, does not cover all DIPG | Yes 76 (34) No 151 (66) | Yes 13 (35) No 24 (65) | .84 |

**Appendix B.** Full Survey Results by HDI. Participant No. (% within HDI) *(continued)*

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q7_Do you believe there is an entity of DIPG, H3K27 WILDTYPE, WHO IV? | Yes | 281 (71) | 50 (83) | .15 |
| | No | 91 (23) | 9 (15) | |
| | No information given | 22 (6) | 1 (2) | |
| Q8_How would you treat a child (3 years and older) with a diffuse astrocytoma WHO grade II of the pons, H3K27 WILDTYPE, which fulfills clinical/radiological criteria of DIPG? | Not answered (% total HDI) | 23 (6) | 1 (2) | |
| | a. Like a low grade glioma | Yes 36 (10)<br>No 335 (90) | Yes 6 (10)<br>No (90) | .91 |
| | b. Like a diffuse midline glioma, H3k27M mutant | Yes 92 (25)<br>No 279 (75) | Yes 16 (27)<br>No 43 (73) | .70 |
| | c. Like other high grade gliomas, H3K27M | Yes 47 (13)<br>No 324 (87) | Yes 16 (27)<br>No 43 (73) | **.004** |
| | d. Individually, depending on other genetic findings including methylation | Yes 196 (53)<br>No 175 (47) | Yes 21 (36)<br>No 38 (64) | **.01** |
| Q9_Do you think there is a need to introduce a new tumour entity of "Diffuse midline glioma of the pons, H3K27 WILDTYPE (WHO grade IV)" with typical neuroradiological features of a DIPG? | Yes | 221 (56) | 42 (70) | .11 |
| | No | 152 (39) | 17 (28) | |
| | No information given | 21 (5) | 1 (2) | |
| Q10_Do you think there is a need to introduce a new tumour entity of "infantile glioma" for histologically diagnosed high grade gliomas in infants younger than 3 years? | Yes | 237 (60) | 44 (73) | .11 |
| | No | 147 (37) | 16 (27) | |
| | No information given | 10 (3) | 0 | |

**Appendix B.** Full Survey Results by HDI. Participant No. (% within HDI) *(continued)*

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q11_If you answered YES to the previous question, please specify why (multiple answers possible) | Not answered (% total HDI) | 152 (39) | 14 (23) | |
| | a. Prognosis is usually better | Yes 131 (54)<br>No 111 (46) | Yes 16 (35)<br>No 30 (65) | **.02** |
| | b. Genetic findings including methylation suggest a tumour entity of its own | Yes 151 (62)<br>No 91 (38) | Yes 30 (65)<br>No 16 (35) | .72 |
| | c. Therapy is usually from high grade gliomas of older children and adults | Yes 132 (55)<br>No 110 (45) | Yes 19 (41)<br>No 27 (59) | .10 |
| Q12_If you think that there is indeed a need for a new tumour entity of "infantile glioma" would you classify this new entity as; | Not answered (% total HDI) | 106 (27) | 10 (17) | |
| | a. WHO grade I | Yes 1 (.3)<br>No 287 (99) | Yes 0 (0)<br>No 50 (100) | 1.0 |
| | b. WHO grade II | Yes 10 (4)<br>No 278 (96) | Yes 3 (6)<br>No 47 (94) | .42 |
| | c. WHO grade III/IV (depending on histological grade like it is now) | Yes 66 (23)<br>No 222 (77) | Yes 18 (36)<br>No 32 (64) | **.05** |
| | d. Individually depending on genetic findings including methylation signature | Yes 154 (54)<br>No 134 (46) | Yes 16 (32)<br>No 34 (68) | **.01** |
| | e. Without a defined WHO grade | Yes 57 (20)<br>No 231 (80) | Yes 13 (26)<br>No 37 (74) | .32 |
| Q13_What do you think about routine analysis of IDH status in paediatric anaplastic astrocytoma and glioblastoma? | Not answered (% total HDI) | 2 (.5) | 0 | |
| | a. Not adequate because of low percentage (<10%) of IDH mutant paediatric HGG | Yes 46 (12)<br>No 346 (88) | Yes 26 (43)<br>No 34 (57) | **<.001** |
| | b. Obligatory for all cases | Yes 176 (45)<br>No 216 (55) | Yes 12 (20)<br>No 48 (80) | **<.001** |
| | c. Only if sufficient tumour material is available | Yes 81 (21)<br>No 311 (79) | Yes 12 (20)<br>No 48 (80) | .91 |
| | d. I don´t know | Yes 44 (11)<br>No 348 (89) | Yes 8 (13)<br>No 52 (87) | .63 |

**Appendix B.** Full Survey Results by HDI. Participant No. (% within HDI) *(continued)*

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q14_Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytoma and glioblastoma in children (3 years and older) and adolescents/young adults? | Yes | 268 (68) | 44 (73) | .63 |
| | No | 117 (30) | 16 (27) | |
| | No information given | 9 (2) | 0 | |
| Q15_If you answered YES to the previous question, please specify why (multiple answers possible) | Not answered (% total HDI) | 120 (30) | 14 (23) | |
| | a. Prognosis is usually better than in adults. | Yes 82 (30)<br>No 192 (70) | Yes 20 (44)<br>No 26 (56) | .07 |
| | b. Genetic findings including methylation suggest specific paediatric subtypes of anaplastic astrocytoma/ glioblastomas | Yes 246 (90)<br>No 28 (10) | Yes 34 (74)<br>No 12 (26) | **.003** |
| Q16_Do you think there is a need to introduce a new tumour entity of "Anaplastic pilocytic astrocytoma (WHO grade III)" or "Anaplastic astrocytoma with piloid features (WHO grade III)", respectively, for pilocytic astrocytoma with anaplastic features? | Yes | 245 (62) | 46 (77) | .08 |
| | No | 133 (34) | 12 (20) | |
| | No information given | 16 (4) | 2 (3) | |
| Q17_Do you think there is still a need for diagnosis of gliomatosis cerebri with typical neuroradiological features of diffuse growth pattern involving two and more cerebral lobes? | Yes | 224 (57) | 42 (70) | .17 |
| | No | 165 (42) | 18 (30) | |
| | No information given | 5 (1) | 0 | |

**Appendix B.** Full Survey Results by HDI. Participant No. (% within HDI) *(continued)*

| | | **Very High HDI No. (%) N=394** | **High and Medium HDI No. (%) N=60** | **P value** |
|---|---|---|---|---|
| Q18_If you answered YES to the previous question, please specify (multiple answers possible) | Not answered (% total HDI) | 159 (40) | 18 (30) | |
| | a. Diagnosis in the renaming of a SPECFIC PHENOTYPE of an underlying glioma. | Yes 134 (57)<br>No 101 (43) | Yes 16 (38)<br>No 26 (62) | **.02** |
| | b. Diagnosis in the renaming of a SPECIFIC TUMOUR SUBTYPE of its own for an underlying glioma histology | Yes 44 (19)<br>No 191 (81) | Yes 16 (38)<br>No 26 (62) | **.01** |
| | c. Diagnosis in the renaming of a TUMOUR ENTITY of its own independently of an underlying glioma | Yes 46 (20)<br>No 189 (80) | Yes 8 (19)<br>No 34 (81) | .94 |
| Q19_In summary, has the implementation of the revised WHO Classification caused any problems? | Yes | 220 (56) | 43 (72) | .07 |
| | No | 169 (43) | 17 (28) | |
| | No information given | 5 (1) | 0 | |
| Q20_If you answered YES to the previous question, please specify your relevant issues (multiple answers possible) | | | | |
| | Not answered (% total HDI) | 156 (40) | 15 (25) | |
| | a. Introduction of new tumour entities. | Yes 82 (35)<br>No 156 (65) | Yes 15 (33)<br>No 30 (67) | .89 |
| | b. Abolishment of tumour entities | Yes 70 (29)<br>No 168 (71) | Yes 11 (24)<br>No 34 (76) | .50 |
| | c. Renaming of tumour entities | Yes 77 (32)<br>No 161 (68) | Yes 16 (36)<br>No 29 (64) | .68 |
| | d. Insufficient diagnostic definitions of tumour entities. | Yes 108 (45)<br>No 130 (55) | Yes 16 (36)<br>No 29 (64) | .22 |
| | e. Diagnostic definitions are less relevant for paediatric than for adult neurooncology. | Yes 92 (39)<br>No 146 (61) | Yes 16 (36)<br>No 29 (64) | .70 |
| | f. Diagnostic definitions are sometimes hard to explain to patients/parents | Yes 72 (30)<br>No 166 (70) | Yes 17 (38)<br>No 28 (62) | .32 |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | G7 Count (%) N=261 | Non-G7 Count (%) N=193 | P value |
|---|---|---|---|---|
| Q1_Are you aware of the revision of the WHO Classification of Tumors of the Central Nervous System that occurred in 2016? | Yes | 246 (94) | 187 (97) | .19 |
| | No | 15 (6) | 6 (3) | |
| Q2_Do you use the revised WHO Classification in your daily practice? | Yes | 240 (92) | 181 (94) | .38 |
| | No | 19 (7) | 9 (5) | |
| | No information given | 2 (1) | 3 (1) | |
| Q3_Are you aware of the newly introduced tumour entity "diffuse midline glioma, H3K27M mutant (WHO grade IV)"? | Yes | 253 (97) | 183 (95) | .11 |
| | No | 6 (2) | 10 (5) | |
| | No information given | 2 (1) | 0 | |
| Q4_Do you use the diagnosis of diffuse midline glioma, H3K27M mutant? | Yes | 246 (94) | 160 (83) | **<.001** |
| | No | 13 (5) | 32 (17) | |
| | No information given | 2 | 1 | |
| Q5_Do you still prefer DIPG as radiological/clinical diagnosis instead of diffuse midline glioma, H3K27M mutant, when located within the pons? | Yes | 111 (42) | 101 (52) | **.05** |
| | No | 148 (57) | 92 (48) | |
| | No information given | 2 (1) | 0 | |
| Q6_If you answered YES to the previous question, please specify why or when you still use the term DIPG. (multiple answers possible) | Not answered (% total HDI) | 120 (46) | 70 (36) | |
| | a.DIPG is a well defined and established diagnosis/diagnostic term | Yes 62 (44) No 79 (56) | Yes 56 (45) No 67 (55) | .80 |
| | b.I use both terms depending on the respective context | Yes 89 (63) No 52 (37) | Yes 58 (47) No 65 (53) | **.01** |
| | c.Patients can better understand DIPG as diagnosis than diffuse midline glioma, H3K27M mutant | Yes 50 (35) No 91 (65) | Yes 31 (25) No 92 (75) | .07 |
| | d. Diffuse midline glioma, H3K27M mutant, does not cover all DIPG | Yes 45 (32) No 96 (68) | Yes 44 (36) No 79 (64) | .51 |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | **G7 Count (%) N=261** | **Non-G7 Count (%) N=193** | **P value** |
|---|---|---|---|---|
| Q7_Do you believe there is an entity of DIPG, H3K27 WILDTYPE, WHO IV? | Yes | 180 (69) | 151 (78) | **.03** |
| | No | 69 (26) | 31 (16) | |
| | No information given | 12 (5) | 11 (6) | |
| Q8_How would you treat a child (3 years and older) with a diffuse astrocytoma WHO grade II of the pons, H3K27 WILDTYPE, which fulfills clinical/radiological criteria of DIPG? | Not answered (% total HDI) | 16 (6) | 13 (7) | |
| | a. Like a low grade glioma | Yes 23 (9)<br>No 227 (91) | Yes 19 (11)<br>No 161 (89) | .64 |
| | b. Like a diffuse midline glioma, H3k27M mutant | Yes 70 (28)<br>No 180 (72) | Yes 38 (21)<br>No 142 (79) | .10 |
| | c. Like other high grade gliomas, H3K27M | Yes 27 (11)<br>No 223 (89) | Yes 36 (20)<br>No 144 (80) | **.01** |
| | d. Individually, depending on other genetic findings including methylation | Yes 130 (52)<br>No 120 (48) | Yes 87 (48)<br>No 93 (52) | .45 |
| Q9_Do you think there is a need to introduce a new tumour entity of "Diffuse midline glioma of the pons, H3K27 WILDTYPE (WHO grade IV)" with typical neuroradiological features of a DIPG? | Yes | 144 (55) | 119 (62) | .20 |
| | No | 106 (41) | 63 (33) | |
| | No information given | 11 (4) | 11 (5) | |
| Q10_Do you think there is a need to introduce a new tumour entity of "infantile glioma" for histologically diagnosed high grade gliomas in infants younger than 3 years? | Yes | 143 (55) | 138 (72) | **.001** |
| | No | 111 (42) | 52 (27) | |
| | No information given | 7 (3) | 3 (2) | |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | **G7 Count (%) N=261** | **Non-G7 Count (%) N=193** | **P value** |
|---|---|---|---|---|
| Q11_If you answered YES to the previous question, please specify why (multiple answers possible) | Not answered (% total HDI) | 114 (44) | 52 (27) | |
| | a. Prognosis is usually better | Yes 79 (54)<br>No 68 (46) | Yes 68 (48)<br>No 73 (52) | .35 |
| | b. Genetic findings including methylation suggest a tumour entity of its own | Yes 97 (66)<br>No 50 (34) | Yes 84 (60)<br>No 57 (40) | .26 |
| | c. Therapy is usually from high grade gliomas of older children and adults | Yes 81 (55)<br>No 66 (45) | Yes 70 (50)<br>No 71 (50) | .35 |
| Q12_If you think that there is indeed a need for a new tumour entity of "infantile glioma" would you classify this new entity as; | Not answered (% total HDI) | 78 (30) | 38 (20) | |
| | a. WHO grade I | Yes 1 (.5)<br>No 182 (99) | Yes 0<br>No 155(100) | .36 |
| | b. WHO grade II | Yes 6 (3)<br>No 177 (97) | Yes 7 (5)<br>No 148 (95) | .56 |
| | c. WHO grade III/ IV (depending on histological grade like it is now) | Yes 50 (27)<br>No 133 (73) | Yes 34 (22)<br>No 121 (78) | .25 |
| | d. Individually depending on genetic findings including methylation signature | Yes 94 (51)<br>No 89 (49) | Yes 76 (49)<br>No 79 (51) | .67 |
| | e. Without a defined WHO grade | Yes 32 (17)<br>No 151 (83) | Yes 38 (24)<br>No 117 (76) | .11 |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | **G7 Count (%) N=261** | **Non-G7 Count (%) N=193** | **P value** |
|---|---|---|---|---|
| Q13_What do you think about routine analysis of IDH status in paediatric anaplastic astrocytoma and glioblastoma? | Not answered (% total HDI) | 1 (.4) | 1 (5) | |
| | a. Not adequate because of low percentage (<10%) of IDH mutant paediatric HGG | Yes 27 (10)<br>No 233 (90) | Yes 45 (23)<br>No 147 (77) | **<.001** |
| | b. Obligatory for all cases | Yes 119 (46)<br>No 141 (54) | Yes 69 (36)<br>No 123 (64) | **.04** |
| | c. Only if sufficient tumour material is available | Yes 54 (21)<br>No 206 (79) | Yes 39 (20)<br>No 153 (80) | .91 |
| | d. I don´t know | Yes 28 (11)<br>No 232 (89) | Yes 24 (13)<br>No 168 (87) | .60 |
| Q14_Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytoma and glioblastoma in children (3 years and older) and adolescents/young adults? | Yes | 174 (67) | 138 (71) | .30 |
| | No | 83 (32) | 50 (26) | |
| | No information given | 4 (2) | 5 (3) | |
| Q15_If you answered YES to the previous question, please specify why (multiple answers possible) | Not answered (% total HDI) | 83 (32) | 51 (26) | |
| | a. Prognosis is usually better than in adults. | Yes 58 (33)<br>No 120 (67) | Yes 44 (31)<br>No 98 (69) | .76 |
| | b. Genetic findings including methylation suggest specific paediatric subtypes of anaplastic astrocytoma/ glioblastomas | Yes 158 (89)<br>No 20 (11) | Yes 122(86)<br>No 20 (14) | .44 |
| Q16_Do you think there is a need to introduce a new tumour entity of "Anaplastic pilocytic astrocytoma (WHO grade III)" or "Anaplastic astrocytoma with piloid features (WHO grade III)", respectively, for pilocytic astrocytoma with anaplastic features? | Yes | 165 (63) | 126 (65) | .65 |
| | No | 87 (33) | 58 (30) | |
| | No information given | 9 (4) | 9 (5) | |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | **G7 Count (%) N=261** | **Non-G7 Count (%) N=193** | **P value** |
|---|---|---|---|---|
| Q17_Do you think there is still a need for diagnosis of gliomatosis cerebri with typical neuroradiological features of diffuse growth pattern involving two and more cerebral lobes? | Yes | 140 (54) | 126 (65) | **.004** |
| | No | 120 (46) | 63 (33) | |
| | No information given | 1 | 4 (2) | |
| Q18A_If you answered YES to the previous question, please specify (multiple answers possible) | Not answered (% total HDI) | 112 (43) | 65 (34) | |
| | a. Diagnosis in the renaming of a SPECFIC PHENOTYPE of an underlying glioma. | Yes 85 (57)<br>No 64 (43) | Yes 65 (51)<br>No 63 (49) | .30 |
| | b. Diagnosis in the renaming of a SPECIFIC TUMOUR SUBTYPE of its own for an underlying glioma histology | Yes 29 (19)<br>No 120 (81) | Yes 31 (24)<br>No 97 (76) | .34 |
| | c. Diagnosis in the renaming of a TUMOUR ENTITY of its own independently of an underlying glioma | Yes 25 (17)<br>No 124 (83) | Yes 29 (23)<br>No 99 (77) | .22 |
| Q19_In summary, has the implementation of the revised WHO Classification caused any problems? | Yes | 146 (56) | 117 (61) | .58 |
| | No | 112 (43) | 74 (38) | |
| | No information given | 3 (1) | 2 (1) | |

**Appendix C.** Full Survey Results G7 vs Non-G7. Participant No. (% within HDI) *(continued)*

| | | **G7 Count (%) N=261** | **Non-G7 Count (%) N=193** | **P value** |
|---|---|---|---|---|
| Q20_If you answered YES to the previous question, please specify your relevant issues (multiple answers possible) | | | | |
| | Not answered (% total HDI) | 105 (40) | 66 (34) | |
| | a. Introduction of new tumour entities. | Yes 45 (29)<br>No 111 (71) | Yes 52 (41)<br>No 75 (59) | **.03** |
| | b. Abolishment of tumour entities | Yes 49 (31)<br>No 107 (69) | Yes 32 (25)<br>No 95 (75) | .25 |
| | c. Renaming of tumour entities | Yes 47 (30)<br>No 109 (70) | Yes 46 (36)<br>No 81 (64) | .28 |
| | d. Insufficient diagnostic definitions of tumour entities. | Yes 71 (45)<br>No 85 (55) | Yes 53 (42)<br>No 74 (58) | .52 |
| | e. Diagnostic definitions are less relevant for paediatric than for adult neurooncology. | Yes 56 (36)<br>No 100 (64) | Yes 52 (41)<br>No 75 (59) | .40 |
| | f. diagnostic definitions are sometimes hard to explain to patients/ parents | Yes 54 (35)<br>No 102 (65) | Yes 35 (28)<br>No 92 (72) | .20 |

# 5

# Pediatric High-Grade Gliomas and the WHO CNS Tumor Classification - Perspectives of Pediatric Neuro-oncologists and Neuropathologists in Light of Recent Updates

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## ABSTRACT

**Background:** The WHO Classification of Tumors of the Central Nervous System has undergone major restructuring. Molecularly defined diagnostic criteria were introduced in 2016 (revised 4th edition) and expanded in 2021 (5th edition) to incorporate further essential diagnostic molecular parameters. We investigated potential differences between specialists in perception of these molecularly defined subtypes for pediatric high-grade gliomas (pedHGG).

**Methods:** We designed a 22-question survey studying the impact of the revised 4th edition of the WHO classification on pedHGG. Data were collected and statistically analyzed to examine the spectrum of viewpoints and possible differences between neuro-oncologists and neuropathologists.

**Results:** 465 participants from 53 countries were included; 187 pediatric neuro-oncologists (40%), 160 neuropathologists (34%) and 118 additional experts (26%). Neuro-oncologists reported issues with the introduction of molecularly defined tumor types, as well as the abolishment or renaming of established tumor entities, while neuropathologists did not to the same extent. Both groups indicated less relevant or insufficient diagnostic definitions were available in 2016. Reported issues were classified and assessed in the 2021 WHO classification and a substantial improvement was perceived. However, issues of high clinical relevance remain to be addressed, including the definition of clinical phenotypes for diffuse intrinsic pontine glioma and gliomatosis cerebri.

**Conclusions:** Within the WHO classification of pediatric brain tumors, such as pedHGG, rapid changes in molecular characterization have been introduced. This study highlights the ongoing need for cross talk between pathologist and oncologist to advance the classification of pedHGG subtypes and ensure biological relevance and clinical impact.

## INTRODUCTION

The 5th edition of the WHO Classification of Tumors of the Central Nervous System (CNS5) is now available[1], and its summary has been published.[2] The new edition further increases the role of molecular diagnostics for some CNS tumor types, initialized in the revised 4th edition (CNS4).[3] For pediatric high-grade glioma (pedHGG) in particular, major changes were implemented following advances in the understanding of genomic and epigenomic landscapes, including the discovery of histone H3 mutations.[4,5] In 2016, based on several biopsy studies, the diagnosis of diffuse intrinsic pontine glioma (DIPG), a primarily neuroradiological characterized entity until that point, was molecularly defined as diffuse midline glioma (DMG), H3K27M-mutant. In 2021, this tumor type was expanded to DMG H3K27-altered[2,3], such that H3K27-wildtype DMGs display (like H3K27M-mutant DMGs), loss of H3K27 trimethylation, but carry other underlying molecular events than K27M mutations.[6,7] Such rapid reclassification and fundamental changes in nomenclature have resulted in debates between clinicians and pathologists with regard to the implementation of the WHO classification and its impact on diagnostics and treatment of pedHGG patients in daily routine.

The CNS5 (2021) is a substantial refinement of the revised CNS4 (2016). It was generated over the last three years after extensive evaluation of the current status by an expert panel, 'cIMPACT-NOW' (Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy – Not Officially WHO).[8-14] General feedback from the greater neuro-oncology community on the introduction of molecular diagnoses however is still missing. We therefore conducted a worldwide survey among specialists involved in the diagnosis and therapy of pediatric brain tumors. The survey was created by largely focusing on the CNS4-related issues that were brought up during meetings of the European Society for Paediatric Oncology High Grade Glioma Working Group (SIOPE HGG WG) following the publication of CNS4. The main issues identified by the SIOPE HGG WG were: the introduction of molecularly vs clinically defined DMG and the issue that other pedHGG tumor (sub)types had not been adequately addressed. Furthermore, issues were raised by the SIOPE HGG WG about access to technology and socioeconomic factors involved in molecular diagnostics in pedHGG. These issues have already been covered separately.[15] Here, we bring into focus the clinical and tissue-based diagnostic issues by a comparison of the different perceptions and experiences of pediatric neuro-oncologists and neuropathologists on this subject, providing a representative overview for the specific needs with regard to pedHGG management and WHO classification. Since CNS5 was published in the meantime with further major changes for pedHGG tumor subtypes, we were able to assess and discuss if and how the various issues raised with our survey have been addressed in this update.

## METHODS

The survey was designed and pretested by the European Society for Paediatric Oncology High Grade Glioma Working Group (SIOPE HGG WG). An online version of the survey was created using SurveyMonkey® (San Mateo, Ca, USA). Addressees of this survey study were primarily neuro-pathologists, pediatric neuro-oncologists, neurosurgeons, radiation oncologists, neuroradiologists and other professionals in the field of pediatric neuro-oncology between March 22 and May 8, 2019. These professionals were identified using contact lists from a prior international survey within the International Society of Neuropathology (ISN)[16], from the SIOPE Brain Tumour Group, the German Society of Pediatric Oncology and Hematology (GPOH), the German Neuro-oncology Working Group (NOA), the German Society of Neuropathology and Neuroanatomy (DGNN), as well as other international collaborators in the field of pediatric neuro-oncology. Multiple replies from the same IP and/or email address were excluded.

The survey consisted of twenty-two questions, twelve "Yes" or "No" questions, eight multiple choice questions, and two demographic questions. Within each thematic section we identified one key question. Respondents who failed to answer four out of six predefined key questions (including questions 1, 3, 10, 14, 16 and 17) were excluded. All key questions were dichotomous, "Yes or No". Key questions covered subjects including *(i)* awareness of the revised 2016 WHO classification, *(ii)* awareness of the newly introduced entity diffuse midline glioma (DMG), H3K27M mutant, *(iii)* opinions on the upcoming 5$^{th}$ WHO classification regarding introducing infantile glioma, *(iv)* introducing pediatric subtypes for anaplastic astrocytoma and glioblastoma, *(v)* introducing anaplastic pilocytic astrocytoma grade III and, *(vi)* removing gliomatosis cerebri (see Appendix A). Inclusion and exclusion criteria of respondents for survey analysis were consistent with methods used in Baugh et al.[15] Data were analysed using Pearson's Chi-square and Fisher's Exact Test in IBM SPSS Statistics version 26 (Armonk, NY, USA). Research involving human subjects according to the World Medical Association Declaration of Helsinki did not apply, thus ethics approval was not required for this study. Independent professionals, no patients, were asked for voluntary participation. No personal identifying data were collected and participation did not involve any advantage, disadvantage or any potential harm.

## RESULTS

The survey was completed by 482 participants, of whom 17 (4%) were excluded for not completing the predefined minimum key questions as outlined above. Participants included 187 pediatric neuro-oncologists (40%), 160 neuropathologists (34%) and 118 (26%) other specialists in the field. The latter group included 45 neuroradiologists (10%), 29 radiation oncologists (6%), 20 neurosurgeons (4%), eight adult neuro-oncologists (2%), seven scientists (2%), and nine non-specified specialists (2%). Geographically,

most participants were from Europe (n=291; 62%), followed by North America (n=59; 13%), Asia (n=49; 11%), Latin America (n=36; 8%), Oceania (n=11; 2%), and Africa (n=8; 2%), 11 (2%) respondents could not be geographically allocated (Table 1). In total, 53 different countries were represented in the survey.

Key issues from five specific pedHGG areas from the CNS4, defined by the SIOPE HGG WG, were surveyed: I) DMG/DIPG, II) infantile glioma (referred to as "infant-type hemispheric glioma" in CNS5), III) specific (diffuse) pedHGG subtypes, IV) anaplastic pilocytic astrocytoma WHO grade III, and V) gliomatosis cerebri. Results from questions representing these key areas are displayed in Figure 1. Participating pediatric neuro-oncologists more often prefer using the diagnosis of DIPG than neuropathologists, i.e., 72% vs 15% respectively (survey question 5, p <.001; Figure 1a.). When further asked why and when one would still use the term DIPG, most oncologists stated using both terms, DIPG and DMG, depending on context (survey question 6, answer b) while, interestingly, the majority of pathologists still agreed that "Diffuse midline glioma, H3K27M mutant, does not cover all DIPG" (survey question 6, answer d).

On the need for introduction of infantile (high-grade) glioma as a new tumor entity, 75% of pediatric neuro-oncologists were in support, in comparison to 51% of neuropathologists (survey question 10, p .002; Figure 1b.). Argumentation for introducing infantile glioma varied, as neuropathologists indicated that "genetic findings including methylation suggest a tumor entity of its own" (survey question 11, answer b), whereas oncologists argued that "therapy is usually different from high-grade gliomas of older children and adults" (survey question 11, answer c). The term "infantile hemispheric glioma" corresponds to a DNA methylation class. The corresponding tumor type was finally named "infant-type hemispheric glioma" in the WHO CNS5.

Concerning specific pediatric high-grade glioma subtypes (distinct from adult high-grade glioma) and the presumed need to introduce a subtype for anaplastic astrocytoma and glioblastoma in children (3 years and older), both specialties were in support, 68% and 72% of oncologists and pathologists, respectively (survey question 14, p .237; Figure 1c.). Agreement was also found on the reasoning, with a majority from each group selecting "genetic findings including methylation suggest specific pediatric subtypes of anaplastic astrocytomas/glioblastomas" (survey question 15, answer b). However, regarding adding a new tumor type for "anaplastic pilocytic astrocytoma, WHO grade III", pathologists were more in favor with 76% in support, in comparison to 56% oncologists (survey question 16, p .001; Figure 1d).

On the topic of a diagnosis for gliomatosis cerebri, neuro-oncologists were more in support than neuropathologists, i.e., 72% vs 41% respectively (survey question 17, p <.001; Figure 1e). The majority in support of the diagnosis from both groups selected their reasoning as, "diagnosis for a specific phenotype of an underlying glioma, but not as a tumor subtype or entity of its own" (survey question 18, answer a).

**Table 1.** Survey participants by specialization and location.

| Specialty No. (%) | Africa | Asia | Europe | Latin America | North America | Oceania | Not Specified | Total |
|---|---|---|---|---|---|---|---|---|
| Neuro-oncologists | 3 | 12 | 121 | 12 | 27 | 6 | 6 | **187** (40%) |
| Neuropathologists | 4 | 21 | 91 | 16 | 25 | 3 | 0 | **160** (34%) |
| Other[a] | 1 | 16 | 79 | 8 | 7 | 2 | 5 | **118** (26%) |
| **Total** | **8** (2%) | **49** (11%) | **291** (62%) | **36** (8%) | **59** (13%) | **11** (2%) | **11** (2%) | **465** |

[a]Neurosurgeons, radiation oncologist, neuroradiologists, adult neurooncologists, scientists, and not specified.

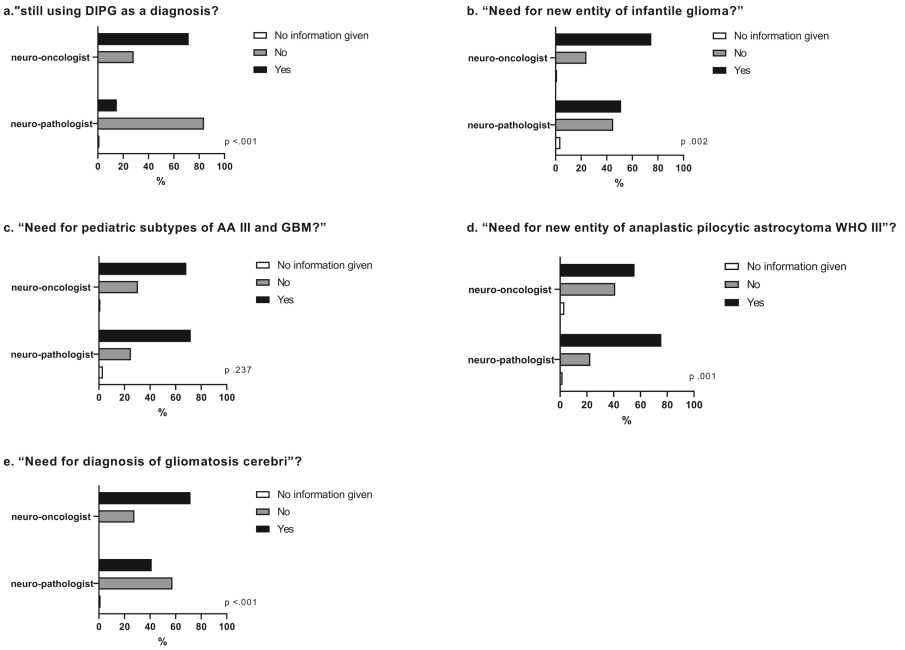
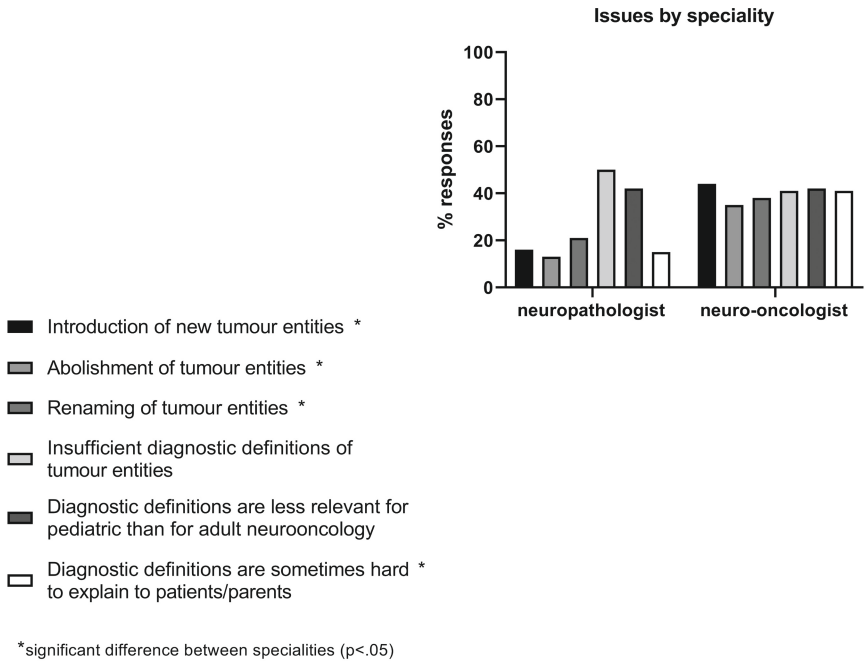



**Figure 1.** Participant feedback on key questions regarding the revised CNS4.

Finally, overall experiences with the revised 4th edition was collected from 57% of all participants, who reported having issues with the classification. The specific issues surveyed are displayed in Figure 2. Neuro-oncologists significantly more often stated that "the introduction of new tumor entities" caused issues, 44% vs 16% of neuropathologists (survey question 20, answer a; p<.001), followed by difficulty

with “the abolishment of tumor entities”, 35% vs 13% (survey question 20, answer b; p<.001), and the “renaming of tumor entities”, 38% vs 21% (survey question 20, answer c; p .004). Neuro-oncologists also reported that “diagnostic definitions are sometimes hard to explain to patients/parents”, 41% vs 15% (survey question 20, answer f; p<.001). Feedback was not significantly different on the topics including; “insufficient diagnostic definitions of tumor entities”, 50% of pathologist in support and oncologists 41% (survey question 20, answer d; p .20), and lastly, for “diagnostic definitions are less relevant for pediatric than for adult neuro-oncology”, 42% for both groups (survey question 20, answer e; p .90) (Figure 2).

**Figure 2.** Participant feedback on issues with the revised CNS4.




## DISCUSSION

Five editions of the WHO CNS Tumor Classification are now available, with the first edition published in 1979.[17] Later editions followed in 1993, 2000, 2007, 2016 and 2021.[18-20, 1] The pace of discoveries in recent decades has greatly improved our understanding of pediatric brain tumor pathogenesis. This has led to the invention, reinvention, and fine-tuning of a classification system that is now largely based on molecular genetics. The 2016 revised 4th edition was the first large scale molecular restructuring of the WHO CNS Tumor classification, with the introduction of an “integrated approach” utilizing both

pheno- and genotype.[3] This new approach affected diffuse glioma as well as embryonal tumors. This system was devised from the ISN-Haarlem Consensus Guidelines in 2014. During development, particular focus was placed on balancing molecular advances with practical issues arising from molecular classifications being incorporated into patient management and diagnosis.[21]

Our present study, underlines the ongoing need to balance molecular advances with meaningful clinical impact in pedHGG. Here, we compared the respective perspectives of the two key players at both ends of this balance, i.e. the neuropathologists as representatives for the focus on the scientific state of the art diagnostics, and the pediatric neuro-oncologists with their special focus on clinical needs. Among the participating specialists, particularly neuro-oncologists reported having issues with the introduction of new tumor types, renaming or abolishment of established tumor types, while neuropathologists did not. Neuro-oncologists also cited diagnostic definitions being difficult to explain to patients and families. Neuro-oncologists and neuropathologists however agreed on the points that insufficient diagnostic definitions were available for molecular-based entities in 2016 and that these entities were less relevant for pediatric cases (Figure 2).

Interestingly, many of the issues raised in our survey are mirrored by the changes made in the 2021 CNS5. In 2016 CNS4, some arguably clinically relevant pedHGG tumor types like non-diffuse pilocytic astrocytoma, IDH-wildtype diffuse pedHGG and diffuse pedHGG in infants younger than 3 years of age were not included, but are now specifically addressed (Table 2). “Entities” not included in the CNS5, DIPG and gliomatosis cerebri, are both imaging-defined. In our survey, generally more pathologists accepted the removal of the designation “gliomatosis cerebri“ than oncologists. This was also the case with DIPG. Neuro-oncologists were in favor of re-establishing the option of the previous clinical radiological diagnosis of DIPG, in addition to the sole option of setting the DMG diagnosis by biopsy only. It should be noted that in the CNS5, DIPG is listed in a new section entitled ‘related terminology’, as an acceptable definition.

For DIPG/DMG, there remains no curative treatment approach with radiation as the palliative therapeutic mainstay. Prognostic differences within DMG subtypes have emerged, with H3.1 K27M-mutant tumors conferring a relative survival advantage over H3.3 K27M-mutant and H3K27-wildtype tumors.[22] However, outcomes remain universally poor with an 11-month median overall survival.[23] Tumor subtyping requires a biopsy to be performed in specialized centers, and preferably in the context of clinical trials, given targeted therapies are purely investigative at this point.[24] Moreover, imaging exams are also generally more available to clinicians than to pathologists, forming a routine part of their clinical decision making. Oncologists will at some point find themselves in the situation where a treatment decision needs to be made, and if no

definite molecular-based diagnosis could be rendered, at least an imperfect surrogate (i.e. imaging) can support decision making.

Why imaging defined tumor types like DIPG are not incorporated in the CNS5 is based on the decision that the WHO classification follows a tissue-based approach. When molecular analysis could not (or not successfully) be performed and therefore diagnosis is histology-based only, the classification system advises to add the term "NOS" (not otherwise specified). Imperfect surrogates to molecular classification are required particularly in the context of no biopsy and/or when advanced molecular analyses are not possible. In such a situation for DIPG, a limited immunohistochemistry (IHC) stain for mutant H3 K27M protein or loss of H3 K27 trimethylation can be performed. IHC demonstration of loss of H3 K27 trimethylation may also enable detection of the newly introduced CNS5 DMG diagnoses, with wildtype H3 K27 and absent H3 K27 trimethylation associated with EZHIP protein overexpression and/or EGFR alterations.[6] IHC staining for H3 K27 trimethylation and H3 K27M appears sufficiently indicative in comparison to molecular sequencing, beyond it is cost-effective and efficient.[25]

**Table 2.** Comparisons Between Participant Feedback on the Revised CNS4 in 2016 and Changes Implemented in the CNS5 in 2021

| **Paediatric HGG WHO 2016** | **Relevant survey questions addressing the issue** | **Problem confirmed by survey results** | **Addressed by WHO 2021?** | **Pediatric HGG WHO 2021** |
|---|---|---|---|---|
| 1. Diffuse midline glioma, H3K27M mutant<br>2. DIPG removed as neuroradiological diagnosis | Neuroradiologically defined DIPG diagnosis still needed? | Yes: 46.9%<br>No: 52.7% | No | No change |
| | H3 wildtype DIPG with poor prognosis as own subtype needed? | Yes: 73.3%<br>No: 21.5% | Yes | Two subtypes of DMG, H3 wildtype with loss of H3K27 trimethylation defined:<br>DMG, EZHIP overexpressed<br>DMG, EGFR mutant |

**Table 2.** Comparisons Between Participant Feedback on the Revised CNS4 in 2016 and Changes Implemented in the CNS5 in 2021 *(continued)*

| Paediatric HGG WHO 2016 | Relevant survey questions addressing the issue | Problem confirmed by survey results | Addressed by WHO 2021? | Pediatric HGG WHO 2021 |
|---|---|---|---|---|
| Anaplastic astrocytoma, IDH wildtype and Glioblastoma, IDH wildtype | Pediatric subtypes of anaplastic astrocytoma and glioblastoma needed? | Yes: 68.6%<br>No: 29.5% | Yes | Two new entities of pediatric diffuse high grade glioma: Diffuse pediatric high grade glioma, IDH/H3 wildtype<br>Diffuse hemispheric glioma, H3.3G34 mutant |
| | New entity "infantile glioma" for high grade gliomas in infants < 3 years needed? | Yes: 61.7%<br>No: 35.9% | Yes | Infant-type hemispheric glioma as new entity of diffuse high grade glioma in infants |
| Pilocytic astrocytoma with anaplastic features analogous to WHO III | "Anaplastic pilocytic astrocytoma WHO III" needed? | Yes: 63.4%<br>No: 32.3% | Yes | Pilocytic astrocytoma with anaplasia is still present. The new entity "high grade astrocytoma with piloid features" does not represent the pediatric anaplastic pilocytic astrocytoma |
| Gliomatosis cerebri removed as a neuroradiological diagnosis | Neuroradiological defined diagnosis of gliomatosis cerebri still needed? | Yes: 58.7%<br>No: 40.0% | No | No change |

For less advanced national health systems where molecular analyses may not be available, the clinical radiological diagnosis of DIPG, as performed for more than 20 years, represents an affordable and clinically meaningful surrogate test for the diagnosis of pontine DMG.[15] This consideration is supported by a lack of effective therapies available, based on the presence of H3 K27M mutation. And, when there are H3 K27M-specific therapies in future, clinical radiological diagnosis of DIPG would still include most, if not all H3 K27M mutant DIPG.[26] Furthermore, it remains unclear if all DIPG diagnosed by clinical radiological criteria are indeed sufficiently covered by the CNS5 diagnoses of DMG. According to von Bueren et al., up to 15% of DIPGs display H3 K27 wildtype, with a similarly poor prognosis as H3.3 K27M mutant DIPG. [27] By now, it remains speculative if these 15% of DIPG are all characterized by loss of H3K27 trimethylation and really fitting into the present range of DMG, H3K27-altered. If neuroradiologically defined DIPG with a similarly poor prognosis of DMG are indeed not fully covered by CNS5, then the consideration of introducing an additional neuroradiological layer for WHO CNS tumor classification might be helpful in future.[26]

The tension between clinical relevance and keeping pace with advances in science and technology has been evident in the development of prior versions of the WHO CNS Tumor Classifications. The WHO grade I-IV system for CNS tumors for example was controversial at the time of development. Derived in the concept of "clinical malignancy", it sought to associate meaningful clinical prognosis, with histologic parameters. This numeric grading was seen as imperfect and of limited utility by some contributors, yet in practice verbal grading was already being carried out, necessitating a formalized grading system. [28] The challenge today to correlate molecular findings with meaningful clinical significance is much the same. It is well demonstrated that genotype and epigenetics are of clinical significance in pediatric high-grade glioma, but should not eliminate clinical phenotyping, as both provide relevant complementary information. For example, meaningful new predictors in the future could include information about immune status or tumor microenvironment, when single cell sequencing or liquid biopsies are more commonly performed.

Future research will surely help discern whether clinical correlates with biology result in improved therapeutic response and outcome and inform new iterations of the WHO CNS Tumor Classification. Increased multidisciplinary representation within working groups such as the cIMPACT-NOW, with more neuro-oncologists, neuroradiologists, and others involved in the treatment of brain tumor patients could help improve clinical translation. Importantly, representation from countries with a limited access to molecular diagnostics can help inform adaptation of the WHO CNS tumor classification to resource-limited settings. Furthermore, inclusion of patients from sites in middle and low income countries will be required to enable robust and powered clinical trials utilizing stratification by pediatric tumor subtype.[29] Without inclusion of these patients into large international trials, there is a concern that clinical studies will be hindered by

too small biological groups.[30] The challenge remains to improve molecular diagnostic capabilities within low resourced settings and in turn improve the applicability of the WHO classification for CNS tumors.

## CONCLUSIONS

In the quest to classify pediatric high-grade gliomas utilizing the most up to date research, the WHO CNS classification has made substantive improvements in incorporating molecular information into the diagnosis of several tumor types. Our study underlines the ongoing need to balance advances in the understanding of the biology of CNS tumors with meaningful clinical impact, but also reassures the substantial improvement for definition and diagnostics of pedHGG within the latest WHO classification. Many points of criticism in the revised CNS4 have been addressed in CNS5. Nevertheless, upcoming WHO CNS Tumor Classifications should continuously work towards improved molecular stratification with a meaningful emphasis on clinical pathological correlation in a multidisciplinary fashion.

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# SUPPLEMENTARY DATA

## Appendix A. Full Length Survey

**Pediatric HGG and YOUR experience with the revised WHO classification**

1. Are you aware of the revision of the WHO Classification of Tumours of the Central Nervous System that occurred in 2016?
*If you are a neuropathologist who needs to work with the revised classification please don't feel offended and*
*continue :) ...*

- No
- Yes

2. Do you use the revised WHO Classification in your daily practice?

- No
- Yes

3. Are you aware of the newly introduced tumour entity "diffuse midline glioma, H3K27M mutant (WHO grade IV)"?

- No
- Yes

4. Do you use the diagnosis of diffuse midline glioma, H3K27M mutant?

- No
- Yes

5. Do you still prefer DIPG ("diffuse intrinsic pontine glioma") as neuroradiological/clinical diagnosis instead of diffuse midline glioma, H3K27M mutant, when located within the pons?

- No
- Yes

6. **If you answered YES to the previous question** (*"Do you still prefer DIPG as neuroradiological/clinical diagnosis instead of diffuse midline glioma ...?"*), please specify why or when you still use the term DIPG (several answers are possible):

- DIPG is a well defined and established diagnosis/diagnostic term
- I use both terms depending on the respective context
- Patients can better understand DIPG as diagnosis than diffuse midline glioma, H3K27M mutant
- Diffuse midline glioma, H3K27M mutant, does not cover all DIPG
- Any other answer?_____

7. Do you believe there is an entity of DIPG, **H3K27 WILDTYPE**, WHO IV?
- No
- Yes

8. How would you treat a child (3 years and older) with a diffuse astrocytoma WHO grade II of the pons, **H3K27 WILDTYPE**, which fulfils clinical/neuroradiological criteria of DIPG?
- Like a low grade glioma
- Like a diffuse midline glioma, H3K27M mutant
- If using different protocols for diffuse midline gliomas, H3K27M mutant, and other high grade gliomas: Like other high grade
- gliomas
- Individually, depending on other genetic findings including methylation

9. Do you think there is a need to introduce a new tumour entity of **"Diffuse midline glioma of the pons, H3K27 WILDTYPE (WHO grade IV)**" with typical neuroradiological features of a DIPG?
- No
- Yes



10. Do you think there is a need to introduce a new tumour entity of "infantile glioma" for histologically diagnosed high grade gliomas in infants younger than 3 years?
- No
- Yes

11. **If you answered YES to the previous question** (*"Do you think there is a need to introduce a new tumour entity of "infantile glioma" …"*), please specify why (several answers are possible):
- Prognosis is usually significantly better
- Genetic findings including methylation suggest a tumour entity of its own
- Therapy is usually different from high grade gliomas of older children and adults
- Any other reason? _____

12. If you think that there is indeed a need for a new tumour entity of "infantile glioma" would you classify this new entity as
- WHO grade I
- WHO grade II
- WHO grade III/IV (depending on histological grade like it is now)
- Individually depending on genetic findings including methylation signature
- Without a defined WHO grade

13. What do you think about routine analysis of IDH status in paediatric anaplastic astrocytomas and glioblastomas?
- Not adequate because of low percentage (<10%) of IDH mutant paediatric HGG
- Obligatory for all cases
- Only if sufficient tumor material is available
- I don´t know
- Any other comment? _____

14. Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytomas and glioblastomas in children (3 years and older) and adolescents/young adults?
- No
- Yes

15. **If you answered YES to the previous question** (*"Do you think there is a need to introduce new "paediatric subtypes" for anaplastic astrocytomas and glioblastomas ..."*), please specify why (several answers are possible):
- Prognosis is usually better than in older adults
- Genetic findings including methylation suggest specific paediatric subtypes of anaplastic astrocytomas/glioblastomas
- Any other reason? _____

16. Do you think there is a need to introduce a new tumour entity of "Anaplastic pilocytic astrocytoma (WHO grade III)" or "Anaplastic astrocytoma with piloid features (WHO grade III)", respectively, for pilocytic astrocytomas with anaplastic features?
- No
- Yes

17. Do you think there is still a need for diagnosis of gliomatosis cerebri with typical neuroradiologcal features of diffuse growth pattern involving two and more cerebral lobes ?
- No
- Yes

18. **If you answered YES to the previous question** (*"Do you think there is still a need for diagnosis of gliomatosis cerebri ..."*), please specify (several answers are possible):
- Diagnosis in the meaning of a SPECIFIC PHENOTYPE of an underlying glioma, but not as a tumour subtype or entity of its own
- Diagnosis in the meaning of a SPECIFIC TUMOUR SUBTYPE of its own for an underlying glioma histology
- Diagnosis in the meaning of a TUMOUR ENTITY of its own independently of an underlying glioma histology

- Any other suggestions? _____

19. In summary, has the implementation of the revised WHO Classification caused any problems?
- No
- Yes

20. **If you answered YES to the previous question** (*"In summary, has the implementation of the revised WHO Classification caused any problems?"*), please specify your relevant issues (several answers are possible):
- Introduction of new tumour entities
- Abolishment of tumour entities
- Renaming of tumour entities
- Insufficient diagnostic definitions of tumour entities
- Diagnostic definitions are less relevant for pediatric than for adult neurooncology
- Diagnostic definitions are sometimes hard to explain to patients/parents
- Any other problems? _____

21. What is your field of expertise?
- Paediatric Oncologist/Paediatric Neurooncologist
- Neuropathologist
- Neurosurgeon
- Radiotherapist
- Radiologist/Neuroradiologist
- Scientist/Biologist/Physician Scientist
- Any other field of expertise? _____

22. In which country are you working?
and_____ YOUR experience

# 6

# Clinical, radiological, and 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

### *Purpose*

Diffuse intrinsic pontine glioma (DIPG) is a brainstem malignancy with a median survival of < 1 year. The International and European Society for Pediatric Oncology DIPG Registries collaborated to compare clinical, radiologic, and histomolecular characteristics between short-term survivors (STSs) and long-term survivors (LTSs).

### *Materials and Methods*

Data abstracted from registry databases included patients from North America, Australia, Germany, Austria, Switzerland, the Netherlands, Italy, France, the United Kingdom, and Croatia.

### *Results*

Among 1,130 pediatric and young adults with radiographically confirmed DIPG, 122 (11%) were excluded. Of the 1,008 remaining patients, 101 (10%) were LTSs (survival ≥ 2 years). Median survival time was 11 months (interquartile range, 7.5 to 16 months), and 1-, 2-, 3-, 4-, and 5-year survival rates were 42.3%(95%CI, 38.1%to 44.1%), 9.6%(95% CI, 7.8% to 11.3%), 4.3% (95% CI, 3.2% to 5.8%), 3.2% (95% CI, 2.4%to 4.6%), and 2.2% (95%CI, 1.4%to 3.4%), respectively. LTSs, compared with STSs, more commonly presented at age <3 or >10 years (11%v 3% and 33% v 23%, respectively; P <.001) and with longer symptom duration (P <.001). STSs, compared with LTSs, more commonly presented with cranial nerve palsy (83%v 73%, respectively; P = .008), ring enhancement (38% v23%, respectively; P = .007), necrosis (42%v 26%, respectively; P = .009), and extrapontine extension (92%v 86%, respectively; P = .04). LTSs more commonly received systemic therapy at diagnosis (88% v 75% for STSs; P = .005). Biopsies and autopsies were performed in 299 patients (30%) and 77 patients (10%), respectively; 181 tumors (48%) were molecularly characterized. LTSs were more likely to harbor a HIST1H3B mutation (odds ratio, 1.28; 95% CI, 1.1 to 1.5; P = .002).

### *Conclusion*

We report clinical, radiologic, and molecular factors that correlate with survival in children and young adults with DIPG, which are important for risk stratification in future clinical trials.

## INTRODUCTION

Diffuse intrinsic pontine glioma (DIPG) is a malignant brainstem tumor of childhood for which median survival is < 1 year.[1] Longterm survival, historically defined as overall survival (OS) >2

years, is reported in <10% of patients.[1] Characteristics associated with longer survival include younger age, longer symptom latency, and absent ring enhancement on diagnostic magnetic resonance imaging.[1,2] Up to 90% of DIPGs harbor a pathognomonic point mutation in H3F3A (65% of tumors) or HIST1H3B (25% of tumors); the latter seems 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 multinational collaboration between the International DIPG Registry (IDIPGR) and European Society for Pediatric Oncology DIPG Registry (SIOPE-DIPGR) [6,7] was to define clinical, radiologic, histologic, and molecular factors associated with short- and long-term survival in the largest cohort of centrally reviewed DIPGs to date.



## MATERIALS AND 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 radiographically confirmed DIPG diagnosed from 1990 to 2015. IDIPGR patients (n = 409) were age 0 to 27 years from the United States, Canada, and Australia. SIOPE-DIPGR patients (n = 721) were age 0 to 21 years from the Netherlands, Germany, Austria, Switzerland, Italy, France, the United Kingdom, and Croatia. Patients were referred to the registries as previously described.[6,7] Exclusion criteria are listed in Figure 1. No patients with neurofibromatosis type 1 were included.

### *Clinical Variables*

Clinical data were abstracted (J.B., B.C., S.E.M.V.v.Z., and N.C.) using standardized case report forms. Cerebellar signs included dysmetria, ataxia, dysarthria, or nystagmus. Pyramidal tract signs included mono-, hemi-, or quadriparesis; hyperreflexia; or positive Babinski sign. Because over survival (OS), defined as the time from diagnosis

to death or last follow-up, is regarded as the most reliable outcome variable for DIPG, progression-free survival (PFS) was not reported. Short-term survivors (STSs), long-term survivors (LTSs), and very long–term survivors (VLTSs) had OS times of <24, ≥ 24, and ≥ 60 months, respectively. Two LTSs (patients DIPG-0016 and DIPG-0081) lost to follow-up at our data cutoff (January 1, 2017) were included in primary statistical analyses.

### *Radiologic Variables*

Anonymized diagnostic magnetic resonance imaging was centrally reviewed (M.W., B.B., E.S., R.C., J.L., and B.J.) and classified as typical or unlikely DIPG; 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 LTSs and 10% of STSs was cross-validated by a neuroradiologist from the other registry. Metastatic disease, defined as noncontiguous tumor in the brain or spine, was reported by individual sites but not centrally reviewed.

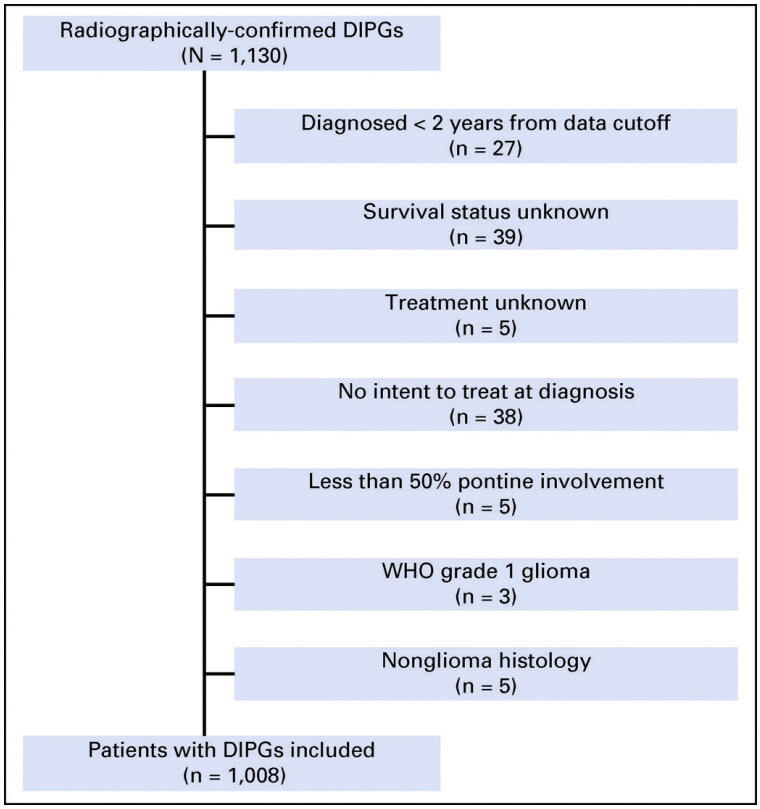



**Fig 1.** Flowchart of patients excluded from this study. DIPG, diffuse intrinsic pontine glioma.

### *Histopathologic and Molecular Variables*

Histology was defined according to 2007 WHO criteria[8]; based on availability of tissue in the registries, 61 tumor specimens were centrally reviewed (C.F. and C.H.). Databases were queried for common genomic alterations in DIPG. Histone mutations were assessed by Sanger sequencing, whole-exome sequencing, or whole-genome sequencing, polymerase chain reaction, or immunohistochemistry to detect H3K27M-mutant protein or H3K27 trimethylation (H3K27me3).Mutations in H3F3A (H3.3 K27M) or HIST1H3B (H3.1 K27M) were considered mutually exclusive even if both were not evaluated.

### *Statistical Analyses*

Patient characteristics were summarized using medians and ranges or frequencies and percentages. Univariable analyses were performed using the Fisher's exact test or Wilcoxon rank sum test. Multivariable logistic regression was performed on variables with < 15% missing data and univariable P < .1; however, transverse tumor dimension was excluded as a result of high correlation with craniocaudal dimension. For subgroup analyses, multivariable logistic regression models were used to determine subgroup significance and adjusted for confounding factors. Survival was estimated using the Kaplan-Meier method. Statistical evaluation was performed using R (Version 3.1.3). P < .05 was considered significant.



## RESULTS

### *Survival*

A total of 1,008 patients met inclusion criteria (IDIPGR, n = 374; SIOPE-DIPGR, n = 634). Median survival time was 11 months (interquartile range, 7.5 to 16 months), and 1-, 2-, 3-, 4-, and 5-year OS rates were 42.3% (95% CI, 38.1% to 44.1%), 9.6% (95% CI, 7.8% to 11.3%), 4.3% (95% CI, 3.2% to 5.8%), 3.2% (95% CI, 2.4% to 4.6%), and 2.2% (95% CI, 1.4% to 3.4%), respectively. Characteristics of 101 LTSs (10%) and 16 VLTSs (1.6%) are shown in Figure 2 and Appendix Figure A1 (online only), respectively. Kaplan- Meier survival analyses for age, symptom duration, systemic therapy, histology, and molecular status are shown in Figure 3.

### *Clinical Presentation*

Median age was 6.8 years (range, 0 to 26.8 years); 4% of patients were age < 3 years at diagnosis. Of patients with available data, 755 (82%) of 917, 468 (51%) of 915, and 567 (62%) of 920 patients presented with one or more cranial nerve (CN) palsy, pyramidal

tract, or cerebellar sign, respectively. On univariable analysis (Table 1), LTSs were more likely to be age < 3 years (28% v 3% of STSs) or > 10 years (33% v 23% of STSs; P< .001) and had longer symptom duration at diagnosis. LTSs were less likely to present with CN palsy (72% v 83% of STSs; P = .008). Multivariable analyses (Table 2) confirmed association of age and symptom duration with long-term survival but failed to associate CN palsy with short-term survival.

### *Therapy*

Thirty-eight patients (3%) who did not receive therapy at diagnosis (Appendix Fig A2A, online only) were excluded. Untreated patients were more often < 3 years old at diagnosis. Eleven patients underwent biopsy or autopsy. At progression, one patient received chemotherapy; no patients received RT. Median OS of untreated patients was 1month (range, 0 to 135 months). Two patients were LTSs (both infants), including one who was alive 135 months after diagnosis (Appendix Fig A2B, online only).

The status of RT and systemic therapy was known for 968 patients; 721 patients (74%) received both RT and systemic therapy, 231 patients (24%) received RTalone, and 16 patients (2%) received systemic therapy alone. In univariable and multivariable analyses, LTSs more commonly received systemic therapy at diagnosis (88% v 75% for STSs; P = .005; odds ratio [OR], 3; 95% CI, 1.46 to 7.3; P = .01). Systemic therapy type was known for 702 patients (70%);

350 patients (50%) received cytotoxic therapy only, 193 patients (27%) received targeted therapy only, and 159 patients (23%) received both cytotoxic and targeted. On univariable analysis, type

of targeted therapy yielded no survival difference (Table 1). However, multivariable logistic 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; 95%CI, 1.1 to 4.82; P = .03) or bevacizumab (OR, 2.67; 95%CI, 1.09 to 6.55; P = .03), an anti–vascular endothelial growth factor (VEGF) antibody, at diagnosis (Table 2). Seventy-two patients (7%) underwent reirradiation at first or subsequent progression (as reported by individual sites). The rate of first progression recordedwithin 1 year of diagnosis was significantly lower in patients who underwent reirradiation compared with patients who did not (74% v 88%, respectively; P = .007).

### *Imaging*

Table 1 lists diagnostic imaging characteristics. STSs demonstrated larger craniocaudal tumor dimension (43 v 40 mm for LTSs; P = .04) and higher rates of extrapontine extension (92% v 85% for LTSs; P = .04), tumor necrosis (45% v 26% for LTSs; P = .009),

and ring enhancement (38% v 23% for LTSs; P = .007). Metastatic disease at diagnosis was reported in 18 STSs (2%) and no LTSs.

### *Histology and Molecular Characteristics*

More SIOPE-DIPGR patients (39%) than IDIPGR patients (14%) underwent biopsy, and more IDIPGR patients (16%) than SIOPE-DIPGR patients (4%) underwent autopsy (Appendix Table A1, online only). LTSs from both registries were more often biopsied than STSs (38% v 28%, respectively; P = .04). Histology and WHO grade were known for 288 biopsy and 76 autopsy samples. WHO grade did not influence survival. Biopsy specimens included glioblastoma multiforme (GBM; n = 80), anaplastic astrocytoma (n = 76), anaplastic oligodendroglioma (n = 10), diffuse astrocytoma (n = 37), fibrillary astrocytoma (n = 4), oligodendroglioma (n = 2), low-grade astrocytoma (n = 8), and unknown (n = 71). Histology of autopsy tissue included GBM (n = 48), anaplastic astrocytoma (n = 12), diffuse astrocytoma (n = 3), and unknown (n = 13).

Of 376 patients from whom tissue was obtained, genomic data were available for 181 (48%) of patients (18% of the entire cohort; Data Supplement), including 21 LTSs (Fig 4). Global molecular assessment was undertaken for 44 patients (whole-genome sequencing, n = 16; whole-exome sequencing, n = 25; 450k methylation array, n = 3), whereas 98 patients underwent limited genomic sequencing (Sanger, n = 80; other targeted platform, n = 18), and 36 patients underwent immunohistochemistry alone. H3.1 K27M was associated with longer median OS (15 months) and long-term survival in multivariable analysis (OR, 1.28; 95% CI, 1.1 to 1.5; P = .002). In contrast, H3.3 K27M was associated with short-term survival (OR, 0.88; 95% CI, 0.78 to 0.99; P = .04; median survival, 10.4 months). Patients with H3 wild-type tumors (n = 26) had a median OS of 10.5 months. WHO grade did not correlate with histone mutation status. TP53 and ACVR1 mutations were not associated with survival. Of the 50 patients age >10 years at diagnosis, who as a group demonstrated higher likelihood of long-term survival, 38 (78%) harbored H3.3 K27M, nine (18%) were H3 wild-type, and only three (6%) had H3.1 K27M.

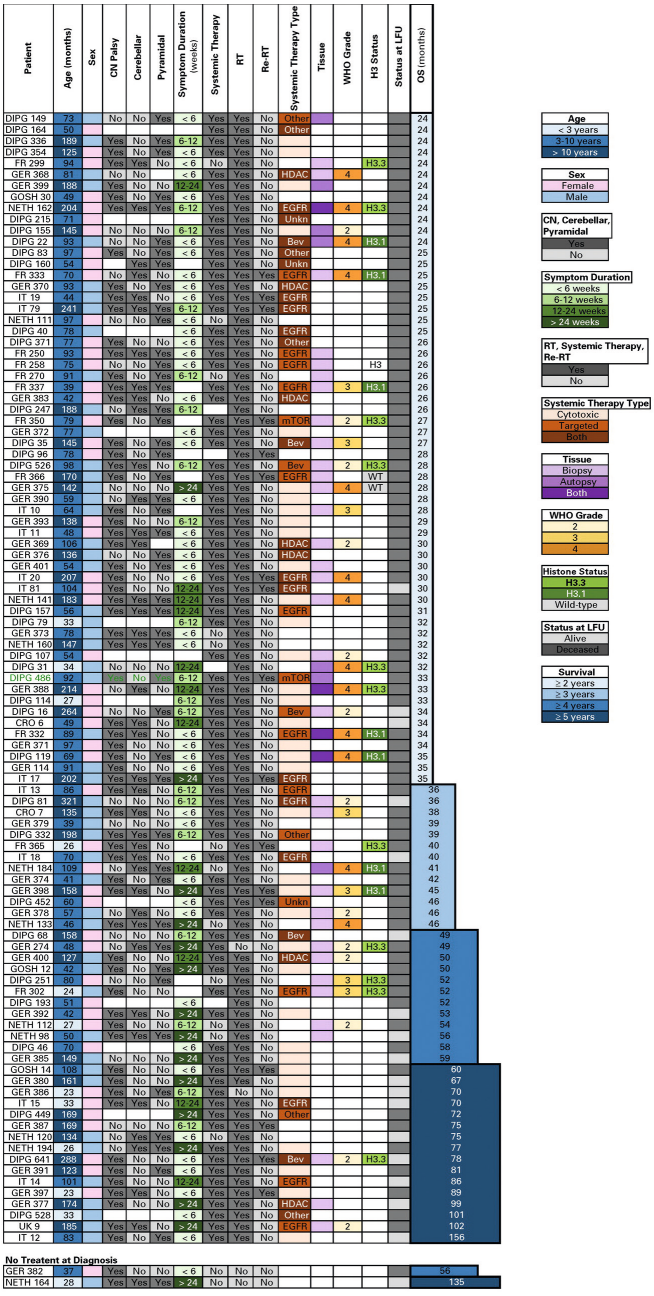
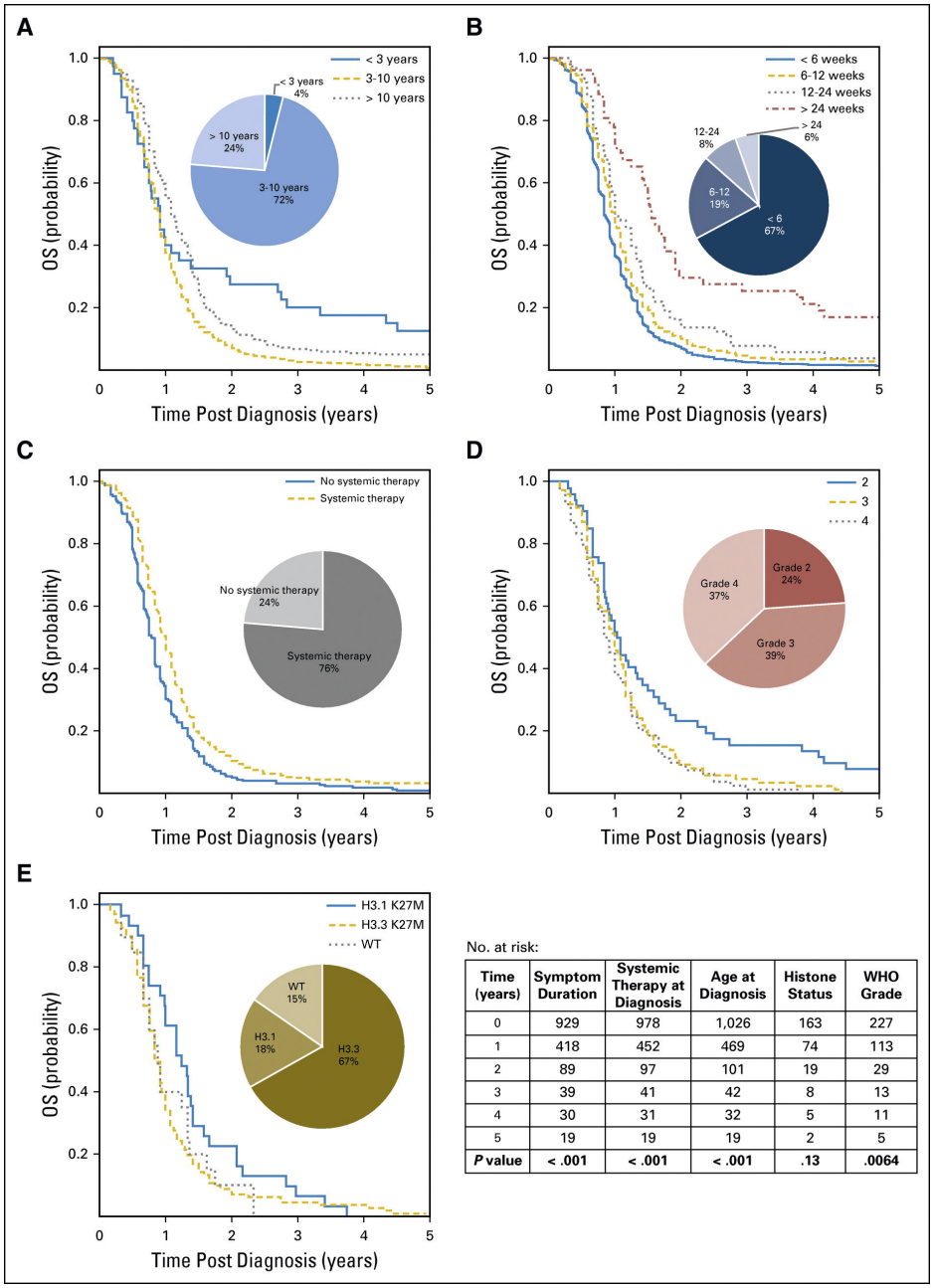



**Fig 2.** Clinical, histologic, and molecular characteristics of long-term survivors of diffuse intrinsic pontine glioma. Bev, bevacizumab; CN, cranial nerve; CRO, Croatia; DIPG, International DIPG Registry; EGFR, epidermal growth factor receptor; FR, France; GER, Germany, Switzerland, Austria; GOSH, Great Ormond Street Hospital; HDAC, histone deacetylase inhibitor; IT, Italy; LFU, last follow-up; mTOR, mammalian target of rapamycin inhibitor; NETH, the Netherlands; OS, overall survival;Re-RT, reirradiation;RT, radiation therapy; UK, United Kingdom; Unkn, unknown.




No. at risk:

| Time (years) | Symptom Duration | Systemic Therapy at Diagnosis | Age at Diagnosis | Histone Status | WHO Grade |
|---|---|---|---|---|---|
| 0 | 929 | 978 | 1,026 | 163 | 227 |
| 1 | 418 | 452 | 469 | 74 | 113 |
| 2 | 89 | 97 | 101 | 19 | 29 |
| 3 | 39 | 41 | 42 | 8 | 13 |
| 4 | 30 | 31 | 32 | 5 | 11 |
| 5 | 19 | 19 | 19 | 2 | 5 |
| ***P* value** | **< .001** | **< .001** | **< .001** | **.13** | **.0064** |

**Fig 3.** Kaplan-Meier curves representing overall survival (OS) based on (A) patient age (years), (B) symptom duration (weeks), (C) systemic therapy at diagnosis, (D) WHO grade, or (E) histone status. WT, wild type.

**Table 1.** Results of Univariable Analyses Comparing Clinical, Radiologic, and Histologic Characteristics of Long- and Short-Term Survivors of Diffuse Intrinsic Pontine Glioma

| Characteristic | LTSs (n = 101) | STSs (n = 907) | *P* |
|---|---|---|---|
| Clinical | | | |
| Registry, No. (%) | | | .39 |
| International | 33 (9) | 341 (91) | |
| SIOPE | 68 (11) | 566 (89) | |
| Sex, No. (%) | | | .46 |
| Male | 51 (50) | 420 (46) | |
| Female | 50 (50) | 485 (54) | |
| Race, No. (%) | | | .43 |
| African | 4 (9) | 43 (12) | |
| Asian | 2 (4) | 14 (4) | |
| White | 36 (80) | 237 (69) | |
| Other | 3 (7) | 50 (15) | |
| Median age, years (range) | 7.2 (1.9-26.8) | 6.8 (0-26.5) | .61 |
| Age, years, No. (%) | | | < .001 |
| < 3 | 11 (11) | 29 (3) | |
| 3-10 | 57 (56) | 668 (74) | |
| > 10 | 33 (33) | 205(23) | |
| Symptom duration, weeks, No. (%) | | | < .001 |
| < 6 | 45 (51) | 564 (69) | |
| 6-12 | 19 (21) | 156 (19) | |
| 12-24 | 11 (12) | 62 (8) | |
| > 24 | 14 (16) | 35 (4) | |
| Symptoms at diagnosis, No. (%) | | | |
| Cranial nerve palsy | | | .008 |
| Yes | 63 (73) | 692 (83) | |
| No | 25 (27) | 137 (17) | |
| Pyramidal tract sign | | | .5 |
| Yes | 39 (44) | 429 (52) | |
| No | 50 (56) | 397 (48) | |
| Cerebellar sign | | | .08 |
| Yes | 46 (53) | 521 (63) | |
| No | 41 (47) | 312 (37) | |
| CSF diversion, No. (%) | | | 1.00 |
| Yes | 22 (22) | 196 (22) | |
| No | 79 (78) | 709 (78) | |
| Systemic therapy at diagnosis, No. (%) | | | .005 |
| Yes | 85 (88) | 644 (75) | |
| No | 12 (12) | 214 (25) | |
| Category of systemic therapy, No. (%) | | | .07 |
| Cytotoxic chemotherapy | 36 (44) | 314 (51) | |
| Targeted chemotherapy | 19 (23) | 174 (28) | |
| Both | 27 (33) | 132 (21) | |
| Chemotherapy type, No. (%) | | | |
| Cytotoxic | 63 (56) | 446 (60) | .43 |
| EGFR inhibitor | 21 (19) | 114 (15) | .14 |
| HDAC inhibitor | 8 (7) | 54 (7) | .68 |
| mTOR inhibitor | 2 (2) | 14 (2) | 1.00 |
| Bevacizumab | 8 (7) | 44 (6) | .37 |
| Other targeted agent | 10 (9) | 88 (12) | .74 |
| Radiologic | | | |
| Median tumor size, mm (range) | | | |
| AP | 36 (18-57) | 36 (14-70) | .98 |
| Transverse | 43 (15-76) | 45 (17-81) | .08 |
| CC | 40 (20-88) | 43 (16-107) | .04 |
| Median pons size, mm (range) | | | |
| AP | 36 (21-50) | 35 (20-58) | .12 |
| Transverse | 49 (31-62) | 48 (22-78) | .62 |
| Extrapontine extension, No. (%) | | | .04 |
| Yes | 78 (86) | 739 (92) | |
| No | 13 (14) | 60 (8) | |
| Hemorrhage, No. (%) | | | .35 |
| Yes | 11 (14) | 136 (19) | |
| No | 68 (86) | 588 (81) | |
| Necrosis, No. (%) | | | .009 |
| Yes | 20 (26) | 306 (42) | |
| No | 56 (74) | 424 (58) | |
| Hydrocephalus, No. (%) | | | 1.00 |
| Yes | 14 (18) | 136 (18) | |
| No | 65 (82) | 632 (82) | |
| Tumor margin, No. (%) | | | .14 |
| Ill defined | 64 (75) | 605 (82) | |
| Well defined | 21 (25) | 132 (18) | |
| Ring enhancement, No. (%) | | | .007 |
| Yes | 19 (23) | 281 (38) | |
| No | 63 (77) | 457 (62) | |
| Histologic | | | |
| Biopsy, No. (%) | | | .03 |
| Yes | 38 (38) | 249 (28) | |
| No | 61 (62) | 652 (72) | |
| Autopsy, No. (%) | | | .04 |
| Yes | 11 (18) | 65 (10) | |
| No | 49 (82) | 597 (90) | |
| WHO grade, No. (%) | | | .08 |
| 2 | 12 (41) | 40 (21) | |
| 3 | 9 (31) | 76 (40) | |
| 4 | 8 (28) | 73 (39) | |

Abbreviations: AP, anterior-posterior; CC, craniocaudal; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; LTSs, long-term survivors; mTOR, mammalian target of rapamycin; SIOPE, European Society for Pediatric Oncology; STSs, short-term survivors.

**Table 2.** Results of Multivariable Cox Proportional Analysis of Clinical, Radiologic, and Molecular Variables Predicting Survival

| Variable | Odds Ratio (95% CI) | *P* |
|---|---|---|
| Clinical | | |
| Age, years | | .02 |
| < 3 | 2.82 (1.06 to 10.28) | |
| 3-10 | 1.0 | |
| > 10 | 2.24 (1.27 to 3.96) | |
| Symptom duration, weeks | | < .001 |
| < 6 | 1.0 | |
| 6-12 | 1.49 (0.76 to 2.92) | |
| 12-24 | 2.43 (1.04 to 5.75) | |
| > 24 | 5.7 (2.77 to 14.54) | |
| Cranial nerve palsy | | .08 |
| Yes | 0.57 | |
| No | 1.0 | |
| Systemic therapy at diagnosis | | .01 |
| Yes | 3 (1.46 to 7.3) | |
| No | 1.0 | |
| Category of systemic therapy | | .14 |
| Cytotoxic chemotherapy | 1.0 | |
| Targeted chemotherapy | 1.03 (0.51 to 2.09) | |
| Both | 1.84 (0.99 to 3.41) | |
| Systemic therapy type | | |
| Cytotoxic | 1.59 (0.73 to 3.45) | .24 |
| EGFR inhibitor | 2.32 (1.1 to 4.82) | .03 |
| HDAC inhibitor | 1.49 (0.62 to 3.6) | .38 |
| mTOR inhibitor | 0.98 (0.11 to 8.66) | .98 |
| Bevacizumab | 2.67 (1.09 to 6.55) | .03 |
| Other targeted agent | 0.71 (0.22 to 2.28) | .56 |
| Radiologic | | |
| Tumor dimension, mm | | .58 |
| AP | — | |
| Transverse | 0.99 (0.96 to 1.02) | |
| CC | — | |
| Extrapontine extension | | .91 |
| Yes | 0.95 (0.36 to 2.43) | |
| No | 1.0 | |
| Molecular | | |
| *H3F3A* mutation | | .04 |
| Yes | 1.0 | |
| No | 1.14 (1.01 to 1.28) | |
| *HIST1H3B* mutation | | .002 |
| Yes | 1.0 | |
| No | 0.78 (0.67 to 0.91) | |
| *ACVR1* mutation | | .09 |
| Yes | 1 | |
| No | 0.75 (0.54 to 1.03) | |
| *TP53* mutation | | .36 |
| Yes | 1 | |
| No | 0.92 (0.76 to 1.1) | |

NOTE. Necrosis, enhancement, and WHO grade were excluded because > 15% of data for these variables were missing. Types of systemic therapy are not mutually exclusive and were not excluded for multiple therapies.
Abbreviations: AP, anterior-posterior; CC, craniocaudal; EGFR, epidermal growth factor receptor; HDAC, histone deacetylase; mTOR, mammalian target of rapamycin.

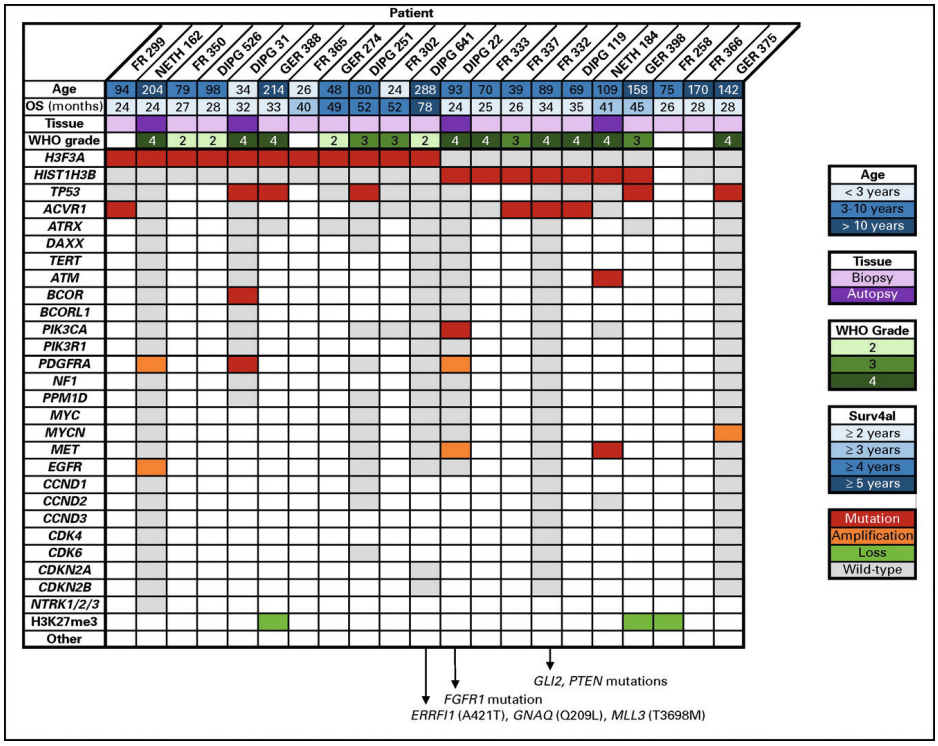



**Fig 4.** Genomic aberrations in long-term survivors of diffuse intrinsic pontine glioma (DIPG). DIPG, International DIPG Registry; FR, France; GER, Germany, Switzerland, Austria; NETH, the Netherlands; OS, overall survival.



## DISCUSSION

This study confirms the relevance of some previously reported survival-associated factors in patients with DIPG and offers unique insight into 101 LTSs (including 16 VLTSs). Median survival for all 1,008 patients was 11 months.[1,5] Median survival times of LTSs and VLTSs were 33 months (range, 24 to 156 months) and 78 months (range, 60 to 156 months), respectively. Of 16 surviving patients, two were lost to follow-up but were LTSs at the time of last contact (patients DIPG-0016 and DIPG-0081; OS, 33 and 36 months). The 2-year OS rate of 9.6% in this study was consistent with large retrospective studies[2,5] that reported 9.2% and 9% 2-year OS rates in 153 and 316 patients with DIPG, respectively. The 1-year OS rate in our study (42.3%) is comparable to that reported by Hassan et al[9] in a meta-analysis of 2,336 pediatric patients with high-grade brainstem glioma (41%); however, the 2- and 3-year OS rates of 15.3% (95% CI, 12% to 20%) and 7.3% (95% CI, 5.2% to 10%) in their study were higher than those in our study (9.6% and 4.3%, respectively), likely reflecting the heterogeneity of their cohort, some whom may not have true DIPGs.

Previously, 43 VLTSs had been reported in the literature.[1,10-15] In Appendix Figure A1, we compare the characteristics of 22 previously published VLTSs to our 16 VLTSs, including eight (0.02% of the total cohort) who are alive with a median follow-up time of 6.5 years (range, 5 to 13 years). Our 5-year OS rate of 2.3% is comparable to the rate of 2.6% reported by Jackson et al1 in 191 patients with DIPG; however, two of their five VLTSs would have been excluded from our study for atypical magnetic resonance imaging features. Freeman et al[12] reported nine VLTSs (6.9%) among 130 patients with DIPG treated with hyperfractionated RT (Pediatric Oncology Group 8495 trial), although only four of these patients (3%) would have met inclusion criteria in our study.

Age <3 or >10 years, longer symptom latency, lack of CN palsy, and systemic therapy at diagnosis were predictors of longterm survival. Of 41 patients age <3 years at diagnosis, 36 received first-line RT with or without systemic therapy and five received systemic therapy alone. Although median OS for children age < 3 years (11 months) was the same as the entire cohort, a greater proportion was LTSs or VLTSs. Other studies have reported similar findings.[1,2,5,16] Broniscer et al[17] described 10 DIPG patients age < 3 years who received RTwith or without chemotherapy (n = 8) or chemotherapy only (n = 2) at diagnosis (n = 6) or progression (n = 4). Five patients (50%) were LTSs, including one treated without RT. Wagner et al[5] similarly reported higher median survival in 13 children with DIPG age < 4 years compared with older children (13.6 v 10months); only eight patients (61%) received RT. Although limitations to our data precluded making conclusions about biologic differences in this young age group, we postulate that unique mechanisms, such as potently oncogenic NTRK fusions described in infantile midline high-grade gliomas,[18] may underlie this observed survival advantage.

Patients age >10 years at diagnosis had longer median OS (13 months) and were more likely to be LTSs. Bailey et al[19] similarly reported five LTSs (all > 9 years old) among 43 patients with radiographically confirmed DIPG. In contrast, Veldhuijzen van Zanten et al[16] reported no difference in OS between patients age 9 to 18 years versus younger patients. 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. Clinical and molecular characteristics for patients age > 18 years (n = 13) were also similar to their younger counterparts (AppendixFig A3, online only). Consistent with prior reports,[1,2] the presence of symptoms for > 24 weeks at diagnosis was strongly associated with longer survival in univariable and multivariable analyses. CN palsy at diagnosis predicted shorter survival in univariable but not multivariable analysis. Previous studies reporting association of CN palsy with shorter survival included all brainstem tumors, not just DIPG, and/or diagnosis based on computed tomography scan, making comparison difficult.[20]

Neoadjuvant or adjuvant systemic therapy correlated with long-term survival in both univariable and multivariable analyses. This finding differs from the long-standing view that systemic therapy provides no survival benefit for DIPG, a principle largely based on small, nonrandomized clinical trials. Effective crosscomparison of therapeutic studies for DIPG has been hindered by wide variation in inclusion criteria, as demonstrated in studies by Hargrave et al[21] and Jansen et al[22] in which only six of 29 DIPG specific therapeutic trials between 1984 and 2012 had comparable eligibility. In a randomized trial, Wagner et al[5] reported better median OS in patients with DIPG treated with adjuvant chemotherapy after RT (11.3 months) compared with patients treated with RT alone (9.5 months; P = .03). Similarly, others have reported superior median OS with use of adjuvant or neoadjuvant chemotherapy.[4]

Multivariable logistic regression demonstrated higher odds of long-term survival with use of EGFR inhibitors (eg, gefitinib, erlotinib, nimotuzumab, rindopepimut, cetuximab) or bevacizumab at diagnosis. A phase II study of gefitinib with RT in newly diagnosed patients with DIPG noted 2-year OS of 19.6%with PFS.36 months in three patients.[23] In a biopsy mandated phase I study of erlotinib with RT, EGFR overexpression trended toward longer PFS (10.1 months v 6.3 months in patients without EGFR overexpression; P = .058) but not OS.24 Despite only modest activity of nimotuzumab in progressive DIPG, two patients lived for 663 and 481 days from the start of therapy.[25]

Despite efficacy in adult GBM, bevacizumab has shown little activity in pediatric trials for newly diagnosed[26] or progressive DIPG[27] (median PFS, 2.3 months). However, in a phase I trial of vandetanib, a selective vascular endothelial growth factor receptor receptor 2 (VEGFR2) and EGFR inhibitor, in newly diagnosed DIPG, Broniscer et al[28] reported 2-year OS of 21.4%, and higher levels of plasma VEGF were associated with longer PFS (P = .02). Although numbers were too small to assess patient outcomes based on genomically matched targeted therapy, our findings support prospective assessment of biopsy tissue to define potential therapeutic targets, as recently undertaken in two multi-institution, multinational trials (ClinicalTrials.gov identifiers: NCT01182350 andNCT02233049).

Janssens et al[29] reported improved OS in 31 children with DIPG who received reirradiation at first progression (13.7 months) compared with a matched control cohort (10.3 months) despite similar PFS (8.2 v 7.7 months, respectively). Progression was not defined or centrally reviewed in our study; however, we noted that the proportion of patients with recorded progression within 1 year of diagnosis was significantly lower among patients who underwent reirradiation compared with those who did not, suggesting potential clinician bias to recommend reirradiation to patients with a more indolent disease course or potentially greater sensitivity to initial RT in patients who ultimately received reirradiation. As postulated by others,[30] increased RT sensitivity may be a manifestation of distinct biology. We did not report reirradiation-based

outcomes given limitations conferred by analysis of registry data; more robust analysis of the effect of reirradiation in patients with DIPG would be best assessed prospectively in the context of a clinical trial.

On the basis of the radiographic definition of DIPG by Barkovich et al,[31] patients with , 50% pontine involvement (n = 5) were excluded. Similar to a prior report,[5] these patients had better median OS (20 months), and two patients were LTSs. Greater craniocaudal tumor dimension and extrapontine extension were associated with shorter survival; the former finding contrasts with a report by Poussaint et al,[32] in which larger tumor at diagnosis was associated with longer survival.

As previously described,[32] tumor necrosis and ring enhancement were associated with short-term survival in univariable analysis. Multivariable analysis was not performed because > 15% of data were missing for each variable, precluding comparison of our findings to the validated multiparametric prediction model published by Jansen et al.[2]

DIPG biology has been intensely studied since discovery of first-in-human histone mutations in 2012.[15] Our findings confirm the independent association of H3.1 K27M and H3.3 K27M with long- and short-term survival, respectively.[3,15] Median OS did not significantly differ between histone wild-type and mutant DIPGs; this contrasts with the report by Khuong-Quang et al1[5] of longer median OS (4.59 years) for patients with histone wild-type tumors.

In univariable analysis, WHO grade did not differ between LTSs and STSs (Table 1), but on Kaplan-Meier analysis, WHO grade 2 was associated with longer survival (Fig 3D). In the most recent WHO classification of CNS tumors,[33] K27M-mutant midline gliomas are classified as WHO grade 4 regardless of histology, making this point less relevant. Tumors classified as primitive neuroectodermal tumors (now called embryonal tumor not otherwise specified) may represent true embryonal mimics of DIPG or result from sampling error in the context of intratumoral heterogeneity. Embryonal pontine tumors often demonstrate sharp margination and eccentric location, whereas others have radiologic characteristics indistinguishable from DIPG,[34] like those excluded from our study (Appendix Table A2, online only).

A limitation of this study is use of disease-specific registry data, which are 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 between countries and institutions may have also influenced findings. Anonymity of registry data makes some overlap of registry patients with those previously reported possible, biasing our findings toward similarity with published literature because they are not completely independent cohorts. The primary strength of this study is mandated central review of diagnostic imaging with cross-validation by highly

experienced pediatric neuroradiologists and use of standardized case report forms. To our knowledge, 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 subgroups and emphasizes patient subsets from whom the most could be learned from analyzing pretreatment biopsy tissue. Understanding biologic differences that confer survival advantage in DIPG paves the road toward development of subgroup-specific therapies that, when implemented in the context of clinical trials, may improve outcomes for this devastating disease.

## ACKNOWLEDGEMENTS

We thank the children and families who have suffered from diffuse intrinsic pontine glioma (DIPG) for their invaluable contribution to this research. Special thanks also to KPMG for the pro bono support of this project and for validation of design and security of the European Society for Pediatric Oncology (SIOPE) DIPG Registry and Imaging Repository.

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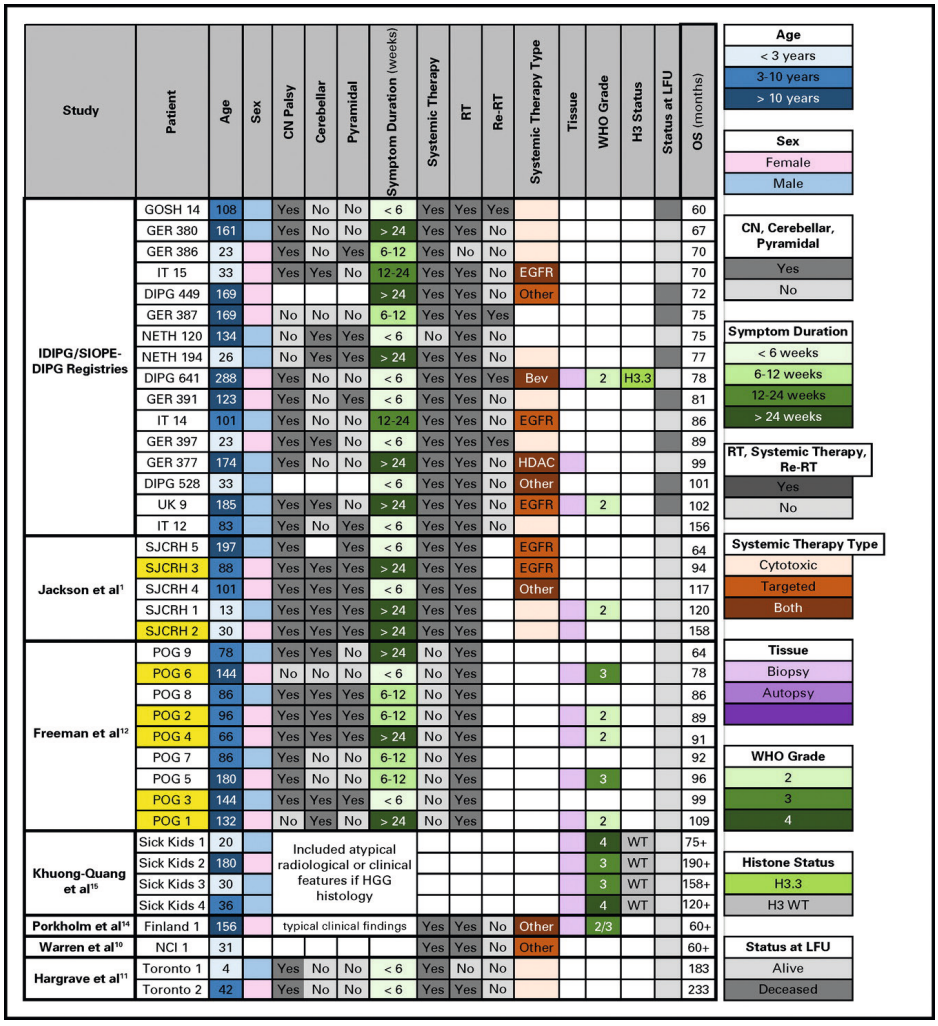
# APPENDIX




| Study | Patient | Age | Sex | CN Palsy | Cerebellar | Pyramidal | Symptom Duration (weeks) | Systemic Therapy | RT | Re-RT | Systemic Therapy 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 | | 2 | 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 | | 2 | | | 102 |
| | IT 12 | 83 | | Yes | No | Yes | < 6 | Yes | Yes | No | | | | | | 156 |
| Jackson et al[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 | | | | 2 | | | 120 |
| | SJCRH 2 | 30 | | Yes | Yes | Yes | > 24 | Yes | Yes | | | | | | | 158 |
| Freeman et al[12] | POG 9 | 78 | | Yes | Yes | No | > 24 | No | Yes | | | | | | | 64 |
| | POG 6 | 144 | | No | No | No | < 6 | No | Yes | | | | 3 | | | 78 |
| | POG 8 | 86 | | Yes | Yes | Yes | 6-12 | No | Yes | | | | | | | 86 |
| | POG 2 | 96 | | Yes | Yes | Yes | 6-12 | No | Yes | | | | 2 | | | 89 |
| | POG 4 | 66 | | Yes | Yes | Yes | > 24 | No | Yes | | | | 2 | | | 91 |
| | POG 7 | 86 | | Yes | No | No | 6-12 | No | Yes | | | | | | | 92 |
| | POG 5 | 180 | | Yes | No | No | 6-12 | No | Yes | | | | 3 | | | 96 |
| | POG 3 | 144 | | Yes | Yes | Yes | < 6 | No | Yes | | | | | | | 99 |
| | POG 1 | 132 | | No | Yes | No | > 24 | No | Yes | | | | 2 | | | 109 |
| Khuong-Quang et al[15] | Sick Kids 1 | 20 | | Included atypical radiological or clinical features if HGG histology | | | | | | | | | 4 | WT | | 75+ |
| | Sick Kids 2 | 180 | | | | | | | | | | | 3 | WT | | 190+ |
| | Sick Kids 3 | 30 | | | | | | | | | | | 3 | WT | | 158+ |
| | Sick Kids 4 | 36 | | | | | | | | | | | 4 | WT | | 120+ |
| Porkholm et al[14] | Finland 1 | 156 | | typical clinical findings | | | | Yes | Yes | No | Other | | 2/3 | | | 60+ |
| Warren et al[10] | NCI 1 | 31 | | | | | | Yes | Yes | No | Other | | | | | 60+ |
| Hargrave et al[11] | Toronto 1 | 4 | | Yes | No | No | < 6 | Yes | No | No | | | | | | 183 |
| | Toronto 2 | 42 | | Yes | No | No | < 6 | Yes | Yes | No | | | | | | 233 |

**Fig A1.** Very long–term survivors of diffuse intrinsic pontine glioma in the current study compared with those described in the literature. Yellow highlight indicates atypical radiologic features that would have been excluded in the current study. Bev, bevacizumab; CN, cranial nerve; DIPG, diffuse intrinsic pontine glioma; EGFR, epidermal growth factor; GER, Germany, Switzerland, Austria; GOSH, Great Ormond Street Hospital; HDAC, histone deacetylase inhibitor; HGG, high-grade glioma; IDIPGR, International Diffuse Intrinsic Pontine Glioma Registry; IT, Italy; LFU, last follow-up; NCI, National Cancer Institute; NETH, the Netherlands; OS, overall survival; POG, Pediatric Oncology Group; Re-RT, reirradiation; RT, radiation therapy; SIOPE, European Society for Pediatric Oncology; SJCRH, St Jude Children's Research Hospital; UK, United Kingdom; WT, wild type.

**A**

| Clinical Variables | Untreated (n = 38) | Treated (n = 1,008) |
|---|---|---|
| **LTS, No. (%)** | | |
| 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, No. (%)** | | |
| 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, months (range)** | 1 (0-135) | 11 (0-167) |

**B**

| Patient | GER 382 | NETH 164 |
|---|---|---|
| Age (months) | 37 | 28 |
| Sex | Female | Male |
| CN palsy | Yes | Yes |
| Cerebellar | No | Yes |
| Pyramidal | No | Yes |
| Symptom duration (weeks) | < 6 | > 24 |
| Chemotherapy | No | No |
| RT | No | No |
| Re-RT | No | No |
| Status at LFU | Deceased | Alive |
| OS (months) | 56 | 135 |

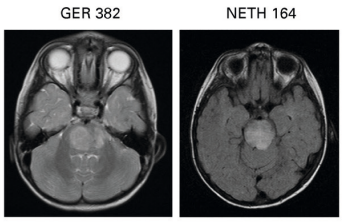



**Fig A2.** (A) Comparison of characteristics of patients who received therapy or did not receive therapy at diagnosis. (B) Magnetic resonance images and clinical characteristics of two long-term survivors (LTSs) of diffuse intrinsic pontine glioma who did not receive therapy. CN, cranial nerve; GER, Germany, Switzerland, Austria; LFU, last follow-up; NETH, the Netherlands; OS, overall survival; Re-RT, reirradiation; RT, radiation therapy.

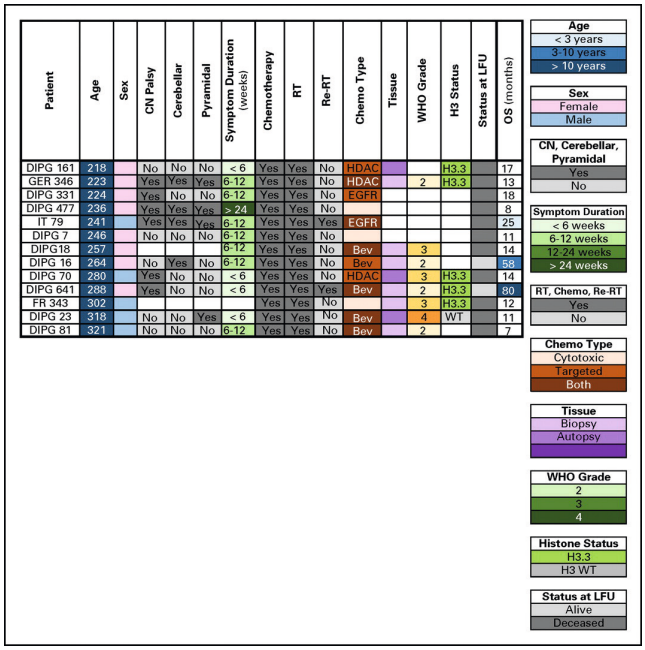



**Fig A3.** Clinical, radiologic, and molecular characteristics of patients with diffuse intrinsic pontine glioma age > 18 years. Bev, bevacizumab; CN, cranial nerve; DIPG, International DIPG Registry; EGFR, epidermal growth factor; FR, France; GER, Germany, Switzerland, Austria; HDAC, histone deacetylase inhibitor; IT, Italy; LFU, last follow-up; OS, overall survival; Re-RT, reirradiation; RT, radiation therapy; WT, wild type.

**Table A1.** Biopsies and Autopsies Performed by Country or Region

| Country | No./Total No. (%) Biopsy | Autopsy |
|---|---|---|
| SIOPE-DIPGR | | |
| 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) |

Abbreviations: IDIPGR: International Diffuse Intrinsic Pontine Glioma Registry; SIOPE-DIPGR, European Society for Pediatric Oncology Diffuse Intrinsic Pontine Glioma Registry.

**Table A2.** Clinical, Radiologic, and Molecular Characteristics of Patients With Primitive Neuroectodermal Tumor

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

Abbreviations: CN, cranial nerve; DIPG, International DIPG Registry; OS, overall survival; RT, radiation therapy; WT, wild type.

# 7

# Treatment-Related Survival Patterns in Diffuse Intrinsic Pontine Glioma (DIPG): A Report from the European Society for Pediatric Oncology DIPG/DMG Registry

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## ABSTRACT

**Background:** Frontline radiotherapy (RT) for diffuse intrinsic pontine glioma (DIPG) is generally considered the only proven effective, albeit palliative, treatment. Our aim is to examine six treatment paths and investigate their association with survival.

**Patients and methods:** Data were collected retrospectively on 409 patients using the GPOH HIT-HGG trial database and the SIOPE DIPG/DMG Registry. All patients were radiologically centrally reviewed DIPG. Survival outcomes were estimated using the Kaplan Meier method and a landmark analysis for survival after relapse. Cox proportional hazard models were estimated to study treatment effect.

**Results:** Median overall survival (OS) was 11.2 months (95%CI, 10.5-11.9), estimated from diagnosis. OS with no frontline treatment was 3.0 months (95%CI, 2.0-4.0), 10.4 months (95%CI, 9.1-11.8) with RT alone, and 11.7 months (95%CI, 10.8-12.6) with RT-chemotherapy. Median survival after first progression (PPS) was 4.1 months (95%CI, 3.5-4.7). PPS was 2.2 months (95%CI 1.8-2.6) with no relapse treatment, 4.4 months (95%CI 3.7-5.0) with chemotherapy alone and 6.6 months (95%CI 5.3-8.0) with reirradiation +/- chemotherapy. The hazard ratio (HR) for OS with no treatment, estimated with a Cox model from diagnosis, was 3.65 (95%CI, 2.3-5.8), and RT alone 1.34 (95%CI, 1.1-1.7), relative to RT-chemotherapy. From relapse, OS with no treatment had a HR of 1.44 (95%CI, 1.12-1.85), and reRT +/- chemotherapy a HR of 0.72 (95%CI, 0.53-0.98), relative to chemotherapy.

**Conclusions:** This study suggests what survival benefits may be gained by which general therapeutic approach, as a first step to quantifying survival differences observed in a historical DIPG cohort.

## INTRODUCTION

Due to the very poor prognosis of patients with diffuse intrinsic pontine glioma (DIPG), despite intensive therapeutic research efforts for the last decades, pediatric oncologists often consider no oncological therapy an acceptable option. In the largest DIPG series published to date, 3% of patients received no oncological treatment.[1] This may be an underestimate, as most patients were included in clinical trials. Epidemiological studies from the Netherlands and Canada, report 14% and 8% of patients with DIPG, respectively, without any oncological treatment.[2,3] The absence of untreated or minimally treated patients from observational studies, inflates survival estimates towards prognostically better patients who are eligible for clinical trials.[4] Our study is the first to include patients with no tumor directed therapy in a contemporary survival analysis.

Frontline radiotherapy (RT) is standard of care in DIPG treatment, conferring 3-4 additional months of survival compared to no tumor directed therapy.[5,6] The role of systemic chemotherapy, both concomitant and/or adjuvant to radiotherapy however is a subject of debate. In the European context, 54% of treating physicians indicate using radiotherapy only, and 45% combining with chemotherapy.[7] Given many patients receive therapy beyond radiation at diagnosis, it is important to evaluate if there is a survival advantage. Furthermore, in a large series of 1100 patients with DIPG, Hoffman et al. found longer overall survival (i.e., greater than two years) correlated with neoadjuvant and adjuvant systemic chemotherapy.[1] This warrants further investigation into the survival benefit of additional treatments beyond standard RT.



Immortal time bias poses a significant challenge in the assessment of DIPG treatments, particularly in the relapse setting. This form of selection bias is highly prevalent in observational studies published in leading journals.[8] In such a scenario, patients are classified retrospectively using treatment status at the time of study completion, which is not known at baseline when the analysis is performed. Median overall survival is then calculated from diagnosis, regardless of the timing of therapy initiation. This erroneous inclusion of a covariate in the analysis at baseline, which is only known in the future, has the effect of underestimating the death rate in the treated group and overestimating the death rate in the untreated group. If many patients die early, as in the case of DIPG, the bias can be quite large. In our study design, we correct for immortal time bias using the landmark method.[9]

We report survival in patients with no treatment, radiation only at diagnosis and no treatment at relapse and compare these limited treatments with more intensive treatment. This allows for comparison to a more diverse set of patient outcomes. To conduct this project, survival outcomes were examined across six treatment modalities, three in the frontline setting and three at relapse. At diagnosis, modalities included I)

no treatment, II) RT alone, and III) RT-chemotherapy. At relapse, modalities included I) no additional treatment, II) chemotherapy, and III) re-RT, +/- chemotherapy.

Our aim is to investigate associations to survival among these six 'treatment paths' and quantify the effect of individual treatment modalities on survival. Prognostic information on clinical course and survival without any treatment or radiotherapy alone, versus progressive oncologic treatment, will be helpful for patients and families who are considering all available treatment options.

## PATIENTS AND METHODS

### *Study population*

Data were collected retrospectively on 409 patients using the German Society of Pediatric Oncology and Hematology (GPOH) HIT-HGG trial database and the European Society for Pediatric Oncology (SIOPE) DIPG/DMG Registry. The SIOPE-DIPG Registry has been reviewed and medical research involving human subjects act (WMO) does not apply, protocol reference number 22/724. This study was also approved by the IRB at University Medical Center Göttingen.

All patients had radiologically centrally reviewed DIPG, mostly not biopsied. Inclusion criteria for the SIOPE DIPG Registry were based on protocol version 1.0. These criteria for patient inclusion included; patients with DIPG, defined as a T1-weighted hypointense and T2-weighted hyperintense tumor with at least 50% involvement of the pons (DIPG) on T2, and as confirmed by expert neuroradiologists via the central radiology review procedure. Furthermore, at least one of the following typical brainstem symptoms should be present, cranial nerve deficits, long tract signs or ataxia. Onset of symptoms should be short, preferably less than 3 months, at maximum 6 months. If duration of symptoms was longer than 6 months, a biopsy was usually performed to confirm high grade glioma. Nevertheless, due to the nature of a retrospective cohort, in some cases the duration of symptoms before diagnosis was not clearly defined. All GPOH-HIT-HGG patients in the present study were trial patients and underwent confirmation of DIPG diagnosis by central neuroradiological review. Only patients between ≥3 and <18 years of age at diagnosis were included in this study. No patients were excluded based on the year of diagnosis.

In the frontline "untreated" group, treatment refusal was voluntary in all 20 patients. Clinical records were checked to ensure these patients did not forgo treatment after diagnosis because of rapid deterioration or poor performance/clinical condition. The "radiotherapy only" group was either a voluntary treatment decision or based on recommended national standards at the time of diagnosis. Patients on whom we did not have reliable data on the relapse situation and/or no centrally reviewed data were

excluded. Detailed treatment information on individual treatment modalities was limited in this study due to the retrospective design. Information on systemic therapy regimens is available in supplementary Table 1.

### *Statistical analysis*

The Kaplan Meier methodology was used to analyze survival data. Median overall survival (OS) time was computed from date of diagnosis to death; OS is reported at three months, six months, one year, two years, and five years. To study the effect of different treatments upon progression, the landmark method was also used.[9,10] A landmark point was set from first relapse time for patients who experienced relapse. Patients with no documented relapse were not included. Date of first relapse/progression was reported by the enrolling center. An univariable Cox proportional hazard regression model was used to quantify the effect of each treatment on survival. Two Cox models were estimated: one from diagnosis and one from relapse. To examine differences in sex and age distribution, at baseline t-test and Pearson's Chi Square were used. IBM SPSS Statistics version 26 (Armonk, NY, USA) was used to perform the statistical analysis.

## RESULTS

### *Patient Characteristics*

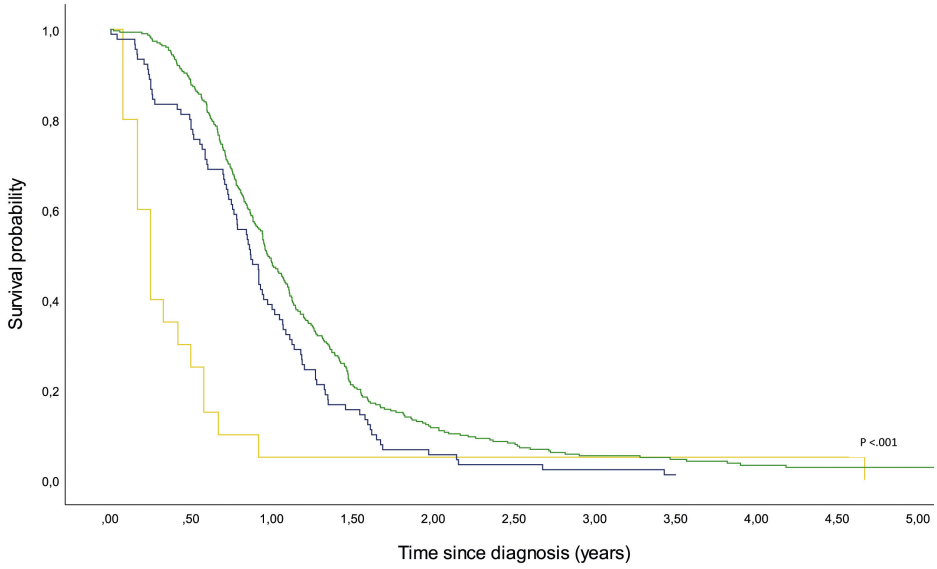
At baseline among 409 patients (Table 1), the median age was 7.5 years (95%CI, 6.8–7.8) with a range of 3 to 17.8 years. Median age of the three treatment groups I) no treatment (5.7 yr.), II) radiotherapy alone (7.0 yr.), III) radio-chemotherapy (7.7 yr.) was significantly different (p=.05). Most patients (72 %) were between 3 and 10 years of age and 28% between 10 and 18 years. The sex distribution was 52% female and 48% male and not significantly different between treatment groups (.98). The biopsy rate was 23%. Most patients had a symptom duration <6 weeks (63.3%), followed by 6–12 weeks (19.6%), 12-24 weeks (8.6%), >24 weeks (5.6%), unknown (2.9%) and not significantly different between baseline treatment groups (p=.10).

**Table 1.** Baseline patient characteristics (*N*=409).

| **Characteristics** | ***n* (%)** | ***P*** |
|---|---|---|
| Sex | | .96 |
| Male | 196 (48) | |
| Female | 213 (52) | |
| Age at diagnosis | | |
| Range | 3.0-17.8 yr. | |
| Median age (95%CI) | 7.5 yr. (6.8-7.8) | .05 |
| No treatment | 5.7 yr. | |
| Radiotherapy alone | 7.0 yr. | |
| Radiochemotherapy | 7.7 yr. | |
| Age by group | | |
| 3-10 years | 293 (72) | |
| >10-18 years | 116 (28) | |
| Biopsy | | |
| Yes | 92 (23) | |
| No | 317 (77) | |
| Symptom duration | | .10 |
| <6 weeks | 260 (63.3) | |
| 6-12 weeks | 80 (19.6) | |
| 12-24 weeks | 35 (8.6) | |
| >24 weeks | 23 (5.6) | |
| Unknown | 12 (2.9) | |

## *Survival Outcomes*

From diagnosis, median overall survival (OS) was 11.2 months (95% CI, 10.5–11.9) for the whole cohort (*n*=409). For the different treatment groups, median OS was 3 months (95% CI, 2.0–4.0) for patients who received no treatment, versus 10.4 months (95% CI, 9.1–11.8) for those who were treated with radiotherapy alone, and 11.7 months (95% CI, 10.8–12.6) for patients receiving radio-chemotherapy (Figure 1a, p<.001). For patients who received no treatment, OS at 6 months and 1 year was 25% (95% CI, 6–44%) and 5% (95% CI, 0–15%) respectively, in comparison to patients treated with RT only, showing an OS of 80% (95% CI, 72–88%) at 6 months and 39% (95% CI, 29–49%) at 1 year. Radio-chemotherapy patients had a 6-month OS of 88% (95% CI, 84–92%) and 49% (95% CI, 43–54%) at 1 year (Table 2a).



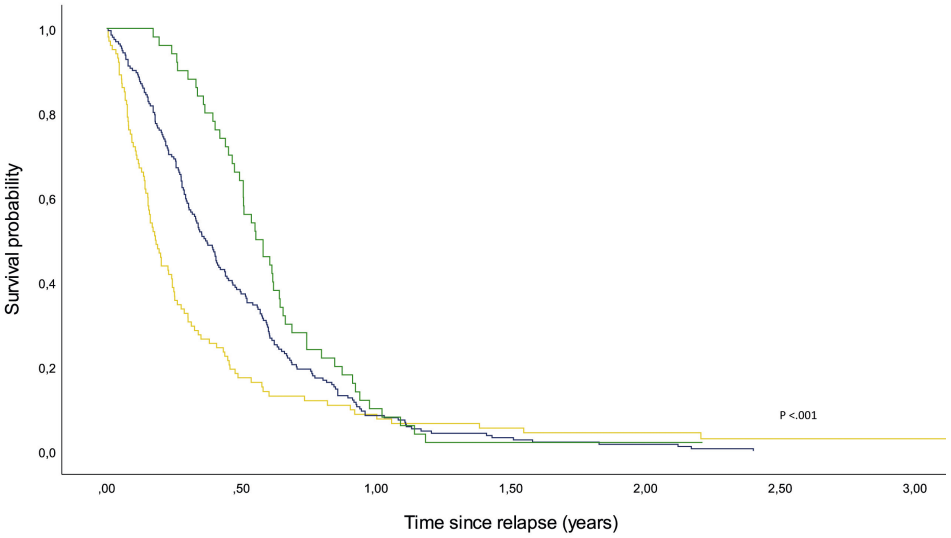

| No. at risk | 0 | 0.25 yr. | 0.5 yr. | 1 yr. | 2 yr. | 5 yr. |
|---|---|---|---|---|---|---|
| No treatment | 20 | 11 | 5 | 1 | 1 | 0 |
| Radiotherapy | 90 | 79 | 71 | 34 | 4 | 0 |
| Radiochemotherapy | 299 | 293 | 262 | 143 | 33 | 5 |

**Figure 1a.** Estimated Kaplan-Meier survival time from diagnosis (*N*=409).

**Table 2a.** Survival time from diagnosis with 95% confidence interval.

| **First-line Treatment** (*N*=409) | **Group I: None** (*n*=20) | **Group II: Radiotherapy** (*n*=90) | **Group III: Radiochemotherapy** (*n*=299) |
|---|---|---|---|
| **Median Survival** | **3.0** mo. (2.0-4.0) | **10.4** mo. (9.1-11.8) | **11.7** mo. (10.8-12.6) |
| Survival at 6 mo. | 25% (6-44%) | 80% (72-88%) | 88% (84-92%) |
| Survival at 1 yr. | 5% (0-15%) | 39% (29-49%) | 49% (43-54%) |
| Survival at 2 yr. | 5% (0-15%) | 6% (1-10%) | 12% (8-15%) |
| Survival at 5 yr. | 0% | 0% | 3% (1-5%) |
| **Median Overall Survival** **11.2** mo. (10.5-11.9) | | | |

In the relapse setting, among patients that experienced a relapse (*n*=342), median post progression survival (PPS) was 4.1 months (95% CI, 3.5–4.7). For the respective treatment groups, PPS was 2.2 months (95% CI, 1.8–2.6) for patients with no relapse treatment vs 4.4 months (95% CI, 3.7–5.0) for patients that received relapse chemotherapy, and 6.6 months (95% CI, 5.3–8.0) for patients receiving reirradiation, +/-relapse chemotherapy (Figure 1b, p= <.001). For patients with no additional treatment after relapse, survival at 6 months was 17% (95% CI, 10–25%). With chemotherapy, survival at 6 months was 37% (95% CI, 31–44%), and for patients receiving reirradiation, +/-relapse chemotherapy, 6-month survival was 64% (95% CI, 51–77%, Table 2b). The number of events from both relapse and diagnosis is available in supplementary Table 2.




| No. at risk | 0 | 0.25 yr. | 0.5 yr. | 1 yr. | 2 yr. | 5 yr. |
|---|---|---|---|---|---|---|
| No additional treatment | 100 | 36 | 15 | 7 | 2 | 1 |
| Chemotherapy | 191 | 131 | 71 | 15 | 2 | 0 |
| Reirradiation +/- chemotherapy | 51 | 46 | 32 | 4 | 1 | 0 |

**Figure 1b.** Estimated Kaplan-Meier survival time from relapse (*N*=342).

**Table 2b.** Survival time from relapse with 95% confidence interval.

| **Relapse Treatment** (*N*=342) | **Group I: None** (*n*=100) | **Group II: Chemotherapy** (*n*=190) | **Group III: Reirradiation, +/-chemotherapy** (*n*=51) |
|---|---|---|---|
| **Median Survival** | **2.2** mo. (1.8-2.6) | **4.4** mo. (3.7-5.0) | **6.6** mo. (5.3-8.0) |
| Survival at 6 m. | 17% (10-25%) | 37% (31-44%) | 64% (51-77%) |
| Survival at 1 yr. | 8% (2-13%) | 8% (4-12%) | 10% (2-18%) |
| Survival at 2 yr. | 4% (0.2-8%) | 1% (0-2%) | 2% (0-6%) |
| Survival at 5 yr. | 3% (0-6%) | 0% | 0% |
| **Median Post-Progression Survival** **4.1 mo.** (3.5-4.7) | | | |

## *Treatment Effect*

For patients who received no treatment and radiotherapy at diagnosis, hazard ratios (HRs) for OS of 3.65 (95%CI, 2.30–5.81), and 1.34 (95%CI, 1.05–1.70), were found, respectively, relative to radiochemotherapy. From relapse, patients with no additional treatment had a HR for OS of 1.44 (95%CI, 1.12–1.85) and reirradiation, +/- relapse chemotherapy a HR of 0.72 (95%CI, 0.52–0.98), relative to chemotherapy (Table 3).

**Table 3.** Estimated univariable Cox proportional hazard regression models. Hazard Ratio (HR) along with 95% confidence interval (CI).

| | | **HR (95%CI)** |
|---|---|---|
| **Diagnosis:** | No treatment | 3.65 (2.30-5.81) |
| | Radiotherapy alone | 1.34 (1.05-1.70) |
| | Radiochemotherapy* | |
| **Relapse:** | No additional treatment | 1.44 (1.12-1.85) |
| | Reirradiation +/- chemotherapy | 0.72 (0.52-0.98) |
| | Chemotherapy* | |

* Reference category (i.e. largest group)

## DISCUSSION

Although not evidenced in a randomized fashion, our data suggest treating patients with DIPG with radiotherapy is beneficial, and additional chemotherapy to RT, both concomitant and/or as post-radiotherapy maintenance treatment, seems to prolong survival. The association between systemic therapy and better survival observed in our study is supported by other large-scale assessments using historical cohorts.[1,11,12,13] The survival benefit of irradiation in DIPG is well documented over the last 50 years, and in the absence the disease progresses quickly.[14] Atac et al. documented cases of three-week survival in children too sick to receive irradiation.[15] We excluded patients too sick to receive therapy.

Unique to our survival analysis is the inclusion of patients who received limited treatments (i.e., no treatment, irradiation only at diagnosis and no additional treatment at relapse). A note of caution must be used to compare patient outcomes in the absence of treatment. However, better defined outcome measures observed in historical controls, such as post-progression survival, can provide a reference value for future clinical trials and drug development.[16] We describe what survival benefits may be gained by which general therapeutic approach, as a first step to quantifying survival differences observed in a historical DIPG reference cohort. Differences in survival, albeit small in scale, are essential to document for patients/families and clinicians alike. By knowing how long patients with DIPG survive broadly without treatment or radiotherapy alone, treating physicians have more detailed information to employ when talking to patient families.

Novel to our study design is the use of the landmark methodology, in which the date of first relapse was used as a landmark time point.[9,10] Post progression survival (PPS), rather than OS from diagnosis was then used to evaluate relapse treatments. This design is both clinically relevant and easily transferable to other observational DIPG studies investigating relapse treatments.[16] By utilizing the landmark method, patients were grouped using new treatment information known at relapse to evaluate the effect of relapse therapies on survival. There may be a short gap between date of relapse and start of relapse treatment, but this is considered minimal in DIPG.[17] This method allowed us to gain more insight into what survival can be attributed to relapse therapies and, consequently, better define overall survival for frontline therapies.

Our survival data suggest combination therapy is most effective in extending survival in both the primary and relapse settings. Patients receiving no frontline treatment had an increased risk of death, three and a half times that of patients receiving radiochemotherapy. Patients receiving radiotherapy alone had 34% increase in the expected hazard of death relative to radiochemotherapy. At relapse, patients with no additional treatment had a 44% increase in the expected hazard of death, relative

to maintenance chemotherapy alone, while reirradiation plus or minus relapse chemotherapy was protective, with a 28% reduction (Table 3). Caution is required in the interpretation of the results due to the observational retrospective nature of the data. Survival rates were also higher with combination therapy. In the frontline setting, at six months and one year from diagnosis, 8% and 10% more patients respectively, were alive in the radiochemotherapy group than in the radiotherapy group (Table 2a.). Similarly, from relapse, at six months 27% more patients in the reirradiation plus or minus relapse chemotherapy group were alive than in the relapse chemotherapy group (Table 2b.).

Importantly all survival differences disappeared within one year after relapse, aside from a few outliers, with the disease remaining almost uniformly fatal. Data availability and sample sizes precluded any subgroup analysis on specific drugs or protocols and was outside the scope of this study. Information on available systemic therapy regimens can be found in Supplementary Table 1. Durable response and long-term survival, as suspected is not feasible using existing treatment modalities. Given the mostly palliative nature of current systemic therapy regimens, the use of chemotherapy must be carefully balanced with risk of toxicity in an effort to maintain optimal quality of life.[18] To what extent specific therapies contribute to survival and their effect on quality of life should be subject to further investigation ideally in the context of a randomized controlled trial.

A limitation of this study is the observational and retrospective design, in which indication bias might be present. In such case, an association between an individual treatment modality and survival could be a marker of favorable prognosis, rather than treatment efficacy.[19] To investigate potential bias by indication, using available data we examined age at diagnosis and symptom duration as surrogate markers of prognosis.[13] Median age at diagnosis differed between the frontline groups, namely due to the younger age of the untreated group (median 5.7 yr.), relative to the median of 7.5 yr. across all patients. This could portend that younger patients are less likely to receive treatment. Although infants were specifically excluded in this study to lessen the potential for younger age to act as a confounder.[20,21] In addition, the vast majority (72%) of patients were between 3-10 years of age, minimizing the impact of older age.[22] For symptom duration, most patients had a short symptom duration of less than six weeks (63%) and only 6% a long symptom duration of greater than 24 weeks (Table 1). This suggests our cohort is a robust representation of the DIPG patient population and comparable to other historical cohorts.[1,2,3]

Therapeutic efficacy has been difficult to discern in DIPG, given the heterogeneous comparisons between trials caused by inconsistent inclusion and exclusion criteria, unclear/differing endpoints, and small sample sizes.[23] Prior to the discovery of histone mutations and widespread utilization of biopsy, DIPG diagnoses in clinical studies, without MRI central review, included less aggressive pediatric brainstem

gliomas, artificially inflating survival estimates and largely explaining initial survival differences.[24] Central review by MRI, as performed in this study, has been proven to eliminate low grade gliomas and is consistent at eliminating atypical cases when performed by an experienced neuroradiologist.[11,25] The International and SIOPE DIPG/DMG Registries enable population-based research to be done for the first time in DIPG utilizing centrally reviewed MRI data.[26,27]

Despite the absolute need for randomized trials, historical controls remain relevant due to still low patient numbers in the vast majority of future DIPG trials. Large scale retrospective studies like the present one remain important in defining the setting and survival references of such future trials. In the pathway forward to cure primary brain tumors, it is required to rethink the design of clinical trials in general.[28] Recent innovation in adaptive clinical trial design, coupled with the introduction of the FDA "breakthrough therapy designation", holds the promise to greatly accelerate the regulatory approval process for promising agents/strategies found in early phase clinical trials.[29,30] By such an approach, historical control data from registry-based studies, like the present one, can aid the adaptive design and compensate for the lack of standard therapy in DIPG. The incorporation of historical control data, if comparable to trial participants, reduces variance and increases power in clinical studies.[31]

## CONCLUSIONS

For the first time, in a large retrospective analysis we show by using the landmark method how to deal with immortal time bias and provide robust estimate survival outcomes in DIPG. Population-based registries, such as the SIOPE and International DIPG/DMG Registries, that include trial and non-trial patients, are essential to identify patterns of response in these rare cancers. Multi-arm randomized clinical trials in an international, multi-institutional setting are needed to finally improve the fatal prognosis. Studies like the present one which better define survival outcomes, will help to avoid reiterative DIPG clinical trials by providing a representative historical reference point. Furthermore, survival data presented here may be helpful for treating physicians communicating with patient families who are considering a clinical course without any treatment or radiotherapy alone, versus progressive oncological treatment. Future studies should strive to incorporate quality of life parameters and balance the extension of survival with optimal quality of life.

## ACKNOWLEDGEMENTS

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# APPENDIX

**Supplementary Table** 1. Systemic therapy regimens (n=409).

| Regimen | *n* (%) |
|---|---|
| HIT-HGG-2007 | 145 (35.5) |
| HIT-GBM-D | 67 (16.4) |
| HIT-GBM-C | 36 (8.8) |
| HIT-GBM-B | 11 (2.7) |
| Temozolomide | 10 (2.4) |
| HIT-GBM-A | 6 (1.5) |
| Other chemotherapy | 6 (1.5) |
| Nimotuzumab | 3 (0.7) |
| Temozolomide+Nimotuzumab | 3 (0.7) |
| Erlotinib | 2 (0.5) |
| Sirolimus | 2 (0.5) |
| Vincristine | 2 (0.5) |
| Vinorelbine+Nimotuzumab | 2 (0.5) |
| Everolimus | 1 (0.2) |
| Methotrexate | 1 (0.2) |
| Temozolomide+Vinorelbine | 1 (0.2) |
| Temozolomide+Valproate | 1 (0.2) |
| N/A | 110 (26.9) |

**Supplementary Table** 2a. Number of events from diagnosis

| Status | No treatment | Radiotherapy | Radiochemotherapy |
|---|---|---|---|
| Alive | 0 | 1 | 11 |
| Deceased | 20 | 89 | 288 |
| Total | 20 | 90 | 299 |

**Supplementary Table** 2b. Number of events from relapse

| Status | No treatment | Chemotherapy | Reirradiation, +/-chemotherapy |
|---|---|---|---|
| Alive | 4 | 0 | 2 |
| Deceased | 96 | 191 | 49 |
| Total | 100 | 191 | 51 |

# 8

# General Discussion and Future Directions

## PROMISING DEVELOPMENTS

Diffuse intrinsic pontine glioma (DIPG), nowadays better known as H3K27M-altered diffuse midline glioma (DMG) of the pons, is an insidious disease. It continues to be an elusive tumor to treat with 2-yr survival rates under 10%.[1] Despite significant investment in clinical time and resources over the last decades, with over 200 clinical trials performed, survival has not increased.[2,3] Pediatric high-grade glioma (pedHGG) located in the brainstem continues to portend the worst survival.[4] The majority, up to 80% of pedHGGs are located in the midline, largely represented by DMGs.[5] Recently developed biologically and immunotherapy driven approaches show promise in early phase studies to begin changing this narrative.[6,7,8,9]

Panobinostat is a potent histone deacetylase (HDAC) inhibitor with strong preclinical evidence of restoring H3K27-methylation and normalizing gene expression.[10] However results published on the first phase 1 clinical trial in DIPG/DMG, PBTC-047, preliminarily demonstrate poor efficacy at achievable doses with a median OS of 11.8 months. The authors report poor brain penetrance as the likely cause of inefficacy.[11] PNOC-015 on the contrary delivered panobinostat locally via convection enhanced delivery (CED) and demonstrated an improved median OS of 26.1 months but only in a small cohort (N=7).[12] To overcome acquired treatment resistance to panobinostat a multipronged approach will likely be needed. One proposed synergistic approach is to combine panobinostat with an agent targeting the dysregulated energy metabolism, a likely player in acquired treatment resistance.[13]

ONC201 is the next agent in line showing very promising early-stage clinical efficacy, with an approximate doubling in survival relative to historical controls of 21.7 months.[14] DMG has been shown to be dopaminergic on $^{18}$F-DOPA PET.[15] The drug ONC201 or dordaviprone, is the first of its kind in a new class of targeted therapies called imipridones, which targets the dopamine receptor. This brain penetrant small molecule inhibitor demonstrates potential to be the first monotherapy to significantly improve survival in DIPG/DMG.[14] A phase 3 randomized, double-blinded, placebo-controlled trial is now underway internationally in children and adults (NCT05580562).[16] Pooled results from early phase DMG trials in the recurrent setting (excluding pontine tumors) show ONC 201 is well tolerated with a median duration of response from progression of 11 months, granted only an overall response rate of 20% was observed, and in predominantly H3K27M adult patients.[17]

Immunotherapy using CAR T cells and oncolytic viruses has also shown promise in early phase trials.[6,7] Clinical trials with CAR T cells are on-going, so far B7-H3- and GD2-specific CAR T cells show early signs of clinical efficacy however, the responses have not been universal.[6,18,19] In addition, acute neurotoxicity has been observed due to tumor associated inflammation within the brainstem.[20] Importantly with these

immunotherapy approaches, all patients still succumb to their disease, as with the other above-mentioned experimental approaches. Durable response is elusive. Acquired therapeutic resistance, tumor heterogeneity and drug delivery remain barriers to overcome in the development of curative therapeutics. Combination therapy will likely be required, yet there are now "tools in the tool belt".

More and more early phase clinical trials are building upon 'promising' preclinical research funded by a groundswell of financial support from patient family foundations. This funding has correlated with a reinvigorated field of scientific research into DIPG since 2012, as measured by the exponential increase in peer reviewed publications (Figure 1).[21] Much has changed in our understanding of DIPG tumor biology, diagnostic criteria, prognosis, response assessment and treatment approaches as a result.

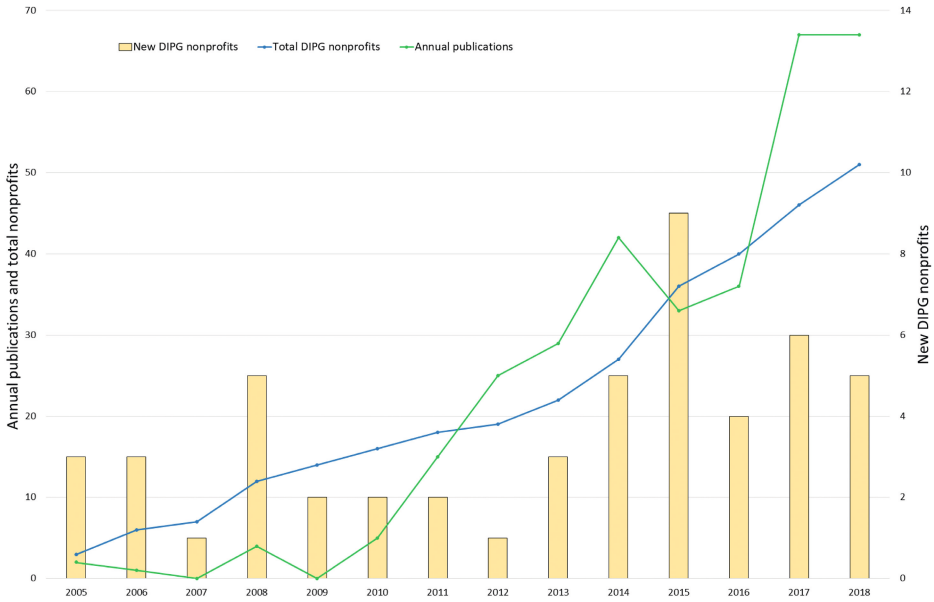


**Fig 1.** Establishment of DIPG focused non-profits and correlation with peer reviewed publications, as published in: Kuzan-Fischer CM, et al. J. Neurosurgery Pediatrics. 2019[21]

Another key factor in the acceleration of DIPG research is the formation of (inter) national trial consortia and collaborations connecting diverse researchers and clinicians in pediatric high-grade glioma to enable the accrual of adequate patient numbers, data and tissue resources to perform research in a rare disease. Clinical trial consortia investigating novel therapeutics in DIPG/DMG include PNOC, CONNECT, SIOPE, ITCC-Brain, PBTC and COG.

The SIOPE DIPG Registry is an example of such international collaboration. The project was constructed under the preexisting umbrella organization of the European Society for Paediatric Oncology (SIOPE) Brain Tumour Group, as part of the SIOPE DIPG Network. These early collaborations initiated the comprehensive collection of data on patients with DIPG across Europe to support a wide spectrum of interdisciplinary and translational projects.

## ESTABLISHMENT AND EXPANSION OF THE INTERNATIONAL AND EUROPEAN SOCIETY OF PEDIATRIC ONCOLOGY (SIOPE) DIPG REGISTRIES

In 2012, the SIOPE and International DIPG/DMG Registries were established jointly with the goal of improving the diagnosis, biological understanding, and treatment of DIPG, by providing an international research infrastructure, data and tumor resources. The SIOPE Registry collects retrospective patient data and enrolls patients from countries across Europe, as well as Russia, Turkey and Mexico, while the International Registry includes patients from North America, Africa, Asia, Australia, and South America. Together we developed a standardized set of case report forms (CRFs), which form the foundation of our clinical and imaging data collection and enable research at scale. Furthermore, in close collaboration we setup parallel research infrastructures to support the Registries in their North American and European contexts respectively. We then jointly published our Registry establishment papers (Chapters 2 & 3), to display our unified approach and joint aims.

To date, there are 1106 patients enrolled in the SIOPE DIPG/DMG Registry (634 retrospective and 472 prospective) from 23 sites, representing sixteen countries. The organizational structure laid out in the original publications remains intact and international research collaborations between the two Registries are on-going. At the time of the Registry establishment in 2012 there were few models available for creating a registry, much less a rare disease registry. The Breast Cancer Family Registry served as our model organizational structure, due to its proven capacity to support a wide spectrum of collaborative translational research projects.[22]

The IDIPGR and SIOPE DIPG Registries now together comprise the largest set of DIPG specific clinical, imaging and correlative molecular data available to researchers in the world, with a combined ~2200 patients as of August 2023.[23] This represents an enduring research data infrastructure capable of aiding the next generation development of therapeutics and ultimately the improvement of outcomes in patients with DIPG. Furthermore, it now serves as a model for enhancing clinical and translational research into a rare orphan disease.

## ROLE OF DIPG REGISTRIES

### Improved Characterization of DIPG

The joint collection of a uniform set of clinical, imaging and biologic data by the IDIPGR and SIOPE Registry has enabled high powered population-based research to be done for the first time, outside the context of a single country, institution or clinical trial analysis. This reduces selection bias by providing a more diverse representation of DIPG patient outcomes than those solely found in clinical trials, which tend to favor prognostically better patients (i.e., highly selective).[24] To avoid inclusion of non-DIPGs we employ radiological central review performed by a panel of experienced neuroradiologist, which has been shown to eliminate low grade gliomas and eliminate atypical cases not deemed to be DIPGs.[25,26]

So far, we have discovered or verified the following variables to influence prognosis. Positive prognostic factors at diagnosis include age less than 3 and greater than 10, longer symptom duration (i.e. greater than six months) prior to diagnosis (i.e., initial slow progression of the disease), H3.1 mutation status and systemic chemotherapy. Poor prognostic factors at diagnosis include a cranial nerve palsy, H3.3 mutation and on MRI, presence of ring enhancement, necrosis and extrapontine extension (Chapter 6).

Future studies with increasingly larger datasets and more power, can expand upon our mostly univariable models and investigate additional prognostic factors, particularly the emerging biologic factors implicated in tumor progression and treatment response. Since DIPG is a developmental disease in which biological/deterministic factors change over time and new mutations are acquired in response to treatment, it can be postulated prognostic models for DIPG need to be dynamic.[26,28,29] Dynamic biologic factors that are modeled as such, can perhaps better elucidate the high-level associations between treatment and survival in DIPG (particularly in the relapse setting) and improve prognostic model performance in DIPG.[30]



A retrospective analysis with matching or stratification using known clinical, imaging and biologic prognostic factors as mentioned above can also be performed to adjust statistically for confounders and strengthen evidence of a causal relationship.[31] Yet, evidence for prognostic factors is still developing and adjusting for variables potentially involved in the causal path can bias the results.[32] A stratified analysis using prognostic factors was outside the scope of this thesis work, however it should be performed in future analysis.

## Survival Outcomes

One key finding from our survival analyses is that systemic therapy is significantly associated with increased survival, relative to radiotherapy alone, which is often considered to be standard of (palliative) care (Chapter 6 & 7). A role for systemic chemotherapy has recently also been supported in adult H3K27M-altered DMG.[33] Interestingly, our data suggest that there may be a beneficial effect of chemotherapy for primary, as well as for relapse treatment in DIPG. Wolff et al. in one of the few studies investigating DIPG patient survival at relapse, also found a survival benefit in a portion of patients receiving relapse chemotherapy.[34] The overall ability to induce a treatment effect however seems to be lower after relapse. This could be explained by the lower cumulative doses of radiation that can be tolerated at relapse.[35] In addition, recent developments in cancer neuroscience postulate neural integration by malignant gliomas increases over time and aids tumor growth and resistance, making DIPG more difficult to treat at relapse.[36]

Therapeutic efficacy has been difficult to discern in DIPG/DMG, given the heterogeneous comparisons between trials caused by inconsistent inclusion and exclusion criteria, unclear/differing endpoints, and small sample sizes.[37] Key reference studies in pedHGG such as DIPG/DMG report difficultly discerning between progression and pseudoprogression on MRI, challenging the assessment of treatment response.[26,38-40] Clinical reviews have stated chemotherapy has no effect citing differing historical clinical trial reference points, contemporary to the time of publication.[41-44]

During this time the clinical definition of DIPG has evolved from a clinicoradiologic diagnosis to a molecularly defined tumor entity and subsequently with it, trial inclusion/exclusion criteria (Chapter 4). Prior to the discovery of histone mutations and widespread utilization of biopsy, DIPG diagnoses in clinical studies, without MR central review, included less aggressive pediatric brainstem gliomas, artificially inflating survival estimates and largely explaining initial survival differences.[45] The issue of misdiagnosis is also evident in the epidemiological literature.[46]

Pediatric pontine gliomas diagnosed by CT and treated with standard of care radiation until 1986, survived 9 months and untreated 5 months.[47] With the advent of MRI in the 1990s, and incorporation of neuroradiologic definitions, (e.g. >50% pontine involvement) patients diagnosed until 2005, had a median OS of 8-11 months, suggesting small survival differences by treatment strategy.[36] From 2005 until 2012 median OS ranged from 7–14 months.[48] And until 2018 in a recent systematic review by Gallitto et al. encompassing all radiation regimens, patients with concomitant systemic therapy had an OS of 11.5 months, in comparison to 9.4 months for radiation only patients.[49] All studies limited inclusion to patients with >50% pontine involvement, as a minimal neuroradiologic definition.

Using the SIOPE DIPG Registry, we can better define historical survival outcomes (Chapter 7). Median survival times observed in our cohort provide a baseline reference value (or historical control) for commonly used treatment modalities, such as temozolomide, from which new investigational therapies can be compared. Furthermore, we report survival in patients who elect not to receive therapy and those who die very shortly after diagnosis. These patient populations are not captured in clinical trials. Prior reference values for survival outcomes were largely based on small non-randomized, mostly single institution clinical trials.[37,50]

## Historical Controls for Clinical Trial Development

The use of chemotherapeutics in the field has not advanced substantially, over the last three decades, in part due to the issues mentioned above, but also a lack of innovation in clinical trial design. Randomized controlled trials are needed to determine to what extent specific chemotherapies may contribute to survival and their effect on quality of life. Yet randomization to a control arm outside of standard of care is unethical in the context of a fatal prognosis. For these reasons, single-arm trials without a concurrent control arm have made up the majority of early phase trials in DIPG.

An over reliance on single-arm trial designs has been suggested as a leading factor for the lack of successful trial development in neuro-oncology. Single-arm designs do not account for differences between populations or different standards to assess outcomes across trials, nor do they control for biases.[51] External controlled clinical trials using historical control data, offer an alternative design. The incorporation of historical controls, if the comparable to trial participants, can reduce variance, increase power and improve trial efficiency, thereby reducing the number of patients needed.[52] External control data from Registries should be used in the design of several externally augmented trial designs outlined below (Figure 2). Perhaps the most applicable design to the current DIPG/DMG trial environment is the externally controlled single-arm design.



Using an externally controlled single-arm design (Figure 2c), statistical adjustments like matching can be used to account for baseline differences between the historical control and experimental group. This reduces bias in comparison to standard single-arm trials. As an example, the aforementioned early phase 'successes' for ONC201 and CAR-T cell therapies should be considered with caution, as these studies will be subject to different biases, given their single arm designs. Using Registry data, a matched-pair control analyses can be performed to potentially reduce some of this bias. Furthermore, during the analysis, treatment effects can be estimated directly between the experimental and external controls using patient level data rather than extrapolating using a published benchmark. Historical controls can also be used to inform the interim trial analysis, but these designs are still in the exploratory phase.[53,54]

In the pathway forward to curing primary brain tumors, it is required to systematically confront existing faults within the research pipeline. It entails rethinking the design of clinical DIPG trials in general.[55] Due to the lack of an adequate standard treatment and the high desire for improvement of the fatal prognosis, the use of innovative designs using historical controls is ideally suited. Historical control data from registries-based studies (Chapter 7) demonstrate the potential to inform trial design and compensate for the lack of standard therapy in DIPG.

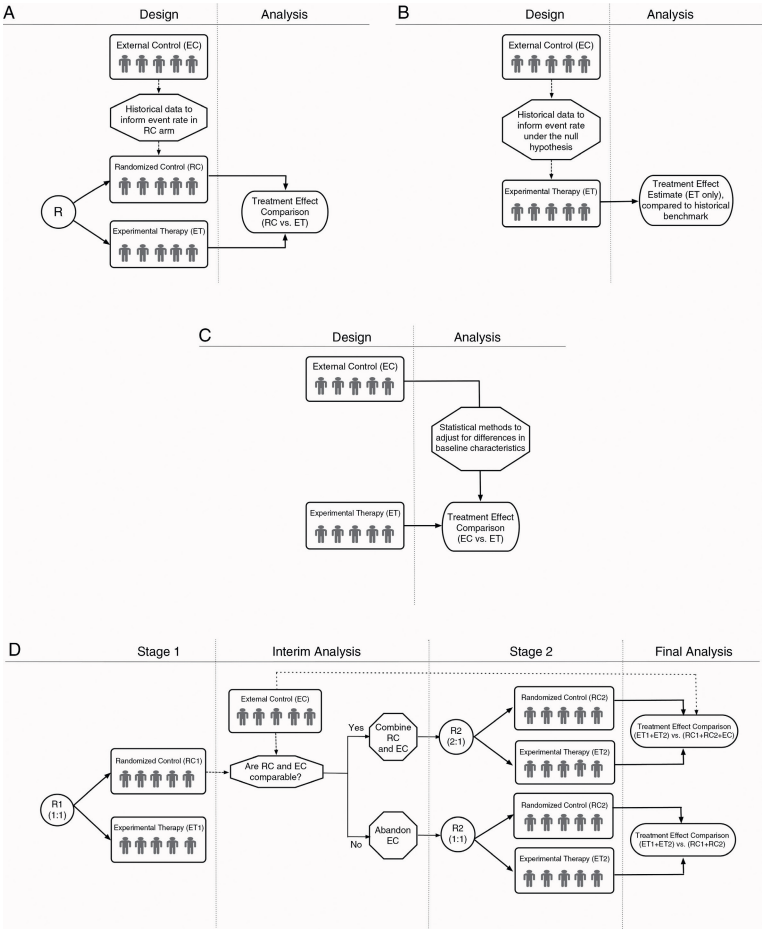


**Fig 2.** Clinical Trial Designs Using Historical Controls, including A) randomized control, B) single-arm design, C) externally controlled single-arm design, D) externally augmented design as published in: Polley MC et al. Neuro-Oncology 2024.[53]

Future trials will mandate biological subgrouping based on the presence of histone mutations. However, the IDIPGR and SIOPE Registries now also incorporate DMGs and capture available pathogenomic data from these patients. This allows our historical

cohorts to remain relevant in informing on-going and future DIPG/DMG clinical trials. Historical controls in the absence of biopsy remain relevant as well. At present 70% of pediatric patients with brainstem high grade glioma are confirmed radiographically in the United States.[5] A biopsy still requires a delicate and invasive surgery to be performed in a specialized center, and preferably in the context of a clinical trial.[56] Minimally invasive liquid biopsy techniques using circulating tumor DNA are currently in development.[57,58]

Until there are effective therapies for DMG based on the presence of the H3K27M mutation a biopsy is purely investigative. The neuroradiological classification of DIPG therefore remains clinically significant and diagnostically relevant, particularly in a resource limited setting without access to specialized neurosurgical suites and molecular diagnostics. By capturing the full spectrum of the disease, both phenotypic DIPG and genotypic DMG, the Registries are uniquely positioned to investigate the diagnostic transition and resulting survival trends.

## Reducing Global Health Disparities

Despite having an estimated 80% of the global cancer burden, low- and middle-income countries account for only 5% of global spending on cancer care.[59] In 2020, it was estimated there were 413,000 cases of childhood cancer worldwide, of which 181,000 were undiagnosed. Between 2020 and 2050, models estimate a total of 11.1 million children will die from cancer, and 9.3 million (84%) will be from low-income and lower-middle-income countries.[60] Underdiagnosis and late diagnosis are key contributors to disparities in pediatric cancer outcomes (Figure 3).[61,62]

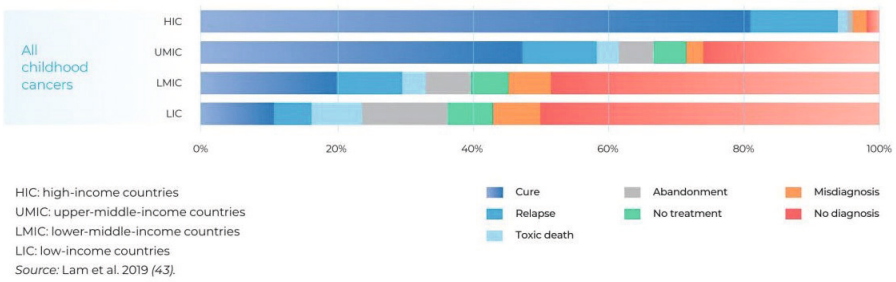


**Fig 3.** Factors contributing to lower survival in low-middle income countries., as published in: CureAll framework: WHO Global Initiative for Childhood Cancer. Increasing access, advancing quality, saving lives. Geneva: World Health Organization; 2021[62]

Access to effective diagnostics is a well-documented problem in low- and middle-income countries.[63] A 2016 survey by the International Society of Neuropathology (ISN) found

25% of the countries participating and 79/314 neuropathology centers (25%) declared not to have access to molecular diagnostics for brain tumors. Furthermore, 12% of the neuropathologists surveyed claimed to be unfamiliar with molecular techniques.[64] A lack of diagnostics is similar to a lack of technology. It stems from a lack of availability, accessibility or acceptability.[65]

Lack of access to advanced diagnostics and neurosurgical techniques, particularly in under resourced settings, necessitates a bifurcation in terminology between DIPG and DMG (Chapter 4). Many children suspected of a brainstem tumor centered in the pons, in the absence of a biopsy, are still diagnosed as a DIPG based on clinical presentation and MR-imaging alone.[56] Changes in diagnostic criteria can have important implications for global health in terms of disease surveillance, assessment, and delivery of timely and evidenced based clinical care.

Advanced classification systems alienate the pediatric populations lacking access to diagnostic technology at a time when they are desperately needed to be recruited into international trials. Emerging diagnostic technologies, such as Nanopore Sequencing during surgery will allow for cheap and real-time genomic sequencing, but we are in a transition phase.[66] Much work needs to be done to bridge this diagnostic gap and include a large part of the world where the majority of future cancers will occur. Inclusion of patients from non-Western countries in trials is needed.

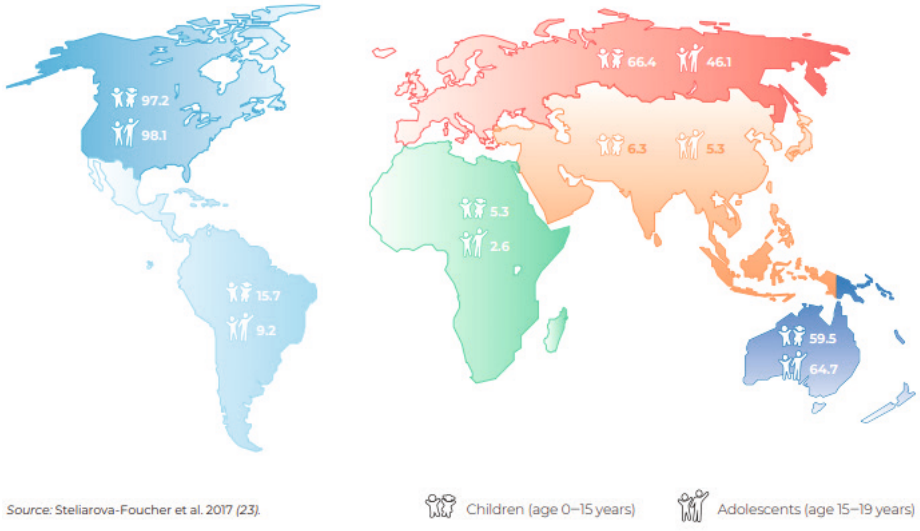


**Fig 4.** Percentage of childhood cancer registration around the world as published in: CureAll framework: WHO Global Initiative for Childhood Cancer. Increasing access, advancing quality, saving lives. Geneva: World Health Organization; 2021.[62]

For global health, registries represent a pillar of evaluation and monitoring in health systems. At present in most countries around the world, outside of very high-income countries, cancer registries do not exist (Figure 4).[62] To bridge disparities in cancer care and control worldwide, bold research, financing, and implementation agendas are needed.[59] Two major actions, which can lessen pediatric cancer outcome disparities, include expanding cancer networks and population-based cancer registries to include low- and middle-income countries. Participation in these organizations increases access to diagnostic services, treatments, and fosters research.[60]

In pediatric HGG, rare disease registries that also function as networks, such as the SIOPE DIPG Registry and the International DIPG/DMG Registry, provide promising avenues to increase inclusion of low- and middle-income countries. These organizations provide an infrastructure and international network of neuro-oncology expertise. In collaboration with organizations like the WHO, cancer registries/networks can aid the rapid deployment of neuropathological expertise, molecular diagnostics, and treatments for high grade gliomas.

## FUTURE DIRECTIONS

### Enhance Data Sharing Collaborations

The exponential creation of health care data coupled with the development of machine learning and AI tools has revolutionized the ability to query large complex amounts of data, garnering the interest of research institutions, industry, and technology companies alike, who seek to abstract value from these data. As a result, in recent years there has been a proliferation of so called "health data collaborations". These collaborations are part of the broader movement towards data sharing to empower research gathered in diverse information systems with a standardized set of criteria and central review process.[67]



Within pediatric high-grade glioma, data collaboratives now include CBTN, primarily collecting genomic data (but also correlative clinical information) and Primage, focused on applying AI to medical imaging (but also correlative clinical information).[68,69] These initiatives should be symbiotic, offering useful expertise in adjacent fields to enhance the utility of the Registry's clinical and imaging data, but we have yet to join forces. There are now more than 20 glioma specific registries, with their own research aims and resources.[70]

In pediatric cancer, registries are mostly sponsored by pediatric societies and funded by non-profits or government agencies due to the rarity of the disease. When compared to adult cancer, pediatric cancer research is drastically underfunded.[71] And pediatric

cancer registries, are commonly grouped into a bucket with non-essential research protocols, given their indirect and long-term impact on patients. The need to work together and avoid duplication of resources and efforts is especially pertinent in pediatric neuro-oncology.

## Maturation of a Rare Disease Registry into a Learning Health System

To maximize the impact of the SIOPE DIPG Registry, data sharing activities need to be enhanced both externally with partners such as those mentioned above but also internally. The Registry should be embedded within a "learning health system".[72] In such a model the database is at the center of a research network or clinical trial consortium, providing the data infrastructure to support a wide array of clinical and translational research. Each patient is prospectively enrolled (if consented) and their treatment and patient journey is captured in real-time, whether on or off trial. These patient experiences are then aggregated into learnings disseminated back to the clinical teams to actively improve the quality of their care (Figure 5).[73,74]

An example of learning health system is the ImproveCareNow Network (ICN) aimed at improving outcomes for children and adolescents with inflammatory bowel disease. The ICN Registry supports the network by collecting standardized data at enrollment, all follow-up visits, hospitalization, and when the patient discontinues participation. Data are then used to generate patient management reports and to monitor the quality of care throughout the patient's journey with comparative performance metrics, such as patient outcomes and patient reported quality of life. These metrics are actively monitored by quality improvement specialists to identify areas of improvement. As a result, the ICN Network has improved remission rates for patients with Crohn's disease from 55-68% and in ulcerative colitis from 61-72%.[75]

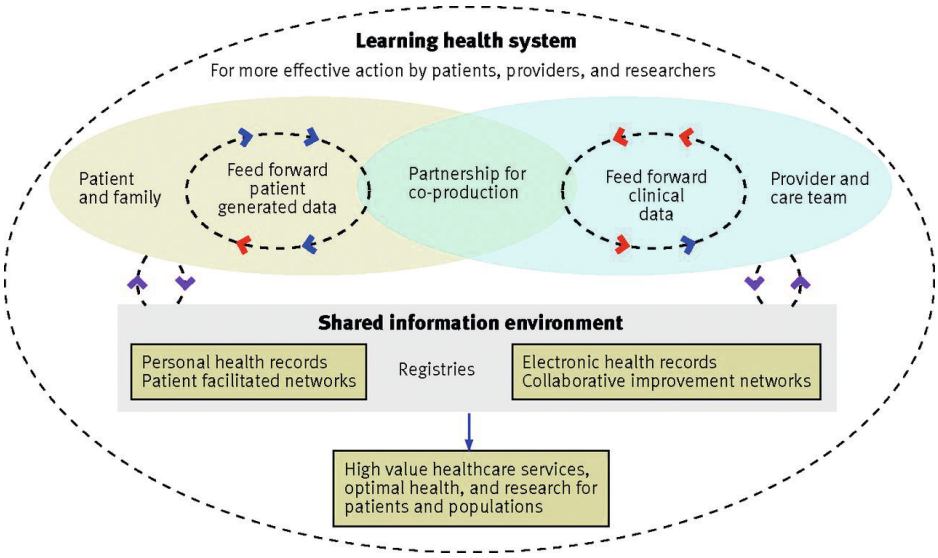


**Fig 5.** A model learning health system as published in Nelson et al. BMJ 2016[74]

Data from the ICN Registry are used to support wide array of clinical research, including pragmatic randomized clinical trials. The COMBINE study is one such example. This multicenter, randomized, double-blind, placebo-controlled pragmatic trial compared the effectiveness of two commonly used treatments for Crohn's disease with and without the addition of methotrexate was recently published.[76] Results of this study will directly affect clinical care in the more than 75 centers participating in the ICN Network. Furthermore, by using the network's registry to support the trial, funding for the COMBINE trial helped bolster development of the database and improved quality assurances measures.



The learning health systems model offers a proven methodology for improving patient outcomes in a pediatric population and can be emulated in DIPG. The maturation of a rare disease network and registry into a comprehensive translational research infrastructure (i.e., learning health system) enabling clinical studies, capturing clinical outcomes and identifying areas for improvement is a logical evolution for the SIOPE DIPG/DMG Registry.

Relying solely on centers to input their data without offering learnings in return, as is now the case, is an unsustainable model for growth. Institutions will continue to withhold sending their data until it is no longer useful for their research purposes, by which point patients with DIPG/DMG are typically deceased and interventions and novel studies are impossible. If adjustments are not made, the SIOPE DIPG Registry

will remain a retrospective observational database, and not realize the potential of a prospective Registry/network capable of improving outcomes.

Ideally creation of a new network would not be required given the long and arduous funding and legal process. Preexisting pediatric high-grade glioma networks could be used such as PNOC or SIOPE in the European context and CBTN or CONNECT in the United States, Canada and Australia. Or the SIOPE DIPG Network, already the umbrella platform in principle for the SIOPE DIPG Registry, could suffice but it would need to become a legal entity. Alternatively, development of a joint network/database, shared between the International and SIOPE DIPG Registry, could service the larger international community and their collaborative partners. Due to privacy law these data could still be physically housed separately but then merged seamlessly in the cloud. Technology to enable such a data construct is already under development in collaboration with the SIOPE DIPG Registry.[77,78]

## Areas for Improvement

Over the years since the development of the SIOPE Registry, a core team was established including, additional to non-profit input by researchers, a data manager and project manager. Now, enrolling across 23 sites in 16 countries, the core team is limited in capacity. To facilitate further improvement of the SIOPE Registry it is essential to expand the core team with help of institutional support and adequate funding, as running a large international, multi-institutional Registry requires a wide range of expertise and assistance in data management, monitoring, regulatory, contracts, finance, project management, coordination and execution.

Staffing issues still need to be resolved for the Registry to realize its ambition. The adoption of newer database technology can drastically cut the effort required for data quality checks and reporting through automation. Furthermore, increased pooling of resources and expertise through collaboration with the International DIPG Registry can greatly enhance the speed and research impact of both DIPG Registries.

To re-establish close collaborations a 5-year update of the long-term survivor study (Chapter 6) is underway, with potential inclusion of approximately 2200 patients. This study seeks to further elucidate prognostic factors associated with increased survival, determine the role of re-radiation in survival and examine the effect of socioeconomic status. We have also established quarterly shared executive committee meetings to discuss on-going issues and opportunities. A shared scientific advisory committee and centralized review process for research proposals is under development to avoid research duplication and find synergies.

## Concluding Remarks

To make clinically meaningful steps towards curing primary brain tumors, we must systematically confront existing faults within the research pipeline. This includes improving upon an incomplete understanding of brain tumor biology, enhancing drug delivery, and bridging the divide between preclinical drug development and testing in clinical trials.[55] It entails rethinking DIPG/DMG clinical trial design and improving the scale of supportive Registries.

If empowered, rare disease registries like the IDIPGR and SIOPE Registry, offer an essential piece of research infrastructure, by sourcing data and tumor resources capable of supporting innovations to improve the diagnosis, biological understanding and treatment of DIPG and DMG internationally. With adequate support and evolution, these projects can improve access to care and the quality of outcomes achievable in pediatric high-grade glioma and aid the reduction of global health disparities.

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# 9

# English Summary/ Nederlandse Samenvatting

This thesis describes the clinical and translational landscape of DIPG, viewed primarily through the lens of the DIPG/DMG Registries. I aim to 1) describe the development and establishment of these Registries, 2) discuss the diagnostic evolution of DIPG to DMG, a transition with clinical implications and divisions along socioeconomic lines, and 3) to demonstrate the capability of the Registry to characterize the prognostic factors underlying survival in DIPG.

## PART I: ESTABLISHMENT OF THE SIOPE AND INTERNATIONAL DIPG/ DMG REGISTRIES

In Part I, I describe the development and establishment of the DIPG Registries, as a model for accelerating research into a rare disease. Collectively these registries represent an important piece of a research infrastructure mechanism capable of "assessing the landscape" of this rare disease.

**Chapter 2**: In a rare orphan disease like DIPG, scientific progress and development of effective therapies have often been impeded by the lack of large scale, well-annotated, clinico-radiologic and biologic data available about the disease. In 2012, international investigators banded together to establish the International DIPG Registry (IDIPGR) and a parallel European SIOPE Registry. In this paper, we report the logistical challenges, pitfalls, and successes of developing this registry, which we hope will serve as a model for other orphan disease registries.

The IDIPGR consists of the operations center, a steering committee, scientific advisory committee, research ethics panel, quality assurance group, and collaborating institutions. Cincinnati Children's Hospital Medical Center (CCHMC) is the Operations Center and repository for all clinical and neuroimaging data and pathology specimens from collaborating institutions. The steering committee serves as the governing board, providing oversight of the IDIPGR.

There are two principal mechanisms for identification and recruitment of participants (a) self-referral by patients and their families via the DIPG Registry website or (b) procurement of deceased patient records from participating institutions, after Institutional Review Board (IRB) approval or non-human subjects' determination.

From April 2012 to December 2016, 670 patients diagnosed with DIPG were enrolled from 55 participating institutions in the US, Canada, Australia and New Zealand. The radiology repository contained 3558 studies from 448 patients. The pathology repository contained tissue on 81 patients with another 98 samples available for submission.

The IDIPGR provides the infrastructure for acquisition of biological specimens, imaging, and correlative clinical and genomics data to facilitate basic and translational

research studies in this rare disease. The increased availability and centralization of data and specimens from DIPG patients, and the effective collaboration among clinical, translational and basic researchers as well as philanthropic foundations represent a welcome paradigm shift in DIPG research in which data and tumor specimens are no longer rate-limiting resources.

**Chapter 3**: Collaboration and data sharing are promising strategies for tackling rare diseases, by facilitating uniform and hypothesis-driven research. To overcome the current lack of data and improve the integration, speed, quality, and coherence of research, we aimed to create a DIPG research-infrastructure consortium and initiate collaborative collection of comprehensive data on DIPG patients. This paper describes the methodology of the set-up of an international research network infrastructure, the SIOPE DIPG Network and SIOPE DIPG Registry, including legal and IT aspects, as well as preliminary patient inclusion data.

The SIOPE DIPG Network was established as a sub-committee of the high-grade glioma (HGG) working group of International Society of Paediatric Oncology Europe (SIOPE). The SIOPE DIPG Network is comprised of (i) an executive committee, (ii) a group of scientific advisors, (iii) National Coordinators (NCs) and (iv) members. The Executive Committee (i) manages and controls the DIPG Network and abides by and enforces the mission and the core values of the Network. The establishment of a DIPG registry was set as first project of the Network, with the purpose to include clinical, biological and centrally reviewed radiology data of patients with DIPG, both in and outside clinical trials.

The SIOPE DIPG Registry is composed of an online database for clinical data, and an imaging repository. To allow for the inclusion of uniform data, standardized electronic case report forms (CRFs) were developed by the SIOPE DIPG Network, in coordination with colleagues from the International DIPG Registry. Each country represented in the SIOPE DIPG Network is responsible for delivering their own data to the SIOPE DIPG Registry and Imaging Repository upon ethical approval. As of April 2016, six countries have submitted retrospective data of 694 patients to the SIOPE DIPG Registry and Imaging Repository.



## PART II: THE SHIFTING DIPG/DMG LANDSCAPE

The diagnostic evolution of DIPG to DMG represents a transition from a reliance on clinico-radiographic factors (phenotypic) to a molecular based (genotypic) diagnosis. I investigate the implications for global health of implementing advanced molecular diagnostics and furthermore the clinical implications, as described by specialists in pediatric neuro-oncology.

**Chapter 4**: We sought to address if the implementation of molecularly defined entities into clinical practice is adequately and equally perceived to be of added clinical benefit and supported by neurooncological professionals worldwide. We collected input from nearly 500 neurooncological experts treating patients with pediatric high-grade glioma (pedHGG). These specialists represented 53 countries and eight disciplines.

Our findings demonstrated an overall greater reliance and favorability among very high HDI (human development index) country participants to genetic testing. The process of adoption and adaptation has not been the same in countries with a highly developed national health system, as it has been in countries with much fewer financial and medical resources. Results of this survey are the first to document international differences in implementation along socioeconomic lines of the 2016 revised 4$^{th}$ edition, where molecular diagnostics were introduced for the first time.

In the context of molecular diagnostics for CNS tumors, evident in our survey is that they are in fact available and accepted, however not internationally accessible. We demonstrate the mechanisms to introduce a genetic layer of neuropathological diagnoses have not been sufficient so far to bridge the resource gap in a large part of the world. As a result, many centers in lower income settings cannot adequately diagnose pediatric high grade glioma patients *per* the WHO 2016 criteria. Suggestive from our survey, the WHO Classification of CNS Tumors can play a role in perpetuating or eliminating disparities within the neuro-oncology community. We document how disparities in access to molecular diagnostics can shape the implementation of the WHO 2016 tumor classification, and how perspectives towards diagnosis and treatment can differ in resource constrained settings during the molecular era.

**Chapter 5**: We performed an additional analysis on the survey results from Chapter 4, regarding implementation of molecular diagnostics in the WHO CNS4, to specifically examine the different perceptions and experiences between two key players in pedHGG management, pediatric neuro-oncologists and neuropathologists. We then assessed and discussed if and how the various issues raised within our survey we have been addressed in the 5$^{th}$ edition of the WHO CNS Tumor Classification (CNS5), introduced in 2021.

Our results highlight neuropathologists as representatives for the focus on the scientific state of the art diagnostics, and the pediatric neuro-oncologists with their special focus on clinical needs. Neuro-oncologists reported having issues with the introduction of new tumor types, renaming or abolishment of established tumor types, while neuropathologists did not. Neuro-oncologists also cited diagnostic definitions being difficult to explain to patients and families. Neuro-oncologists and neuropathologists however agreed on the points that insufficient diagnostic definitions were available for

molecular-based entities in 2016 and that these entities were less relevant for pediatric cases.

Interestingly, many of the issues raised in our survey are mirrored by the changes made in the 2021 CNS5. In 2016 CNS4, some arguably clinically relevant pedHGG tumor types like non-diffuse pilocytic astrocytoma, IDH-wildtype diffuse pedHGG and diffuse pedHGG in infants younger than 3 years of age were not included but are now specifically addressed. "Entities" not included in the CNS5, DIPG and gliomatosis cerebri, are both imaging-defined. In our survey, generally more pathologists accepted the removal of the designation "gliomatosis cerebri" than oncologists. This was also the case with DIPG. Neuro-oncologists were in favor of re-establishing the option of the previous clinical radiological diagnosis of DIPG, in addition to the sole option of setting the DMG diagnosis by biopsy only. Why imaging defined tumor types like DIPG are not incorporated in the CNS5 is based on the decision that the WHO classification follows a tissue-based approach.

In the quest to classify pediatric high-grade gliomas utilizing the most up to date research, the WHO CNS classification has made substantive improvements in incorporating molecular information into the diagnosis of several tumor types. Our study underlines the ongoing need to balance advances in the understanding of the biology of CNS tumors with meaningful clinical impact, but also reassures the substantial improvement for definition and diagnostics of pedHGG within the latest WHO classification. Increased multidisciplinary representation within working groups such as the cIMPACT-NOW, with more neuro-oncologists, neuroradiologists, and others involved in the treatment of brain tumor patients could help improve clinical translation.

## PART III: IMPROVED CHARACTERIZATION OF DIPG USING THE SIOPE DIPG/DMG REGISTRY



Using the SIOPE DIPG Registry as a high-level epidemiological tool to "survey the land", capable of improving the characterization of prognostic factors underlying survival in DIPG/DMG

**Chapter 6:** Long-term survival (LTS) in DIPG is historically defined as overall survival (OS) >2 years and characteristics associated with longer survival include younger age, longer symptom latency, and absent ring enhancement on diagnostic magnetic resonance imaging. In the first large-scale collaborative Registry we sought to further define clinical, radiologic, histologic, and molecular factors associated with short- and long-term survival among 1008 patients, the largest cohort of centrally reviewed DIPGs to date.

Median overall survival time was 11 months (interquartile range, 7.5 to 16 months). Median survival times of LTSs were 33 months (range, 24 to 156 months). Age <3 or >10 years, longer symptom latency, lack of CN palsy, and systemic therapy at diagnosis were predictors of long-term survival. Our findings also confirm the independent association of H3.1 K27M and H3.3 K27M with long- and short-term survival, respectively.

Neoadjuvant or adjuvant systemic therapy correlated with long-term survival in both univariable and multivariable analyses. This finding differs from the long-standing view that systemic therapy provides no survival benefit for DIPG, a principle largely based on small, nonrandomized clinical trials. Effective cross comparison of therapeutic studies for DIPG has been hindered by wide variation in inclusion criteria.

To our knowledge, 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 subgroups and emphasizes patient subsets from whom the most could be learned from analyzing pretreatment biopsy tissue. Understanding biologic differences that confer survival advantage in DIPG paves the road toward development of subgroup-specific therapies that, when implemented in the context of clinical trials, may improve outcomes for this devastating disease.

Chapter 7: Frontline radiotherapy (RT) for diffuse intrinsic pontine glioma (DIPG) is generally considered the only proven effective, albeit palliative, treatment. The role of systemic chemotherapy, both concomitant and/or adjuvant to radiotherapy however is a subject of debate. Our aim is to examine six historical treatment paths and investigate their association with survival. Data were collected on 409 patients using the German Society of Pediatric Oncology and Hematology (GPOH) HIT-HGG trial database and the SIOPE DIPG/DMG Registry.

Unique to our survival analysis is the inclusion of patients who received limited treatments (i.e., no treatment, irradiation only at diagnosis and no additional treatment at relapse). We report survival in patients with no treatment, radiation only at diagnosis and no treatment at relapse and compare these limited treatments with more intensive treatment. This allows for comparison to a more diverse set of patient outcomes. To conduct this project, survival outcomes were examined across six treatment modalities, three in the frontline setting and three at relapse. At diagnosis, modalities included I) no treatment, II) RT alone, and III) RT-chemotherapy. At relapse, modalities included I) no additional treatment, II) chemotherapy, and III) re-RT, +/- chemotherapy.

Median overall survival (OS) was 11.2 months (95%CI, 10.5-11.9), estimated from diagnosis. OS with no frontline treatment was 3.0 months, 10.4 months with RT alone,

and 11.7 months with RT-chemotherapy. Median survival after first progression (PPS) was 4.1 months (95%CI, 3.5-4.7). PPS was 2.2 months with no relapse treatment, 4.4 months with chemotherapy alone and 6.6 months with reirradiation +/- chemotherapy. The hazard ratio (HR) for no treatment, estimated with a Cox model from diagnosis, was 3.65, and RT alone 1.34, relative to RT-chemotherapy. From relapse, no treatment had a HR of 1.44, and reRT +/- chemotherapy a HR of 0.72, relative to chemotherapy.

Although not evidenced in a randomized fashion, our data suggest treating patients with DIPG with radiotherapy is beneficial, and additional chemotherapy to RT, both concomitant and/or as post-radiotherapy maintenance treatment, seems to prolong survival. In addition, our survival data suggest combination therapy is most effective in extending survival in both the primary and relapse settings. Importantly, all survival differences disappeared within one year after relapse, aside from a few outliers, with the disease remaining almost uniformly fatal. This study suggests what survival benefits may be gained by which general therapeutic approach, as a first step to quantifying survival differences observed in a historical DIPG cohort.

Novel to our study design is the use of the landmark methodology, in which the date of first relapse was used as a landmark time point. Post progression survival (PPS), rather than OS from diagnosis was then used to evaluate relapse treatments. This design is both clinically relevant and easily transferable to other observational DIPG studies investigating relapse treatments. This method allowed us to gain more insight into what survival can be attributed to relapse therapies and, consequently, better define overall survival for frontline therapies.

For the first time, in a large retrospective analysis we show by using the landmark method how to deal with immortal time bias and provide robust estimate survival outcomes in DIPG. Studies like this one which better define survival outcomes, will help to avoid reiterative DIPG clinical trials by providing a representative historical reference point. Furthermore, survival data presented here may be helpful for treating physicians communicating with patient families who are considering a clinical course without any treatment or radiotherapy alone, versus progressive oncological treatment.

## NEDERLANDSE SAMENVATTING

Dit proefschrift beschrijft het klinische en translationele landschap van DIPG, voornamelijk bekeken door de lens van de DIPG/DMG-registers. Mijn doel is om 1) de ontwikkeling en opzet van deze registers te beschrijven, 2) de diagnostische evolutie van DIPG naar DMG te bespreken, een transitie met klinische implicaties en verdeeldheid langs sociaaleconomische lijnen, en 3) de potentie van het register om prognostische factoren voor overleving in DIPG te karakteriseren, aan te tonen.

### DEEL I: OPZET VAN DE SIOPE EN INTERNATIONALE DIPG/DMG-REGISTERS

In Deel I beschrijf ik de ontwikkeling en opzet van de DIPG-registers als een model om onderzoek naar een zeldzame ziekte te versnellen. Gezamenlijk vormen deze registers een belangrijk onderdeel van een onderzoeksinfrastructuur dat in staat is om "het landschap" van deze zeldzame ziekte te karakteriseren.

**Hoofdstuk 2**: Bij een zeldzame weesziekte zoals DIPG zijn wetenschappelijke vooruitgang en de ontwikkeling van effectieve therapieën vaak belemmerd door het gebrek aan grootschalige, goed geannoteerde, clinicoradiologische en biologische gegevens over de ziekte. In 2012 sloegen internationale onderzoekers de handen ineen om het 'International DIPG Registry' (IDIPGR) en een parallele Europese SIOPE Register op te zetten. In dit artikel doen we verslag van de logistieke uitdagingen, valkuilen en successen bij de ontwikkeling van dit register, waarvan we hopen dat het als voorbeeld zal dienen voor andere registers voor weesziekten.

Het IDIPGR bestaat uit het operationeel centrum, een stuurgroep, een wetenschappelijk adviescomité, een onderzoeksethisch panel, een kwaliteitsborgingsgroep en samenwerkende instellingen. Het 'Cincinnati Children's Hospital Medical Center' is het operationeel centrum en het opslagpunt voor alle klinische en neuroradiologische gegevens en biologisch materiaal van patiënten uit elk van de samenwerkende instellingen. De stuurgroep fungeert als het bestuursorgaan en houdt toezicht op het IDIPGR.

Identificatie en werving van deelnemers verloopt via twee hoofdroutes: (a) zelfverwijzing door patiënten en hun families via de website van het DIPG-register of (b) verwerving van dossiers van overleden patiënten van deelnemende instellingen, na goedkeuring van de Medisch Ethische Toetsingscommissie (METC) of na niet-WMO (Wet Medisch-wetenschappelijk Onderzoek met mensen) verklaring.

Van april 2012 tot december 2016 werden 670 patiënten met de diagnose DIPG geïncludeerd vanuit 55 deelnemende instellingen in de VS, Canada, Australië en Nieuw-

Zeeland. Het radiologische archief bevatte 3558 onderzoeken van 448 patiënten. Het pathologische archief bevatte weefsel van 81 patiënten met daarnaast 98 beschikbare monsters voor indiening.

Het IDIPGR biedt de infrastructuur voor het verzamelen van biologische materiaal, beeldvorming en bijbehorende klinische en genomische gegevens, waarmee fundamenteel en translationeel onderzoek naar deze zeldzame ziekte kan worden gefaciliteerd. De toegenomen beschikbaarheid en centralisatie van gegevens en biologisch materiaal van DIPG-patiënten, en de effectieve samenwerking tussen klinische, translationele en fundamentele onderzoekers, evenals filantropische stichtingen, vormen een welkome paradigma verschuiving in DIPG-onderzoek waarbij beschikbaarheid van gegevens en weefsel monsters niet langer een beperkende factor zijn.

**Hoofdstuk 3**: Samenwerking en gegevensuitwisseling zijn veelbelovende strategieën voor het aanpakken van zeldzame ziekten, door het faciliteren van uniform en hypothese-gedreven onderzoek. Om het huidige gebrek aan gegevens te overkomen en de integratie, snelheid, kwaliteit en samenhang van onderzoek te verbeteren, beoogden we een DIPG onderzoeksinfrastructuur consortium op te zetten en de gezamenlijke verzameling van uitgebreide gegevens van DIPG-patiënten te initiëren. Dit artikel beschrijft de methodologie van het opzetten van een internationaal onderzoeksnetwerk, het SIOPE DIPG-netwerk en het SIOPE DIPG-register, inclusief juridische en IT-aspecten, evenals voorlopige patiëntgegevens.

Het SIOPE DIPG-netwerk werd opgericht als een subcommissie van de werkgroep voor hooggradige gliomen (HGG) van de 'International Society of Pediatric Oncology Europe' (SIOPE). Het SIOPE DIPG-netwerk bestaat uit (i) een uitvoerend comité, (ii) een groep wetenschappelijke adviseurs, (iii) nationale coördinatoren (NC's) en (iv) leden. Het uitvoerend comité (i) beheert en controleert het DIPG-netwerk en volgt en handhaaft de missie en de kernwaarden van het netwerk. De oprichting van een DIPG-register werd ingesteld als het eerste project van het netwerk, met als doel om klinische, biologische en centraal beoordeelde radiologische gegevens van patiënten met DIPG, zowel binnen als buiten klinische trials, te includeren.



Het SIOPE DIPG-register bestaat uit een online database voor klinische gegevens en een beeldbank. Om uniforme gegevensverzameling te bevorderen, werden gestandaardiseerde elektronische 'case report forms' (CRF's) ontwikkeld door het SIOPE DIPG-netwerk, in samenwerking met collega's van het Internationale DIPG-register. Elk land dat vertegenwoordigd is in het SIOPE DIPG-netwerk is verantwoordelijk voor het aanleveren van eigen gegevens aan het SIOPE DIPG-register en de beeldvorming database, na ethische goedkeuring. Vanaf april 2016 hebben zes landen retrospectieve

gegevens van 694 patiënten ingediend bij het SIOPE DIPG-register en de beeldvorming database.

## DEEL II: HET VERANDERENDE DIPG/DMG-LANDSCHAP

De diagnostische evolutie van DIPG naar DMG weerspiegelt een overgang van een op clinicoradiologische factoren berustende (fenotypische) naar een op moleculair profiel gebaseerde (genotypische) diagnose. Ik onderzoek de implicaties van het implementeren van geavanceerde moleculaire diagnostiek voor de mondiale gezondheid en bovendien de klinische implicaties hiervan, zoals beschreven door specialisten in de pediatrische neuro-oncologie.

**Hoofdstuk 4**: We hebben geprobeerd vast te stellen of de implementatie van moleculair gedefinieerde entiteiten in de klinische praktijk op adequate en gelijkwaardige wijze als van toegevoegde waarde wordt beschouwd en wordt ondersteund door neuro-oncologische professionals wereldwijd. We hebben input verzameld van nagenoeg 500 neuro-oncologische experts die patiënten behandelen met pediatrische hooggradige gliomen (pedHGG). Deze specialisten vertegenwoordigden 53 landen en acht disciplines.

Onze bevindingen toonden over het algemeen een groter beroep op en voorkeur voor genetische testen aan onder deelnemers uit landen met een zeer hoog HDI ('Human Development Index'). Het proces van implementatie en aanpassing is in landen met een zeer ontwikkeld nationaal gezondheidssysteem niet hetzelfde geweestals in landen met veel minder financiële en medische middelen. De resultaten van deze enquête zijn de eerste die internationale verschillen langs sociaaleconomische lijnen rapporteren in de implementatie van de herziene 4e editie van de Wereldgezondheidsorganisatie (World Health Organization; WHO) classificatie van hersentumoren (Central Nervous System; CNS) uit 2016, waarin moleculaire diagnostiek voor het eerst werd geïntroduceerd.

Uit onze enquête blijkt dat moleculaire diagnostiek voor CNS-tumoren beschikbaar en erkend is, maar niet internationaal toegankelijk. We laten zien dat de manieren om een genetische laag van neuropathologische diagnoses te introduceren tot nu toe onvoldoende zijn geweest om de kloof in beschikbaarheid van middelen in een groot deel van de wereld te overbruggen. Als gevolg hiervan kunnen veel centra in settings met een lager inkomen pediatrische hooggradige glioompatiënten niet adequaat diagnosticeren volgens de criteria van de WHO 2016. Onze enquête suggereert dat de WHO-classificatie van CNS-tumoren een rol kan spelen bij het in stand houden of elimineren van ongelijkheid binnen de neuro-oncologie gemeenschap. We documenteren hoe ongelijkheid in de toegang tot moleculaire diagnostiek de implementatie van de WHO 2016-tumorclassificatie kan beïnvloeden, en hoe perspectieven ten aanzien van diagnose en behandeling kunnen verschillen in omgevingen met beperkte middelen in het moleculaire tijdperk.

**Hoofdstuk 5**: We hebben een aanvullende analyse uitgevoerd op de enquêteresultaten uit Hoofdstuk 4, met betrekking tot de implementatie van moleculaire diagnostiek in de WHO CNS4, om specifiek de verschillen in percepties en ervaringen te onderzoeken tussen twee belangrijke gebruimakers van het classificatiesysteem, te weten, kinderneuro-oncologen en neuropathologen. Vervolgens hebben we onderzocht en besproken of en hoe de verschillende kwesties die in onze enquête naar voren kwamen, zijn aangepakt in de 5e editie van de WHO CNS Tumor Classification (CNS5), geïntroduceerd in 2021.

Onze resultaten tonen dat neuropathologen, van focus houden op wetenschappelijke tot stand gekomen classificatie, als vertegenwoordigers van de kunst diagnostiek, maar dat kinderneuro-oncologen met hun speciale focus op klinische behoeftenproblemen ervaren met de introductie van nieuwe tumortypen, het hernoemen of afschaffen van gevestigde tumortypen. Neuro-oncologen noemden dat het bijvoorbeeld lastig is om veranderende diagnostische definities uit te leggen zijn aan patiënten en families. Neuro-oncologen en neuropathologen waren het echter eens over het feit dat er onvoldoende diagnostische definities beschikbaar waren voor moleculair gedefinieerde entiteiten in 2016 en dat deze entiteiten minder relevant waren voor pediatrische casus.

Opvallenderwijs worden veel van de kwesties die in onze enquête naar voren waren gebracht, weerspiegeld in de wijzigingen die zijn doorgevoerd in de CNS5 van 2021. In de CNS4 van 2016 waren sommige aantoonbaar klinisch relevante pedHGG-tumortypen zoals non-diffuus pilocytair astrocytoom, IDH-wildtype diffuus pedHGG en diffuus pedHGG bij kinderen jonger dan 3 jaar niet opgenomen, terwijl deze nu specifiek worden behandeld. “Entiteiten” die niet zijn opgenomen in de CNS5, DIPG en gliomatosis cerebri, zijn beiden op basis van beeldvorming gedefinieerd. In onze enquête gingen over het algemeen meer pathologen akkoord met het afschaffen van de aanduiding “gliomatosis cerebri” dan oncologen. Dit was ook het geval bij DIPG. Neuro-oncologen waren voorstander van het herintroduceren van de eerdere clinicoradiologische diagnose optie van DIPG, naast de enige optie om de diagnose DMG alleen door middel van een biopsie te stellen. De reden waarom op basis van beeldvorming gedefinieerde tumortypen zoals DIPG niet zijn opgenomen in de CNS5, is gebaseerd op het besluit dat de WHO-classificatie een op weefsel gebaseerde benadering volgt.

In de zoektocht naar classificatie van pediatrische hooggradige gliomen met behulp van het meest actuele onderzoek, heeft de WHO CNS-classificatie substantiële verbeteringen aangebracht in het opnemen van moleculaire informatie in de diagnose van verschillende tumortypen. Onze studie benadrukt de voortdurende noodzaak om een balans te vinden tussen vooruitgang in het begrip van de biologie van CZS-tumoren en betekenisvolle klinische impact, maar bevestigt ook de aanzienlijke verbetering van de definitie en diagnostiek van pedHGG binnen de nieuwste WHO-classificatie. Een grotere multidisciplinaire vertegenwoordiging binnen werkgroepen zoals de cIMPACT-

NOW, met meer neuro-oncologen, neuroradiologen en anderen die betrokken zijn bij de behandeling van patiënten met hersentumoren, zou kunnen bijdragen aan het verbeteren van klinische translatie.

## DEEL III: VERBETERDE KARAKTERISERING VAN DIPG MET BEHULP VAN HET SIOPE DIPG/DMG-REGISTER

Het gebruik van het SIOPE DIPG-register als een hoogwaardig epidemiologisch instrument om "het land te verkennen", heeft de potentie om karakterisatie van prognostische factoren onderliggend aan de overleving in DIPG/DMG te verbeteren.

**Hoofdstuk 6**: Langetermijnoverleving (LTS) bij DIPG wordt gedefinieerd als een algehele overleving (OS) van >2 jaar en kenmerken die geassocieerd zijn met een langere overleving zijn onder andere jongere leeftijd, langere symptoomlatentie en afwezigheid van ringaankleuring op diagnostische 'magnetic resonance imaging'. In het eerste grootschalige register hebben we geprobeerd klinische, radiologische, histologische en moleculaire factoren die geassocieerd zijn met korte- en langetermijnoverleving verder te definiëren bij 1008 patiënten, het grootste cohort van centraal beoordeelde DIPG's tot nu toe.

De mediane OS was 11 maanden (interkwartielafstand, 7,5 tot 16 maanden). De mediane overleving van LTS patienten was 33 maanden (bereik, 24 tot 156 maanden). Leeftijd <3 of >10 jaar, langere symptoomlatentie, afwezigheid van hersenzenuwuitval en systeemtherapie bij diagnose waren voorspellers van lange termijn overleving. Onze bevindingen bevestigen ook de onafhankelijke associatie van H3.1 K27M en H3.3 K27M met lange- en korte termijn overleving respectievelijk.

Neoadjuvante of adjuvante systeemtherapie correleerde met langetermijnoverleving in zowel univariabele als multivariate analyses. Deze bevinding verschilt van de lang bestaande opvatting dat systeemtherapie geen overlevingsvoordeel biedt voor DIPG, een opvatting dat grotendeels gebaseerd is op kleine, niet-gerandomiseerde klinische onderzoeken. Een effectieve vergelijking van therapeutische studies voor DIPG wordt bemoeilijkt door brede variatie in inclusiecriteria.

Voor zover bij ons bekend, vertegenwoordigt deze studie de grootste, meest uitgebreid geannoteerde cohort van radiologisch bevestigde DIPG's die tot nu toe is gerapporteerd, en biedt het de meest nauwkeurige cijfers rondom langdurige en zeer langdurige overleving in deze zeldzame tumor. De identificatie van robuuste, met overleving geassocieerde factoren in deze studie is essentieel voor de ontwikkeling van prognostische subgroepen en licht subgroepen patiënten uit waarvan het meest kan worden geleerd door analyse van weefselbiopten vóór behandeling. Het begrijpen van biologische verschillen die een overlevingsvoordeel opleveren bij DIPG baant

een weg naar de ontwikkeling van op subgroepen gerichte therapieën die, wanneer geïmplementeerd in de context van klinische onderzoeken, de uitkomsten in deze verwoestende ziekte kunnen verbeteren.

**Hoofdstuk 7**: Eerstelijns radiotherapie (RT) voor DIPG wordt over het algemeen beschouwd als de enige bewezen effectieve, zij het palliatieve, behandeling. De rol van systemische chemotherapie, zowel concomitant als adjuvant aan radiotherapie, is echter onderhevig aan discussie. Ons doel is om zes historische behandeltrajecten en de associatie ervan met overleving te onderzoeken. Gegevens werden verzameld van 409 patiënten met behulp van de HIT-HGG-trialdatabase van de Duitse Vereniging voor Kinderoncologie en Hematologie (GPOH) en het SIOPE DIPG/DMG-register.

Uniek aan onze overlevingsanalyse is de inclusie van patiënten die beperkte behandelingen hebben gekregen (d.w.z. geen behandeling, alleen bestraling bij diagnose en geen aanvullende behandeling bij progressie van ziekte). We rapporteren de overleving bij patiënten zonder behandeling, alleen bestraling bij diagnose en geen behandeling bij progressie, en vergelijken deze beperkte behandelingen met intensievere behandeling. Om dit project uit te voeren, werden overlevingsresultaten onderzocht voor zes behandelmodaliteiten, drie in de eerstelijns setting (primaire behandeling bij diagnose) en drie bij recidief. Modaliteiten bij diagnose betreffen I) geen behandeling, II) alleen RT, en III) RT-chemotherapie. Modaliteiten bij recidief zijn I) geen aanvullende behandeling, II) chemotherapie, en III) re-RT, +/- chemotherapie.

De mediane algehele overleving (OS) was 11,2 maanden (95% CI, 10,5-11,9), geschat vanaf de diagnose. OS zonder eerstelijnsbehandeling was 3,0 maanden, met alleen RT 10,4 maanden, en met RT-chemotherapie 11,7 maanden. De mediane overleving na de eerste progressie (PPS) was 4,1 maanden (95% CI, 3,5-4,7). PPS was 2,2 maanden zonder recidiefbehandeling, 4,4 maanden met alleen chemotherapie en 6,6 maanden met bestraling +/- chemotherapie. De hazard ratio (HR) voor geen behandeling, geschat met een Cox-model vanaf de diagnose, was 3,65, en voor alleen RT 1,34, ten opzichte van RT-chemotherapie. Bij progressie had geen behandeling een HR van 1,44, en reïrradiatie +/- chemotherapie een HR van 0,72, ten opzichte van chemotherapie.



Hoewel niet aangetoond in een gerandomiseerde setting, suggereren onze gegevens dat het behandelen van patiënten met DIPG met radiotherapie gunstig is, en dat aanvullende chemotherapie bij RT, zowel gelijktijdig met als onderhoudsbehandeling na radiotherapie, de overleving lijkt te verlengen. Daarnaast suggereren onze overlevingsgegevens dat combinatietherapie het meest effectief is om de overleving te verlengen, zowel bij de primaire behandeling als bij progressie. Belangrijk is dat alle overlevingsverschillen binnen een jaar na progressie verdwenen, afgezien van enkele uitschieters, waarbij de ziekte bijna uniform fataal blijft. Deze studie toont overlevingsverschillen in een historische DIPG-cohort op basis van therapeutische

benaderingen, als een eerste stap naar het kwantificeren van overlevingsvoordelen die middels verschillende therapie vormen behaald kunnen worden.

Nieuw in onze onderzoeksopzet is het gebruik van de landmark-methodologie, waarbij de datum van het eerste recidief werd gebruikt als landmark-tijdstip. Post-progressie overleving (PPS), in plaats van OS vanaf de diagnose, werd vervolgens gebruikt om behandelingen ten tijde van progressie te evalueren. Deze opzet is zowel klinisch relevant als gemakkelijk over te nemen in andere observationele studies in DIPG die recidiefbehandelingen onderzoeken. Deze methode stelde ons in staat om een beter inzicht te krijgen in welke overleving kan worden toegeschreven aan tweedelijnstherapieën, waardoor we ook en beter de algehele overlevingsverschillen door eerstelijnstherapieën beter konden definiëren.

Voor het eerst laten we in een grote retrospectieve analyse zien hoe om te gaan met ‘immortal time bias’ door gebruik te maken van de landmark-methode en bieden we robuust geschatte overlevingsresultaten bij DIPG. Studies zoals deze, die overlevingsresultaten beter definiëren, zullen helpen om herhaling van klinische DIPG-studies te vermijden door een representatief historisch referentiepunt te bieden. Bovendien kunnen de hier gepresenteerde overlevingsgegevens nuttig zijn voor behandelend artsen die communiceren met families van patiënten die een klinisch traject overwegen zonder enige behandeling of alleen radiotherapie, versus progressieve oncologische behandeling.

# Appendices

# CURRICULUM VITAE

Joshua Norman Baugh was born in Lafayette, Indiana, USA on June 2, 1988, the middle of three children, to Jerry and Stacy Baugh. He graduated from Frankfort Senior High School in 2007 and attended DePauw University majoring in Biology. Upon graduation he first pursued a career in Ecology, working at a marine field station in Costa Rica and teaching marine science in the Florida Keys. In 2013, he began a career transition into medicine, largely sparked by his mother's cancer diagnosis. Until 2019 he worked as a clinical research coordinator at Cincinnati Children's Hospital, first in Oncology and later in the Anderson Center for Health Systems Excellence. During his five years working in Oncology he helped establish the International DIPG Registry. In the Anderson Center he managed study site start up and quality improvement initiatives to improve recruitment for the COMBINE study, the largest clinical trial ever conducted in pediatric IBD.

In 2016, he began a part-time Master of Public Health (MPH) at the University of Cincinnati College of Medicine focused on Global Health. During his MPH he received a fellowship to work with the Center for Closing the Health Gap, an organization focused on eliminating health disparities among Cincinnati's vulnerable populations. For his MPH thesis project, he traveled to Guatemala with the University of Cincinnati Family Medicine Residency Program to develop a program evaluation of their primary care field clinics, conducted in close collaboration with the Mayan Health Alliance, an NGO providing health care to the indigenous Mayan population.

After graduating with his MPH, he moved to the Netherlands to pursue a PhD in the research group of dr. van Vuurden at the Princess Maxima Center. This provided an opportunity to pursue his lifelong dream of becoming an independent research scientist and expand upon his research experience with the International DIPG Registry. The results of his research on the European Society for Paediatric Oncology (SIOPE) DIPG Registry are presented in this thesis.

# PUBLICATIONS

## This thesis

Gielen GH, **Baugh JN**, van Vuurden DG, Veldhuijzen van Zanten SEM, Hargrave D, Massimino M, Biassoni V, Morales la Madrid A, Karremann M, Wiese M. Pediatric high-grade gliomas and the WHO CNS Tumor Classification-Perspectives of pediatric neuro-oncologists and neuropathologists in light of recent updates. *Neuro-oncology Advances 2022* May 20.

**Baugh JN**, Gielen GH, van Vuurden DG, Veldhuijzen van Zanten SEM, Hargrave D; Massimino M, Biassoni V, Morales la Madrid A, Karremann M, Wiese M. Transitioning to molecular diagnostics in pediatric high-grade glioma: experiences with the 2016 WHO classification of CNS tumors. *Neuro-oncology Advances 2021* August 18.

Hoffman LM, Veldhuijzen van Zanten SEM, Colditz N, **Baugh J**, Chaney B, Hoffmann M, Lane A, Fuller C, Miles L, Hawkins C, Bartels U, Bouffet E, Goldman S, Leary S, Foreman NK, Packer R, Warren KE, Broniscer A, Kieran MW, Minturn J, Comito M, Broxson E, Shih CS, Khatua S, Chintagumpala M, Carret AS, Escorza NY, Hassall T, Ziegler DS, Gottardo N, Dholaria H, Doughman R, Benesch M, Drissi R, Nazarian J, Jabado N, Boddaert N, Varlet P, Giraud G, Castel D, Puget S, Jones C, Hulleman E, Modena P, Giagnacovo M, Antonelli M, Pietsch T, Gielen GH, Jones DTW, Sturm D, Pfister SM, Gerber NU, Grotzer MA, Pfaff E, von Bueren AO, Hargrave D, Solanki GA, Jadrijevic Cvrlje F, Kaspers GJL, Vandertop WP, Grill J, Bailey S, Biassoni V, Massimino M, Calmon R, Sanchez E, Bison B, Warmuth-Metz M, Leach J, Jones B, van Vuurden DG, Kramm CM, Fouladi M. Clinical, Radiologic, Pathologic, and Molecular Characteristics of Long-Term Survivors of Diffuse Intrinsic Pontine Glioma (DIPG): A Collaborative Report From the International and European Society for Pediatric Oncology DIPG Registries. *Journal of Clinical Oncology 2018* July 1.

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**Baugh J**, Bartels U, Leach J, Jones B, Chaney B, Warren KE, Kirkendall J, Doughman R, Hawkins C, Miles L, Fuller C, Hassall T, Bouffet E, Lane A, Hargrave D, Grill J, Hoffman LM, Jones C, Towbin A, Savage SA, Monje M, Li XN, Ziegler DS, van Zanten SV, Kramm CM, van Vuurden DG, Fouladi M. The international diffuse intrinsic pontine glioma registry: an infrastructure to accelerate collaborative research for an orphan disease. *J Neurooncol. 2017* Jan 16.

## Other Publications

Hoogendijk R, van der Lugt J, **Baugh J**, Kline C, Kranendonk M, Hoving E, Kremer L, Wesseling P, Karim-Kos H, van Vuurden, DG. Sex-related incidence and survival differences in pediatric high-grade glioma subtypes: A population-based cohort study. *iScience 2023* September 17.

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van Binsbergen, TL, Kebede MG, **Baugh J**, Van Engers T, van Vuurden DG. Dynamic generation of access control policies from social policies. *Procedia Computer Science 2021* November 1.

Leach JL, Roebker J, Schafer A, **Baugh J**, Chaney B, Fuller C, Fouladi M, Lane A, Doughman R, Drissi R, DeWire-Schottmiller M, Ziegler DS, Minturn JE, Hansford JR, Wang SS, Monje-Deisseroth M, Fisher PG, Gottardo NG, Dholaria H, Packer R, Warren K, Leary SES, Goldman S, Bartels U, Hawkins C, Jones BV. MR Imaging features of Diffuse Intrinsic Pontine Glioma (DIPG) and Relationship to Overall Survival: Report from the International DIPG Registry. *Neuro Oncology 2020* June 6.

Cooney T, Lane A, Bartels U, Bouffet E, Goldman S, Leary S, Foreman N, Packer R, Broniscer A, Minturn J, Schin Shih C, Chintagumpala M, Hassall T, Gottardo N, Dholaria H, Hoffman L, Chaney B, **Baugh J**, Doughman R, Leach J, Jones B, Fouladi M, Warren K, and Monje M. Contemporary Survival Endpoints: An International Diffuse Intrinsic Pontine Glioma Registry Study. *Neuro Oncology 2017* July 31.

Veldhuijzen van Zanten SEM, Lane A, Heymans MW, **Baugh J**, Chaney B, Hoffman LM, Doughman R, Jansen MH, Sanchez E, Vandertop WP, Kaspers GJL, van Vuurden DG, Fouladi M, Jones BV, Leach J. External validation of the diffuse intrinsic pontine glioma survival prediction model: a collaborative report from the International DIPG Registry and the SIOPE DIPG Registry. *J Neurooncol. 2017* May 30.

# PHD PORTFOLIO

| | |
|---|---|
| **Name:** | Joshua Baugh |
| **PhD period:** | October 2019-October 2023 |
| **Research School:** | Utrecht University, Graduate School of Life Sciences (GSLS) |
| **Program:** | Clinical and Translational Oncology (CTO) |
| **Department:** | Neuro-Oncology, Princess Máxima Center for Pediatric Oncology |
| **Promoter:** | Prof. dr. Eelco W. Hoving |
| **Co-promoters:** | Dr. Dannis G. Van Vuurden, Dr. Sophie E.M. Veldhuijzen van Zanten |

Courses (ECTS)

| PhD Training | Year |
|---|---|
| The Art of Presenting Science, GSLS 1.4 | 2022 |
| Writing a Scientific Paper, GSLS 1.5 | 2022 |
| Adobe Illustrator – Scientific Artwork and Infographics, GSLS 1.2 | 2022 |
| Clinical Trial Development, CTO 1.5 | 2022 |
| CTO Peer to Peer Sessions, CTO .3 | 2022 |
| This Thing Called Science, GSLS 2.0 | 2021 |
| Clinical & Translational Oncology Intro Course, CTO 1.5 | 2021 |
| Introductory Biostats for Researchers, GSLS 4.5 | 2021 |
| Responsible conduct of research: how to do it right, UU .45 | 2020 |
| Study Design in Etiologic Research, GSLS 3.0 | 2020 |

Seminars and Workshops

| PhD Training | Year |
|---|---|
| Weekly Máxima Research Seminars | 2019-2023 |
| Preclinical Van Vuurden Research Group Meetings | 2022-2023 |
| Neuro-oncology Translational Research Meetings | 2019-2023 |
| Monthly Neuro-Oncology Department Clinical Research Meetings | 2021-2023 |
| Neuro-oncology Research Retreat | 2022, 2023 |
| CTO PhD Retreat | 2020, 2022, 2023 |
| UMC Utrecht Brain Center X-talks Neuro-oncology | 2019, 2023 |
| Research Retreat Princess Máxima Center | 2021, 2022 |
| UMC Utrecht Brain Center Neuro-oncology Masterclass | 2019 |
| Joint KiTZ & Máxima Research Retreat | 2020 |

## Conferences

| PhD Training | Year |
|---|---|
| DIPG/DMG and Medulloblastoma Symposium. Lexington, Kentucky. | 2023 |
| SIOPE High Grade Glioma Working Group. Milan, Italy. *Oral presentation*. | 2022 |
| 20th International Symposium on Pediatric Neuro-Oncology (ISPNO). Hamburg, Germany. *Poster.* | 2022 |
| SIOPE High Grade Glioma Working Group, virtual. *Oral presentation*. | 2021 |
| 19th International Symposium on Pediatric Neuro-Oncology (ISPNO), virtual. *Poster.* | 2021 |
| SIOPE High Grade Glioma Working Group, Utrecht, Netherlands. *Oral presentation*. | 2020 |

## Teaching Activities

| PhD Training | Year |
|---|---|
| Supervising Medical Student, Nada Mohammed, Honors Bachelor Thesis: *Hydrocephalus Treatment and the Impact on Survival in Children with DIPG* | 2022 |

## Other Activities

| PhD Training | Year |
|---|---|
| Steering Committee Member, SIOPE DIPG Registry | 2019-2023 |
| International Student Representative, Maxima PhD Survey Committee | 2021-2023 |
| Chair of Buddy Program, Máxima International Community 2020-2022 | |
| Buddy, Máxima Prima PhD Group | 2022 |
| Committee Member, Princess Máxima Diversity and Inclusion Group | 2020-2022 |
| PhD Student Talent Program Representative, Princess Máxima SEP Site Visit | 2021 |
| Project site visits to Göttingen, Germany | 2019, 2020 |

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With Grace Foundation, Jeffrey Thomas Hayden Foundation, Cure Brain Cancer Foundation, The Jones Family Foundation, Musella Foundation for Brain Tumor Research and Information, The Pray, Hope Believe Foundation, Benny's World, Smiles for Sophie Forever, Aiden's Avengers, Love Chloe Foundation, A Cure From Caleb Society, Operation Grace White Foundation, Ryan's Hope, American Childhood Cancer Organization, Juliana Rose Donnelly Trust, Joshua's Wish, The Ellie Kavalieros DIPG Research Fund, Voices Against Brain Cancer, Wayland's Warriors, Sheila Jones & Friends and The Cure Starts Now Foundation.

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