CHAPTER 1

General introduction and thesis outline

This chapter provides a general background on diffuse intrinsic pontine glioma (DIPG) and its historical perspective to identify the reigning hypotheses and gaps in knowledge in 2012, at the start of the research projects described in this thesis. This chapter concludes with a detailed outline of the projects and aims.

NOMENCLATURE

The most commonly used definition of DIPG describes four aspects of the tumor: diffuse, intrinsic, pontine and glioma.

"*Diffuse*" describes its growth characteristic: tumor cells diffusely infiltrate adjacent and distant brain parenchyma, as opposed to displacing it like focal tumors do. This growth pattern precludes the possibility to surgically resect DIPGs.

As opposed to extra-axial or exophytic tumors, "*intrinsic*" refers to the intra-axial growth pattern: within the brain parenchyma. The massive infiltration of tumor cells causes elevated pressure, dysfunction and possibly destruction of the normal neuronal structures.

"*Pontine*" refers to the location in the brainstem (Fig. 1). The pons, first described by anatomist Constantius Varolius in the 16th century, is also known as the "bridge of Varolius"

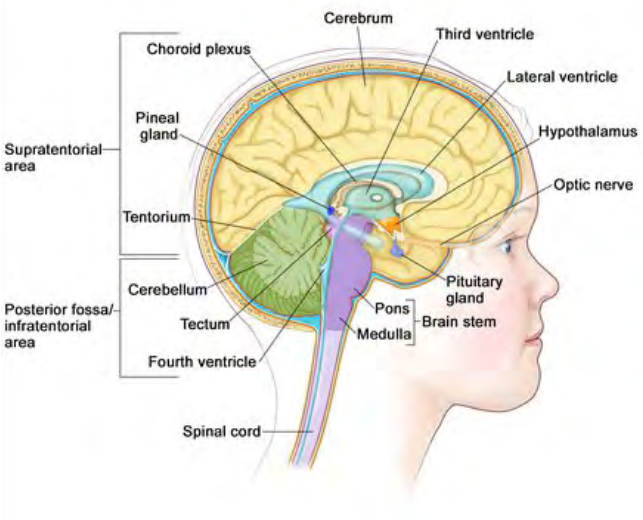

FIGURE 1 | Localization of the pons; copy from [1].

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This delicate and vital area literally 'bridges' higher brain structures with lower nervous centers by longitudinal tracts (separating the ventral and dorsal elements of the pons), and with the cerebellum by transverse tracts that form the cerebellar peduncles [2]. Autonomic functions necessary for life, such as respiratory depth and rate are regulated in the pons. The pons also contains motor and sensory nuclei of several cranial nerves, including the trigeminal nerve (n.V), abducens nerve (n. VI), facial nerve (n.VII), and the vestibulocochlear nerve (n.VIII).

"*Glioma*" finally, refers to the glial origin of the tumor cells. In normal brain development, glial precursor cells have the ability to differentiate into astrocytes, oligodendrocytes, ependymal cells and microglia. They form non-neuronal supportive tissue that provide nerve cell homeostasis, myelin insulation and help maintain the blood-brain barrier (BBB). Upon malignant transformation, different glioma types may arise: astrocytomas, oligodendrogliomas, or ependymomas. DIPGs typically have an astrocytic morphology, although oligodendroglial or rarely a mixed oligodendroglial-astrocytic morphology has also been recognized [3].

It is unknown why and how glial (precursor) cells of the pons undergo malignant transformation to DIPG. Since DIPGs almost exclusively occur in children and have a peak incidence in middle childhood [4], a relationship with early brain development has been suggested [5]. It is also not known if and to what extent malignant transformation changes the function of glial cells, such as maintenance of the BBB, or the local tissue homeostasis of the pontine microenvironment.

EPIDEMIOLOGY, CLASSIFICATION AND REGISTRATION

Each year approximately 700 children and young adolescents in the Netherlands are diagnosed with cancer among 3.8 million individuals aged 0–20 years [6]. Central nervous system (CNS) tumors are the most common type of solid childhood cancers. Although childhood cancer is rare, it remains the main cause of death in children in our Western society.

Childhood brain tumors represent an extremely heterogeneous group of diseases with prognosis depending on age, tumor histology and anatomical localization. Epidemiological data from the Central Brain Tumor Registry of the United States (CBTRUS) show that gliomas account for approximately 47.0% of tumors, and the majority of brain tumors, in children and adolescents age 0-19 years (Fig. 2A). Locations most frequently affected by a childhood brain tumor are the cerebellum (18.5%) and the brainstem (12.4%) (Fig. 2B). Brainstem gliomas, of which 80% grow diffusely (i.e., are DIPGs), cause the largest proportion (±38%) of brain tumor-related death in children.

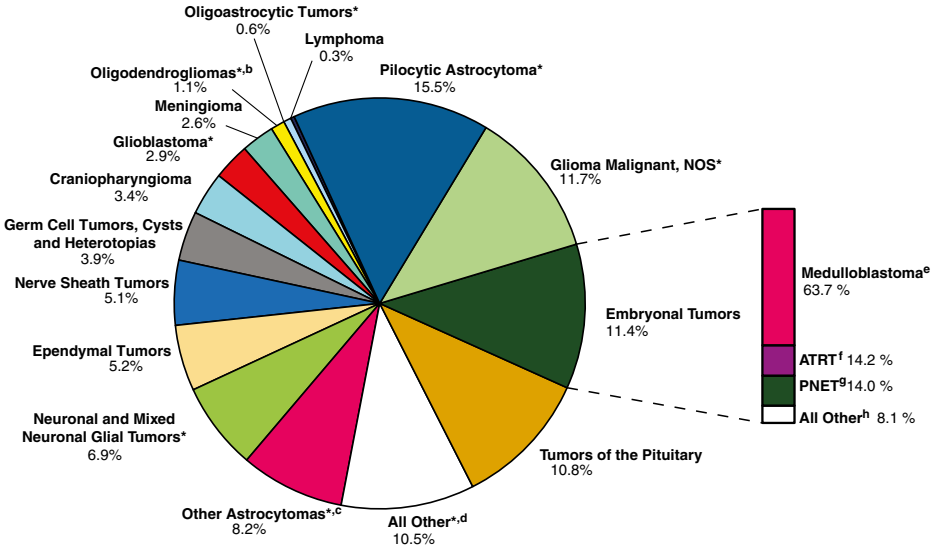

FIGURE 2A | Distribution in Children and Adolescents (Age 0–19 years) of Primary Brain and CNS Tumors by histology (n = 23.522); copy from [7].
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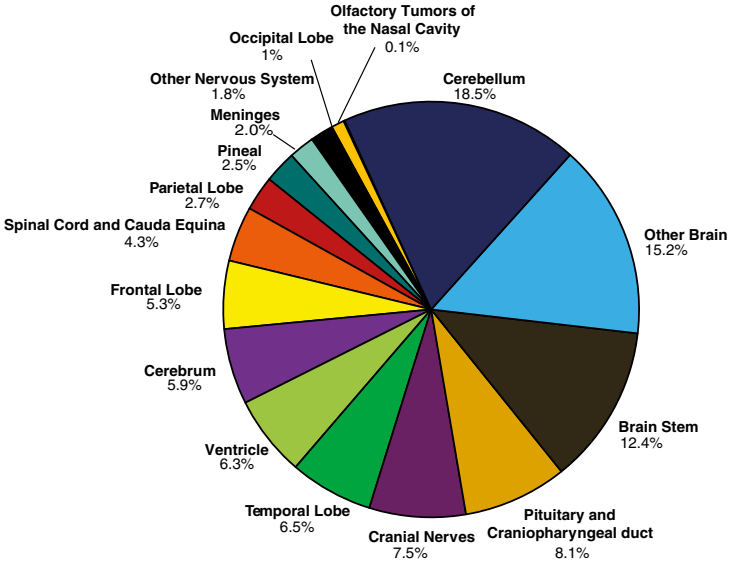

FIGURE 2B | Distribution in Children and Adolescents (Age 0–19 years) of Primary Brain and CNS Tumors by location (n = 23.522); copy from [7].
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In the Netherlands, the incidence and survival rates of DIPG are not known. This also applies to most other countries. Over the past decades, various classification systems for brainstem tumors have been proposed, utilizing the best diagnostic modalities available at the time. For years, DIPG patients have been diagnosed based on a typical clinical presentation in combination with specific neuro-imaging findings. The diagnosis did not include pathology and DIPG was no self-contained biological entity: it had no specific code in the International Classification of Diseases for Oncology (ICD-O) [3], potentially having resulted in misinterpretation, misclassification and under-registration.

CLINICAL PRESENTATION

The clinical presentation of DIPG patients reflects the tumors' origin within the delicate brainstem. Symptoms are caused by either direct tumor invasion and destruction of critical pontine structures, or by tumor- and edema-induced compression. At the time of diagnosis, patients usually present with (uni- or bilateral) cranial nerve dysfunction, long tract signs (e.g., increased tone, hyperreflexia, clonus, Babinski sign, motor deficit, etc.) and cerebellar signs (e.g., ataxia, dysmetria, dysarthria). These symptoms may occur solitary, or as a classic triad [4]. Symptoms usually preceed presentation by several weeks, but it is not unusual to have mild symptoms present for several months [4]. Parents often report odd eye movements (with or without the patient reporting double vision), an asymmetric smile or drooping of one side of their child's face, slurred speech, drooling, difficulty swallowing, clumsiness or trouble to maintain balance [8]. Pathological laughter has also been reported [9].

Literature provides only one meta-analysis reviewing symptoms in DIPG patients [10]. This paper, published in 2007, solely addresses symptoms at the time of diagnosis. During the disease course, however, progressive tumor growth, tumor spread, edema formation, bleeding, and/or hydrocephalus cause gradual or sudden neurological deterioration, which severely affects the child's daily functioning and quality of life. No data have been published on the occurrence symptoms towards end-stage disease.

IMAGING

The earliest description of radiology used for the diagnosis of DIPG dates back to 1946 where Lysholm described air-ventriculography X-rays, showing an "*upward displacement of the posterior part of the third ventricle, a bow-shaped upward and backward displacement of the aqueduct and fourth ventricle, and a narrowing of the cisterna pontis*" [11]. In a 1967 Lancet report by Lassman et al., air-venriculography was described as the most helpful radiological investigation, alongside plain X-rays of the skull, to detect signs of increased

intracranial pressure [12]. In the 1970s, computerized tomography (CT) revolutionized the field of anatomical imaging, for the first time allowing direct visualization of intracranial structures in a relatively non-invasive fashion [13]. A downside to this technique is that it provides limited differentiation in soft tissue contrast, especially in the posterior fossa (due to beam hardening artifacts of the petrous bone). Patients with any type of tumor in the brainstem were therefore initially uniformly classified and treated as having a brainstem glioma (BSG). The introduction of magnetic resonance imaging (MRI) in the 1980s, allowed for better differentiation in soft tissue contrast and resulted in more specified classification systems [14].

Magnetic Resonance Imaging (MRI)

In 1990, Barkovich et al. were the first to publish a classification system for BSG based on MRI. This classification made use of new variables, such as a more detailed topography (midbrain, pons, and/or medulla oblongata), the degree of enlargement of anatomic segment(s), the direction and extent of tumor spread (exophytic, longitudinal and/or axial), and tumor characteristics such as focality (focal versus diffuse), signal intensity as compared to surrounding healthy brain structures (hypo-, iso-, hyperintensity), and the presence of hemorrhage, necrosis, cysts and hydrocephalus [15].

Some of these radiologic variables were found to be significantly associated with survival [15,16]. A primary tumor site in the pons and a diffuse growth pattern were found to correlate with the least favorable survival (Fig. 3A and 3B).

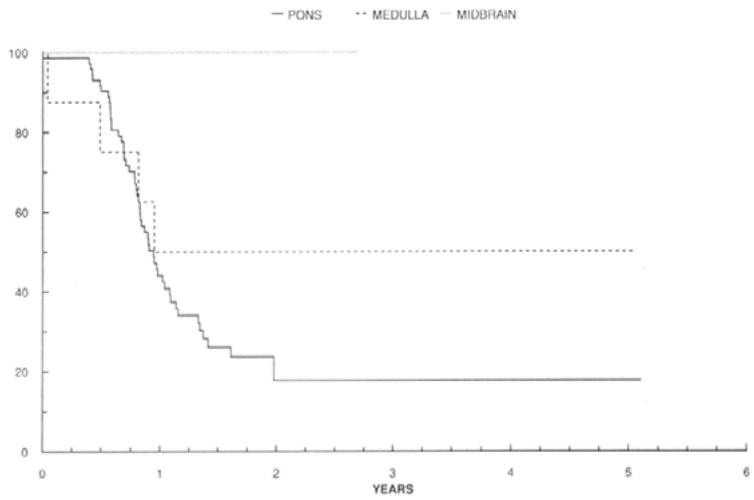

FIGURE 3A | Survival by primary site; copy from [15].
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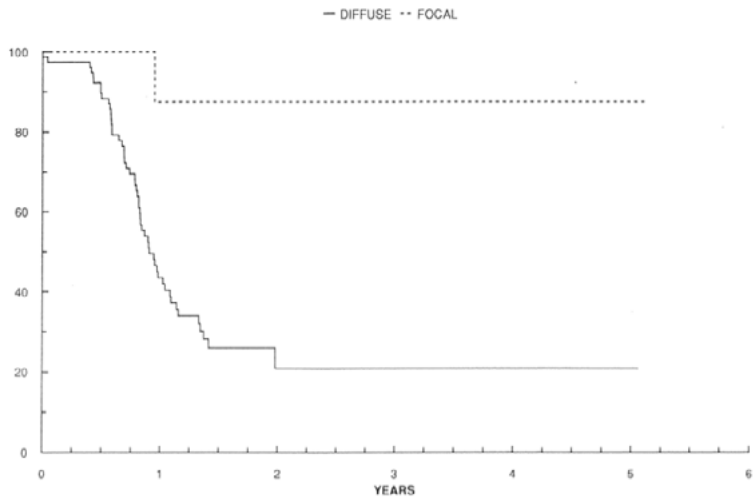

FIGURE 3B | Survival by tumor focality; copy from [15].
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Over the past two decades, MRI has become the gold standard diagnostic tool in case a patient presents with symptoms suggestive for DIPG. At the start of this research, the most commonly used definition of a DIPG is based on Barkovich' classification system, being a T1-weighted hypointense and T2-weighted hyperintense tumor occupying at least 50% of the pons on T2-images (Fig. 4). DIPGs are herewith separated from focal tumors (often occupying less than 50% of the pons), exophytic tumors, tumors which are sharply demarcated, and other diffuse tumors that occur elsewhere in the midline structures [15].

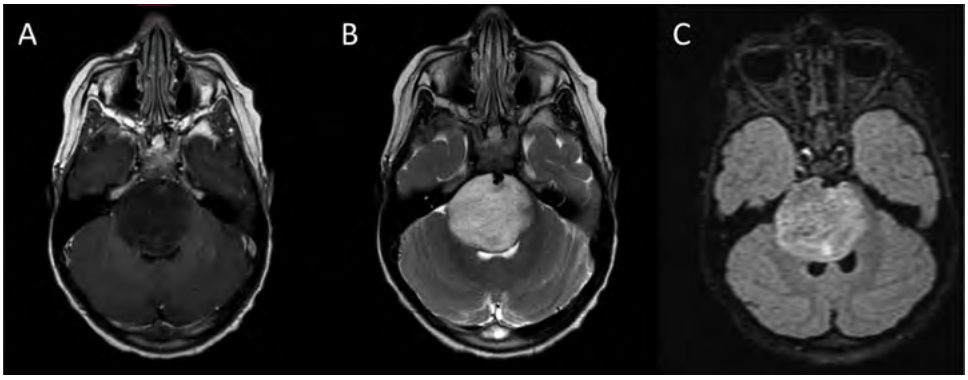

FIGURE 4 | Typical MRI appearance of DIPG: on **(A)** T1-weighted post-contrast images, **(B)** T2-weighted images, and **(C)** FLAIR images; copy from [4].
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Typically, DIPGs are poorly demarcated tumors often accompanied by edema. DIPGs originate from the ventral pons, resulting in encasement of the basilar artery and generally extend continuously along the longitudinal and transverse tracts of the brainstem. Strikingly, only a minority of patients develop hydrocephalus, although DIPGs often compress the aqueduct and fourth ventricle. Distant parenchymal, subependymal, and leptomeningeal metastases in the brain and/or spine are thought to occur in only 13–17% of patients [17,18]. After gadolinium contrast administration, 38% of MR-images obtained at the time of diagnosis show enhancement, which is usually restricted to only a small part of the tumor. The occurrence of hemorrhage and necrosis within the tumor, reflected by ring-like contrast enhancement, seems variable [19].

Contrast enhancement generally reflects extravasation of gadolinium through an altered BBB [20]. In DIPG literature it is hypothesized that limited contrast enhancement reflects an intact BBB [21]. This might also prevent systemically applied chemotherapeutics from reaching larger parts of the tumor properly, which may explain the lack of success of systemic cytotoxic treatment strategies. The relationship between gadolinium contrast enhancement and treatment response or survival, however, has not extensively been studied.

In recent years, more advanced MR-techniques have been developed that enable the visualization of (patho)physiological and biochemical processes of the brain, in addition to solely anatomical imaging. Examples are Perfusion Weighted Imaging (PWI), which visualizes blood perfusion, susceptibility-weighted imaging (SWI), showing (micro) hemorrhages [19,22], diffusion-weighted imaging (DWI), which quantifies the number of water molecules [22], diffusion tensor imaging (DTI) which maps the direction of the water molecule diffusion [23–28], and magnetic resonance spectroscopy (MRS), which visualizes the presence and concentration of various metabolites [29–35]. The additional value of these techniques in the classification and prognostication of DIPG patients is yet to be determined.

Positron Emission Tomography (PET)

Another potentially useful technique in the classification, prognostication and response assessment of DIPG patients is positron emission tomography (PET). Imaging of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) distribution provides information on normal brain and brain tumor glucose metabolism. A stronger ^{18}F-FDG PET signal at the site of a tumor has been suggested to correlate with higher grades of malignancy in childhood brainstem tumors [36]. In DIPG patients, the first ^{18}F-FDG-PET-imaging studies are being conducted [18,36–40]. However, normative values for pontine ^{18}F-FDG uptake in children are lacking, which hampers the interpretation of the results.

PET-technology also enables imaging of radiolabeled drugs, especially monoclonal antibodies and tyrosine kinase inhibitors [41]. By this non-invasive *in vivo* quantification of drug distribution and tumor uptake, therapeutic potential, as well as toxicity, can be predicted. Especially for DIPG, molecular drug imaging might be of importance, since for most drugs, currently investigated in early phase trials, BBB passage is largely unknown. More in general, children with brain tumors and other solid cancers are particularly likely to benefit from molecular drug imaging, as drugs without therapeutic effect (based on a lack of drug-uptake in the tumor) may only cause (life-long) side effects. Despite a recent boost of molecular drug imaging in adults, showing promising results, to date no molecular drug imaging studies have been performed in children.

DIPG TUMOR BIOPSIES AND AUTOPSIES

In the pre-imaging eras, up until the early 90's, biopsies were routinely performed as diagnostic confirmation of DIPG. In addition to determining a glial cell type, grading was generally applied to describe the degree of tumor abnormality. Following the 2007 World Health Organization (WHO) classification of CNS tumors that was commonly used in 2012, about 90% of DIPGs were graded as high-grade glioma. Of these, 65% showed anaplasia, mitotic activity, as well as (foci of) microvascular proliferation and/or necrosis consistent with WHO Grade IV glioblastoma. The other 25% contained solely anaplasia and mitotic activity, consistent with WHO Grade III anaplastic astrocytoma. The remaining 10% of DIPGs lacked high-grade features and were thus consistent with WHO grade II low-grade diffuse astrocytoma [3]. Of note in this respect, it is important to emphasize that in 1985, Epstein et al. showed that DIPGs are heterogeneous tumors, with areas varying from high-grade (WHO III and IV) to low-grade (WHO II). These regional differences may result in sampling error if only one area of the tumor is biopsied (Fig. 5).

In the early 1990s, routine biopsy was questioned based on (*i*) the observed heterogeneity of DIPGs, (*ii*) the fact that histological grading did not alter therapy or outcome, (*iii*) the possible morbidity associated with the procedure and (*iv*) the availability of advanced imaging techniques [14,43–45].

In 1993, Albright et al. proclaimed that "*MR-scans provide images that are virtually diagnostic and yield prognostic information equivalent to that obtainable from biopsies*...". As a consequence, for almost a decade, performing a biopsy in case of a suspected DIPG was controversial, resulting in scarcity of tumor material for research purposes. Biopsies were mainly performed in case of a non-typical clinical or radiological presentation. The resulting one-sided selection of only "atypical tumor material" and pollution by autopsy data (i.e., end-stage disease, and/or post-radiation and/or post-chemotherapy

material), misrepresented the disease, and limited the understanding of DIPG biology, etiology and pathogenesis [46].

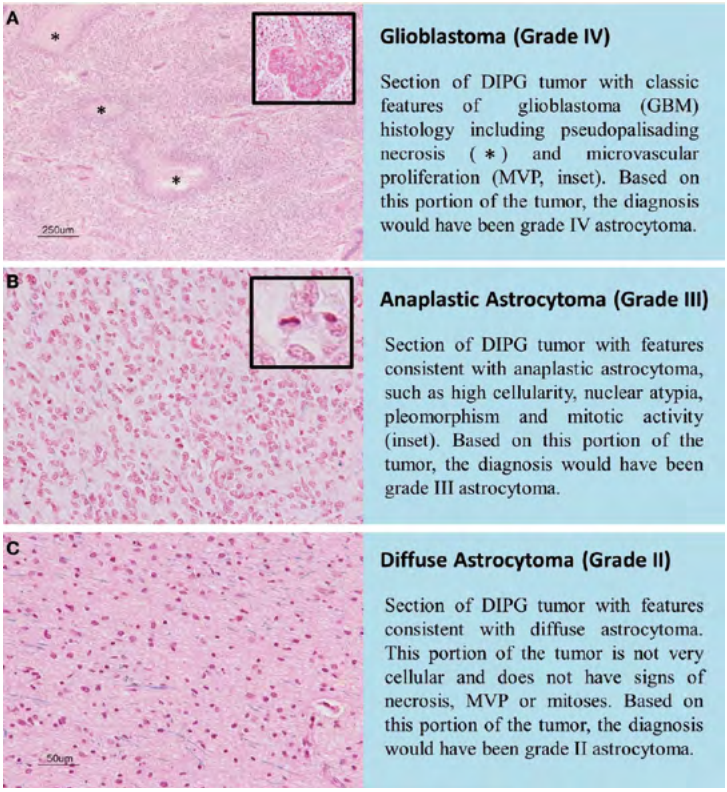

FIGURE 5 | Example of possible sampling error from single biopsy in DIPG; copy from [42]. With permission.

In recent years, French colleagues reintroduced biopsies in DIPG and showed that taking biopsies in DIPG patients is relatively safe [47,48]. The procedure is now more frequently considered, especially in the context of clinical trials [49–51]. In addition to taking biopsies, the number of autopsy studies is increasing [52–54]. Autopsy studies have the advantage of providing considerably larger amounts of material than biopsy. Also, it enables the collection of normal brain tissue, which is useful as internal control. Finally, the discrepancy between material obtained from biopsy and autopsy might provide hints about tumor evolution over time caused by the natural course of the disease and/or therapy-induced changes. The recent paradigm shift towards the collection of tumor material opens a whole new area of opportunities to unravel the underlying pathophysiology and find novel effective treatment options.

TREATMENT AND TRIALS

Novel and effective treatment options for DIPG are urgently needed. To date, there is no curative treatment. The gold standard approach is conventional focal radiotherapy, which is seen as the only effective, albeit palliative, treatment option. Over the past decades, a wide variety of clinical trials have been performed aimed at improving the effect of radiotherapy, but no major differences were observed between hypo- normo- and hyperfractionated schedules [55]. The addition of neo-adjuvant, concurrent or adjuvant chemotherapy, showed no significant clinical benefit [56].

In 2006, a review was published assessing the methods and results of 29 clinical trials conducted between 1984 and 2005 [55]. This review brought to light great intra-trial variability in eligibility criteria, response criteria and trial endpoints. Only three studies were randomized controlled trials (RCTs), as opposed to 26 single-arm trials. The number of patients per trial differed greatly (range 6–130), as did the yearly accrual rates. For the latter, significant differences were shown between studies done by institutions (median accrual of 3 patients per year; range 1–10) and those done by cooperative groups (median 17; range 3–51). In the beginning of 2012, an update was published, in essence reiterating these findings [56]. The conduction of non-standardized, single-arm, and largely underpowered clinical trials may have led to biased results. It became clear that DIPG research needed more collaboration and standardization to acquire comprehensive and comparable data on potentially effective treatments.

SURVIVAL

In DIPG patients, progression-free survival ranges from 5–9 months, and the median overall survival (OS) ranges from 7–14 months, based on studies using the most common definition of DIPG [55,56]. The majority of DIPG studies report a 2-years' survival rate of less than 10% and provide Kaplan-Meier curves that have not improved since the first DIPG specific curves published by Barkovich et al. in 1990 (Fig. 4).

SUPPORTIVE AND PALLIATIVE CARE

Despite the dismal prognosis of DIPG patients, no data have been published on quality of life and the specific needs for palliative and active end-of-life care, including the use of steroids. Steroids are widely prescribed as supportive or palliative treatment, although they are known to cause numerous, sometimes severe, side effects. In addition to the severe symptoms caused by the disease itself, this form of symptom management may also reduce the quality of life of DIPG patients. These issues, so important to patients, urgently need to be addressed in DIPG research.

CONCLUSION

At the initiation of the research described in this thesis, comprehensive knowledge on DIPG with regard to epidemiology, risk predictors, possible patient subgroups, DIPG biology, potentially effective treatments, and supportive and palliative care, was largely lacking. The conduction of non-standardized, single-arm, and largely underpowered clinical trials may have led to biased results and has certainly led to a lack of comprehensive and comparable data. Despite a wide variety of treatment strategies that have been explored over the past decades, no significant improvement in survival has been established with this strategy.

AIMS AND OUTLINE OF THIS THESIS

This thesis aims to provide more insight into DIPG epidemiology, risk predictors, patient subgroups, DIPG biology, potentially effective treatments, and supportive and palliative care. Another aim, on an organizational level, is to optimize the efficiency of DIPG research in order to support the search for a cure by collaboration and the establishment of comprehensive and comparable data.

This thesis is subdivided into three parts, going from studies on a national level (part I) via international retrospective studies (part II) to the establishment of an international DIPG research infrastructure and registry built for future research (part III).

Part I - First DIPG-specific studies conducted in the Netherlands

Part I of this thesis (Chapters 2–6) encompasses the first clinical studies for patients with DIPG in the Netherlands. These studies cover multiple aspects of DIPG, ranging from clinical symptoms, diagnostics and treatment strategies, to autopsy. The results of the studies are presented in analogy to the patient journey, starting at the time of diagnosis and ending with death and autopsy.

Chapter 2 described the first clinical trial for DIPG in the Netherlands: The DIPG study VUmc 01 - phase A. This study aims to determine the tolerability of radiosensitizer gemcitabine added to standard radiotherapy in patients with newly diagnosed DIPG, and to explore the preliminary efficacy in terms of clinical and radiological response.

Chapter 3 describes a molecular imaging study to determine the biodistribution and tumor uptake of systemically applied zirconium-89(^{89}Zr)-labeled bevacizumab by means of PET.

Chapter 4 describes a functional imaging study to visualize fluor-18 fluorodeoxyglucose (^{18}F-FDG) distribution by means of PET, in order to produce normative values for ^{18}F-FDG

uptake in the pons in children with a non-affected brainstem, and to compare this to ^{18}F-FDG uptake in DIPG tumors.

In **Chapter 5** a multi-institutional whole-brain autopsy study is described in which comprehensive morphologic and molecular characterization of multiple affected and non-affected brain samples of nine DIPG patients is performed, with special focus on intratumoral heterogeneity (ITH) and histone 3 K27 trimethylation (H3 K27me3).

Chapter 6 describes the case of a 12-year-old patient of whom comprehensive data from biopsy, ^{89}Zr-bevacizumab PET imaging, and autopsy were obtained in short succession.

Part II - Expanding the scope: historical and international research initiatives

In Part II of this thesis (Chapters 7–13), the research perspective is expanded to a larger scope, both in time and in scale. Starting with historical cohort studies and extensive literature reviews to learn from the past, we reach out to our colleagues at a national, European and global level.

In **Chapter 7** the incidence of DIPG in the Netherlands between 1990 and 2010 is determined using a population-based retrospective cohort. Additionally, all treatment strategies that have been applied are reviewed.

In **Chapter 8** a theoretical model is developed to predict whether chemotherapeutics are suitable for passive diffusion over an intact BBB, or whether local administration via convection-enhanced-delivery (CED) may increase their potential to more efficiently treat these tumors.

In **Chapter 9** the needs of DIPG patients at end-stage disease are identified through a retrospective cohort study, including all children that received palliative treatment under the care of two London hospitals. In addition, a global questionnaire-study among healthcare professionals is conducted to ascertain information on the (multi) institutional and (multi)national approach to palliative care for DIPG patients, the availability of clinical guidelines, and possible gaps in the current organization of care.

Chapter 10 reviews the current use of steroids to reduce peritumoral edema-induced symptoms in DIPG patients. An extensive literature review and global questionnaire-study among health care professionals is performed to ascertain information on the current (multi)institutional and (multi)national use of steroids, the availability of clinical guidelines, and the need for improvements in prescribing steroids to DIPG patients.

In **Chapter 11** the results of a first European multicenter retrospective cohort study are presented. The aim of this study is to determine the predictive value of multiple clinical and radiological variables for survival, and to develop a DIPG survival prediction model.

Chapter 12 builds on chapter 11 and presents the results of an external validation study, in which the validity of the DIPG survival prediction model is determined through external validation in an independent cohort of patient from the United States, Canada, Australia and New Zealand.

Chapter 13 presents a critical appraisal on a French DIPG cohort study in which the histone H3 mutation is shown to have stronger predictive value for survival than the DIPG survival prediction model described in chapter 11 and validated in chapter 12. We dispute whether this is a valid conclusion.

Part III - A new era for DIPG research: large-scale, collaborative studies

Part III of this thesis (Chapters 14 and 15) is focused on the organizational level and looks at the future of DIPG research.

In **Chapter 14** the methodology and initiation of the SIOPE DIPG Registry and Imaging Repository is described, which is a result of the establishment of an international research infrastructure of biomedical experts: the SIOPE DIPG Network. The aim is to facilitate standardized, large-scale data collection for future collaborative research projects.

Chapter 15 builds on Chapter 14. This chapter describes the first worldwide retrospective DIPG patient cohort study, which emerged from the establishment of comprehensive and comparable data within the SIOPE and International DIPG Registries. The aim of this study is to compare the characteristics of long-term survivors (e.g. the few patients that have lived ≥24 months after diagnosis) and compare these to patients with shorter survival.

Chapter 16 provides a general discussion, covering all subjects that are assessed in this thesis. The findings of the individual research studies are placed in the context of recent developments in the field of DIPG research. Current challenges and the implications for future perspectives are discussed.

Chapter 17 provides an English summary of the work presented in this thesis.

Chapter 18 provides a Dutch summary of the work presented in this thesis.

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