# CHAPTER 17

## English summary

This thesis aims to increase the knowledge on diffuse intrinsic pontine glioma (DIPG), and aims to enhance the efficiency of research into DIPG. It is divided into three parts, starting with the first DIPG-specific clinical studies conducted in the Netherlands (Part I). Since DIPG is a rare disease, these studies have an order of magnitude of maximum nine patients. To enable larger and faster studies, the perspective of the research was expanded to a larger scope. Historical cohort studies and literature reviews were conducted at national, European and global level, reaching a maximum of 316 patients (Part II). This formed the foundation for the establishment of an international infrastructure for collaborative research-initiatives and the development of a DIPG Registry for large-scale uniform data collection, leading in 2016 to the first global study including over 1100 DIPG patients (Part III).

**Chapter 1** describes the general background of DIPG and its historical perspective to identify the reigning hypotheses and gaps in knowledge in 2012, before the start of the research described in this thesis. This chapter concludes with a detailed outline of the projects and aims of the subsequent chapters.

## PART I – FIRST DIPG-SPECIFIC STUDIES CONDUCTED IN THE NETHERLANDS

Up until March 2012, DIPG research was mostly regionally organized, small-scaled, and geographically scattered. In the Netherlands, no prospective clinical DIPG trial had yet been conducted. Thanks to the financial support of Stichting Semmy, the opportunity was provided to initiate the first DIPG-specific single- and multi-center trials in The Netherlands. The studies that were initiated at VU University Medical Center cover multiple aspects of DIPG, ranging from clinical symptoms, diagnostics and treatment strategies, to pre-clinical laboratory research on tumor material obtained via autopsy. Part I of this thesis (Chapters 2–6) describes the results of these studies, which are presented in analogy to a patient's journey.

**Chapter 2** describes the results of the first DIPG-specific clinical trial in the Netherlands: the DIPG study VUmc 01 (Dutch Trial Register: NTR2391) - phase A. In this Phase I/II trial, the feasibility and preliminary efficacy of gemcitabine added to standard radiotherapy (30 x 1.8 Gy) was investigated via a 3-step dose escalation in patients with newly diagnosed DIPG. The results show that addition of gemcitabine at doses of 140, 175 and 200 mg/m$^2$ is safe and well tolerated. All patients experienced reduction of tumor-related symptoms and quality of life tended to improve during treatment. Progression-free survival and overall survival were not significantly different from literature. This study creates a foundation for future clinical trials investigating the safety, tolerability, optimal dose and efficacy of gemcitabine when added to radiotherapy in DIPG.

In **Chapter 3** the results of the first molecular imaging study in childhood oncology patients are described. In this study, tumor-uptake and biodistribution of zirconium-89($^{89}$Zr) labeled bevacizumab (Avastin®) was visualized by means of PET imaging performed at 1, 72 and 144 hours post-injection. The results show that molecular imaging is feasible and safe in DIPG patients above 6 years of age, without the use of anesthetics. No adverse events occurred during any of the procedures. Based on the tumor-to-blood standard uptake value (SUV) ratio, which increased over time ($p = 0.045$), 144 hours post-injection was found to be the optimal moment of imaging. Among seven DIPG patients, marked inter- and intratumoral heterogeneity of $^{89}$Zr-bevacizumab-uptake was observed. Results from the biodistribution analysis show a toxicity profile with relatively high organ-uptake in the liver, kidneys, lungs, and bone marrow, and a mean effective dose per patient of 0.9 ± 0.3 mSv/MBq. The results suggest considerable variability in the efficiency (i.e., drug delivery and targeting) of bevacizumab in DIPG patients, possibly related to variable integrity of the blood-brain-barrier (BBB) and/or heterogeneous expression of vascular endothelial growth factor (VEGF). This study shows that imaging of $^{89}$Zr-bevacizumab-uptake by PET may be of value for the selection of potential candidates for bevacizumab treatment in DIPG. At the same time it will prevent unnecessary toxicity in patients that show no tumor-uptake.

**Chapter 4** describes the results of a PET-imaging study of fluor-18 fluorodeoxyglucose ($^{18}$F-FDG). In order to produce normative values of $^{18}$F-FDG-uptake in the pons in children with a non-affected brainstem, SUV ratios of the pons versus the cerebellum ($SUVr_{p/c}$) and versus the occipital lobe ($SUVr_{p/o}$) were determined in 36 non-DIPG patients who underwent $^{18}$F-FDG PET scans for epilepsy surgery planning. Both $SUVr_{p/c}$ (0.65 ± 0.054 and $SUVr_{p/o}$ 0.51 ± 0.056) values were shown to be strikingly constant between subjects, irrespective of sex, age, or pontine volume and may therefore be well used as a reference value for future $^{18}$F-FDG PET studies in pontine disorders. Six DIPG patients were compared to these newly derived normative values. In these patients, the mean SUV ratios were higher ($SUVr_{p/c}$ 0.74 ± 0.20 and $SUVr_{p/o}$ 0.65 ± 0.30), although not statistically significant, probably due to the small sample size. Future research should determine whether $^{18}$F-FDG PET is of value in the classification, prognostication and response evaluation of DIPG patients.

In **Chapter 5** the results of a multi-institutional whole-brain autopsy study in DIPG patients are described. Multiregional sampling of DIPG tumor material revealed extensive heterogeneity in intratumoral morphology, with areas showing high-grade (WHO Grade IV) and low-grade (even WHO-grade I like pilocytic astrocytoma- and/or subependymoma-like) tumor histopathology. In seven out of nine patients the tumor harbored a histone H3 K27M mutation (67% H3.3, 11% H3.1). As expected, these tumors

showed loss of H3 K27 trimethylation (H3 K27me3) immunoreactivity in all tumor cells, irrespective of histological phenotype and grade. Interestingly, in the two patients with a H3 wildtype DIPG, the tumor showed focal loss of H3 K27me3, which was not clearly related to local tumor morphology or grade. The results show that histologic phenotype and immunohistochemical staining for H3 K27M/H3 K27me3 status in small DIPG biopsies can be deceptive. Hence, we suggest mutation analysis of the histone H3 gene, additional to the current clinic-radiological approach, since this will correctly classify 80-90% of tumors. Future research investigating the origin and effect of heterogeneous loss of H3 K27me3 in H3 K27 wildtype DIPG should increase the knowledge on this particular DIPG subgroup.

**Chapter 6** discusses the case of a 12-year old patient who died shortly after participation in the $^{89}$Zr-bevacizumab PET study and for whom permission to perform an autopsy was granted by the parents. At autopsy, multiple tumor and non-affected brain samples were obtained for *ex vivo* $^{89}$Zr-bevacizumab measurement, and analysis of local histopathology and vascular morphology. Only tumor areas with extensive vascular proliferation showed high $^{89}$Zr-bevacizumab-uptake, suggesting vascular proliferation to be a major determinant for bevacizumab-uptake in DIPG.

## PART II – EXPANDING THE SCOPE: HISTORICAL AND INTERNATIONAL RESEARCH INITIATIVES

Since DIPG is so rare, the regionally organized, small-scaled research initiatives provide insufficient data to answer the many questions raised. Therefore, in Part II of this thesis (Chapters 7–13), the research perspective was expanded to a larger scope, both in time and in scale.

**Chapter 7** presents the results of a nation-wide, population-based retrospective cohort study, in which all children diagnosed with DIPG in the Netherlands between 1990 and 2010 were evaluated. The incidence of DIPG in the Netherlands was determined to be nine patients per year (0.54 per 1,000,000; 2.32 per 1,000,000 aged 0–20 years), with annual variations ranging from 5–13 per year. It was shown that in this time period only 18% of DIPG patients were included in clinical trials, resulting in a large heterogeneity of incomparable treatment schedules, which were mostly based on single-center guidelines or individualized therapy due to lack of evidence-based guidelines.

**Chapter 8** describes the results of a literature search identifying all chemotherapeutics applied to DIPG patients around the world. A model was developed, that takes into account all physicochemical properties that affect the degree of passive diffusion across an intact BBB (upon intravenous administration). Likewise, the likelihood of adequate

convection upon local intratumoral administration by convection-enhanced delivery (CED) of these drugs was ascertained. The model shows that most drugs previously administered to DIPG patients are molecularly charged, hydrophilic, and/or relatively large, and therefore less suitable for passive diffusion over the BBB. Local administration of chemotherapeutics via CED is less dependent on drug size and hydro/lipophilicity and only excludes positively charged drugs. Application of the model to the list of chemotherapeutic agents that historically were administered via the systemic route to DIPG patients showed that for many of these drugs, CED may increase their potential to reach therapeutic concentrations. This study may lead to a more efficient choice of chemotherapeutics in the treatment of DIPG patients in the future, especially when combination therapies with various routes of administration are considered.

**Chapter 9** describes the results of a retrospective cohort study of 63 DIPG patients who received palliative and end-of-life care in two large London Children's hospitals. Symptoms, interventions and services applied during the 12 weeks before death were analyzed. The results show that over 80% of the patients suffered from problems concerning mobility, swallowing, communication, consciousness, and breathing during end-stage disease. Supportive drugs were widely prescribed, in contrast to medical aids (such as a speech-computer) that were prescribed to only 15% of patients. In addition, a global questionnaire-study among healthcare professionals was conducted to ascertain information on the (multi) institutional and (multi)national approach to palliative care for DIPG patients. It was shown that palliative and end-of-life care was mostly based on the health care professional's experience; only 21% of the respondents reported to have a disease-specific palliative care guideline available. This study forms the basis for the development of international disease-specific guidelines for palliative care in DIPG patients.

In **Chapter 10,** the current use of corticosteroids for symptom management in DIPG patients was reviewed. A global questionnaire-study among health care professionals was performed. The vast majority of respondents, 140 out of 150, reported not to have a guideline for the prescription of these drugs. Analysis of current corticosteroid prescription policies showed great heterogeneity and over 85% of health care professionals reported to observe serious side effects in their patients. A review of the literature yielded only 14 low-level evidence articles describing the use of corticosteroids in pediatric brain tumor patients. Clinical trials investigating optimal dose or regimens of corticosteroid use in DIPG and other pediatric brain tumors are lacking. This study forms the basis for the establishment of an international trial with the aim of developing a disease-specific guideline for the use of steroids in DIPG patients.

In **Chapter 11,** the results of the first European multicenter retrospective cohort study are presented, including 316 centrally-reviewed and radiologically-confirmed DIPG

patients from the Netherlands, United Kingdom, and Germany. To better understand the variables influencing the outcome of DIPG patients, a multivariable DIPG survival prediction model was developed. The model identified age at diagnosis below 3 years and longer symptom duration at time of diagnosis, and receipt of any chemotherapy at any time during the disease course as favorable predictors, and presence of ring enhancement on diagnostic MR-imaging as unfavorable predictor. Based on a calculated risk score, the model was able to distinguish patients with shorter, average, and increased survival (with medians of 7.0, 9.7, and 13.7 months, respectively).

In **Chapter 12** the validity of the DIPG survival prediction model was determined through external validation in a similar, but independent patient cohort including 249 patients from the United States, Canada, and Australia. Model performance was evaluated by analyzing the discrimination and calibration abilities. The results show that the DIPG survival prediction model has adequate calibration abilities and is able to discriminate patients with shorter, average, and increased survival in the independent validation cohort. The DIPG survival prediction model is herewith validated for use in clinical practice. The model may perform a useful role, especially for "retrospective risk classification", enabling stratification of patients by disease risk category in the re-evaluation of historical clinical trial efficacy.

**Chapter 13** presents a critical appraisal on a French DIPG cohort study in which the histone H3 mutation was shown to have strong predictive value for survival, and suggested to be better than the DIPG survival prediction model. We dispute whether this was a valid conclusion.

## PART III – A NEW ERA FOR DIPG RESEARCH: LARGE-SCALE, COLLABORATIVE STUDIES

Part III of this thesis (Chapters 14 and 15) focuses on the organizational level and looks at the future of DIPG research with the purpose to increase the efficiency.

**Chapter 14** describes the initiation and implementation of the SIOPE DIPG Registry and Imaging Repository, which is a result of the establishment of an international research infrastructure of biomedical experts from different countries in- and outside Europe: the SIOPE DIPG Network. Since April 2016, standardized clinical data as well as MRI-scans of 694 patients from multiple European countries have been registered in the SIOPE DIPG Registry and Imaging Repository. For the first time, large datasets have become available to identify important aspects of clinical characteristics, diagnostics and treatment strategies, as well as important aspects regarding diagnostics, treatment, quality of life and supportive care.

**Chapter 15** describes the first multi-national collaborative study into DIPG, using combined data from the SIOPE DIPG Registry and the International DIPG Registry. It represents the largest and most comprehensive analysis of centrally reviewed DIPG patients worldwide, and includes the largest patient cohort with known histone mutation status. Among 1130 patients with confirmed DIPG, 101 (10%) were long-term survivors, historically defined as patients with overall survival (OS) greater than two years. Median survival for the entire cohort was 11 months (range 0-167 months), and 1-, 2-, 3-, 4- and 5-year OS were 42.3%, 9.6%, 4.3%, 3.2%, and 2.2%, respectively. Using univariate and multivariate analyses, significant predictors of long-term survival were identified, including age <3 or >10 years, longer symptom duration, absence of cranial nerve palsy, smaller cranio-caudal tumor dimension and absence of extra-pontine extension, necrosis, or ring enhancement on diagnostic MRI. This study also confirms the previously reported prognostic significance of the *HIST1H3B* mutation for long-term survival. And finally, this study shows that chemotherapy or targeted therapy, in combination with radiotherapy, does confer a survival advantage compared to radiotherapy alone.

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