In chapter 1, the introduction, an overview is given of the status of DIPG at the start of this thesis. Less than 10% of the patients with DIPG survive beyond two years from diagnosis. Thirty years of clinical trials with increasing radiotherapy dose schedules, several combinations of chemotherapy and single-agent targeted therapy regimens have not improved the prognosis, although there is some variation in outcome between trials. It is unclear whether this variation is due to real treatment effects or to selection bias, i.e. inclusion of patients with distinct disease courses. An important hurdle in improving the selection of potential drugs for DIPG is the lack of tumor tissue, which has hampered preclinical research thus far. In addition, drug delivery might be an issue in these tumors, but data to support this were lacking at the start of this thesis. The main aims of this thesis are to establish the incidence of DIPG in the Netherlands, to develop preclinical models from autopsy material, to define predictors of prognosis based on clinical and imaging criteria and to make the first steps in monitoring drug delivery in DIPG.

Chapter 2 provides a systematic review of all trials from 2005 until 2011. In total, 26 prospective clinical trials, including 561 children with newly-diagnosed DIPG, were published in this period. Although restricted to DIPG, there was considerable inter-study variability in eligibility criteria, including performance state, life expectancy, symptoms at diagnosis and laboratory findings and a minority of the studies further specified the MRI criteria. The prognosis of DIPG has not improved during the past six years. The high expectations of temozolomide have not been realized. Only one study clearly showed an improvement in median OS applying pre-irradiation therapy consisting of high-dose methotrexate, BCNU, cisplatin and tamoxifen, but selection bias cannot be excluded in this study and the long-term outcome was poor.

In chapter 3 we report on the patients treated in Netherlands between 1990 and 2010. The incidence is 0.54/1,000,000, which means that nine patients with DIPG were newly diagnosed each year. More than 20 different radiotherapy and chemotherapy regimens were applied in 157 patients and unfortunately the majority of the patients was treated off-trial. These results emphasize the need to treat patients with rare diseases as DIPG in (inter)national trials.

In chapter 4 we present the development of a survival prediction tool for DIPG in a multinational cohort. Multivariate Cox analysis yielded five prognostic variables. Age ≤ 3 years, longer symptom duration at diagnosis, and use of oral and intravenous chemotherapy were favorable predictors, while ring enhancement on MRI at diagnosis was an unfavorable predictor. The combination of these variables results in a DIPG risk score. The DIPG risk score of individual patients estimates the overall survival time and, in non-randomized trials, can explain a change in overall survival due to selection bias that would otherwise be attributed to the study drug. In future studies, patients can be stratified according to the DIPG risk score in a standard, medium and high-risk group.
In chapter 5, we aimed to establish a reference for glucose metabolism of the pons. We show that the $^{18}$F-FDG SUV ratios of the normal pons versus cerebellum and occipital lobe are very constant amongst controls, independent of sex, age and pontine volume and are therefore suitable as a reference for $^{18}$F-FDG PET studies in DIPG. The reference allows detecting subtle changes in glucose metabolism in DIPG.

In chapter 6, we used molecular imaging to study the biodistribution of $^{89}$Zr-bevacizumab in mouse models with an E98 DIPG- or striatal glioma versus an E98 subcutaneous tumor. We found neither significant uptake of $^{89}$Zr-bevacizumab in the brain, nor in both intracranial tumors. In contrast, high accumulation of $^{89}$Zr–bevacizumab was observed in the subcutaneous E98-xenograft. We initially hypothesized that this was solely due to poor bevacizumab distribution over the blood-brain barrier. However, also the target expression (VEGF) of the E98 glioma cells was different; gliomas in both striatum and pons were VEGF-negative, while the subcutaneous E98 tumors were VEGF-positive. This is probably due to a lack of necrosis in the intracranial tumor models.

In chapter 7 we studied the first three children worldwide with immuno-PET. We show that immuno-PET is feasible in children without the use of anesthetics. $^{89}$Zr-bevacizumab uptake was observed in the contrast-enhancing part of the tumor in two patients at best at 144 hours post-infusion, while the third tumor had no significant uptake. It seems that bevacizumab uptake is related to gadolinium uptake in the tumor, and thus to blood-brain barrier disruption. However, the uptake clearly differed between the two PET positive tumors, probably due to a local difference in VEGF expression.

In chapter 8 we describe the results of a matched-pair analysis of patients who underwent hypofractionated radiotherapy versus conventional radiotherapy. With this radiotherapy schedule we aimed to reduce the burden for patients, by limiting the radiation time to three instead of six weeks, without causing a decrease in survival. We showed that the median overall survival is 9.0 versus 9.4 months, respectively, which was not significantly different ($P=.84$). Although not statistically significantly different either, there was a trend to a decreased median time to progression (PFS) between hypofractionation and conventional radiotherapy: 5.0 versus 7.6 months, respectively ($P=.24$). Overall, hypofractionation therapy seems to be a reasonable alternative for parents who choose not to participate in experimental clinical trials, although studies with higher patient numbers should investigate a possible reduction in PFS.

In chapter 9 we report the results of the first five DIPG patients who underwent autopsy in the Netherlands. The death-autopsy interval was short (three hours) compared to other autopsy protocols in North America. It enabled us to develop and characterize one of the first DIPG cell-
cultures. Evaluation by the parents revealed that no one regretted their choice to participate and yielded several useful recommendations that we integrated in our procedure.

In chapter 10 our findings are discussed and ongoing and future research (perspectives) is described.