Summary

This thesis comprises eight chapters, including a general introduction, six original research manuscripts and a general discussion. All chapters focus on preclinical and translational research in diffuse intrinsic pontine glioma (DIPG), nowadays also referred to as diffuse midline glioma, H3K27M mutated or not otherwise specified (NOS). DIPG is a devastating disease that is diagnosed 8-10 times per year in the Netherlands in children from 1 to 18 years old. Median survival is 9 months and 2-year survival is 10% or less. DIPG grows diffusely in the pons, complicating treatment with traditional surgical and radiotherapeutic methods. Moreover, drug delivery appears inadequate due to a (partly) functional blood brain barrier, which helps explain the lack of effective chemotherapy. Because no therapy can currently cure DIPG, new treatment strategies are needed, and preclinical research using DIPG in vitro and in vivo models can be of aid in rational clinical trial design. It is currently known that 80% of DIPG tumors harbour mutations in the histone 3 gene, leading to epigenetic deregulation. Other genetic changes identified in DIPG are mutations in ACVR1 (20-32%), TP53 (42-71%), PPM1D (10-12%), ATRX (9-13%) and PIK3CA (12-23%), amplifications in PDGFRA (36%) and deletions in PTEN (14%), NF1 (7%) and RB1 (16%). With insights in DIPG biology and the availability of preclinical model systems, new potential drug targets have emerged and targeting those is currently being investigated in clinical trials. Still many questions remain regarding disease initiation and progression as well as efficacy of drug delivery. This thesis focuses on DIPG disease models and on preclinical drug delivery. In chapter 2 we describe an unexpected phenomenon occurring after direct injection of DIPG autopsy material in the brainstem and striatum of mice. All tumors, formed after injection of material from three different patients, proved to be composed of murine and not of human cells. The murine pontine tumors exhibited a histology and immunophenotype similar to human DIPG, suggesting that direct injection of human DIPG cells in vivo can give rise to malignant murine tumors. In contrast, an initial in vitro culture step allows the establishment of human orthotopic xenografts. The mechanisms underlying this phenomenon are not completely understood, but further study could provide valuable information on the oncogenesis of DIPG.

Chapters 3-5 are focused on preclinical convection-enhanced delivery (CED) studies. CED uses both fluid convection and diffusion to infuse large (tumor) areas in the brain with local chemotherapeutics. The study in chapter 3 aims to develop a method to perform CED into the murine brainstem and to test this method using the chemotherapeutic agent carmustine (BiCNU). After determination of safety and distribution, mice bearing
VU-DIPG-3 and adult high-grade glioma derived Eg8FM-DIPG-like brainstem tumors were treated with 15µl carmustine delivered via CED in 30 minutes. Our results showed that CED in the murine brainstem can be performed safely, is well tolerated and can be used to study the efficacy of chemotherapeutic agents orthotopically. CED of carmustine increased median survival of mice with VU-DIPG-3 and Eg8FM tumors in the brainstem by 35% and 25% respectively. This technique allows for more preclinical CED studies in mice to obtain data to make a successful translation to the clinic. In chapter 4 we determine the efficacy of anthracycline drugs against DIPG and pediatric high-grade glioma (pHGG) cells in vitro, and we subsequently study the feasibility and efficacy of performing CED with free doxorubicin and pegylated liposomal doxorubicin (PLD) to treat orthotopic DIPG models. Methods used in preclinical CED are the same as described in chapter 3. Both pHGG and DIPG cells were sensitive to anthracyclines in vitro, but the maximum tolerated dose (MTD) of CED with free doxorubicin and PLD in the pons was very low (0.02 mg/ml) and doxorubicin or PLD via CED was ineffective against HSJD-DIPG-007 (grown in vivo from a primary DIPG cell line) and Eg8FM-DIPG (grown in vivo from an adult high-grade glioma cell line) in the pons. MTD in the thalamus was 10 times higher and when applied in the thalamus, 0.2 mg/ml PLD slowed down tumor growth and increased survival in a subset of animals with small tumors. We conclude that local delivery of doxorubicin causes severe toxicity when delivered to the brainstem. As a result, we could not establish a therapeutic window for treating orthotopic brainstem tumors in mice. For tumors in the thalamus, therapeutic concentrations could be reached to slow down tumor growth. This suggests that anatomical location determines the severity of toxicity after local delivery of therapeutic agents, and that caution should be used when translating data from supratentorial CED studies to treat infratentorial tumors. In chapter 5 we study long-term, high-volume CED in rat pons using delivery of irinotecan and SN38 Poly Lactic-co-Glycolic Acid (PLGA) nanoparticles (NPs) via a subcutaneously implanted Alzet pump attached to an intratumorally-placed infusion catheter. First, the stability and release of SN-38 from NPs was tested in a period of seven days at 37°C in our selected vehicle (NaCl 0.9%). Release was tested using a dialysis chamber and sequential samples were analysed by HPLC. Stability of SN-38NPs and release from the Alzet pump was tested via an in vitro setup and analysis of released fluid using HPLC. After these experiments had confirmed slow release of SN-38 from nanoparticles and stability of SN-38NPs in the Alzet pump for at least 24 hours, long-term CED in vivo was optimized. Adequate distribution was assessed using SN-38NPs containing fluorescein isothiocyanate (FITC) and toxicity of CED was determined by
Rota-rod neurological function tests. Long-term, high-volume CED (200µl in 24 hours) using NaCl 0.9% vehicle caused only transient neurological deficits (measured by Rota-rod function tests) and weight loss in naïve rats. Long-term CED using irinotecan and SN38NPs caused severe neurological symptoms at medium and high doses (4 mg/ml and 0.2 mg/ml respectively) and MTD (0.4 mg/ml and 6µg/ml respectively) was again much lower than previously described in the literature. This low dose was insufficient to effectively treat diffusely growing HSJD-DIPG-007 tumors when CED treatment was begun 18 days after injection of HSJD-DIPG-007 cells.

Chapters 6 and 7 focus on intra-tumoral distribution of vascular endothelial growth factor (VEGF)-inhibitor bevacizumab. In both studies we use injection of zirconium-89 (\(^{89}\text{Zr}\))-labeled bevacizumab followed by positron emission tomography (PET) scanning and ex vivo measurements. In chapter 6 we use different high-grade glioma and DIPG mouse models to study distribution in the tumor. Adult E98FM, U251-FM glioma cells and pediatric HSJD-DIPG-007-Fluc primary DIPG cells were injected into the subcutis, pons or striatum of nude mice. Tumor growth was monitored by bioluminescence imaging (BLI) and visualized by Magnetic Resonance Imaging (MRI). Seventy-two to 96 hours after \(^{89}\text{Zr}\)-bevacizumab injections, mice were imaged by Positron Emitting Tomography (PET) and biodistribution was analyzed ex vivo. Analyzing the data, no significant \(^{89}\text{Zr}\)-bevacizumab uptake could be detected in xenografts located in the pons and striatum at an early or late stage of the disease. The E98FM, and to a lesser extent the U251-FM and HSJD-DIPG-007 subcutaneous tumors, showed high accumulation of \(^{89}\text{Zr}\)-bevacizumab. VEGF expression could not be demonstrated in the intracranial tumors by in situ hybridization (ISH) but was clearly present in the perinecrotic regions of subcutaneous E98FM tumors. The poor uptake of \(^{89}\text{Zr}\)-bevacizumab in xenografts located in the brain suggests that VEGF-targeting with bevacizumab has limited efficacy for diffuse infiltrative parts of glial brain tumors in mice. In chapter 7 we describe a unique case report involving a DIPG patient in whom an MRI, a PET scan after administration of \(^{89}\text{Zr}\)-bevacizumab and an autopsy allowing for collection of tissue for ex vivo \(^{89}\text{Zr}\) measurement and histological analysis took place in short succession. In this study, we correlate \(^{89}\text{Zr}\) –bevacizumab uptake measured ex vivo in tumor samples obtained at autopsy, to histological features and vascular morphology. We observed that \(^{89}\text{Zr}\)-bevacizumab uptake in end-stage DIPG is heterogeneous, and only substantially high in an area with profound vascular proliferation, which suggests that vascular proliferation is in important determinant for bevacizumab targeting. Since histology of DIPG is heterogeneous and not each part of the tumor is characterized by florid vascular proliferation, it is likely many patients will fail
on bevacizumab treatment. This hypothesis is strengthened by the disappointing results from clinical trials with bevacizumab in pediatric DIPG and high-grade glioma patients. Translating results from the studies described in chapters 6 and 7 to the clinic would imply that treatment with bevacizumab in DIPG patients is only justified after targeting of VEGF has been demonstrated by $^{89}$Zr-bevacizumab immuno-PET. In chapter 8, particular findings mentioned in previous chapters are discussed and appraised by the most recent literature, and suggestions are made for further research.