CHAPTER 8
General Discussion
References
Summary
Samenvatting
In the previous chapters we described different preclinical approaches to gain knowledge that can be used to reach a better outcome for patients with diffuse intrinsic pontine glioma (DIPG). We either used disease model systems (chapters 2 – 6) or a unique patient case presentation (chapter 7) to answer research questions related to disease initiation (chapter 2), drug delivery (chapters 3, 4 and 5) or drug distribution (chapters 6 and 7).

### Oncogenic transformation in DIPG mouse models

Up until now, the exact reason that children develop a DIPG is insufficiently understood. We know that almost all DIPG tumors have some form of epigenetic deregulation, most clearly illustrated by the frequent occurrence of H3K27M mutations. It was recently suggested that the mutation in histone 3 requires a mutually exclusive partner mutation in either cell cycle (TP53 or PPM1D) or growth factor activation pathways (ACVR1 or PIK3R1) 208. Knowledge about tumor initiation and progression can provide insights into potential therapeutic and preventative strategies. In chapter 2 we described how human DIPG cells can cause horizontal oncogenic transformation in the murine brain. Studying this phenomenon helps us to increase our knowledge of tumor initiation and progression.

Horizontal oncogenic transformation has long been described in the literature in various cancer types and hosts, with the first report dating back to 1905 119. A wide range of explanations has been suggested over time, but with one important exception, this has not led to a comprehensive understanding of this intriguing phenomenon. This exception is the identification of the so-called oncoviruses, such as for instance HPV-related cervical carcinoma and hepatitis-related hepatocellular carcinoma 253. This discovery led to new and unique possibilities to prevent cancer. The role of (retro)viral sequences and other retro-elements in the initiation of brain tumors is a topic of much controversy 254,255. Especially the role of human cytomegalovirus (hCMV) has been much debated in recent years. In 2012 a consensus statement from various scientists was issued in Neuro-Oncology stating that “there was sufficient evidence to conclude that hCMV sequences and viral gene expression exist in most, if not all, malignant gliomas,” that “hCMV could modulate the malignant phenotype in glioblastomas by interacting with key signaling pathways,” and that “hCMV could serve as a novel target for a variety of therapeutic strategies” 256. Subsequently, a clinical trial was published in 2013 reporting a spectacular effect of valganciclovir treatment in glioblastoma patients 257. Even though the trial included only few patients and had a complex study design, analysis showed a median overall survival of 24.1 months in patients receiving more than six months of valganciclovir versus 13.1 months in patients who did not receive this particular therapy.
It is unknown whether hCMV plays a role in the initiation or progression of DIPG. Considering our observation that horizontal oncogenic transformation is possible after injection of DIPG cells into the murine pons, detection of a viral agent in DIPG could be of great importance. Truly determining a causal relation between an infective agent and oncogenic transformation is challenging, but we think a comprehensive and unbiased study of our samples is warranted and these studies are currently ongoing.

**Preclinical convection-enhanced delivery**

Deciphering tumor-initiating events can be of great importance for scientific research, but it might not directly influence patient outcomes. At the moment, identifying new successful treatment strategies is still essential, and preclinical research helps to identify promising strategies. In chapters 3 and 5 we described the development of a new method to study the efficacy of drugs *in vivo* after local convection-enhanced delivery in mice. The value of preclinical CED experiments in predicting the efficacy of this treatment modality in patients is topic of some debate. Distribution after delivery is key, and due to the small size of the murine pons and the difference in delivery technique between murine and human CED, external validity is diminished for some of the research questions studied. However, we argue that preclinical CED experiments can aid substantially in identifying the therapeutic potential of drugs. When a drug is not successful in treating relatively small tumors in mice, as illustrated in chapter 4, it is unlikely to be successful in treating extensive tumors in the human pons.

However, our murine CED model system should be used with the correct research question, as it is inadequately suited to study technical issues such as differences in flow rate, catheter design and timing of (repeated) delivery. In addition, functional toxicity studies that assess subtle differences in neurological functioning are difficult to perform in mice. Technical aspects and toxicity of CED could also be studied in naïve non-human primates and pigs, but because these models lack brain tumors, they do not allow for efficacy studies. Using rat-DIPG models, as suggested in chapter 5, can be of aid to study technical aspects, toxicity and efficacy of CED using various agents, but still a large gap exists from rat to human brain in terms of size and complexity. Knowledge of the strengths and weaknesses of the different preclinical model systems is essential when designing preclinical research projects.
Translation of CED to the clinical situation

Since the first publication of convection-enhanced delivery of targeted toxin IL13-PE into the pons of a DIPG patient in 2007\(^7\), the potential for CED in the treatment of DIPG remains unclear. In theory, CED should allow for intratumoral drug concentrations that surpass therapeutic concentrations by manifold. In practice, tumor cells escape treatment due to: 1 – the fast efflux of the drug into the tumor parenchyma, leading to inadequate exposure time of tumor cells to the drug\(^\text{188}\); 2 – a small therapeutic window due to sensitivity of innate brain cells to toxic agents (chapter 3,4); and 3 – the inadequate distribution after CED as tumor cells may already grow outside the therapeutic infusion area\(^\text{154,208}\). To maximize therapeutic potential of CED in DIPG, all three aspects should be addressed in preclinical studies followed by clinical trials. Efflux can be prevented by using nanoparticle formulations\(^\text{76,169}\), by choosing drugs with the correct chemical properties, or by co-infusion of drugs with inhibitors of drug efflux pumps. Using drugs with little toxicity to normal brain cells would allow for high drug concentrations, whereas repeated infusions would prevent toxic peak concentrations. Distribution can be improved by using multiple infusion catheters, brain-penetrating nanoparticles\(^\text{75}\) and sophisticated mathematical modeling to ensure adequate targeting of tumor areas\(^\text{190}\).

But even when addressing all these issues, treatment of DIPG patients will most likely require a multi-modality approach, which combines both systemic and local therapeutic strategies. Up until now, 10 cases of CED in DIPG patients (and one in a high-grade glioma infiltrating the brainstem) have been published using classic chemotherapeutic agents (lomustine, carboplatin, topotecan,\(^\text{65,71,72}\), targeted toxins (IL13-PE\(^\text{73}\)) or radioactive-iodine labeled antibodies (I\(^\text{124-8H9, clinicaltrials.gov}\)). Prolonged survival\(^\text{71}\) and radiological response\(^\text{72}\) have both been described in one patient. Recently, a clinical series of eight DIPG patients treated with carboplatin infusions via CED was reported by colleagues from Bristol University Hospital (personal communication ISPNO 2016, Edwards & Singelton). In this small case series, patients received 2 – 18 infusions with carboplatin. Median follow-up time, or survival from diagnosis at time of the interim analysis, was 11.8 months and six out of eight patients were still alive (personal communication ISPNO 2016, Edwards & Singelton). However, these patients represent a selected population and it can therefore not yet be concluded that CED with carboplatin is an effective treatment strategy for DIPG. But these results are among the first to suggest a treatment effect of CED in DIPG patients, and a well-designed, controlled, clinical trial should be performed as soon as possible to confirm these findings. In the meantime, these encouraging
results call for more preclinical efforts to further improve the pharmacological and technical aspects of CED.

**Drug distribution in DIPG; conclusions from $^{89}$Zr- bevacizumab distribution studies**

In chapters 5 and 6 we explored the possibilities to study drug distribution after systemic delivery. $^{89}$Zr-bevacizumab plays a central role in these chapters, but our presented data and literature references suggest that the role for bevacizumab itself in the treatment of DIPG is limited. High VEGF expression is observed in end-stage DIPG and high VEGF expression has been correlated to poor outcome in high grade glioma. Unfortunately, clinical studies with VEGF-inhibiting antibody bevacizumab in children with DIPG and in adults with high grade glioma have been disappointing. Our data suggest that in diffusely growing E98FM and HSJD-DIPG-007 orthotopic xenografted tumors, very little uptake of $^{89}$Zr-bevacizumab can be detected in the tumor with either preclinical PET-imaging or ex vivo measurements (chapter 5). Similar results were seen in our clinical PET studies and ex vivo measurements (chapter 6). $^{89}$Zr-bevacizumab uptake was present in selected tumor areas but there was clear heterogeneity and only the area with extensive vascular proliferation showed substantially high bevacizumab-targeting. This suggests that even in end-stage, high-grade DIPG, high bevacizumab-targeting is only present in limited areas of the tumor due to intratumoral heterogeneity. This is in line with the disappointing results from clinical trials so far. However, more limited VEGF-inhibition can also lead to vascular normalization and subsequent improved distribution of chemotherapeutic agents and improved response to radiotherapy due to better oxygenation of the tissue. Exploiting these secondary effects of VEGF-inhibition would require a different strategy. Timing of VEGF-therapy and treatment combinations need to be studied in more detail. To this end, our preclinical models are only partly suited because bevacizumab only binds weakly to murine VEGF, and murine elements form an important part of the tumor micro-environment.

The tumor micro-environment in gliomas is made up of tumor-associated parenchymal cells, such as vascular cells, microglia, peripheral immune cells and neural precursor cells. Because diffuse gliomas grow infiltratively, i.e. in between normal brain structures, non-transformed resident supportive cells can be influenced by the tumor, and tumor-associated astrocytes have been demonstrated to mediate glioblastoma cell invasion. This underlines the importance of using orthotopic tumor models to study...
the efficacy of new treatment strategies, but it also illustrates an important weakness of murine tumors when studying species-specific (targeted) agents. Again, knowing the strengths and weaknesses of the disease models used is essential to correctly interpret the data obtained from preclinical studies.

**Molecular drug imaging – identifying the right drug for the right patient**

Even though only a limited number of studies have been performed to directly study the BBB in DIPG, it is assumed that the BBB plays an important role in therapy resistance. Currently, many trials in DIPG and brain tumors in general are conducted without knowing what proportion of the drug will actually reach the tumor cells. Phase 0 studies, aimed at obtaining comprehensive pharmacodynamic and pharmacokinetic information using patient tumor samples, are rarely performed but are needed to make informed decisions for subsequent trials. Especially in rare diseases with limited patients available to participate in clinical trials, optimal trial design is essential. Molecular drug-imaging techniques can provide information on the distribution and targeting of drugs after systemic administration and should therefore be an integral part of preclinical drug development and clinical trial design, especially in brain tumors. Immuno-PET studies have been shown to be feasible in children and to have potential to determine the distribution of both targeted antibodies and small molecule inhibitors.

With the development of advanced targeted agents to influence very specific cellular pathways that play a role in cancer progression, identification of the right drug for the right patient will become a mandatory part of the diagnostic process. Failure to identify the right patient will lead to unacceptably high health care costs, unnecessary and potentially toxic side effects and failure of the agent to deliver significant treatment effects in unselected patient populations. There are several strategies to identify what pathways should be targeted and which patients should therefore be treated with which drug(s). Tissue obtained with tumor resection or biopsy can be studied for specific mutations or pathway (de)activation by using (RNA)sequencing techniques, gene expression or methylation profiling. The new WHO 2016 classification, which uses not only histological but also molecular data to classify diseases, is an example of integrating molecular biology into clinical practice. Even though biopsies have been shown to be safe, they are still not routinely performed in DIPG because they constitute an invasive procedure with, at the moment, few therapeutic implications. Taking small biopsies can lead to sampling error, whereas repeated sampling to determine tumor adaptation...
to treatment is not readily feasible. Taking “liquid biopsies” and for instance gathering information from the tumor by sequencing RNA present in “tumor-educated platelets” might provide an attractive alternative to obtaining tumor material in the near future. These techniques will need to be validated in a pediatric population before they can be of use in the diagnosis and follow-up of DIPG and other pediatric brain tumor patients. Molecular drug imaging using immune-PET is a validated and acceptable technique in children. It can be used to study not only pathway activation but also target engagement after systemic administration of a drug, and it can provide information over time by performing repeated scans. Using all three approaches mentioned (studying tumor material, taking liquid biopsies and performing molecular drug imaging studies) should provide comprehensive knowledge useful for designing a personal treatment plan for each individual patient, and this could be essential in treating complex, treatment-resistant tumors such as DIPG.

**International collaboration and support**

Generous financial support from parent organizations has initiated the start of research projects worldwide. In the Netherlands, “Stichting Semmy” played an invaluable role and supported our research group at the VU University Medical Center from the start. The first projects in DIPG research were aimed at obtaining tumor material, and this was done by the initiation of autopsy studies. The VUMC-DIPG-autopsy study was initiated in 2007 and was followed by the opening of a phase I/II clinical trial in 2010 (NTR2391) including a voluntary biopsy, a phase 0 trial studying distribution using PET (NTR3518) and the initiation of the European DIPG registry. In the last five years, the number of research papers and doctoral theses published on DIPG has multiplied. International collaboration has enabled the exchange of material, knowledge and experience, which has contributed to many of the breakthroughs in understanding of DIPG biology and availability of preclinical models. Because DIPG is such a rare disease, sharing of patient material, cell lines, patient and imaging data is crucial for the advancement of both preclinical and clinical studies. The identification of panobinostat as a novel, potentially therapeutic agent targeting epigenetic deregulation is illustrative of what can be achieved with effective collaboration. But international collaboration is no less essential when performing high-quality clinical studies within an acceptable timeframe. Only 8-10 patients are diagnosed with DIPG each year in the Netherlands, and only a small percentage of those patients are enrolled in clinical trials. To obtain clinical and imaging data on enough patients to perform (retrospective and
especially prospective) studies, the European DIPG network and European registry was initiated, running parallel to the international DIPG registry including patients from the USA, Canada and Australia. Soon the first papers from this network will be published and we envision that in the future the network will provide the logistical framework necessary to perform efficient clinical studies and exchange both clinical and biological information to enable high-quality translational research.

**Closing remarks**

In the past years there have been many developments in DIPG research, but prognosis is still very poor and no current treatment schedule exists that can provide evidence-based hope to even a small subgroup of patients. Some factors are out of the control of researchers and doctors: the tumor is located in a vital, and surgically inaccessible, part of the brain, has a diffuse growth pattern, with an at least partially intact blood brain barrier, and tumor cells are very chemo- and radio-resistant. Other factors, however, are being addressed successfully by the international scientific community: the lack of knowledge regarding tumor biology, scant biological material to study, few patients included in clinical trials, no comprehensive registry system to perform (retrospective) clinical studies and no or very few disease model systems available to perform translational research. In the past few years, these gaps and limitations are slowly being addressed. We now have a substantial number of preclinical models to study new therapies, several drug-imaging techniques to study the targeting of therapeutics to the tumor, and an international network to study clinical, radiological and biological features of many patients, all of which can provide the logistical support to perform clinical trials.

All of these developments allow researchers and clinicians to work towards the same goal: an effective therapy for DIPG patients with acceptable side effects on the short and long-term.
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