Chapter 1

General introduction and outline of this thesis

Adapted from: ‘Hersentumoren op kinderleeftijd’
Tijdschrift voor Kindergeneeskunde 2011; 79: 146-155
EPIDEMIOLOGY OF CENTRAL NERVOUS SYSTEM TUMORS IN CHILDHOOD

Although malignancies are rare in childhood, pediatric cancer is the main cause of death in the age group of 0-15 years in the Netherlands. After leukemia, brain tumors constitute the largest group of malignancies in children and form the most common type of solid tumors (Figure 1).

The incidence of malignant tumors of the central nervous system (CNS) in children aged 0-14 years is about 3.5 per 100,000 children. This incidence has increased gradually over time, partly due to improved diagnostic facilities and a better registration. In the Netherlands in recent years, on average 120 children per year are diagnosed with a CNS tumor, and more than 90 percent of these are located intracranially. The five-year overall survival (OS) of children with low-and high-grade brain tumors has increased over past decades from 58% in the 1970’s to 75% in 2008. However, childhood brain tumors are an extremely heterogeneous group of biologically different tumors with more than 130 subentities and with large differences in survival per brain tumor subtype (Figure 2).

**Figure 1. Overview of the types of childhood oncological diseases**  
(Source: Dutch Childhood Oncology Group (DCOG) Patient Registration 2004-2011)
Figure 2. Overview of the types of childhood brain tumors (Source: Dutch Childhood Oncology Group (DCOG) Patient Registration 2004-2011)

CLINICAL PRESENTATION

A child with a brain tumor can exhibit a wide spectrum of symptoms, which are largely determined by the location of the tumor. In contrast to a mainly supratentorial localization of brain tumors in adults, 50% of childhood brain tumors are localized infratentorially (posterior fossa / brainstem). Specific symptoms result from local infiltrative tumor growth or compression of adjacent structures, causing loss of specific functions or epileptic seizures. Non-specific symptoms, such as headache, nausea and vomiting, are usually caused by increased intracranial pressure and can be misleading and misinterpreted as gastro-intestinal problems, leading to a diagnostic delay. These non-specific symptoms frequently occur in children, as infratentorial tumors often cause obstructive hydrocephalus by compression of the cerebral aqueduct or obstruction of the fourth ventricle. Children under four years of age frequently present with macrocephaly, agitation and delayed developmental milestones. The low incidence of childhood brain tumors and non-specificity of symptoms frequently cause a delay in the diagnostic process. A brain tumor should therefore be considered in the differential diagnosis when one or more of the symptoms mentioned above are present, especially if these persist.
IMAGING

Nowadays, magnetic resonance imaging (MRI) is routinely used for diagnosing tumors of the CNS\textsuperscript{10}. Increasingly, new MRI modalities, such as MR spectroscopy, diffusion- and perfusion-weighted imaging, functional MRI and diffusion tensor imaging (DTI) are being used. These techniques allow for better discrimination of healthy brain tissue from tumor tissue\textsuperscript{11}. Additionally, nuclear imaging, such as fluoro-deoxy-glucose (FDG) and methionine positron emission tomography (PET), may be used, although its role for most brain tumors still needs to be established. With advanced imaging techniques, more information can be provided about the biological behavior of the tumor and the response to therapy\textsuperscript{10,12}.

PATHOLOGY

Pathological classification of childhood brain tumors is performed according to the 2007 World Health Organization (WHO) classification of CNS tumors. CNS tumors are divided into seven groups: neuro-epithelial tumors, cranial and paraspinal nerve tumors, meningeal tumors, CNS lymphomas and hematopoietic malignancies, germ cell tumors, sellar tumors and metastases\textsuperscript{6}. Ninety percent of childhood brain tumors are of neuro-epithelial origin. Tumors are classified into low-grade (WHO grade I/II) or high-grade tumors (WHO grade III/IV), based on features such as mitotic activity, endothelial proliferation and necrosis, and this classification reflects the biological aggressiveness and prognosis of these tumors. The most common high-grade brain tumors of childhood, subject of this thesis, medulloblastoma (WHO grade IV), anaplastic ependymoma (WHO grade III), anaplastic astrocytoma (grade III) and glioblastoma multiforme (grade IV), are discussed below.

HIGH-GRADE CHILDHOOD BRAIN TUMORS

Medulloblastoma

Medulloblastoma (MB), a WHO grade IV tumor, belongs to the group of embryonal tumors and is, by definition, apart from its metastases, localized exclusively in the cerebellum\textsuperscript{6}. MB constitutes 15-20\% of all childhood brain tumors, with a peak incidence between four and seven years of age\textsuperscript{13}. Histopathologically, MB is subclassified into ‘classical MB’, ‘desmoplastic/nodular MB (DNMB)’, ‘MB with extensive nodularity (MBEN)’ and ‘large cell anaplastic MB’\textsuperscript{6}. Historically, patients have been divided into standard- and high-risk groups depending on histopathological subclassification, extension of the disease (Chang stage)\textsuperscript{14} and the volume of residual tumor following surgical resection\textsuperscript{15}. Fifty to sixty-two percent of patients have Chang stage M0, with no evidence of metastases in the cerebrospinal fluid or on MRI, whereas disseminated disease is defined as malignant cells in the cerebrospinal fluid (M1 stage),
intra-neural metastases, visible on MRI (M2 and M3 stage) or extra-neural metastases (M4 stage)\textsuperscript{15,16}. High-risk patients have an M1 or higher stage and/or a tumor residue greater than 1.5 cm\textsuperscript{2} in the maximal transverse plane on postoperative MRI and/or large cell anaplastic histology\textsuperscript{14,16,17}. The treatment of MB consists of surgical resection followed by craniospinal radiotherapy with adjuvant chemotherapy. Children in the high-risk group receive intensive radio- and chemotherapy. Due to the increased risk of serious damage to the developing brain, very young children are not irradiated initially, but treated with intensive chemotherapy, followed by delayed radiotherapy at the age of three to five years. The five-year event-free survival (EFS) of children three years and older with M0 stage MB is 81-91\%\textsuperscript{18-21}. In the event of metastatic disease, lower survival rates are reported, with a five-year EFS of 67\% and 32\% for M1 and M2/M3 stage, respectively\textsuperscript{21}.

MB patients under the age of three years have a less favorable prognosis, with a ten-year OS of 59-66\% for children with M0 stage, partly caused by the impossibility to deliver higher doses of craniospinal radiotherapy in view of devastating late effects\textsuperscript{22}. DNMB and MBEN have a more favorable prognosis in this younger age group.

**Anaplastic ependymoma**

Ependymomas, WHO grade II/III tumors, concern 8-10\% of brain tumors in children, with a peak incidence in children below the age of three years\textsuperscript{2,23,24}. Fifty-four percent of all ependymomas are anaplastic (WHO grade III)\textsuperscript{25}. Ependymomas originate from the ventricular ependymal cells. The majority of these tumors arise infratentorially, in the fourth ventricle\textsuperscript{24}. In 9-20\% of ependymoma patients, metastatic disease in the subarachnoid space is detected at diagnosis, which is prognostically unfavorable\textsuperscript{26}. The mainstay of treatment is maximal surgical tumor resection, followed by adjuvant, conformal radiotherapy. Although ependymomas are generally insensitive to chemotherapy, it is used with some success in children with unresectable and/or residual tumors, and metastatic or recurrent disease. In younger children, chemotherapy is given to postpone radiotherapy. Children with completely resected anaplastic ependymomas have a five-year OS of 50-60\%\textsuperscript{25,27,28}. The most important prognostic indicator is the achievement of a gross total resection, which can be achieved in 40-60\% of cases\textsuperscript{25,28}. If this is impossible, survival is very poor\textsuperscript{25}.

**High-grade astrocytoma**

Anaplastic astrocytoma (WHO grade III) and glioblastoma multiforme (GBM, WHO grade IV) are the most commonly observed high-grade astrocytic tumors in children. These tumors constitute 4-7\% of all brain tumors in children and are usually localized supratentorially\textsuperscript{2,23,24}. Children above the age of three years are treated with surgery, radiotherapy with concomitant chemotherapy, and chemotherapeutic maintenance therapy\textsuperscript{29,30}. The five-year OS is 20-46\%\textsuperscript{30,31}. The extent of surgical resection is the main prognostic factor\textsuperscript{30,32-34}. In addition, children with anaplastic astrocytoma have a slightly better prognosis than those with GBM. Children younger than four years have a better survival than older children, possibly caused by a different biology of
these tumors, especially when one considers that radiotherapy is often omitted or postponed in these patients\textsuperscript{30,31}.

High-grade astrocytomas in the brainstem are usually diffuse intrinsic pontine gliomas (DIPG) and constitute 3-13\% of brain tumors in children\textsuperscript{2,7}. As opposed to astrocytomas elsewhere in the brain, WHO grade in DIPG does not correlate with prognosis\textsuperscript{35}. Given the location and diffuse nature of DIPG, surgical resection is not possible. Corticosteroids and radiotherapy usually lead to temporary clinical improvement or stabilization. The prognosis is very poor with a median OS of eight months and a 1- and 2-year OS of 25-53\% and 5-23\%, respectively\textsuperscript{36,37}.

**LATE EFFECTS**

Besides a high mortality rate in high-grade childhood brain tumors in patients surviving from their disease, a striking 82\% suffer from several, sometimes severe, long-term consequences as a result of their disease and treatment. As a result of these long-term sequelae, quality of life is significantly reduced compared to the normal population or other childhood cancer survivors, in whom quality of life usually improves with age\textsuperscript{38}.

Childhood brain tumor survivors experience significant physical and mental health problems and suffer from reduced emotional and social functioning\textsuperscript{39}. Important neurocognitive functions such as concentration, attention, information processing, memory, emotional regulation and organizational capacities are reduced in 10-40\% of survivors. This results in a lower average intelligence score (IQ), a lower educational level, a higher risk of unemployment and in severe mental retardation in five percent of cases. The majority of survivors from a high-grade brain tumor are able to function reasonably independent, but a fully independent life is often difficult\textsuperscript{38,40,41}.

In general, neurosurgical resection and postoperative chemotherapy have limited influence on later neurocognitive functioning. Location of the tumor and associated problems, such as increased intracranial pressure and postoperative ventriculitis have a negative impact on cognitive outcome\textsuperscript{42}. Whole brain, but also only posterior fossa radiotherapy is a major determinant of reduced neurocognitive development\textsuperscript{40,41,43,46}. Higher radiation dose, larger volume of the supratentorial target area and younger age at the time of radiotherapy all negatively influence this outcome. After radiotherapy, the risk of stroke at higher age is increased 100-fold\textsuperscript{47}.

Forty-three percent of brain tumor survivors suffer from one or more endocrine disorders, mainly caused by radiotherapy and increased intracranial pressure at diagnosis\textsuperscript{48,49}. Furthermore, chemotherapy and craniospinal irradiation may also affect various other organ systems leading to late toxicity, such as fertility problems, reduced kidney function, loss of hearing and vision, and cardiotoxicity. Proper follow-up of survivors of brain tumors, even into adulthood, is essential to detect these late effects in time.
NEED FOR INNOVATIVE TREATMENTS

Compared to most other pediatric cancers high-grade brain tumors still carry a high mortality and the development of new treatment strategies is therefore urgently needed, also in view of severe late effects caused by current treatment protocols. In recent decades, neurosurgical and radiotherapy procedures have improved, also by better MR- and PET-imaging techniques. Neuro-navigation techniques, such as intra-operative MRI or ultrasound, and electrophysiological monitoring have increased the likelihood of a complete resection with lower risk of neurological morbidity. Thus far, extent of resection has proven to be the most important prognostic factor in childhood brain tumors.

Radiotherapy is an effective treatment for many childhood brain tumors. A major disadvantage of radiotherapy however, is the damage to healthy, developing brain tissue, resulting in late effects in a large proportion of survivors. Conformal and intensity-modulated radiotherapy (IMRT), and more recently proton beam radiotherapy, specifically irradiate tumor volume, and minimize the radiation dose to surrounding healthy tissues. These techniques are especially relevant in younger children in general and in children harboring tumors in close vicinity to important structures that need to be protected, such as the cochlea, hypothalamus, pituitary gland, optic system, brainstem and hippocampus.

In the past three decades, the role of chemotherapy in children with brain tumors has been extensively investigated. In medulloblastoma, adjuvant chemotherapy, as explored in randomized studies in the 1970s and 1980s, resulted in higher survival rates in patients with extensive tumors. In standard-risk MB, although not investigated in a randomized fashion, the addition of chemotherapy seems to facilitate reduction of craniospinal radiotherapy without affecting survival. Furthermore, patients below four years of age without metastases seem to benefit from chemotherapy, although this was investigated in non-randomized studies. In high-grade gliomas and ependymomas, survival still remains poor, and chemotherapy has, so far, led to an - at best - modest improvement of survival. In DIPG, none of the multiple systemic therapies which have been investigated in the past decades has resulted in long-term survival in these patients.

Novel cytotoxic and tumor cell-targeted agents are being investigated in these diseases. Targeted agents (small molecules or monoclonal antibodies) are drugs which are specifically directed (preferentially exclusively) against proteins expressed on, or in, tumor cells. By targeting proteins that are tumor-specific and tumor-driving, these drugs harm normal cells to a lesser extent, and are therefore more likely to be effective and less toxic. Targeted agents could also improve current treatments. By specifically enhancing the sensitivity of tumor cells to radio- and chemotherapy, the therapeutic index can be increased.
Phase I and II clinical trials have investigated the role of targeted agents in progressive or recurrent pediatric brain tumors, both as single agents and in combination with conventional cytostatic chemotherapy. A list of studies performed up to 2008, when the research for this thesis was initiated, is depicted in Table 1. Many agents investigated in these studies were not specifically developed for use in brain tumors. Designed for treatment of other cancers, these drugs were often engineered not to cross the blood-brain barrier (BBB), preventing them from being neurotoxic, but also rendering them ineffective for treatment of brain tumors. There is therefore a clear need to identify new, brain tumor-directed targets for therapy in high-grade brain tumors, which can ultimately be translated into new clinical trials. This thesis aims to explore new drug targets for future treatment.

Table 1: Overview of phase I and II clinical trials in pediatric high-grade brain tumors performed up to 2008.

<table>
<thead>
<tr>
<th>Year</th>
<th>NCT Number</th>
<th>Inclusion</th>
<th>Phase</th>
<th>Targeting agent</th>
<th>Target</th>
<th>Additional Therapy</th>
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<tbody>
<tr>
<td>1996</td>
<td>NCT00001502</td>
<td>Brain Tumors</td>
<td>1</td>
<td>Lobradimil</td>
<td>Bradykinine</td>
<td>Carboplatin</td>
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<td>1996</td>
<td>NCT00001565</td>
<td>Refractory BT</td>
<td>1</td>
<td>Phenylbutyrate</td>
<td>HDAC</td>
<td></td>
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<td>1998</td>
<td>NCT00003241</td>
<td>Recurrent or Progressive BT</td>
<td>2</td>
<td>Phenylacetate</td>
<td>HDAC</td>
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<td>1999</td>
<td>NCT00004200</td>
<td>Newly Diagnosed GBM</td>
<td>2</td>
<td>Prinomastat</td>
<td>MMP</td>
<td>TMZ</td>
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<td>2000</td>
<td>NCT00006247</td>
<td>Recurrent or Progressive BT</td>
<td>2</td>
<td>Phenylbutyrate</td>
<td>HDAC</td>
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<td>2001</td>
<td>NCT00005602</td>
<td>Newly Diagnosed BSG</td>
<td>1</td>
<td>Carboquin</td>
<td>Bradykinine</td>
<td>Carboplatin / RT</td>
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<td>2001</td>
<td>NCT00011414</td>
<td>Solid Tumors</td>
<td>1-2</td>
<td>Imatinib</td>
<td>PDGFR</td>
<td>Docetaxel / Doxorubicin / Vinorelbine</td>
</tr>
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<td>2001</td>
<td>NCT00021229</td>
<td>Recurrent or Progressive GBM</td>
<td>1-2</td>
<td>Imatinib</td>
<td>PDGFR</td>
<td>Local RT</td>
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<td>2002</td>
<td>NCT00038389</td>
<td>BSG</td>
<td>1</td>
<td>Vioxx</td>
<td>COX2</td>
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<td>2002</td>
<td>NCT00015899</td>
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<td>1</td>
<td>Lonafarnib</td>
<td>Farnesyltransferase</td>
<td></td>
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<tr>
<td>2002</td>
<td>NCT00036959</td>
<td>Refractory Solid Tumors</td>
<td>1</td>
<td>ABT-751</td>
<td>Microtubules</td>
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<td>2002</td>
<td>NCT00142991</td>
<td>Newly Diagnosed Gliomas</td>
<td>2</td>
<td>Gefitinib</td>
<td>EGFR</td>
<td>Local RT</td>
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<td>2002</td>
<td>NCT00050986</td>
<td>Recurrent and Progressive GBM</td>
<td>2</td>
<td>Tipifarnib</td>
<td>Farnesyltransferase</td>
<td>TMZ</td>
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<tr>
<td>2003</td>
<td>NCT00063973</td>
<td>Refractory Primary BT</td>
<td>1</td>
<td>Cilengitide</td>
<td>αvβ3integrin</td>
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<td>2003</td>
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<td>Tipifarnib</td>
<td>Farnesyltransferase</td>
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<td>2003</td>
<td>NCT00050466</td>
<td>Anaplastic Glioma Patients</td>
<td>2</td>
<td>Celecoxib</td>
<td>COX2</td>
<td>Capecitabine / TMZ / CCNU / 6-TG</td>
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<td>2004</td>
<td>NCT00077454</td>
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<td>1</td>
<td>Erlotinib</td>
<td>EGFR</td>
<td>TMZ</td>
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<td>2004</td>
<td>NCT00079339</td>
<td>BSG</td>
<td>1-2</td>
<td>Tipifarnib</td>
<td>Farnesyltransferase</td>
<td>Local RT</td>
</tr>
<tr>
<td>2004</td>
<td>NCT00124667</td>
<td>Recurrent or Refractory Tumors</td>
<td>1</td>
<td>Everolimus</td>
<td>MTOR</td>
<td></td>
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<tr>
<td>2005</td>
<td>NCT000357500</td>
<td>Relapsed or Progressive Cancer</td>
<td>2</td>
<td>Celecoxib</td>
<td>COX2</td>
<td>CPM / VP-16 / Fenofibrate / Thalidomide</td>
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<tr>
<td>2005</td>
<td>NCT001756989</td>
<td>BSG and Thalamic Tumors</td>
<td>2</td>
<td>Celecoxib</td>
<td>COX2</td>
<td>Thalidomide / VP-16</td>
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<td>2005</td>
<td>NCT00095940</td>
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<td>2</td>
<td>Laptinib</td>
<td>EGFR/HER2</td>
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<td>2005</td>
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<td>Erlotinib</td>
<td>EGFR</td>
<td></td>
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<td>2005</td>
<td>NCT00360854</td>
<td>Refractory or Relapsed Malignant BT or Newly Diagnosed BSG</td>
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<td>Erlotinib</td>
<td>EGFR</td>
<td></td>
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<tr>
<td>2005</td>
<td>NCT00107458</td>
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<td>Valproic Acid</td>
<td>HDAC</td>
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<tr>
<td>2005</td>
<td>NCT001418327</td>
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<td>Erlotinib</td>
<td>EGFR</td>
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<td>2005</td>
<td>NCT003303940</td>
<td>Relapsed or Refractory BT or Other Solid Tumors</td>
<td>1</td>
<td>Talabostat</td>
<td>DDP4, 8 and 9</td>
<td>Carboplatin / TMZ</td>
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<td>2006</td>
<td>NCT00032666</td>
<td>Refractory, Progressive, or Refractory Primary BT</td>
<td>1</td>
<td>Cediranib</td>
<td>VEGFR2, AZD2171</td>
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<td>2006</td>
<td>NCT00381797</td>
<td>Recurrent, Progressive, or Refractory Glioma, MBL, Ep, or LGG</td>
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<td>Bevacizumab</td>
<td>VEGF</td>
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<td>Vandetanib</td>
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<td>2007</td>
<td>NCT00513162</td>
<td>Neuronal Tumors and Brain Metastases</td>
<td>1</td>
<td>Valproate</td>
<td>HDAC</td>
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TARGET DISCOVERY

Based on the above, it is clear that new treatments are needed to improve survival, as well as the quality of survival, of children suffering from a high-grade brain tumor. To this aim, preclinical research is essential to define and investigate therapeutic targets and translate these into clinical trials. This thesis is aimed at identifying proteins that are specifically overexpressed, and/or potentially targetable, in (pediatric) high-grade brain tumors (high-grade astrocytoma, ependymoma and medulloblastoma), so as to ultimately facilitate translation into new therapies, or enhancement of existing therapies.

To this purpose, AMPA-type glutamate receptors were investigated as promising targets for anti-glioma treatment, as is described in chapter 2. By using in silico analysis on high-grade pediatric brain tumors datasets, poly-ADP-ribose polymerase 1 (PARP1) and the ErbB-family of receptors were selected and investigated as targets for radiosensitization in chapters 3 and 4, respectively. In silico proteomics, using on-line immunohistochemical proteomic data, revealed pre-B leukemia homeobox interacting protein 1 (PBXIP1) as a potential therapeutic target, overexpressed in high-grade gliomas, which we described in chapter 5. In chapter 6, based on studies in other types of cancer, we investigated signal-regulatory protein alpha (SIRPα) in medulloblastoma.
OUTLINE OF THIS THESIS

Chapter 1  
Gives a general introduction on pediatric high-grade brain tumors, discussing epidemiology, pathology, treatment, late effects and the need for new therapies, using innovative methods of target discovery.

Chapter 2  
Describes the role of AMPA-type glutamate receptors in GBM, previously suggested as potential treatment targets. GBM cells produce large amounts of the neurotransmitter glutamate, killing neurons by overstimulation. In this chapter we investigate how GBM cells are able to survive this high glutamate micro-environment by modification of AMPA receptors.

Chapter 3  
Is a study on the DNA repair enzyme poly ADP-ribose polymerase (PARP) in high-grade glioma, ependymoma and medulloblastoma. PARP-inhibition potentially enhances sensitivity of tumor cells to radio- and chemotherapy. The study described in this chapter therefore determines the radiosensitizing properties of the PARP-inhibitor olaparib as a proof-of-principle in these tumors.

Chapter 4  
Investigates ErbB family (EGFR, ErbB2,3 and 4) expression in normal brain and pediatric and adult HGG in silico and determines radiosensitizing properties of pan-ErbB inhibitor CI-1033, in HGG cells in vitro.

Chapter 5  
Describes the discovery of a new protein, pre-B-cell leukemia homeobox (PBX) interacting protein 1 (PBXIP1), overexpressed in glioma and ependymoma. The effects of shRNA-mediated knockdown of PBXIP1 on in vitro viability and motility of glioma cells is described. With immunohistochemical studies, PBXIP1-expression in human astrocyte progenitor cells during early human brain development is investigated.

Chapter 6  
Is a study on SIRPα as a potential treatment target in medulloblastoma. Epigenetic therapies in MB cells, aiming at SIRPα re-expression, are discussed.

Chapter 7  
Provides the summary of this thesis

Chapter 8  
Presents the discussion of this thesis and provides suggestions for future research and perspectives for innovative treatment of pediatric high-grade brain tumors.
REFERENCES
Chapter 1

General introduction and outline of this thesis


