



## UvA-DARE (Digital Academic Repository)

### Angiogenesis inhibition in high grade glioma

Verhoeff, J.J.C.

**Publication date**  
2009

[Link to publication](#)

#### **Citation for published version (APA):**

Verhoeff, J. J. C. (2009). *Angiogenesis inhibition in high grade glioma*. [Thesis, fully internal, Universiteit van Amsterdam].

#### **General rights**

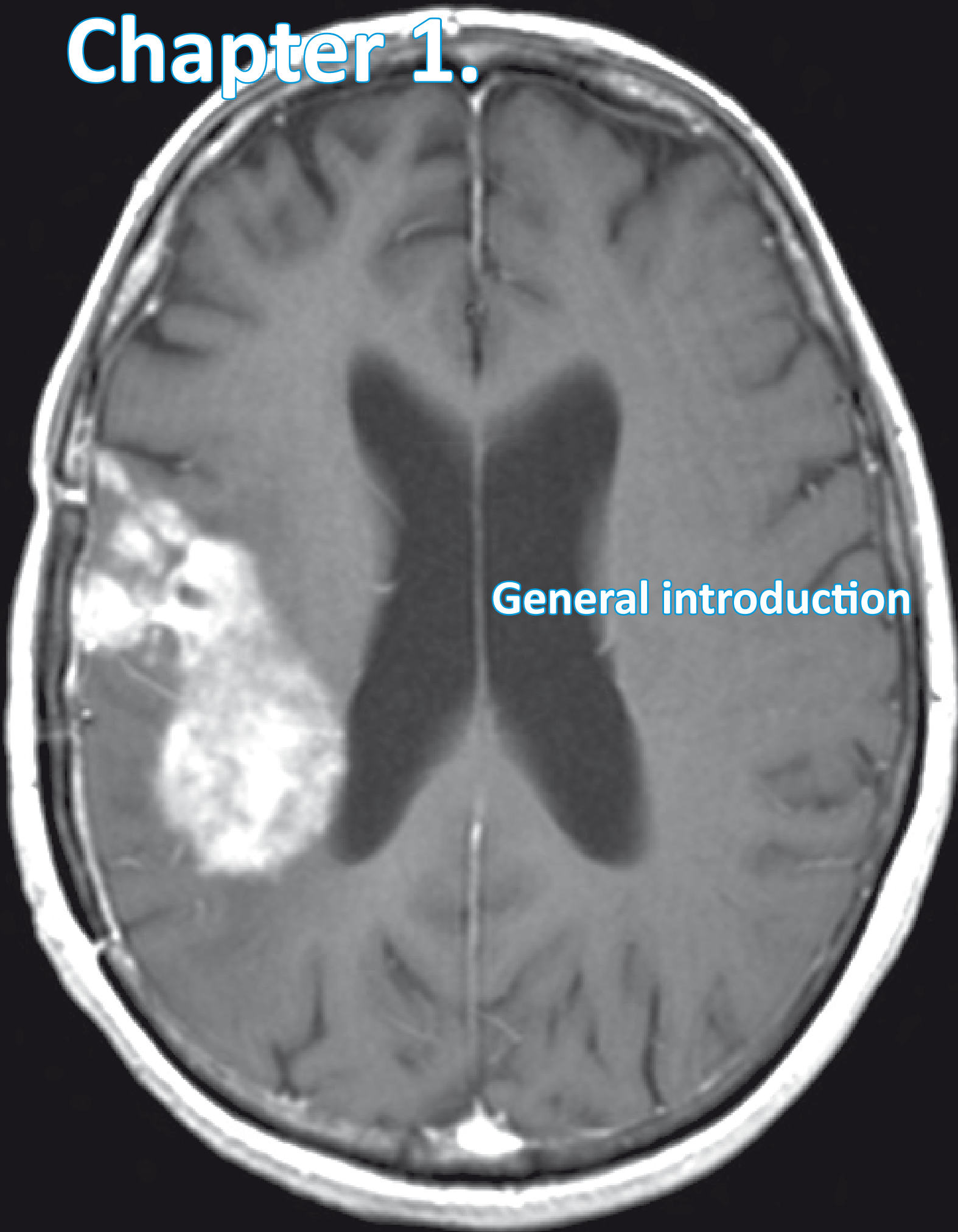
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

#### **Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

# Chapter 1.

General introduction



## Thesis Outline

High grade glioma (HGG) is a devastating disease. An important way by which these brain tumors sustain themselves is by abundantly exploiting angiogenesis, i.e. the formation and re-arrangement of blood vessels. Vascular endothelial growth factor (VEGF) is the key regulator of angiogenesis. Furthermore, VEGF protects cells against apoptosis-inducing agents and stimulates proliferation. Glioma cancer cells frequently secrete large amounts of VEGF to stimulate endothelial cells to expand the vascular network by proliferation and attraction of new endothelial cells by which they fulfill their need for extra blood supply. This trait of malignant glioma may offer a unique treatment opportunity. The abundant production of VEGF is often even further increased following irradiation treatment. Consequently, in several solid tumors VEGF inhibition combined with chemotherapy resulted in improved survival. Therefore VEGF-pathway inhibition combined with irradiation and/or chemotherapy is a potential treatment strategy to influence the poor prognosis for GBM patients.

### Central hypothesis of the thesis:

*Selective therapeutic VEGF pathway inhibition reduces gliomagenesis and prolongs patient survival in combination with irradiation and/or chemotherapy*

This thesis covers a project *from bench to bed side*, from the laboratory to the clinical implementation of angiogenesis inhibitors for malignant glioma. In short, in **Chapter 2** we exploit the latest advances in *in vitro* expansion of malignant glioma cells to study the intimate relationship between glioma cells and tumor associated endothelial cells. **Chapters 3, 4 and 5** describe several effects of VEGF-pathway inhibition in animal glioma models. Subsequently, we discuss the clinical results we obtained with VEGF inhibitors and we put special emphasis on the potential pitfalls the use of these drugs halt on various imaging techniques in **Chapters 6, 7 and 8**. Finally, the joined results are discussed in **Chapter 9**.

First, this general introduction zooms-in on malignant glioma; it will discuss several perspectives of glioma and its standard and experimental treatment modalities. Next, the features of angiogenesis (inhibition) are discussed, in general and more specific its interaction with (chemo-) radiation. Eventually we state that VEGF(R) inhibition in combination with radiotherapy and/or chemotherapy is a promising treatment strategy to improve survival outcome of patients with high grade gliomas.

## Perspectives on malignant glioma

### Tumors in the human brain

In the western population brain tumors occur approximately in 70 per 100.000 persons each year (Oncoline, 2007). Ninety percent of these tumors are metastases of solid tumors elsewhere in the body, e.g. breast or lung (Oncoline, 2007). Beside these metastatic tumors, 10 percent arise from the brain tissue itself, the so-called primary brain tumors. Their incidence is 5-7 per 100.000 persons per year. For the Netherlands in 2006, 1006 new patients with primary brain tumors were registered (IKCnet, 2006).

Eighty percent of the primary brain tumors originate from the glial tissue component, e.g. astrocytes, oligodendrocytes, ependym, and plexus choroideus (Louis et al., 2007). Brain tumors are often initially diagnosed when patients present with neurological symptoms, headache, nausea, vomiting, papilledema, character changes and/or epilepsy, caused by tumor progression leading to increased intracranial pressure, and impression and damage of vital neurological structures (DeAngelis, 2001). However, ongoing improvement of imaging techniques leads to earlier recognition of brain tumors.

### Human brain tumors from glial origin

Low grade gliomas, grade I and II, are well differentiated tumors characterized by a low growth rate and a relatively mild clinical behavior, whereas grade III and IV high grade gliomas (HGG) are poorly differentiated, aggressive tumors with a high growth rate and a tendency to invade the normal brain. Grade IV, or Glioblastoma Multiforme (GBM) is discriminated from grade III gliomas by abundant vascular proliferation. Dr. Hans-Joachim Scherer was the first neuropathologist to describe GBM in detail, during the period 1934 - 1941 (Peiffer and Kleihues, 1999). Both grade III and IV gliomas display a high degree of vascular permeability around a necrotizing tumor core displaying fields of palisading necrosis and have the tendency to furtively invade normal brain tissue, although contrast-enhanced MRI suggests the presence of a discrete border to the lesion. Tumor cells typically extend microscopically several centimeters away from the visible contrast-enhanced area and, in some cases, can extend throughout the entire hemisphere or large portions of the brain— a condition known as gliomatosis cerebri (DeAngelis, 2001).

Grade III tumors, or anaplastic astrocytomas, show little histogenetic differentiation. Patients of less than 50 years of age have an estimated median Overall Survival (OS) of almost 5 years, while this is around 2 years in patients over age 50 (Curran, Jr. et al., 1993; Stupp et al., 2007). Grade IV tumors (malignant glioma, glioblastoma multiforme, GBM, i.e. more than one shape or appearance) are the most devastating malignant disease of the brain (Wen and Kesari, 2008). Since 2005 standard treatment, consisting of surgery, irradiation and chemotherapy, yields an OS of 14.6 months, even in selected patient populations (Stupp et al., 2005).

Grade IV tumors mostly present as *de novo* malignancies not preceded by an earlier stage of low grade lesions. Therefore these tumors are called primary GBM

(Ohgaki and Kleihues, 2007). Patients are typically of middle to elder age with a short clinical history and rapid disease progression. The more rare secondary GBMs, roughly 5% of the cases, arise from low grade glial tumors (Ohgaki et al., 2004). These patients tend to be younger at presentation and show a higher overall survival.

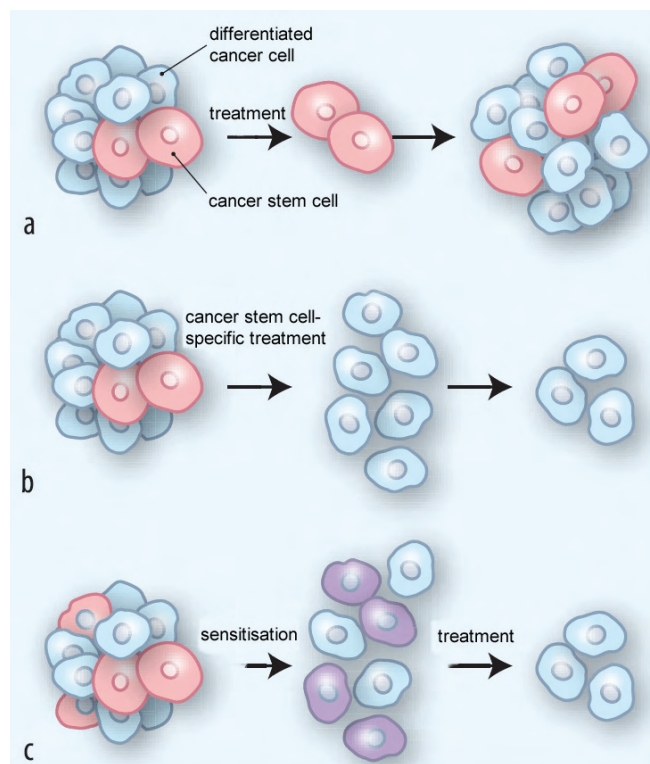
### A new paradigm for cancer

During the past century, the scientific approach to cancer was based on the paradigm that cancer cells within one tumor differ in morphology, marker expression, proliferation capacity and tumorigenicity based on the existence of genetically distinct clones (Nowell, 1976). Availability of new techniques such as immunofluorescent flow cytometry (FACS) shed new light on an alternative model, favoring a hierarchical organization within the tumor with a stem cell compartment, similar to normal organs (Clarke et al., 2006). While being highly fashionably in the beginning of the 21<sup>st</sup> century, this so-called *Cancer Stem Cell (CSC) theory* has already been suggested in 1867 by Julius Friedrich Cohnheim, in his work *Ueber Entzündung und Eiterung* (Cohnheim, 1867).

First serious attempts to gain insight into the nature of hierarchical tumor organization were made in the 1970's (Hamburger and Salmon, 1977). With 21<sup>st</sup> century techniques available, evidence is compiling that cancer indeed does not consist of a homogenous cell population, but contains several cell compartments, including a small subpopulation of multi-potent CSCs. This relatively small subset of slowly cycling cells undergoes self renewal for an unlimited period of time and gives rise to a larger bulk population of cells that have committed to a particular fate and have finite division capacity (Reya et al., 2001). The gold standard for this so-called 'stemness' of CSCs is the ability to generate a phenocopy of the original malignancy in immunocompromised mice, that displays the variety of differentiated cells of the original tumor (Clarke et al., 2006). Furthermore, serial transplantation of this xenotransplanted tumor into new recipient mice confirms self-renewal potential of the CSC population.

Importantly, the CSC paradigm greatly influences the approach for development of new cancer treatments, because it predicts that only eradicating the CSC component will stop tumor growth and let the tumor regress (**Figure 1**). It also offers a comprehensible explanation why tumors recur under present anti-cancer treatment regimens. The present strategies are directed against the tumor bulk, and not to the CSC fraction that is much more resistant to irradiation and chemotherapy (Dean et al., 2005; Vermeulen et al., 2008). With this in mind it is even very well possible that experimental treatments were rejected in clinical trials because of irresponsive tumor bulk, while drug activity against the small CSC fraction was unnoticed (Huff et al., 2006).

As one of the first solid malignancies following shortly after the discovery of a CD44+/CD24- CSC population in breast cancer, GBM showed to contain a CSC fraction positive for marker CD133 (Singh et al., 2004; Al-Hajj et al., 2003). While non-CSC containing tumor lines do not re-grow tumors even with 100.000 cells injected, transplanting as



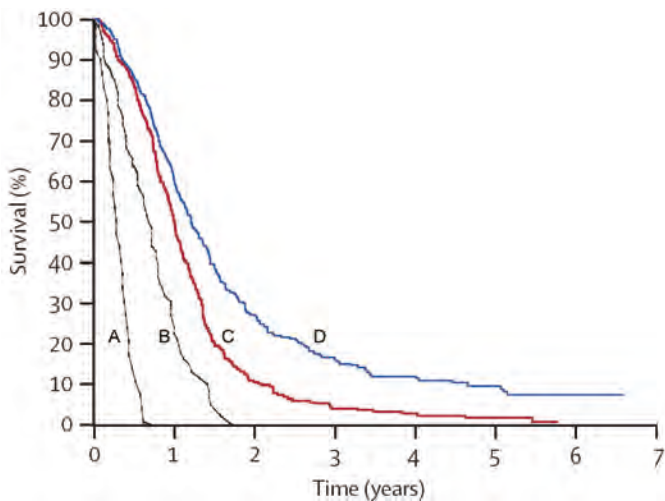
**Figure 1. CSC paradigm and treatment response.**

a) Minimal residual disease: CSCs are less sensitive to treatment compared with differentiated cancer cells. Treatment reduces differentiated bulk, but CSC fraction repopulates the tumor after treatment. b) Treatment directed against CSCs does not reduce tumor size initially; it will regress after treatment. c) Sensitization of CSCs prior to conventional treatment (induction of sensitization, interference with the niche, interference with signaling) (adapted from Vermeulen et al., 2009).

little as 100 CD133<sup>+</sup> cells into immune-deficient mice give rise to tumors that mirrored the original patients' tumors (Singh et al., 2004). For research purposes, glioma CSCs can be expanded under specialized culture conditions, i.e. serum-free suspension culture with the addition of bFGF and EGF. Compared with classic serum-cultured cell lines, not only the gene expression pattern of serum-free cultures more closely mirrors the primary tumor, but also the xenotransplanted tumors are more similar to the original patient tumors in their *in vivo* biology (Lee et al., 2006).

### Tumor environment

For tumor growth and support cancer cells rely on their environment, additionally this support from the microenvironment significantly influences resistance to treatment (Becher et al., 2008). Moreover, it is suggested that the microenvironment is a key component in the maintenance of CSCs (Gilbertson and Rich, 2007). For GBM it is proposed that endothelial cells shape a perivascular niche for the CSC fraction (Calabrese et al., 2007). Following, obstruction of communication between niche cells and CSCs can be an attractive treatment modality for GBM (Christensen et al., 2008; Hambardzumyan et al., 2008). To investigate this interaction, complex techniques are needed to model the vascular niche for CSCs *in vitro* and *in vivo*. First, the regularly cultured human endothelial cells derived from umbilical



**Figure 2. Survival curves of GBM patients.**

Surgery alone (A, 1978); surgery followed by radiotherapy (B, 1978) and (C, 2009); and resection, radiotherapy plus TMZ (D, 2009). Merged graphs from Walker et al., 1978 and Stupp et al., 2009.

veins (HUVECs) differ greatly in gene-expression patterns from the endothelial cells that are derived from human GBM (Charalambous et al., 2005). Second, the intimate relationship with the tumor cells is further expressed by the changes in phenotype of endothelial cells inside a tumor (Charalambous et al., 2006). Therefore, an improved *in vitro* system is welcome that enables co-culturing of both tumor-derived endothelial cells and CSCs (**Chapter 2**).

## Treatment modalities for GBM

### Surgery

In the Netherlands, in 1889 the first brain tumor was removed surgically by J.A. Guldenarm, guided by psychiatrist C. Winkler (Koekkoek and Pieters, 2008). During the first decades almost half of the procedures ended fatal for the patient. From 1910, arise of antisepsis and narcosis, new imaging techniques (e.g. plain X-ray of the skull and cerebral ventriculography) and knowledge about brain functioning and brain structure cleared the way for a more precise surgical treatment of brain tumors. With the introduction of carotid angiography in the 1950's and Computed Tomography (CT) in 1973 more complex surgery was feasible. These radiologic improvements extended survival of GBM patients in the 1970's to median 4 - 6 months (Walker et al., 1978; Walker et al., 1980). From then on, further improvement of techniques focused on imaging like MRI-guided neuro-navigation, intra-operative MRI, functional MRI and fluorescence-guided surgery (Stummer et al., 2006; Asthagiri et al., 2007).

### Irradiation

Of all GBM treatments available up to today, the biggest survival increasing break-through came from application of ionizing radiation following surgery (Walker et al., 1980). Irradiation of brain tumors started in the 1920's when 200KeV X-rays enabled treatment of tumors located up to a few cm under the skin. In 1935 first reports on irradiation of brain tumors were published. Since then, irradiation techniques

and treatment planning were further optimized, and normal tissues were better spared (Leibel et al., 1975). In the 1980's surgery followed by 30x 1.8 - 2Gy irradiation increased the median overall survival to 11 months (Walker et al., 1980). This became the standard irradiation schedule for GBM. Strategies to further increase tumor dose are brachytherapy and stereotactic radiosurgery (Selker et al., 2002; Tsao et al., 2005). Recently, retrospective analysis revealed that when surgical resection is performed and less than 98% of the enhancing tumor is removed, the patient survival is not better than that of irradiation alone (Lacroix et al., 2001). The mean extent of tumor resection in all 416 patients was 89%. In 197 patients a resection of more than 98% of the tumor was achieved as shown by comparing tumor volumes between preoperative and postoperative MR images.

### Chemotherapy

The last 4 decades, numerous chemotherapeutic agents have been tested in GBM, e.g. carmustine (BCNU), lomustine (CCNU), irinotecan (CPT-11) and carboplatin; all agents displayed disappointing activity in GBM, except for temozolomide (TMZ) (Nieder et al., 2006a; Wong et al., 1999). After demonstrating anti-cancer activity in relapsed gliomas, temozolomide was tested in the primary treatment of GBM as part of a combination schedule comprising surgery and radiotherapy. Added to irradiation, as radiosensitizer after surgery and as monotherapy during 6 months after chemoradiation, this schedule extended median overall survival in a selected patient population with approximately 3 more months to 14.6 months compared to surgery and radiotherapy alone (**Figure 2**). This schedule is therefore the current standard adjuvant therapy for primary GBM (Stupp et al., 2005; Stupp et al., 2009).

### Failing primary treatment: recurrent GBM

Despite these developments in treatment strategies, GBM almost always recur. Recurrent GBM is genotypically almost identical to its preceding tumor (van Niftrik et al., 2006), although CSCs harvested from the recurred tumor are more aggressive than those cultured from the primary tumor of the same patient (Huang et al., 2008).

Re-resection, systemic chemotherapy, and sometimes re-irradiation are used in second line (Nieder et al., 2006a). Despite second line treatment, the progression free survival after 6 months (PFS6) is approximately 20% for recurrent disease (Wong et al., 1999). Therefore no standardized treatment is established due to marginal results of these experimental treatments.

### Experimental regimens for (recurrent) GBM

Best OS results for recurrent GBM are reached with TMZ. This treatment was demonstrated to increase survival as second-line therapy in TMZ naive patients when compared to historical controls (Wong et al., 1999). However, because most patients nowadays received TMZ as initial treatment, a rechallenge with TMZ at the same treatment schedule in second line is often not as effective as in naive patients, with a median OS ranging from 25 - 61 weeks in different studies (Wick et al., 2009).

To overcome resistance, alternative TMZ schedules (other than 150-200 mg/m<sup>2</sup> on 5 days of each 28-day cycle) are deployed with continuous low dose administration (**chapter 7**). Protracted TMZ regimens may deplete O6-methylguanine-methyltransferase (MGMT), a DNA repair protein that is an important factor in TMZ resistance (Tolcher et al., 2003), and offer a higher dose intensity per month of delivery. Several preclinical studies demonstrated that continuous daily administration of cytotoxic drugs at low dose have potential anti-angiogenic activity, even below the maximum tolerated dose (Browder et al., 2000; Bertolini et al., 2003; Kerbel and Kamen, 2004; Tuettenberg et al., 2005; Kim et al., 2006; Zhou et al., 2007). Although several responses have been reported in TMZ non-naive patients receiving second line regimens with daily low dose TMZ regimens, the duration is short and a survival advantage has never been demonstrated clearly (Kong et al., 2006; Perry et al., 2008a; Perry et al., 2008b; Jauch et al., 2008; Kesari et al., 2009; Wick et al., 2009).

Better understanding of cancer biology due to fundamental research efforts has led to the exploration of target-directed cancer treatments. Last years numerous clinical trials have been performed, targeting specific aberrant functioning molecules in cancer cells and abnormal angiogenesis in the micro-environment (Wen and Kesari, 2008). According to several recently performed clinical studies, angiogenesis inhibition seems also to be an attractive approach in patients with recurrent GBM. The phenotypical features of GBM (high pro-angiogenic activity) cued researchers in the late 1990's to experiments with anti-angiogenic treatment. This strategy proved to be successful in multiple in vivo model systems (Lund et al., 2000; Kozin et al., 2001; Hess et al., 2001; Winkler et al., 2004; Sarkaria et al., 2006; Tabatabai et al., 2007). These first results strongly stimulated the neuro-oncology community to thoroughly explore vascular endothelial growth factor (VEGF) inhibition in the clinical setting.

## Angiogenesis inhibition and its potential in GBM

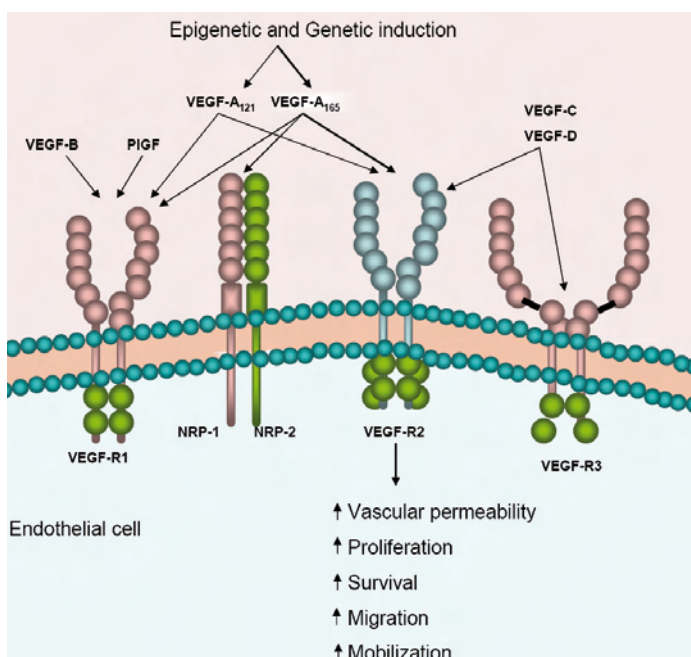
### Drugs targeting angiogenesis

In angiogenesis, i.e. the formation and re-arrangement of blood vessels, numerous molecules are involved like VEGF, angiopoietin, neuropilins, integrins, PDGF and fibroblast growth factor (Norden et al., 2008). VEGF has been identified as key mediator of angiogenesis and is influencing cancer cell survival, local tumor growth and development of metastasis (Folkman, 1986). VEGF protects against apoptosis-inducing agents, stimulates proliferation and increases angiogenic potential (Gorski et al., 1999; Geng et al., 2001; Harney and Bouchier-Hayes, 2002; Gupta et al., 2002).

The VEGF pathway comprises of a family of ligands VEGF-a to VEGF-d and placental growth factor (PlGF) (**Figure 3**). VEGF is secreted by all human cells, but in larger amounts by tumor cells, and the signal is read out by VEGF-receptors, from which VEGFR-2 has a pivotal function. This receptor is predominantly present on endothelial cells and on circulating bone marrow-derived endothelial progenitor cells that contribute to newly formed vessels (Cross et al., 2003).

Significantly elevated levels of VEGF are found in the tissue and cyst fluid of GBM and VEGF concentrations clearly correlate with the vascularity of these tumors (Takano et al., 1996). Even in GBM tumor cells infiltrating the surrounding brain tissue, a high expression of VEGF is found (Johansson et al., 2002). VEGF plays an important role in aberrant tumor angiogenesis in GBM, characterized by structurally and functionally abnormal blood vessels leading to edema formation, high intra-tumoral interstitial pressure, tissue hypoxia and further stimulation of angiogenesis. Vasogenic brain edema and high interstitial pressure of the cerebrum contribute to hypoxia-induced tumor progression and resistance to both radiotherapy and chemotherapy (Jain et al., 2007). Anti-VEGF treatment results in a rapid "normalization" of the tumor vasculature by passively pruning the immature and leaky vessels and actively remodeling the remaining vasculature so that it more closely resembles the normal vasculature. A decrease of interstitial pressure in the tumor, and a depletion of tumor vessels have been reported after long-term anti-VEGF treatment (Batchelor et al., 2007).

Another feature of anti-angiogenesis treatment could be the interference with the communication of cancer stem cells (CSCs) and their presumed vascular niche (Calabrese et al., 2007). This feature further increases the



**Figure 3. The VEGF pathway.**

The binding of VEGF-A to VEGFR-2 leads to a cascade of different signaling pathways, resulting in the up-regulation of genes involved in mediating the proliferation and migration of endothelial cells and promoting their survival and vascular permeability. For example, it leads to intracellular activation of the MAPK pathway and subsequent initiation of DNA synthesis and cell growth, whereas activation of the PI3K-Akt pathway leads to increased endothelial-cell survival. Activation of src can lead to actin cytoskeleton changes and induction of cell migration. The role of VEGFR-1 in sprouting angiogenesis is much less clear. Binding of VEGF-C to VEGFR-3 mediates lymphangiogenesis. VEGF<sub>165</sub> can bind to neuropilin (NRP) receptors, which can act as co-receptors with VEGFR-2 (horizontal arrow) to regulate angiogenesis. Adapted from Kerbel, 2008.

attractiveness of anti-angiogenic treatment for GBM.

The VEGF pathway can be blocked by ligand inactivation (bevacizumab / Avastin®) and by VEGFR inhibition by small molecules targeting VEGF receptor 2, e.g. cediranib (Recentin®), vatalanib (PTK787/ZK 222584), sunitinib (Sutent®) and vandetanib (Zactima®). Some compounds target the floating VEGF-receptor, e.g. aflibercept (VEGF Trap®). Targeting the ligand VEGF itself is most commonly used in clinical trials, e.g. bevacizumab (Avastin®). Bevacizumab in combination with chemotherapy is registered for treatment of advanced non small-cell lung cancer, breast cancer and colorectal cancer, and in combination with  $\alpha$ -interferon for renal cell cancer. In May 2009, the FDA also approved bevacizumab in recurrent GBM; this accelerated approval was based on results from a non-placebo-controlled clinical trial (Kreisl et al., 2008).

#### **Side effects of angiogenesis inhibitors in clinical trials**

Although anti-angiogenic drugs are generally well-tolerated, rare life-threatening complications are reported including thrombo-embolism, hemorrhage, and gastrointestinal perforation (Dietrich et al., 2008). In addition, more frequently observed adverse effects include hypertension and proteinuria, wound repair delay, vomiting and myelosuppression. For metastatic colon cancer, in the definitive phase III trial that led to first FDA approval for bevacizumab the rate of GI perforation was 1.5% in patients treated with bevacizumab and chemotherapy, as compared to none in patients treated with chemotherapy alone (Hurwitz et al., 2004). For unclear reasons, GI perforation appears to be more common among bevacizumab-treated patients with colorectal and renal cancers as compared to other solid tumors (Becher et al., 2008).

#### **Combining irradiation and angiogenesis inhibition**

Irradiation is the pivotal treatment for GBM. Since angiogenesis inhibitors are thought to normalize tumor vessels resulting in improved blood flow and oxygenation (Jain, 2005), they might potentiate the cell kill and sensitize the effects of irradiation (Nieder et al., 2006b). In vivo modeling of combined irradiation and angiogenesis inhibition has its limitations. High-dose external beam irradiation of the mouse brain is restricted by acute irradiation toxicity to the esophagus and trachea, therefore single fraction or low-dose short-term irradiation is usually applied (Lund et al., 2000; Kozin et al., 2001; Hess et al., 2001; Winkler et al., 2004; Sarkaria et al., 2006; Tabatabai et al., 2007). These pre-clinical GBM models predominantly show an additive effect of combined irradiation and angiogenesis. Rationale behind this effect is that radiation therapy not only inflicts damage to GBM cells, but more important also to the tumor micro-environment, e.g. endothelial cells (Fuks and Kolesnick, 2005). In response to irradiation, endothelial cells and tumor cells start to produce VEGF which may induce apoptosis resistance

in endothelial cells and decreased oxygenation of the tumor by promoting aberrant vessel formation, hyperpermeability, and increased interstitial pressure. This treatment-induced VEGF-production has also been described for temozolomide (Fisher et al., 2007).

#### **Combined modalities, combined side effects?**

Several clinical studies in colorectal, and head neck cancer have demonstrated that radiotherapy in combination with bevacizumab is a feasible and safe approach (Willett et al., 2005; Willett et al., 2009; Seiwert et al., 2008; Czito et al., 2007; Crane et al., 2006). Recently, results of combined bevacizumab, radiotherapy and chemotherapy (TMZ) have been presented of patients with GBM (Narayana et al., 2008; Lai et al., 2008).

These phase II trials showed that the combined approach (radiotherapy in combination with bevacizumab) is feasible and relatively safe in patients with GBM, although toxicities (e.g. intratumoral hemorrhage, wound dehiscence, and bowel perforation) and patterns of relapse (e.g. satellite formation) need to be monitored closely (Lai et al., 2008; Narayana et al., 2009; Gutin et al., 2009).

Furthermore, the anti-edema effect of angiogenesis inhibition may improve neurological function and allow better clinical tolerance and efficacy of fractionated radiation, since well-known adverse effects of radiation are brain/tumor necrosis and exacerbation of vasogenic edema with increased interstitial pressure (Gonzalez et al., 2007).

#### **Imaging modalities for GBM**

MRI is the most important imaging modality for GBM. On T1-weighted sequences the enhancing tumor rim can be distinguished from the surrounding hypo-intense regions with vasogenic edema or microscopic tumor infiltration. Nowadays, new techniques like functional imaging, high-resolution spectroscopic imaging and diffusion techniques may contribute to pre-therapeutic grading and characterization of gliomas (Lemort et al., 2007). Diffusion techniques hold promise in predicting survival in malignant astrocytoma and could help to define areas for biopsy. Perfusion-weighted MRI techniques offer potential markers of tumor angiogenesis and capillary permeability, and correlate well with vascular endothelial growth factor expression in grade II and grade III tumors (Law et al., 2003). Recently, the value of Apparent Diffusion Coefficient (ADC) as predictor for progression free survival of recurrent glioma treated with angiogenesis inhibitors was shown (Pope et al., 2009). Techniques that quantify perfusion and permeability of tumors are regarded to give insight into anti-angiogenic treatment responses. Earlier it was shown that glioma capillary blood volumes assessed by DSC-MRI and vessel permeability assessed by DCE-MRI correlated with tumor grade (Law et al., 2004). Its value for evaluation of treatment responses is examined in **chapter 7**.

## References

- Al-Hajj, M., Wicha, M.S., Ito-Hernandez, A., Morrison, S.J., and Clarke, M.F. (2003). Prospective identification of tumorigenic breast cancer cells. *Proc. Natl. Acad. Sci. U. S. A* 100, 3983-3988.
- Asthaqiri, A.R., Pouratian, N., Sherman, J., Ahmed, G., and Shaffrey, M.E. (2007). Advances in brain tumor surgery. *Neurol. Clin.* 25, 975-9ix.
- Batchelor, T.T., Sorensen, A.G., di Tomaso, E., Zhang, W.T., Duda, D.G., Cohen, K.S., Kozak, K.R., Cahill, D.P., Chen, P.J., Zhu, M., Ancukiewicz, M., Mrugala, M.M., Plotkin, S., Drappatz, J., Louis, D.N., Ivy, P., Scadden, D.T., Benner, T., Loeffler, J.S., Wen, P.Y., and Jain, R.K. (2007). AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. *Cancer Cell* 11, 83-95.
- Becher, O.J., Hambardzumyan, D., Fomchenko, E.I., Momota, H., Mainwaring, L., Bleau, A.M., Katz, A.M., Edgar, M., Kenney, A.M., Cordon-Cardo, C., Blasberg, R.G., and Holland, E.C. (2008). Gli activity correlates with tumor grade in platelet-derived growth factor-induced gliomas. *Cancer Res.* 68, 2241-2249.
- Bertolini, F., Paul, S., Mancuso, P., Monestiroli, S., Gobbi, A., Shaked, Y., and Kerbel, R.S. (2003). Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. *Cancer Res.* 63, 4342-4346.
- Browder, T., Butterfield, C.E., Kraling, B.M., Shi, B., Marshall, B., O'Reilly, M.S., and Folkman, J. (2000). Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res.* 60, 1878-1886.
- Calabrese, C., Poppleton, H., Kocak, M., Hogg, T.L., Fuller, C., Hamner, B., Oh, E.Y., Gaber, M.W., Finklestein, D., Allen, M., Frank, A., Bayazitov, I.T., Zakharenko, S.S., Gajjar, A., Davidoff, A., and Gilbertson, R.J. (2007). A perivascular niche for brain tumor stem cells. *Cancer Cell* 11, 69-82.
- Charalambous, C., Chen, T.C., and Hofman, F.M. (2006). Characteristics of tumor-associated endothelial cells derived from glioblastoma multiforme. *Neurosurg. Focus.* 20, E22.
- Charalambous, C., Hofman, F.M., and Chen, T.C. (2005). Functional and phenotypic differences between glioblastoma multiforme-derived and normal human brain endothelial cells. *J. Neurosurg.* 102, 699-705.
- Christensen, K., Schroder, H.D., and Kristensen, B.W. (2008). CD133 identifies perivascular niches in grade II-IV astrocytomas. *J. Neurooncol.* 90, 157-170.
- Clarke, M.F., Dick, J.E., Dirks, P.B., Eaves, C.J., Jamieson, C.H., Jones, D.L., Visvader, J., Weissman, I.L., and Wahl, G.M. (2006). Cancer stem cells--perspectives on current status and future directions: AACR Workshop on cancer stem cells. *Cancer Res.* 66, 9339-9344.
- Cohnheim, J. (1867). Ueber Entzündung und Eiterung. *Virchows Arch* 40, 1-79.
- Crane, C.H., Ellis, L.M., Abbruzzese, J.L., Amos, C., Xiong, H.Q., Ho, L., Evans, D.B., Tamm, E.P., Ng, C., Pisters, P.W., Charnsangavej, C., Delclos, M.E., O'Reilly, M., Lee, J.E., and Wolff, R.A. (2006). Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. *J. Clin. Oncol.* 24, 1145-1151.
- Cross, M.J., Dixelius, J., Matsumoto, T., and Claesson-Welsh, L. (2003). VEGF-receptor signal transduction. *Trends Biochem. Sci.* 28, 488-494.
- Curran, W.J., Jr., Scott, C.B., Horton, J., Nelson, J.S., Weinstein, A.S., Fischbach, A.J., Chang, C.H., Rotman, M., Asbell, S.O., Krisch, R.E., and . (1993). Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J. Natl. Cancer Inst.* 85, 704-710.
- Czito, B.G., Bendell, J.C., Willett, C.G., Morse, M.A., Globe, G.C., Tyler, D.S., Thomas, J., Ludwig, K.A., Mantyh, C.R., Ashton, J., Yu, D., and Hurwitz, H.I. (2007). Bevacizumab, oxaliplatin, and capecitabine with radiation therapy in rectal cancer: Phase I trial results. *Int. J. Radiat. Oncol. Biol. Phys.* 68, 472-478.
- Dean, M., Fojo, T., and Bates, S. (2005). Tumour stem cells and drug resistance. *Nat. Rev. Cancer* 5, 275-284.
- DeAngelis, L.M. (2001). Brain tumors. *N. Engl. J. Med.* 344, 114-123.
- Dietrich, J., Norden, A.D., and Wen, P.Y. (2008). Emerging antiangiogenic treatments for gliomas - efficacy and safety issues. *Curr. Opin. Neurol.* 21, 736-744.
- Fisher, T., Galanti, G., Lavie, G., Jacob-Hirsch, J., Kventsel, I., Zeligson, S., Winkler, R., Simon, A.J., Amariglio, N., Rechavi, G., and Toren, A. (2007). Mechanisms operative in the antitumor activity of temozolomide in glioblastoma multiforme. *Cancer J.* 13, 335-344.
- Folkman, J. (1986). How is blood vessel growth regulated in normal and neoplastic tissue? G.H.A. Clowes memorial Award lecture. *Cancer Res.* 46, 467-473.
- Fuks, Z. and Kolesnick, R. (2005). Engaging the vascular component of the tumor response. *Cancer Cell* 8, 89-91.
- Geng, L., Donnelly, E., McMahon, G., Lin, P.C., Sierra-Rivera, E., Oshinka, H., and Hallahan, D.E. (2001). Inhibition of vascular endothelial growth factor receptor signaling leads to reversal of tumor resistance to radiotherapy. *Cancer Res.* 61, 2413-2419.
- Gilbertson, R.J. and Rich, J.N. (2007). Making a tumour's bed: glioblastoma stem cells and the vascular niche. *Nat. Rev. Cancer* 7, 733-736.
- Gonzalez, J., Kumar, A.J., Conrad, C.A., and Levin, V.A. (2007). Effect of bevacizumab on radiation necrosis of the brain. *Int. J. Radiat. Oncol. Biol. Phys.* 67, 323-326.
- Gorski, D.H., Beckett, M.A., Jaskowiak, N.T., Calvin, D.P., Mauceri, H.J., Salloum, R.M., Seetharam, S., Koons, A., Hari, D.M., Kufe, D.W., and Weichselbaum, R.R. (1999). Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. *Cancer Res.* 59, 3374-3378.
- Gupta, V.K., Jaskowiak, N.T., Beckett, M.A., Mauceri, H.J., Grunstein, J., Johnson, R.S., Calvin, D.A., Nodzinski, E., Pejovic, M., Kufe, D.W., Posner, M.C., and Weichselbaum, R.R. (2002). Vascular endothelial growth factor enhances endothelial cell survival and tumor radioresistance. *Cancer J.* 8, 47-54.
- Gutin, P.H., Iwamoto, F.M., Beal, K., Mohile, N.A., Karimi, S., Hou, B.L., Lymberis, S., Yamada, Y., Chang, J., and Abrey, L.E. (2009). Safety and Efficacy of Bevacizumab with Hypofractionated Stereotactic Irradiation for Recurrent Malignant Gliomas. *Int. J. Radiat. Oncol. Biol. Phys.*
- Hambardzumyan, D., Becher, O.J., Rosenblum, M.K., Pandolfi, P.P., Manova-Todorova, K., and Holland, E.C. (2008). PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. *Genes Dev.* 22, 436-448.
- Hamburger, A.W. and Salmon, S.E. (1977). Primary bioassay of human tumor stem cells. *Science* 197, 461-463.
- Harmey, J.H. and Bouchier-Hayes, D. (2002). Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for anti-angiogenic therapy. *Bioessays* 24, 280-283.
- Hess, C., Vuong, V., Hegyi, I., Riesterer, O., Wood, J., Fabbro, D., Glanzmann, C., Bodis, S., and Pruschy, M. (2001). Effect of VEGF receptor inhibitor PTK787/

- ZK222584 [correction of ZK222548] combined with ionizing radiation on endothelial cells and tumour growth. *Br. J. Cancer* 85, 2010-2016.
- Huang,Q., Zhang,Q.B., Dong,J., Wu,Y.Y., Shen,Y.T., Zhao,Y.D., Zhu,Y.D., Diao,Y., Wang,A.D., and Lan,Q. (2008). Glioma stem cells are more aggressive in recurrent tumors with malignant progression than in the primary tumor, and both can be maintained long-term in vitro. *BMC. Cancer* 8, 304.
- Huff,C.A., Matsui,W., Smith,B.D., and Jones,R.J. (2006). The paradox of response and survival in cancer therapeutics. *Blood* 107, 431-434.
- Hurwitz,H., Fehrenbacher,L., Novotny,W., Cartwright,T., Hainsworth,J., Heim,W., Berlin,J., Baron,A., Griffing,S., Holmgren,E., Ferrara,N., Fyfe,G., Rogers,B., Ross,R., and Kabbinavar,F. (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* 350, 2335-2342.
- IKCnet. NKR cijfers. [http://www.ikcnet.nl/cijfers/index.php?taal=nl&frequentie=1&regio=Landelijk&soort\\_kanker=41,42&soort\\_morfologie=&periode=2006&inputRegioLandelijk=on&inputRegioMZ=&fnvasief=1&uitkomstmaat=1&periode\\_input=2006&leeftijdsklassen=2](http://www.ikcnet.nl/cijfers/index.php?taal=nl&frequentie=1&regio=Landelijk&soort_kanker=41,42&soort_morfologie=&periode=2006&inputRegioLandelijk=on&inputRegioMZ=&fnvasief=1&uitkomstmaat=1&periode_input=2006&leeftijdsklassen=2).
- Jain,R.K. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307, 58-62.
- Jain,R.K., di Tomaso,E., Duda,D.G., Loeffler,J.S., Sorensen,A.G., and Batchelor,T.T. (2007). Angiogenesis in brain tumours. *Nat. Rev. Neurosci.* 8, 610-622.
- Jauch,T., Hau,P., and Bogdahn,U. (2008). Re-challenge with temozolomide (TMZ) at recurrence in high-grade gliomas (HGG). *J. Clin. Oncol.* 25, 2034.
- Johansson,M., Brannstrom,T., Bergenheim,A.T., and Henriksson,R. (2002). Spatial expression of VEGF-A in human glioma. *J. Neurooncol.* 59, 1-6.
- Kerbel,R.S. and Kamen,B.A. (2004). The anti-angiogenic basis of metronomic chemotherapy. *Nat. Rev. Cancer* 4, 423-436.
- Kesari,S., Schiff,D., Drappatz,J., LaFrankie,D., Doherty,L., Macklin,E.A., Muzikansky,A., Santagata,S., Ligon,K.L., Norden,A.D., Ciampa,A., Bradshaw,J., Levy,B., Radakovic,G., Ramakrishna,N., Black,P.M., and Wen,P.Y. (2009). Phase II study of protracted daily temozolomide for low-grade gliomas in adults. *Clin. Cancer Res.* 15, 330-337.
- Kim,J.T., Kim,J.S., Ko,K.W., Kong,D.S., Kang,C.M., Kim,M.H., Son,M.J., Song,H.S., Shin,H.J., Lee,D.S., Eoh,W., and Nam,D.H. (2006). Metronomic treatment of temozolomide inhibits tumor cell growth through reduction of angiogenesis and augmentation of apoptosis in orthotopic models of gliomas. *Oncol Rep.* 16, 33-39.
- Koekkoek,J.A. and Pieters,T. (2008). [Development of concepts of disease, diagnostics and neurosurgical treatment of brain tumours in The Netherlands (1890-1950)]. *Ned. Tijdschr. Geneesk.* 152, 2810-2816.
- Kong,D.S., Lee,J.I., Kim,W.S., Son,M.J., Lim,d.H., Kim,S.T., Park,K., Kim,J.H., Eoh,W., and Nam,D.H. (2006). A pilot study of metronomic temozolomide treatment in patients with recurrent temozolomide-refractory glioblastoma. *Oncol. Rep.* 16, 1117-1121.
- Kozin,S.V., Boucher,Y., Hicklin,D.J., Bohlen,P., Jain,R.K., and Suit,H.D. (2001). Vascular endothelial growth factor receptor-2-blocking antibody potentiates radiation-induced long-term control of human tumor xenografts. *Cancer Res.* 61, 39-44.
- Kreisli,T.N., Kim,L., Moore,K., Duic,P., Royce,C., Stroud,I., Garren,N., Mackey,M., Butman,J.A., Camphausen,K., Park,J., Albert,P.S., and Fine,H.A. (2008). Phase II Trial of Single-Agent Bevacizumab Followed by Bevacizumab Plus Irinotecan at Tumor Progression in Recurrent Glioblastoma. *J. Clin. Oncol.*
- Lacroix,M., Abi-Said,D., Fourney,D.R., Gokaslan,Z.L., Shi,W., DeMonte,F., Lang,F.F., McCutcheon,I.E., Hassenbusch,S.J., Holland,E., Hess,K., Michael,C., Miller,D., and Sawaya,R. (2001). A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. *J. Neurosurg.* 95, 190-198.
- Lai,A., Filka,E., McGibbon,B., Nghiemphu,P.L., Graham,C., Yong,W.H., Mischel,P., Liau,L.M., Bergsneider,M., Pope,W., Selch,M., and Cloughesy,T. (2008). Phase II pilot study of bevacizumab in combination with temozolomide and regional radiation therapy for up-front treatment of patients with newly diagnosed glioblastoma multiforme: interim analysis of safety and tolerability. *Int. J. Radiat. Oncol. Biol. Phys.* 71, 1372-1380.
- Law,M., Yang,S., Babb,J.S., Knopp,E.A., Golfinos,J.G., Zagzag,D., and Johnson,G. (2004). Comparison of cerebral blood volume and vascular permeability from dynamic susceptibility contrast-enhanced perfusion MR imaging with glioma grade. *AJNR Am. J. Neuroradiol.* 25, 746-755.
- Law,M., Yang,S., Wang,H., Babb,J.S., Johnson,G., Cha,S., Knopp,E.A., and Zagzag,D. (2003). Glioma grading: sensitivity, specificity, and predictive values of perfusion MR imaging and proton MR spectroscopic imaging compared with conventional MR imaging. *AJNR Am. J. Neuroradiol.* 24, 1989-1998.
- Lee,J., Kotliarova,S., Kotliarov,Y., Li,A., Su,Q., Donin,N.M., Pastorino,S., Puroow,B.W., Christopher,N., Zhang,W., Park,J.K., and Fine,H.A. (2006). Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer Cell* 9, 391-403.
- Leibel,S.A., Sheline,G.E., Wara,W.M., Boldrey,E.B., and Nielsen,S.L. (1975). The role of radiation therapy in the treatment of astrocytomas. *Cancer* 35, 1551-1557.
- Lemort,M., Canizares-Perez,A.C., Van der Stappen,A., and Kampouridis,S. (2007). Progress in magnetic resonance imaging of brain tumours. *Curr. Opin. Oncol.* 19, 616-622.
- Louis,D.N., Ohgaki,H., Wiestler,O.D., Cavenee,W.K., Burger,P.C., Jouvet,A., Scheithauer,B.W., and Kleihues,P. (2007). The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol.* 114, 97-109.
- Lund,E.L., Bastholm,L., and Kristjansen,P.E. (2000). Therapeutic synergy of TNP-470 and ionizing radiation: effects on tumor growth, vessel morphology, and angiogenesis in human glioblastoma multiforme xenografts. *Clin. Cancer Res.* 6, 971-978.
- Narayana,A., Golfinos,J.G., Fischer,I., Raza,S., Kelly,P., Parker,E., Knopp,E.A., Medabalmi,P., Zagzag,D., Eagan,P., and Gruber,M.L. (2008). Feasibility of using bevacizumab with radiation therapy and temozolomide in newly diagnosed high-grade glioma. *Int. J. Radiat. Oncol. Biol. Phys.* 72, 383-389.
- Narayana,A., Kelly,P., Golfinos,J., Parker,E., Johnson,G., Knopp,E., Zagzag,D., Fischer,I., Raza,S., Medabalmi,P., Eagan,P., and Gruber,M.L. (2009). Antiangiogenic therapy using bevacizumab in recurrent high-grade glioma: impact on local control and patient survival. *J. Neurosurg.* 110, 173-180.
- Nieder,C., Adam,M., Molls,M., and Grosu,A.L. (2006a). Therapeutic options for recurrent high-grade glioma in adult patients: recent advances. *Crit Rev. Oncol Hematol.* 60, 181-193.
- Nieder,C., Wiedenmann,N., Andratschke,N., and Molls,M. (2006b). Current status of angiogenesis inhibitors combined with radiation therapy. *Cancer Treat. Rev.* 32, 348-364.
- Norden,A.D., Drappatz,J., and Wen,P.Y. (2008). Novel anti-angiogenic therapies for malignant gliomas. *Lancet Neurol.* 7, 1152-1160.
- Nowell,P.C. (1976). The clonal evolution of tumor cell populations. *Science* 194, 23-28.

- Ohgaki,H., Dessen,P., Jourde,B., Horstmann,S., Nishikawa,T., Di Patre,P.L., Burkhard,C., Schuler,D., Probst-Hensch,N.M., Maiorka,P.C., Baeza,N., Pisani,P., Yonekawa,Y., Yasargil,M.G., Lutolf,U.M., and Kleihues,P. (2004). Genetic pathways to glioblastoma: a population-based study. *Cancer Res.* *64*, 6892-6899.
- Ohgaki,H. and Kleihues,P. (2007). Genetic pathways to primary and secondary glioblastoma. *Am. J. Pathol.* *170*, 1445-1453.
- OncoLine. Landelijke richtlijn Gliomen, versie 2.0. [http://www.oncoline.nl/richtlijn/item/pagina.php?id=26720&richtlijn\\_id=572](http://www.oncoline.nl/richtlijn/item/pagina.php?id=26720&richtlijn_id=572).
- Peiffer,J. and Kleihues,P. (1999). Hans-Joachim Scherer (1906-1945), pioneer in glioma research. *Brain Pathol.* *9*, 241-245.
- Perry,J.R., Mason,W., Belanger,K., Kavan,P., Fulton,D., Easaw,J., Kirby,S., Macdonald,D., Shields,C., and Pouliot,J.F. (2008a). The temozolomide RESCUE study: A Phase II trial of continuous (28/28) dose-intense temozolomide (TMZ) after progression on conventional 5/28 day TMZ in patients with recurrent malignant glioma. *Neuro-Oncology* *10*, 1063.
- Perry,J.R., Rizek,P., Cashman,R., Morrison,M., and Morrison,T. (2008b). Temozolomide rechallenge in recurrent malignant glioma by using a continuous temozolomide schedule: the "rescue" approach. *Cancer* *113*, 2152-2157.
- Pope,W.B., Kim,H.J., Huo,J., Alger,J., Brown,M.S., Gjertson,D., Sai,V., Young,J.R., Tekchandani,L., Cloughesy,T., Mischel,P.S., Lai,A., Nghiemphu,P., Rahmanuddin,S., and Goldin,J. (2009). Recurrent glioblastoma multiforme: ADC histogram analysis predicts response to bevacizumab treatment. *Radiology* *252*, 182-189.
- Reya,T., Morrison,S.J., Clarke,M.F., and Weissman,I.L. (2001). Stem cells, cancer, and cancer stem cells. *Nature* *414*, 105-111.
- Sarkaria,J.N., Carlson,B.L., Schroeder,M.A., Grogan,P., Brown,P.D., Giannini,C., Ballman,K.V., Kitange,G.J., Guha,A., Pandita,A., and James,C.D. (2006). Use of an orthotopic xenograft model for assessing the effect of epidermal growth factor receptor amplification on glioblastoma radiation response. *Clin. Cancer Res.* *12*, 2264-2271.
- Seiwert,T.Y., Haraf,D.J., Cohen,E.E., Stenson,K., Witt,M.E., Dekker,A., Kocherginsky,M., Weichselbaum,R.R., Chen,H.X., and Vokes,E.E. (2008). Phase I study of bevacizumab added to fluorouracil- and hydroxyurea-based concomitant chemoradiotherapy for poor-prognosis head and neck cancer. *J. Clin. Oncol* *26*, 1732-1741.
- Selker,R.G., Shapiro,W.R., Burger,P., Blackwood,M.S., Arena,V.C., Gilder,J.C., Malkin,M.G., Mealey,J.J., Jr., Neal,J.H., Olson,J., Robertson,J.T., Barnett,G.H., Bloomfield,S., Albright,R., Hochberg,F.H., Hiesiger,E., and Green,S. (2002). The Brain Tumor Cooperative Group NIH Trial 87-01: a randomized comparison of surgery, external radiotherapy, and carmustine versus surgery, interstitial radiotherapy boost, external radiation therapy, and carmustine. *Neurosurgery* *51*, 343-355.
- Singh,S.K., Hawkins,C., Clarke,I.D., Squire,J.A., Bayani,J., Hide,T., Henkelman,R.M., Cusimano,M.D., and Dirks,P.B. (2004). Identification of human brain tumour initiating cells. *Nature* *429*, 396-401.
- Stummer,W., Pichlmeier,U., Meinel,T., Wiestler,O.D., Zanella,F., and Reulen,H.J. (2006). Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol* *7*, 392-401.
- Stupp,R., Hegi,M.E., Mason,W.P., van den Bent,M.J., Taphoorn,M.J., Janzer,R.C., Ludwin,S.K., Allgeier,A., Fisher,B., Belanger,K., Hau,P., Brandes,A.A., Gijtenbeek,J., Marosi,C., Vecht,C.J., Mokhtari,K., Wesseling,P., Villa,S., Eisenhauer,E., Gorlia,T., Weller,M., Lacombe,D., Cairncross,J.G., and Mirimanoff,R.O. (2009). Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* *10*, 459-466.
- Stupp,R., Mason,W.P., van den Bent,M.J., Weller,M., Fisher,B., Taphoorn,M.J., Belanger,K., Brandes,A.A., Marosi,C., Bogdahn,U., Curschmann,J., Janzer,R.C., Ludwin,S.K., Gorlia,T., Allgeier,A., Lacombe,D., Cairncross,J.G., Eisenhauer,E., and Mirimanoff,R.O. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* *352*, 987-996.
- Stupp,R., Reni,M., Gatta,G., Mazza,E., and Vecht,C. (2007). Anaplastic astrocytoma in adults. *Crit Rev. Oncol Hematol.* *63*, 72-80.
- Tabatabai,G., Frank,B., Wick,A., Lemke,D., von Kurthy,G., Obermuller,U., Heckl,S., Christ,G., Weller,M., and Wick,W. (2007). Synergistic antiglioma activity of radiotherapy and enzastaurin. *Ann. Neurol.* *61*, 153-161.
- Takano,S., Yoshii,Y., Kondo,S., Suzuki,H., Maruno,T., Shirai,S., and Nose,T. (1996). Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. *Cancer Res.* *56*, 2185-2190.
- Tolcher,A.W., Gerson,S.L., Denis,L., Geyer,C., Hammond,L.A., Patnaik,A., Goetz,A.D., Schwartz,G., Edwards,T., Reyderman,L., Statkevich,P., Cutler,D.L., and Rowinsky,E.K. (2003). Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br. J. Cancer* *88*, 1004-1011.
- Tsao,M.N., Mehta,M.P., Whelan,T.J., Morris,D.E., Hayman,J.A., Flickinger,J.C., Mills,M., Rogers,C.L., and Souhami,L. (2005). The American Society for Therapeutic Radiology and Oncology (ASTRO) evidence-based review of the role of radiosurgery for malignant glioma. *Int. J. Radiat. Oncol Biol. Phys.* *63*, 47-55.
- Tuettenberg,J., Grobholz,R., Korn,T., Wenz,F., Erber,R., and Vajkoczy,P. (2005). Continuous low-dose chemotherapy plus inhibition of cyclooxygenase-2 as an antiangiogenic therapy of glioblastoma multiforme. *J. Cancer Res. Clin. Oncol* *131*, 31-40.
- van Nifterik,K.A., Elkhuzen,P.H., van Andel,R.J., Stalpers,L.J., Leenstra,S., Lafleur,M.V., Vandertop,W.P., Slotman,B.J., Hulsebos,T.J., and Sminia,P. (2006). Genetic profiling of a distant second glioblastoma multiforme after radiotherapy: Recurrence or second primary tumor? *J. Neurosurg.* *105*, 739-744.
- Vermeulen,L., Sprick,M.R., Kemper,K., Stassi,G., and Medema,J.P. (2008). Cancer stem cells--old concepts, new insights. *Cell Death. Differ.* *15*, 947-958.
- Walker,M.D., Alexander E Jr, Hunt,W.E., MacCarty,C.S., Mahaley,M.S., Jr., Mealey,J., Jr., Norrell,H.A., Owens,G., Ransohoff,J., Wilson,C.B., Gehan,E.A., and Strike,T.A. (1978). Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J. Neurosurg.* *49*, 333-343.
- Walker,M.D., Green,S.B., Byar,D.P., Alexander E Jr, Batzdorf,U., Brooks,W.H., Hunt,W.E., MacCarty,C.S., Mahaley,M.S., Jr., Mealey,J., Jr., Owens,G., Ransohoff,J., Robertson,J.T., Shapiro,W.R., Smith,K.R., Jr., Wilson,C.B., and Strike,T.A. (1980). Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N. Engl. J. Med.* *303*, 1323-1329.
- Wen,P.Y. and Kesari,S. (2008). Malignant gliomas in adults. *N. Engl. J. Med.* *359*, 492-507.
- Wick,A., Pascher,C., Wick,W., Jauch,T., Weller,M., Bogdahn,U., and Hau,P. (2009). Rechallenge with temozolomide in patients with recurrent gliomas. *J. Neurol.* *256*, 734-741.
- Willett,C.G., Boucher,Y., Duda,D.G., di,T.E., Munn,L.L., Tong,R.T., Kozin,S.V., Petit,L., Jain,R.K., Chung,D.C., Sahani,D.V., Kalva,S.P., Cohen,K.S., Scadden,D.T., Fischman,A.J., Clark,J.W., Ryan,D.P., Zhu,A.X., Blaszkowsky,L.S., Shellito,P.C., Mino-Kenudson,M., and Lauwers,G.Y. (2005). Surrogate markers for antiangiogenic therapy and dose-limiting toxicities for bevacizumab with radiation and chemotherapy: continued experience of a phase I trial in rectal cancer patients. *J. Clin. Oncol* *23*, 8136-8139.

Willett,C.G., Duda,D.G., di,T.E., Boucher,Y., Ancukiewicz,M., Sahani,D.V., Lahdenranta,J., Chung,D.C., Fischman,A.J., Lauwers,G.Y., Shellito,P., Czitovics,B.G., Wong,T.Z., Paulson,E., Poleski,M., Vujaskovic,Z., Bentley,R., Chen,H.X., Clark,J.W., and Jain,R.K. (2009). Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. *J. Clin. Oncol* 27, 3020-3026.

Winkler,F., Kozin,S.V., Tong,R.T., Chae,S.S., Booth,M.F., Garkavtsev,I., Xu,L., Hicklin,D.J., Fukumura,D., di Tomaso,E., Munn,L.L., and Jain,R.K. (2004). Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiotensin-1, and matrix metalloproteinases. *Cancer Cell* 6, 553-563.

Wong,E.T., Hess,K.R., Gleason,M.J., Jaeckle,K.A., Kyritsis,A.P., Prados,M.D., Levin,V.A., and Yung,W.K. (1999). Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J. Clin. Oncol.* 17, 2572-2578.

Zhou,Q., Guo,P., Wang,X., Nuthalapati,S., and Gallo,J.M. (2007). Preclinical pharmacokinetic and pharmacodynamic evaluation of metronomic and conventional temozolomide dosing regimens. *J. Pharmacol. Exp. Ther.* 321, 265-275.