

File ID 135590  
Filename Chapter 1: Introduction, aim and outline  
Version Final published version (publisher's pdf)

---

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation  
Title Mutational profiling of glioblastoma  
Author F.E. Bleeker  
Faculty Faculty of Medicine  
Year 2009  
Pages 176  
ISBN 978-90-9024310-8

FULL BIBLIOGRAPHIC DETAILS:

<http://dare.uva.nl/record/305743>

---

*Copyright*

*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.*

---

# ... 1

**Introduction, aim and outline**

## **Glial tumors**

Gliomas are the most common primary neoplasms of the central nervous system, comprising over 50% of all such tumors in adults. Besides ependymomas, gliomas can essentially be divided into three categories: astrocytoma, oligodendroglioma and a mixed form which is called oligoastrocytoma. These categories can be subdivided into different grades (from II to IV) according to the WHO classification.<sup>1</sup> Prognosis is dependent on both tumor type and malignancy grade: oligodendroglioma components and lower grades correspond with a better prognosis. Glioblastoma, WHO grade IV with predominant astrocytic differentiation, is the most frequently occurring and most aggressive type of primary brain cancer in human. Glioblastoma is preferentially located in the cerebral hemispheres. Glioblastomas are histopathologically characterized by nuclear atypia, high mitotic activity, microvascular proliferation (angiogenesis) and necrosis.<sup>1</sup>

## **Epidemiology of glioblastoma**

The incidence of glioblastoma is about 3.5 per 100.000 people per year, with a slight male predominance consistently observed between countries.<sup>2,3</sup> The incidence has shown only minor trends in the past decades; most variation can be explained by better detection, improving diagnostic precision and changing attitude towards the elderly. Glioblastoma may manifest at any age, but preferentially affects adults, with a peak of incidence between 45 and 75 years.<sup>2</sup> Most glioblastomas are sporadic; however, occasionally they occur in hereditary syndromes, such as Neurofibromatosis types 1 and 2, Li-Fraumeni and Turcot's syndromes.<sup>1</sup> The only proven risk factor for gliomas is therapeutic irradiation of the head.<sup>4</sup>

## **Clinical aspects of glioblastoma: diagnosis, treatment and prognosis**

The clinical history of a patient presenting with a glioblastoma is in more than 50% of cases less than three months. Patients with a short history prior to diagnosis typically are older patients (age >40 years). Younger patients can have a much longer history, in particular in those cases of a prior low-grade glioma. Symptoms can either be generalized such as headache, nausea/vomiting and papilledema as a result of raised intracranial pressure, or focal such as hemiparesis, aphasia and visual deficits depending on the location of the tumor. Up to one third of the patients will experience an epileptic seizure. The clinical diagnosis of brain tumors is preferentially made by magnetic resonance imaging (MRI) with gadolinium enhancement. To obtain a histological diagnosis and to reduce the space occupying effect, resection of most of the tumor (gross total removal) is the initial preferred intervention. Complete removal however, is impossible due to widespread invasion of tumor cells in the surrounding brain.<sup>1</sup> If the tumor is located in the proximity of an eloquent area, or if the patient's condition does not allow an extensive surgical intervention, a subtotal resection or only a biopsy will be performed to obtain a histological diagnosis. There is mounting evidence that there is a correlation between the extent of resection and the prognosis.<sup>5</sup> Surgical treatment is followed by radiotherapy which is administered in 30 daily fractions of 1.8-2 Gy.<sup>6</sup> Since 2005, temozolomide or Temodal®, an alkylating cytostatic drug, is given concomitant with and adjuvant to radiotherapy. This so called 'chemoradiation' is now widely accepted as standard treatment for newly diagnosed glioblastoma patients.<sup>7</sup>

Differences between patients and their performance status cause variation in survival, which can be calculated for individual patients based on nomograms.<sup>8</sup> A better prognosis is associated with younger age, more extensive resection and chemoradiation following resection.<sup>8</sup> Although a rare group (2-5%) of long-term survivors, generally characterized by young age and good condition, shows a survival longer than three years without recurrence,<sup>8,9</sup> the median overall survival is still limited to only 15 months.<sup>7</sup> Further improvement in survival is therefore urgently required. No standardized treatment is available for recurrent tumors and most patients who are selected for treatment enter clinical trials. Trials for both primary and recurrent tumors usually include small molecules and antibodies aimed to inhibit the oncogenic pathways which are activated in glioblastoma. For a rational drug design, it is essential to further unravel the etiology of glioblastoma. Here, the present understanding of cancer is summarized to form a basis for the studies described in this thesis.

### **Molecular alterations in cancer**

Cancer is a genetic disease, as it is caused by a series of genetic mutations. Although some of these mutations may be inherited, hereditary germline mutations cause only a small part of the cancer cases. The majority of cancer cases however, are sporadic, in which case mutations are acquired during life. Errors in the DNA can either be fatal or beneficial for the cell, depending on the function of the protein that the gene encodes, or have no consequences, when the mutated sequence is present in a non-coding region in the DNA or encodes a protein that is not crucial for that particular cell.

A single mutation is not enough to cause cancer. A normal cell can turn into a cancer cell by an accumulation of mutations that progressively alters its phenotype leading to an escape of the various control mechanisms that normally prevent malignant transformation. These include mutations that inactivate tumor suppressor genes and mutations that transform proto-oncogenes into oncogenes. Tumor suppressor genes encode proteins that regulate the cell cycle or induce programmed cell death by apoptosis, whereas oncogenes encode proteins that induce cell proliferation and survival. In addition, mutations can occur in genes involved in replication, recombination and repair of DNA damage, which can cause genetic instability, and gives rise to subsequent genetic errors in the genome without repair or apoptosis. Accumulation of these DNA alterations may finally induce malignant transformation.

In addition to the genetic changes, several other mechanisms play a role in the contribution to malignant transformation of cells. For example, epigenetic silencing of tumor suppressor genes is a common motif of genomic instability in cancer.<sup>10</sup> Epigenetics are inheritable characteristics of gene expression, not due to any alteration in the DNA sequence. Epigenetic examples are promoter hypermethylation, histone deacetylation, histone methylation and other histone modifications which can alter chromatin structure (in)directly.<sup>10</sup>

Recently, RNA silencing mechanisms, such as RNA interference and microRNA regulation, have also been linked to cancer. MicroRNAs (miRNAs or miRs) are short non-coding RNAs, consisting of approximately 22 nucleotides, which regulate gene expression in normal tissue and cancer. MicroRNAs are located in introns or intergenic regions, transcribed and processed so that they can target genes with complementary sites in the 3' or 5'-untranslated region. MicroRNAs usually inhibit expression of target genes, either by inhibition of translation or by triggering cleavage of the target mRNA. Over 500 miRNAs have been described in human. Differences in miRNA expression have been observed between normal tissue and tumors.<sup>10</sup>

Cancer is a disease in which cells undergo transformation and show features such as invasion and uncontrolled cell division. Normally, cell proliferation and apoptosis are well-balanced processes in normal cells, but in cancer cells proliferation is increased and apoptosis is decreased. Of utmost importance are the cell division checkpoints which control the cell cycle and allow DNA repair systems to repair mutations or other genetic alterations before a cell can divide into two daughter cells. When DNA damage cannot be repaired, the cell goes into programmed cell death (apoptosis). However, when this mechanism fails, genetic instability develops and DNA alterations are passed to daughter cells. In addition, new genetic alterations may develop due to failure of the control mechanisms. Some of these alterations may give rise to growth advantage over other –neighboring- cells. Clones of this genetically altered and instable cell will form and the cells of these clones will dominate in the region where they are formed. These clones then continue to acquire genetic errors in the DNA without repair and eventually may become a malignant tumor.<sup>11</sup>

### **Cancer stem cells**

Recently, the theory of cancer stem cells (CSCs) has been introduced, and for most tumor types, including glioblastoma, CSCs have been identified.<sup>12</sup> CSCs form a small subpopulation of the cancer cells in a tumor and possess the features of stem cells. The CSCs exhibit a capacity for self renewal, can differentiate<sup>13</sup> and form tumors in NOD/SCID mice which exactly recapitulate the original tumor.<sup>12</sup> Questions about the origin of these CSCs are still unanswered, and for glioma CSCs the difficulty is whether they arise from developmentally stalled neural progenitor cells or from dedifferentiated astrocytes.<sup>14</sup>

### **Cancer treatment**

Until a decade ago, cancer treatment consisted of surgery, radiotherapy and classical cytotoxic chemotherapy interfering with cell division. These chemotherapeutic drugs do not only kill the fast dividing cancer cells, but all other dividing cells in the body as well, leading to many side effects. Nowadays, cancer treatment is moving towards so-called targeted treatment. These therapies do not interact systemically with cell division, but interfere with a target preferably specific to the cancer cells, and therefore should have fewer side effects. Specifically those genes and signaling pathways are targeted that are (over-)activated in cancer.

### Kinases as treatment targets

Constitutive activation of the phosphorylation of proteins or lipids involved in intracellular signaling pathways is one of the biochemical hallmarks of cancer. Therefore, the phosphorylation proteins, the so-called kinases, are very suitable for ‘targeted’ treatment. Kinases are a family of approximately 550 enzymes<sup>15</sup> that transfer phosphate groups to other proteins or molecules (phosphorylation). Phosphorylation usually activates the target proteins. Many kinases play a role in signaling cascades, usually by activation. Constitutive activation may transform signaling cascades in oncogenic pathways. A cancer cell is in need of constitutively activated kinases for its oncogenic properties, and this dependence (so-called oncogene addiction), provides an Achilles heel for tumors to be exploited in cancer therapy.<sup>16</sup> Inhibition of kinases can be achieved by designed drugs that can block specific enzymes (small molecules) or specifically block receptors (antibodies), which both can inhibit phosphorylation of specific proteins or lipids. This blocking consequently results in inhibition of downstream signaling of relevant pathways. Kinases have been identified as potential therapeutic targets in various types of cancer and kinase inhibition therapies are used nowadays in clinical settings with meaningful responses. For example, approximately 30% of the breast cancer cases shows *ERBB2* amplification and/or overexpression.<sup>17</sup> Drugs that specifically block the *ERBB2* protein, such as the humanized monoclonal antibody trastuzumab (Herceptin), can inhibit cell growth.<sup>18</sup> In gastrointestinal stromal tumor (GIST), activating mutations in *KIT* are often detected,<sup>19</sup> which respond to kinase inhibitors such as imatinib (Gleevec).<sup>20</sup>

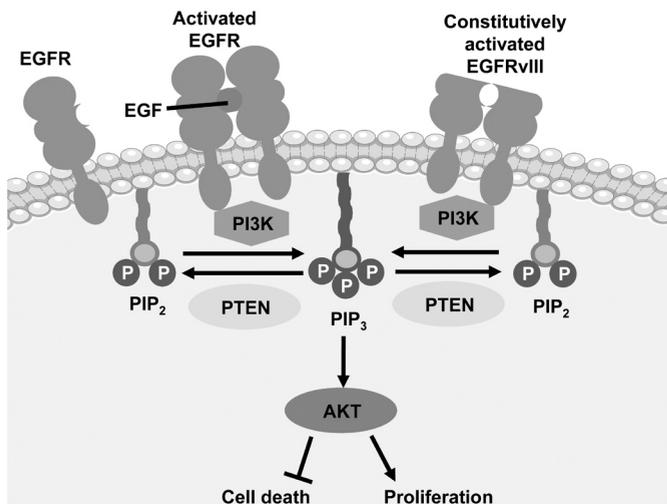


Figure 1. Simplified representation of the PI3K-AKT signaling pathway

### Targeting the EGFR kinase in glioblastoma

To date, targeted therapies are not part of the standard treatment of glioblastoma, although the tyrosine kinase epidermal growth factor receptor (EGFR) might be an interesting target. *EGFR* is amplified in 40% of the glioblastomas,<sup>21</sup> and activating mutations in *EGFR* are found as point mutations or large deletions in the extracellular part of the receptor (*EGFRvIII*).<sup>22</sup>

The response to EGFR inhibitors however, is limited to 15-20% of glioblastoma patients.<sup>23</sup> This limited response is likely caused by molecular events downstream of EGFR, leading to activation of the oncogenic PI3K-AKT signaling pathway (**Figure 1**). EGFR signals indirectly to PI3K, which phosphorylates PIP2 into PIP3, in turn, activating the downstream oncogene AKT. The tumor suppressor PTEN inhibits the activation of AKT by dephosphorylation of PIP3 into PIP2.<sup>24</sup> However, it has been found that in 40% of glioblastomas, the tumor suppressor gene *PTEN* is mutationally or transcriptionally inactivated,<sup>25</sup> causing activation of the PI3K-AKT pathway. As a result, the effect of therapeutic EGFR inhibition can be neutralized by loss of PTEN. This explains the correlation between the response to EGFR inhibitors and the co-expression of EGFRvIII and PTEN proteins.<sup>23,26</sup>

## Mutation analysis

Several mechanisms underlie the changes in the DNA of genes involved in tumorigenesis and tumor progression. These include: amplification or deletion, overexpression and mutation. Various technical approaches are nowadays available to identify deregulated cancer genes and proteins, and one of these is mutation analysis of tumor DNA. Mutational profiling is an expensive but robust technique that can be performed on a large scale. Mutations are digital (they are present or not), occur rarely and are often localized in cancer genes. On the basis of the type and the location of a mutation in a gene, the effect of a mutation on protein activity can sometimes be predicted. DNA mutations in oncogenes can give rise to constitutively activated proteins, which can consequently lead to altered signal transduction in cells and thus provide therapeutic targets in cancer. DNA mutations in tumor suppressor genes, on the other hand, often form truncated proteins with abnormal function. In this way, mutation analysis may lead to potential therapeutic targets on one hand and give indications for prognosis and response treatment in a given case on the other hand. To clarify the spectrum of mutations that can be found in large scale analysis, the nomenclature of different types of mutations is presented in **Table 1**.

## History of mutation analysis

The first transforming somatic mutation was described in the *HRAS* gene in human bladder cancer.<sup>27</sup> Since then, transforming somatic mutations in different types of malignant tumors have been identified in numerous genes.<sup>28</sup> Of special interest is the fact that genes encoding kinases are overrepresented in the group of cancer genes that have been found mutated.<sup>28</sup> After the completion of the initial working draft sequence of the 3.1 billion base pairs of the human genome in 2001,<sup>29,30</sup> the 'kinome' encoding 518 protein kinases was described.<sup>15</sup> Other kinases, mainly lipid kinases, have been identified afterwards. The total number of kinases is now estimated at 550. This provided the possibility for systematic mutation analysis of protein and lipid kinases in colon cancer.<sup>31,32</sup> Subsequently, mutation profiling studies were performed on the protein kinome in other cancer types,<sup>33</sup> including glioblastoma.<sup>34</sup> Although glioblastoma did not reveal any frequently mutated kinases, mutated kinase candidates might easily have been missed as the study was performed on a limited number of nine tumors.<sup>34</sup>

Type of mutation	Description
Mutation	A genetic alteration in the nucleotide sequence of DNA.
Somatic mutation	A mutation that occurs in any cell of the body except germ cells. Therefore, the mutation is not hereditary and cannot be passed on to the next generation.
Germline mutation	A mutation that occurs in all cells of the body including the germ cells. Therefore, the mutation is hereditary and can be passed on to the next generation.
Synonymous or silent mutation	A mutation that does not result in a change of the amino acid sequence of a protein.
Non-synonymous or non-silent mutation	A mutation that results in a change of the amino acid sequence of a protein.
Point mutation	A single nucleotide change causing a synonymous or non-synonymous mutation.
Deletion	A mutation in which part of a chromosome or a sequence of DNA is missing.
Insertion	A mutation in which part of a chromosome or a sequence of DNA is inserted.
Missense mutation	A point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid.
Nonsense mutation	A mutation causing a premature stop codon, and therefore results in truncation of the transcript and protein.
Frameshift mutation	A mutation caused by insertion or deletion of a number of nucleotides, resulting in a translation completely different from the original sequence.
Driver mutation	A mutation that confers an oncogenic potential towards a (cancer) cell and causes cancer cells to grow.
Passenger or bystander mutation	A mutation that is observed in cancer cells, but does not contribute to cancer development.
Gain of function mutation	A mutation that activates the cellular activity or the protein corresponding to the gene in which the mutation is occurring. This type of mutation occurs generally in proto-oncogenes.
Loss of function mutation	A mutation that inactivates the cellular activity or the protein corresponding to the gene in which the mutation is occurring. This type of mutation occurs generally in tumor suppressor genes.
Transition	A mutation that substitutes a purine for another purine nucleotide (A ↔ G) or a pyrimidine for another pyrimidine nucleotide (C ↔ T).
Transversion	A mutation that substitutes a purine for a pyrimidine or a pyrimidine for a purine.

Table 1. Nomenclature of mutations

## Aims of this thesis

The aim of the studies described in this thesis is to identify genetic changes in glioblastoma in order to reveal new therapeutic targets. The focus lies specifically on the identification of somatic mutations that play a role in gliomagenesis and/or progression. Furthermore, mutations that can be predictive with regard to the response to treatment or prognostic factors are searched for. In this thesis, different mutation analyses are described to find novel genetic mutations in glioblastoma and other types of cancer.

## Outline of the thesis

First, a general overview of the genotyping of cancer genomes and its prospects for treatment is presented (**Chapter 2**). Second, different mutational studies performed in glioblastoma and other types of cancer are discussed in **Chapters 3 to 11**. From a therapeutic point of view, the most promising candidates seem to be the genes that code for kinases. Therefore, many kinases have been included in a large scale mutation analysis in glioblastoma which is described in **Chapter 3**. Cancer candidate genes selected from sequencing studies that have been performed in colon and breast carcinoma were subjected to mutation analysis in glioblastoma, melanoma, and pancreatic carcinoma and this is described in **Chapters 4 and 5**. The hotspot mutation *AKT1*<sup>E17K</sup>, of utmost importance in cancer development, was assessed in a panel of eight human solid tumors (**Chapter 6**). In addition, the complete *AKT1* gene was mutationally profiled in glioblastoma (**Chapter 7**). Then, the frequently reported *PLXNB1* mutations in prostate cancer were investigated and this is reported in **Chapter 8**. Next, the prevalence of *IDH1*<sup>R132</sup> mutations was assessed in various types of solid cancer types, including glioblastoma (**Chapter 9**) and other types of brain tumors (**Chapter 10**). In addition, metabolic mapping experiments were performed to examine the activity of isocitrate dehydrogenases in *IDH1*<sup>R132</sup> mutated and wild-type tumors (**Chapter 10**). An up to date overview of the molecular hallmarks known in glioblastoma is presented in **Chapter 11**. Finally, the studies described in this thesis are discussed and future perspectives are presented (**Chapter 12**).

## References

1. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK. WHO Classification of Tumors of the Central Nervous System, 2007.
2. Ohgaki H, Dessen P, Jourde B, et al. Genetic pathways to glioblastoma: a population-based study. *Cancer Res* 2004; 64: 6892-99.
3. The Central Brain Tumor Registry of the United States (CBTRUS). <http://www.cbtrus.org>
4. Ron E, Modan B, Boice JD, Jr., et al. Tumors of the brain and nervous system after radiotherapy in childhood. *N Engl J Med* 1988; 319: 1033-39.
5. Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. *Neurosurgery* 2008; 62: 753-64; discussion 264-46.
6. Walker MD, Strike TA, Sheline GE. An analysis of dose-effect relationship in the radiotherapy of malignant gliomas. *Int J Radiat Oncol Biol Phys* 1979; 5: 1725-31.
7. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352: 987-96.
8. Gorlia T, van den Bent MJ, Hegi ME, et al. Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981-22981/CE.3. *Lancet Oncol* 2008; 9: 29-38.
9. Krex D, Klink B, Hartmann C, et al. Long-term survival with glioblastoma multiforme. *Brain* 2007; 130: 2596-606.
10. Esteller M. Epigenetics in cancer. *N Engl J Med* 2008; 358: 1148-59.
11. Nowell PC. The clonal nature of neoplasia. *Cancer Cells* 1989; 1: 29-30.
12. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumor initiating cells. *Nature* 2004; 432: 396-401.
13. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. *Cancer Res* 2004; 64: 7011-21.
14. Stiles CD, Rowitch DH. Glioma stem cells: a midterm exam. *Neuron* 2008; 58: 832-46.
15. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S. The protein kinase complement of the human genome. *Science* 2002; 298: 1912-34.
16. Weinstein IB. Cancer. Addiction to oncogenes--the Achilles heel of cancer. *Science* 2002; 297: 63-64.
17. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987; 235: 177-82.
18. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344: 783-92.
19. Rubin BP, Singer S, Tsao C, et al. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001; 61: 8118-21.
20. Verweij J, Casali PG, Zalcberg J, et al. Progression-free survival in gastrointestinal stromal tumors with high-dose imatinib: randomised trial. *Lancet* 2004; 364: 1127-34.
21. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. *Nature* 1985; 313: 144-47.
22. Lee JC, Vivanco I, Beroukhi R, et al. Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. *PLoS Med* 2006; 3: e485.
23. Mellinger IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 2005; 353: 2012-24.
24. Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002; 296: 1655-57.
25. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997; 275: 1943-47.
26. Cloughesy TF, Yoshimoto K, Nghiemphu P, et al. Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. *PLoS Med* 2008; 5: e8.
27. Reddy EP, Reynolds RK, Santos E, Barbacid M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. *Nature* 1982; 300: 149-52.
28. Futreal PA, Coin L, Marshall M, et al. A census of human cancer genes. *Nat Rev Cancer* 2004; 4: 177-83.
29. Venter JC, Adams MD, Myers EW, et al. The sequence of the human genome. *Science* 2001; 291: 1304-51.
30. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. *Nature* 2001; 409: 860-921.
31. Bardelli A, Parsons DW, Silliman N, et al. Mutational analysis of the tyrosine kinome in colorectal cancers. *Science* 2003; 300: 949.
32. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004; 304: 554.
33. Greenman C, Stephens P, Smith R, et al. Patterns of somatic mutation in human cancer genomes. *Nature* 2007; 446: 153-58.
34. Hunter C, Smith R, Cahill DP, et al. A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. *Cancer Res* 2006; 66: 3987-91.