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PROGNOSTIC AND PREDICTIVE BIOMARKERS OF GLIAL TUMORS OF THE CENTRAL NERVOUS SYSTEM IN THE CONTEXT OF PERSONALIZED MEDICINE

The self report of the dissertation

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Abstract

The glial tumors, so called gliomas, represent the largest group of the primary central nervous system malignancies. Gliomas remain generally an incurable disease progressing from the lower grades of malignancy to the more aggressive tumors in the course of time. This finally leads to the rapid patient's clinical deterioration and eventually the death. Recently there has been a significant expansion of knowledge in the neuro-oncology domain regarding the onset and development of neoplastic disease at the genetic as well as epigenetic level. Novel prognostic and predictive molecular genetic biomarkers are emerging that can be used for more precise diagnosis, for more accurate assessment of a patients´ prognosis, or for better selection of therapy and prediction of therapeutic response. The fundamental view of the histological-based classification of central nervous system tumors is gradually changing and the molecular biomarkers are incorporating in addition to histopathology to refine the diagnoses of many tumor entities at the moment. The recent findings from molecular genetics of gliomas together with the results from clinical trials incorporating the various biomarkers are discussed in this thesis.

In the first study the biomarker isocitrate dehydrogenases 1 (IDH1) R132H mutation was examined in the tumor tissue from patients with glioblastoma multiforme and the results were correlated with the clinical characteristics of patients. The prognostic value of this biomarker was proved. Patients with IDH1 R132H mutation in the tumor tissue had significantly longer survival than patients with IDH1 wild-type tumors. The presented results were included into the large recently published meta-analysis that confirmed positive prognostic effect of the IDH mutations on both overall survival and progression-free survival in patients with gliomas.

The second study examined the chromosomal aberration 1p/19q co-deletion in patients with anaplastic oligodendroglioma who were treated with the combined radiotherapy and chemotherapy (procarbazine, lomustine and vincristine regime - PCV). The results were correlated with the clinical characteristics of patients. The prognostic value of 1p/19q co-deletion was proved. The strong positive predictive value of this biomarker for overall survival was also shown for patients with co-deletion treated with neurosurgery and radiotherapy plus PCV chemotherapy by comparison with neurosurgery and radiotherapy alone.

The enormous advances in the molecular genetics of central nervous system tumors especially gliomas bring completely new opportunities for the optimization of the treatment strategies for an individual patient with these diagnoses. The analysis of molecular genetics in central nervous system tumors is now recommended in order to implement the principles of personalized medicine into the clinical management of these malignancies.

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List of abbreviations

2-HG	2-hydroxyglutarate
AODG	anaplastic oligodendroglioma
ATRX	alpha-thalassemia/mental retardation syndrome
CI	confidence interval
CIC	homolog of the drosophila gene capicua
CNS	central nervous system
COSMIC	catalogue of somatic mutations in cancer
CpG	cytosine-guanine
DNA	deoxyribonucleic acid
DNET	dysembryoplastic neuroepithelial tumors
EORTC	European organization for research and treatment of cancer
FFPE	formalin-fixed, paraffin-embedded
FISH	fluorescence in situ hybridization
FUBP1	far upstream element binding protein
GBM	glioblastoma multiforme
G-CIMP	glioma cytosine-guanine islets methylator phenotype
HGG	high grade glioma
HR	hazard ratio
CHT	Chemotherapy
IDH1/2	isocitrate dehydrogenases 1 and 2
KPS	Karnofsky performance status
LGG	low grade glioma
MGMT	O-6-methylguanine-methyltransferase
MRI	magnetic resonance imaging
MYC	myelocytomatosis viral oncogene homolog
NADP+	nicotinamide adenine dinucleotide phosphate
NOS	non-otherwise specified
ODG	oligodendroglioma
OS	overall survival
PCR	polymerase chain reaction
PCV	procarbazine, lomustine and vincristine chemotherapy
PDGF	platelet-derived growth factor
PFS	progression-free survival
PI3K	phosphoinositide 3-kinase
RT	Radiotherapy
RTOG	radiation therapy oncology group
SD	standard deviation
TCGA	the cancer genome atlas research network
TERT	telomerase reverse transcriptase
WHO	world health organization
α-KG	alpha-ketoglutarate

1. Theoretical introduction

1.1 Biomarkers and personalized medicine in neurooncology

Personalized medicine represents new model of an individual patient's medical care [1,2]. The main goal of personalized medicine is the shift from the concept of "one medicine fits to all patients with the same disease" to individual treatment of each patient - "the right treatment to the right patient in the right time" [3–5]. Personalized medicine is based on the evolving knowledge about the human genome, gene functions as well as the genetic basis of the individual differences in responses to a treatment. The main strategy of personalized medicine is to provide an individualized approach to each patient, based on his/her personal genetic profile and combining information from omics disciplines (genomics, epigenomics, proteomics, transcriptomics, metabolomics and others) with innovative preventive and therapeutic strategies that are more efficient, safe and cost-effective [6–9].

Central nervous system (CNS) tumors account for about 2% of all cancers with the annually incidence 9.5 cases out of 100,000 people [10,11]. The widely used World Health Organization (WHO) classification from 2007 recognized more than 130 different histopathological units of primary CNS tumors [12]. This represents a very extensive and markedly heterogeneous group of diseases, with individual types of tumors exhibiting various biological behaviors.

Recently there has been a significant expansion of knowledge in the neuro-oncology domain regarding the onset and development of neoplastic disease at the genetic as well as epigenetic levels [13]. Novel prognostic and predictive biomarkers are emerging and the fundamental view of the histological-based classification of CNS tumors is gradually changing. Moreover, even in the given histopathological units, further segmentation is starting to establish based on molecular genetic profiles resulting from the international integrative multiplatform studies of the CNS tumors [14–16]. The huge progress in genetic and epigenetic findings led to the very recent update of WHO CNS tumors classification in 2016 [17]. For the first time, the molecular biomarkers are incorporated in addition to histopathology to refine the diagnoses of many tumor entities. The updated classification presents a new perspective for how CNS tumor diagnoses should be structured in the era of molecular medicine.

The largest group among the primary CNS tumors (about 50%) are formed from supporting glial cells and are called gliomas [12,18]. Gliomas are the most diverse group of CNS tumors differing in their typical localization, age predisposition, morphology, grade and the inclination to progression. To date, gliomas are classified mainly based on their histopathological characteristics. The most important classes are astrocytomas, oligodendrogliomas, ependymomas and mixed type of gliomas such as oligoastrocytomas.

Gliomas can be also categorized according to morphological features of anaplasia into the grade of malignancy with a range of WHO grades I to IV. This classification is closely linked to the distinct disease behavior, ranging from slow progression in lower grade tumors, to extremely

poor prognosis for patients with WHO grade IV glial tumors (glioblastoma multiforme - GBM). However, it is not time independent during the disease course. Low-grade tumors (WHO grade II) progress to high-grade (anaplastic) gliomas (WHO grade III) and finally also to secondary GBM over time, which is now explained in detail on molecular genetic level [19]. The progression to GBM leads to rapid clinical deterioration and eventually to the patient's death within 15 months despite the complex treatment [20,21]. The only exception are WHO grade I gliomas (the most important representative - pilocytic astrocytoma) representing biologically entirely different type of tumors also called "circumscribed". These tumors are potentially curable with surgical resection only and do not progress to the higher grades over the disease time course [22]. Schematically CNS gliomas are subdivided into the lower grade tumors (low grade glioma - LGG) representing the WHO grades I and II tumors and high grade tumors (high grade glioma - HGG) with the WHO grade III and IV tumors (anaplastic gliomas and GBM). This sub-classification has strong clinical significance because of the substantial differences in treatment strategies.

In the near future it is likely to be necessary to integrate various molecular genetic biomarkers together with the principles of personalized medicine into standard clinical care for patients suffering from neurological cancers. The most recent and clinically relevant examples of the use of personalized medicine approaches in the management of the glioma patients will be discussed in this dissertation focusing on glioblastoma multiforme, oligodendroglioma and the group of low grade gliomas.

1.2 Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most common and most malignant primary brain tumor in adults with an incidence of 3-4/100,000/year [23,24]. GBM is extremely invasive and difficult to treat surgically, characterized by intense and aberrant vascularization and high resistance to radiotherapy (RT) and chemotherapy (CHT). The current standard of care for patients with newly diagnosed GBM is neurosurgery followed by fractionated external beam RT and CHT with systemic temozolomide [25]. The median survival of GBM patients is 12.1-14.6 months and only 3-5% of patients survive longer than 3 years [26]. The progress in the knowledge of GBM genetics over the past 10 years has revealed several abnormalities in a diversity of mutated genes and cellular signaling pathways. The importance of the GBM microenvironment, especially of tumor angiogenesis, has also been studied. New knowledge regarding the diversity of GBM on molecular and genetic level could lead to the deep analysis of the tumor and the refinement of management personalized to the individual patient in the near future.

1.2.1 Prognostic and predictive glioblastoma biomarkers

The huge progress in the genetics as well as epigenetics of gliomas in the recent years revealed some particularly important molecular biomarkers that significantly change the approach to

clinical management of patients with these primary CNS tumors. The most important examples of prognostic and/or predictive biomarkers and their clinical relevance in the treatment of GBM patients is discussed in this section, such as the mutations in isocitrate dehydrogenases 1 and 2 (IDH1/2), the glioma cytosine-guanine (CpG) islets methylator phenotype (G-CIMP) or the promoter methylation status of the O-6-methylguanine-methyltransferase (MGMT) gene.

1.2.2 Mutations in IDH1/2 as a glioblastoma biomarker

The isocitrate dehydrogenases mutations are the important glioma biomarkers close to clinical application that are able to contribute to determining the patient's prognosis. IDH is an important Krebs cycle enzyme that converts isocitrate into alpha-ketoglutarate (α-KG) and reduces nicotinamide adenine dinucleotide phosphate (NADP+) to the reduced form NADPH. IDH thus acts in one of the critical steps of carbohydrate, lipid as well as amino acid metabolism [27]. Human IDH enzyme has three different isoforms - IDH1 (found in the cytoplasm and peroxisomes) and IDH2 and 3 (presented in the mitochondria).

Recurrent mutations in IDH were systematically described in patients with GBM, although only in about 5-10% of the tumors (predominantly secondary GBM). In contrast, IDH1/2 mutations were subsequently found with high frequency in diffuse astrocytomas (70-80%) and anaplastic astrocytomas (up to 50%) [28,29]. Mutations in IDH1 show conservative amino acid substitution R132H in 90%. R132C, R132G, R132S and R132L substitutions are also known but uncommon. Mutations in IDH2 are much rarer and primarily involve R172 amino acid substitution [29,30].

The real breakthrough in the understanding of IDH1/2 mutations for glioma oncogenesis was the discovery of completely new function of the mutant enzyme. Instead of NADP+ dependent production of α-KG, mutant IDH catalyzes the NADPH-dependent reduction of α-KG to 2-hydroxyglutarate (2-HG). Gliomas with IDH1/2 mutations therefore contain the high concentration of 2-HG, unlike tumors without such mutations [31]. Potential onco-metabolite 2-HG is closely related to cancer initiation and progression. 2-HG serves as an potent inhibitor of alpha-ketoglutarate-dependent dioxygenases, which leads to genome-wide epigenetic changes [32]. Cells with mutations in IDH1/2 thus undergo massive epigenetic alterations including DNA and histone hypermethylation that leads to chromatin remodeling and extensively influences gene expression [33–35].

From the perspective of personalized medicine the marked impact of these mutations on GBM prognosis is especially important, regardless of the therapy intervention. Patients with GBM and mutations in IDH1/2 are generally younger and have a significantly longer median OS than patients without these mutations (IDH-wild type). Across several studies, better prognosis for patients with IDH1/2 mutated GBM than IDH-wild type GBM were observed with the longer median OS of 3.8 vs. 1.1 years, 2.6 vs. 1.3 years, 2.3 vs. 1.2 years and 3 vs. 1 year [16,30,36,37]. Even more significant differences in OS were found in patients with anaplastic astrocytomas; 5.4 vs. 1.7 years, 6.8 vs. 1.6 years and 7 vs. 2 years [30,36,37] as well as diffuse astrocytoma

12.6 vs. 5.5 years [36]. Recent meta-analysis of 55 observational studies has shown that patients with gliomas positive for IDH1/2 mutations have improved both overall survival and progression-free survival [38].

The growing importance of IDH mutations in clinical practice also requires the development of standardized and validated methods for analyzing of this biomarker in the tumor tissues with high sensitivity and specificity. IDH mutations can be assessed by immunohistochemistry or molecular biology techniques from the resected tumor tissue or biopsy [39–42]. These can be complemented or even replaced with non-invasive in-vivo determination of onco-metabolite 2-HG in the tumor tissue by MRI-spectroscopy [43–46]. This approach detecting the resulting product of mutated enzyme is also independent from sequential type of IDH1/2 mutations. It represents unique case in oncology when the specific mutation in the tumor tissue can be assessed by accessible radiology method with high sensitivity and specificity.

Further research will clarify the potential therapeutic effect of inhibition of mutated enzyme or depletion of onco-metabolite 2-HG accumulated in glial tumors. Inhibition of mutant IDH shows promise in phase I/II clinical trials with hematologic malignancies and further development is ongoing in solid tumors including gliomas [47].

1.2.3 MGMT promoter methylation as a glioblastoma biomarker

The current standard of care for GBM patients includes neurosurgery, RT and the use of the temozolomide-based chemotherapy. Temozolomide is an oral alkylating agent that causes DNA damage by alkylation of the 0-6 position of guanine and the production of DNA interstrand cross-links [25]. In a large, randomized, phase III trial in newly diagnosed patients with GBM conducted by Roger Stupp, RT and concurrent daily temozolomide followed by adjuvant temozolomide provided a median survival benefit of 2.5 months and the proportion of 2-year survivors increased from 10.4% to 26.5% in comparison with RT alone. 5-year OS was also higher in combined treatment arm (9,8 vs. 1,9%) [48]. The Stupp's regime has become a gold standard of care in the treatment of patients with newly diagnosed GBM and is still valid today. There exists a subset of patients who have better response to temozolomide, but the majority of GBM patients become rapidly resistant.

One of the strongest predictive biomarkers for the chemotherapy response is the alteration in the MGMT gene [49]. The enzyme MGMT is able to repair the DNA damage caused by temozolomide. The presence of MGMT leads to reduction in the effect of temozolomide-based chemotherapy. The silencing of MGMT can be caused by epigenetic mechanisms, such as the DNA hypermethylation of CpG islands in the promoter region. This alteration leads to a decrease in the transcription of MGMT and to worse ability of tumor to repair damage caused by temozolomide which means a better therapeutic response [50]. Methylation of the MGMT promoter was observed in more than 40% of patients with GBM (more in the subgroup with secondary GBM) [51,52].

The subset analysis of the Stupp´s clinical trial showed that the patients with hypermethylated MGMT promoter had a significantly longer median OS after therapy with RT plus temozolomide compared with RT alone (21.7 vs. 15.3 months) [25,51]. There was no statistically significant difference in OS between the treatment arms in the subgroup without methylation of the MGMT promoter. In another study, MGMT promoter hypermethylation was predictive for a better response to RT independently of treatment with temozolomide [53]. Therefore, the MGMT methylation status could be potentially considered as a general biomarker of better therapeutic response in GBM.

But what is the real predictive value of MGMT promoter methylation in everyday clinical practice? The substantial limitation of the use of this biomarker in choosing the most appropriate therapy for an individual patient is the lack of an alternative effective treatment for patients with newly diagnosed GBM. Moreover, the randomized phase III clinical trial radiation therapy oncology group (RTOG) 0525 which compared dose-intense temozolomide (75-100 milligrams per square meter of body surface on days 1 to 21 of a 28-day cycle) versus standard dose temozolomide (150-200 milligrams per meter squared on days 1 to 5 of a 28-day cycle) didn´t reveal a benefit of dose-intense regime overall, or in the subgroups of MGMT hypermethylated or unmethylated patients [54]. However the prognostic effect of this biomarker was also proven in this trial.

The MGMT promoter methylation could be incorporated into clinical practice as a predictive biomarker in some particular scenario, such as in the treatment of patients with the higher age and/or poorer performance status. Patients with age of more than 65 years and/or Karnofsky performance status (KPS) less than or equal to 60 often develop significant toxicity which limits the applicability of the standard treatment regime with RT and temozolomide.

Two independent clinical trials in elderly patients with GBM (NOA-08 and Nordic trial) randomized subjects into the RT alone (standard RT vs. hypofractionated RT in Nordic trial) versus temozolomide alone (dose-intense temozolomide in NOA-08) arms as an initial treatment [55,56]. Patients with MGMT promoter methylated tumors showed better outcome with temozolomide in both trials. Whereas those with MGMT unmethylated tumors had reduced survival when treated with temozolomide by comparison with RT alone. These results strongly support the predictive role of MGMT biomarker for the choosing of the optimal therapy in elderly GBM patients who are not commonly eligible for the combined modality treatment [13]. Currently, the optimal treatment strategy for elderly patients with GBM should be selected in a multi-disciplinary setting taking into account the KPS, extent of tumor resection and MGMT promoter methylation status. Based on the results from clinical trials mentioned above, it is now recommended to use temozolomide monotherapy after surgery in GBM patients with age more than 70 years and/or KPS less than or equal to 60 with tumor positive for MGMT promoter methylation also in the Czech Republic [48].

The prognostic as well as predictive role of MGMT biomarker has a close relation to the presence or absence of IDH mutations in the tumor tissue. In the recent study with 98 GBM patients the combined analyses of IDH mutations together with MGMT promoter methylation

outperforms either IDH1 mutations or MGMT methylation assessment alone in predicting survival [57]. The best prognosis was observed for those patients with IDH mutated MGMT methylated tumors followed by IDH mutated MGMT unmethylated and IDH wild-type MGMT methylated GBM. The worst prognosis was found in patients with IDH wild-type MGMT unmethylated tumors. The subanalyses of 183 anaplastic glioma patients from the NOA-04 clinical trial revealed the predictive effect of MGMT promoter methylation for benefit from alkylating agent chemotherapy only in patients with IDH1-wild-type, but not IDH1-mutant tumors [58]. The analysis of various biomarkers and their combinations will probably become the gold standard in the treatment planning for GBM patients in the near future.

1.2.4 G-CIMP as a glioblastoma biomarker

Another molecular genetic biomarker with possible clinical relevance for GBM patients is the glioma cytosine-guanine (CpG) islets methylator phenotype (G-CIMP). Hypermethylation of the CpG islets in glioma genome was studied mainly as a prognostic biomarker for GBM patients. The subanalysis of 272 GBM from the TCGA dataset demonstrated that patients with G-CIMP positive tumors were of younger age and experienced significantly improved OS [59]. Moreover, the vast majority of the G-CIMP positive tumors had also IDH1 mutations and belonged to proneural pattern of gene expression. The direct relationship between the mutations in IDH1/2 and occurrence of G-CIMP in tumor tissue was subsequently found [60]. The presence of IDH1/2 mutations and an accumulation of onco-metabolite 2-HG seems to be the sufficient factor for the establishment of G-CIMP in glioma genome.

1.3 Oligodendrogliomas

Oligodendrogliomas (ODG) represent approximately 5% of primary brain tumors. They have more favorable response to radiotherapy and chemotherapy than other types of CNS gliomas [61]. According to the updated 2016 WHO classification of CNS tumors, they are characterized by a histopathological finding with an oligodendroglial component together with the presence of distinct molecular genetic profile [17].

The huge progress in the research of ODG molecular genetics offers new knowledge in the diagnosis and treatment of these tumors that has, together with recent results from clinical trials, the direct impact on the management of ODG patients. The analysis of molecular genetics in ODG and the use of specific biomarkers are now well-established and recommended as an important part of treatment-decision algorithms in clinical practice.

1.3.1 Co-deletion of 1p/19q as an oligodendroglioma biomarker

Oligodendroglial tumors are characterized by frequent co-deletions of chromosome 1p and 19q (1p/19q co-deletion). This chromosomal aberration was discovered in 1994 and became the first biomarker in neuro-oncology [62]. 1p/19q co-deletion means the loss of genetic material

from both the short arm of chromosome 1 (1p) and the long arm of chromosome 19 (19q). The unbalanced translocation t(1;19)(q10;p10) and formation of derived chromosome 1p/19q was identified later as the mechanism of this aberration [63]. 1p/19q co-deletion is present almost exclusively in oligodendroglial tumors (80% to 90% of grade II ODG; 50% to 70% of AODG) [64,65].

Mutations in two important tumor suppressor genes, CIC (a homolog of the Drosophila gene capicua) located on 19q13.2, and far upstream element binding protein (FUBP1) on the 1p chromosome, were recently discovered in the majority of ODG with 1p/19q co-deletion (50-70% and 15% for CIC and FUBP1 mutations, respectively) [66,67]. Mutations in these genes are involved in the ODG formation and progression. CIC protein binds to regulatory regions and blocks gene transcription. FUBP1 mutations are closely related to a myelocytomatosis viral oncogene homolog (MYC) activation. Currently, 1p/19q co-deletion serves as an important diagnostic, prognostic and predictive biomarker in oligodendroglial tumors, as is discussed in detail further in this section.

1.3.2 Other oligodendroglioma biomarkers

Recurrent IDH1/2 mutations were first demonstrated in GBM. However, the frequent occurrence of mutations in IDH1 and IDH2 genes were also reported in ODG (up to 69%-94% tumors) [14,30,68]. The presence of the IDH1/2 mutations is a significant positive prognostic biomarker for patients with glioma including ODG [36,61]. Patients with ODG positive for both the 1p/19q co-deletion and IDH1/2 mutations experienced the best prognosis that shows the necessity of incorporating a combination of multiple biomarkers in the management of glioma patients [14,69].

The MGMT promoter methylation was discovered as a significant prognostic as well as predictive biomarker in patients with glioblastoma. This aberration was also found in 80% of AODG and in 73% of anaplastic oligoastrocytomas [70,71]. MGMT promoter methylation serves mainly as a positive prognostic biomarker for ODG patients treated with chemotherapy as was proven in the EORTC 26951 as well as NOA-4 clinical trials [72,73].

Hypermethylator phenotype of cytosine-guanine islets in the glioma genome is another important molecular characteristic of ODG. Positivity for G-CIMP is not an entirely independent biomarker as it is closely related to the presence of the IDH1/2 mutations also in ODG [59,60]. G-CIMP is approximately two-times more frequently presents in oligodendrogliomas (93%) than astrocytomas (45%) and is an important positive prognostic factor for all types of glioma including ODG [59].

1.3.3 The relevance of biomarker 1p/19q co-deletion in the clinical management of oligodendroglioma

The 1p/19q co-deletion status can be used in clinical practice as an important diagnostic, prognostic, as well as predictive biomarker in patients with oligodendroglial tumors. According to the WHO 2016 classification of CNS tumors, the diagnosis of oligodendroglioma is supported by the presence of 1p/19q co-deletion in tumor tissue, especially in cases where the histological findings are atypical or non-conclusive [17,74]. There are other tumor types that can mimic oligodendrogliomas by histopathological diagnosis such as dysembryoplastic neuroepithelial tumors (DNET), neurocytomas, clear cell ependymomas, and small cell anaplastic astrocytomas. Unlike ODG, these tumors do not have 1p/19q co-deletion, so as this biomarker is a useful diagnostic aid in these cases [74].

The 1p/19q co-deletion also has a role as an important positive prognostic ODG biomarker. Retrospective and prospective studies showed that ODG patients with 1p/19q co-deletion treated with standard therapy had significantly better survival outcomes than patients without 1p/19q co-deletion [74,75].

The 1p/19q co-deletion also acts as an important predictive biomarker for patients with ODG, especially AODG, in relation to combined treatment with RT plus chemotherapy. As early as 1998 it was found that patients with AODG positive for 1p/19q co-deletion are more sensitive to chemotherapeutic regimen containing the combination of procarbazine, lomustine and vincristine (PCV regime) [76]. The evidence-based proof of the significantly longer survival in patients with oligodendrogliomas and 1p/19q co-deletion treated with combined chemotherapy and radiotherapy did not exist for a long time. However, the long-term follow-up of two important phase III randomized clinical trials that incorporated 1p/19q co-deletion analyses (RTOG 9402 and EORTC 26951) evaluating RT and PCV regime in patients suffering from AO brought substantial results and led to a paradigm shift of the AODG treatment [77,78].

The RTOG study 9402 randomized 291 anaplastic oligodendroglial tumors (anaplastic oligodendrogliomas and oligoastrocytomas) into two treatment arms: PCV with follow up RT, and RT-alone. In the EORTC 26951 study, 368 patients with anaplastic oligodendroglial tumors (anaplastic oligodendrogliomas and oligoastrocytomas) were randomized into two arms: RT-alone and RT followed by PCV chemotherapy. The 1p/19q status was determined through fluorescent in situ hybridization in both studies. In RTOG 9402 study, 1p/19q co-deletion was found in 46% of the patients. Over the course of the study, 80% of the patients randomized for radiotherapy subsequently received PCV therapy due to the progression of the disease. After a minimum three-year follow-up in 2006, the median PFS was different for the RT plus PCV arm compared with the RT alone arm (2.6 vs. 1.7 years, $P = 0.004$). However, the median OS was similar in both study arms (4.9 vs. 4.7 years, $P = 0.26$). The OS in both treatment arms was not significantly different based on the presence of 1p/19q co-deletion, therefore the positive predictive effect of this biomarker in relation to PCV chemotherapy was not proven [79].

Similar results were observed from EORTC 26951 study in 2006 after an average five-year follow up. 25% of patients had tumors positive for 1p/19q co-deletion. The median PFS was different for the RT plus PCV arm compared with the RT alone arm (23 vs. 13.2 months, P = 0.0018). However the medial OS was similar in both study arms (40.3 vs. 30.6 months, P = 0.23) [80]. Patients with 1p/19q co-deletion had longer OS than patients without co-deletion, irrespective of the therapy arm. The results of both studies were considered rather negative in 2006. They did not prove the significance of 1p/19q co-deletion as a predictive biomarker in relation to chemotherapy, but rather showed the significance of 1p/19q co-deletion as a prognostic biomarker.

However, the decisive results came in 2013 following the long-term patient monitoring when the positive effect of combined oncological treatment (RT plus PCV) for anaplastic oligodendroglial tumors was proven. In the RTOG 9402 study, the median OS in patients without 1p/19q co-deletion remained similar to the results in 2006 in both groups receiving RT plus PCV and RT alone (2.6 vs. 2.7 years, P = 0.39) [77]. On the contrary, patients with 1p/19q co-deletion had significantly longer median OS in the RT plus PCV arm than in the RT alone arm (14.7 vs. 7.3 years respectively, P = 0.03). In multivariate analysis including co-deletion status, the OS for all patients was prolonged by RT plus PCV treatment (HR = 0.67; CI 0.50 to 0.91; P = .01). Likewise in the EORTC 26951 trial after more than 10 years' follow up, the OS of patients without 1p/19q co-deletion in tumor tissue was similar in the groups receiving RT plus PCV and RT alone (25 vs. 21 months, P = 0.19) [78]. However, the median OS was not reached for patients with co-deletion in the RT plus PCV arm, whereas it was just 9.3 years in patients primarily receiving only RT.

The benefit in OS resulting from combined oncological treatment (RT plus PCV) in patients with 1p/19q co-deletion positive tumors was present in both clinical studies, irrespective of which type of therapy was started first. Even in patients who, due to the occurrence of adverse effects to therapy, received lower doses of PCV than planned. These results led to an important paradigm shift in the treatment algorithm of patients with AODG tumors positive for 1p/19q co-deletion. Nevertheless, the positive effects of combined treatment is negatively impacted by the adverse effects such as late radiotherapy toxicity (post-radiation necrosis, dementia) or toxic effects of PCV chemotherapy [81,82]. It is necessary to carefully monitor patients and detect the toxic effects of the treatment as early as possible.

Another important clinical question is the administration of combined oncological treatment in patients with anaplastic oligodendroglial tumors that do not have 1p/19q co-deletion. The results from the RTOG 9402 and EORTC 26951 studies showed that RT plus PCV treatment had a positive effect on PFS even among patients without 1p/19q co-deletion. To answer this question the phase III CATNON study randomized patients with anaplastic gliomas without 1p/19q co-deletion to the RT alone treatment or RT plus temozolomide in three different regimens (RT with concurrent daily 75 mg/m2 temozolomide, RT followed with 12 cycles of 150-200 mg/m2 adjuvant temozolomide, and RT with both concurrent temozolomide and 12 cycles of adjuvant temozolomide). The primary endpoint was OS. Recent interim analysis

showed the OS benefit for patients in the temozolomide arms by comparison with RT alone arm (HR 0.645; CI 0.450 - 0.926, P = 0.0014) [83]. The 5-year OS rate was 56% when temozolomide was added to RT compared with 44% survival rate in patients treated with RT alone. The analysis of another glioma biomarker MGMT promoter methylation showed that patients with tumors positive for this biomarker had the OS advantage (HR 0.54; CI 0.38 - 0.77, P = 0.001). However, MGMT promoter methylation did not predict improved outcome with adjuvant temozolomide as was previously determined in GBM.

To evaluate the effect of temozolomide on treatment of AODG patients with 1p/19q co-deletion, the CODEL study (NCT00887146) was opened with three parallel arms: RT plus temozolomide, RT alone, and temozolomide alone. Based on the results of RTOG 9402 and EORTC 26951 trials, the RT-alone arm was abolished and the study is continuing in a two-arm design comparing the RT plus temozolomide with RT plus PCV regimes. The final results are planned up to 2018 that should give definitive answer for the best therapeutic strategies in patients with 1p/19q co-deletion positive anaplastic oligodendroglial tumors.

The 1p/19q co-deletion status is currently recommended to be determined in all patients with AODG [61,84]. The PCV chemotherapeutic regimen in combination with RT should be implemented for all patients with AODG positive for 1p/19q co-deletion. The analysis of molecular genetics in ODG is now recommended as an important part of the management of these tumors and together with the novel chemotherapeutic regimes means a paradigm shift in current clinical practice in neurooncology, which demonstrates another example of the integration of the personalized medicine principles and molecular biomarkers into the management of glioma patients.

1.4 Low grade gliomas

Low grade gliomas (LGG) form a heterogeneous group of neuroepithelial tumors of the CNS. LGG primarily consist of astrocytomas, oligodendrogliomas, oligoastrocytomas and a rare group of mixed glioneural tumors. LGG are histologically characterized by hypercellularity, nuclear atypia, pleomorphism and the lack of significant mitotic activity [17,85]. These tumors also have lower proliferative index and don´t comprise necrosis and vascular proliferation as gliomas of higher degrees of malignancy.

LGG occur mainly at a younger age with a maximum between the third and fourth decade [86]. The clinical manifestations are mostly epileptic seizures (80%), less frequently changes in cognition, behavior, focal neurological symptoms or headaches. Neurological symptoms significantly impair patient´s quality of life. LGG may also be asymptomatic with an incidental diagnosis with imaging methods indicated for another reason. They grow infiltrative and often affect eloquent areas of the brain parenchyma. Although LGG are considered relatively benign tumors they progress gradually to the higher grade and the median OS of patients after diagnosis is only 7.5 years [86,87]. Therefore an intensive LGG research is needed in order to optimize the clinical management and improve the quality of life and prolong survival of patients.

1.4.1 Prognostic and predictive biomarkers of low grade gliomas

Also in patients with LGG both the IDH1/2 mutations as well as 1p/19q co-deletion are the most important molecular aberrations in relation to clinical practice. IDH1 is mutated in high portion of diffuse astrocytomas (70-80%) and grade II oligodendrogliomas (up to 80%). IDH2 mutations are rare, occurring in 1-2% of diffuse astrocytomas and in 4.5% of grade II oligodendrogliomas [35,88,89]. Mutations in IDH1/2 detected in tumor tissue significantly correlate with better prognosis of patients with gliomas across all grades of malignancy including LGG [30,36–38,90].

The 1p/19q co-deletion was detected in 80-90% of low grade ODG and up to 10% of low-grade astrocytomas [65,91]. The recent meta-analysis showed prognostic and predictive significance of this biomarker in patients with gliomas [92]. The data from 28 studies were analyzed including 3408 patients with glial tumors of which 898 (26.3%) patients had confirmed diagnosis of LGG. Compared with patients with wild-type tumors, co-deletion of 1p and 19q was associated with a better PFS (HR = 0.63; CI 0.52-0.76) and OS (HR = 0.43; CI 0.35-0.53) irrespective of the grades and subtypes of gliomas. 1p/19q co-deletion was also demonstrated to be a positive predictive biomarker of responses to combined RT and chemotherapy (PCV regime) in patients with anaplastic oligodendroglioma and anaplastic oligoastrocytoma (grade III tumors) as was discussed in detail in the previous section. However, similar relation of this biomarker to treatment response in patients with LGG has not been confirmed yet. Thus 1p/19q co-deletion is the strong positive prognostic biomarker in patients with glial tumors including LGG.

The interrelations of individual LGG molecular genetic biomarkers seems to be more important for the clinical practice. Recently it has been shown that there exist at least three genetically as well prognostically heterogeneous groups of gliomas (having significant homogeneity within the groups) that can be distinguished by the presence of IDH1/2 mutations, 1p/19q co-deletion and mutual combination of these biomarkers in the tumor tissue [14]. The international consortium TCGA conducted an extensive multi-platform analyses of 293 patients with grade II and III gliomas. Data processing by Cluster of Clusters analysis and OncoSign integrated methods revealed three genetically distinct categories of gliomas. These categories strongly correlated with tumor subtypes determined based on the presence of IDH1/2 mutations, 1p/19q co-deletion and their combinations, but only weakly correlated with the histological type of tumors (R = 0.79 vs. R=0.19, respectively).

Gliomas in the first group were characterized by the presence of both IDH1/2 mutations and 1p/19q co-deletion. Activating mutations in the telomerase reverse transcriptase (TERT) gene promoter region, also identified in primary GBM, occurred in 96% of tumors classified into this group [14,93]. Other frequent aberrations identified in this glioma group were activating mutations in PI3K (20%), or inactivating mutations in tumor suppressor genes CIC (62%) and FUBP1 (29%) that were identified previously in 1p/19q co-deleted ODG [67]. This group mostly comprised of gliomas with oligodendroglial component (82% of oligodendrogliomas

and 16% of oligoastrocytomas). The patients exhibited the best prognosis with the longest median OS of 8 years. It is necessary to emphasize that in this group of patients with the most favorable prognosis, there were 43% of patients with grade III gliomas who should have a significantly worse prognosis if classified by the histopathological criteria alone without the use of molecular genetic biomarkers (especially IDH 1/2 mutations and 1p/19q co-deletion).

The second group included patients with gliomas positive for IDH1/2 mutations, but without the presence of 1p/19q co-deletion [14]. Moreover, 94% of the tumors had inactivating mutations in the tumor suppressor gene p53 and 86% in alpha - thalassemia/mental retardation syndrome (ATRX) gene. Tumors in this group comprised various gliomas without a clear predominance in the histological type and patients in this category had worse prognosis with shorter median OS of 6.3 years.

The last group comprised gliomas without the presence of IDH1/2 mutations, so-called IDH 1/2 wild-type tumors [14]. None of these tumors had 1p/19q co-deletion. Molecular genetic profile and biological behavior of these tumors were considerably closer to the primary GBM. Likewise the survival of patients with a median OS of just 1.7 years was similar to GBM. More than a half of these tumors were astrocytoma (56%). It is necessary to emphasize that almost one quarter of patients (24%) had histopathologic diagnosis of grade II gliomas that should expect a much better prognosis. Therefore the molecular genetic biomarkers incorporated into the classification of CNS gliomas provide an additional information to simple histopathological diagnosis that could improve the clinical care of patients with these tumors.

However, TCGA study was not the only one that tried to subdivide gliomas including LGG into prognostically different subcategories using several molecular genetic biomarkers and their combinations. The research group from Mayo Clinic/University of California San Francisco analyzed 1,087 patients with gliomas (grades II-IV) and defined five distinct subgroups of tumors according to the combination of three molecular genetic biomarkers (IDH1/2 mutations, 1p/19q co-deletion and mutations in TERT promoter region) [94]. Patients with grade II and III tumors had significant differences in median OS among the groups, which was not the case for GBM. The worst prognosis among patients with grade II and III gliomas had TERT positive and IDH and 1p/19q-negative tumors, where the OS was similar with GBM patients. On the contrary, the best prognosis was observed in the group of patients with IDH and TERT positive tumors.

There are also other studies trying to classify gliomas into different subgroups according to combinations of various biomarkers. For example Japanese research group subdivided 332 grade II and III gliomas using the IDH1/2 mutations and 1p/19q co-deletion [95], or German study of 405 adult patients with gliomas which analyzed IDH1 mutations 1p/19q co-deletion and ATRX expression [96] and others [97].

2 Thesis objectives and hypotheses

The fundamental aim of this thesis was to obtain and discuss new knowledge on the molecular genetics and biological behavior of the most common CNS tumors - gliomas in relation to their clinical management. Molecular genetic biomarkers should be used to more accurately determine patients´ prognosis or to predict better the treatment efficacy and outcome.

The practical part of this thesis contains two studies dealing with the important molecular genetic biomarkers in patients with two types of CNS gliomas. The experimentally obtained data were statistically analyzed and discussed in relation to clinical characteristics and outcome of patients.

In the first study the occurrence of the biomarker IDH1 R132H mutation was examined in the tumor tissue from patients with glioblastoma multiforme who were treated with the standard protocol and subsequently monitored in the Faculty Hospital in Pilsen. The mutation was assessed by the quantitative real time polymerase chain reaction (PCR) method and the results were correlated with the clinical characteristics of GBM patients.

In the second study the chromosomal aberration 1p/19q co-deletion was observed in patients with anaplastic oligodendroglioma who were treated with the combined radiotherapy and chemotherapy (procarbazine, lomustine and vincristine - PCV regime) and subsequently monitored in the Faculty Hospital in Pilsen. The 1p/19q co-deletion was assessed by the fluorescence in situ hybridization (FISH) method and the results were correlated with the patients´ clinical characteristics.

Hypotheses:

1. The IDH1 R132H mutation will be observed in a subset of patients with glioblastoma multiforme, predominantly with secondary glioblastomas.
2. Patients with the IDH1 R132H mutation detected in tumor tissue will have a better prognosis and longer survival than patients with wild-type tumors. Therefore this mutation will serve as a positive prognostic biomarker for patients with glioblastoma multiforme.
3. Patients with anaplastic oligodendroglioma positive for the chromosomal aberration 1p/19q co-deletion will have a better prognosis with longer survival than patients with wild-type tumors. Therefore this mutation will serve as a positive prognostic biomarker for patients with anaplastic oligodendroglioma.
4. Patients with anaplastic oligodendroglioma positive for the chromosomal aberration 1p/19q co-deletion will have a better response to the combined RT plus PCV treatment with longer survival than to the RT alone. Therefore this mutation will serve as a positive predictive biomarker for the treatment with combined RT plus PCV regimen in this subset of patients.

3 Materials and methods

3.1 The assessment of IDH1 R132H mutation in tumor tissue from patients with glioblastoma multiforme

3.1.1 Study participants

The study enrolled 44 patients diagnosed with WHO grade IV astrocytoma - glioblastoma multiforme (GBM) in the Faculty Hospital in Pilsen, who had available complete clinical data as well as tissue samples of the tumors. There were 22 males and 22 females among the patients. The median age of the entire study group was 64.3 years. Patients were treated (total or subtotal tumor resection or tumor biopsy, radiotherapy, chemotherapy with temozolomide) in the Faculty Hospital in Pilsen between the years 2009 and 2011. The formalin-fixed, paraffin-embedded (FFPE) tissue samples were obtained from the archives of the Sikl´s institute of pathology, Faculty of Medicine in Pilsen and Faculty Hospital in Pilsen. The complete clinical data were obtained from the medical information system of the Faculty Hospital in Pilsen. The study protocol was approved by the ethics committee. Written informed consent was obtained from all participants in this study. The description of the entire study group with important patients´ clinical characteristics is given in Table 1.

Table 1 - The study group demographics and clinical characteristics

Patients characteristics	
Sex	
Male-to-female ratio	1
Male	22
Female	22
Age, years	
Median	64.3
Range	35 - 87
KPS	
Median	77.5
Range	30 - 100
Postoperative treatment	
RT (±CHT)	29
CHT alone	1
None	15
Abbreviations KPS, Karnofsky performance score; RT, radiotherapy; CHT, chemotherapy	

3.1.2 DNA isolation

DNA was extracted from 10 μm FFPE sections following macrodissection of tumor tissue and normal brain tissue using the QIAamp® DNA FFPE Tissue kit (Qiagen, Hilden, Germany). The 10 μm sections corresponded to the representative hematoxylin eosin slide with tumor tissue verified by pathologist.

3.1.3 Mutation detection

For detection of mutant allele IDH1 c.395G>A (p.R132H, COSMIC ID 28746) the TaqMan® Mutation Detection Assays (Assay Name: IDH1 28746 mu and IDH1 rf) was used with the TaqMan® Mutation Detection IPC Reagent Kit (Life Technologies, Carlsbad, California, U.S.). Mutant allele detection was performed in the laboratory of the department of biology at the Faculty of Medicine in Pilsen according to the recommended procedure and reaction conditions found in the manual. For the amplification the Stratagene Mx3000P real-time PCR system instrument was used (Agilent Technologies, Inc., Santa Clara, California, U.S.). Detection of mutant alleles was performed in duplicates in a reaction volume of 20 μl. Likewise detection of reference gene. Detection of samples with high values of cycle threshold (Ct) of the reference gene were repeated. The analyses of the normal brain tissue samples were done for detection of cut-off amplification curve before analyzes of tumor samples. No amplifications of mutant allele were present in normal brain tissue samples. On the base of these results and the shape of amplification curve of positive tumor samples the 25 deltaCt cut-off value was determined.

3.1.4 Statistical analysis

Overall survival (OS) was defined as the time between the diagnosis and death or last follow up. Progression-free survival (PFS) was defined as the time between the diagnosis and recurrence or last follow up. Kaplan-Meier survival curves were plotted and the survival distributions were compared with the use of the Wilcoxon test. Reported P values are two-sided. P values of less than 0.05 were considered to indicate statistical significance. All statistical analyzes were performed in software SPSS Statistics (IBM, Armonk, New York U.S.).

3.2 The examination of chromosomal aberration 1p/19q co-deletion in tumor tissue from patients with anaplastic oligodendroglioma

3.2.1 Study participants

The study enrolled 23 patients diagnosed with WHO grade III oligodendroglioma - anaplastic oligodendroglioma (AODG) in the Faculty Hospital in Pilsen, who had available complete clinical data as well as tissue samples of the tumors. There were 13 males and 10 females among the patients. The median age of the entire study group was 55.4 years. Ten patients were treated with the neurosurgery followed by radiotherapy (RT) plus chemotherapy (procarbazine,

lomustine and vincristine - PCV regime), thirteen patients were treated with the neurosurgery followed by RT alone. The formalin-fixed, paraffin-embedded (FFPE) tissue samples were obtained from the archives of the Sikl´s institute of pathology, Faculty of Medicine in Pilsen and Faculty Hospital in Pilsen. The complete clinical data were obtained from the medical information system of the Faculty Hospital in Pilsen. The study protocol was approved by the ethics committee. Written informed consent was obtained from all participants in this study. The description of the entire study group with important patients´ clinical characteristics is given in Table 2.

Table 2 - The study group demographics and clinical characteristics

Patients Characteristics	
Sex	
Male-to-female ratio	1.3
Male	13
Female	10
Age, years	
Median	55.4
Range	25 - 72
mRS	
Median	3.35
Range	0 - 6
Postoperative treatment	
RT alone	10
RT + CHT (PCV)	13
Abbreviations mRS, modified Rankin Scale; RT, Radiotherapy; CHT, Chemotherapy	

3.2.2 Mutation detection

Deletion of 1p and 19q in FFPE tumor tissue samples were primarily determined by the fluorescence in situ hybridization (FISH) with locus-specific probes (10µl mixture) LSI 1p36/1q25 or LSI 19q13/19p13 (Vysis/Abbott, Downers Grove, IL, USA) in the laboratory of the Sikl´s institute of pathology, Faculty of Medicine in Pilsen and Faculty Hospital in Pilsen. The positive result for the 1p/19q co-deletion was assessed as the loss of 1p36 or 19q13 signal in more than 50% of nuclei (±3 SD in negative control).

3.2.3 Statistical analysis

Overall survival (OS) was defined as the time between the diagnosis and death or last follow up. Progression-free survival (PFS) was defined as the time between the diagnosis and recurrence or last follow up. Kaplan-Meier survival curves were plotted and the survival distributions were compared with the use of the Wilcoxon test. Reported P values are two-sided. P values of less than 0.05 were considered to indicate statistical significance. All statistical analyzes were performed in software SPSS Statistics (IBM, Armonk, New York U.S.).

4 Results and discussion

4.1 The assessment of IDH1 R132H mutation in tumor tissue from patients with glioblastoma multiforme

4.1.1 Results

The mutation IDH1 R132H was observed in 20 from 44 GBM patients´ tumor samples. Therefore the IDH1 mutation was identified in more than 45.4% of glioblastomas. The separation of primary and secondary glioblastomas (GBM that progressed from the low-grade glioma) was done on the basis of clinical information, where possible. The IDH1 R132H mutation occurred in 4 from 26 primary GBM (15.3%). Whereas the majority 16 from 18 (89.9%) of secondary GBM was mutated (Table 3).

Table 3 - The representation of IDH1 R132H mutation in primary versus secondary glioblastomas.

Glioblastoma type	**Primary GBM (n=26)**	**Secondary GBM (n=18)**
Mutation status	**[n]**	**[n]**
IDH1 R132H	4 (15.3 %)	16 (89.9 %)
IDH1 wild-type	22 (84.7 %)	2 (11.1 %)

The significant relations between the IDH1 mutation status and clinical characteristics such as PFS and OS were also observed (Table 4). Patients with IDH1 R132H mutation had longer PFS than patients with wild-type IDH1 (136 vs. 51 days, $P < 0.021$, Wilcoxon test) (Figure 1). Significantly longer OS was observed as well for patients with IDH1 R132H mutation than for patients without the mutation (270 vs. 130 days, $P < 0.024$, Wilcoxon test) (Figure 2).

Table 4 - Results for progression-free survival and overall survival differences in patients with GBM in the relation to IDH1 mutation status.

Glioblastoma patients results	**n**	**Median [days] (95% Cl)**	**P (Wilcoxon)**
Overall Survival (OS)			
IDH1 R132H	20	270 (139-400)	0.024
IDH1 wild-type	24	130 (87-172)	
Progression-free Survival (PFS)			
IDH1 R132H	20	136 (22-249)	0.021
IDH1 wild-type	24	51 (19-82)	

Figure 1 - Progression-free survival of patients with glioblastoma with (red line) or without (blue line) IDH1 R132H mutation (P = 0.021, Wilcoxon test).

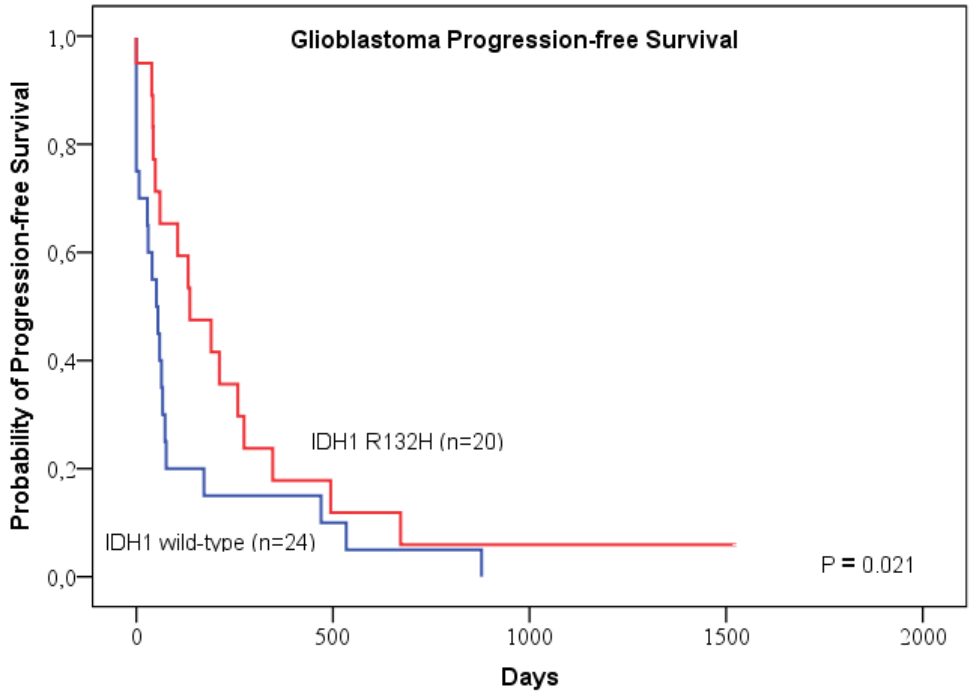
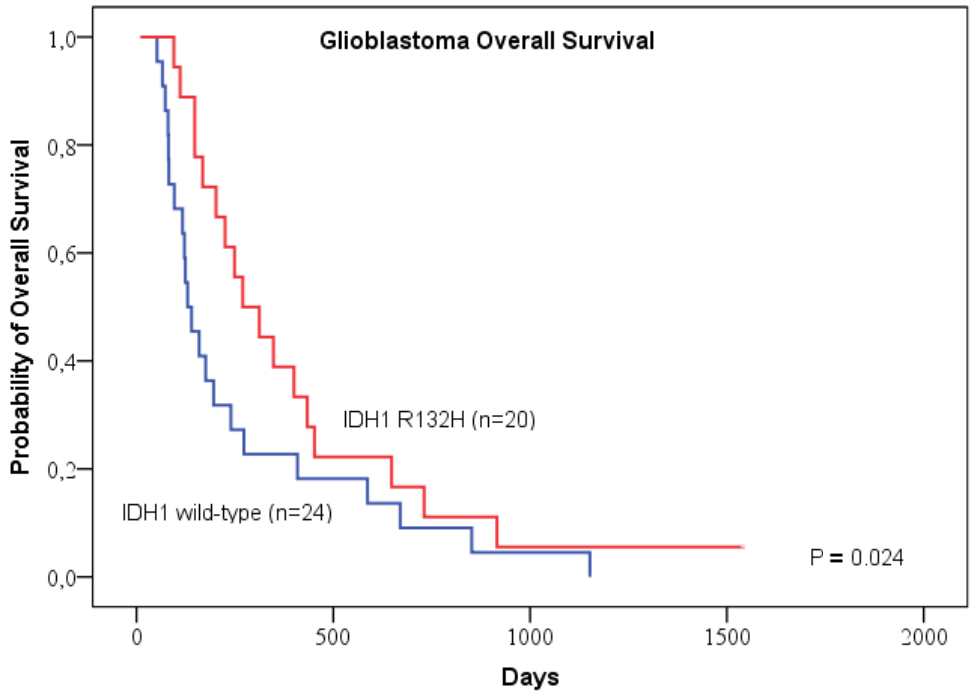


Figure 2 - Overall survival of patients with glioblastoma with (red line) or without (blue line) IDH1 R132H mutation (P = 0.024, Wilcoxon test).

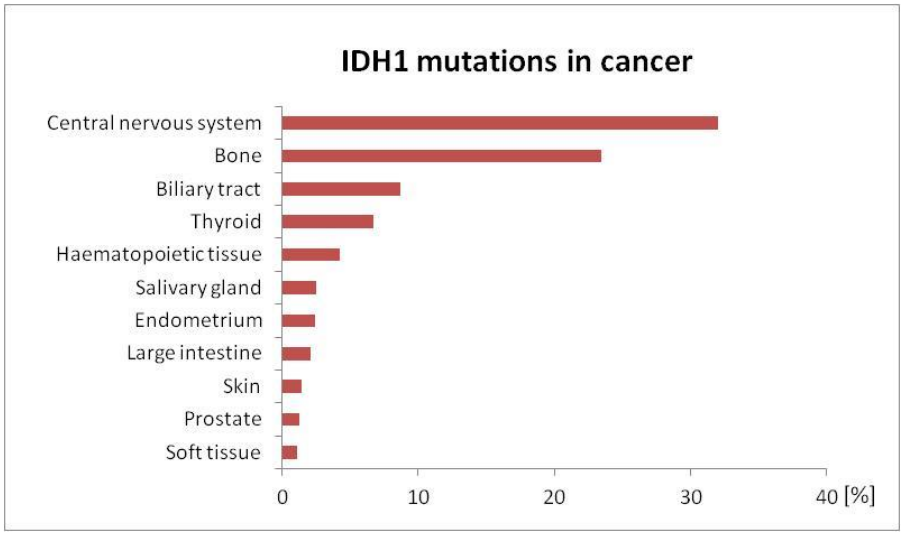


4.1.2 Discussion

Recurrent IDH1/2 mutations and their role in oncogenesis and tumor progression were systematically described first in GBM [16]. This observation led to new insights into the biology of cancer including GBM. Alterations in cancer cell metabolism are now well accepted as one of the principal hallmarks of the process of cancerogenesis and tumor progression [98].

Mutations in IDH1 were also identified in other tumor types. The data from the Sanger Institute Cancer Genome Project - Catalogue of Somatic Mutations in Cancer (COSMIC) revealed the presence of IDH1 mutations in more than 32% of central nervous system tumors, 23% of bone tumors, 8% of biliary tract tumors, 6% of thyroid cancer and many other tumor types [89] (Figure 3). In the primary brain tumors, IDH1 mutations are presented mostly in diffuse astrocytomas, anaplastic astrocytomas, glioblastomas or oligodendrogliomas as was discussed in detail in the theoretical part of this thesis [89]. The R132H amino acid substitution is the most common form of IDH1 mutations with the prevalence of 90% among IDH1-mutant tumors. Less common mutants such as R132C, R132G, R132S, and R132L are also known [29,30].

Figure 3 - The representation of IDH1 mutations in various types of cancer [89].



The fundamental shift in the understanding of mutated IDH and its role in cancer progression came with the observation of the neomorphic function of the mutated enzyme. Instead of the production of alpha-ketoglutarate, mutated IDH produced novel onco-metabolite 2-hydroxyglutarate (2-HG) that were highly accumulated in the cancer cells [31]. It was subsequently discovered that 2-HG inhibits the functions of the alpha-ketoglutarate dependent superfamily of dioxygenases. These enzymes have diverse cellular functions including, but not limited to histone demethylation and demethylation of hypermethylated DNA [32,33]. Moreover, IDH mutations and 2-HG production were identified to be sufficient steps in the process leading to glioma hypermethylator phenotype. That observation was important for understanding of glioma oncogenesis and highlighted the interplay between genetic and epigenetic changes in human cancers [60,99].

Mutations in IDH are important also for their clinical consequences as was discussed in more detail in the theoretical part of this thesis. Recent studies revealed the important role of mutated IDH in the assessment of astrocytoma patient prognosis. Across several studies, the better prognosis for patients with IDH 1/2 mutated GBMs were observed with the longer OS of 3.8 vs. 1.1 years, 2.6 vs. 1.3 years, 2.3 vs. 1.2 years and 3 vs. 1 year [16,30,36,37]. Even more significant differences in OS were found in patients with anaplastic astrocytomas; 5.4 vs. 1.7 years, 6.8 vs. 1.6 years and 7 vs. 2 years [30,36,37] as well as diffuse astrocytoma 12.6 vs. 5.5 years [36]. These data highlighted the major impact of IDH1/2 mutation status on glioma patient survival and support the incorporation of this biomarker into the clinical management. Mutations in IDH1/2 and production of onco-metabolite 2-HG could be used as well for therapeutic intervention in the near future [100].

The results from this study also support the IDH1 R132H mutation to be the strong prognostic biomarker for patients with GBM. However, the differences in median PFS and OS between patients with IDH1 mutated and IDH1 wild-type tumors were not as big as in other studies. The

reason for the relatively small differences in median survival between both groups could be the heterogeneity of the treatment protocols. The standard treatment with neurosurgery and concomitant chemo-radiotherapy with temozolomide was implemented in only 29 patients. 1 patient had radiotherapy alone and 15 patients were treated neither with radiotherapy nor with chemotherapy. The proportion of IDH1 mutated tumors was also higher than in other similar studies. The IDH1 mutations in glioblastomas were originally identified predominantly in secondary GBM that progressed from the low grade tumors [101]. The distinction between the primary and secondary GBM in this study was done on the basis of clinically relevant information from the patient history, although it was not possible absolutely exactly. Only 5 patients had previously assessed low-grade glioma (surgery in 2 cases, tumor biopsy in 3 cases). Other patients with tumor´s corresponding neurological symptomatology (epileptic seizures, focal neurological deficit) present at least 6 month before the final diagnosis were considered as likely secondary GBM. Moreover, the primary-like glioblastomas could be in fact secondary without the symptoms of low-grade tumors.

The recent study of mutations in the promoter of TERT gene has revealed the high incidence of these aberrations in a large portion of primary GBM (about 80%) [102]. In the further research the TERT promoter mutations will be used in addition to clinically relevant information for the separation of primary and secondary glioblastomas. The assessments of other IDH1 mutations as well as the analysis of IDH2 mutations are also planned together with their quantification using digital PCR methods (digital droplet PCR).

Despite the drawbacks of this study, IDH1 R132H mutation still served as a strong prognostic biomarker for the patients with GBM treated in the Faculty Hospital in Pilsen. The results from this work became a part of an important and recently published meta-analysis of 55 observational studies that confirmed improved both overall survival and progression-free survival in patients with gliomas positive for IDH1/2 mutations and helped with the incorporation of IDH1/2 mutations status into the updated 2016 WHO classification of CNS tumors [17,38].

4.2 The examination of chromosomal aberration 1p/19q co-deletion in tumor tissue from patients with anaplastic oligodendroglioma

4.2.1 Results

The biomarker 1p/19q co-deletion was identified in 12 out of 23 patients´ tumor samples (52.2%) (Table 5). Patients with tumors positive for co-deletion had a significantly longer median OS than patients without 1p/19q co-deletion (587 vs. 132 weeks, P = 0.012, Wilcoxon test) (Figure 4). There was also the trend for longer median PFS in patients with 1p/19q co-deleted tumors than in those without this biomarker (321 vs. 43 weeks, P = 0.075, Wilcoxon test) (Figure 5).

Table 5 - Progression-free survival and overall survival differences in patients with anaplastic oligodendroglioma in relation to 1p/19q co-deletion.

Anaplastic oligodendroglioma patients´ results	**n**	**Median [weeks] (95% CI)**	**P (Wilcoxon)**
Overall survival			
1p/19q co-deleted	12	587 (466 - 707)	0.012
1p/19q negative	11	132 (0 - 271)	
Progression-free survival			
1p/19q co-deleted	12	321 (21 - 620)	0.075
1p/19q co-deletion-negative	11	43 (0 - 150)	

Figure 4 - Overall survival of patients with anaplastic oligodendroglioma in relation to 1p/19q co-deletion status (P = 0.012, Wilcoxon test).

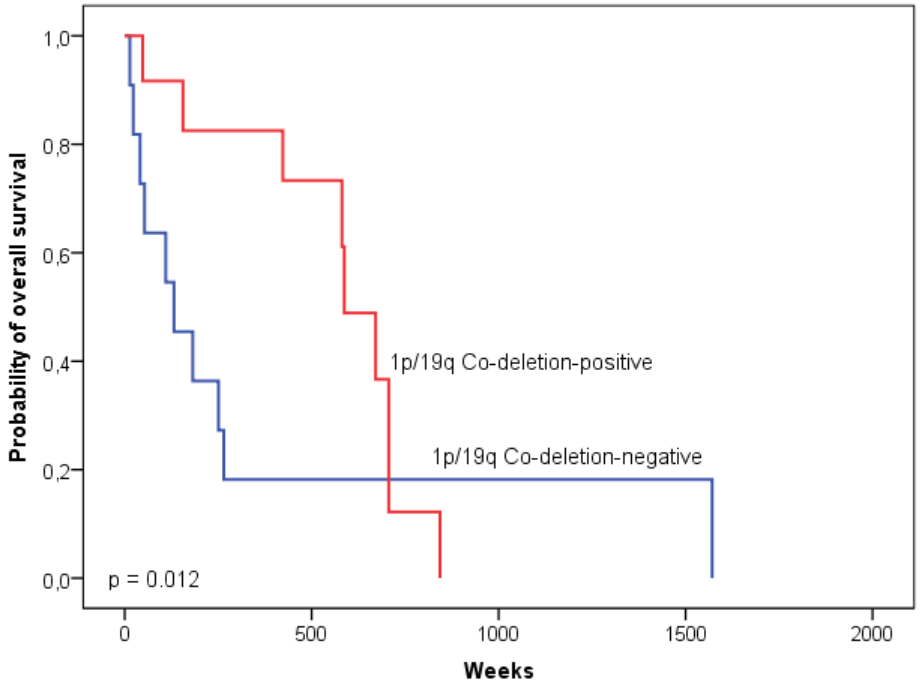
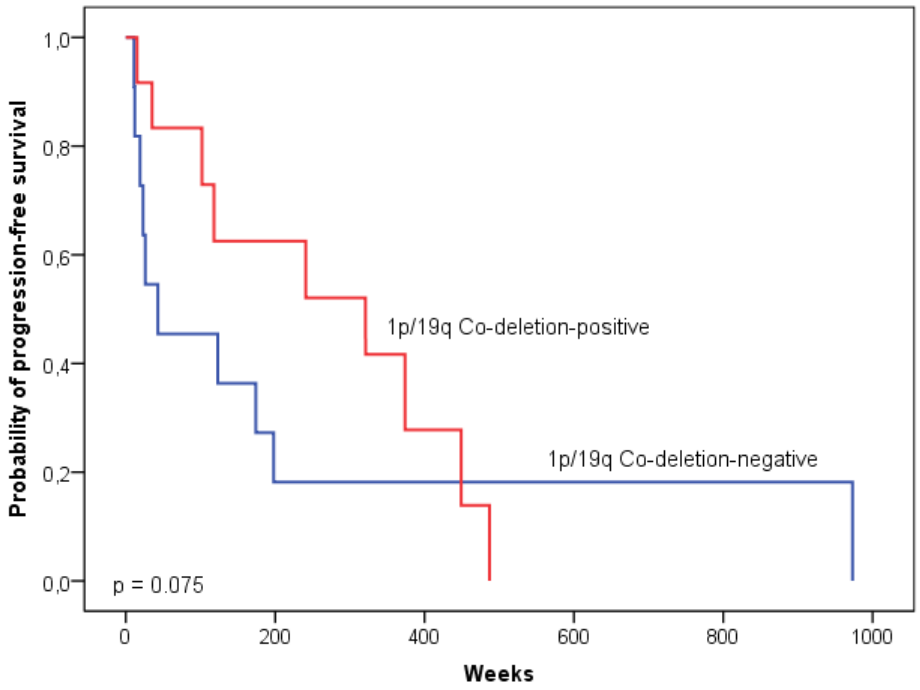


Figure 5 - Progression-free survival of patients with anaplastic oligodendroglioma in relation to 1p/19q co-deletion status (P = 0.075, Wilcoxon test).



In the subgroup of patients with 1p/19q co-deleted tumors (n=12), the median OS was significantly longer in those treated with neurosurgery plus RT and PCV (n=7) by comparison with patients that were treated with neurosurgery followed by RT alone (n=5) (706 vs. 423 weeks, P = 0.008, Wilcoxon test) (Table 6 and Figure 6). On the other hand, there was no significant difference in median PFS in the subgroup of patients treated with neurosurgery plus RT and PCV vs. those treated with neurosurgery plus RT alone (374 vs. 321 weeks, P = 0.626, Wilcoxon test) (Table 6 and Figure 7).

Table 6 - Progression-free and overall survival differences in patients with anaplastic oligodendroglioma treated with neurosurgery plus radiotherapy (NRT) or with neurosurgery plus radiotherapy and procarbazine, lomustine and vincristine (NRT-PCV) in relation to 1p/19q co-deletion.

1p/19q co-deletion status	**Median OS [weeks] (95% CI)**	**P (Wilcoxon)**	**Median PFS [weeks] (95% CI)**	**P (Wilcoxon)**
Co-deletion (n=12)				
NRT-PCV (n=7)	706 (675 - 736)	0.008	374 (129 - 618)	0.626
NRT (n=5)	423 (0 - 996)		321 (67 - 574)	
Without co-deletion (n=11)				
NRT-PCV (n=6)	182 (12 - 351)	0.223	43 (0 - 224)	0.523
NRT (n=5)	53 (0 - 117)		26 (10 - 41)	

Figure 6 - Overall survival of patients with anaplastic oligodendroglioma positive for 1p/19q co-deletion in relation to the treatment protocol [neurosurgery plus radiotherapy (RT) vs. neurosurgery plus RT and procarbazine, lomustine and vincristine (PCV)] (P = 0.008, Wilcoxon test).

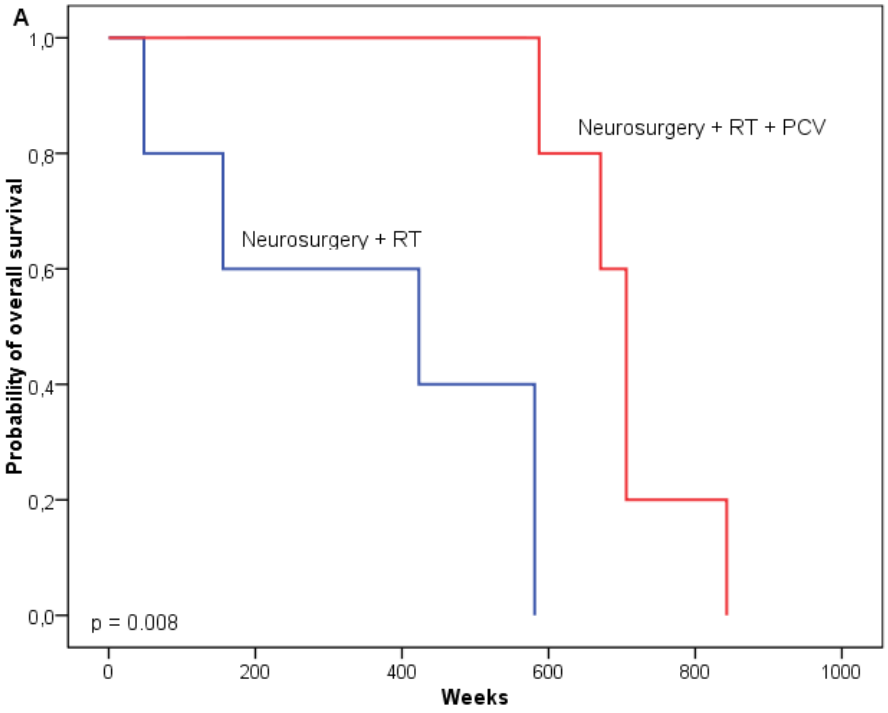
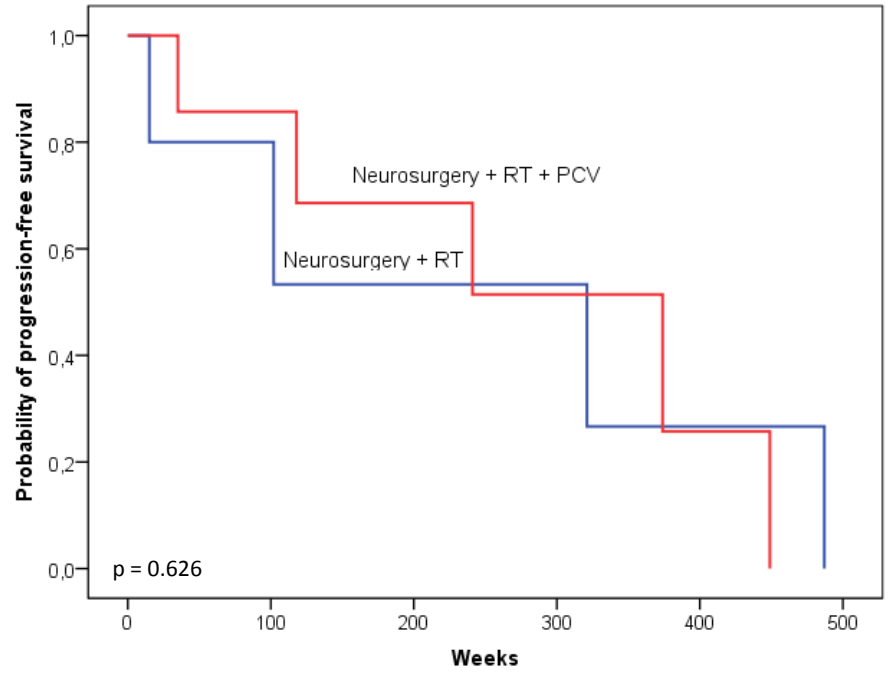


Figure 7 - Progression-free survival of patients with anaplastic oligodendroglioma positive for 1p/19q co-deletion in relation to the treatment protocol [neurosurgery plus radiotherapy (RT) vs. neurosurgery plus RT and procarbazine, lomustine and vincristine (PCV)] (P = 0.626, Wilcoxon test).



In contrast, in the subgroup of patients without 1p/19q co-deletion (n=11) the median OS was not significantly different in those treated with neurosurgery plus RT and PCV (n=6) by comparison with those treated with neurosurgery followed by RT alone (n=5) (182 vs. 53 weeks, P = 0.223, Wilcoxon test) (Table 6 and Figure 8). There was also no significant difference in the median PFS in this subgroup of patients (43 vs. 26 weeks, P = 0.523) (Table 6 and Figure 9).

Figure 8 - Overall survival of patients with anaplastic oligodendrogliomas without 1p/19q co-deletion in relation to the treatment protocol [neurosurgery plus radiotherapy (RT) vs. neurosurgery plus RT and procarbazine, lomustine and vincristine (PCV)] (P = 0.223, Wilcoxon test).

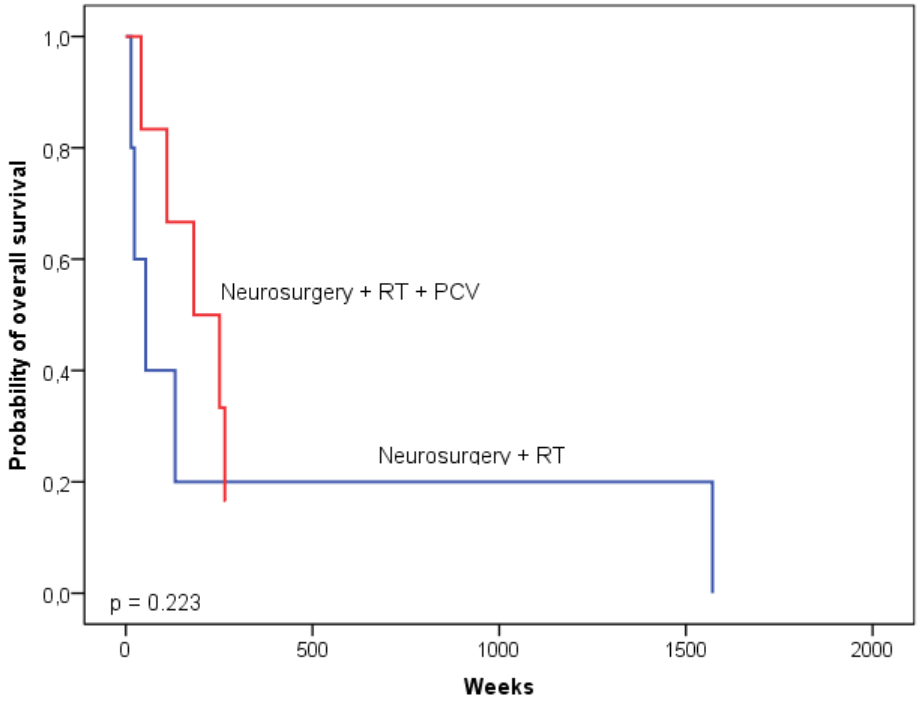
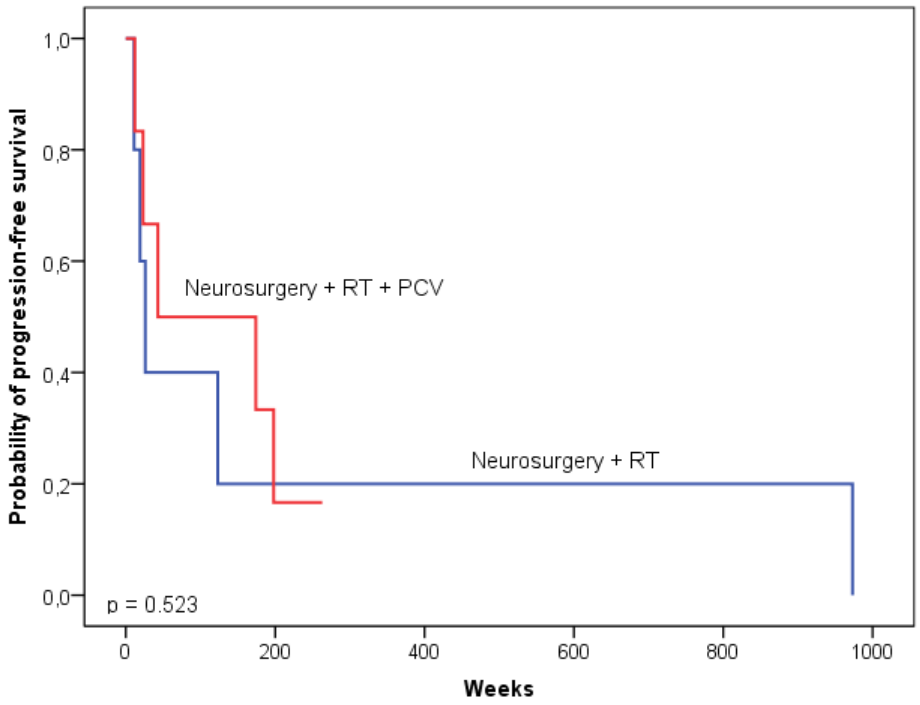


Figure 9 - Progression-free survival of patients with anaplastic oligodendrogliomas without 1p/19q co-deletion in relation to the treatment protocol [neurosurgery plus radiotherapy (RT) vs. neurosurgery plus RT and procarbazine, lomustine and vincristine (PCV)] (P = 0.523, Wilcoxon test).



4.2.2 Discussion

The molecular genetic characteristic of oligodendroglial tumors is the frequent co-deletion of chromosome 1p and 19q. This chromosomal aberration was identified in 1994 and became the first biomarker in neuro-oncology [62]. The mechanism of 1p/19q co-deletion is the unbalanced translocation t(1;19)(q10;p10) [63]. Recently, the mutations in two important tumor-suppressor genes, capicua homolog drosophila (CIC) located on 19q13.2, and far upstream element-binding protein (FUBP1) on the 1p chromosome, was discovered in the majority of oligodendrogliomas with 1p/19q co-deletion [66,67]. Mutations in these genes are probably involved in the formation and progression of oligodendrogliomas. However, their true significance in neoplastic diseases remains to be verified.

Co-deletion of 1p/19q appears almost exclusively in oligodendroglial tumors (80% to 90% of grade II oligodendrogliomas and 50% to 70% of AO) [64,65]. This chromosomal aberration can be used in clinical practice as an important diagnostic, prognostic, as well as predictive biomarker. From the diagnostic point of view, the presence of 1p/19q co-deletion supports the diagnosis of oligodendroglioma, especially in cases where the histological findings are not clear.

The 1p/19q co-deletion is also an important positive prognostic biomarker of the disease. Several studies found significantly better survival outcome for patients with oligodendroglioma with 1p/19q co-deletion than for those without [61,74,75,77–80].

Co-deletion of 1p/19q was found to have substantial clinical significance also as a strong predictive biomarker for patients with anaplastic oligodendroglial tumors. Its detection predicts longer survival of patients with the combined RT plus PCV treatment by comparison with RT alone that was recently shown by the long-term follow-up of two important phase III clinical trials RTOG 9402 and EORTC 26951 [77,78]. These trials produced substantial results and led to a paradigm shift in anaplastic oligodendroglioma treatment as was discussed in detail in the theoretical part of this thesis.

In this study, the 1p/19q co-deletion served as the strong prognostic biomarker for OS for all patients with anaplastic oligodendroglioma irrespective of the treatment regime. Moreover, the positive predictive value of 1p/19q co-deletion was demonstrated for the subgroup of patients treated with the combination of neurosurgery and RT plus PCV. These results are in concordance with the results from the recently published long-term follow up of two phase III clinical trials RTOG 9402 and EORTC 26951. The major weakness of this work remains the relatively small number of patients and the retrospective study design. The limited number of patients in this study is caused mainly by the rare incidence of anaplastic gliomas among cancer patients. The future research will expand the assessment of other molecular biomarkers in the patient cohort such as mutations in IDH1/2 or the PI3K signaling pathway and the correlation of these mutations with 1p/19q co-deletion and patients´ clinical characteristics and outcome.

5 Conclusions

The IDH1 R132H mutation was observed in the interestingly higher number of patients with glioblastoma multiforme that was previously published. On the other hand the majority of mutated tumors in the cohort were probably secondary glioblastomas. The prognostic value of the IDH1 R132H mutation was also observed. Patients with this mutation in the tumor tissue had significantly longer PFS as well as OS than patients with IDH1 wild-type tumors.

The presented results were included into the large recently published meta-analysis that confirmed positive prognostic effect of the IDH mutations on both overall survival and progression-free survival in patients with CNS gliomas. These findings helped with the incorporation of IDH mutations assessment into the updated 2016 WHO classification of CNS tumors.

The prognostic value of 1p/19q co-deletion in the cohort of patients with anaplastic oligodendroglioma was proved. The strong positive predictive value of this biomarker for OS was also shown for patients with co-deletion who were treated with neurosurgery and RT plus PCV by comparison with neurosurgery and RT alone. Patients with anaplastic oligodendrogliomas who have tumor positive for 1p/19q co-deletion should be treated intensively with combined RT and chemotherapy (PCV) regime.

The results of this thesis were also practically applied during the formation of the multidisciplinary neurooncology center, which was set up in the Faculty of Medicine in Pilsen and Faculty Hospital in Pilsen under the patronage of the neurooncology section of the Czech oncological society. The main objective of this center is to help to patients as well as treating physicians with the decision-making process during the whole treatment procedure.

The enormous advances in the molecular genetics of CNS tumors and especially gliomas that were made over the past decade bring completely new opportunities for the optimization of the treatment strategies for and individual patient with these diagnoses. The important molecular biomarkers were discovered and validated in the clinical studies. These biomarkers can be used in the clinic for more precise diagnosis, for the more accurate assessment of a patients´ prognosis, or for the better selection of therapy and prediction of therapeutic response.

Together with the implementation of the molecular genetics in the recently updated 2016 WHO classification of CNS tumors it will likely be necessary to integrate molecular biomarkers and personalized medicine principles into standard clinical care of patients suffering from neurological cancers.

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8 The publication activity of the applicant

Monographs and chapters in monographs

Tonar Z, Eberlova L, Polivka J, Daum O, Witter K, Kralickova A, Gregor T, Nedorost L, Kochova P, Rohan E, Kalusova K, Palek R, Skala M, Glanc D, Kralickova M, Liska V. Stereological methods for quantitative assessment of hepatic microcirculation. Current Microscopy Contributions to Advences in Science and Technology. Vol. 1. Microscopy Book Series - 2012 Edition. Formatex Research Center, Badajoz, Spain, pp. 737-748. ISBN 978-84-939843-6-6.

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9 The curriculum vitae of the applicant

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Highest Education

2007 University of West Bohemia in Pilsen, Faculty of Applied science, Master study in Applied Sciences, Physical Engineering, Physical Mathematical Simulations

2009 Czech Technical University in Prague, Masaryk Institute of Advanced Studies, Master study in Entrepreneurship and Commercial Engineering in Industry and Management

Occupation

2008 – now, Department of Computer Science and Engineering, Faculty of Applied Sciences, University of West Bohemia in Pilsen

2012 – now, Department of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University, also postgraduate study

2013 – now, Laboratory of Tumor Biology, Biomedical Center, Faculty of Medicine in Pilsen, Charles University

Membership

EPMA – YPS, European Association for Predictive, Preventive and Personalized Medicine - Section of Young Professionals – representative of the Czech Republic

European Association for Cancer Research

Czech Society for Oncology, Section of Neurooncology

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