Chemotherapy in Glioma

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Chapter 1
General introduction

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Walter Taal, Jacoline E.C. Bromberg and Martin J. van den Bent

ABSTRACT

The treatment of glial brain tumors begins with surgery, and standard adjuvant treatment at the end of the past millennium for high-grade glioma and high-risk low-grade glioma was radiotherapy and chemotherapy was given at recurrence. However, over the past 10 years much has changed regarding the role of chemotherapy in gliomas and it is now clear that chemotherapy has a role in the treatment of almost all newly diagnosed diffuse gliomas (WHO grade II–IV). This is the result of several prospective studies that showed survival benefit after combined chemoradiotherapy with temozolomide in glioblastoma (WHO grade IV) or after procarbazine, CCNU (lomustine) and vincristine chemotherapy in diffuse low-grade (WHO grade II) and anaplastic (WHO grade III) glioma. The current standard of treatment for diffuse gliomas is described in this overview and in addition some attention is given to targeted therapies.

INTRODUCTION

Every year around 1000-1200 patients with a primary brain tumor are diagnosed in the Netherlands. They include astrocytomas, oligodendrogliomas, and mixed oligo-astrocytomas (figure 1). These tumors arise from the supporting tissues of the brain, the glia, and are called glioma. Besides this classification into different types, these tumors are graded according to the presence of anaplastic features in low-grade (or WHO grade II) and high-grade tumors (either WHO grade III or anaplastic and WHO grade IV or glioblastoma). Grade I gliomas are well-demarcated gliomas, and if completely resectable can be cured. The grade II-IV gliomas are among the so-called diffuse gliomas for which a curative treatment is not available. Over the past 10 years much has changed regarding the place of chemotherapy in glial brain tumors. The purpose of this overview is to describe the current standard of treatment for gliomas and in addition some attention is given to targeted therapies, e.g. angiogenesis inhibitors. Currently there is a lot of attention for this category, but the role for this class of agents in diffuse gliomas has by no means been defined.

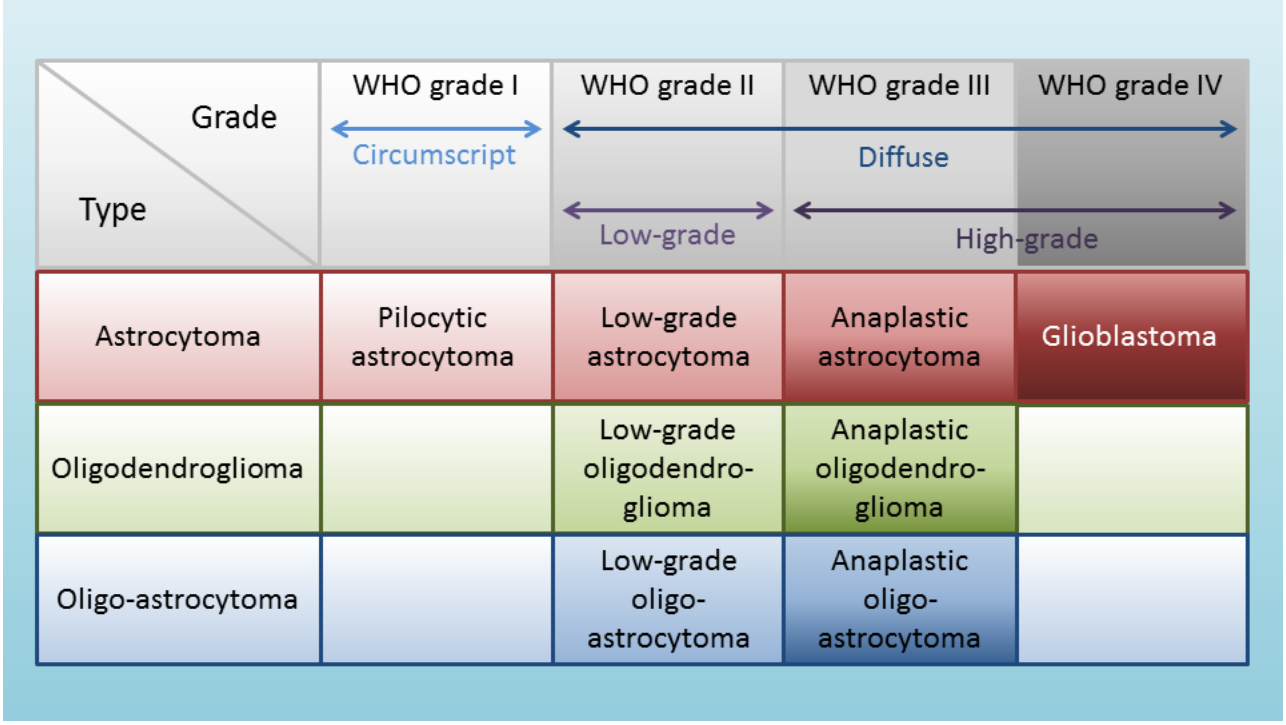

Figure 1. Classification of glioma in types and WHO grades.

PATHOPHYSIOLOGY

Most brain tumors occur sporadically and no obvious environmental causes of brain tumors are known. Occasionally gliomas arise as a part of rare genetic syndromes (e.g. Neurofibromatosis type I, Li-Fraumeni syndrome and Turcot syndrome type I).(1) The various types of diffuse glioma are characterized by different molecular abnormalities, such as loss of chromosome 10, EGFR amplification, PTEN mutations and TERT mutations in glioblastoma, combined 1p/19q loss and ATRX mutations in oligodendroglial tumors, TP53 mutations and TERT mutations in grade II and III astrocytic tumors and mutations in isocitrate dehydrogenase (IDH) genes in grade II and III diffuse glioma and secondary glioblastoma (figure 2).(2, 3, 4) Epigenetic factors such as methylation of the MGMT gene and other genes are involved in the pathogenesis, but their role is far from understood.

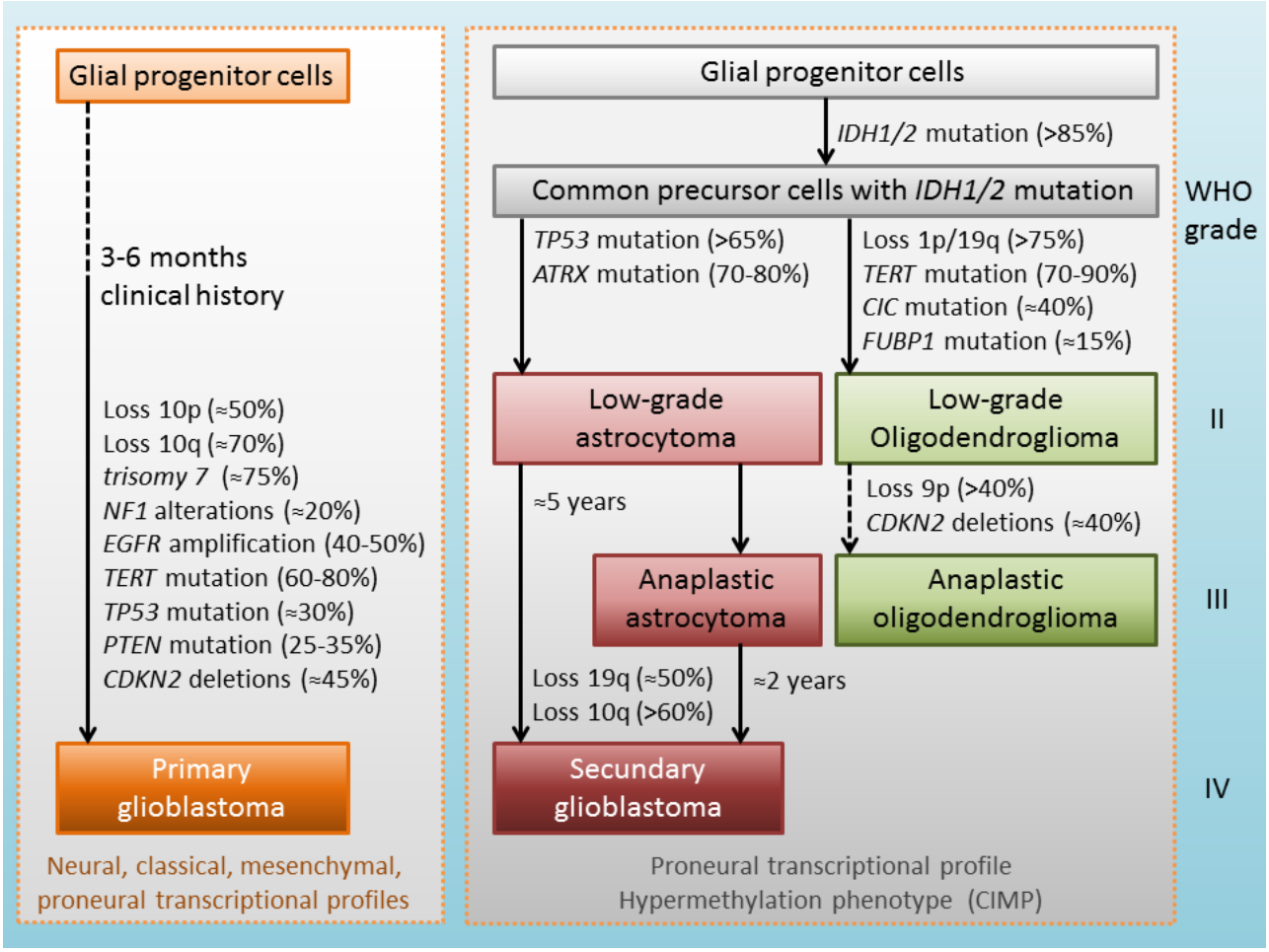

Figure 2. Possible genetic pathways in glioma. Glioblastomas (WHO grade IV) can be differentiated into de novo glioblastomas (primary) or secondary glioblastomas, which originate from low-grade astrocytomas (WHO grade II) directly or via malignant transformation from anaplastic astrocytomas (WHO grade III). It has been demonstrated that the two glioblastoma pathways show different genetic alterations. Mutations of IDH1/2 are almost exclusively present in WHO grade II and III gliomas and are early events occurring before co-deletion of chromosomes 1p and 19q in oligodendrogliomas or TP53 mutation in astrocytomas.

DIAGNOSIS AND PROGNOSIS OF PRIMARY BRAIN TUMORS.

The MRI scan is sensitive for the diagnosis of a brain tumor, but it is nonspecific for the type of tumor. The diagnosis of glial tumors is therefore based on the histology of the tumor after biopsy or resection. The histological distinction between different types of glial tumors, such as astrocytomas, oligodendroglial tumors, and mixed oligo-astrocytoma (with characteristics of both oligodendrogliomas and astrocytomas) is difficult, and is subject to 'interobserver variation'.(5) Specific markers for these tumors do not exist. The hallmark of glioblastoma is the presence of endothelial proliferation and/or necrosis in an astrocytic tumor. The prognosis of brain tumors depends on tumor grade and type: high-grade tumors and astrocytic tumors have a worse prognosis than low-grade tumors and oligodendrogliomas. Also, genetic defects have an important influence on the prognosis. For example, the presence of combined 1p/19q loss and/or IDH1 mutations are correlated with a favorable prognosis. Other non-treatment related prognostic factors are the age of the patient and the clinical condition: the prognosis is worse in older patients in a poor condition (usually expressed as performance status). The median survival of glioblastoma (the most aggressive glioma)patients treated with combined chemo-radiotherapy is

approximately 15 months.(6) For grade III tumors without 1p/19q loss the median survival is 2 to 3 years and for anaplastic oligodendrogliomas with combined 1p/19q loss more than 12 to 14 years. The prognosis of patients with low-grade glioma varies in most series between 5 and 15 years.

OUTCOME ASSESSMENT IN GLIOMA STUDIES

Response rate, progression free survival and survival are used for the assessment of outcome in studies on chemotherapy in gliomas. The objective response or response rate (ORR) is the percentage of patients with a complete or partial response, usually measured using Magnetic Resonance Imaging (MRI) of the brain in conjunction with clinical assessment and corticosteroid dose.

Progression-free survival (PFS) is the time between the start of treatment (or randomization) until progression or death. PFS is particularly used in phase II studies on recurrent glioblastoma as the primary endpoint, in which it is usually expressed as the percentage of patients that are alive and do not have progression after 6 or 12 months (resp. 6mo-PFS or 12 mo-PFS).

Both the ORR and PFS are largely dependent on the MRI findings, but changes in the enhancing lesion on an MRI and CT scan does not necessarily reflect changes in tumor activity. The phenomenon of pseudoprogression after chemo-radiotherapy in glioblastoma patients is now well established.(7, 8) Similarly, pseudoresponses occur after treatment with vascular endothelial growth factor receptor signaling pathway inhibitors.(9) Both these phenomena confuse the assessment of ORR and PFS in clinical trials.(10) Because of the above, alternative endpoints and response criteria have been developed by an international working group (Response Assessment in Neuro-Oncology (RANO).(11, 12)

Survival or overall survival (OS) is defined by the time between the start of treatment until death, in trials usually measured from the time of randomization. OS is not influenced by imaging. In oncology OS is usually seen as the golden endpoint, although salvage treatments given at progression may have an influence on this endpoint.

TREATMENT

Surgery

The treatment begins with surgery, to resect as much of the tumor as safely possible. The surgery of brain tumors performs three functions: obtaining a histological diagnosis, improving the condition of the patient by a rapid reduction in tumor volume and mass effect, and reduce the increased intracranial pressure, and finally improve the prognosis. If no meaningful resection is possible a biopsy must be performed to obtain tissue for diagnosis. Although no randomized studies exist showing a survival benefit from extensive resection, uncontrolled studies have suggested that more extensive resection contribute to survival.(13) Safe extensive resections are more often possible with advanced surgical and imaging techniques.

Radiotherapy

Radiotherapy was the first treatment proven in randomized trials to be effective for patients with high-grade gliomas. Postoperative radiotherapy of 60 Gray (Gy) in 30-33 fractions of 1.8 - 2.0 Gy improved the median overall survival (mOS) from 4-5 months to 8-10 months for glioblastoma.(14, 15) The use of a higher radiotherapy dose, either with conventional techniques or high-dose techniques (i.e. interstitial brachytherapy or stereotactic radiotherapy has not improved survival. Many centers give a short radiotherapy schedule (e.g. 14 fractions of 3 Gy; category 1C) to older patients with a poor prognosis (age over 65-70 years, moderate clinical condition, only a biopsy).(16) In very poor prognosis patients (e.g. older patients with a poor performance status),

when it is assumed the burden of treatment does not outweigh the limited survival benefit, a palliative supportive strategy is often appropriate.

Chemotherapy

Until recently, in the Netherlands chemotherapy was mainly reserved for recurrent high-grade (WHO grade III and IV) tumors after radiotherapy, in which case temozolomide or a combination of procarbazine, CCNU (=lomustine) and vincristine (PCV) chemotherapy was used. Recent results of several trials are discussed below, showing an early role for classical cytotoxic chemotherapy in glioma.

In recent years inhibitors of blood vessel formation (angiogenesis inhibitors) have been extensively evaluated in glioblastoma, in particular inhibitors of the Vascular Endothelial Growth Factor (VEGF) signaling pathway. VEGF is expressed by hypoxic tumor cells and released into the bloodstream, leading to angiogenesis in the tumor. Especially bevacizumab (a humanized monoclonal antibody against circulating VEGF) and cediranib (receptor tyrosine kinase inhibitor of VEGF receptor)) have been extensively investigated.

Temozolomide

Temozolomide is an alkylating cytostatic, which has the same active metabolite as dacarbazine (DTIC). It is a relatively small molecule (194 daltons) and it penetrates the blood-brain barrier easily due to its lipophilic character. Unlike dacarbazine, temozolomide does not require hepatic activation: a spontaneous conversion to the active ingredient MTIC finds place at a physiological pH. After oral administration the bioavailability is almost 100%, minimally (10%) affected by taking food.(17) Elimination occurs in the liver and excretion is renal. Temozolomide is registered for use in gliomas. The standard schedule of temozolomide when used as monotherapy is 150-200 mg/m2 on days 1-5 every four weeks. There are many alternative "dose dense" regimens in use, resulting in a 2-fold greater dose intensity.

Temozolomide is generally well tolerated. Side effects are mainly nausea and vomiting (well avoidable with 5HT3 antagonists), bone marrow depression (in particular leukopenia and thrombocytopenia; nadir after 21-28 days), lymphopenia (with a decrease in the CD4+ population) and hepatotoxicity. Sporadically allergic skin reactions are seen with the use of temozolomide. Pregnancy and lactation are contraindications. In the continuous dosing schedules a relative lymphopenia occurs, with a risk of opportunistic infections, especially Pneumocystis carinii (PCP) infections. PCP prophylaxis, for example cotrimoxazole, is indicated in these dose dense schedules.

Lomustine

Due to their good penetration across the blood brain barrier, the nitrosoureas (especially CCNU (lomustine), BCNU (carmustine), ACNU (nimustine) and fotemustine) have been investigated early on for their efficacy in brain tumors.(15) In particular lomustine is used, which is in the Netherlands registered for the treatment of high-grade gliomas. Lomustine is also used as part of the PCV combination regimen. PCV was widely used in the nineties, especially in oligodendroglial tumors. The PCV schedule is currently used less frequently, due to the more favorable side effect profile and easier dosing schedule of temozolomide.

Recently, a number of studies in recurrent glioblastoma used single agent lomustine as a control arm and proved that lomustine is at least as effective as temozolomide in that setting. This has led to widespread use in recurrent glioblastoma patients. For single agent use, the recommended dose is 110-130 mg/m2 with a maximum of 200 mg, after combined chemo-radiotherapy with temozolomide the maximum recommended dose of lomustine is 110 mg/m2. In the Netherlands only 40 mg capsules are available, which makes individual dosing more difficult. One of the main side effects of lomustine is cumulative bone marrow suppression, with a relatively late nadir

occurring at 4 to 6 weeks. Therefore, nitrosourea are given in 6 to 8-week cycles. Other side effects are nausea, vomiting and hepatotoxicity. The use of prophylactic 5HT3 antagonist is recommended with lomustine. The 6-weekly PCV combination regimen consists of lomustine 110 mg/m2 on day 1, procarbazine (60 mg/m2 on days 8-21) and intravenous vincristine (1.4 mg/m2, maximum 2 mg on days 8 and 29) . The added value of vincristine in this schedule is questionable, and other combinations schedules have been used. Other side effects of the PCV chemotherapy schedule are the vincristine associated peripheral neurotoxicity, loss of appetite, weight loss and malaise symptoms, including fatigue. These side effects seem largely due to procarbazine. Furthermore, the myelosuppression of the PCV schedule is more prominent compared to that of lomustine alone. Pregnancy and lactation are contraindications.

Carmustine wafers

Carmustine (BCNU)-containing wafers are registered in Europe for use in newly diagnosed high-grade gliomas and in recurrent glioblastoma.(18, 19) However, the possible survival benefit is limited and not statistically significant in newly diagnosed glioblastoma.

Mechanism of action

The nitrosoureas cause in particular chloroethyl-adducts at the O^6 position of guanine. This results in N^1-guanine, N^3-cytosine DNA interstrand cross-links which are cytotoxic. Temozolomide causes single-and double-stranded DNA breaks by adding methyl groups to N^7 guanine (70% of total number of adducts), N^3 adenine (9%), and O^6 guanine (5%). The cytotoxic effects of temozolomide are mainly attributed to the O^6-methylguanine adducts. The DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) removes both methyl and 2-chloroethyl adducts from the O^6 position of guanine, and is an important mechanism of resistance against these agents.(20) Removing the MGMT protein, for example with O^6- benzylguanine leads to a higher cytotoxicity of nitrosourea and temozolomide. Expression of the MGMT gene is affected by epigenetic changes such as DNA methylation of the promoter gene, thus inhibiting the expression of MGMT. The presence of MGMT promoter methylation results in an increased effectiveness of temozolomide chemotherapy in glioma and perhaps also of PCV chemotherapy.(21, 22) Once O^6-methylguanine adducts are present an intact mismatch repair (MMR) system is necessary for the induction of apoptosis. Temozolomide is not effective in cells with MMR deficiency.(23) A defect in the MMR system results in microsatellite instability and tolerance to O^6-methylguanine DNA adduct mismatch. More than 80% of the lesions induced by temozolomide are N-methylated bases, recognized by DNA glycosylases and not by MGMT. Therefore, the resistance to temozolomide is also determined by the base excision repair (BER) system. The enzyme poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme that recognizes both double and single-stranded DNA breaks and plays a central role in the activity of the BER system and the removal of methylated N^3 and N^7 adducts. Possibly PARP inhibitors may disrupt temozolomide resistance by blocking BER, through which N^3- and N^7-methyl adducts are still becoming cytotoxic. The combination of temozolomide and PARP inhibitors is therefore potentially interesting.

Chemotherapy in glioblastomas

Newly diagnosed glioblastoma

The EORTC 26981/NCIC CE3 randomized phase III study in 563 glioblastoma patients, showed that radiotherapy combined with temozolomide chemotherapy (60 Gy radiotherapy in 30 daily fractions of 2 Gy in combination with daily 75 mg/m2 temozolomide followed by adjuvant 6 cycles of temozolomide at a dose of 150-200 mg / m 2 on days 1-5 every four weeks) significantly improves survival compared to treatment with radiotherapy alone (Table 1).(6) A small randomized phase II study showed a similar result.(24) The long term follow up of the EORTC

study confirmed the results of the initial publication (Hazard ratio (HR) for death with chemotherapy: 0.6 [95% confidence interval (CI) 0.5 to 0.7], see Table 1).(25) Combined chemo-radiotherapy increases to two-year survival to 27% vs. 11% after radiotherapy alone. The five-year survival was 10% in the combination arm vs. 2% in the radiotherapy only arm. With these results chemo-radiation with temozolomide became the world-wide accepted standard treatment for glioblastoma patients.

Table 1. Survival in months measured from the date of randomization in EORTC study 26981 of the combined chemo-radiotherapy and temozolomide in glioblastoma

		Survival			
	n	**Median (mo)**	**2-year (%)**	**3-year (%)**	**5-year (%)**
Radiotherapy	286	12.1 [11.2-13.0]	10.9 [7.6-14.8]	4.4 [2.4-7.2]	1.9 [0.6-4.4]
Chemo-radiotherapy	287	14.6 [13.2-16.8]	27.2 [22.2-32.5]	16.0 [12.0-20.6]	9.8 [6.4-14.0]

mo: months, CI: confidence interval, 95% confidence interval between brackets

The methylation status of the MGMT gene promoter was found to be of prognostic significance for survival in the EORTC 26981 study and appeared to be of of predictive significance but the study was not powered to show this. In tumors without methylation of the MGMT gene promoter the 2-year survival was 14.8% whereas it was 49% in the MGMT methylated tumors (and 24% after radiotherapy alone).(26) Predictive value of MGMT promoter methylation status was found in two studies on elderly glioblastoma patients (see below). The survival benefit from the combined chemo-radiotherapy was less in patients with a worse prognosis (only biopsy, older patients, poorer performance status).(27)

Newly diagnosed glioblastoma in elderly patients

Elderly patients in a poor clinical condition are often treated with a short radiotherapy schedule.(16, 28) The main consideration for this choice is the limited survival in this group compared to younger patients. Subgroup analyses of EORTC 26981 show that even in elderly patients (>60 years) with favorable prognostic factors the median survival is more than 12 months. Therefore, a longer radiotherapy schedule, combined with chemotherapy, can be considered in patients over 60 with favorable prognostic factors (macroscopic total resection and with a good performance status (KPS>70, MMSE>27)). Two trials have investigated treatment with temozolomide alone in an elderly population. The 'Nordic' trial showed that a long schedule radiotherapy leads to shorter survival compared to a short radiotherapy schedule or temozolomide alone.(29) In both this 'Nordic´ and in the German 'NOA-08' trial a better survival was found after temozolomide monotherapy compared to a short schedule radiotherapy in patients with a methylated MGMT gene promoter, whereas MGMT promoter methylation status did not impact outcome to radiotherapy.(29, 30) Therefore, temozolomide can be considered in elderly patients with MGMT promoter methylation instead of a short radiotherapy schedule. However, neither the Nordic nor the NOA-08 trial investigated the combination of a short schedule radiotherapy and temozolomide chemotherapy. Such a trial was conducted by the EORTC/NCI (ClinicalTrials.gov identifier: NCT00482677). In this trial on elderly glioblastoma patients hypofractionated radiotherapy is compared to hypofractionated radiotherapy with temozolomide. Results are expected by the end of 2015.

Recurrent glioblastoma

In Europe a randomized phase II study in recurrent glioblastoma led to the registration of temozolomide. In this study, a significant improvement in 6-month progression free survival (6mo-PFS) was found after treatment with temozolomide compared with the procarbazine treated control group (Table 2, HR 1.47 (95% CI, 1.11-1.95%; category 2b).(31) Although the median survival in the temozolomide arm was 1.5 months longer, this difference was not significant. A single arm phase II study with temozolomide showed similar results.(32) The 6mo-PFS of 19-21% also shows that temozolomide chemotherapy in this situation is only marginally effective, with an objective response rate in glioblastoma of only 5-10%. Although today most relapsed glioblastoma patients will have received prior treatment with temozolomide, a recent Canadian study has shown that temozolomide may still be effective for recurrent glioblastoma after chemo-irradiation with a temozolomide therapy-free interval of >3 months.(33)

Recent phase III studies using lomustine as a control arm showed that nitrosoureas may be a useful treatment alternative in recurrent glioblastoma, with 20-30% 6mo-PFS (Table 2).(34, 35) A large British randomized phase III study in recurrent glioblastoma patients after radiotherapy alone showed no difference between temozolomide and the PCV regimen (HR = 0.89 95% CI (0.73-1.08).(36)

Table 2. Phase II and phase III studies in recurrent glioblastoma

Chemotherapy	**n**	**6mo-PFS**	**Median PFS [months]**	**Median OS [months]**
Temozolomide (31)	112	21%	3	
Procarbazine	113	8%	2	
Temozolomide (32)	138	19%	2.1	
Lomustine (34)	84	19%	1.6	7.1
Lomustine (35)	65	25%	1.5	9.8
Lomustine/cediranib	129	35%	4	9.4
Cediranib	131	16%	3	8.0
PCV(36)	224		3.6	6.7
Temozolomide	223		4.7	7.2
PCV(37)	63	29%	3	7.5
Bevacizumab (38)	85	43%	4.2	9.2
Bevacizumab/irinotecan	82	50%	5.6	

PFS: progression free survival, 6m-PFS: percentage of patients free of progression 6 months after the start of the chemotherapy, OS: overall survival, PCV: procarbazine, CCNU (=lomustine) and vincristine chemotherapy

Dose intensified temozolomide schedules

The use of continuous or 'dose dense' temozolomide regimens were based on theoretical considerations that these schedules may overcome MGMT dependent resistance to temozolomide.(39) Some uncontrolled studies suggested relatively favorable results with these intensified regimens for recurrent glioblastoma.(40) Many studies however failed to show such a promising results.(41, 42, 43) In line with the latter studies was a British randomized phase III study in chemotherapy naive patients with a recurrent glioblastoma. This study showed a better survival of the standard 1-5 days every four weeks temozolomide schedule compared to temozolomide given in a three weeks on / one week off dose intensified regimen (HR 1.32, 95% CI, 0.99 to 1.75).(36) Furthermore, a large international RTOG lead study in newly diagnosed

glioblastoma showed no survival benefit of a dose-intensified adjuvant schedule in newly diagnosed glioblastoma after radiotherapy, regardless of the MGMT promoter status.(44) With the currently available data it can be concluded that there is no evidence for superior efficacy of dose-intensive temozolomide regimens, and that retreatment with temozolomide can be considered in carefully selected patients.

Chemotherapy in newly diagnosed grade II and III gliomas

Anaplastic tumors including oligodendrogliomas

In the late eighties of the past century the first reports emerged that recurrent anaplastic oligodendrogliomas were sensitive to PCV chemotherapy, with response rates in the 55-65% range and a median duration of response of 12-18 months.(45, 46, 47) In particular, tumors with combined 1p/19q loss were found to be responsive, with 90-100% of these patients showing a response to the PCV regimen or temozolomide.(48, 49) These studies in recurrent tumors were the reason to investigate whether outcome would improve if PCV chemotherapy was given early in the treatment of these patients. Two open randomized controlled phase III trials (EORTC 26951: 368 patients, RTOG 9402: 289 patients), investigated the value of adjuvant PCV immediately before or after radiotherapy in newly diagnosed anaplastic oligodendroglial tumors compared with radiotherapy only and further chemotherapy at the time of progression.(50, 51) At the time of their first report both studies did not show an overall survival benefit from early adjuvant PCV chemotherapy although both studies showed a significant improvement in PFS after adjuvant chemotherapy.(50, 51) However, the long-term results (median follow-up > 11 years) of both studies showed survival benefit in anaplastic oligodendroglial tumors with combined 1p/19q loss after adjuvant PCV chemotherapy (category 1b; see table 3) despite of cross over treatment with chemotherapy at the time of progression in 70% of patients randomized to radiotherapy only.(52, 53) Both studies have shown that the presence of combined 1p/19q loss in oligodendrogliomas is of important prognostic significance, and identifies patients with more benefit from adjuvant PCV chemotherapy. The median survival is over 10-14 years in patients with tumors with combined 1p/19q loss as opposed to 2-3 years for tumors without combined 1p/19q loss. Correlative side studies conducted within the context of these clinical trials have suggest that the CpG island hypermethylated phenotype (CIMP), a methylated MGMT gene promoter and mutations of IDH in anaplastic oligodendroglioma can also be used to identify patients that may benefit from adjuvant PCV chemotherapy.(22, 54)

Table 3. Median overall survival (months) and median progression free survival (months) in relation to the 1p/19q status. EORTC study 26951 and RTOG 9402 study with (neo-) adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors.(52, 53)

	Median OS (months)		**Median PFS (months)**	
Chromosomal status	**RT/PCV**	**RT**	**RT/PCV**	**RT**
Combined 1p/19q loss				
EORTC	NR	111.8 [75.7, 134.3]	156.8 [68.1, NR]	49.9 [27.8, 101.8]
RTOG	176.4	87.6	100.8	34.8
No 1p/19q loss				
EORTC	25 [18, 37]	21 [18, 29]	14.8 [9.9, 21.1]	8.7 [7.1, 11.7]
RTOG	31.2	32.4	14.4	12.0

Between brackets: 95% confidence interval. OS: overall survival, PFS: progression free survival, NR: not reached, EORTC: European Organization of Research and Treatment of Cancer, RTOG: Radiotherapy and Oncology Group, RT: Radiotherapy, PCV: procarbazine, CCNU (=lomustine) and vincristine chemotherapy

A randomized German study in 318 patients with grade III gliomas compared initial treatment with radiotherapy with initial treatment with chemotherapy, and randomized patients in the chemotherapy arm between PCV and temozolomide.(55) While previous studies consistently showed a better survival when high-grade tumors were treated with radiotherapy compared to chemotherapy, the first report of the study with still rather immature survival data showed no difference in PFS between patients treated with one line of chemotherapy followed at progression by radiotherapy or patients treated with radiotherapy followed at progression by one line of chemotherapy. This suggests that in grade III tumors there is no difference between initial treatment with chemotherapy or with radiotherapy, as long as patients are being surveyed carefully and further treatment is given at the time of progression. This study and the EORTC study 26951 in anaplastic oligodendrogliomas revealed that grade III tumors have a methylation of the MGMT gene promoter in 70-80% of the cases, and that this is also a favorable prognostic factor after radiotherapy only. This was subsequently found to be related to hypermethylation of CpG islands (CIMP) in IDH mutated tumors. In these tumors methylation is induced by metabolic alterations that are the consequence of the altered substrate affinity of the IDH1 mutation product resulting in an increased 2HG glutarate production.(56, 57, 58, 59) This shows that the clinical significance of MGMT promoter methylation is determined by the molecular background of the tumor. An ongoing randomized trial (CATNON) investigates the value of combined chemo-radiotherapy with temozolomide in grade III tumors without combined 1p/19q loss (anaplastic astrocytomas).

Low-grade gliomas

The role of chemotherapy in newly diagnosed low-grade gliomas is slowly being clarified. Uncontrolled studies with up-front temozolomide and PCV showed favorable results, especially in tumors with combined 1p/19q loss. In a study of 149 patients treated with upfront temozolomide for a low-grade glioma, the median time to progression was 28 months and significantly longer in the group with combined 1p/19q loss.(60) Similar observations have been made with PCV.(61) A first and still early analysis of the European Organization for Research and Treatment of Cancer (EORTC) study 22033 presented at the ASCO meeting in 2013 suggests that PFS does not differ between patients with 1p loss receiving upfront temozolomide versus patients receiving radiotherapy, but radiotherapy may provide a superior PFS in patients without 1p loss ($p = 0.06$).(62) The median OS was not yet reached in that study.

An updated report from an American randomized study (RTOG 9802) in 251 patients with newly diagnosed low-grade gliomas with a relatively unfavorable prognostic profile (less than gross total resection and/or over 40 years of age) showed that adjuvant PCV chemotherapy increased both PFS and OS.(63) With 55% of the patients having died, the median survival was 7.8 years after radiotherapy alone and 13.3 years after radiotherapy followed by PCV (HR 0.59, $p = 0.002$). This implies that radiotherapy followed by adjuvant chemotherapy should now perhaps be standard therapy in high-risk low-grade glioma. Unfortunately, molecular data from RTOG 9802 are not available yet and it is not clear whether all molecular subgroups benefit equally from combined treatment. This suggest that some fundamental questions on how to best select patients for adjuvant PCV chemotherapy in this population may remain unanswered.

Another unsolved question is whether temozolomide will provide the same OS advantage as PCV. Although the tolerability of temozolomide is better than that of PCV, no comparable trials with temozolomide have been performed.

Another important and unanswered question is whether it is safe to use only up-front chemotherapy in low-grade glioma patients with a large chemotherapy sensitive tumor (e.g.

1p/19q loss), in which is a large radiation field is required is unclear. Such a policy is used by some to delay radiotherapy as this has been associated with cognitive deficits.(64, 65)

Chemotherapy in recurrent grade II and III gliomas

Recurrent grade II and III tumors and in particular anaplastic oligodendroglioma with 1p/19q loss are significantly more sensitive to chemotherapy than glioblastoma (Table 4). In oligodendrogliomas with combined loss of 1p/19q a response is seen in 90-100% of the tumors, with a median response duration of 1-2 years. However, even in these tumors the response to a second line of chemotherapy is limited, with an objective response in 20-25% of cases and a 6 months PFS of approximately 50%.(66, 67) Both temozolomide and nitrosoureas (or PCV) are considered standard treatment in this situation.

Table 4. Studies with first line temozolomide or PCV chemotherapy in recurrent grade II and III gliomas

Histology (reference)	n	Drugs	Response	6mo-PFS	12mo-PFS	mOS [months]
AII(68)	70	TMZ	47%	63%		13
AA/AOA(69)	65	TMZ	43%	50%		11,5
AOD/AOA(70)	67	TMZ	46%		50%	31
AOD/AOA(47)	38	TMZ	54%	71%	40%	NR
AO/AOA(46)	52	PCV	63%		50%	20
AOD(71)	37	PCV	59%	72%	52%	30,7

6mo-PFS: progression free survival at 6 months, 12mo-PFS: progression free survival at 12 months, mOS: median overall survival, AII: low-grade (grade II) astrocytoma, AA: anaplastic (grade III) astrocytoma, AOA: anaplastic oligoastrocytoma, AOD: anaplastic oligodendroglioma, TMZ: temozolomide, PCV: procarbazine, CCNU and vincristine chemotherapy, Response: percentage of patients with a an objective response (partial and complete response)

Angiogenesis inhibitors, integrin inhibitors and other targeted agents

In recent years there is much interest in treatment of glioblastoma with substances inhibiting the signal transduction by the Vascular Endothelial Growth Factor (VEGF) system, either by catching circulating VEGF (such as bevacizumab, a humanized monoclonal antibody against circulating VEGF) or by blocking the receptor (such as cediranib, an orally tyrosine kinase inhibitor of VEGF receptor 2 and 3). Uncontrolled phase II studies showed high response rates and 6-month progression-free survival with bevacizumab (Table 2).(38, 72) Based on the randomized phase II BRAIN trial (bevacizumab versus bevacizumab with irinotecan), the U.S. Food and Drug Administration (FDA) registered bevacizumab for use in recurrent glioblastoma ("conditional approval") in the U.S. The lack of a control arm without bevacizumab was the reason for the European Medicines Agency (EMEA) to reject the registration of bevacizumab. Nonetheless, bevacizumab is currently used in many European countries for this indication.

An unsolved issue of the BRAIN trial is the use of imaging endpoints: the percentage of patients showing an objective response and or 6 months progression-free survival).(73) Inhibition of the VEGF signaling system results also in a normalization of the abnormal permeability of the tumor blood vessels, reducing contrast enhancement on MR scans even in the absence of a true tumor response.(74) The occurrence of these "pseudo-responses', makes the interpretation of the MRI scans troublesome: it is questionable whether the reduction of contrast uptake on MRI scans in anti-VEGF agents treated glioblastoma patients indeed reflects a real tumor reduction or only a

decrease in contrast uptake and edema. Indeed, a large placebo-controlled phase III study on cediranib in recurrent glioblastoma (the REGAL trial) showed no survival benefit of cediranib alone or in combination with lomustine compared to only lomustine single agent in recurrent glioblastoma, despite a >20% response rate in an uncontrolled phase II study.(35, 75) While the progression-free survival in this trial was slightly better in patients treated with cediranib this did not translate into a survival benefit. Though initial reports suggested that inhibition of tumor angiogenesis might induce an infiltrative growth pattern by co-opting existing blood vessels some more recent studies failed to find evidence for this.(76)

Two large phase III trials (AVAglio trial, RTOG 0825) on bevacizumab in newly diagnosed glioblastoma have failed to show an improvement of overall survival despite a major improvement of PFS.(77, 78) Theoretically, a longer PFS could translate into an improved quality of survival, as progression is often accompanied by neurological deterioration. Therefore in both studies a quality of life assessment was included as a mandatory secondary endpoint. Surprisingly the AVAglio trial showed maintenance of the quality of life in the bevacizumab treated patients prior to progression, whereas the RTOG 0825 trial showed a decreased quality of life despite using similar questionnaires. The present results do not justify the use of Bevacizumab in newly diagnosed glioblastoma, and the role of bevacizumab in recurrent glioblastoma is still unclear. The Dutch randomized phase II BELOB trial is the only study with a control arm without bevacizumab in recurrent glioblastoma.(79) This trial also failed to show a survival benefit of bevacizumab single agent, although the results within the combination of lomustine and bevacizumab with a OS at 9 months of 59% met the pre-specified criterion for further phase III studies. The ongoing phase III EORTC trial 26101 in recurrent glioblastoma is designed to demonstrate whether there is indeed survival benefit of the combination bevacizumab/lomustine compared to lomustine alone (ClinicalTrials.gov identifier: NCT01290939).

CONCLUSION

In the last decade a shift has occurred from chemotherapy for recurrent disease towards the use of adjuvant chemotherapy for newly diagnosed tumors. In addition, there is a better understanding of molecular factors that predict responsiveness to adjuvant chemotherapy.

Chemo-radiotherapy with temozolomide is currently the standard of care for newly diagnosed glioblastoma and ongoing studies investigate whether this treatment also improves outcome in elderly patients with glioblastoma treated with a short schedule of radiotherapy. Two studies have showed that chemotherapy is more effective than radiotherapy alone in elderly glioblastoma patients with a methylated MGMT promoter. Treatment with lomustine can be considered in recurrent glioblastoma. In patients with a longer therapy-free interval, retreatment with temozolomide can also be considered. Still, treatment results in this setting are of rather modest effectivity, and it is recommended that these patients are enrolled in clinical trials aiming at the improvement of outcome.

Bevacizumab added to standard treatment in newly diagnosed glioblastoma does not improve survival. The combination of bevacizumab and lomustine may play a role in recurrent glioblastoma and is currently being investigated in an adequately powered phase III trial (EORTC 26101). Until now other “targeted” therapies have shown poor results.

Adjuvant PCV chemotherapy is part of standard of care in newly diagnosed WHO grade III oligodendroglial tumors at least in the tumors with combined loss of 1p/19q. Adjuvant PCV also improves survival in newly diagnosed high risk LGG. Clearly, more patients benefit from chemotherapy, but the optimal way of identifying these patients (IDH mutated? CIMP or MGMT promoter methylated tumors?) remains to be established.

The value of combined chemo-radiotherapy with temozolomide for grade III astrocytoma without 1p/19q loss is currently being investigated, it will take however many years before the results of this study become available. Whether in large grade II and III tumors, initial treatment with chemotherapy alone can be considered is at present unclear, the prevailing data suggest that this approach may decrease survival.

For recurrent grade II and grade III tumors, chemotherapy is the standard and commonly temozolomide is used instead of lomustine or PCV due to better tolerance. Although in about half of the patients a response is achieved, it is often limited in duration, with the exception of oligodendroglial tumors with combined 1p/19q loss.

With the current data available, early chemotherapy is now part of the management of nearly all diffuse glioma. It is unlikely that with the currently available cytotoxic drugs outcome can be further enhanced, although patient selection can still be improved. This implies that for further improvement of outcome new drugs are needed. This should be a high priority.

Practice Points:

- Chemoradiotherapy with temozolomide is currently the standard of care for newly diagnosed glioblastoma.
- Temozolomide chemotherapy is more effective than radiotherapy alone in elderly glioblastoma patients with a methylated MGMT promoter.
- Treatment with lomustine or retreatment with temozolomide can be considered in recurrent glioblastoma.
- Bevacizumab combined with lomustine may play a role in recurrent glioblastoma.
- Other 'targeted' therapies have shown poor results in diffuse glioma until now.
- Adjuvant PCV (procarbazine, CCNU [lomustine] and vincristine) chemotherapy is currently the standard of care in newly diagnosed anaplastic oligodendroglioma with combined loss of 1p/19q.
- Adjuvant PCV clearly improves survival in newly diagnosed high-risk low-grade glioma.
- Chemotherapy is the standard for recurrent low-grade WHO grade II and high-grade gliomas grade III.
- Temozolomide is commonly used instead of nitrosoureas containing regimen (e.g., PCV) due to better tolerance.
- It is unlikely that outcome in glioma can be further enhanced with the currently available cytotoxic drugs and new (targeted) drugs are needed.

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Chapter 2

The incidence of early pseudo-progression in a cohort of malignant glioma patients treated with chemo-irradiation with temozolomide

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ABSTRACT

Background. Radiotherapy (RT) plus concomitant and adjuvant temozolomide (TMZ) is now the standard of care for patients with newly diagnosed glioblastoma. The occurrence of pseudo-progression directly after RT is a recognized phenomenon, but to the authors' knowledge its incidence after combined RT/ TMZ is unknown. The occurrence of early pseudo-progression was retrospectively assessed in a cohort of malignant glioma patients treated with RT/TMZ.

Methods. The pre-RT and post-RT brain scans from patients treated with RT/ TMZ for a malignant glioma were reviewed. Scans were made before the start of RT, 4 weeks after the end of RT, and every 3 months thereafter. In addition, information was collected regarding clinical signs and symptoms, dexamethasone dose, histology, and survival.

Results. Eighty-five patients were identified. In 36 patients (42%) the first follow-up scan 4 weeks after the end of RT indicated disease progression. Of these 36 patients, 18 (50%) were diagnosed with pseudo-progression. None of the patients received additional treatment other than TMZ. Six of 18 patients with pseudo-progression and 12 of the 18 patients with real tumor progression developed new clinical signs and symptoms during RT or in the first 4 weeks thereafter.

Conclusions. Up to 50% of malignant glioma patients treated with RT/TMZ and progression immediately after RT develop pseudo-progression. The current study data support the idea to continue TMZ in the case of progressive lesions immediately after RT/TMZ. Surgery should be considered in symptomatic cases. The inclusion of patients with progressive lesions developing directly after chemo-radiation in studies regarding recurrent gliomas will lead to an overestimation of the results.

INTRODUCTION

Radiotherapy (RT) and concomitant temozolomide (TMZ), followed by adjuvant TMZ has become the standard of care for patients with glioblastomas since the European and Canadian randomized trial was published in 2005.(1) Despite the improved outcome with combined modality treatment, the overall outcome of this disease remains dismal, with many patients progressing early after RT or during adjuvant TMZ chemotherapy. In recent years we and other clinicians observed the occurrence of progressive MRI lesions immediately after the end of concurrent chemo-irradiation with TMZ (RT/TMZ), with spontaneous improvement without further treatment other than adjuvant TMZ. In an earlier study this phenomenon of early pseudoprogression was investigated in 32 patients with a malignant glioma who received RT only.(2) In that study 3 out of 9 patients (33%) with a progressive lesion immediately after RT demonstrated a stabilized or improved lesion during at least 6 months on subsequent scans, without additional treatment. To our knowledge, the incidence of early pseudo-progression in malignant glioma patients treated with RT/TMZ is unknown, nor is it known whether this is clinically symptomatic. (At the time of publication, Brandes et al have since published data regarding the incidence of pseudo-regression and the correlation with the MGMT promoter methylation status in glioblastoma patients.(3)) We investigated the incidence of early pseudo-progression and its clinical features by reviewing a cohort of patients with newly diagnosed malignant gliomas who were treated with RT plus concomitant and adjuvant TMZ.

MATERIALS AND METHODS

For this study, all malignant glioma patients treated between 2000 and July 2006 with RT/TMZ in the Daniel den Hoed Cancer Center in Rotterdam, The Netherlands were reviewed. Furthermore all patients who participated in the European Organization for Research and Treatment of Cancer (EORTC) 22981 study and received chemo-irradiation in the University Medical Center Utrecht, The Netherlands were also reviewed. No patients were excluded. Treatment was comprised of fractionated irradiation at a dose of 2.0 grays (Gy) per fraction given once daily on weekdays over a period of 6 weeks to a total dose of 60 Gy and concomitant TMZ (75 mg/m2/day on all days), followed after 4 weeks by six 28 day-cycles of adjuvant TMZ (Day 1-5 every 28 days at a dose of 150-200 mg/m2/day). None of the patients received other treatment, such as gliadel or a focal radiotherapy boost.

Clinical records were reviewed concerning the type of surgery performed, histology, radiation field, neurologic signs and symptoms, dexamethasone dose, and survival. Per treatment protocol, brain imaging was performed before RT (median interval of 14 days between the brain scan and the initiation of RT), 4 weeks after the end of RT (median interval of 30 days between the end of the RT/concomitant TMZ) and thereafter every 3 months. Two independent reviewers reviewed all brain scans (HGdB and WT). The evaluation was based on precontrast and postcontrast images and primarily on the changes in the contrast enhancing area. In the case of disagreement the scans were jointly re-evaluated. The response criteria developed by Macdonald et al were used to quantify all changes of the enhancing lesions on the scan, clinical status and dexamethasone dose.(4)

Early disease progression was defined as progression (≥25% increase) noted on the MRI-scan 4 weeks after RT and concomitant TMZ, with or without neurologic deterioration and on a stable or higher dose of dexamethasone. Real-early-progression was scored if the patient with early progression developed additional disease progression within the following 6 months. Pseudo-early-progression was scored if the patient with early progression: 1) had at least a 50% decrease of the enhancing lesion during further follow-up, while remaining neurologically stable and on a stable or decreasing dose of dexamethasone (a ‘partial response’ according to the Macdonald

criteria) or 2) remained clinically and radiologically stable with a stable or decreased dosage of steroids for at least 6 months after RT/TMZ without any further treatment other than adjuvant cycles of TMZ. Clinical features of the patients with real-early-progression and pseudo-early-progression were compared. Kaplan-Meier survival curves were used to analyze survival in the patients with real-early-progression and pseudo-early-progression.

RESULTS

Eighty-five patients were treated with RT plus concomitant and adjuvant TMZ. The majority of the patients had a glioblastoma multiforme (GBM). Table 1 summarizes the demographic and clinical features of these patients.

Table 1. Characteristics at baseline of 85 malignant glioma patients treated with radiotherapy plus concomitant and adjuvant temozolomide.

Characteristic	**Patients**
Median age (range), y	50 (18-68)
Male/Female, %	66/34
Histopathology, n (%)	
Glioblastoma multiforme	68 (80%)
Anaplastic astrocytoma	11 (13%)
Anaplastic oligodendroglioma	3 (3.5%)
Anaplastic oligo-astrocytoma	3 (3.5%)
Performance score 0-1 versus 2, %	89/11
Complete or partial resection versus biopsy, %	69/31

WHO: World Health Organization

In 39 patients the pre-RT/TMZ scan was a computed tomography (CT-) scan; in the other 46 patients MRI was used. All follow-up scans were MRI-scans.

Thirty-six of the 85 patients (42%; 95% confidence interval [95%CI], 31.5-52.5%) were identified as having early progression on the first follow-up scan 4 weeks after RT and concomitant TMZ compared with the pre-RT imaging (Table 2). Thirty-one of the 68 patients (45%; 95%CI, 33.2-56.8%) with a GBM and 5 of the 17 patients (29%; 95%CI, 7.4-50.6%) with an anaplastic glioma had early disease progression. In only 1 patient could the decreased dose of dexamethasone explain the observed progression noted on the MRI scan (Patient 17). Three patients did not continue with adjuvant TMZ, because of neurologic deterioration (Patients 22, 31, and 34). All of the remaining patients continued with adjuvant TMZ. Eighteen out of 36 patients (50%; 95%CI, 33.7-66.3%) with early disease progression were diagnosed with pseudo-early-progression. Pseudo-early-progression was noted in 15 of the 31 patients (48%; 95%CI, 30.4-65.6%) with a GBM and in 3 of the 5 patients (60%; 95%CI, 17.0-100%) with an anaplastic glioma.

Table 2. Description of demographics, type of scan pre-RT/TMZ*, DXM dosage on the day of pre- and post-RT/TMZ scan, and outcome in the 36 patients with an increasing lesion at the time of the first follow-up MRI scan after RT/TMZ.

Patients with early progression							DXM dose, mg		Scan changes compared to prior scan				
No.	Age	Sex	Histology	Surgery	Pre-RT/TMZ scan	WHO score	Pre-RT/TMZ	Post-RT/TMZ	Post-RT/TMZ	At 3 mo	At 6 mo	At 9 mo	Survival, mo†
1	31	M	GBM	Resection	CT	1	0	0	PD	PR	SD	CR	25+
2	51	M	AOD	Biopsy	CT	1	0	0	PD	SD	PR	SD	13+
3	39	M	GBM	Resection	MRI	0	0	0	PD	PD/SO	PR	SD	22+
4	57	F	GBM	Resection	CT	1	0	0	PD	SD	SD	SD	15+
5	58	M	GBM	Resection	MRI	1	0	3.5	PD	SD	SD	SD	13+
6	34	M	GBM	Biopsy	CT	0	0	0	PD	PR	SD	SD	38+
7	48	F	GBM	Resection	MRI	1	4	16	PD	PR	PD	DIED	10
8	50	F	GBM	Resection	MRI	1	5	4	PD	SD	PR	SD	21
9	44	F	GBM	Resection	MRI	0	0	0	PD	SD	PR	SD	32+
10	34	M	AOD	Resection	MRI	0	0	0	PD	SD	PR	CR	17+
11	47	M	GBM	Biopsy	CT	2	10	16	PD	SD	SD	SD	25
12	38	M	GBM	Resection	MRI	0	0	0	PD	SD	PR	SD	31+
13	32	F	AOA	Biopsy	CT	0	0	0.5	PD	SD	SD	SD	27+
14	19	M	GBM	Biopsy	MRI	1	0	1.5	PD	SD	SD	SD	17
15	59	M	GBM	Biopsy	CT	0	0	0	PD	NA	SD	SD	19
16	53	M	GBM	Resection	CT	1	0	0	PD	PR	PD	NA	17
17	42	F	GBM	Resection	CT	1	3	0	PD	SD	PR	CR	23+
18	55	M	GBM	Resection	CT	1	0	6	PD	PR	NA	PD	12
19	58	M	GBM	Resection	CT	1	0	0	PD	SD	NA	DIED	11
20	18	M	GBM	Resection	MRI	1	0	0	PD	PD	DIED		7
21	37	M	GBM	Biopsy	MRI	1	8	8	PD	SD	PD	DIED	11
22	67	F	AA	Biopsy	MRI	1	0	16	PD	DIED			3
23	61	F	GBM	Resection	CT	1	4	24	PD	NA	NA	NA	14
24	45	F	GBM	Resection	CT	1	4	1.5	PD	PD	DIED		6
25	50	F	GBM	Resection	MRI	0	4	6	PD	PD	DIED		6
26	68	M	GBM	Resection	CT	1	7.5	3	PD	PD	PD	DIED	8
27	47	F	GBM	Biopsy	MRI	1	4	6	PD	SD	PD	NA	15
28	57	M	GBM	Resection	CT	1	4	0.5	PD	SD	DIED		6
29	57	M	AOA	Biopsy	MRI	1	0	8	PD	DIED			4
30	59	M	GBM	Biopsy	MRI	1	0	0	PD	PD	NA	NA	16+
31	54	F	GBM	Biopsy	MRI	2	0	0	PD	DIED			4
32	54	M	GBM	Resection	CT	2	0	1	PD	DIED			5
33	62	M	GBM	Biopsy	CT	1	2	8	PD	SD	PD	OT	16+
34	37	M	GBM	Resection	MRI	0	1	1	PD	DIED			2
35	55	F	GBM	Resection	CT	1	4	4	PD	PD	PD	NA	14
36	60	M	GBM	Resection	CT	0	0	1.5	PD	PD	DIED		8

RT/TMZ: radiotherapy and concomitant temozolomide, DXM: dexamethasone, GBM: glioblastoma multiforme, AA: anaplastic astrocytoma, AOA: anaplastic oligo-astrocytoma, AOD: anaplastic oligodendroglioma, CR: complete remission of lesion, PR: decreasing lesion, SD: stable lesion, PD: progressive lesion, SO: second operation, NA: not available, OT: other therapy, mo: months

*all follow-up scans were MRI scans

†survival is measured in months from the first day of RT/TMZ on

In 17 of the 36 patients with early disease progression, the enhancing lesion on subsequent MRI-scans stabilized for at least 6 months (Patients 4, 5, 11, 13, 14, and 15), decreased (Patients 2, 6, 7, 8, 9, 12, 16, and 18) (Figure 1) or disappeared completely (Patients 1, 10, and 17). Because these 17 patients were also clinically stable or improved and were receiving a stable or decreasing dose of dexamethasone, they were scored as having pseudo-early-progression. One of the 36 patients with early progression underwent a re-resection 3 months after RT and concomitant TMZ because of further deterioration; at surgery, only necrosis was found (Patient 3). The patient continued with 3 more cycles of adjuvant TMZ and remained stable for another 15 months.

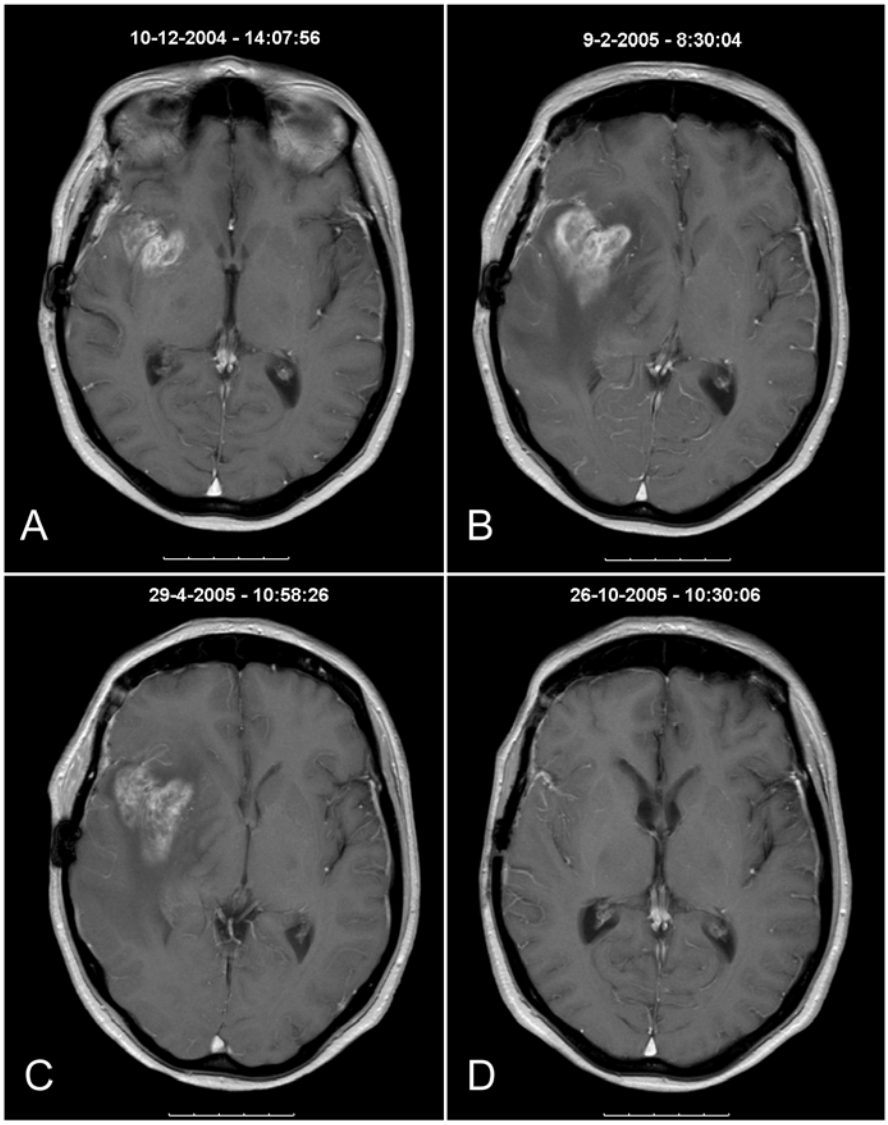

Figure 1. Patient 8: A 50-year-old female was diagnosed with a right temporal glioblastoma. Debulking surgery was followed by radiotherapy and concomitant temozolomide (RT/TMZ). Compared to the pre-RT/TMZ scan (A) the scan of the brain 4 weeks after RT/TMZ showed progression of the area of gadolinium uptake (B). She remained clinically stable and was on a stable dose of dexamethasone. She continued with 3 cycles of adjuvant TMZ and the dexamethasone was gradually lowered and stopped. The MRI scan 3 months after the RT/TMZ was unchanged (C) and she received another 3 cycles of TMZ. The MRI scans 6 and 9 months after RT/TMZ showed a diminishing lesion (D). She progressed 12 months after the RT/TMZ.

To investigate whether the percentage of patients with pseudo-early-progression was artificially high because of the use of a CT scan rather than an MRI scan before RT, we separately analyzed

the patients diagnosed with early disease progression who had been evaluated with MRI scans both before and after RT. In this group 8 of 17 patients (47%) were subsequently diagnosed with pseudo-early-progression compared with 10 of 19 patients (53%) who had a pre-RT CT scan.
The individual charts of the patients with pseudo-early-progression were re-examined for other explanations of disease remission, but none were found. In particular, no new treatments had been initiated other than adjuvant TMZ.

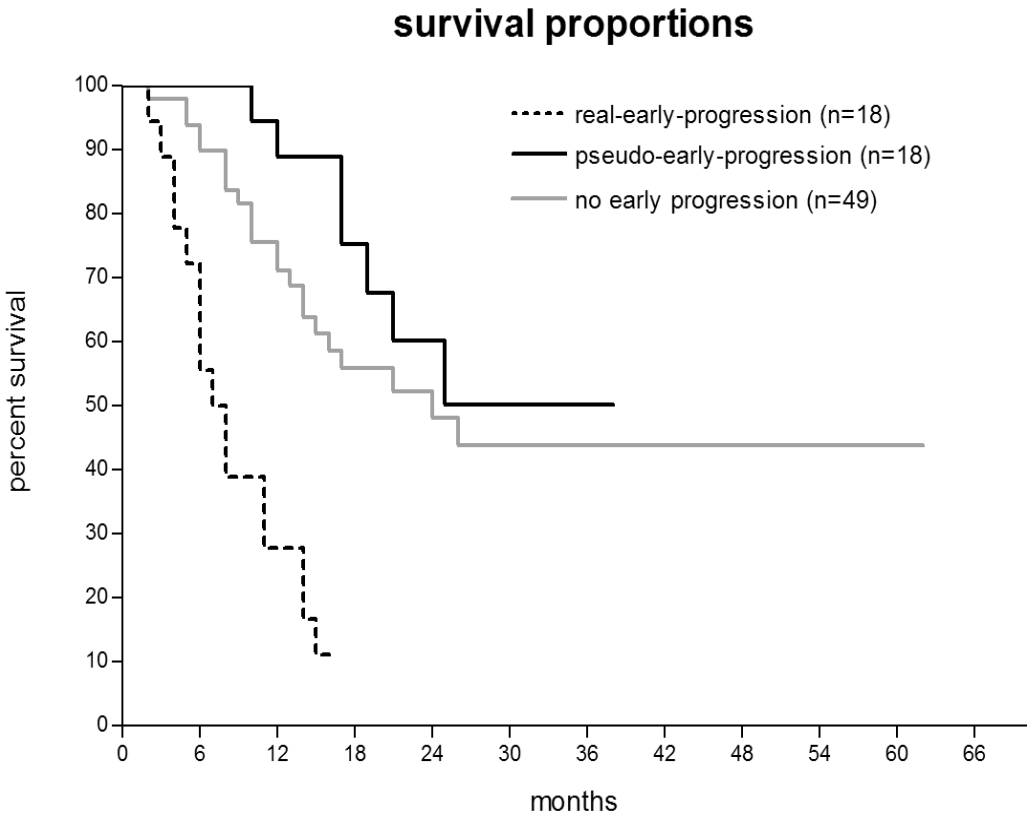

Figure 2. Kaplan-Meyer survival curves of malignant glioma patients treated with chemo-irradiation with temozolomide. Survival curves of patients developing disease progression 4 weeks after the radiotherapy and concomitant temozolomide, split into patients with further progression (real-early-progression), patients who remained stable for at least 6 months or improved (pseudo early progression) and patients without early progression (no early progression), are shown.

Neurologic deterioration was found in 6 of the 18 patients (33%) with pseudo-early progression and in 12 of the 18 patients (67%) with real early progression during RT or in the first 4 weeks thereafter. The mean age of the patients with real early progression was significantly higher compared with that of the patients with pseudo–early progression (55 years vs 46 years, respectively; P = .0342). The World Health Organization (WHO) performance status was not found to be significantly different between the patients with real early progression and those with pseudo early progression (Table 2; P = .313, chisquare) The volume of the radiation field was not found to be significantly different between the patients with real early progression, pseudo-early progression, and no early progression (data not shown). Pseudo–early progression was observed in 6 of 26 patients who underwent a biopsy (23%; 95% CI, 6.8–39.2%) and in 13 of 59 patients who underwent a partial or complete surgical resection (22%; 95% CI, 11.4–32.6%) The extent of surgical resection could not be taken into consideration because no direct postoperative scans were made. The survival curves of the patients with early disease progression (split between those

with pseudo early progression and those with real early progression) and patients with no early progression are shown in Figure 2.

DISCUSSION

In the past decades, the sporadic occurrence of early clinical deterioration with increasing imaging abnormalities immediately after RT with spontaneous recovery have been described.(5) Since the introduction of chemo-irradiation with TMZ for GBM, there has been an increasing awareness of this phenomenon. From our cohort of 85 patients treated with chemo-irradiation with TMZ, the progressive enhancement was not found to be because of tumor progression in 18 of the 36 patients (50%) with a progressive lesion at the time of first tumor evaluation after chemo-irradiation. Although the pre-RT/concomitant TMZ scan was a CT scan in approximately half of these patients, it is unlikely that this influenced the results because the outcome was the same in patients who underwent an initial MRI scan. In addition, the patients with pseudo-early progression were found to have a similar survival compared with patients without early progression (Fig. 2). A recent report examining surgery performed within 6 months from RT/concomitant TMZ in patients with GBM corroborated our findings regarding the frequency of non-tumoral increase in enhancement.(6) In that study, 26 of 51 GBM patients demonstrated disease progression within 6 months after the completion of RT/ concomitant TMZ. Fifteen of these 26 patients underwent surgery again and 7 of them were diagnosed with radiation necrosis. Although we hypothesized that pseudo–early progression would occur more frequently after RT/ concomitant TMZ compared with RT only, the incidence we observed (18 of 85 patients [21%]; 95% CI, 12.5–29.9%) is still within the range de Wit et al. observed after the use of RT only (3 of 32 patients [9%]; 95% CI, 20.7–19.5%).(2) One possible explanation for the increased awareness of the phenomenon of pseudo–early progression could be that most GBM patients are now treated with RT/TMZ and therefore are more closely followed. Conversely, in cell lines, synergy between TMZ and RT has been demonstrated in MGMT promoter gene methylated tumors.(7) It may well be that this synergistic antitumoral effect causes more profound tumor necrosis and inflammation with vascular changes, leading to a deficient blood-brain barrier mimicking enhancing tumor on a scan. Further research will explore whether there is indeed a correlation between MGMT methylation status and the occurrence of pseudo–early progression. (At the time of publication, Brandes et al have since published data regarding the incidence of pseudoprogression and the correlation with the MGMT promoter methylation status in glioblastoma patients.(3)) The increase in radiation necrosis noted to occur if chemotherapy is given after RT in patients with brain tumors also suggests that more intensified treatments cause more severe local reactions.(7) This is also the likely explanation for the earlier occurrence of radiation necrosis noted after combined chemo-irradiation with TMZ.(6, 8) Most likely, pseudo–early progression and early radiation necrosis are a continuum, with more severe local reactions leading to new focal signs and symptoms and true radiation necrosis. The precise mechanism of this early post-RT/ concomitant TMZ deterioration is unknown. The underlying mechanism may be varied and in addition to the above-mentioned radiation-induced (and perhaps vascular endothelial growth factor [VEGF] signaling-dependent) vascular and necrotic changes, tumor progression during the first part of RT and subsequent response could also be an explanation. Although to our knowledge the exact nature of this pseudo-early progression is unknown, these observations have important consequences for trials of recurrent malignant glioma. Of the 36 patients in the current study with early progression (according to the response criteria of Macdonald et al.), 3 achieved a complete response and 8 achieved a partial response, whereas 6 fulfilled the criteria for stable disease at 6 months. Six-month progression-free survival (PFS) is currently considered the most valid endpoint for phase II studies of recurrent GBM.(9) If the presently reported patients with immediate disease

progression would all have been entered in a phase II trial of recurrent GBM, this would have led to a false-positive study result with a 6-month PFS rate of 50%.

From the clinical perspective, an important question is how to differentiate between pseudo– early progression and real early progression immediately after RT. Because of the inherent risks and invasiveness of a stereotactic biopsy, it is not very attractive to obtain histologic proof of these lesions. Moreover, it is not clear whether all cases with pseudo-early progression will show only necrosis at biopsy because tumor cells may still be present. Clinical deterioration during or within the 4 weeks after RT/concomitant TMZ cannot be used to distinguish between these entities because clinical deterioration was also observed in the group of patients with pseudo-early progression, although less frequently (33% vs 67%). WHO performance score, biopsy versus surgical resection, and volume of the radiation field also cannot be used to discriminate between pseudo–early progression and real early progression. The median age in the group of patients with real early progression was found to be higher (56 years vs 46 years), which could simply reflect the higher likelihood of disease progression in elderly patients. Again, this finding is also of no value in individual patients. Furthermore, pseudo–early progression was also noted in 3 of 17 patients with anaplastic glioma (17%; 95% CI, 20.9–34.9%) versus 15 of 68 GBM patients (22%; 95% CI, 12.2–31.9%). To our knowledge, to date it has been unclear whether modern imaging techniques such as positron emission tomography, magnetic resonance spectroscopy, or diffusion-weighted and perfusion imaging can be used to make a distinction, although some data appear to be promising.(10) Patients with real early progression have a terrible outcome associated with continuing standard TMZ (Fig. 2); future efforts to better identify these patients are critical so that a potential opportunity to salvage them with an alternative therapeutic intervention is not lost. Until then, we advise continuing adjuvant TMZ in patients with early disease progression and not to include these patients in studies of malignant gliomas that recur within 3 months after RT/TMZ. Surgery should be considered in the case of patients who develop early clinical signs and symptoms and a progressive lesion. If mainly or only necrosis is found at the time of surgery, treatment with TMZ should be continued.

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Chapter 3

IDH1 mutations in low-grade astrocytomas predict survival but not response to temozolomide

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ABSTRACT

Background. Mutations in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) have been implicated in tumorigenesis of gliomas. Patients with high-grade astrocytomas with IDH1 or IDH2 mutations were reported to have a better survival, but it is unknown if this improved survival also holds for low-grade astrocytoma and whether these mutations predict outcome to specific treatment.

Methods. We retrospectively investigated the correlation of IDH1 and IDH2 mutations with overall survival and response to temozolomide in a cohort of patients with dedifferentiated low-grade astrocytomas treated with temozolomide at the time of progression after radiotherapy.

Results. IDH1 mutations were present in 86% of the 49 progressive astrocytomas. No mutations in IDH2 were found. Presence of IDH1 mutations were early events and significantly improved overall survival (median survival 48 vs 98 months), but did not affect outcome of temozolomide treatment.

Conclusion. These results indicate that IDH1 mutations identify a subgroup of gliomas with an improved survival, but are unrelated to the temozolomide response.

INTRODUCTION

Mutations in the isocitrate dehydrogenase 1 (IDH1) gene appear to occur frequently and selectively in glial tumors of the CNS.(1, 2, 3, 4, 5) The incidence of IDH1 mutations in glial tumors ranges from 12% in primary glioblastoma multiforme (GBM) to 70% in anaplastic astrocytomas (AA) and 85%–90% in low-grade astrocytomas (LGA) and secondary GBM.(1, 2, 3, 4, 5) Mutations in the related IDH2 gene were found in less than 3% of glial tumors.(5) IDH1 mutations were observed in tumors with TP53 mutations as well as in tumors with the 1p/19q codeletion. Because TP53 mutation and loss of 1p/19q are mutually exclusive aberrations, IDH1 mutations seem to occur very early on in glial tumor development at the time when the stem cell can still give rise to both types of glial cell lineages.

In retrospective series, patients with high-grade astrocytomas with IDH1 or IDH2 mutations were reported to have a better survival.(5) It is at present unclear if this improved survival also holds for LGA and, perhaps even more importantly, whether the mutation predicts outcome to specific treatment. We therefore analyzed retrospectively the clinical significance of IDH1 or IDH2 mutations in a cohort of LGA patients treated with temozolomide (TMZ) at the time of progression after prior radiotherapy (RT) for the outcome to TMZ treatment.

METHODS

Data of all patients from 5 hospitals in the Netherlands (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI and VUMC, Amsterdam; RUNMC, Nijmegen) with progressive LGA after RT treated with TMZ in the period of 1995 to 2006 were retrospectively collected. The study was approved by the local institutional review board and conducted according to national regulations. Patients were eligible for this study if they had 1) a histologically confirmed LGA or a histologically confirmed high- grade astrocytoma after a “wait and see” policy for a low-grade nonenhancing lesion for more than 1.5 years and 2) a progressive lesion after prior RT.

All patients received 200 mg/m2/day TMZ on day 1 to 5 of a 28-day cycle up to 12 cycles or until progression. Response was assessed with MRI using Macdonald’s criteria; complete and partial responses were considered objective responses.(6) The histology of all tumor samples was centrally reviewed (J.M.K.). DNA was extracted from formalin-fixed paraffin-embedded tissues as previously described from selected areas enriched for a high tumor cell percentage.(7)

IDH1 and IDH2 alterations of the mutational hotspot codons R132 and R172 were assessed by bidirectional cycle sequencing of PCR-amplified fragments. Primers used were IDH1-forward 5’-CTCCTGATGAGAAGAGGGTTG-3’ and IDH1-reverse 5’-TGGAAATTTCTGGGCCATG-3’ and IDH2-forward 5’-TGGAACTATCCGGAACATCC-3’ and IDH2- reverse 5’-AGTCTGTGGCCTTGTACTGC-3’. TP53 exon 4-9 mutation analysis was performed by bidirectional sequencing of PCR products as described elsewhere.(8) Chromosomal arms 1p and 19q copy number were determined by fluorescence in situ hybridization with locus-specific probes for 1p36 and 19q13 as described elsewhere.(9) Overall survival (OS) was calculated from the first symptom of the tumor and also from the start of the TMZ treatment to the date of death. Progression-free survival (PFS) was calculated from the start of the TMZ until the date of progression. Kaplan-Meier survival curves were plotted and the survival distributions were compared with the use of the log-rank test. Because of the multiple comparisons, p values of less than 0.01 were considered to indicate significance.

RESULTS

Seventy patients were treated with TMZ for a progressive LGA after RT. Median follow-up time was 13 months (range 2– 85 months). From 49 patients tumor tissue was available for molecular analysis; from 27 patients tissue from 2 consecutive operations was available. The table shows the

patient characteristics. Original pathology of the tumor tissue from the first operation showed LGA in 39 (80%), AA in 8 (16%), and GBM in 2 (4%) of the 49 samples. All 10 patients with a high-grade astrocytoma had their first operation after a "wait and see" policy for a median of 49.5 months (range 21–138 months) for a low-grade glioma-like lesion. Central review pathology on the tumor tissue from the first operation suggested an oligodendroglial phenotype in 2 of the samples, although combined 1p/19q loss was found in only 1 of the 49 samples.

Table. Characteristics of patients treated with temozolomide for a progressive low-grade astrocytoma after radiotherapy at the start of the temozolomide.

Characteristic	**No (%) of patients, n=49**
Age, y	
Median	38
Range	25-59
Sex	
Male	29 (59%)
Female	20 (41%)
First symptom	
Epilepsy	44 (90%)
Other	5 (10%)
Surgery at 1st operation	
Biopsy	15 (31%)
Resection	34 (69%)
WHO-PS	
0-1	39 (80%)
2	10 (20%)
Temozolomide as	
1st line of chemotherapy	42 (86%)
2nd line of chemotherapy after PCV	7 (14%)

PCV: Procarbazine, CCNU and Vincristine chemotherapy, WHO-PS: World Health Organization – Performance Score

IDH1 mutations were present in 42 (86%) of the 49 samples from the last operation before the start of TMZ treatment and in 81% of the samples from the first operation. None of the tumor samples showed an IDH2 mutation. In 76% of the samples (n = 46; in 3 cases no data on p53 status could be obtained) from the last operation before the start of TMZ treatment a TP53 mutation was found (see figure 1). Nine tumors showed an IDH1, but no TP53 mutation, 4 a TP53 mutation, but no IDH1 mutation (Fisher exact test 0.619).

OS, measured from the date of the first symptom, was worse (p = 0.003) in patients without IDH1 mutations (median survival 48 vs 98 months; figure 2A). There were no differences in median age at the start of TMZ treatment (median age of 39 and 38 years), median age at the time of the first symptom (30 and 35 years), or the presenting clinical symptom (seizures or focal deficits/increased intracranial pressure) between patients with or without IDH1 mutations.

Of the 49 patients, 5 had no measurable disease after a re-resection prior to the start of TMZ chemotherapy. PFS at 6 months was 63% and PFS at 12 months was 27%. The objective response rate of the 44 patients evaluable for response was 45% (95% confidence interval 30.3%–59.7%). Median OS from the start of TMZ treatment was 11 months. There was no difference in outcome to TMZ treatment between patients with and without IDH1 mutations with respect to response rate (Fisher exact test 0.428), PFS (p = 0.423; figure 2B), or OS (p = 0.650).

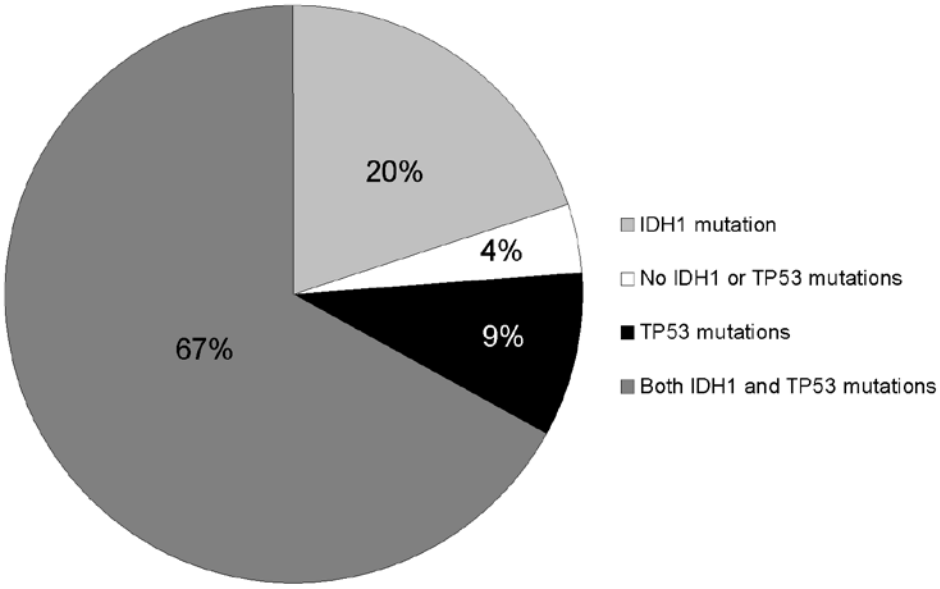

Figure 1. Frequencies of IDH1 and TP53 mutations in 46 tumor samples from the last operation before the start of temozolomide treatment for progression after radiotherapy in patients with a low-grade astrocytoma.

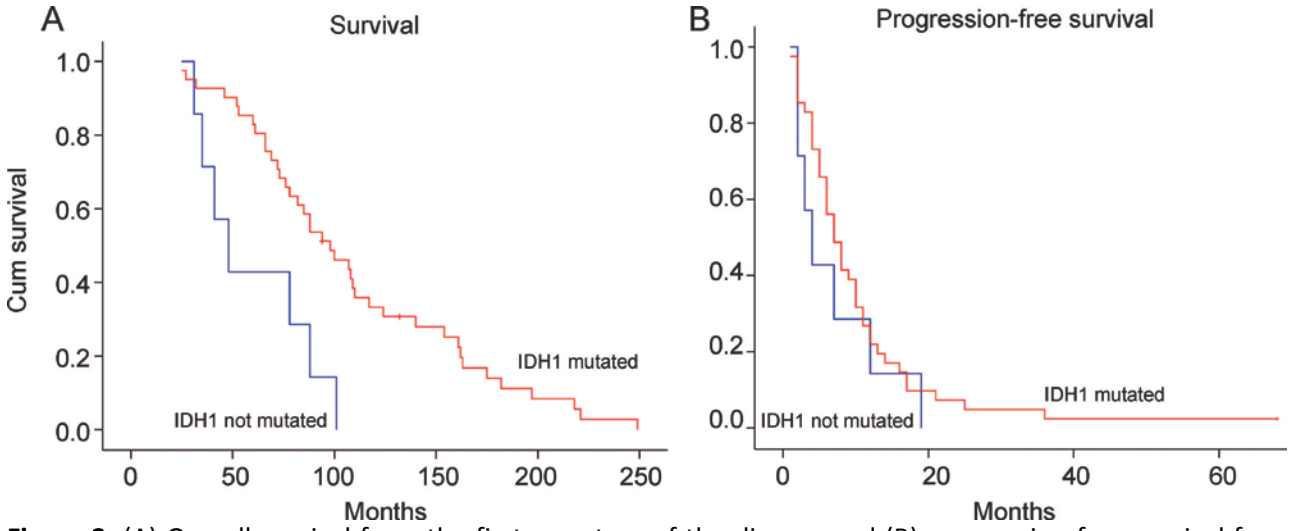

Figure 2. (A) Overall survival from the first symptom of the disease and (B) progression free survival from the start of the chemotherapy in 49 patients treated with temozolomide for a progressive low-grade astrocytoma after radiotherapy compared by IDH1 mutations.

DISCUSSION

The strong association of mutations in IDH family members with glial tumors and the prognostic significance of IDH1 mutations in high-grade glioma have recently been firmly established. (1, 2, 3, 4, 5) Our results show for the first time that IDH1 mutations in LGA are correlated with longer OS, but not with outcome to TMZ treatment at the time of tumor progression. In addition, we confirm that IDH1 mutations occur frequently (81%) and appear to be early events in LGA. We did not find

IDH2 mutations, either in early or in late stage LGA, which is in accordance with the recently described low frequency of IDH2 mutations in LGA.(5)
As expected, the incidence of TP53 mutations was also high in our series, but no correlation was found between IDH1 mutations and TP53 mutations in LGA. Although all presently available data suggest that IDH1 mutations are an early event in the gliomatogenesis, mutation of IDH1 seems not required for the genesis of TP53 mutated tumors. However, it cannot be excluded that the function of this gene or pathway in non-IDH1 mutated tumors is altered through other (epigenetic?) mechanisms or due to functional alterations up or downstream of this gene.
Similar to high-grade gliomas with IDH1 or IDH2 mutations, our results show a better prognosis for patients with IDH1-mutated LGA as compared to patients without an IDH1 mutation. (5)
The data corroborate the notion that IDH1 and IDH2 mutations are linked with improved outcome irrespective of tumor lineage or genotype (astrocytic/TP53 mutation or oligodendroglial/1p and 19q codeletion).
Despite the high clinical activity of TMZ in this study, the presence of IDH1 mutations did not identify a particularly responsive or unresponsive group of patients. It will be interesting to investigate if IDH1 mutations are related to the rate of spontaneous growth, or with response to RT.
At present, it is unclear why IDH1 and IDH2 alterations may result in selective growth advantages of gliomas. Functional studies are warranted to elucidate the underlying mechanism for this tissue selectivity and to investigate whether the presence of mutated catalytic sites of IDH1 and IDH2 could provide novel therapeutic targets for gliomas.

AUTHOR CONTRIBUTIONS
Statistical analysis was conducted by Dr. W. Taal.

DISCLOSURE
Dr. Dubbink, Dr. Taal, R. van Marion, Dr. Kros, I. van Heuvel, and Dr. Bromberg report no disclosures. Dr. B.A. Zonnenberg has received fund- ing for travel from Novartis and has received honoraria from Sanofi- Aventis. Dr. C.B.L. Zonnenberg, Dr. Postma, Dr. Gijtenbeek, Dr. Boogerd, Dr. Groenendijk, Dr. Sillevis Smitt, and Dr. Dinjens report no disclosures. Dr. van den Bent has served on scientific advisory boards for Schering-Plough Corp., Siena Biotech, Merck Serono, Bristol-Myers Squibb, and Exelixis Inc.; received speaker honoraria and funding for travel from Merck Serono; serves on the editorial boards of the European Journal of Cancer and Neuro-Oncology; receives royalties from publishing in Up-to-Date (2008–09); serves on a speakers' bureau for Schering- Plough Corp; and receives research support from Novartis, Schering- Plough Corp., and the Dutch Cancer Society.

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Chapter 4

First-line temozolomide chemotherapy in progressive low-grade astrocytomas after radiotherapy: molecular characteristics in relation to response

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ABSTRACT

Only a few studies examined the effect of temozolomide (TMZ) in recurrent low-grade astrocytoma (LGA) after surgery, none of which included a homogeneous and sufficiently sized group of patients with progression after radiotherapy (RT). We evaluated a cohort of 58 patients treated with TMZ for progression after RT of a previous LGA and investigated the relation between outcome and mutations in the IDH1, IDH2, and TP53 genes, O6-methylguanine-methyltransferase (MGMT) promoter methylation, trisomy of chromosome 7, and loss of chromosomes 1p and 19q. All patients received first-line TMZ 200 mg/m2/day on days 1–5 every 4 weeks or a progressive LGA with a contrast-enhancing lesion on MRI after RT. Six months progression-free survival (PFS) was 67%, and the median overall survival was 14 months. An objective response was obtained in 54%. TP53 mutations and loss of chromosome 19q showed a borderline association with PFS, but none of the other molecular characteristics were correlated with the outcome to TMZ. Both a methylated MGMT promoter gene and IDH1 mutations were found in 86% of the tumor samples. A correlation was found between IDH1 mutations and MGMT promoter methylation ($P < .001$). Neither MGMT promoter methylation nor IDH1 mutations correlated with PFS, but the interval between the very first symptom of the LGA and the start of the TMZ was significantly longer in the patients with IDH1 mutations ($P = .01$) and a methylated MGMT promoter ($P = .02$). We conclude that MGMT promoter methylation and IDH1 mutations seem to predict survival from the time of diagnosis, but not PFS to TMZ.

INTRODUCTION

Diffuse infiltrating low-grade glioma (LGG; WHO grade II tumors) constitute approximately 10 – 20% of primary brain tumors in adults and primarily occur in young adults.(1) The majority of LGGs are low-grade diffuse astrocytomas (LGAs). Although LGAs are usually well-differentiated and slow-growing tumors, no curative treatment exists. In most patients, progression into higher grade tumors (WHO grade III – IV) occurs during the course of this ultimately fatal disease. The standard treatment for LGA is surgical resection and radiotherapy (RT). Although the role of early surgery and extent of resection has never been proven in randomized studies, retrospective studies suggest that extensive resection may improve the outcome.(2) Radiotherapy is usually offered to patients with symptomatic and/or progressive disease or to patients with poor prognostic factors.(3, 4) As LGAs are less sensitive to chemotherapy than low-grade oligodendroglioma, chemotherapy is currently primarily used for recurrent disease. Temozolomide (TMZ) has become the mainstay of treatment in this setting. Only a few studies, however, have systematically examined the effect of TMZ in progressive LGA after surgery and RT.(5, 6) These studies are difficult to interpret because they included heterogeneous groups of patients, with both low-grade lesions and de-differentiated tumors, and with a variety of prior treatments.

Furthermore, there are limited data on molecular correlates with the outcome to chemotherapy in patients with progressive LGA after RT. At the molecular level, LGAs frequently show TP53 mutations, isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) gene mutations, trisomy of chromosome 7, and O6-methylguanine-methyltransferase (MGMT) promoter methylation. It is at present unclear whether the different molecular subtypes respond differently to TMZ. TP53 mutations are found in 39 – 43% of the patients with LGA.(7, 8) Recent studies have shown that 90% of LGA have mutations in the IDH1 gene and that patients with IDH1 mutations have a better survival.(9, 10) Additionally, IDH1 mutations were found not to predict the outcome in recurrent LGA to TMZ treatment.(10) Polysomy of chromosome 7 was observed in 66% of LGAs and correlated with survival.(11) The nuclear enzyme MGMT is a key factor in the resistance against alkylating and methylating chemotherapy. Clinical evidence suggests that the methylation of the MGMT gene promoter (mMGTM) is related to the outcome to TMZ treatment in newly diagnosed glioblastoma.(12) However, data on mMGMT in LGA are scarce, and the relevance of MGMT promoter methylation of recurrent LGA in the response to TMZ is unclear.

We conducted a retrospective multicenter study in a cohort of patients with LGA treated with TMZ at the time of progression into a high-grade glioma after prior RT to evaluate the therapeutic efficacy of the standard TMZ regimen with respect to response, survival, and toxicity. Outcome was correlated with 19q loss, trisomy of chromosome 7, MGMT promotor methylation, and IDH1 and TP53 mutations.

METHODS

Data of all patients from 5 hospitals in the Netherlands (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI and VUMC, Amsterdam; RUNMC, Nijmegen) with a progressive LGA after RT, treated with TMZ since TMZ became available until 2006, were retrospectively collected. The study was approved by the local institutional review board. Patients were eligible for this study if they had (i) either a histologically confirmed LGA or a histologically confirmed high-grade astrocytoma after a "wait and see" policy for a low-grade non-enhancing lesion for >1.5 years and (ii) a progressive and enhancing lesion at least 3 months after prior RT. The clinical details in relation to IDH1 mutations in these patients have been published elsewhere.(10) For the present study, 12 patients who received TMZ after prior chemotherapy were excluded from further analysis.

Patients received 200 mg/m^2/day TMZ on days 1–5 of a 28-day cycle up to 12 cycles or until progression with dose reductions as described elsewhere.(13) The primary objectives of the study

were the assessment of progression-free survival (PFS) and objective response rate (ORR) (complete and partial response), with overall survival (OS) as a secondary objective measured from the day of the start of the TMZ. Response was assessed with 3 monthly MRIs using Macdonald's criteria and evaluating the enhancing area of the tumor.(14) Patients treated with TMZ after prior surgery were not evaluable for response but only for (progression-free) survival. Response to treatment and the histology of all tumor samples were centrally reviewed (respectively, by W.T. and J.M.K.).

Molecular studies

DNA was extracted from formalin-fixed paraffinembedded tissues as previously described from selected areas enriched for a high tumor cell percentage.(15) IDH1 and IDH2 alterations of the mutational hotspot codons R132 and R172 were assessed by bidirectional cycle sequencing of PCR-amplified fragments. Primers used were IDH1-forward 5'-CTCCTGATGAGAAGAGGGTTG-3' and IDH1-reverse 5'-GGAAATTTCTGGGCCATG-3' and IDH2-forward 5'-TGGAACTATCCGGAACATCC-3' and IDH2-reverse 5'-AGTCTGTGGCCTTGTACTGC-3'. MGMT promoter methylation was assessed using methylation-specific multiplex ligation – dependent probe amplification (MS-MLPA).(16, 17) The MLPA kit contains 3 methylation-sensitive probes that recognize CpG dinucleotides within the MGMT promoter (MGMT 1: 2239-L1261; MGMT 2: 5670-L5146; and MGMT 3: 7188-L5144). The MS-MLPA results were normalized by dividing the peak height of each MGMT probe signal by the mean peak height of the 8 control fragments within the same sample. To estimate the degree of methylation, normalized values of each MGMT probe within digested DNA samples were divided by normalized values of corresponding undigested samples. This resulted in methylation ratios for the individual MGMT probes, which were averaged (MGMTav). Methylation analyses were performed in duplicate, and the average ratios of each experiment and for each probe were calculated. Spearman rank correlations were used to compare the outcome of the two analyses. For the analyses with outcome, the MGMTav score was used. Analyses were done with MGMTav score as a binary variable using cutoff >0.30 as indicative of methylation.

TP53 exon 4–9 mutation analysis was performed by bidirectional sequencing of PCR products as described elsewhere.(18) The copy numbers of chromosomal arms 1p and 19q were determined by fluorescence in situ hybridization (FISH) with locus-specific probes for 1p36 and 19q13 as described elsewhere.(19) Determination of trisomy 7 was assessed with FISH as described elsewhere.(20)

Statistical Analysis

Overall survival was calculated from the start of the TMZ treatment to the date of death. PFS was calculated from the start of the TMZ until the date of progression or death, whichever came first. Kaplan–Meier survival curves were constructed, and the survival distributions between subgroups (19q loss, no vs yes; trisomy 7, no vs yes; TP53, no vs yes; IDH1, no vs yes; and MGMTav ≤ 0.3 vs >0.3) were compared using the log-rank test. All reported P values were two sided; in this exploratory analysis, no adjustments were made for multiple testing.

RESULTS

Fifty-eight patients were treated with first-line TMZ for a progressive LGA after RT. Twelve of the 58 TMZ-treated patients had no measurable disease after a prior resection. Median follow-up time was 14 months (range, 4–104 months). Table 1 shows the patient characteristics of the 58 patients.

Table 1. Characteristics of the 58 patients treated with first line temozolomide for a progressive low-grade astrocytoma after radiotherapy.

Characteristic	**No. (%) of patients, *n = 58***
Age, years	
Median	38
Range	25-59
Sex	
Male	34 (59)
Female	24 (41)
First symptom	
Epilepsy	53 (91)
Other	5 (9)
Surgery	
Biopsy	22 (38)
Resection	36 (62)
Interval between first surgery and start of TMZ	
Median	47 months
Range	8-210 months
WHO performance score	
0-1	53 (91)
2	5 (9)

TMZ: temozolomide

For 54 of 58 patients, tumor tissue was available for central review and molecular analysis; for 53 patients, tumor tissue of the first operation was available; for 1 patient, the tumor tissue of the second surgery before the start of the TMZ was available, and for 28 patients, tissue from 2 consecutive operations were available. Original pathology of the tumor tissue from the first operation showed LGA in 83% (Table 2).

All 10 patients with a high-grade astrocytoma had their first operation after a “wait and see” policy for an LGG-like lesion for >1.5 years (median, 50 months; range, 21–138 months). Central review pathology (Table 2) on the tumor tissue from the first operation suggested an oligodendroglial phenotype in 3 of the samples, but 1p and/ or 19q loss was not found in any of these samples. In 3 other cases, combined 1p/19q loss was found, although all 3 showed astrocytic morphology at central review. These 3 patients with combined 1p/19q loss had a complete response on the TMZ treatment and had a PFS between 12 and 17 months. The median number of cycles of TMZ was 8 (range, 1–18 cycles). Ten patients had transient grade III/IV hematological toxicity, but none of the patients had to discontinue TMZ because of toxicity. An objective response was obtained in 25 (54%) of the 46 patients evaluable for response (complete response 26%, partial response 28%). The median PFS of the 58 patients treated with TMZ was 8 months, PFS at 6 months was 67% (95% confidence interval [CI] 54– 78%), and PFS at 12 months was 25% (CI 15–37%). Median OS was 14 months; OS at 12 months was 60% (CI 46–71%) and 23% (CI 13–34%) at 2 years.

Table 2. Original and central pathology review of the samples from the first operation in patients treated with first line temozolomide for a progressive astrocytoma after radiotherapy

Original pathology (*n = 58*)			**Central review pathology (*n = 53*)**		
	n	***%***		***n***	***%***
Astrocytoma	48	83	Astrocytoma	36	68
Anaplastic astrocytoma*	8	14	Anaplastic astrocytoma	9	17
Glioblastoma*	2	3	Glioblastoma	5	9
			Oligodendroglioma	1	2
			Anaplastic oligo-astrocytoma	2	4

***** All high-grade histologies had been followed for >1.5 years for an nonenhancing lesion suggestive of a low-grade glioma

For the molecular studies, we used the 54 tumor samples from the last operation before the start of the TMZ; 29 samples were from the second operation, and 25 samples were from the first operation. Not all molecular characteristics could be determined in all samples, mainly because of technical problems (in particular, small tissue samples). Molecular characteristics of the tumor samples are shown in Table 3.

Table 3. Molecular characteristics of the tumor samples from the last operation before the start of the TMZ in patients with a progressive astrocytoma after radiotherapy

Molecular characteristic	**Number of patients (%)**
IDH1 (n=42)	
Wild type	6 (14)
Mutated	36 (86)
IDH2 (n=42)	
Wild type	42 (100)
Mutated	0 (0)
1p loss (n=49)	
Lost	3 (6)
Intact	46 (94)
19q loss (n=47)	
Lost	9 (19)
Intact	38 (81)
Combined 1p/19q loss (n=47)	3 (6); all astocytoma at review
Trisomy 7 (n=48)	
No	17 (35)
Yes	31 (65)
TP53 (n=40)	
Wild type	9 (22)
Mutated	31 (78)
MGMT status (n=42)	
Methylated	36 (86)
Unmethylated	6 (14)

TMZ: temozolomide, IDH1: isocitrate dehydrogenase 1, Trisomy 7: trisomy of chromosome 7, MGMT: O6-methylguanine-methyltransferase promotor

The MLPA provided stable results, and test-retest results were highly correlated ($P < .0001$). A very strong correlation was found between IDH1 mutations and a methylated MGMT promotor ($n = 40$; $P < .001$); except for one case, all tumors with mMGMT had IDH1 mutations. No significant associations were found between the other investigated molecular characteristics. In 3 out of 20

patients with a second tissue sample from a second operation, the MGMT promoter status changed in time; in 2 patients, it changed from unmethylated to methylated (MGMTav 0.29 vs 0.50 and MGMTav 0.21 vs 0.77); and in 1 patient, it changed from methylated to unmethylated (MGMTav 0.35 vs 0.23).

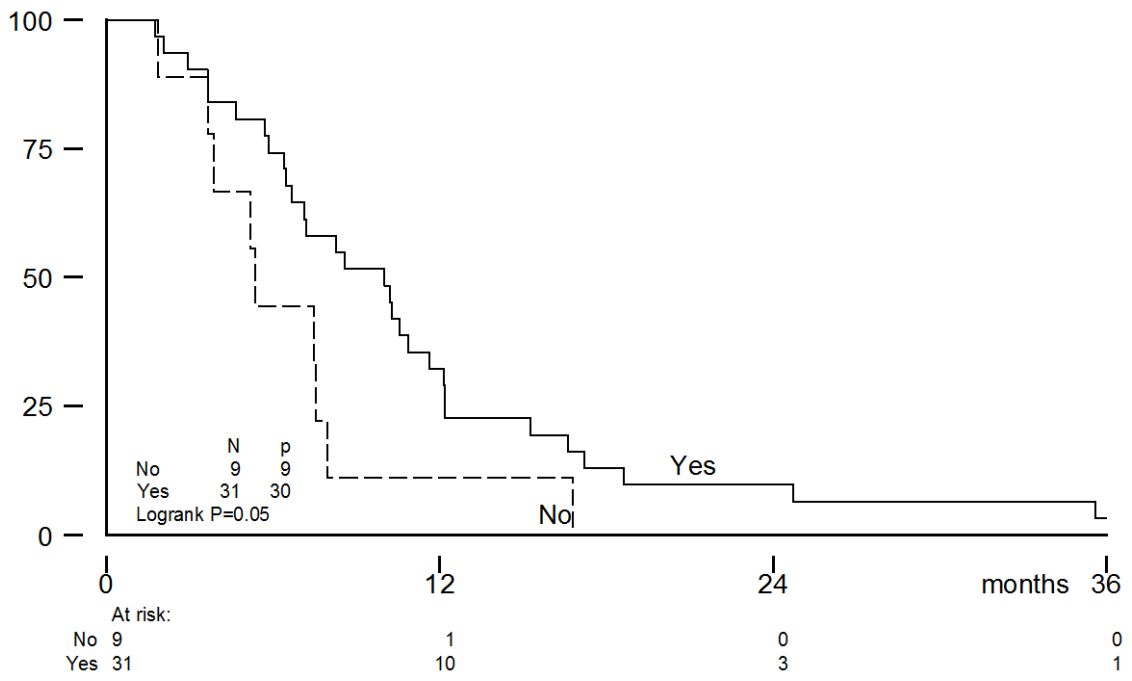

Figure 1. Progression free survival according to *TP53* mutations in patients (n=40) treated with first line chemotherapy with temozolomide for a progressive astrocytoma after radiotherapy

Table 4. Six- and 12 month PFS and median OS related to molecular characteristics in patients treated with first line TMZ for a progressive astrocytoma after radiotherapy.

Molecular characteristic	6 mo-PFS (%)	12 mo-PFS (%)	Hazard ratio (95%-CI)	Median OS (Months)	Hazard ratio (95%-CI)
IDH1 (n=42)					
Wild type	50	33	0.83 (0.34-1.98)	16	0.86 (0.36-2.06)
Mutated	67	22		12	
Trisomy 7 (n=48)					
No	59	24	1.00 (0.55-1.81)	14	1.07 (0.58-1.98)
Yes	74	23		14	
TP53 (n=40)					
Wild type	44	11	0.48 (0.22-1.03)	11	0.84 (0.39-1.80)
Mutated	74	32		14	
MGMT status (n=42)					
Methylated	72	25	0.55 (0.21-1.45)	12	0.73 (0.28-1.89)
Unmethylated	20	20		5	
19q loss (n=47)					
Lost	89	44	0.50 (0.23-1.09)	17	0.66 (0.30-1.42)
Intact	61	18		12	

PFS: progression free survival, TMZ: temozolomide, OS: overall survival, 95%-CI: 95% confidence interval, IDH1: isocitrate dehydrogenase 1, Trisomy 7: trisomy of chromosome 7, MGMT: O6-methylguanine-methyltransferase promoter

There was a trend toward longer PFS in patients harboring a tumor with TP53 mutations (P = .054, Fig. 1, Table 4), with a 6-month PFS of 74% in the TP53-mutated patients vs 44% in the non-TP53-mutated patients. Although the median age in the non-TP53–mutated patients was somewhat higher (42 vs 38 years), it was not statistically significant (P = .123; Wilcoxon rank-sum test). No association was found between TP53 mutations and histology grade at original pathology (P = .254; Fisher's exact test) or central review (P = 1.000; Fisher's exact test), and 9 of 11 patients with a high-grade astrocytoma (central review pathology) at first surgery had TP53 mutations, consistent with a de-differentiation from a low-grade precursor lesion.

There was no difference in the outcome to TMZ treatment between patients with mMGMT or an unmethylated MGMT gene promoter (uMGMT) with respect to PFS or OS (Table 4), although the interval between the very first symptom of the LGA and the start of the TMZ was longer in the patients with mMGMT (P = .02), with a median interval of 3.3 years in the unmethylated patients vs 6.0 years in the methylated patients. This interval was also longer in the patients with IDH1 mutations, with a median interval of 2.8 years in the nonmutated patients vs 7.0 years in the mutated patients (P = .01). Trisomy of chromosome 7 and loss of chromosome 19q was not associated with PFS and OS (Table 4), although a borderline association was noted for 19q loss and PFS (P = .07).

DISCUSSION

In this cohort of patients treated with first-line TMZ for a progressive and enhancing LGA after prior RT, the 6-month PFS was 67%, with an ORR of 54%. This 67% 6-month PFS lies between the 6-month PFS of anaplastic astrocytoma as reported in the pivotal TMZ trial and the outcome of recurrent anaplastic oligodendroglioma; still OS was in the same range as in the anaplastic astrocytoma trial.(13, 21) The most striking finding with respect to the molecular characteristics was the strong correlation between mMGMT and IDH1 mutations and the longer interval between the very first symptom of the LGA and the start of the TMZ in these patients. However, none of the molecular characteristics was clearly correlated with the outcome to TMZ treatment, although a trend toward improved PFS was observed in patients with tumors with TP53 mutations.

The improved PFS in patients with TP53 mutations does not seem to be related to age or tumor grade, although our sample size is limited. Little is known about the relation between TP53 and the response to TMZ in LGA, and the preclinical data are conflicting. In vitro it has been shown that TP53 makes glioma cells more sensitive to TMZ, by the down regulation of MGMT.(22) However, another study with glioma cell lines showed that abrogation of TP53 wild-type function strongly attenuates TMZ cytotoxicity.(23) In addition, melanoma cell lines expressing wild-type TP53 were more resistant to TMZ.(24) More research on this subject and confirmation in an independent dataset is needed, but two explanations are possible: (a) there is indeed a functional relation between TP53 mutations and the sensitivity to TMZ and (b) TP53 mutations identify a more sensitive LGA independent of the TP53 status.

Our results confirm a higher percentage (86%) of mMGMT in LGA as observed by others.(25, 26, 27, 28) Preliminary studies have yielded contradictory results on the predictive value of MGMT methylation status with respect to survival after and an objective response to chemotherapy. In the study by Nakasu et al.(28) the methylation status of MGMT did not affect the OS, whereas Komine et al.(29) unexpectedly reported that mMGMT was an independent predictor of shortened PFS in the absence of treatment. Everhard et al.(27) investigated 68 LGGs treated with TMZ and reported that the presence of mMGMT correlated with a longer PFS. In agreement with these results, Levin et al.(30) observed a correlation between response and MGMT expression as measured by immunohistochemistry in a small cohort of only 9 patients with progressive LGG treated with TMZ. In our cohort, we did not find a correlation between MGMT status and outcome

to TMZ in progressive LGA after RT, but our results suggest that mMGMT in LGA is correlated with longer survival from the time of diagnosis, with a longer interval between the first symptom and the start of salvage TMZ for progression after RT. However, these findings may need to be interpreted differently in view of the very strong correlation between MGMT promoter methylation and IDH1 mutations in this series. A correlation between IDH1 mutations and MGMT promoter methylation was also noted in studies of anaplastic gliomas.(31, 32) Other studies found a better outcome to RT in mMGMT anaplastic gliomas, suggesting that mMGMT is an epiphenomenon for a molecular glioma subtype.(17, 31, 33) This now appears to be related to IDH1 mutations. In a previous study on the IDH1 mutations in LGG that in part included the patients from the present report, we showed that IDH1 mutations in LGA predict survival but not response to TMZ at the time of tumor progression.(10) In view of the correlation between IDH1 and mMGMT, the current finding of a longer time to progression in the mMGMT cases appears to be related to the presence of an IDH1 mutation. Levin et al.(30) observed a correlation between the absence of 1p loss and high levels of MGMT expression, but it is as yet unclear whether this also correlates with the presence of IDH1 mutations. The strong correlation between IDH1 mutations and MGMT promoter methylation raises questions as to how IDH1 mutations are related to mMGMT and how the improved outcome of these tumors may be explained at a more mechanistic level. Recently, a glioma-CpG island methylator phenotype (G-CIMP) has been indentified, with hypermethylation at a large number of CpG loci, including the MGMT promoter-associated CpG island.(34) The G-CIMP was more prevalent among LGGs and was tightly associated with IDH1 mutations. Furthermore, patients with G-CIMP were younger at the time of diagnosis and displayed a significantly improved survival. These results hint that mMGMT may be part of a more extensive hypermethylation, but the question remains as to why this occurs in IDH1-mutated tumors.

Our study suggests that the evaluation of MGMT promoter methylation status and IDH1 mutation analysis in LGA patients are not predictive of the outcome to TMZ chemotherapy, although they may be used to identify patients with good prognosis. Prospective studies will be required to expand the reach of our observations and to clarify the links between IDH1 mutations and MGMT promoter methylation.

Conflict of interest statement. W.T., H.J.D., C.B.L.Z., B.A.Z., T.J.P., J.M.., W.B., F.H.G., J.M.K., M.C.M.K., R.M., I.H., B.H., J.E.C.B., P.A.E.S.S., and W.N.M.D. report no disclosures. M.J.B. has served on scientific advisory boards and on the speakers' bureau of Schering-Plough.

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Chapter 5

Dose dense 1 week on/1 week off temozolomide in recurrent glioma: a retrospective study

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ABSTRACT

Alternative temozolomide regimens have been proposed to overcome O6-methylguanine-DNA methyl- transferase mediated resistance. We investigated the efficacy and tolerability of 1 week on/1 week off temozolomide (ddTMZ) regimen in a cohort of patients treated with ddTMZ between 2005 and 2011 for the progression of a glioblastoma during or after chemo-radiation with temozolomide or a recurrence of another type of glioma after radiotherapy and at least one line of chemotherapy. Patients received ddTMZ at 100–150 mg/m^2/d (days 1–7 and 15–21 in cycles of 28-days). All patients had a contrast enhancing lesion on MRI and the response was assessed by MRI using the RANO criteria; complete and partial responses were considered objective responses. Fifty-three patients were included. The median number of cycles of ddTMZ was 4 (range 1–12). Eight patients discontinued chemotherapy because of toxicity. Two of 24 patients with a progressive glioblastoma had an objective response; progression free survival at 6 months (PFS-6) in glioblastoma was 29%. Three of the 16 patients with a recurrent WHO grade II or III astrocytoma or oligodendroglioma or oligo-astrocytoma without combined 1p and 19q loss had an objective response and PFS-6 in these patients was 38%. Four out of the 12 evaluable patients with a recurrent WHO grade II or III oligodendroglioma or oligo- astrocytoma with combined 1p and 19q loss had an objective response; PFS-6 in these patients was 62%. This study indicates that ddTMZ is safe and effective in recurrent glioma, despite previous temozolomide and/or nitrosourea chemotherapy. Our data do not suggest superior efficacy of this schedule as compared to the standard day 1–5 every 4 weeks schedule.

INTRODUCTION

Gliomas are the most common primary brain tumors in adults and are usually classified and graded according to the World Health Organisation (WHO). Currently, chemotherapy is standard of care for all diffuse gliomas, either at first diagnosis or at first recurrence.(1, 2, 3, 4, 5) The most frequently used agents are temozolomide (TMZ) and nitrosoureas. Treatment options for patients failing radiotherapy and a first line of alkylating or methylating chemotherapy are limited. The cytotoxic effect of TMZ is mediated primarily via methylation at the O6 position of guanine. One of the main mechanisms of tumor resistance to TMZ is thought to be mediated by O6-methylguanine-DNA methyltransferase (MGMT).(6) Evidence supporting this role of MGMT comes from clinical studies indicating that hypermethylation of the promoter of MGMT is associated with improved tumor response and survival in patients with GBM.(7, 8) Because of the more continuous exposition with ddTMZ it has been assumed that dose dense temozolomide (ddTMZ) schedules could over- come MGMT dependent resistance against TMZ by a more effective depletion of deplete intracellular levels of the DNA repair enzyme, MGMT.(9) Studies using ddTMZ show it is well tolerated and generally safe, also when given in higher monthly doses and in patients who have previously received TMZ.(10, 11, 12, 13, 14, 15, 16, 17, 18) We used the 1 week on/1 week off TMZ regimen (ddTMZ) for patients with relapsing GBM or other glioma after prior TMZ or nitrosourea chemotherapy to study the efficacy and toxicity of ddTMZ in heavily pre-treated patients with high-grade glioma.

MATERIAL AND METHODS

Data of all diffuse glioma patients treated with ddTMZ after prior chemotherapy in our center were retrospectively collected. The study was approved by the local institutional review board. Patients were included in this study if they had a histologically confirmed low-grade glioma or high-grade glioma, with a progressive and measurable enhancing tumor on the MRI (diameter [2 cm), relapsing after prior radiotherapy and at least one line of chemotherapy, and had concluded RT at least 3 months prior to the diagnosis of progression. We collected data about patient characteristics, tumor characteristics, prior treatment, number of ddTMZ cycles, use of dexamethasone, adverse effects, reason of discontinuation, and further treatments. According to histology three categories of patients were distinguished: patients treated with ddTMZ for a progressive primary GBM after radiotherapy and TMZ (group A); patients with recurrent astrocytoma WHO grade II or III (group B), or recurrent oligo-astrocytoma WHO grade II or III, without 1p and 19q loss; and patients with progressive WHO grade II or III oligodendroglioma or oligo-astrocytoma with 1p and 19q loss (group C). WHO grade II tumors were combined with WHO grade III tumors because all patients had contrast enhancing lesions on the MRI scan at the time of treatment with ddTMZ, suggesting malignant dedifferentiation of the WHO grade II tumors. Furthermore, a previous study with TMZ in recurrent WHO grade II astrocytoma, with enhancement on the MRI-scan, at our institution has shown similar results to the pilot trial of TMZ in recurrent WHO grade III gliomas (PFS at 12 months 25% vs. 24%).(3, 4)

Patients received TMZ on day 1–7 and on day 15–21 of a 28-day cycle for up to 12 cycles or until documented disease progression or unacceptable toxicity. The starting dose of the TMZ was 100 mg/m^2/day. In the absence of toxicity or only CTCAE grade 1 toxicity during the first two treatment weeks the dose was escalated, in two steps to dose level 1 (150 mg/m^2/day; Table 1 for dose levels). Toxicity was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE; version 3.0). In case of hematological toxicity grade 4 or non-hematological toxicity grade 3, the dosage TMZ of the next cycle was reduced with 1 dose level. In case of CTCAE grade 4 non-hematological toxicity, the patient stopped treatment. In case of a grade 4 hematological toxicity or a grade 3 non-hematological toxicity at dose level 4 (75 mg/m^2/day), the patient went off

treatment. In case of dose reductions, dose re-escalation was not allowed. Blood examinations were done on day 15 and day 29 and when platelets were above $100*10^9$/l and neutrophils counts above $1.5*10^9$/l, the following 7 days TMZ was administered. Otherwise treatment was postponed until recovery to BCTCAE grade 1 and/or platelets were above $100*10^9$/l. The treatment was stopped if it had to be postponed for more than 2 weeks.

Table 1. Dose levels of dose dense temozolomide

dose level	Daily temozolomide dose	Dose temozolomide per cycle
1	150 mg/m^2/day	2100 mg/m^2
2	125 mg/m^2/day	1750 mg/m^2
3	100 mg/m^2/day	1400 mg/m^2
4	75 mg/m^2/day	1050 mg/m^2

The objectives of the study were the assessment of progression free survival at 6 (PFS-6) and 12 (PFS-12) months, objective response rate (ORR), overall survival (OS), and toxicity. OS was calculated from the start of the TMZ treatment to the date of death. PFS was calculated from the start of the TMZ until the date of progression or death. Response was assessed using RANO criteria.(19, 20) Both complete and partial responses were considered objective responses. Clinical evaluation was done every 4 weeks and MRI was made every 12 weeks or in case of neurological deterioration. Response to treatment was reviewed as part of this analysis (W.T.). In this exploratory analysis, no adjustments were made for multiple testing.

Table 2. Characteristics of patients treated with dose dense temozolomide for a progressive glioma after radiotherapy and 1 or 2 lines of chemotherapy

Characteristic	All patients No. (%) n=53	Group A No. (%) n=24	Group B No. (%) n=16	Group C No. (%) n=13
Age, years				
Median	49	52	43	44
Range	31-74	31-74	32-61	33-60
Sex				
Male	38 (72%)	14 (58%)	13 (81%)	11(85%)
Female	15 (28%)	10 (42%)	3 (19%)	2 (15%)
First symptom				
Epilepsy	36 (68%)	11 (46%)	13 (81%)	12 (92%)
Other	17 (32%)	13 (54%)	3 (19%)	1 (8%)
WHO-PS				
0	15 (28%)	6 (25%)	5 (31%)	4 (31%)
1	27 (51%)	16 (67%)	7 (44%)	4 (31%)
2	11 (21%)	2 (8%)	4 (25%)	5 (38%)
WHO histology grade at first operation				
2			10 (63%)	6 (46%)
3			6 (37%)	7 (54%)

WHO: World Health Organisation, PS: Performance Score, Group A: patients with recurrent primary glioblastoma, Group B: patients with recurrent WHO grade II or III astrocytoma or oligodendroglioma or oligo-astrocytoma without combined 1p and 19q loss and with a contrast enhancing lesion on MRI, Group C: patients with recurrent WHO grade II or III oligodendroglioma or oligo-astrocytoma with combined 1p and 19q loss and with a contrast enhancing lesion on MRI

RESULTS

Between June 2005 and June 2011, 53 patients were treated with ddTMZ for the progression of a glioma in our center. Twenty-four patients were treated for a recurrent GBM (group A), 16

patients were treated for a recurrence of a WHO grade II or III astrocytoma, oligodendroglioma, or oligo-astrocytoma, without combined 1p and 19q loss, with a contrast enhancing lesion on MRI (group B) and 13 patients were treated for a recurrence of a WHO grade II or III oligodendroglioma or oligo-astrocytoma with combined 1p and 19q loss, with a contrast enhancing lesion on MRI (group C).

Tables 2 and 3 show the patient characteristics of the 53 patients. All GBM patients progressed after chemo-irradiation with TMZ, except for one patient who had progression after radiotherapy and during the 6th cycle of 1st line standard day 1–5 every 4 weeks schedule TMZ chemotherapy. Five GBM patients treated with chemo-irradiation progressed directly after six cycles of adjuvant TMZ; all other patients had a TMZ free interval, before the start of ddTMZ. Six patients with a recurrent primary GBM received a second line of therapy after chemo-irradiation: dendritic-cell therapy (1), cediranib (1), lomustine combined with cediranib (2), and, imatinib combined with hydroxyurea (2).

Table 3. Previous treatments of patients treated with dose dense temozolomide for a progressive glioma after radiotherapy and 1 or 2 lines of chemotherapy

Characteristic	All Patients No.(%); *n=53*	Group A No.(%); *n=24*	Group B No.(%); *n=16*	Group C No.(%); *n=13*
ddTMZ as 2nd line of CT	40 (75%)	19 (79%)	11 (69%)	10 (77%)
ddTMZ as 3rd line of CT	13 (25%)	5 (21%)	5 (31%)	3 (23%)
Second operation	15 (28%)	3 (13%)	6 (38%)	6 (46%)
Third operation	1 (2%)	0	0	1 (8%)
Time between start last RT and start ddTMZ, median (range), mo	24 (5-197)	19 (8-75)	18 (5-197)	45 (10-93)
Time between last CT and start ddTMZ, median (range), mo	10 (0-94)	5 (0-67)	13 (1-92)	17 (1-94)
Prior 1st line treatment	53 (100%)	24 (100%)	16 (100%)	13 (100%)
RT/TMZ	27 (51%)	23 (96%)	3 (19%)	1 (8%)
TMZ	6 (11%)	1 (4%)	5 (31%)	0
PCV	20 (38%)	0	8 (50%)	12 (92%)
Prior 2nd line treatment	13 (25%)	5 (21%)	5 (31%)	3 (23%)
RT/TMZ	1 (2%)	0	1 (6%)	0
TMZ	5 (9%)	0	3 (19%)	2 (15%)
PCV	2 (4%)	0	1 (6%)	1 (8%)
Other	5 (9%)	5 (21%)	0	0

ddTMZ: dose dense temozolomide; CT: chemotherapy, RT: radiotherapy, Group A: patients with recurrent primary glioblastoma, Group B: patients with recurrent WHO grade II or III astrocytoma or oligodendroglioma or oligo-astrocytoma without combined 1p and 19q loss and with a contrast enhancing lesion on MRI, Group C: patients with recurrent WHO grade II or III oligodendroglioma or oligo-astrocytoma with combined 1p and 19q loss and with a contrast enhancing lesion on MRI

The median number of cycles of ddTMZ was 4 (range 1–12), three patients completed 12 cycles. Most patients stopped because of tumor progression. One patient stopped because of unrelated cholecystitis and elevated transaminases. Eight patients discontinued ddTMZ because of toxicity: grade 4 thrombocytopenia (1), persistent grade 2 or grade 3 fatigue (5), grade 3 elevated transaminases (1), and grade 3 allergic skin reaction (1). Five patients who stopped because of fatigue continued TMZ in regular regimen of day 1–5 in a 28 day cycle and tolerated this well. In 25 patients, CD4+ lymphocytes counts were monitored; 14 (56%) of these patients developed a grade 3 CD4+ lymphopenia (<0.2*10^9/l) and 6 (24%) of these patients developed a grade 4 CD4+ lymphopenia (<0.05*109/l). All patients with grade 3/4 CD4+ lymphopenia received prophylactic cotrimoxazol. None of these patients developed a pneumocystis carinii pneumonia. Two of the

patients with a grade 4 CD4+ lymphopenia developed a pneumocystis carinii pneumonia, prior to routine monitoring of CD4+ counts, from which they fully recovered.
Fifty-two patients were evaluable for response. In the patient with cholecystitis, no follow-up imaging was done. The PFS-6, the ORR (complete and partial response) and median OS for the three groups of patients are shown in Table 4. The median interval (and range) between the prior chemotherapy and the start of the ddTMZ was 5 months (0–67 months) in group A, 12.5 months (range 1–92) in group B and 17 months (range 1–94) in group C. The patients without 1p and 19q loss (group A and B) that started with the ddTMZ within 3 months of the previous chemotherapy (12 out of 40 patients) had a lower PFS-6 compared to the patients with a chemotherapy free interval of more than 3 months (PFS-6 8% vs. 43%; Fisher exact test 0.033).

Table 4. Outcome of patients treated with dose dense temozolomide for a progressive glioma after radiotherapy and 1 or 2 lines of chemotherapy.

Outcome	All patients n=53	Group A n=24	Group B n=16	Group C n=13
progression free survival at 6 months	40%	29%	38%	62%
progression free survival at 12 months	13%	13%	13%	15%
Median overall survival	9 months	6 months	9 months	19 months
Complete + partial responses	17%	8%	19%	33% (4:12)

Group A: patients with recurrent primary glioblastoma, Group B: patients with recurrent WHO grade II or III astrocytoma or oligodendroglioma or oligo-astrocytoma without combined 1p and 19q loss and with a contrast enhancing lesion on MRI, Group C: patients with recurrent WHO grade II or III oligodendroglioma or oligo-astrocytoma with combined 1p and 19q loss and with a contrast enhancing lesion on MRI

DISCUSSION

In this group of 53 chemotherapy and radiotherapy pre-treated gliomas, ddTMZ showed activity. Although this is a retrospective study with a limited sample size, our results are also comparable to other studies on dose-dense TMZ in recurrent gliomas.(12, 13, 14, 15, 16, 17)
However, in all our groups the observed activity is well within the range of previous reports on standard dosing TMZ. The PFS-6 of 29% (95%-CI 11–47%) (Table 4; group A) in GBM is within the range of the pivotal standard dose phase II TMZ trials in recurrent GBM (PFS-6: 19–24%).(21, 22, 23) More in particular, Brandes et al. described a 24% (95% CI 14–42%) for 2nd line standard dosing TMZ in recurrent GBM. The results in group B (recurrent WHO grade II or III astrocytoma or oligodendroglioma without 1p and 19q loss) are comparable to the 2nd line results in the pivotal phase II trial in recurrent anaplastic astrocytoma or anaplastic oligo-astrocytoma (PFS-6 44% versus PFS-6 38% in the present series, Table 4).(4)
The PFS-6 in group B and C (All recurrent non-primary GBM's, with a contrast enhancing lesion on MRI at the start of the ddTMZ) is higher than the PFS-6 found in the EORTC study 26972 in recurrent oligodendroglioma, with or without combined 1p/19q loss after first line chemo-therapy (Table 4: PFS-6 38–62% vs. 29%), although the PFS-12 is comparable (Table 4: PFS-12 13–15% vs. 11%).(24) Data on second line TMZ in recurrent oligodendroglial tumors with combined 1p/19q loss are scarce, Kouwenhoven et al. reported only one responder in nine patients treated after prior procarbazine, lomustine, and vincristine chemotherapy, but PFS-6 was not reported.(25)
Almost none of the patients with a primary GBM or WHO grade II or III glioma without combined 1p/19q loss (group A and B) with a chemotherapy free interval of 3 months or less before the start of the ddTMZ had a good outcome (PFS-6 8% vs. 43%; Fisher exact test 0.033). Similar to the results of Perry et al. on metronomic TMZ, ddTMZ is not effective in patients with progressive disease within 3 months of previous chemotherapy.(10) The patients with a dedifferentiated

glioma with combined 1p/ 19q loss were left out of this analysis, because these patients have a completely different prognosis and response to chemotherapy and only two patients in this group had progressive disease within the 3 months before the start of the ddTMZ. The single patient relapsing during standard TMZ and responding to ddTMZ had a progression free survival of 48 months up till now, suggesting he didn't have real tumor progression at the time of ddTMZ. Probably the enhancement on MRI in this patient was caused by radionecrosis 45 months after RT. Since all patients started ddTMZ relatively long after (chemo-) irradiation (median time between start last RT and start ddTMZ 24 months, range 5–197; Table 3), it is unlikely that pseudoprogression played a role in this study.(26)

Dose dense TMZ appears more toxic than the standard dosing regimen of TMZ. Five patients were switched to the standard day 1–5 every 4 weeks TMZ because of fatigue. After switch their fatigue improved. Two patients developed PCP infections before routine monitoring of CD4? counts, none of the monitored patients (who received PCP prophylaxis with cotrimoxazol in case CD4+ counts decreased below $0.2*10^9$/l) developed a PCP infection. Data from available phase II trials investigating ddTMZ in gliomas indicate a high incidence of lymphopenia, especially in patient treated with the 3 weeks on/1 week off regimen.(12, 14, 16, 17, 27) In melanoma patients treated with daily TMZ for 6 weeks out of every 8-week cycle, a high incidence of lymphopenia and an increased risk of opportunistic infections were reported.(28) Clearly, patients who receive ddTMZ are at risk to develop Pneumocystis carinii pneumonia, and prophylaxis is indicated in patients who develop lymphopenia or low CD4+ counts.

Although this study has a limited number of patients and is retrospective, it however seems from these results that ddTMZ is an effective treatment for patients with a recurrence of GBM or otherwise heavily pre-treated gliomas, albeit with an increase in toxicity. Whether it is more effective than the standard 5 of 28-day regimen remains unclear.

Although administration of ddTMZ regimens causes more pronounced depletion of MGMT in peripheral blood mononuclear cells, the effects of ddTMZ on MGMT activity in brain tumor tissue and its impact on clinical outcome remain unclear.(9) A study from the United Kingdom, comparing standard day 1–5 every 4 weeks TMZ with a ddTMZ schedule, (given in a 3 weeks on/1 week off schedule) failed to show any benefit of ddTMZ in high-grade glioma recurrent after RT only in comparison to the standard day 1–5 every 4 weeks schedule.(29) Of note, although these patients were chemotherapy naive, one may assume that two-thirds of patients would have an unmethylated MGMT promoter. Thus, if ddTMZ would have been effective in overcoming that resistance, one would expect at least some trend toward a more favorable outcome in ddTMZ treated patients. The recently reported RTOG 0525 trial on newly diagnosed GBM also failed to produce superior outcome of ddTMZ in newly diagnosed GBM (and regardless of the MGMT promoter status).(30) This casts further doubt on the usefulness of intensified dosing regimen. Future trials into ddTMZ regimens require a control arm with a standard dosing regimen.

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Chapter 6

Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase II trial

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Taal W, Oosterkamp HM, Walenkamp AM, Dubbink HJ, Beerepoot LV, Hanse MC, Buter J, Honkoop AH, Boerman D, de Vos FY, Dinjens WN, Enting RH, Taphoorn MJ, van den Berkmortel FW, Jansen RL, Brandsma D, Bromberg JE, van Heuvel I, Vernhout RM, van der Holt B and van den Bent MJ.

ABSTRACT

Background Treatment options for recurrent glioblastoma are scarce, with second-line chemotherapy showing only modest activity against the tumor. Despite the absence of well controlled trials, bevacizumab is widely used in the treatment of recurrent glioblastoma. Nonetheless, whether the high response rates reported after treatment with this drug translate into an overall survival benefit remains unclear. We report the results of the first randomized controlled phase II trial of bevacizumab in recurrent glioblastoma.

Methods The BELOB trial was an open-label, three-group, multicenter phase II study undertaken in 14 hospitals in the Netherlands. Adult patients (≥18 years of age) with a first recurrence of a glioblastoma after temozolomide chemo-radiotherapy were randomly allocated by a web-based program to treatment with oral lomustine 110 mg/m2 once every 6 weeks, intravenous bevacizumab 10 mg/kg once every 2 weeks, or combination treatment with lomustine 110 mg/m2 every 6 weeks and bevacizumab 10 mg/kg every 2 weeks. Randomization of patients was stratified with a minimization procedure, in which the stratification factors were center, performance status, and age. The primary outcome was overall survival at 9 months, analyzed by intention to treat. A safety analysis was planned after the first ten patients completed two cycles of 6 weeks in the combination treatment group. This trial is registered with the Nederlands Trial Register (www.trialregister.nl, number NTR1929).

Findings Between Dec 11, 2009, and Nov 10, 2011, 153 patients were enrolled. The preplanned safety analysis was done after eight patients had been treated, because of hematological adverse events (three patients had grade 3 thrombocytopenia and two had grade 4 thrombocytopenia), which reduced bevacizumab dose intensity; the lomustine dose in the combination treatment group was thereafter reduced to 90 mg/m2. Thus, in addition to the eight patients who were randomly assigned to receive bevacizumab plus lomustine 110 mg/m^2, 51 patients were assigned to receive bevacizumab alone, 47 to receive lomustine alone, and 47 to receive bevacizumab plus lomustine 90 mg/m^2. Of these patients, 50 in the bevacizumab alone group, 46 in the lomustine alone group, and 44 in the bevacizumab and lomustine 90 mg/m^2 group were eligible for analyses. 9-month overall survival was 43% (95% CI 29–57) in the lomustine group, 38% (25–51) in the bevacizumab group, 59% (43–72) in the bevacizumab and lomustine 90 mg/m^2 group, 87% (39–98) in the bevacizumab and lomustine 110 mg/m^2 group, and 63% (49–75) for the combined bevacizumab and lomustine groups. After the reduction in lomustine dose in the combination group, the combined treatment was well tolerated. The most frequent grade 3 or worse toxicities were hypertension (13 [26%] of 50 patients in the bevacizumab group, three [7%] of 46 in the lomustine group, and 11 [25%] of 44 in the bevacizumab and lomustine 90 mg/m^2 group), fatigue (two [4%], four [9%], and eight [18%]), and infections (three [6%], two [4%], and five [11%]). At the time of this analysis, 144/148 (97%) of patients had died and three (2%) were still on treatment.

Interpretation The combination of bevacizumab and lomustine met pre-specified criteria for assessment of this treatment in further phase III studies. However, the results in the bevacizumab alone group do not justify further studies of this treatment.

INTRODUCTION

Glioblastoma is the most common primary brain tumor in adults, and affects 600–800 people every year in the Netherlands. Standard care for newly diagnosed glioblastoma is surgical resection as extensively as is safely possible, followed by radiotherapy with concurrent temozolomide and six monthly cycles of adjuvant temozolomide.(1) However, the prognosis remains poor, with a median survival of only 12–16 months and only 10% of patients surviving for 4–5 years.(2) At tumor progression, treatment options are scarce and of poor effectiveness. On the basis of the results of recent phase III trials in which lomustine was used as the comparator, this drug is now one of the first-choice salvage treatment regimens.(3, 4) However, its use is limited by cumulative bone marrow suppression that might be clinically significant in patients pretreated with temozolomide. Moreover, less than 10% of patients respond and only roughly 20% of patients remain free from disease progression at 6 months. More effective treatments are urgently needed.

Glioblastomas are highly vascularized tumors in which the VEGF signaling pathway is upregulated. Bevacizumab is a humanized monoclonal antibody against circulating VEGF, and is registered by the European Medicines Agency for use in colorectal, lung, ovarian, renal, and breast cancer. Following a retrospective report showing an interesting high response rate in recurrent glioblastoma to treatment with a combination of bevacizumab and irinotecan,(5) several phase II studies have reported high responses and 6-month progression-free survival (PFS) with bevacizumab.(6, 7, 8) These findings led to the accelerated approval of bevacizumab for use in recurrent glioblastoma in the USA. However, the randomized phase II BRAIN trial that investigated bevacizumab alone and in combination with irinotecan did not show a convincing overall survival benefit of bevacizumab compared with historical series.(8) Phase III trials in patients with newly diagnosed glioblastoma also did not show an overall survival benefit with the addition of bevacizumab to chemo-radiotherapy with temozolomide, despite an impressive PFS benefit.(9, 10) Such findings mean that the overall survival benefit of bevacizumab treatment in glioblastoma remains unclear. A drawback of the many phase II trials of bevacizumab in recurrent glioblastoma is that they do not include adequate control groups of patients who are not given the drug. Owing to this absence of properly controlled trials, the registration application for recurrent glioblastoma was rejected by the European Medicines Agency. However, bevacizumab is nonetheless used widely in several European countries.

Additional reports have suggested that bevacizumab and other anti-VEGF agents might induce an infiltrative non- enhancing growth pattern (gliomatosis cerebri) at disease progression.(11, 12) Others have described a rapid normalization of abnormally permeable tumor blood vessels with a reduction of enhancement of the tumor on contrast-enhanced MRI, even in the absence of a true tumor response (i.e., a pseudo-response).(13) The clinical significance of these findings is unclear, again because of the uncontrolled nature of the studies in which these results were recorded. Moreover, other investigators did not report this different pattern of progression in patients with recurrent glioblastoma treated with bevacizumab.(14, 15, 16) Nonetheless, these early observations questioned the classical assessment of response and progression in neuro-oncology, and led to a revision of the response criteria used in trials for recurrent glioblastoma (the RANO criteria).(17, 18) These criteria give more emphasis to changes on T2-weighted or fluid-attenuated inversion recovery (FLAIR) magnetic resonance (MR) images. These observations also clearly showed that the classical outcomes of 6-month PFS and the proportion of patients who achieve an objective response, which are used routinely in trials of recurrent glioblastoma, are not the best possible endpoints for trials of bevacizumab in recurrent glioblastoma.(17, 19) We report the

results of the BELOB study, the first randomized phase II trial of the role of bevacizumab in the treatment of recurrent glioblastoma that includes a bevacizumab-free control group.

METHODS

Study design and participants

This multicenter trial was undertaken in 14 academic hospitals in the Netherlands (five university hospitals and nine community hospitals). Patients were eligible for inclusion in the trial if they had histologically proven glioblastoma with a first progression after previous chemo-radiotherapy with temozolomide, documented by MRI with at least one bi-dimensionally measurable target lesion with one diameter of at least 10 mm, visible on two or more axial slices 5 mm apart; had not received previous chemotherapy for recurrent disease; had not previously received treatment with an anti-VEGF agent or nitrosoureas; were on a stable or decreasing dose of steroids for 7 days before the baseline MRI scan; had not received radiotherapy within the 3 months before the diagnosis of progression; had not received chemotherapy in the past 4 weeks; were at least 18 years of age; had WHO performance status of 0–2; and had adequate bone marrow, renal, and hepatic function. Exclusion criteria were uncontrolled hypertension (systolic blood pressure >150 mm Hg or diastolic blood pressure >100 mm Hg), any arterial or venous thrombosis up to 6 months before registration, evidence of recent hemorrhage on brain MRI, substantial cardiac disease (e.g., history of myocardial infarction within 6 months before inclusion or unstable angina), or use of therapeutic doses of oral or parenteral anticoagulants or thrombolytic drugs. Patients undergoing surgery at the time of the recurrence were eligible if surgery had confirmed the nature of the lesion, and for these patients an immediate postoperative MRI scan done within 48 h after the operation was advised. If no measurable disease was present after surgery, patients were still eligible but were only considered for progression and survival analyses, not for response assessment. Re-operated patients could not start treatment until 4 weeks after surgery.

The applicable national and local institutional review board of each participating institution approved the study protocol. Central data management, database development and maintenance, statistical analysis, site monitoring, safety management and trial management were done by the Clinical Trial Center of the Erasmus MC Cancer Institute (Rotterdam, Netherlands). All patients provided written informed consent according to national regulations.

Randomization and masking

The study was initially designed as a two-group, open-label randomized phase II study to assess the activity of bevacizumab alone or in combination with lomustine. Eligible patients were randomized by a web-based program and stratified by a minimization procedure to ensure balance within each group and overall balance. Stratification factors were center, Eastern Cooperative Oncology Group performance status, and age. After the negative ruling of the European Medicines Agency regarding the use bevacizumab in recurrent glioblastoma,(20) the trial was modified into a three-group study by the addition of a lomustine control group. At that point, seven patients had already been enrolled and randomized. Following approval of this protocol amendment, patients were randomly allocated on a 1:1:1 basis to bevacizumab in combination with lomustine, single-agent bevacizumab, or single-agent lomustine. Because no safety data were available for the combination of bevacizumab and lomustine 110 mg/m^2, a safety analysis was preplanned for when the first ten patients had received treatment in the combination group. This safety review resulted in a dose modification of lomustine in the combination group to 90 mg/m^2. The randomization and stratification algorithm was reset to adjust for the imbalance caused by this amendment to ensure a roughly equal number of patients in each of the three treatment groups.

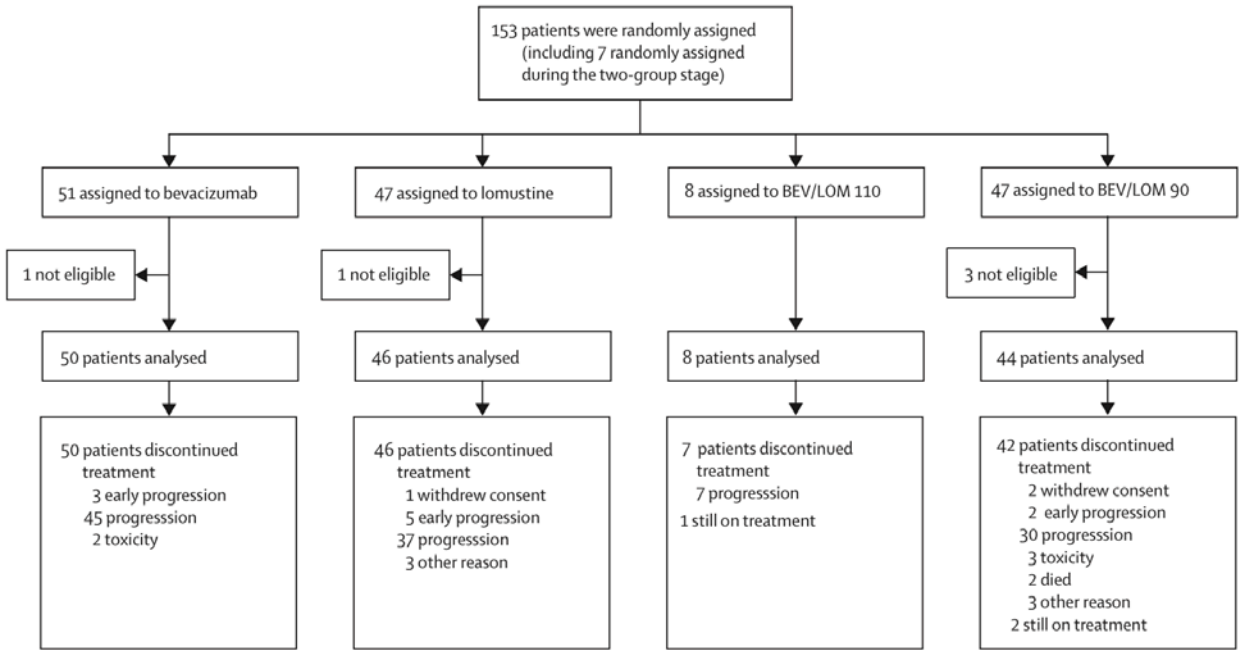

Figure 1. Trial profile
BEV/LOM110: bevacizumab plus lomustine 110 mg/m^2, BEV/LOM90: bevacizumab plus lomustine 90 mg/m^2

Procedures

Single-agent lomustine was given orally at a dose of 110 mg/m^2 (in 40 mg capsules, up to a maximum dose of 200 mg) on day 1 every 6 weeks with prophylactic anti-emetic drugs, for a maximum of six treatment cycles (in which one treatment cycle was defined as 6 weeks). Single-agent bevacizumab was given intravenously at a dose of 10 mg/kg every 2 weeks until disease progression. In the combination group, lomustine was initially given at 110 mg/m^2 every 6 weeks, with a maximum lomustine dose of 200 mg per cycle of 6 weeks. After the preplanned safety review, we reduced the lomustine dose for the rest of the patients in the combination group to 90 mg/m^2, with a maximum lomustine dose of 160 mg per cycle of 6 weeks.

We assessed hematological parameters in all patients every 2 weeks, and serum chemistry every 6 weeks. For the next cycle of lomustine to be administered, recovery of platelets to more than 100 × 10^9 per L and neutrophil count higher than 1.5 × 10^9 cells per L was needed. In cases where treatment delays exceeded 4 weeks, the patients were taken off treatment. The protocol allowed two dose reductions for lomustine (to either 90 mg/m^2 or 70 mg/m^2 for patients starting on 110 mg/m^2, and to 70 mg/m^2 or 50 mg/m^2 for those starting on 90 mg/m^2). Bevacizumab was withheld if diastolic blood pressure was 100 mg or higher or systolic blood pressure was 150 mm Hg or higher. In cases of platelet counts lower than 50 × 10^9 per L, bevacizumab was discontinued until platelets had recovered to 75 × 10^9 per L. The protocol provided detailed guidelines for discontinuation of treatment in case of toxicity, and for management of bevacizumab toxicity, including allergic reactions, proteinuria, and hypertension. If in the combination group, one of the drugs (either lomustine or bevacizumab) had to be discontinued for toxicity reasons, the patient could continue to receive the other drug. The relative dose intensity was calculated as the ratio of the dose intensity of chemotherapy that was actually delivered relative to the standard dose intensity of the 6-week cycles.

Patients were followed with MRI scans after every cycle for the first four treatment cycles, and thereafter following every other cycle (or earlier if clinically indicated). The local investigator assessed response and disease progression using the RANO criteria, and complete and partial responses were regarded as an objective response.(18) Response confirmation was not needed. In

cases of clear disease progression before the first planned response assessment at 6 weeks-even in the absence of confirmatory imaging studies-the outcome was defined as early progression and treatment was discontinued.
Toxicities were assessed with the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Health-related quality of life was assessed by the European Organization for the Research and Treatment of Cancer QLQ-C30 and QLQ-BCM20 questionnaires, and will be reported separately. IDH mutational status was established by the use of sequencing as described elsewhere.(21) MGMT promoter methylation status was established by a methylation-specific PCR, as described elsewhere.(22)

Outcomes

The primary outcome was 9-month overall survival. Secondary endpoints were median PFS, PFS at 6 and 12 months, median overall survival, overall survival at 6 and 12 months, the proportion of patients who achieved an objective response, and the association of outcome with MGMT promoter methylation status. In a post-hoc analysis, outcome was also associated with IDH mutational status. PFS was defined as time from randomization until progression or death, whichever occurred first. Overall survival was measured from randomization until death from any cause. Patients who were still alive at the date of last contact were censored. PFS and overall survival were estimated with the Kaplan–Meier method.

Statistical analysis

We used an A'Hern one-stage design to calculate the sample size needed for each of the treatment groups.(23) With P_0 set at 35% (i.e., true overall survival at 9months of 35% was judged to be too low to warrant further investigation) and P_1 set at 55% (i.e., a true overall survival at 9 months of 55% was judged sufficient to warrant further studies with the corresponding regimen), and with α of 0.10 and β of 0.10, a total of 44 patients were needed per group. On the basis of these assumptions, if 20 of 44 or more patients were still alive at 9 months in any of the groups, we would be able to conclude that that specific treatment warrants further investigation in clinical trials. However, the study was not powered to formally compare results between the three treatment groups. All analyses are by intention to treat. As exploratory analyses, the log-rank test was used to assess the effect of IDH mutations and MGMT methylation on PFS and overall survival. We entered the clinical data of the patients into an Access database (version 14.0.7116.5000), and analyzed the data with STATA version 13.1. The primary analysis of the study data was done after database lock on March 10, 2014. This trial is registered in the Nederlands Trial Register (www.trialregister.nl; number NTR1929).

Role of the funding source

The funders of the study (Roche Nederland and KWF Kankerbestrijding [the Dutch Cancer Society]) financially supported the study, and Roche Nederland provided bevacizumab free of charge. As part of the grant approval process, both grant sources approved of the study design, but neither was involved in the study design, data collection, data analysis, preparation of the report, or in the decision to submit the report for publication. Roche Nederland had the right to review the report before publication. The corresponding author had full access to all the data in the study, prepared the report, and had final responsibility for the decision to submit for publication.

RESULTS

Between Dec 11, 2009, and Nov 10, 2011, 153 patients were enrolled (figure 1). Five patients were judged ineligible and were excluded from all analyses, three of whom had major violations of protocol entry criteria (increasing dose of steroids and less than a 4-week interval since previous

chemotherapy in one patient; uncontrolled hypertension in one; and a subdural hemorrhage at baseline in one), one patient died before randomization, and one patient withdrew consent before the start of treatment. Therefore, after these exclusions, 148 patients were judged to be eligible for this study. No patients were lost to follow-up.

Table 1. Baseline characteristics at the time of randomization and MGMT promoter gene /IDH mutational status at the time of initial diagnosis

	BEV/LOM 110 n = 8	**Bevacizumab n = 50**	**Lomustine n = 46**	**BEV/LOM 90 n = 44**
Age (years)				
Median	53	58	56	58
range	29 - 62	37 - 77	28 - 73	24 - 73
Sex, n (%)				
Male	3 (38%%)	32 (64%)	26 (57%)	30 (68%)
Female	5 (63%)	18 (36%)	20 (43%)	14 (32%)
WHO PS, n(%)				
0	3 (38%)	13 (26%)	15 (33%)	11 (25%)
1	4 (50%)	32 (64%)	25 (54%)	28 (64%)
2	1 (13%)	5 (10%)	6 (13%)	5 (11%)
Surgery at the time of recurrence, n (%)				
	1 (13%)	5 (10%)	6 (13%)	5 (11%)
Corticosteroid use, n (%)	2 (25%)	27 (54%)	22 (48%)	21 (48%)
Maximum enhancing tumor diameter at baseline (mm)				
median	35	35	36	34
range	17-54	12-88	11-82	17-93
Days since last radiotherapy				
median (range)	259 (133, 699)	254 (101, 2087)	298 (106, 1092)	272 (69, 1337)
IDH status, n (% of tested)				
Unmutated	6 (100%)	38 (97%)	39 (93%)	36 (90%)
Mutated	-	1 (3%)	3 (7%)	4 (10%)
Not done/unknown	2	11	4	4
MGMT status n (% of tested)				
Unmethylated	4 (66%)	24 (57%)	20 (47%)	22 (54%)
Methylated	2 (33%)	18 (43%)	23 (53%)	19 (46%)
Not done/unknown	2	8	3	3

BEV/LOM 110: bevacizumab plus lomustine 110mg/m^2, BEV/LOM 90: bevacizumab plus lomustine 90 mg/m^2, MGMT: O6-methylguanine-DNA-methyltransferase, IDH: isocitrate dehydrogenase

Table 1 presents the baseline clinical characteristics of the patients. The treatment groups were well balanced in terms of known major prognostic factors (especially age, WHO performance status, surgery for tumor recurrence, corticosteroid use at baseline, and diameter of the recurrent lesion).(24) One patient was enrolled within 3 months of their previous radiotherapy, after a re-resection before study entry had confirmed the presence of a tumor recurrence. The median number of treatment cycles was one (IQR 1–3) for the patients in the lomustine group, two (1–3) in the bevacizumab group, three (2–7) in the bevacizumab and lomustine 90 mg/m^2 group, and six (2–8) in the bevacizumab and lomustine 110 mg/m^2 group. Most patients discontinued study treatment for progressive disease (10/148 [7%] for early progression and 119/148 [80%] for progression), three patients (2%) discontinued because they withdrew consent, five (3%) for toxicity reasons, two (1%) died while on treatment, and six (4%) stopped for other reasons (including completion of treatment by two patients in the single-agent lomustine group). At the end of follow-up (Oct 1, 2013), three patients (2%) were still on treatment and four (3%) were still alive (median follow-up of those four patients: 35.3 months [range 28.9–41.5 months]).

Table 2. Number of patients (and percentage) in all four treatment groups with non-neurological adverse events according to the common toxicity criteria version 3 (worst grade per patient) occurring in more than 5% of patients in all cycles combined

	BEV/LOM 110 n = 8	Bevacizumab n = 50	Lomustine n = 46	BEV/LOM 90 n = 44
Platelets, CTC AE				
Grade 0, 1	2 (25%)	48 (94%	27 (59%)	33 (75%)
Grade 2	1 (13%)	1 (2%)	10 (22%)	7 (16%)
Grade 3	3 (38%)	-	7 (15%)	3 (7%)
Grade 4	2 (25%)	1 (2%)	2 (4%)	1 (2%)
White blood count				
Grade 0, 1	2 (25%)	48 (96%)	30 (65%)	31 (71%)
Grade 2	3 (38%)	2 (4%)	8 (17%)	10 (23%)
Grade 3	3 (38%)	-	7 (15%)	3 (7%)
Grade 4	-	-	1 (2%)	-
Proteinuria				
Grade 1	-	12 (24%	10 (22%)	16 (36%)
Grade 2	2 (25%)	3 (6%)	1 (2%)	9 (20%)
Grade 3	2 (25%)	-	-	1 (2%)
Hypertension				
Grade 1, 2	1 (13%)	15 (30%)	8 (17%)	15 (34%)
Grade 3	3 (38%)	13 (26%)	3 (7)	11 (25%)
Nausea and vomiting				
Grade 1, 2	4 (50%)	5 (10%)	7 (15%)	10 (23%)
Grade 3	1 (13%)	1 (2%)	2 (4%)	-
Fatigue				
Grade 1,2	7 (88%)	30 (60%)	21 (46%)	31 (70%)
Grade 3	-	2(4%)-	3 (7%)	8 (18%)
Grade 4	-		1 (2%)	-
Infection				
Grade 1, 2	2 (25%)	7 (14%)	5 (11%)	13 (30%)
Grade 3	-	3 (6%)	1 (2%)	4 (9%)
Grade 4	-	-	1 (2%)	-
Grade 5	-	-	-	1 (2%)
Pulmonary/upper respiratory tract				
Grade 1,2	1 (!3%)	5 (10%)	1 (2%)	15 (34%)
Grade 3	-	2 (4%)	-	-
Thrombosis				
Grade 3	-	-	-	3 (7%)

BEV/LOM110: bevacizumab plus lomustine 110mg/m^2, BEV/LOM90: bevacizumab plus lomustine 90 mg/m^2

The preplanned safety review was done earlier than anticipated—after eight patients in the bevacizumab and lomustine 110 mg/m^2 group had completed at least one cycle of treatment—because of hematological side-effects. Of these eight patients, three developed a grade 3 and two patients a grade 4 thrombocytopenia (without clinical sequelae). Because of dose reductions required as a result of these adverse events, the dose intensity of bevacizumab in the bevacizumab and lomustine 110 mg/m^2 group was lower than 70% in four of these eight patients. After the dose of lomustine in the combination treatment group was modified, treatment in the combination group was well tolerated and platelet toxicity in patients who received bevacizumab and lomustine 90 mg/m^2 was similar to that in those who received single-agent lomustine 110 mg/m^2 (table 2). The reported toxicities are in accordance with the known toxicities of both drugs (table 2). Hypertension was reported more frequently in patients who received bevacizumab than in those who did not, whereas fatigue and infections were most prevalent in the bevacizumab and lomustine 90 mg/m^2 group (table 2). Notably, patients in the combination treatment group remained on treatment for longer than did those in the other groups and thus spent more time at risk of developing toxicities during treatment. Five (3%) of 148 patients discontinued treatment for

toxicity reasons. One patient in the bevacizumab plus lomustine 90 mg/m^2 group died from pneumonia while on treatment, which was judged to be more likely to be related to the disease than to treatment.

The median relative dose intensity (RDI) of lomustine and bevacizumab tended to be higher in the single-agent groups than in the bevacizumab plus lomustine 90 mg/m^2 group. The median RDI of bevacizumab was 99% in the single-agent group versus 94% in the bevacizumab plus lomustine 90 mg/m^2 group, whereas that of lomustine was 100% in the single-agent group versus 90% in the bevacizumab plus lomustine 90 mg/m^2 group. In the eight patients in the bevacizumab plus lomustine 110 mg/m^2 group, the median RDI of bevacizumab was 71% and of lomustine 63%. Since patients in the combined treatment groups had more treatment cycles and thus stayed on treatment for longer, dose modifications that were done in patients staying longer on treatment mainly occurred in these groups.

9-month overall survival was 43% (95% CI 29–57) in the lomustine group, 38% (25–51) in the bevacizumab group, 59% (43–72) in the bevacizumab plus lomustine 90 mg/m^2 group, 87% (39–98) in the bevacizumab plus lomustine 110 mg/m^2 group, and 63% (49–75) in all patients given the combination of bevacizumab plus lomustine.

Table 3. Objective response rate in the patients evaluable for response (ORR), median and 6 months PFS (95% CI), median and 12 months OS (95% CI) in the three treatment arm and in all patients treated with the combination bevacizumab/lomustine.

	n (evaluable)	ORR	Median PFS	6-mo PFS	Median OS	12 mo OS
Lomustine	46 (41)	5%	1 mo	13% (5, 24)	8 mo	30% (18, 44)
Bevacizumab	50 (48)	38%	3 mo	16% (7, 27)	8 mo	26% (15, 39)
LOM/BEV 90	44 (41)	34%	4 mo	41% (26, 55)	11 mo	45% (30, 59)
LOM/BEV all	52 (49)	39%	4 mo	42% (29, 55)	12 mo	48% (34, 61)

evaluable: number of patients evaluable for objective response, BEV/LOM 110: bevacizumab plus lomustine 110mg/m^2, BEV/LOM 90: bevacizumab plus lomustine 90 mg/m2, BEV/LOM ALL: all patients who received combination treatment of bevacizumab plus lomustine, PFS: progression-free survival

Table 3 lists other relevant secondary endpoints including response rates, 6-month progression-free survival, and median and 12-month overall survival. Progression-free survival and overall survival were more favorable in the bevacizumab and lomustine combination groups than in the single-agent groups. Ten patients were not evaluable for response assessment (table 3), in most cases because they underwent surgery for recurrence. Despite almost two-fifths of patients in the single- agent bevacizumab group having an objective response to treatment, which compared favorably with the lomustine single-agent group, overall survival in the single-agent bevacizumab group was very similar to that in the single-agent lomustine group (figure 2, table 3). Although median progression-free survival was longer in the bevacizumab group than in the lomustine group, 6-month progression-free survival was similar in both single-agent groups (13% [95% CI 5–24] for the lomustine group, 16% [7–27] for the bevacizumab group; figure 2, table 3). Cox regression analysis showed that achievement of an objective response (included as a time-dependent covariate) was associated with improved survival in both the single-agent bevacizumab group (HR 0.43, 95% CI 0.23–0.82) and in all patients who received bevacizumab (HR 0.37, 95% CI 0.23–0.58) in comparison with patients who did not achieve an objective response.

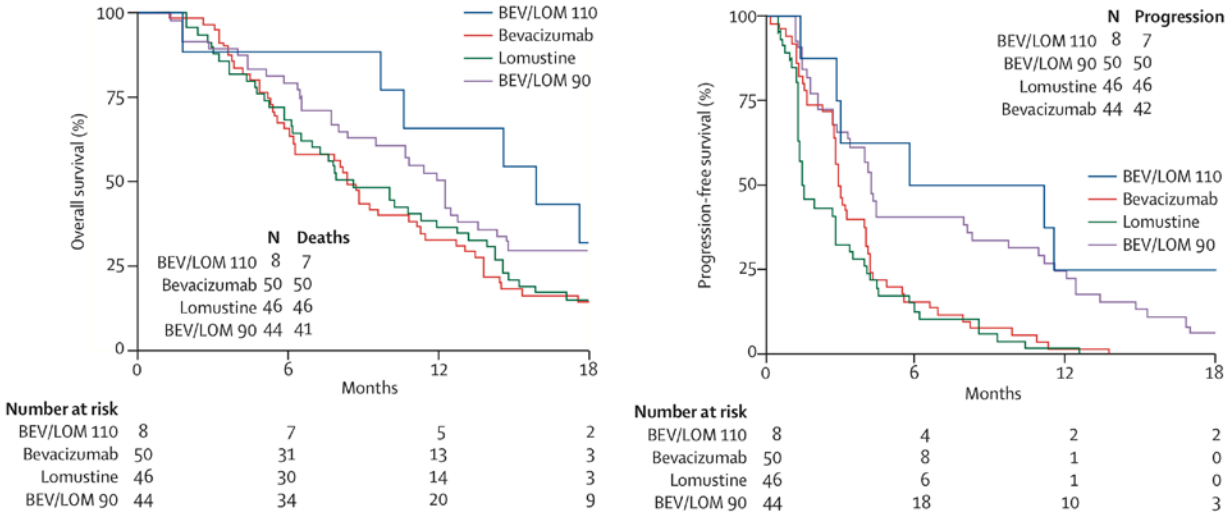

Figure 2. Overall survival and Progression-free survival
BEV/LOM 90: bevacizumab plus lomustine 90 mg/m^2, BEV/LOM 110: bevacizumab plus lomustine 110 mg/m^2

Table 4. Number of patients in each arm that did progress or had died at the time of analysis, and numbers of patients (between brackets: percentage of patients) receiving the various salvage therapies after progression in het BELOB trial.

	Bev/LOM 110	Bevacizumab	Lomustine	Bev/LOM 90
Progressed and/or dead at analysis	7	50	46	42
Some kind of salvage therapy*	4 (57%)	26 (52%)	24 (52%)	16 (38%)
Re-irradiation	-	10 (20%)	11 (24%)	10 (24%)
Surgery	-	-	7 (15%)	1 (2%)
Chemotherapy	3 (43%)	20 (40%)	9 (20%)	3 (7%)
Temozolomide	2	3	8	3
Lomustine	1	19 (38%)	-	-
Other	-	-	1	-
Bevacizumab	-	-	1	-
Other	2	2	5	4
Unknown	-	-	-	1

BEV/LOM 110: bevacizumab plus lomustine 110mg/m2, BEV/LOM 90: bevacizumab plus lomustine 90 mg/m2 *Many patients received more than one line of salvage therapy.

Table 4 shows all salvage therapies given to patients in each study group after disease progression on their assigned treatment. Three patients were still alive and without disease progression at the time of analysis (one in the bevacizumab plus lomustine 110 mg/m2 group and two in the bevacizumab plus lomustine 90 mg/m2 group). The fewest salvage therapies were given to patients in the combined treatment groups (table 4). Salvage chemotherapy was administered most frequently to patients in the bevacizumab group (table 4). Surgery was most common in the lomustine group (table 4). Re-irradiation was given to about a quarter of patients in the three main study groups (table 4). Only one patient (in the single-agent lomustine group) received bevacizumab salvage therapy after disease progression in the BELOB trial.
Tumor material for IDH mutational analysis was available from 127 patients. An IDH1 mutation was identified in eight patients, seven of who also had a methylated MGMT promoter. No IDH2 mutations were identified in any patients. The objective response rate in patients with IDH mutated tumors (38%) was similar to that in patients with IDH wild- type tumors (25%; p=0.427). However, both PFS and overall survival were clearly higher in IDH mutant tumors (median PFS 3 months [95% CI 3–3] in IDH wild-type tumors vs 10 months [1–26] in IDH mutant tumors

[p=0.008]; median overall survival 9 months [95% CI 8–11] in IDH wild-type tumors vs 20 months [4–26] in IDH mutant tumors [p=0.021]). In a sensitivity analysis, we studied outcome in 42 patients with IDH wild-type tumors who received combination treatment at either dose of lomustine; 9-month overall survival in these patients was 60% (95% CI 43–73).
Tumor material was available for MGMT methylation analysis in 132 patients, and the MGMT promoter was methylated in 62 of these tumors (47%; table 5). PFS and overall survival were longer in patients with MGMT promoter methylated tumors than in those with MGMT promoter unmethylated tumors (median PFS 4 months [95% CI 3–7] in those with MGMT methylation vs 3 months [2–3] for those without [p=0.001]; median overall survival 12 months [95% CI 9–14] for those with MGMT methylation vs 8 months [6–8] for those without [p=0.002]). The longer PFS in patients with MGMT promoter methylated tumors occurred irrespective of the assigned treatment, whereas the longer overall survival in such patients occurred in all those who received bevacizumab, with the smallest HR for death recorded in the patients who were given bevacizumab alone (table 5). This improved overall survival in patients with methylated MGMT promoter who received bevacizumab alone is not explained by IDH status, since an IDH1 mutation was only identified in one patient in this group.

Table 5. *MGMT* promoter methylation in relation to PFS at 6 months and OS at 9 months

	n	6-mo PFS	HR PFS (95% CI)	9 mo OS	HR OS (95% CI)
BEV/LOM all					
Unmethylated	26	23%	1	58%	1
Methylated	21	62%	0.41 (0.22-0.77)	67%	0.55 (0.29, 1.02)
Bevacizumab alone					
Unmethylated	24	8%	1	12%	1
Methylated	18	33%	0.43 (0.21, 0.85)	67%	0.27 (0.13, 0.57)
Lomustine alone					
Unmethylated	20	0%	1	40%	1
Methylated	23	26%	0.56 (0.29, 1.09)	52%	0.89 (0.48, 1.64)
All patients					
Unmethylated	70	11%	1	37%	1
Methylated	62	40%	0.54 (0.37, 0.77)	61%	0.57 (0.40, 0.82)

PFS: progression-free survival, HR: hazard ratio

DISCUSSION

To the best of our knowledge, this study is the first well-controlled randomized trial assessing the role of bevacizumab in the treatment of recurrent glioblastoma (panel). To avoid the uncertainties in the assessment of response and progression in patients given bevacizumab, we used overall survival at 9 months as the primary endpoint. The choice for this particular endpoint was based on the median overall survival reported in the BRAIN trial (9.2 months in the bevacizumab alone group and 8.7 months in the bevacizumab–irinotecan combined treatment group).(8) Our results with mature follow-up data show that with a 9-month overall survival of 59%, the bevacizumab plus lomustine 90 mg/m^2 combination warrants further investigation in a phase III study. By contrast, the activity of single-agent bevacizumab in recurrent glioblastoma seems to be low and according to our predefined criteria is insufficient for further studies in recurrent glioblastoma. Salvage treatments given at further progression do not explain the differences in overall survival. As anticipated - since in the Netherlands bevacizumab is not available for use in recurrent glioblastoma - crossover treatment to bevacizumab was virtually absent. By contrast, 19 of the 50 patients (38%) assigned to the bevacizumab group received lomustine after disease progression. The results in the bevacizumab plus lomustine 90 mg/m^2 group are unlikely to be explained by

salvage therapies, since the proportion of patients receiving some type of salvage treatment intensity was lower in this group (38% of patients) than in the other two main study group (52% of patients in both single-agent groups received salvage therapies).
In general, after the lomustine dose reduction in the bevacizumab plus lomustine group, this treatment was well tolerated. Fatigue, infections, and hypertension were more common in the bevacizumab plus lomustine 90 mg/m^2 group, which might be attributable to the longer treatment duration (more cycles of treatment) in this group than in the single-agent groups. Both bevacizumab and lomustine have been associated with thrombocytopenia, and at the initial dose of lomustine 110 mg/m^2 with bevacizumab 10 mg/kg, several patients had grade 3 and 4 thrombocytopenia. Although these hematological toxicities remained uncomplicated, they resulted in a decrease of the bevacizumab dose intensity to below 70% of the intended dose intensity of the first cycle. After reduction of the lomustine dose in the combination group to 90 mg/m^2, the hematological toxicity in the combination group was similar to that recorded in the single- agent lomustine group. The rate of hypertension was higher than previously reported, but all hypertension events were grade 3 and well controlled with (additional) medication. We did not attempt to increase the lomustine dose in the second treatment cycle in the patients in the bevacizumab plus lomustine 90 mg/m^2 group who had no hematological toxicity, but in view of the absence of medical complications in the bevacizumab plus lomustine 110 mg/m2 group, this approach could be justified. Despite the dose reduction of lomustine, the outcome of patients in the bevacizumab plus lomustine 90 mg/m^2 group is more promising than in either of the single-agent groups.

Panel: Research in context

Systematic review
The rationale for this trial was the absence of a randomized trial of bevacizumab in recurrent glioblastoma that includes a proper non-bevacizumab control group. We systematically searched PubMed and ClinicalTrials.gov using the keywords "bevacizumab", "glioblastoma", "recurrent", and "randomized" and we did not identify any other completed randomized trial with a non-bevacizumab group in this setting.

Interpretation
The results of this trial do not support a significant role for single-agent bevacizumab in recurrent glioblastoma. The present study suggests that the combination of lomustine and bevacizumab might have more activity than either drug administered alone; and a phase III trial of this combination treatment is warranted. Until the results of such a phase III trial are available, the activity of this combination in recurrent glioblastoma remains uncertain.

Although the proportion of patients who had a response in both single-agent groups is similar to those reported in published studies, 6-month PFS in both the lomustine and the bevacizumab single-agent groups was lower than those reported in other recent trials.(3, 4, 8, 25) 6-month PFS is often used as the primary endpoint in screening phase II trials on recurrent glioblastoma, and in general drugs showing 6-month PFS of 20% or higher are judged to be of clinical interest. One explanation for the modest 6-month PFS in the single-agent groups in this trial could be that this study might have included a less selected recurrent glioblastoma population compared with early studies on bevacizumab in recurrent glioblastoma or the randomized studies that used lomustine as a comparator.(3, 4, 8) Similar trends towards improved outcome have been reported in phase II studies of novel drugs in newly diagnosed glioblastoma.(26) Whether or not the use of the RANO criteria contributed to the modest 6-month PFS in the single-agent groups is the subject of an ongoing radiology review. Notably, the median overall survival of 8 months in both single-agent groups noted here is no different to other recent trials of these drugs, and the upper limit of the 95% CI for 6-month PFS in the bevacizumab group is 27%, which is in accordance with many other reports. Additionally, this

trial was a randomized study, and major prognostic factors were well balanced between the treatment groups.

The assessment of response and progression in patients with newly diagnosed and recurrent glioblastoma who are given bevacizumab treatment continues to be a controversial topic. Following the early results of the BRAIN trial and other studies of bevacizumab in recurrent glioblastoma without a major improvement in overall survival, the occurrence of pseudo-responses were postulated as a possible explanation for the high response rates to bevacizumab without an obvious improvement in overall survival. This trial report is based on the local assessment of response and progression; a central radiology review including a review of the added value of T2/ FLAIR imaging is ongoing and will be reported separately. Our results so far suggest that responses to bevacizumab in recurrent glioblastoma are indeed of a different type compared to responses obtained with classical cytotoxic agents. Despite 38% of patients having an objective response in the single-agent bevacizumab group, the overall survival in patients who received bevacizumab alone was very similar to that in patients who received lomustine alone (of whom only 5% responded). Similarly, patients in the bevacizumab single-agent group had a longer PFS and remained on treatment for longer than did those in the lomustine single-agent group. Treatment crossover to bevacizumab in the lomustine group does not explain this finding, whereas crossover treatment in the bevacizumab group to lomustine was frequent. The short response duration to bevacizumab alone in most patients is consistent with a rapid normalization of abnormal vessel permeability after treatment with an anti-VEGF agent.(4, 17) Because of these pseudo-responses the classical Macdonald's criteria have been adapted, and the present RANO criteria include assessment of changes on T2/ FLAIR images. Notably, we did not require a confirmatory scan for an objective response. Most bevacizumab trials (e.g., the BRAIN trial) defined one cycle as 4 weeks, whereas this trial defined one cycle as 6 weeks (because of the 6-week cycles of the lomustine treatment).(7, 8) Thus, three cycles in those trials equal two cycles in this trial. In view of the primary endpoint, we used a more relaxed MR follow-up schedule, and we did not ask for confirmatory scans in case of a partial or complete response.

Preliminary evidence from other trials suggests that 5–10% of patients who are treated with bevacizumab for recurrent glioblastoma have evidence of progression on T2/FLAIR images only, without increased contrast uptake on T1 contrast- enhanced MRI.(27, 28) This finding might indeed affect the date of progression, but whether isolated T2/FLAIR progression is associated with decreased overall survival remains unclear.(28) To what extent the incorporation of T2/FLAIR increase into the criteria of progression affects the overall analysis of trials on bevacizumab also remains unclear.(28) Several studies of bevacizumab in recurrent glioblastoma have now shown that response and absence of progression at 3 months is a prognostic factor for survival.(27, 29) Similarly, in our trial, achievement of an objective response was associated with improved overall survival. A first conclusion here is that in patients treated with bevacizumab, objective response is indeed correlated with overall survival, but the proportion of patients with an objective response cannot be compared with that obtained with classic cytostatic agents. Thus, assumptions about objective responses in recurrent glioblastoma used for classic cytotoxic agents to assess activity do not hold true for bevacizumab. This fact has clear implications for the choice of endpoints in trials of drugs that interfere with vessel permeability, and shows that the proportion of patients with an objective response that will be associated with improved overall survival after treatment with VEGF inhibitors is of a different magnitude to that with classic cytotoxic drugs.

As in other studies, bevacizumab was associated with a longer PFS than was lomustine, but overall survival results for the two drugs were similar.(9, 10) Whether or not this improved PFS translates into an improved quality of survival is still unclear. Quality of life and neurological deterioration-

free survival analyses in the BELOB trial are ongoing, but the present study has the same limitation as other trials in neuro-oncology: detailed assessment of functional outcome once radiological progression has occurred is difficult.(30) Moreover, this phase II trial is not powered for comparison, which also limits the power of quality-of-life analyses.

The phase II design of the study is its main limitation, preventing across-group comparisons. Whether or not the outcome of recurrent glioblastoma can indeed be improved with combined treatment with bevacizumab and lomustine therefore needs confirmation in a phase III study. If confirmed, the obvious next question is why this combination is more effective than either drug alone. In preclinical experiments, the addition of bevacizumab to chemotherapy has reportedly improved drug access in the tumor, although other investigators have argued that the vascular normalization might actually reduce drug delivery.(31, 32) The better outcome in the combination group might be a consequence of the restoration the normal vascularization of the tumor, which enables lomustine to penetrate better into the tumor, resulting in an improved cytotoxic effect. It is noteworthy that the phase III trial of cediranib - a pan-VEGF tyrosine kinase inhibitor, given either alone or in combination with Lomustine - did not improve outcome in recurrent glioblastoma.(4)

As part of our exploratory analysis, we assessed IDH mutational status and MGMT promoter methylation status in this trial. IDH mutations are recently discovered point mutations that seem to be an early and oncogenic mutation in grade II and III diffuse glioma.(33) These mutations are also present in glioblastoma that arises from a lower grade precursor lesion (so-called secondary glioblastoma) as opposed to primary (IDH wild-type) glioblastoma. Existing data show that although the glioblastomas are histologically similar, overall survival is better in IDH-mutated secondary glioblastoma than in primary glioblastoma. Data about the effect of IDH mutations on outcome at the time of first recurrence are scarce. In a smaller series of patients (n=63) that included grade II gliomas, a difference in overall survival from first diagnosis between patients with IDH-mutated tumors versus those with IDH wild- type tumors was also recorded; however, although 17 tumors had an IDH mutation, overall survival after recurrence was similar in IDH mutant and IDH wild-type tumours.(34) We anticipated that IDH mutation status would have an effect on outcome and sensitivity to treatment, and we therefore included this analysis to check for prognostic imbalances. Not unexpectedly, the BELOB trial of a more homogeneous patient population suggests that IDH mutations are also associated with outcome when tumors recur after first treatment. Median overall survival was 8 months in patients with IDH wild-type tumors compared with 20 months in the eight patients with IDH mutant tumors. This raises the question of whether trials on recurrent glioblastoma should be limited to patients with IDH wild-type tumors or be analyzed according to IDH mutational status. The clear molecular differences between non-IDH mutant primary glioblastoma and IDH-mutated secondary glioblastoma support such a distinction.(35) In tumors of four of the 44 patients in the bevacizumab plus lomustine 90 mg/m^2 group an IDH mutation was present, compared with only one of the 50 patients treated with bevacizumab only. Nonetheless, overall survival at 9 months was 60% (95% CI 43– 73) for patients with IDH wild-type tumors who were treated with either lomustine dose in the combination group, which fits in with the overall results of the study.

The interpretation of the results of the MGMT promoter methylation status is less straightforward. We used a methylation-specific PCR, and found that 47% of the tumors had a methylated MGMT promoter-similar to the 45% recorded in the pivotal temozolomide chemotherapy irradiation EORTC trial in which a similar assay was used.(36) Recent studies have confirmed the predictive value of MGMT promoter methylation in patients with newly diagnosed glioblastoma who are treated with temozolomide.(36, 37, 38) We recorded improved overall

survival for patients treated with bevacizumab with MGMT methylated tumors, and less effect on overall survival in patients with methylated tumors who received single-agent lomustine. The small sample size resulting in large confidence intervals and the frequent use of temozolomide and lomustine chemotherapy after progression in the bevacizumab group might have affected findings, which require further studies.

After the conditional approval of bevacizumab for use in recurrent glioblastoma, two trials on bevacizumab added to standard chemo-radiotherapy with temozolomide were done in newly diagnosed glioblastoma.(9, 10) Both trials showed that treatment led to a clear increase in PFS but no effect of any significance on overall survival. At present, we have not yet identified a subset of patients that might benefit from this drug, and biomarkers that can predict good outcome after bevacizumab treatment are urgently needed. The results of our trial do not support a role for single-agent bevacizumab in the treatment of recurrent glioblastoma. However, the encouraging results of the combination of lomustine and bevacizumab warrant further investigation in an adequately powered randomized phase III trial. Such a trial has recently been started by the EORTC. This study, EORTC study 26101 (NCT01290939), was originally designed as a randomized phase II trial investigating different sequences of bevacizumab and lomustine, to explore the optimum sequence of these two drugs. Similar to the BELOB trial, this trial included a bevacizumab plus lomustine combination group and a lomustine single-agent group. Following the completion of the enrolment of this phase II trial, the bevacizumab plus lomustine group and a lomustine single-agent group are being continued as a randomized phase III trial. This EORTC trial will hopefully provide the definitive answer as to whether the combination of bevacizumab with lomustine indeed increases overall survival in patients with recurrent glioblastoma.

Contributors

WT, HJD, WND, MJBT, RV, BvdH, and MJvdB designed the study and prepared the protocol. WT, HMO, AW, HJD, LB, MH, JB, AH, DB, FYFdV, WNMD, RHE, MJBT, FWPJvdB, RLHJ, DB, IvH, RV, and MJvdB gathered the data. WT, HJD, WNMD, MJBT, RV, BvdH, and MJvdB analysed and interpreted the data. WT, HJD, WNMD, RV, BvdH, and MJvdB wrote the report. All authors reviewed the report and approved the final version.

Declaration of interests

MJvdB has done paid consultancy for Roche, Abbvie, Celldex, Amgen, and Merck Ag; has received research grants from Roche and AbbVie; and has been on the speakers' bureau for MSD. HMO and MJT have received personal fees from Roche for consultancy. The other authors declare no competing interests.

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Chapter 7

Treatment of large low-grade oligodendroglial tumors with upfront procarbazine, lomustine, and vincristine chemotherapy with long follow-up: a retrospective cohort study with growth kinetics

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ABSTRACT

Background: We treated patients with newly diagnosed and large low-grade oligodendroglial tumors with upfront procarbazine, CCNU and vincristine (PCV) in order to delay radiotherapy.

Methods: Patients were treated with PCV for a maximum of 6 cycles. The response to treatment was defined according to the RANO criteria; in addition change over time of mean tumor diameters (growth kinetics) was calculated.

Results: Thirty-two patients were treated between 1998 and 2006, 18 of which were diagnosed with 1p/19q co-deleted tumors. Median follow-up duration was 8 years (range 0.5 - 13 years). The median overall survival (mOS) was 120 months and the median progression-free survival (mPFS) was 46 months. Growth kinetics showed an ongoing decrease of the mean tumor diameter after completion of chemotherapy, during a median time of 35 months, but an increase of the mean tumor diameter did not herald progression as detected by RANO criteria. 1p/19q co-deletion was associated with a significant increase in OS (mOS 83 months versus not reached for codeleted tumors; p=0.003)) and PFS (mPFS 35 months versus 67 months for codeleted tumors; p=0.024). Patients with combined 1p/19q loss had a 10 year PFS of 34% and the radiotherapy in these patients was postponed for a median period of more than 6 years.

Conclusions: This long-term follow-up study indicates that upfront PCV chemotherapy is associated with long PFS and OS and delays radiotherapy for a considerable period of time in patients with low-grade oligodendroglial tumors, in particular with combined 1p/19q loss.

INTRODUCTION

Early chemotherapy while withholding radiotherapy (RT) is increasingly used for high-risk low-grade glioma (LLG).(1) Early reports showed prolonged stable disease or minor responses in a significant subset of patients, both after treatment with temozolomide (TMZ) and with procarbazine, lomustine (CCNU), and vincristine (PCV) combination chemotherapy.(2, 3, 4, 5, 6, 7, 8, 9) Not unexpectedly, in particular 1p/19q co-deleted low-grade oligodendroglioma (OD) and low-grade oligo-astrocytoma (OA) showed favorable and longer lasting responses.(3, 5) The major rationale for this approach is the wish to postpone RT, which is associated with delayed cognitive disturbances in LGG patients.(10) This strategy is attractive particularly in patients with chemotherapy responsive tumors and with anticipated long-term survival, because these patients are longer at risk to develop clinically significant cognitive disturbances. Several series have been published on this topic, but all with relatively short follow-up or reporting on anaplastic oligodendroglioma (AO).(4, 5, 11)

Because of the expected chemosensitivity and in order to delay RT, we have been treating patients with newly diagnosed and large OD and OA tumors with upfront PCV chemotherapy since 1998. In 2005, we reported initial results of patients with OD and OA treated with PCV as the first line of treatment or at recurrence.(5) At that time we treated 16 patients with upfront PCV, with a median and maximum follow-up of 2 years and 5.5 years respectively. We now report on the long-term follow-up (median follow-up duration: 8 years, maximum follow-up: 13 years) of 32 patients who received upfront PCV and correlate our findings with the 1p/19q and IDH status.

PATIENTS AND METHODS

Patients

All patients receiving chemotherapy for brain tumors in our hospital are prospectively registered in our institutional glioma chemotherapy database. From this database we selected all RT naïve patients with newly diagnosed OD or mixed OA who started first-line treatment with upfront PCV chemotherapy, and we reviewed their records. Patients who received PCV for a recurrence after RT were not selected for this study. This report describes all patients treated between 1998 and 2006 (in 2006 we joined the EORTC study 22033 which employed TMZ in LGG patients). Patients were selected for treatment with upfront PCV if they had an OD or OA on original histology and had large and/or multi-lobe non-enhancing tumors, for which RT would require large treatment volumes (estimated more than 50% of the cerebral hemispheres). Adequate hematologic, hepatic, and renal function and a WHO performance status score of 0-2 were required for chemotherapeutic treatment. In case of a favorable response to chemotherapy further treatment was deferred until disease progression occurred. This retrospective study was conducted according to local and national regulations and is approved of by the Institutional Review Board.

Treatment

All patients received the standard PCV schedule, consisting of lomustine 110 mg/m2 on day 1, procarbazine 60 mg/m2 on days 8–21, and vincristine 1.4 mg/m2 (maximum of 2 mg) on days 8 and 29 in cycles of 6 weeks for a maximum of 6 cycles. Toxicity was assessed with the National Cancer Institute Common Toxicity Criteria Version 2.0. Dose reductions were made as described previously.(12)

Evaluation

Response was assessed with MRI scans made after every second cycle during chemotherapy and routinely thereafter at least every 6 months. All MRI scans (baseline and follow-up) were made with and without gadolinium contrast. For tumor measurements in these non-enhancing tumors

the T2 weighted MRI images were used. The response to treatment and progression was primarily defined using the product of perpendicular diameters on T2-weighted images according to the RANO criteria.(13) In addition, tumor size was calculated by one of the investigators (W.T.) using the three diameters technique (V=(D1xD2xD3)/2) as described elsewhere.(14) With this technique the three tumor diameters (h x w x l) are converted into a single mean tumor diameter (MTD = (2 x V)1/3). MTD measurements were done before and after the chemotherapy and yearly thereafter, until progression occurred according to RANO criteria. The diameter expansion velocity (DEV; the glioma growth curve) was plotted as a function of MTD over time. A negative DEV indicates a tumor volume decrease. Fast responders were defined as patients with a DEV of less than the median DEV during the PCV chemotherapy. The MTD was not used for the classification of response and/or progression.

Histopathology and molecular diagnostics

All tumor specimens were centrally reviewed by J.M.K., who was kept unaware of the clinical data. From each selected tumor block, multiple consecutive 4 muM sections were prepared for molecular diagnostics. For genotyping, the tissue area composed of the highest percentage neoplastic cells was selected. Either fluorescence in situ hybridization (FISH) analysis or loss of heterozygosity (LOH) was used to determine loss of 1p and 19q as described previously, depending on the year of diagnostics.(5, 15)

IDH1 mutational status and MIB-1 labeling was assessed with immunohistochemistry as described earlier.(16, 17, 18) Tumors with clear positive cells on IDH immunohistochemistry were considered IDH mutated.

Statistical Analysis

The primary objectives of this study were the assessment of overall survival (OS) and progression-free survival (PFS) from the start of chemotherapy. Secondary objectives were the objective response rate ((ORR); complete response (CR), partial response (PR) or minor response (MR)) and MTD charts were calculated. The Kaplan-Meier method was used to estimate PFS and OS. PFS and OS were measured in months, from the first day of start of PCV chemotherapy to the date of the event, with censoring at the date of last follow-up for survivors. The survival distributions between the subgroups (combined 1p/19q loss, no vs yes; IDH1 mutational status, mutated vs wild type; and MIB-1 labelling <5% vs >=5%) were compared using the log-rank test. All reported P values are two sided; in this exploratory analysis, no adjustments were made for multiple testing. IBM SPSS statistics 21 software was used for statistics and MS Excel 2010 was used for plotting the MTD charts.

RESULTS

Baseline characteristics

Between July 1998 and November 2006 we treated 32 patients with an OD or OA with upfront PCV chemotherapy. The baseline characteristics are listed in table 1. From 30 patients tumor material was available for genotyping and in 27 patients immunohistochemistry could be performed (see table 1). In tumors of 18 patients the 1p/19q co-deletion was found.

Table 1. Baseline characteristics of patients treated with upfront PCV for large low-grade oligodendroglial tumors.

Characteristic	***No. (%)***
Gender	
Male	23 (72%)
Female	9 (28%)
Median age (range)	46.5 year (28-64 year)
Number of involved brain lobes:	
2 lobes	14 (44%)
3 lobes	5 (16%)
4 lobes	9 (28%)
5 lobes	2 (6%)
6 lobes	1 (3%)
7 lobes	1 (3%)
Initial symptom	
Epilepsy	27 (84%)
Other	5 (16%)
Type of surgery	
Biopsy	19 (59%)
Partial resection	13 (41%)
WHO performance score	
0-1	28 (88%)
2	4 (12%)
Median time from 1st symptom till start chemotherapy (range)	37 months (3-202 months)
Median mean tumor diameter at start chemotherapy (range)	82.5mm (55-122mm)
Histology at central review	
Low-grade oligodendroglioma	17 (53%)
1p/19q loss (no/yes/unknown)	4/12/1
Low-grade oligo-astrocytoma	10 (31%)
1p/19q loss (no/yes/unknown)	6/4/0
Anaplastic oligodendroglioma	3 (10%)
1p/19q loss (no/yes/unknown)	1/2/0
Unspecified low-grade glioma	2 (6%)
1p/19q loss (no/yes/unknown)	1/0/1
Molecular characteristics	
Combined 1p/19q loss	18 out of 30 patients (60 %)
IDH1 positive cells	17 out of 27 patients (63%)
MIB1 labeling of more than 5%	2 out of 27 patients (7%)

PCV: procarbazine, CCNU (lomustine) and vincristine chemotherapy; IDH1: Isocitrate dehydrogenase 1

PCV chemotherapy and toxicity

A total number of 159 PCV cycles were administered; the median number of cycles given was 5.5 (range 1-6). Sixteen patients (50%) completed six cycles. Reasons for premature discontinuation of PCV were hematological toxicity in 9 patients, non-hematological toxicity in 4 patients, tumor progression in 2 patients, and one patient stopped because of an unrelated second malignancy. Grade 3 toxicities were seen in 15 (47%) and grade 4 toxicities in one (3%) out of the 32 patients (see table 2). In four patients in whom PCV was discontinued after 3 cycles because of toxicities (two patients with grade 3 pancytopenia, one patient with grade 3 elevated transaminases and one patient with grade 3 fatigue) chemotherapy was continued with TMZ for a maximum of 12 cycles.

Table 2. Maximum toxicity (grade 3 and 4) in patients treated with upfront PCV for large low-grade oligodendroglial tumors

Toxicity	Grade 3	Grade 4
WBC count	4/32 (13%)	
Neutrophils	3/32 (9%)	1/32 (3%)
Platelets	3/32 (9%)	
Any hematological toxicity	5/32 (16%)	1/32 (3%)
Nausea and vomiting	2/32 (6%)	
Allergic skin reaction	4/32 (13%)	
Hepatotoxicity	1/32 (3%)	
Fatigue	6/32 (19%)	
Neuropathy	1/32 (3%)	
Any (non)-hematological toxicity	15/32 (47%)	1/32 (3%)

PCV: procarbazine, CCNU (lomustine) and vincristine chemotherapy, WBC: white blood cell

Progression free and overall survival

The median follow-up was 94 months (range 6-154 months). Table 3 shows the median PFS (mPFS), median OS (mOS) and ORR. Both the mPFS (p=0.024; see figure 1A) and the mOS (p=0.003; see figure 1B) were significantly longer in patients with 1p/19q loss. The ten years PFS in 1p/19q co-deleted tumors was 34%. The two patients with gliomas in which the MIB-1 labeling index was 10% and 15% (both with 1p/19q loss) had a lower PFS than the patients with a MIB1 labeling index of 0% (mPFS 13 months versus 65 months; p=0.001), but OS was similar. Three patients were diagnosed with an AO at central review, OS and PFS in these patients was similar compared to the patients with WHO grade II tumors at central review. IDH1 did not correlate with either PFS or OS. In patients with the largest tumors (MTD higher than the median MTD of 82.5mm) a trend to a shorter PFS (mPFS 36 versus 63 months; p=0.054) and a significantly shorter OS (mOS 84 months versus not reached; p=0.006) were found in comparison to the rest of the patients. An objective response according to the RANO criteria was seen in 23 patients (18 MR and 5 PR). Eleven of the 12 patients with more than 5-year progression free survival were able to perform daily activities and work at their pre-chemotherapy level until progression. One patient was unable to perform work at baseline because of neurological deficits, but he continued to be able to live independently. In one patient with severe cognitive deficits treatment was discontinued because of lack of clinical improvement after one cycle of PCV.

Table 3. Progression free survival, overall survival and objective response rate in patients treated with upfront PCV chemotherapy for large low-grade oligodendroglial tumors.

	mPFS (mo)	mOS (mo)	ORR (n (%))
All patients (n=32)	46	120	23 (72%)
Combined 1p/19q loss (n=18*)	67	NR (mFU 107 mo)	14 (78%)
No 1p/19q loss (n=12*)	35	83	9 (75%)

mPFS: median progression free survival, mo: months, mOS: median overall survival, ORR: objective response rate according to the RANO criteria, including minor response, mFU: median follow-up

*Tumor material was available for genotyping in 30 out of 32 patients

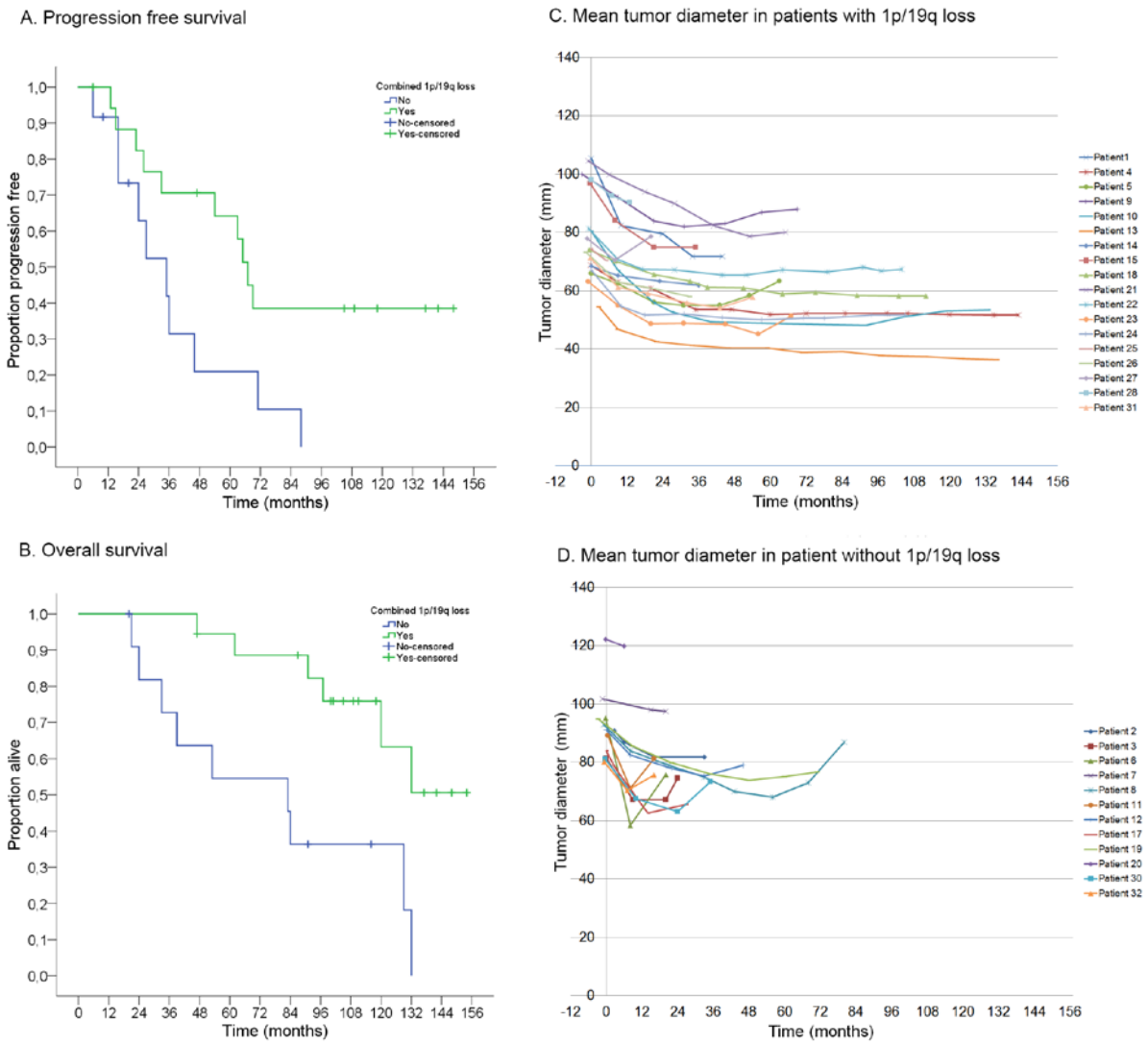

Figure 1. Overall survival, progression free survival and mean tumor diameters plotted over time from the start of the treatment with upfront procarbazine, CCNU (lomustine) and vincristine (PCV) chemotherapy in patients with large low-grade oligodendroglial tumors. A. Progression free survival curves; B. Overall survival curves; C. Mean tumor diameters of patients with combined loss of 1p and 19q. D Mean tumor diameters of patients without combined loss of 1p and 19q.

Volumetric measures

The MTD decreased in all patients during the PCV treatment with a median DEV of -11.7 mm/year (range -1.1 to -55.4 mm/year). After discontinuation of the PCV an ongoing decrease of the MTD was seen for a median duration of 35 months (range 5-136 months) in the 18 patients with combined 1p/19q loss and 15.5 months (range 6-56 months) in the patients without combined 1p/19q loss measured from the start of the treatment. None of the patients developed an increasing MTD during the PCV chemotherapy. The patient in whom treatment was discontinued because of lack of improvement showed a stable MTD. Fast (DEV < -11.7 mm/year during the treatment with PCV) and slow responders (DEV > -11.7 mm/year during the treatment with PCV) did not differ in OS and PFS.

Most patients (15/22; 68%) progressed with enhancement on the T1 weighted MRI after gadolinium. The progression according to the RANO criteria was heralded by an increase of the MTD (positive DEV) in only 3 of 22 (14%) progressive patients (patient 5,8 and 9; figure 1C and 1D).

Treatment at progression

Table 4 shows the details on further treatment after progression. Twenty out of the 22 patients who progressed were further treated with RT. The interval between the start of the PCV chemotherapy and initiation of RT was 31 months in the patients with intact 1p/19q and 75 months in the patients with 1p/19q loss.

Table 4. Treatment after progression in patients treated with upfront PCV chemotherapy for large low-grade oligodendroglial tumors.

Events after PCV chemotherapy	***No. (%)***
Died without further treatment	3 (9%)
Death related to brain tumor	1 (3%)
Death unrelated	2 (6%)
Ongoing response	7 (22%)
Further treatment after progression	22 (69%)
RT only	3 (9%)
RT and one line chemotherapy (mainly TMZ)	6 (19%)
RT and 3 lines of chemotherapy	1 (3%)
RT, TMZ and re-RT	1 (3%)
RT, TMZ, resection and re-RT	1 (3%)
TMZ only	1 (3%)
TMZ and RT	7 (22%)
Resection, TMZ and RT	2 (6%)

PCV: procarbazine, CCNU (lomustine) and vincristine chemotherapy, RT: radiotherapy, TMZ: temozolomide chemotherapy

DISCUSSION

The role of chemotherapy in LGG is gradually being clarified. Initial reports in uncontrolled studies on chemotherapy in LGG showed modest response rates with mostly minor responses but of interesting long duration especially in 1p/19q co-deleted tumors. Data from randomized trials are now becoming available. A first and still early analysis of the European Organization for Research and Treatment of Cancer (EORTC) study 22033 presented at the ASCO meeting in 2013 suggests that PFS does not differ between patients with LGG with 1p loss receiving upfront dose-dense TMZ (75 mg/m² daily x 21 days, q28 days, max. 12 cycles) versus patients receiving RT (50.4 Gy/28 fractions). In contrast RT may provide a superior PFS in patients without 1p loss (p = 0.06).(19) The median OS was not yet reached in that study.

Data on long term follow-up on chemotherapy in LGG are however scarce. It therefore remains unclear whether sustained responses are obtained, especially in patients with oligodendroglial tumors and if so of what duration. Although the present study has several limitations (retrospective design, based on a limited number of patients, no serial neurocognitive tests, no data on the outcome of epileptic seizures and molecular data not available in all patients), the results show a long lasting PFS and OS after treatment with upfront PCV in patients with large low-grade OD or OA. Outcome was particularly favorable in patients with combined 1p and 19q loss, with 34% of these patients still being free from progression after 10 years.

Furthermore this is the first study to show long term follow-up of growth kinetics in these patients. We observed a decrease in the MTD during the PCV treatment in all patients with a median DEV of -11.7 mm/year, very similar to that observed by others.(20) During PCV treatment MTD increase did not occur, and after discontinuation of the PCV an ongoing and prolonged MTD decrease for a median of almost 3 years was observed in the 18 patients with combined 1p/19q loss. In 4 patients the MTD continued to decrease for more than 6 years (figure 1 c).

Outside neuro-oncology interest in tumor growth kinetics has also increased. A recent study shows a near-lineal relationship between growth kinetics and survival in phase I studies, questioning the value of classical categorical responses.(21) In several studies in LGG, the MTD was found to be of use in monitoring growth of both untreated and treated LGG, improving our understanding of the clinical behavior of these tumors.(14, 20, 22, 23, 24) The optimal method of assessing outcome (RANO vs MTD) in LGG patients remains to be defined.(13) Of note, others have shown, with tumor volume measurements, that in untreated LGG an increase in growth rate is a common early indicator of malignant transformation.(25) Somewhat surprisingly, we found an increase of the MTD presaged the progression only in 3 out of 22 patients and most patients progressed with enhancement on the MRI scan suggestive of malignant dedifferentiation without a prior increase in growth rate. Others have demonstrated an increase in mutations in LGG patients progressing after TMZ chemotherapy.(26) This suggests that at a certain point in time after chemotherapy new mutations induce malignant dedifferentiation leading to more aggressive clinical behavior. Another series showed that LGG patients with a fast response after RT had worse survival, but in the present study a fast response (DEV faster than the medium of -11.7 mm/year) was not prognostic.(22)

The rationale for the present choice for upfront chemotherapy was our wish to postpone RT and the associated delayed cognitive disturbances in LGG patients. In our cohort of patients with large OD requiring large RT treatment volumes (estimated more than 50% of the cerebral hemispheres), RT could be delayed for a considerable period of time in all patients. Furthermore, the patients remained in very good condition and most were able to do work at their previous level until progression. Had these patients with large tumors and long survival (especially patients with 1p/19q loss) been treated with RT it is not unlikely that they would have suffered from cognitive deterioration due to the RT. A previous study in long term survivors with LGG showed that long-term survivors who received RT showed a progressive decline in cognition, whereas patients who did not have RT had a stable cognitive status over time.(10) Similarly, neuropsychological evaluation in cohort of 37 long term survivors of the EORTC study 26951 (all irradiated) showed that out of the 27 patients still free from progression since initial treatment 30% were severely cognitively impaired, 41% were employed and 81% lived independently.(27) The impact of RT on the cognitive functioning in long term survivors of LGG remains poorly understood, but the patients in the present cohort that remained free from progression continued to do well even after many years of follow-up. This supports an approach in which RT is delayed as long as this is safely possible. Although, one of the limitations of this study is the lack of data on the outcome of the epileptic seizures, while the control of epilepsy in low-grade glioma is an important issue.(28)

Until recently, standard treatment for LGG requiring postsurgical treatment was either RT or chemotherapy. This may however be changing. An early report of the Radiation Therapy Oncology Group (RTOG) study 9802 on adjuvant PCV chemotherapy in LGG showed that for those patients surviving at least 2 years the addition of PCV to RT conferred a considerable survival advantage, suggesting a delayed OS benefit for the addition of PCV chemotherapy to RT.(29) An update on the more mature data of this study has been announced and it is expected that adjuvant PCV to RT prolongs survival significantly compared with radiation therapy alone.(30) This may imply that RT with adjuvant chemotherapy will become the next standard therapy in high-risk LGG.

These latest results of the RTOG 9802 study results in a complicated dilemma, putting the patient between Scylla and Charybdis. Where on the one hand early RT with adjuvant chemotherapy may prolong survival, on the other hand it could lead to cognitive decline and loss of quality of life in the long run, especially in large LGG with long survival (like the OD with 1P/19 loss in the present study). The alternative approach with chemotherapy alone may compromise survival however.

Further studies are needed to clarify whether patients with large OD or OA with 1p/19q loss are from a quality of survival perspective better off with upfront chemotherapy, and preservation of RT for recurrences after chemotherapy. It needs no further explanation that the phase III studies addressing these issues with their inherent long follow-up are extremely difficult to conduct. This appears however be the most relevant next question in LGG, apart from better drugs to treat these patients.

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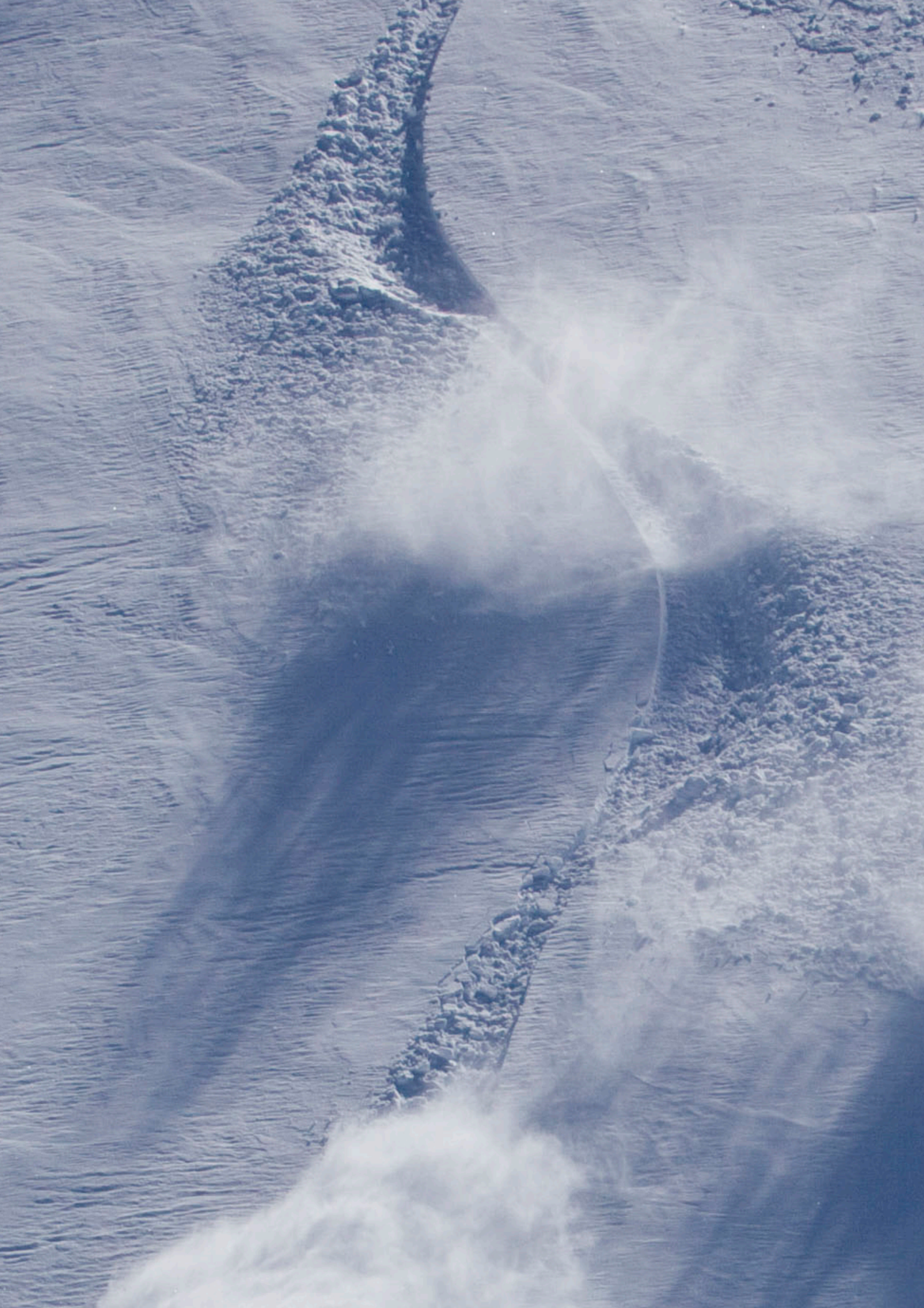

Chapter 8
Summary and future perspectives

INTRODUCTION

Clinical decision-making in patients with gliomas depends on an adequate prognostication and an understanding of the status of the disease of the patient. In this thesis, the diagnosis of tumor progression after combined chemo-irradiation with temozolomide and outcome to chemotherapy in relation to a variety of molecular prognostic factors have been examined.

THE INCIDENCE OF EARLY PSEUDO-PROGRESSION IN A COHORT OF MALIGNANT GLIOMA PATIENTS TREATED WITH CHEMO-IRRADIATION WITH TEMOZOLOMIDE.

With the results of the EORTC 26981 radiotherapy (RT) plus concomitant and adjuvant temozolomide (TMZ) became the standard of care for patients with newly diagnosed glioblastoma.(1) Following the introduction of this treatment we observed the occurrence of progressive Magnetic Resonance Imaging (MRI) lesions immediately after the end of concurrent chemo-irradiation with TMZ, with spontaneous improvement.

This phenomenon had been investigated previously in 32 patients with an anaplastic glioma who received RT only.(2) In that study 33% of the patients with a progressive lesion immediately after RT showed a stabilized or improved lesion on subsequent scans during at least 6 months period, without additional treatment. However, the incidence of this phenomenon and accompanying clinical symptoms in malignant glioma patients treated with chemo-irradiation with TMZ was unknown.

We termed this phenomenon pseudo-progression and investigated its incidence by reviewing a cohort of 85 newly diagnosed malignant glioma patients treated with RT plus concomitant and adjuvant TMZ. The pre- and post-RT brain scans from patients treated with chemo-irradiation with TMZ for a malignant glioma were reviewed. Early progression was defined as progression (≥25% increase) on the MRI-scan 4 weeks after RT and concomitant TMZ, with or without neurological deterioration and on a stable or higher dose of dexamethasone. Real progression was scored if the patient with early progression had further progression within the following 6 months. Pseudo-progression was scored if the patient with early progression had at least a 50% decrease of the enhancing lesion during further follow-up or remained clinically and radiologically stable for at least 6 months after RT/TMZ without any treatment other than adjuvant cycles of TMZ.

From the 85 patients that were identified, 36 patients had early progression. Eighteen out these 36 patients (50%) were diagnosed with pseudo-progression. Six of 18 patients with pseudo-progression and 12 of the 18 patients with real progression developed new clinical signs and symptoms during RT or in the first 4 weeks thereafter.

We were the first to show such high incidence of pseudoprogression in patients treated with chemo-irradiation with TMZ. Because of these findings it became a general treatment policy to continue TMZ in case of progressive lesions immediately after chemo-irradiation especially if asymptomatic, and to consider surgery in symptomatic cases. It was also concluded that inclusion of patients with a progressive lesion directly after chemo-irradiation in studies on recurrent gliomas would lead to an overestimation of the results.

Disappointingly, up till now modern imaging techniques such as PET, magnetic resonance spectroscopy, diffusion-weighted and perfusion imaging have only limited power to make a distinction between real and pseudoprogression in the individual patient.

IDH1 MUTATIONS IN LOW-GRADE ASTROCYTOMAS PREDICT SURVIVAL BUT NOT RESPONSE TO TEMOZOLOMIDE.

Mutations in isocitrate dehydrogenase 1 (IDH1) occur frequently in glial tumors of the central nervous system.(3, 4, 5, 6, 7) The incidence of IDH1 mutations in glial tumors ranged from 12 % in primary GBM to 70% in anaplastic astrocytomas and 85-90% in low-grade astrocytomas and

secondary GBM. IDH1 mutations were observed in tumors with TP53 mutations as well as in tumors with the 1p/19q co-deletion. Because TP53 mutation and loss of 1p/19q are mutually exclusive aberrations, IDH1 mutations seem to occur very early on in glial tumor development at the time when the stem cell can still gives rise to astrocyte and oligodendrocyte glial cell lineages. Patients with high-grade astrocytomas with IDH1 or IDH2 mutations were reported to have a better survival, but at that time it was unknown if this improved survival also holds for low-grade astrocytoma and, perhaps even more importantly, whether these mutations predicted outcome to treatment.

We therefore retrospectively investigated the correlation of IDH1 and IDH2 mutations with overall survival and response in the cohort of 70 patients with dedifferentiated low-grade astrocytomas from the 5 centers (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI and VUMC, Amsterdam; RUNMC, Nijmegen) that were treated with TMZ at the time of progression after prior RT for the outcome to TMZ treatment.

From 49 patients tumor tissue was available for molecular analysis; from 27 patients tissue from two consecutive operations was available. IDH1 mutations were present in 86% of these 49 cases. No mutations in IDH2 were found. The presence of IDH1 mutations was associated with a significantly improved overall survival (median overall survival 98 versus 48 months in IDH1 wild type tumors), but did not correlate with outcome to TMZ treatment. These results showed that IDH1 mutations identified a subgroup of grade II gliomas with an improved survival, but in this study were found unrelated to response to the TMZ given at the time of progression after radiotherapy.

FIRST-LINE TEMOZOLOMIDE CHEMOTHERAPY IN PROGRESSIVE LOW-GRADE ASTROCYTOMAS AFTER RADIOTHERAPY: MOLECULAR CHARACTERISTICS IN RELATION TO RESPONSE.

Only few studies systematically examined the effect of TMZ in progressive low-grade astrocytoma after surgery and RT.(8, 9)These studies are however difficult to interpret because they included small heterogeneous groups of patients, with both low-grade lesions and dedifferentiated tumors, and with a variety of prior treatments. Furthermore, there are limited data on molecular correlates with outcome to chemotherapy in patients with progressive low-grade astrocytoma after RT.

We therefore retrospectively collected data of all patients from 5 hospitals in the Netherlands (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI and VUMC, Amsterdam; RUNMC, Nijmegen) with a progressive low-grade astrocytoma, with a contrast-enhancing lesion on MRI after RT treated with TMZ since TMZ became available (1995) until 2006. Seventy patients were found, but 12 patients received TMZ after prior chemotherapy and were excluded from further analysis. We therefore evaluated a cohort of 58 patients treated with 1st line TMZ after RT of a previous low-grade astrocytoma and investigated the relation between outcome and mutations in the IDH1, IDH2 and TP53 gene, O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation, trisomy of chromosome 7 and loss of chromosome 1p and 19q. All patients received TMZ at a dosage of 200 mg/m2/day on day 1-5 every 4 weeks. Complete and partial responses were considered objective responses.

In this cohort six months progression free survival was 67%, the median overall survival was 14 months. An objective response was obtained in 54%. TP53 mutations and loss of chromosome 19q showed a borderline association with progression free survival, but none of the other molecular characteristics were correlated with outcome to TMZ. Both a methylated MGMT promoter gene and IDH1 mutations were found in 86% of the tumor samples. IDH1 mutations and MGMT promoter methylation were highly correlated ($p<0.001$). Neither MGMT promoter methylation nor IDH1 mutations correlated with progression free survival, but the interval between the very first

symptom of the low-grade astrocytoma and the start of the TMZ was significantly longer in the patients with IDH1 mutations (p=0.01) and in patients with a methylated MGMT promoter (p=0.02).
The 6 months progression free survival in this trial lies in between the 6 months progression free survival of anaplastic astrocytoma as reported in the pivotal TMZ trial and the outcome of recurrent anaplastic oligodendroglioma; still overall survival was in the same range as in the anaplastic astrocytoma trial.(10, 11) Furthermore it was concluded from this study that MGMT promoter methylation and IDH1 mutations are strongly correlated and seem to predict survival from the time of diagnosis, but not progression free survival to TMZ.

DOSE DENSE 1-WEEK ON/ 1-WEEK OFF TEMOZOLOMIDE IN RECURRENT GLIOMA: A RETROSPECTIVE STUDY.

The treatment options for patients with gliomas failing RT and a first line of alkylating or methylating chemotherapy are limited. Furthermore once TMZ became standard of care for newly diagnosed glioblastoma, the pattern of care for relapsing patients had to be redefined. One of the main mechanisms of tumor resistance to TMZ is mediated by MGMT.(12) Evidence supporting this role of MGMT comes from clinical studies indicating that methylation of the promoter of MGMT is associated with improved tumor response and survival in patients with GBM.(13, 14) Because of the more continuous exposition with ddTMZ it has been assumed that dose dense TMZ (ddTMZ) schedules could overcome MGMT dependent resistance against TMZ by a more effective depletion of intracellular levels of MGMT.(15) Since then, a large number of trials have been reported, and some of these generated significant interest because of promising results, with reports of 6 months progression free survival of up to 44% in one trial.(16)
We investigated the efficacy and tolerability of one-week on/ one-week off TMZ (ddTMZ) regimen in a cohort of patients treated with ddTMZ between 2005 and 2011 for progression of a glioblastoma during or after chemo-irradiation with TMZ or a recurrence of another type of glioma after RT and at least one line of chemotherapy. Patients received ddTMZ at 100-150 mg/m2/d (days 1 through 7 and 15 through 21 in cycles of 28-days). All patients had a contrast-enhancing lesion on MRI and response was assessed using the RANO criteria;(17) complete and partial responses were considered objective responses.
Fifty-three patients were included. The median number of cycles of ddTMZ was 4 (range 1-12). Eight patients discontinued chemotherapy because of toxicity. Two of 24 patients with a progressive glioblastoma had an objective response; 6 months progression free survival in glioblastoma was 29%. Three of the 16 patients with a recurrent WHO grade II or III astrocytoma or oligodendroglioma or oligo-astrocytoma without combined 1p and 19q loss had an objective response and 6 months progression free survival in these patients was 38%. Four out of the 12 evaluable patients with a recurrent WHO grade II or III oligodendroglioma or oligo-astrocytoma with combined 1p and 19q loss had an objective response; 6 months progression free survival in these patients was 62%.
This study indicates that ddTMZ is safe and effective in recurrent glioma, despite previous TMZ and/or nitrosourea chemotherapy. Our data do not suggest superior efficacy of this schedule as compared to the standard day 1-5 every four weeks schedule.

SINGLE-AGENT BEVACIZUMAB OR LOMUSTINE VERSUS A COMBINATION OF BEVACIZUMAB PLUS LOMUSTINE IN PATIENTS WITH RECURRENT GLIOBLASTOMA (BELOB TRIAL): A RANDOMISED CONTROLLED PHASE II TRIAL.

Despite the absence of well-controlled trials, bevacizumab is currently widely used in the treatment of recurrent glioblastoma. In the absence of controlled trials it remains unclear if the

high response rates to bevacizumab translate into an overall survival benefit. The BELOB trial was the first randomised controlled phase II trial of bevacizumab in recurrent glioblastoma.
This trial was an open-label, three-group, multicentre phase II study undertaken in 14 hospitals in the Netherlands. Adult patients (≥18 years of age) with a first recurrence of a glioblastoma after chemo-irradiation with TMZ were randomly allocated by a web-based program to treatment with oral lomustine 110 mg/m2 once every 6 weeks, intravenous bevacizumab 10 mg/kg once every 2 weeks, or combination treatment with lomustine 110 mg/m2 every 6 weeks and bevacizumab 10 mg/kg every 2 weeks. Randomisation of patients was stratified with a minimisation procedure, in which the stratification factors were centre, Eastern Cooperative Oncology Group performance status, and age. The primary outcome was overall survival at 9 months, analysed by intention to treat. A safety analysis was planned after the first ten patients completed two cycles of 6 weeks in the combination treatment group.
Between Dec 11, 2009, and Nov 10, 2011, 153 patients were enrolled. The pre-planned safety analysis was done after treating eight patients. Because of haematological adverse events (three patients had grade 3 thrombocytopenia and two had grade 4 thrombocytopenia) the lomustine dose in the combination treatment group was reduced to 90 mg/m2. After the reduction in lomustine dose in the combination group, the combined treatment was well tolerated. Thus, in addition to the eight patients who were randomly assigned to receive bevacizumab plus lomustine 110 mg/m2, 51 patients were assigned to receive bevacizumab alone, 47 to receive lomustine alone, and 47 to receive bevacizumab plus lomustine 90 mg/m2. Of these patients, 50 in the bevacizumab alone group, 46 in the lomustine alone group, and 44 in the bevacizumab and lomustine 90mg/m2 group were eligible for analyses. Nine-month overall survival was 43% (95% CI 29–57) in the lomustine group, 38% (25–51) in the bevacizumab group, 59% (43–72) in the bevacizumab and lomustine 90 mg/m2 group, 87% (39–98) in the bevacizumab and lomustine 110 mg/m2 group, and 63% (49–75) for the combined bevacizumab and lomustine groups.. At the time of this analysis, 144/148 (97%) of patients had died and three (2%) were still on treatment.
The combination of bevacizumab and lomustine met pre-specified criteria for assessment of this treatment in further phase III studies. However, the results in the bevacizumab alone group do not justify further studies of this treatment.

TREATMENT OF LARGE LOW-GRADE OLIGODENDROGLIAL TUMORS WITH UPFRONT PROCARBAZINE, LOMUSTINE, AND VINCRISTINE CHEMOTHERAPY WITH LONG FOLLOW-UP: A RETROSPECTIVE COHORT STUDY WITH GROWTH KINETICS.

In the late nineties of the previous century, our department started to use upfront chemotherapy in patients with large oligodendroglial tumors, to postpone large fields of RT to the brain, with potential long-term toxicities (cognitive deficits).(18) This strategy is attractive particularly in patients with chemotherapy responsive tumors and with anticipated long-term survival, because these patients are longer at risk to develop clinically significant cognitive disturbances. In a first report after limited follow-up we observed that early chemotherapy while withholding RT showed prolonged stable disease or minor responses in a significant subset of patients.(19) Others obtained similar results with TMZ or procarbazine, CCNU and vincristine (PCV) chemotherapy.(8, 20, 21, 22, 23, 24)
In a long-term follow-up study (median follow-up duration: 8 years, maximum follow-up: 13 years) of 32 patients who received upfront PCV we studied clinical outcome and correlated this to the 1p/19q and IDH status. Patients were treated with PCV for a maximum of 6 cycles. The response to treatment was defined according to the RANO criteria and; in addition change over time of mean tumor diameters (growth kinetics) was calculated.

Thirty-two patients were treated between 1998 and 2006, 18 of which were diagnosed with 1p/19q co-deleted tumors. Median follow-up duration was 8 years (range 0.5 - 13 years). The median overall survival was 120 months and the median progression-free survival was 46 months. Growth kinetics showed an ongoing decrease of the mean tumor diameter after completion of chemotherapy, during a median time of 35 months. Unexpectedly, progression was not heralded by an increase of the mean tumor diameter but by the development of enhancement. 1p/19q co-deletion was associated with a significant increase in overall survival (median overall survival 83 months versus not reached for codeleted tumors; p=0.003)) and progression free survival (median progression free survival 35 months versus 67 months for codeleted tumors; p=0.024). Patients with combined 1p/19q loss had a 10 year progression free survival of 34%. Twenty out of the 22 patients who progressed were further treated with RT. The interval between the start of the PCV chemotherapy and initiation of RT was 31 months in the patients with intact 1p/19q and 75 months in the patients with 1p/19q loss.
This long-term follow-up study indicates that in particular in patients with low-grade oligodendroglial tumors with combined 1p/19q loss upfront PCV chemotherapy is associated with long progression free survival and overall survival and delays RT for a considerable period of time.

FUTURE PERSPECTIVES

Currently, treatment of diffuse adult gliomas exists of surgery (safe removal as extensive as safely possible), RT and chemotherapy. Trials conducted in the eighties and the nineties of the past century have demonstrated the role of RT in the management of glioma, and have also demonstrated that optimization of this modality is unlikely to further improve survival. The phase III trials on adjuvant chemotherapy reported in the past decade have shown that chemotherapy is a part of the initial management of all adult diffuse glioma, but also demonstrate that new drugs and modalities are needed for further improvement. Chemotherapy for gliomas today basically consists of either TMZ or PCV, with a still undefined role for bevacizumab.
The current thesis predominantly focused on the evaluation of classical chemotherapy in recurrent diffuse glioma. This thesis has further established the role TMZ in recurrent gliomas, of PCV in newly diagnosed oligodendrogliomas and suggests a role of bevacizumab in combination with lomustine in recurrent glioblastoma. In addition, the chapter on pseudo-progression has demonstrated the need to continue chemotherapy in glioblastoma patients with a relapse within three months of the end of RT/TMZ and exclude them from clinical trials on recurrent glioblastoma.
It seems unlikely that outcome in adult diffuse glioma patients can be further enhanced with the currently available cytotoxic drugs. To achieve this, other approaches are needed. The understandings of the processes that drive growth of glioma have improved considerably. High-throughput screening techniques have given rise to a wealth of new information regarding the aberrant signaling pathways that drive the tumor phenotype. Malignant gliomas are characterized by a wide number of genetic and epigenetic abnormalities that account for their properties: invasion, unregulated growth, and resistance to apoptosis. These include gene mutation, loss, amplification, and rearrangement as well as promoter methylation and other mechanisms controlling gene expression.
The recognition of diverse glial tumor subtypes with different outcome to treatment has led to an interest in the development of personalized medicine strategies for the treatment of glioma. Despite many effort though, to date no clinically interesting results are observed in malignant glioma with single agent targeted therapies. Reasons for this may relate to alternative signalling pathways, molecular heterogeneity and poor penetration of many agents through an intact blood brain barrier.

However, new compounds are under development and existing ones are now being investigated in combinations, both with one another and with other therapeutic modalities including conventional chemotherapy and RT. Without trying to make a comprehensive overview, some compounds that are currently pursued in clinical trials are described below.

IDH as a target

As stated above mutations in the isocitrate dehydrogenase (IDH) genes occur frequently in adult diffuse glioma, especially in low-grade gliomas, anaplastic gliomas and secondary glioblastoma.(3, 4, 5, 6, 7) IDH1 and IDH2 genes encode the enzymes isocitrate dehydrogenase 1 ((NADP+), soluble) and 2 ((NADP+), mitochondrial). The IDH1 enzyme resides outside the mitochondria, while IDH2 resides within mitochondria. IDH1 and IDH2 enzymes catalyze the reaction of isocitrate to alpha-ketoglutarate and vice versa. Mutant IDH enzymes (IDH1 or IDH2) are unable to catalyze these two normal reactions, but instead reduce alpha-ketoglutarate into the oncometabolite 2-hydroxyglutarate (2-HG). It has been shown that IDH mutant 2HG producing glioma cells can prevent DNA and histone demethylation.

Inhibition of DNA demethylation, causing hypermethylation of CpG dinucleotides, may have an impact on gene transcription and genome stability.(25) Histone demethylation is needed for lineage-specific progenitor cells to differentiate into terminally differentiated cells.(26) So, instead of maturing, the cells remain primitive and proliferate quickly, leading to profound epigenetic deregulation and tumorigenesis.

Inhibition of the IDH mutated proteins may lead to clinical benefit for patients with IDH mutations. Oral candidates (IDH305 and AG-120) inhibiting the mutated IDH1 enzyme are identified and phase I trials are ongoing (ClinicalTrials.gov Identifier: NCT02381886 and NCT02073994).

PTEN antagonist

Allelic loss of chromosome 10 has long been known to be common in high grade human glioma.(27) After the identification of PTEN (phosphatase and tensin homolog deleted on chromosome 10) on chromosome 10, PTEN mutations and homozygous deletions were reported frequently in glioblastomas.(28, 29, 30). It has been shown that the phosphatase tumor suppressor PTEN is essential for regulating the highly oncogenic phosphatidylinositol 3-kinase (PI3K) signaling pathway. The PI3K signaling regulates diverse cellular functions, cell survival and growth, proliferation, cellular resilience and repair, cell migration, and angiogenesis.(31) PI3K pathway inhibitors might therefore be a good treatment option in glioblastoma with PTEN mutations and homozygous deletions.(32) A wide spectrum of PI3K pathway inhibitors are currently in clinical development and buparlisibis currently being investigated in a Phase I/II trial in inhibiting in glioblastoma patients with mutations and homozygous deletions of PTEN, together with a c-MET receptor tyrosine kinase inhibitor (ClinicalTrials.gov Identifier: NCT01870726).

c-MET antagonists

The c-Met receptor tyrosine kinase family is structurally distinct from other receptor tyrosine kinase families and is the only known high-affinity receptor for hepatocyte growth factor (HGF), also known as scatter factor (SF).(33) Binding of HGF/SF to the c-Met receptor promotes growth, invasion and migration of both tumor cells and endothelial cells lining the tumor's blood supply.(34) c-Met has been shown to be over-expressed or mutated in gliomas.(34) c-Met receptor tyrosine kinase inhibitors are thus an attractive multi-faceted approach to targeting the glial tumor cells by blocking multiple events involved in disease progression.

Antibody drug conjugates

The success with Epidermal Growth Factor Receptor (EGFR)-directed therapies (i.e. gefitinib, erlotinib and cetuximab) in lung cancer and colon cancer raised expectations that these agents will

show activity against glioblastoma, given the high frequency of oncogenic EGFR alterations in glioblastoma. This expectation has up till now not been fulfilled, questioning the rationale for EGFR inhibiting strategies in glioblastoma patients.(35, 36, 37)
Antibody drug conjugates are a rapidly growing class of cancer drugs that combine the targeting properties of monoclonal antibodies with the anti-tumor effects of potent cytotoxic drugs. ABT-414 is an antibody drug conjugate designed for the treatment of tumors overexpressing EGFR, like glioblastomas. ABT-414 binds to EGFR, is internalized, and then a potent antimicrotubule agent (monomethylauristatin F) is released causing the disruption of critical cellular processes and cell death. ABT-414 is currently being investigated in a Phase I study in glioblastoma patients with overexpression of EGFR (ClinicalTrials.gov Identifier: NCT01800695).

Non-targeted therapies
Gene therapy, immune therapy (vaccination strategies and immunomodulators such as anti-PD1 and anti-PDL1 antibodies) and low intensity alternating electric fields, disrupting mitosis and cytokinesis, seem promising, but are out of scope for this thesis on chemotherapy in gliomas.

CONCLUSION
There is clear promise that in the near future new (combinations of) targeted therapies against the key molecular pathways involved in gliomas and possibly other treatment will become available, which will reduce the mortality and morbidity in glioma patients. Successful exploration of targeted agents will require other ways of the evaluation of compounds, such as the demonstration the target is present before the treatment, the demonstration of actual drug penetration in the tumor, of target inhibition and of subsequent changes in downstream signaling. This will complicate further trials in this disease, but present an important step in the proper evaluation of targeted compounds for gliomas. The molecular heterogeneity of gliomas is another factor that will impact the progress in the treatment of this disease. Major collaborative efforts, and the evaluation of promising treatment in well-designed clinical trials, will be needed to improve outcome.

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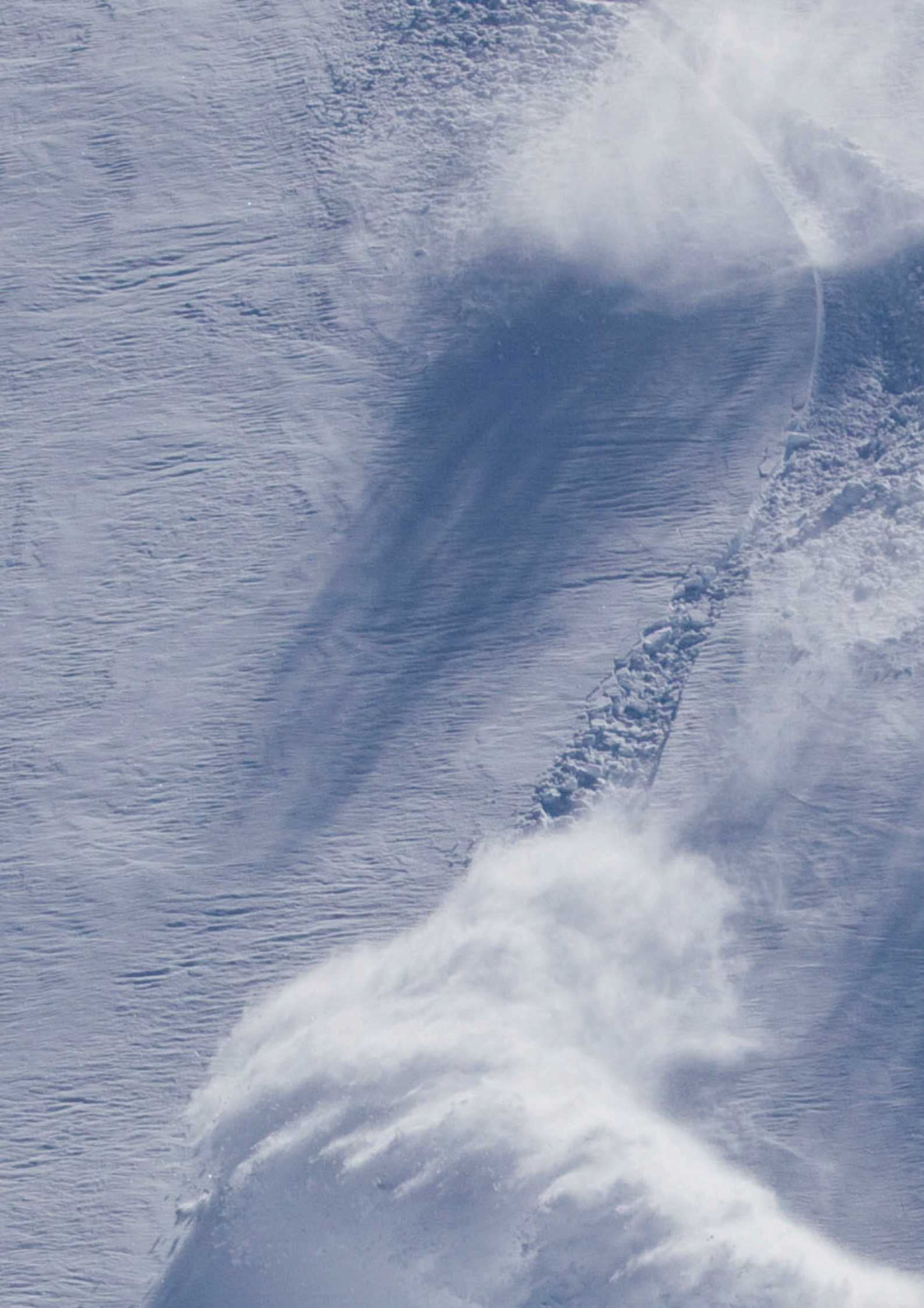

Chapter 9
Samenvatting en toekomstperspectief

INTRODUCTIE

De klinische besluitvorming bij patiënten met een primaire hersentumor (glioom) is afhankelijk van een adequate voorspelling van de prognose en inzicht in de ziektestatus van de patiënt. In dit proefschrift zijn de diagnose van tumorprogressie na gecombineerde chemotherapie /radiotherapie met temozolomide en de effectiviteit van chemotherapie in relatie tot diverse moleculaire prognostische factoren onderzocht bij patiënten met een glioom.

DE INCIDENTIE VAN VROEGE PSEUDOPROGRESSIE IN EEN COHORT VAN MALIGNE GLIOOM PATIËNTEN DIE BEHANDELD ZIJN MET GECOMBINEERDE RADIOTHERAPIE/CHEMOTHERAPIE MET TEMOZOLOMIDE.

Met de resultaten van de EORTC 26981 studie werd radiotherapie (RT) plus gelijktijdige en aanvullende temozolomide (TMZ) de standaard behandeling voor patiënten met een nieuw gediagnosticeerd glioblastoma.(1) Na introductie van deze behandeling zagen we toename van Magnetic Resonance Imaging (MRI) laesies onmiddellijk na afloop van gelijktijdige chemo-radiotherapie met TMZ (RT/TMZ) die spontaan verbeterden.

Dit verschijnsel was eerder onderzocht bij 32 patiënten met anaplastisch glioom die alleen RT kregen.(2) In deze studie kregen 33% van de patiënten een progressieve laesie direct na RT, die stabiliseerde of verbeterde op latere MRI scans gedurende een periode van tenminste 6 maanden zonder verdere behandeling. Echter, de incidentie van dit verschijnsel en bijbehorende klinische symptomen bij patiënten met een maligne glioom, die behandeld worden met RT/TMZ was onbekend.

We noemden dit verschijnsel pseudo-progressie en onderzochten de incidentie bij een cohort van 85 nieuw gediagnosticeerde maligne glioom patiënten die behandeld werden met RT/TMZ. De pre- en post-RT MRI-scans werden beoordeeld. Vroege progressie werd gedefinieerd als progressie (≥25% toename) op de MRI-scan 4 weken na de RT en gelijktijdige TMZ, met of zonder neurologische verslechtering en op een stabiele of hogere dosis dexamethason. Progressie werd gescoord als de patiënt met vroege progressie verdere toename had in de 6 daarop volgende maanden. Pseudo-progressie werd gescoord als de patiënt met vroege progressie een afname had van de aankleurende laesie van ten minste van 50% of klinisch en radiologisch stabiel bleef gedurende tenminste 6 maanden na RT/TMZ zonder verdere therapie dan de adjuvante behandelingscycli met TMZ.

Van de 85 patiënten die werden geïdentificeerd, hadden 36 patiënten vroege progressie. Achttien van deze 36 patiënten (50%) werden gediagnosticeerd met pseudo-progressie. Zes van de 18 patiënten met pseudo-progressie en 12 van de 18 patiënten met echte progressie ontwikkelde nieuwe neurologische klachten en symptomen gedurende de RT of in de eerste 4 weken daarna.

We waren de eersten die een dergelijke hoge incidentie van pseudo-progressie lieten zien bij patiënten die behandeld werden RT/TMZ. Vanwege deze bevindingen werd het algemeen gebruik om, vooral bij asymptomatische patiënten, door te behandelen met adjuvant TMZ bij progressie onmiddellijk na RT/TMZ, en chirurgie te overwegen bij symptomatische patiënten. Daarnaast werd geconcludeerd dat de inclusie van patiënten, met een progressieve laesie direct na RT/TMZ in studies met betrekking tot recief gliomen, zou leiden tot een overschatting van de resultaten.

Helaas hebben de tot nu bekende moderne beeldvormende technieken, zoals PET, magnetic resonance spectroscopy, diffusie-gewogen en perfusie-gewogen beeldvorming, slechts een beperkt vermogen om een onderscheid te maken tussen echte progressie en pseudo-progressie bij een individuele patiënt.

IDH1 MUTATIES IN LAAGGRADIGE ASTROCYTOMEN VOORSPELLEN DE OVERLEVING, MAAR NIET DE RESPONSE OP TEMOZOLOMIDE.

Mutaties in isocitraat dehydrogenase 1 (IDH1) komen frequent en selectief bij gliomen voor.(3, 4, 5, 6, 7) De incidentie van IDH1 mutaties in gliomen varieert van 12% in primaire glioblastomen (WHO graad IV) tot 70% in anaplastische astrocytomen (WHO graad III) en tot 85 - 90% in laaggradige astrocytomen (WHO graad II) en secundaire glioblastomen. IDH1 mutaties worden waargenomen in gliomen met TP53 mutaties, maar ook in tumoren met 1p/19q co-deleties. Aangezien TP53 mutaties en 1p/19q co-deleties elkaar uitsluitende aberraties zijn, lijken IDH1 mutaties zeer vroeg in gliale tumorontwikkeling te ontstaan, op een moment dat de stamcellen zich nog kunnen ontwikkelen tot de astrocytaire- en oligodendrocytaire gliale cellijnen. Bij patiënten met hooggradige astrocytomen (WHO graad II en IV) en IDH1 of IDH2 mutaties werden een betere overleving gerapporteerd, maar op dat moment was het nog onbekend of deze verbeterde overleving ook gold voor laaggradige astrocytomen en, misschien nog belangrijker, of deze mutaties ook de behandelingsuitkomst voorspellen.

Daarom onderzochten wij retrospectief de correlatie van IDH1 en IDH2 mutaties met de overleving en de response op TMZ in een cohort van 70 patiënten met gedifferentieerde laaggradige astrocytomen uit 5 centra (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI en VUMC, Amsterdam; UMC St Radboud, Nijmegen) die werden behandeld met TMZ voor progressie na eerdere RT.

Van 49 patiënten was tumorweefsel beschikbaar voor de moleculaire analyse; van 27 patiënten was weefsel uit twee opeenvolgende operaties beschikbaar. IDH1 mutaties waren aanwezig in 86% van deze 49 gevallen. Er werden geen IDH2 mutaties gevonden. De aanwezigheid van IDH1 mutaties werd geassocieerd met een significant betere overleving (mediane overleving 98 versus 48 maanden in IDH1 wild type tumoren), maar IDH1 mutaties correleerde niet met de uitkomst van de TMZ behandeling. Deze resultaten toonden aan dat IDH1 mutaties een subgroep van WHO graad II gliomen identificeert met een betere overleving, maar in deze studie werd geen verband gevonden met de response op de TMZ chemotherapie op het tijdstip van progressie na RT.

EERSTELIJNS TEMOZOLOMIDE CHEMOTHERAPIE BIJ PROGRESSIEVE LAAGGRADIGE ASTROCYTOMEN NA RADIOTHERAPIE: MOLECULAIRE KARAKTERISTIEKEN IN RELATIE TOT RESPONSE.

Slechts enkele studies hebben systematisch het effect van TMZ bij patiënten met een progressief laaggradig astrocytoom na operatie en RT onderzocht.(8, 9) Deze studies zijn echter moeilijk te interpreteren, aangezien ze kleine en heterogene groepen patiënten includeerden, met zowel laaggradige- als gededifferentieerde gliomen, met een verscheidenheid aan eerdere behandelingen. Verder zijn er beperkte gegevens over de moleculaire karakteristieken en hun correlatie met de response op chemotherapie bij patiënten met progressieve laaggradige astrocytomen na RT.

Wij verzamelde daarom retrospectief gegevens van alle patiënten uit 5 ziekenhuizen in Nederland (Erasmus MC, Rotterdam; UMCU, Utrecht; NCI en VUMC, Amsterdam; UMC St Radboud, Nijmegen) met een progressief laaggradige astrocytoom na RT, met een aankleurende laesie na contrast op de MRI, die behandeld waren met TMZ, sinds TMZ beschikbaar kwam (1995) tot 2006. Zeventig patiënten werden gevonden. Twaalf patiënten kregen TMZ na eerdere chemotherapie en werden uitgesloten van verdere analyse. We hebben daarmee een cohort van 58 patiënten onderzocht die behandeld werden met 1^{e}lijns TMZ chemotherapie na RT voor een oorspronkelijk laaggradige astrocytoom en onderzochten de relatie tussen de respons en mutaties in het IDH1, IDH2 en TP53 gen, O6-methylguanine-DNA-methyltransferase (MGMT) promotor methylering , trisomie van chromosoom 7 en verlies van chromosoom 1p en 19q. Alle patiënten kregen TMZ in

een dosering van 200 mg/m^2 /dag op dag 1-5 elke 4 weken. Een volledige of partiële respons werd beschouwd als objectieve respons.
In dit cohort was de 6 maanden progressie vrije overleving 67%, de mediane overleving was 14 maanden. Een objectieve respons werd gezien bij 54% van de patiënten. TP53 mutaties en verlies van chromosoom 19q toonde een borderline associatie met progressievrije overleving, maar geen van de andere moleculaire eigenschappen konden worden gecorreleerd met de response op TMZ. Een combinatie van een gemethyleerde MGMT gen promoter en IDH1 mutaties werd gevonden in 86% van de tumormonsters. IDH1 mutaties en MGMT promotor methylering waren sterk gecorreleerd (p <0,001). Noch de MGMT promoter methylatie, noch de IDH1 mutaties correleerde met progressie-vrije overleving, maar het interval tussen de eerste symptomen van het laaggradig astrocytoom en het begin van de TMZ chemotherapie was significant hoger in de patiënten met IDH1 mutaties (p = 0,01) en in de patiënten met een gemethyleerde MGMT promotor (p = 0,02).
De 6 maanden progressievrije overleving in deze studie ligt tussen de 6 maanden progressievrije overleving van het anaplastisch astrocytoom, zoals gerapporteerd in de pivotal TMZ studie en de uitkomsten bij het recidief van een anaplastisch oligodendroglioom; echter de overleving was in dezelfde orde van grootte als in de anaplastisch astrocytoom trial. (10, 11) Verder werd uit dit ons onderzoek geconcludeerd dat MGMT promotor methylering en IDH1 mutaties sterk gecorreleerd zijn en de overleving vanaf het moment van de diagnose te voorspellen, maar niet de progressievrije overleving na TMZ chemotherapie.

DOSIS GEÏNTENSIVEERD 1-WEEK OP/ 1-WEEK AF TEMOZOLOMIDE CHEMOTHERAPIE BIJ PATIËNTEN MET EEN RECIDIEF GLIOOM: EEN RETROSPECTIEF ONDERZOEK.

De behandelingsopties voor patiënten met een recidief glioom na RT en een eerstelijns alkylerende of methylerende chemotherapie zijn beperkt. Aangezien TMZ de standaard behandeling werd voor nieuw gediagnosticeerde glioblastomen, moest de behandeling voor recidiverende glioblastoom patiënten worden herzien. Eén van de belangrijkste mechanismen van tumor resistentie tegen TMZ wordt gemedieerd door O6-methylguanine-DNA-methyltransferase (MGMT).(12) Het bewijs voor deze rol van het MGMT is afkomstig uit klinische studies waaruit blijkt dat methylering van de promotor van MGMT geassocieerd is met een verbeterde tumorrespons en overleving bij patiënten met een glioblastoom. (13, 14) Gezien de meer continue expositie met dosis geïntensiveerd (diTMZ) werd aangenomen dat diTMZ schema's MGMT afhankelijke resistentie tegen TMZ zou kunnen overwinnen, door een diepere depletie van de intracellulaire level van MGMT. (15) Sindsdien zijn een groot aantal studies met diTMZ gerapporteerd, waarvan sommige significante en veelbelovende resultaten lieten zien met een 6 maanden progressievrije overleving tot 44%.(16)
Wij onderzochten de werkzaamheid en verdraagbaarheid van een week op / een week af TMZ (diTMZ) schema in een cohort patiënten die behandeld waren met diTMZ tussen 2005 en 2011 voor progressie van een glioblastoom tijdens of na chemo-radiotherapie met TMZ of progressie van een ander type glioom na RT en ten minste één lijn chemotherapie. Patiënten kregen diTMZ in een dosering van 100-150 mg/m^2/dag (dag 1 t/m 7 en dag 15 t/m 21 in cycli van 28 dagen). Alle patiënten hadden een contrast-aankleurende laesie op de MRI-scan en de respons werd geëvalueerd aan de hand van de RANO criteria;(17) complete en partiële responsen werden als objectieve respons beschouwd.
Drieënvijftig patiënten werden geïncludeerd. Het mediane aantal cyclii van diTMZ was 4 (range 1-12). Acht patiënten stopten met chemotherapie vanwege toxiciteit. Twee van de 24 patiënten met een progressief glioblastoom (WHO graad IV) had een objectieve respons; de 6 maanden progressievrije overleving in glioblastoom patiënten was 29%. Drie van de 16 patiënten met een progressief WHO graad II of III astrocytoom of oligodendroglioom of oligo-astrocytoom zonder

gecombineerde 1p en 19q verlies hadden een objectieve respons en 6 maanden progressievrije overleving bij deze patiënten was 38%. Vier van de 12 evalueerbare patiënten met een progressief WHO graad 2 of 3 oligodendroglioom of oligo-astrocytoom met gecombineerd 1p en 19q verlies hadden een objectieve respons; de 6 maanden progressievrije overleving bij deze patiënten was 62%.
Deze studie geeft aan dat diTMZ veilig en effectief is bij patiënten met progressief glioom, ondanks eerdere TMZ en/of nitrosourea chemotherapie. Onze gegevens suggereerden echter geen hogere werkzaamheid van dit dosis geïntensiveerde schema ten opzichte van het standaard dag 1-5 elke vier weken TMZ schema.

MONOTHERAPIE BEVACIZUMAB OF LOMUSTINE VERSUS DE COMBINATIE VAN BEVACIZUMAB PLUS LOMUSTINE BIJ PATIËNTEN MET EEN PROGRESSIEF GLIOBLASTOOM (BELOB TRIAL): EEN GERANDOMISEERDE GECONTROLEERDE FASE 2 STUDIE.
Ondanks de afwezigheid van goed gecontroleerde studies wordt bevacizumab momenteel veel gebruikt bij de behandeling van het progressieve glioblastoom. Door het ontbreken van gecontroleerde studies blijft het echter onduidelijk of de hoge respons op bevacizumab zich vertaald in een betere overleving. De BELOB studie is de eerste gerandomiseerde gecontroleerde fase 2 studie naar het effect van bevacizumab bij het progressieve glioblastoom.
Deze studie is een open-label, driearmige, multicenter fase 2 studie, uitgevoerd in 14 ziekenhuizen in Nederland. Volwassen patiënten (≥18 jaar) met een eerste recidief van glioblastoom, na chemo-radiotherapie met TMZ, werden willekeurig verdeeld door een web-based computerprogramma tussen behandeling met lomustine 110 mg/m^2 eenmalig elke 6 weken, bevacizumab intraveneus 10 mg/kg eenmaal per 2 weken of een combinatiebehandeling met lomustine 110 mg/m^2 elke 6 weken en bevacizumab 10 mg/kg elke 2 weken. Randomisatie van patiënten werd gestratificeerd, waarbij de stratificatiefactoren ziekenhuis, performance status en leeftijd waren. De primaire uitkomstmaat was de totale overleving na 9 maanden, met een “intention to treat” analyse. Een veiligheidsanalyse werd gepland nadat de eerste tien patiënten twee cycli van 6 weken hadden gehad in de combinatie behandelingsgroep.
Tussen 11 december 2009 en 10 november 2011, werden 153 patiënten geïncludeerd. De vooraf geplande veiligheidsanalyse werd uitgevoerd na behandeling van acht patiënten. Vanwege hematologische bijwerkingen (drie patiënten hadden een graad 3 trombocytopenie en twee hadden graad 4 trombocytopenie) werd de dosis lomustine in de combinatie behandelingsgroep daarna verminderd tot 90 mg/m^2. Na de vermindering van de lomustine dosering in de combinatiegroep, werd de gecombineerde behandeling goed verdragen. Naast de acht patiënten, die bevacizumab plus lomustine 110 mg/ m^2 kregen, werden 51 patiënten willekeurig toegewezen voor bevacizumab alleen, 47 voor lomustine alleen en 47 voor bevacizumab plus lomustine 90 mg/m^2. Van deze patiënten kwamen 50 in de bevacizumab groep, 46 in de lomustine groep en 44 in de bevacizumab en lomustine 90 mg/m^2 groep in aanmerking voor analyse. De overleving na 9 maanden was 43% (95% CI 29-57) voor lomustine groep, 38% (95% CI 25-51) in de bevacizumab groep, 59% (95% CI 43-72) in de bevacizumab en lomustine 90 mg/m^2 groep, 87% (95% CI 39-98) in de bevacizumab en lomustine 110 mg/m^2 groep en 63% (95% CI 49-75) voor de gecombineerde bevacizumab en lomustine groepen. Ten tijde van deze analyse waren 144/148 (97%) van de patiënten overleden en drie (2%) nog in behandeling.
De overleving na 9 maanden in de combinatiegroep, die behandeld werd met bevacizumab en lomustine, voldeed aan het vooraf gespecificeerde criterium voor de evaluatie van deze behandeling in een fase 3-studie. De resultaten in de bevacizumab groep rechtvaardigen geen verdere studies naar het effect van bevacizumab alleen.

BEHANDELING VAN GROTE LAAGGRADIGE OLIGODENDROGLIALE TUMOREN MET UPFRONT PROCARBAZINE, LOMUSTINE EN VINCRISTINE CHEMOTHERAPIE MET EEN LANGE FOLLOW-UP: EEN RETROSPECTIEF COHORTONDERZOEK MET GROEIKINETIEK.

In de late jaren negentig van de vorige eeuw, werd op onze afdeling begonnen met upfront chemotherapie bij patiënten met grote oligodendrogliale tumoren, om RT met een groot veld, met potentieel lange termijn bijwerkingen (cognitieve stoornissen), uit te stellen. (18) Deze strategie is vooral aantrekkelijk bij patiënten met chemotherapie responsieve tumoren en met verwachte lange termijn overleving, aangezien deze patiënten langer risico lopen klinisch significante cognitieve stoornissen te ontwikkelen. In een eerste rapport met een beperkte follow-up zagen we dat vroege chemotherapie, en uitstel van RT, langdurige stabiele ziekte of geringe respons liet zien in een aanzienlijk deel van de patiënten.(19) Anderen verkregen soortgelijke resultaten met TMZ of procarbazine, CCNU en vincristine (PCV) chemotherapie. (8, 20, 21, 22, 23, 24)

In een follow-up studie (mediane follow-up duur: 8 jaar, maximale follow-up: 13 jaar) van 32 patiënten die upfront PCV ontvingen, bestudeerden we de klinische uitkomst en gecorreleerde dit aan de 1p/19q- en IDH-status. Patiënten werden behandeld met maximaal 6 PCV cycli. De respons op de behandeling werd gedefinieerd volgens de RANO criteria; bovendien werden de veranderingen van de gemiddelde tumor diameters in de tijd (groeikinetiek) berekend.

Tweeëndertig patiënten werden behandeld tussen 1998 en 2006, waarvan er 18 werden gediagnosticeerd met tumoren met gecombineerd 1p/19q verlies. De mediane follow-up was 8 jaar (range 0,5-13 jaar). De mediane overleving was 120 maanden en de mediane progressievrije overleving was 46 maanden. Groeikinetiek toonde een aanhoudende en continue daling aan van de gemiddelde tumordiameter na voltooiing van de chemotherapie, gedurende een mediane periode van 35 maanden. Opmerkelijk was dat progressie niet begon door een toename van de gemiddelde tumordiameter, maar door het ontstaan van aankleuring. 1p/19q verlies werd geassocieerd met een significante toename van de overleving (mediane overleving 83 maanden versus niet bereikt voor tumoren met 1p/19q verlies; p = 0.003) en progressievrije overleving (mediane progressievrije overleving 35 maanden versus 67 maanden voor tumoren met 1p/19q verlies; p = 0.024). Patiënten met gecombineerde 1p/19q verlies had een 10 jaar progressievrije overleving van 34%. Twintig van de 22 patiënten met een progressieve tumor werden uiteindelijk behandeld met RT. Het interval tussen de start van de PCV chemotherapie en initiatie van RT was 31 maanden bij de patiënten met intacte 1p/19q en 75 maanden bij de patiënten met 1p/19q verlies.

Deze lange termijn follow-up studie laat zien dat upfront PCV chemotherapie geassocieerd is met een lange progressievrije overleving en overleving en een aanzienlijke periode van uitstel geeft van de RT bij patiënten met een laaggradige oligodendrogliale tumoren, en in het bijzonder bij patiënten met gecombineerd 1p/19q verlies.

TOEKOMSTPERSPECTIEVEN

Op dit moment bestaat de behandeling van diffuse gliomen bij volwassen uit chirurgie (zo uitgebreid verwijderen als veilig mogelijk is), RT en chemotherapie. De onderzoeken die uitgevoerd zijn in de jaren tachtig en negentig van de vorige eeuw hebben de rol van RT bij patiënten met een glioom aangetoond, en hebben ook aangetoond dat van deze behandelmodaliteit geen verdere verbetering van de overleving valt te verwachten. Uit de fase III studies met adjuvante chemotherapie die gerapporteerd zijn in het afgelopen decennium, is gebleken dat chemotherapie onderdeel is van de initiële behandeling van alle diffuse gliomen bij volwassen patiënten. Daarmee wordt ook duidelijk dat voor verdere verbetering van het behandelingsresultaat nieuwe geneesmiddelen en behandelmodaliteiten nodig zijn. De huidige

chemotherapie voor gliomen bestaat voornamelijk uit TMZ of PCV, met een nog ongedefinieerde rol voor bevacizumab.
Het huidige proefschrift is voornamelijk gericht op de evaluatie van de klassieke chemotherapie bij progressieve diffuse gliomen. Dit proefschrift bevestigde de rol van TMZ chemotherapie bij progressieve gliomen, van PCV chemotherapie bij nieuw gediagnosticeerde laaggradige oligodendrogliomen en suggereert een rol van bevacizumab in combinatie met lomustine chemotherapie bij het progressieve glioblastoom. Daarnaast toonde het hoofdstuk over pseudo-progressie de noodzaak aan om TMZ chemotherapie te continueren bij glioblastoom patiënten met progressie binnen drie maanden na het einde van RT en gelijktijdige TMZ chemotherapie en deze patiëntengroep uit te sluiten bij studies voor patiënten met een progressief glioblastoom.
Het lijkt onwaarschijnlijk dat de overleving van volwassen diffuse glioompatiënten verder kan worden verbetert met de momenteel beschikbare cytostatica. Daartoe zijn andere benaderingen nodig. Het begrip van de processen die een rol spelen bij de groei en het ontstaan van gliomen zijn aanzienlijk verbeterd. Nieuwe snelle screening technieken hebben aanleiding gegeven tot een schat aan nieuwe informatie over de afwijkende “signaling pathways” welke het in het tumor fenotype bepalen. Maligne gliomen worden gekenmerkt door een groot aantal genetische en epigenetische afwijkingen die verantwoordelijk zijn voor hun eigenschappen, zoals invasie, ongereguleerde groei en resistentie tegen apoptose. Deze omvatten genmutaties, verlies, amplificatie, en reorganisatie evenals promotor methylering en andere mechanismen die de genexpressie coördineren.
De herkenning van diverse glioom subtypes, hun gedrag en resultaten heeft geleid tot een interesse in gepersonaliseerde geneeskunde voor de behandeling van gliomen en heeft nieuwe richtingen gegeven voor klinisch onderzoek naar de behandeling van gliomen. Helaas heeft dit tot op heden met op monotherapie gebaseerde therapieën, geen klinisch interessante resultaten opgeleverd bij patiënten met maligne gliomen. Er is een veelvoud aan verklaringen hiervoor: zoals de aanwezigheid van alternatieve “signaling pathways”, moleculaire heterogeniteit en een slechte penetratie van veel middelen via een intacte bloed-hersenbarrière.
Er zijn echter veel nieuwe gerichte therapieën in ontwikkeling en bestaande worden nu gebruikt in combinatie. Enkele gerichte therapieën, die momenteel in klinische studies worden onderzocht, worden hieronder beschreven, zonder overigens te proberen een volledig overzicht te geven.

IDH als doelwit

Zoals hierboven vermeld komen mutaties in de isocitraat dehydrogenase (IDH) genen vaak voor bij volwassen patiënten met diffuse gliomen, vooral bij laaggradige gliomen, anaplastisch gliomen en secundaire glioblastoom.([3], [4], [5], [6], [7]) IDH1 en IDH2 genen coderen voor de enzymen isocitraat dehydrogenase 1 ([NADP+], oplosbaar) en 2 ([NADP+], mitochondriaal). Het IDH1 enzym verblijft buiten de mitochondriën, terwijl IDH2 zich binnen mitochondriën bevindt. IDH1 en IDH2 enzymen katalyseren de reactie van isocitraat naar alfa-ketoglutaraat en vice versa. Mutant IDH enzymen (IDH1 of IDH2) kunnen beide normale reacties niet katalyseren, maar reduceren alfa-ketoglutaraat in het oncometaboliet 2-hydroxyglutarate (2-HG). Het is aangetoond dat IDH mutante 2HG producerende glioma cellen DNA en histonen demethylering kan voorkomen.
Remming van DNA demethylering, wat hypermethylering van CpG dinucleotiden veroorzaakt, kan gevolgen hebben voor gentranscriptie en stabiliteit van het genoom.([25]) Histonen demethylering is nodig voor lineage-specifieke stamcellen om te differentiëren tot terminaal gedifferentieerde cellen.([26]) Dus in plaats van rijping, blijven de cellen primitief en kunnen zich snel vermenigvuldigen, wat leidt tot epigenetische deregulering en het ontstaan van tumoren.
Remming van de IDH gemuteerde eiwitten zou dus kunnen leiden tot klinisch voordeel voor patiënten met IDH mutaties. Orale middelen (IDH305 en AG-120) welke het gemuteerde IDH1

enzym remmen zijn geïdentificeerd en worden momenteel onderzocht in fase I studies (ClinicalTrials.gov Identifier: NCT02381886 and NCT02073994).

PTEN antagonist

Het is al lang bekend dat allelverlies van chromosoom 10 vaak voorkomt bij hooggradige gliomen.(27) Na de identificatie van het PTEN (phosphatase and tensin homolog deleted on chromosome 10) gen op chromosoom 10 werden PTEN mutaties en homozygote deleties vaak gerapporteerd in glioblastomen.(28, 29, 30) Het fosfatase tumor suppressor PTEN is essentieel voor het reguleren van de zeer oncogene fosfatidylinositol 3-kinase (PI3K) "signaling pathway". De PI3K signalering regelt diverse celfuncties, celoverleving en groei, proliferatie, cellulaire veerkracht en herstel, celmigratie en angiogenese.(31) PI3K remmers kunnen derhalve een goede behandeling optie bij glioblastomen zijn.(32) Een breed spectrum aan PI3K remmers zijn in klinische ontwikkeling en buparlisibis wordt momenteel onderzocht in een fase I / II trial glioblastoom patiënten met homozygote mutaties en deleties van PTEN, samen met een c-Met receptor tyrosine kinase remmer (ClinicalTrials.gov Identifier: NCT01870726).

c-MET-antagonisten

De c-Met receptor tyrosine kinase familie is structureel verschillend van andere receptor tyrosine kinase families en is de enige bekende hoge affiniteit receptor voor hepatocyt groeifactor (HGF), ook bekend als scatter factor (SF).(33) Binding van HGF/SF met de c-Met receptor bevordert groei, invasie en migratie van zowel tumorcellen als endotheelcellen betrokken bij de bloedtoevoer van de tumor. (34) Het is aangetoond dat c-Met vaak tot over-expressie is gebracht of gemuteerd is in gliomen.(34) Mogelijk kunnen deze tumoren met c-Met receptor tyrosine kinase inhibitoren beïnvloed worden.

Gecombineerd antilichamen

Gezien de hoge frequentie van oncogene EGFR veranderingen in glioblastomen, en het succes van epidermale groeifactor receptor (EGFR) gerichte therapieën (bijvoorbeeld gefitinib, erlotinib en cetuximab) bij longkanker en darmkanker, zijn deze middelen lang gezien als potentiële therapie bij het glioblastoom. Aan deze verwachting is tot nu toe echter niet voldaan. Dit zet vraagtekens bij het verder onderzoeken van EGFR remmende strategieën bij glioblastoom patiënten.(35, 36, 37)

Antilichaam-geneesmiddel combinaties zijn een snel groeiende klasse van kanker geneesmiddelen die de gerichte eigenschappen van monoklonale antilichamen combineert met de anti-tumor effecten van krachtige cytotoxische geneesmiddelen. ABT-414 is een gecombineerd antilichaam met een cytostaticum, waarbij het antilichaam zich bindt aan cellen met over-expressie van de EGFR, zoals het glioblastoom. ABT-414 bindt aan EGFR, wordt geïnternaliseerd in de tumorcel, waarna een krachtige anti-microtubulaire stof (monomethylauristatine F) vrijkomt, wat leidt tot celdood. ABT-414 wordt momenteel onderzocht in een Fase I studie bij glioblastoom patiënten met over-expressie van EGFR (ClinicalTrials.gov Identifier: NCT01800695).

Niet-gerichte therapieën

Gentherapie, immuuntherapie (vaccinatiestrategieën en immuunmodulatoren zoals anti-PD1 en anti-PDL1 antilichamen) en mogelijk laagintensiteit alternerende elektrische velden, die de mitose en cytokinese verstoren, lijken veelbelovend, maar vallen buiten het kader van dit proefschrift over chemotherapie bij gliomen.

CONCLUSIE

Er zijn veel nieuwe (combinaties van) gerichte middelen in ontwikkeling die mogelijk effectief zijn tegen de belangrijkste moleculaire "signaling pathways", die betrokken zijn bij het ontstaan en de

progressie van diffuse gliomen, en die mogelijk leiden tot een verbetering van de mortaliteit en morbiditeit bij patiënten met een glioom. Dit zal een andere werkwijze vereisen bij de klinische studies naar deze middelen. Bij deze studies zal aandacht moeten zijn voor de daadwerkelijke aanwezigheid van de moleculaire factoren waartegen het middel gericht is, voor de penetratie van het middel in de tumor, voor de daadwerkelijke binding van het middel op de “target” en voor de veranderingen in de relevante “signaling pathways” in de tumor die door het middel ontstaan. Hoewel dit studies naar ‘targeted agents’ bij diffuse gliomen compliceert vormt dit een belangrijke stap bij de evaluatie van ‘targeted agents’. De moleculaire heterogeniteit van gliomen is een andere factor die van invloed is op de vooruitgang met betrekking tot de behandeling van deze ziekte. Majeure gezamenlijke inspanningen, waarbij nieuwe medicijnen en behandelingen in goed ontworpen klinische studies geëvalueerd worden, zullen nodig zijn om een verbetering van het huidige behandelingsresultaat te bereiken.

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University training

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Employment

1990-1994 Research Trainee, Department of Neuro-Anatomy, Erasmus University Rotterdam, The Netherlands (head: prof. J. Voogd) An ultrastructural-study of the distribution of the inhibitory neurotransmitters in motoneural cell groups of the spinal cord.

1990-1992 Teacher Neuro-Anatomy practicum. Erasmus University, Rotterdam, the Netherlands.

1992-1995 Rotating Internship Academic Hospital, Rotterdam, the Netherlands.

1995-2002 resident neurology, Leiden University Medical Center, Leiden, the Netherlands (head: prof. R.A.C. Roos).

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2002-2002 resident neuro-oncology, Westeinde hospital, 's-Gravenhage (head: dr. C.J. Vecht)

Employment as neurologist

2003-current staff neurologist Neuro-Oncology unit / Erasmus MC Cancer Institute, Rotterdam, The Netherlands

March 2005 Observership Neuro-Oncology MD Anderson Cancer Center Houston (prof. dr. W.K. Yung) Observer out-patient clinic gliomas and neurofibromatosis type 1.

April 2005 Observership Neuro-Oncology Johns Hopkins Baltimore USA (prof. dr. S.A. Grossman) Observer out-patient clinic gliomas and pain and pain consultation team.

2004-current Clinical research coordinator for clinical studies on glioma patients

2012-current Chairman Dutch guideline committee spinal metastases

PhD Portfolio

Summary of PhD training

Courses

March 14-15th 2003	32nd international conference in Neuro-Oncology, Padua, Italy
April 10-11th 2003	Biemond course : Neuro-oncology, Zeist, The Netherlands
October 3rd 2003	EORTC course: One-day introduction to EORTC trials, Brussels, Belgium
January 29th 2004	CPO course: Clinical research training course, Rotterdam, The Netherlands
June 16-18th 2004	EORTC course: Clinical Trial Statistics, Brussels, Belgium
November 24-26th 2004	The 6th EURO-CNS course in Neuropathology, Graz, Austria
June 17-18th 2005	Perspectives in CNS malignancies, Prague, Czech Republic
November 12-16th 2007	CPO course: Good Clinical Practice, Rotterdam, The Netherlands
September 30th 2014	BROK course on Good Clinical Practice , Rotterdam, The Netherlands

Oral presentations at scientific meetings

November 17th 2006	Temozolomide for recurrent low-grade astrocytoma, SNO, Orlando, Fl, USA
June 3rd 2007	The incidence of pseudo-progression in a cohort of high-grade glioma patients treated with chemo-radiation with temozolomide, ASCO, Chicago, IL, USA
November 22nd 2008	MGMT assessed with methylation-specific (MS-) multiplex ligation-dependent probe amplification (MLPA) predicts outcome to temozolomide in progressive low-grade astrocytoma after radiotherapy, SNO, Las Vegas, USA
May 14th 2009	IDH-1 mutations are frequent in low grade glioma and do not corelate with outcome to temozolomide chemotherapy, the 3rd Quadrennial Meeting of the World Federation of Neuro-Oncology (WFNO), Yokohama, Japan
May 14th 2009	Dose dense one week on/ one week off temozolomide in recurrent glioma., the 3rd Quadrennial Meeting of the World Federation of Neuro-Oncology (WFNO), Yokohama, Japan
November 2010	Toxicity of combined bevacizumab-lomustine treatment in patients with recurrent glioblastoma (GBM): report from the randomized phase II study (BELOB), SNO, Montreal, Canada
June 1st 2013	Randomized phase II study of Bevacizumab versus Bevacizumab plus Lomustine versus Lomustine in patients with recurrent Glioblastoma. The BELOB trial (LWNO trial 0901), ASCO's 2013 Annual Meeting, Chicago IL, USA

Invited oral presentations

March 29th 2008	Pseudo-progression after radiotherapy and temozolomide in High-grade Gliomas: Is it of clinical significance., Perspectives in CNS malignancies, Berlin, Germany

May 3rd 2008	Pseudo-progression in High-grade Gliomas., Highlights in Neuro-oncology, Vienna, Germany.
November 27th 2010	Periferal neurotoxicity from cancer treatment.. Networking days dutch oncologists. Goes, The Netherlands
March 13th	Spinal metastasis. Biemond course for Dutch neurologists. Arnhem, The Netherlands.
December 11th 2014	Spinal metastasis. Biemond course for Dutch neurologists. Arnhem, The Netherlands.
November 28th 2014	Final results of the BELOB trial.. GEINO VI symposium: Innovations in Brain Tumor Treatment, Madrid, Spain.
November 19th 2014	Neurosurgical indications and possibilities in oligo metastases of breast cancer. Symposium Breast cancer, Treatment, Beter. Rotterdam, The Netherlands.

Memberships

1995-current	The Dutch Society of Neurology
2003-current	Dutch Neuro-oncology Workgroup
2007-current	European Organisation for Research and Treatment of Cancer - Brain Tumor Group

List of publications

1. **Taal W**, Holstege JC. GABA and glycine frequently colocalize in terminals on cat spinal motoneurons. Neuroreport. 1994 Nov 21;5(17):2225-8
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Stellingen behorend bij het proefschrift

Chemotherapy in Glioma

1. Patiënten met radiologische progressie zonder klinische symptomatologie van een glioblastoom direct na chemo-radiotherapie met temozolomide moeten verder behandeld worden met aanvullende temozolomide.
   (Dit Proefschrift)

2. Het includeren van patiënten in klinische studies naar nieuwe middelen met een op radiologische gronden als progressief beoordeeld glioblastoom direct na chemo-radiotherapie zal leiden tot een overschatting van de resultaten van deze studies.
   (Dit Proefschrift)

3. Methylatie van de O6-methylguanin-DNA-methyltransferase (MGMT) promotor is sterk gecorreleerd met IDH1 mutaties bij patiënten met laaggradige astrocytomen.
   (Dit Proefschrift)

4. Er is geen plaats voor monotherapie bevacizumab bij patiënten met een progressief glioblastoom, maar mogelijk wel in combinatie met lomustine.
   (Dit proefschrift)

5. Bij patiënten met grote laag-gradige oligodendrogliale tumoren is procarbazine, lomustine en vincristine (PCV) chemotherapie geassocieerd met een lange (progressievrije) overleving en geeft een aanzienlijke periode uitstel van de behandeling met radiotherapie en de daarmee geassocieerde cognitieve achteruitgang.
   (Dit proefschrift)

6. Het (anaplastisch) oligodendroglioom met verlies van 1p/19q is een chronische ziekte.

7. Moleculaire tumorkarakteristieken zijn belangrijker dan de histopathologische diagnose bij de klinische besluitvorming bij patiënten met een diffuus glioom.

8. We will eventually be doing whole-body imaging with optical light.
   (Lihong Wang, http://www.nature.com/news/optics-super-vision-1.16877)

9. De werking van het internet is vergelijkbaar met een neuraal netwerk.

10. Technically a space elevator is possible.
    (Arthur C. Clarke, The Fountains of Paradise)

11. Als we verder willen evolueren als mensheid, dan moeten we verder kijken dan moeder aarde.
    (Dr. W. Taal)

Walter Taal
Rotterdam, 30 oktober 2015