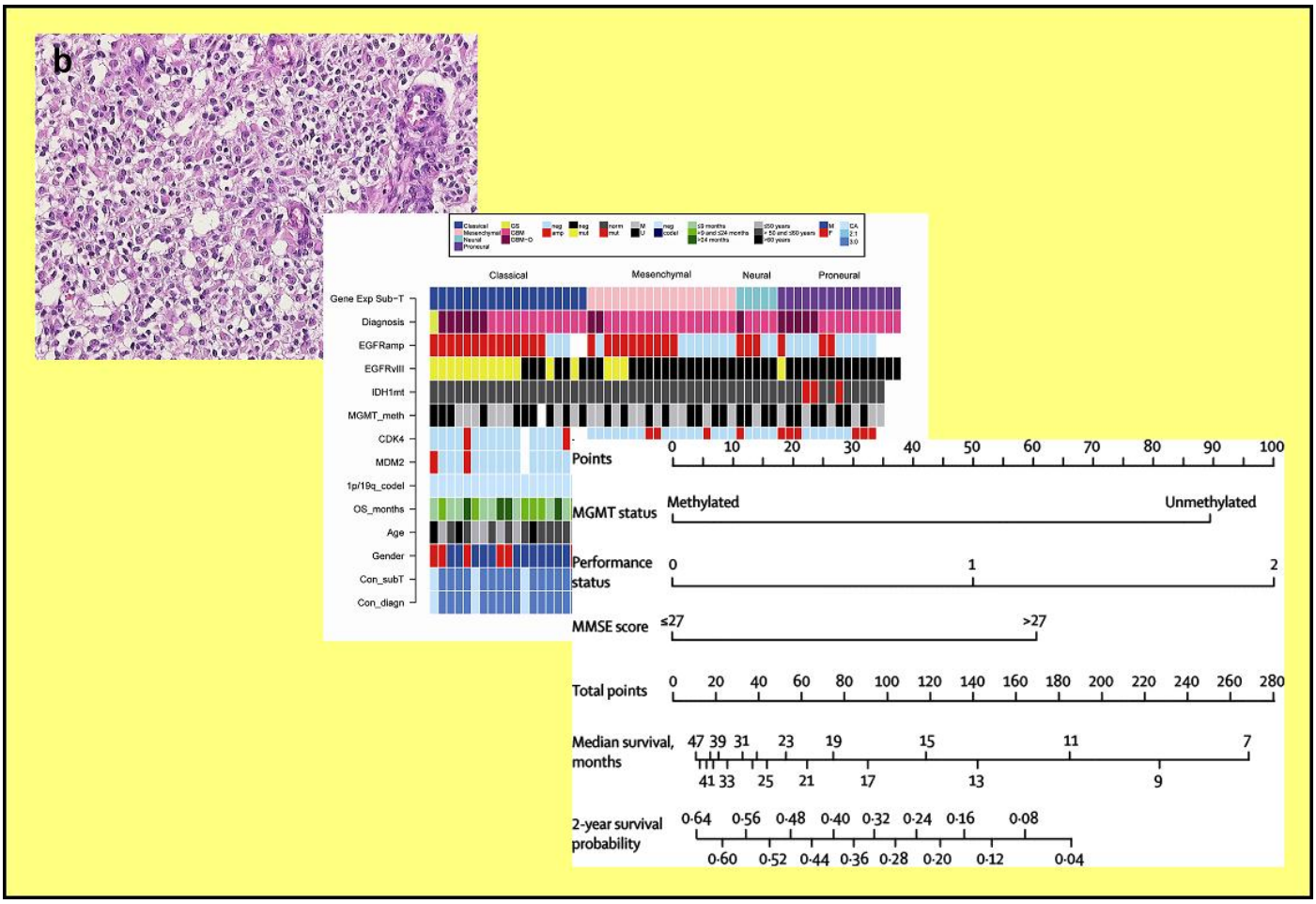
Diagnosis and prognosis of brain tumors in clinical trials



Thierry Gorlia

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Diagnosis and prognosis of brain tumors in clinical trials

Diagnose en prognose van hersentumoren in klinische proeven

Thesis

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By

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Born in Ath, Belgium

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“When you arise in the morning, think of what a precious privilege it is to be alive –
to breathe, to think, to enjoy, to love.“

Marcus Aurelius

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Chapter I. Introduction

1.1 Epidemiology of brain tumors

According to the Central Brain Registry Of The United States (CBTRUS) statistical report (February 2012) the incidence rate of all primary non malignant and malignant brain and central nervous system tumors is 19.89 cases per 100.000 (11.58 for non-malignant tumors and 7.31 for malignant tumors). Malignant brain tumors account for only 1% to 2% of all adult cancers. As a comparison, in 2012, the incidence of women breast cancer was 121.2 (per 100.000). Tumors of neuroepithelial tissue are the most frequent malignant brain tumors with an incidence rate of 6.16. The most common tumor of neuroepithelial tissue is the glioblastoma (GBM) with an incidence of 3.2. Other histologies e.g. astrocytoma, oligodendroglioma or mixed oligoastrocytoma have an incidence rate lower than 0.5. [1,2] The incidence of brain tumors increases with age, with an incidence rate is 8.59 for young patients (age 20-34) and 55.8 for elderly patients (age 65-74). Age distributions also differ by histology and grade. Glioblastoma peaks in incidence at age 65-74 and oligodendroglioma and low grade astrocytoma at age 35-44. Causes of brain tumors are still largely unknown. Various categories of risk factors are investigated in epidemiologic studies: geographic and ethnic, environmental (irradiation, pollution), lifestyle (food, alcohol, smoking bits, use of cell phone), medical treatments and conditions (allergies, infections), familial or hereditary. To date, inherited genetic syndromes, therapeutic ionizing irradiation are the only generally accepted risk factors of glioma genesis.[4,5] In the last decade, advances in microarray and sequencing technologies allowed the realization of large gene expression and genome wide association studies (GWAS). Recently, two GWAS provided more insight into the genetic variants that influence an individual susceptibility to develop gliomas. [6,7,8] In addition to germline genetic risk factors, recent studies showed that acquired somatic genetic changes were associated with treatment response, disease progression and overall survival.[9,10]

1.2 Classification of brain tumors

The international classification of human tumours published by the World Health Organization (WHO) was initiated through a resolution of the WHO Executive Board in 1956 and the World Health Assembly in 1957. The primary goal was to define internationally recognized histopathological and clinical criteria for typing and grading human brain tumors. An ancillary objective was to promote the conduct of epidemiological studies and clinical trials beyond the local institution or national bounderies. The first edition of the WHO "Blue Book" was published in 1979 and included a list of histological types. [11] In the second edition (1993), advances brought by the use of immunohistochemistry were integrated. [12] Anaplastic oligoastrocytoma was recognized as a new entity. The third edition (2000) incorporated the results of epidemiological and clinical studies including prognostic and predictive factors, imaging and genetic profiles. [13] In the fourth edition (2007), new entities and variants were integrated. [14] Anaplastic oligoastrocytoma with necrosis was associated with worse prognosis and was reclassified as "glioblastoma with oligodendroglioma component". New studies showed that most of these tumors had a similar prognosis and genetic profiles compared to standard glioblastoma.[15] Finally, genetic alterations were considered to define new tumor subsets, such as 1p/19q codeletion in oligodendrogliomas although these molecular characteristics are still not integrated in the classification system. [16]

1.3 Inter-observer disagreement in the diagnosis of brain tumors

Despite this huge international effort to harmonize the classification of brain tumours, their diagnosis and grading remain controversial and subject to large inter-observer disagreement. Diagnosis of gliomas is usually performed by local neuro-pathologists in academic or community hospitals. In clinical trials, diagnosis made by local pathologist can be reviewed by one or a panel of central neuro-pathologists. With new and advanced technologies, this review can be more easily performed by virtual microscopy (VM). With VM, histological images can be shared via internet over large distances without physical transfer of the original glass slides.[17] Central pathology review in clinical trials is realized either before or after patient entry into the trial. In the former case, the eligility of a patient to be enrolled a trial can be affected (e.g. if diagnosis is not confirmed by one of two central reviewers), in the latter case, patients is enrolled based on the local diagnosis. In both cases, patient management and/or prognosis can significantly change. Patients with low grade tumor but diagnosed and treated as high grade tumor are overtreated. Conversely, improperly diagnosed high grade tumors can be undertreated. Recent studies showed that disagreement on the diagnosis at central review was higher when the first diagnosis was obtained in community hospitals. Especially in community hospitals without a trained neuropathologist.[18] In a cohort of patients diagnosed with oligodendrogliomas, disagreement was less frequent among neuropathologists than between surgical pathologists. A reason might be the particularity of some pathological features for neuropathology (e.g. microvascular changes) requiring specific training. It was found that only a limited number of features was reproducible and were found to have significant correlation with survival in multivariate Cox models. [19] In another study, inter-observer disagreement was higher for astrocytomas with anaplastic foci (AAF) compared to Glioblastomas (GBM). Patients with AAF reclassified into GBM had GBM-like survival while GBM reclassified as AAF had intermediate survival between confirmed AAF and GBM.[20] Some inter-observer variability might be explained by sampling error or poor quality of slides. More critical is the subjectivity and ambiguity of some definitions in the WHO classifications. [21]

1.4 Role of prognosis in clinical research

Diagnosis, prognosis and therapy of a disease are key in medical practice. Diagnosis is about "examining" disease at a fixed time point with the intent of grouping patients into homogenous disease entities while prognosis is about "predicting" individual disease status or patient outcome and is dynamic over time by nature. Both diagnosis and prognosis are closely related. In neuro-oncology, tumor grade is still widely used to differentiate between groups of disease and prognosis but also to guide treatment decision. Knowledge of both disease diagnosis and patient prognosis should help clinicians to decide the best therapeutic decision for their patient, including giving no anti-tumor treatment. Although it is questionable whether collect data is rational if their use is not relevant for treatment decision, but on the other hand, gather relevant prognostic information may lead to a better understanding of the disease. Prognostic information is only a decision aid and in general will not tell who to treat or not or especially if patients with a worse prognosis will benefit similar to a therapy compared to good prognosis patients. This information will only matter if the prognosis is too poor to justify further treatments. Only properly designed, controlled and if possible, randomized trials can provide answer about patient prognosis and its interaction with treatment. The ultimate objective of a treatment decision will be to significantly improve patient prognosis.[22]

1.5 Prognostic factors

In oncology, although molecular factors are receiving more and more importance, disease diagnosis is still fundamentally guided by tumor site and histology. Additionally, "prognostic factors" are identified to account for some of the heterogeneity associated with the expected course and outcome of the disease. Prognostic factors are useful in patient counseling and for therapeutic decision e.g. to avoid exposing patients with good prognosis to aggressive treatment and vice versa, treating patients that are unlikely to benefit. In clinical research, they can be used to decide the inclusion of patients in clinical trials. When used

as stratification factors, they make the randomization process more efficient by ensuring a better balance of major prognostic variables between treatment groups. With the advancement of science, new molecular prognostic factors are expected to provide a better understanding of disease biology and to direct further research.

1.6 Prognostic factors in brain tumors

Histological grade and type, extent of surgery, age and performance status are the most consistently described prognostic factors in primary brain tumor patients. In addition, several pathological features with prognostic value have been reported (e.g. in diffuse low grade astrocytomas, the presence of gemistocytes is related to more rapid malignant transformation). [23] The fraction of Ki-67 positive tumor cells is a proliferation marker which correlates with higher grade gliomas and poorer survival. [24] Over the past few years, several molecular characteristics have been identified that complement pathological diagnosis and provide a better understanding of disease biology. Among them, the loss of heterozygosity (LOH) on chromosome 1p and 19q is a genetic alteration reported with positive prognostic effect in patients with oligodendroglioma.[25] Many reports have confirmed the value of 1p/19q LOH as a predictor of response to chemotherapy.[26] A large EORTC study showed that GBM patients with *MGMT* promoter gene methylation had a superior clinical outcome when they were treated by chemo-irradiation with temozolomide (TMZ) compared to patients with unmethylated MGMT genes or those treated with radiotherapy alone independently of their MGMT methylation status.[27,28] Fewer reports evaluated the prognostic and/or predictive value of MGMT methylation in other grades and types. Results are controversial and still need confirmation in prospective clinical trials. [29,30] Epidermal Growth Factor Receptor (EGFR) amplification distribution is different between grades and types and might also help better distinguishing between pathological entities when inter-observer variability is high e.g. to differentiate oligoastrocytomas (AOA) from GBM. Recent updates on prognostic and predictive value of molecular markers in neuro-oncology were presented in van den Bent et al (2007).[31]

1.7 Development of statistical prognostic models

Many sources of uncertainty prevent the precise prediction of the outcome of individual patient. At best, is it possible to quantify the chance for a patient to reach or not a certain outcome (e.g. being free of disease progression at a certain time point) based on statistical prognostic models developed on data from groups of patients with the same disease or similar characteristics. The development of a statistical prognostic model is a complex multistep process and finally, it is not sure that such model will provide accurate individual predictions. The most obvious cause of failure is the absence of unknown important predictors in the model but other critical reasons of inaccuracy are the violations of the assumptions underlying the statistical model and in small datasets, the risk of overfitting i.e. of describing random fluctuations rather than true relationships with the outcome. In the current work, the guidelines developed by F. Harrell et al. (1996) were applied.[32] The sections below explain and describe the statistical techniques used in this thesis to analyse survival data.

1.7.1 Cox regression model

Regression modeling is commonly used to establish the relationship between an outcome variable and candidate prognostic factors. The term "regression" was created by Francis Galton in the nineteenth century to describe the phenomenon that the heights of descendants of tall ancestors tend to regress down towards a normal average, a phenomenon also known as regression toward the mean. [33] The selection of a regression model depends on the measurement scale of the outcome variable (i.e. binary, categorical, continuous, time to event,…). Survival models relate the time that passes before some event occurs (e.g. death) to one or more factors that may be associated with that quantity. If the event can't be observed during the study period or if the patient is lost to follow-up, survival times are declared right-censored. The Cox regression model is

a popular model used in survival modeling. It relates possibly influential prognostic factors to survival times through the hazard function defined as the individual rate of failure measured over an infinitely short period of time.[34] In addition, the impact of these factors on important clinical measurements (such as median survival) can be described. In the Cox model, the hazard for individual i is expressed as the product of two functions:

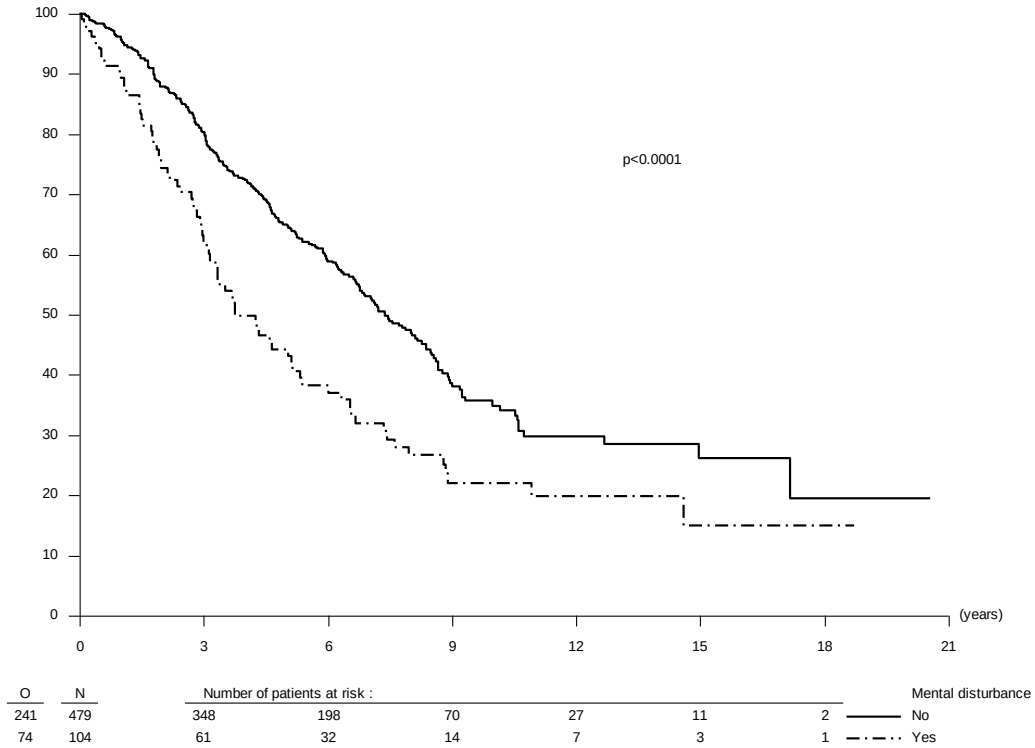
$$h_i(t) = h_0(t)\ \exp(\beta_1 x_{i1} + \beta_2 x_{i2} + \cdots + \beta_k x_{ik})$$

$h_0(t)$ is the baseline hazard function that describes the risk at time t for individuals with all factors equal to 0 (exp(0)=1) which serves as a reference, and $\exp\{\beta x_i\}$ is the relative risk, a proportionate increase or reduction in risk, associated with a set of individual factors x_i. It insures that the hazard function remains positive. In Cox model, the ratio of two individual hazards is proportional and independent of time. The Cox model is therefore often referred to as the proportional hazards (PH) model.

1.7.2 Checking Cox model assumptions

Cox regression model relies on a set of statistical assumptions. Violation of these assumptions can severely invalidate study results. In Cox model, it is assumed that censoring is non-informative. To satisfy this assumption, the design of the underlying study must ensure that the mechanisms giving rise to censoring of individual subjects are not related to the probability of an event occurring i.e. the duration of follow-up does not depend on patient outcome. Other assumptions are the linearity and additivity which are implicit in the linear predictor "βx_i" and finally PH assumption. If this last assumption is severely violated, the Cox model is incorrect and a more sophisticated analysis is required to estimate the factor effects.
The assumption of non-informative censoring can generally be assumed in data collected from randomized control cancer trial where disease is assessed according to pre-specified schedules and/or most patients are followed until death. Other assumptions are evaluated by graphical methods and/or statistical testing. The simplest way to assess proportional hazards assumption is to examine classical Kaplan Meier curves split by the categories of the factor, For instance, for PH assumption to hold, low grade glioma patients enrolled in EORTC 22844 or 22845 trials with mental disturbance caused by their disease with twice the risk of dying one year after enrollment in the trial compared to patients without the mental disturbance, should also have twice the risk of death at any other time (Figure I.1).

Figure I.1: PFS split by mental disturbance status.

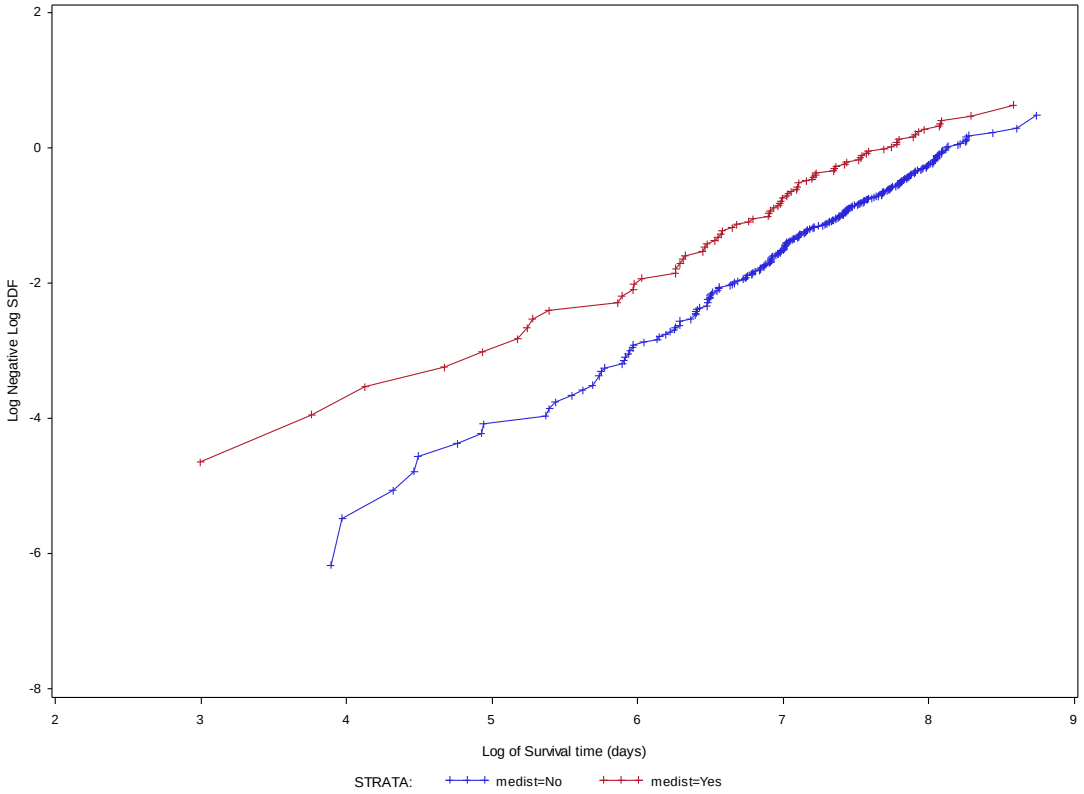


The PH property can be more effectively assessed by examining the log cumulative hazard plot over the log survival time (log(-log)). As can be seen below, under the proportional hazards model, the transformation will result in a straight line.

$$S(t) = S_0(t)\exp(bx)$$
$$H(t) = -\log(S(t)) = \exp(bx)\,(-\log(S_0(t)))$$
$$\log(-\log(S(t))) = bx + \log(-\log(S_0(t)))$$

In case of the two mental disturbance statuses, two (almost) straight and parallel lines indicate that PH assumptions are valid and the factor can enter a Cox model (Figure I.2).

Figure I.2: Log-minus-log SDF vs log survival time plot split by mental disturbance status.

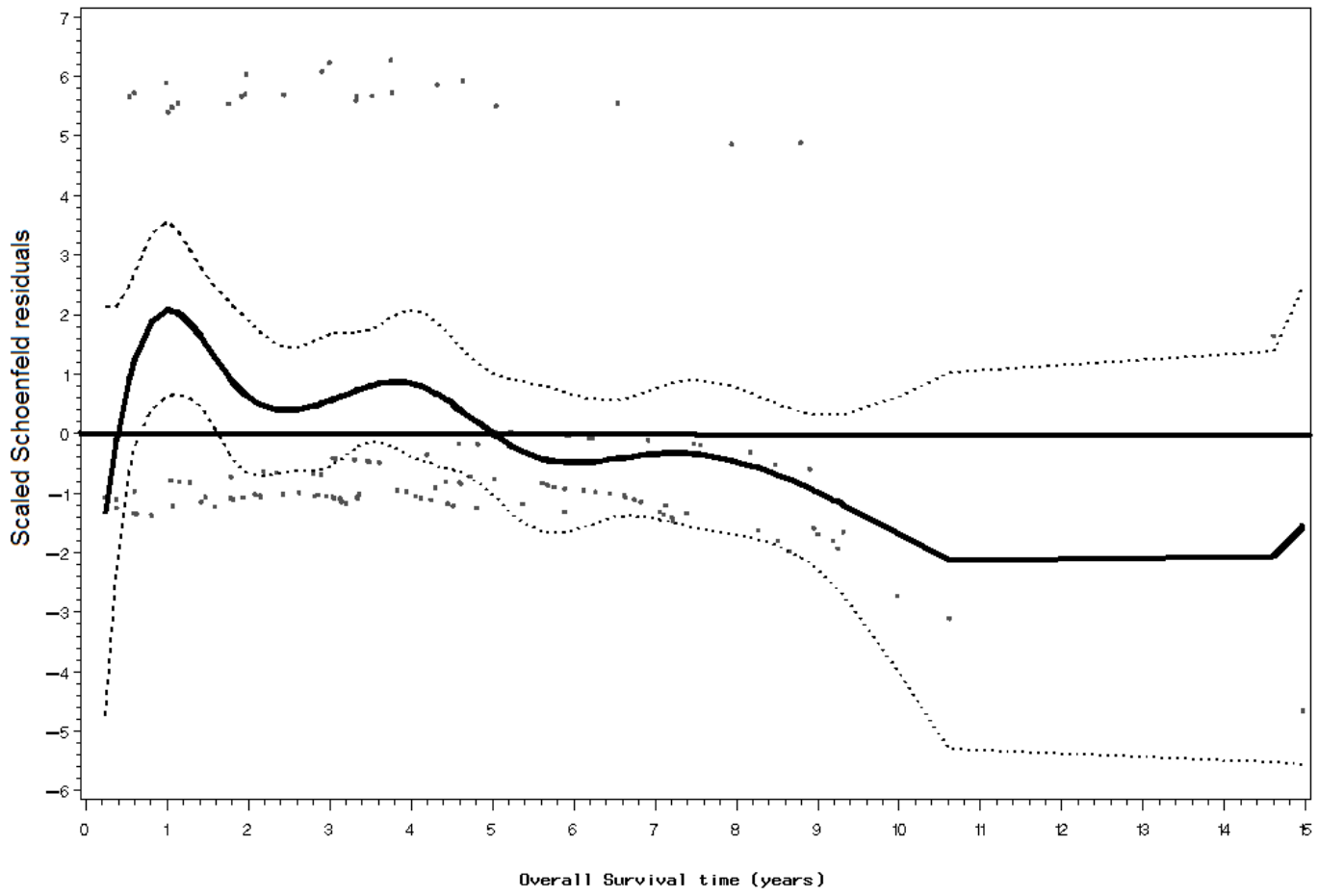


Residuals are quantities which can be used to check model assumptions. In linear or other regression models, residuals are defined as the difference between observed and values predicted by the model. As there is no obvious analog for PH model, different alternative methods have been developed.[35,36] Schoenfeld proposed the first set of residuals for PH models. [37] Schoenfeld residuals are computed for each patient and for each factor based on the individual contributions to the derivative of the log partial likelihood function. The Schoenfeld residual is the value of a factor k for an individual i (x_{ik}) who actually died at time t_i minus the weighted average of the factor expected value for all individual at risk at time t_i. Weights are defined by each individual's likelihood of dying at t_i

$$\text{Schoenfeld residual} = x_{ik} - \sum_{i=1}^{j \in R(t_i)} x_{kj}\, p_j$$

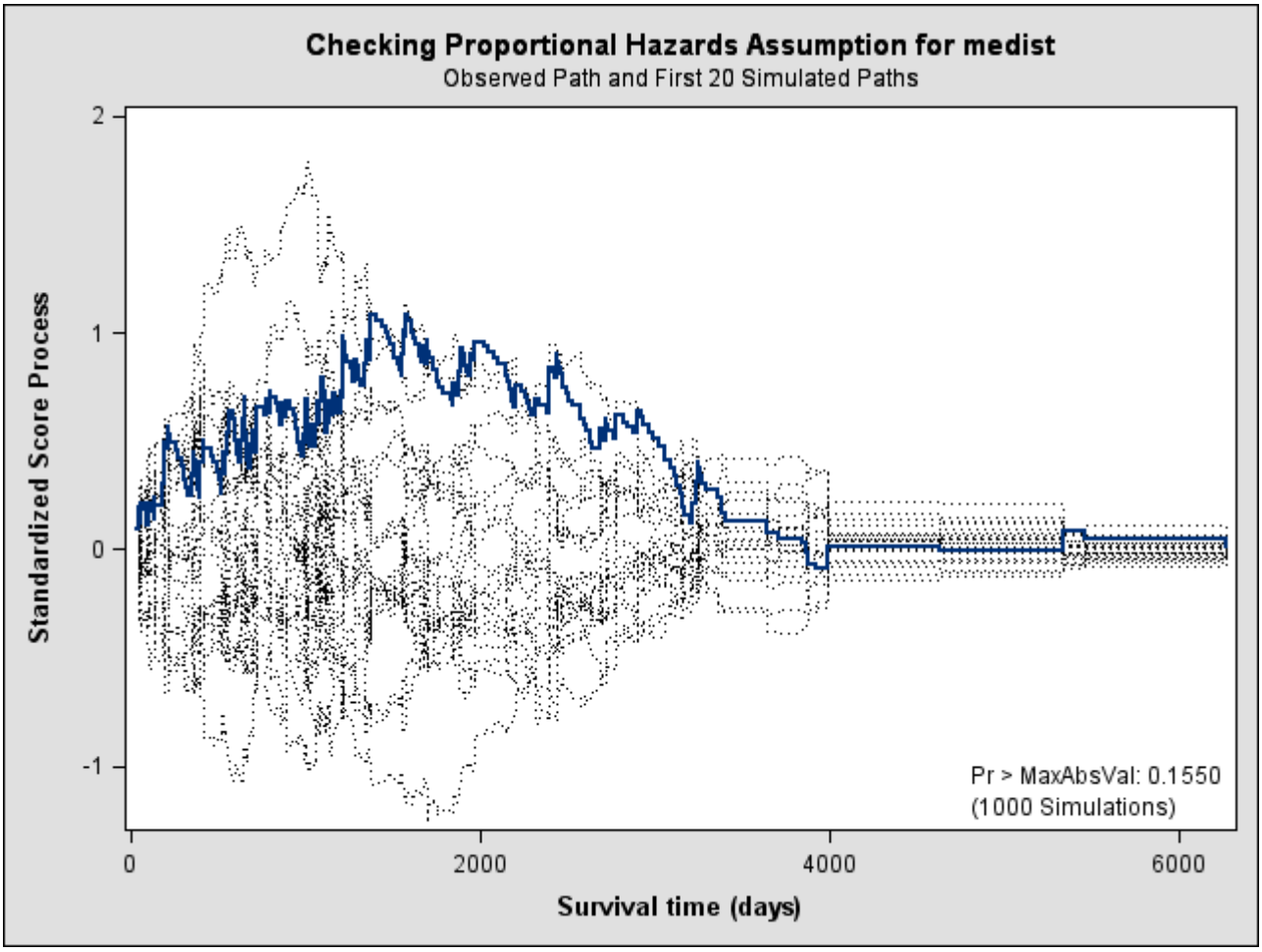
By definition, Schoenfeld residuals are not defined for censored individuals. Gramsch and Therneau proposed to increase the diagnostic power of Schoenfeld residuals by scaling them with an estimator of the variance of the residuals. [38] Since the Schoenfeld residuals are, in principle, independent of time, a plot that shows a non-random pattern against time (a trend) is evidence of PH assumption violation. In practice, if PH assumptions hold then scaled Schoenfeld residuals 95% Confidence Intervals should exclude zero in only small and unrelated period of time. This is illustrated below with the mental disturbance status (Figure I.3).

Figure I.3: Scaled Schoenfeld residuals over time for the mental disturbance status.



Lin, Wei, and Ying developed graphical and numerical methods for checking the PH assumptions of each candidate prognostic factor in a Cox model. The methods are derived from cumulative sums of martingale residuals over follow-up times. A martingale is a sequence of random measurements (i.e. a stochastic process) for which the knowledge of current and previous events does not help to predict future events. On average the future value of the process is equal to the current value of the process. The future is not predictable based on the process history. Martingale residuals are defined for each patient as the difference at time t between the observed and expected number of events under the PH assumption. They can be considered as an estimate of the excess number of events seen in the data but not predicted by the model.
For each factor, the plot of the standardized score process, a function of the martingale residuals, over time of both processes observed from the data and simulated gaussian processes under PH assumption allow assessing departure from PH assumptions.[39] Global statistical significance of this departure is estimated by the Kolmogorov-type supremum test. In the example of the mental disturbance, the observed standardized score process does not significantly differ from the simulated process under the null hypothesis of PH assumptions. This results is confirmed by a non-significant supremum test (p=0.16, figure I.4).

Figure I.4: Standardized Score Process and Supremum test for the mental disturbance status.



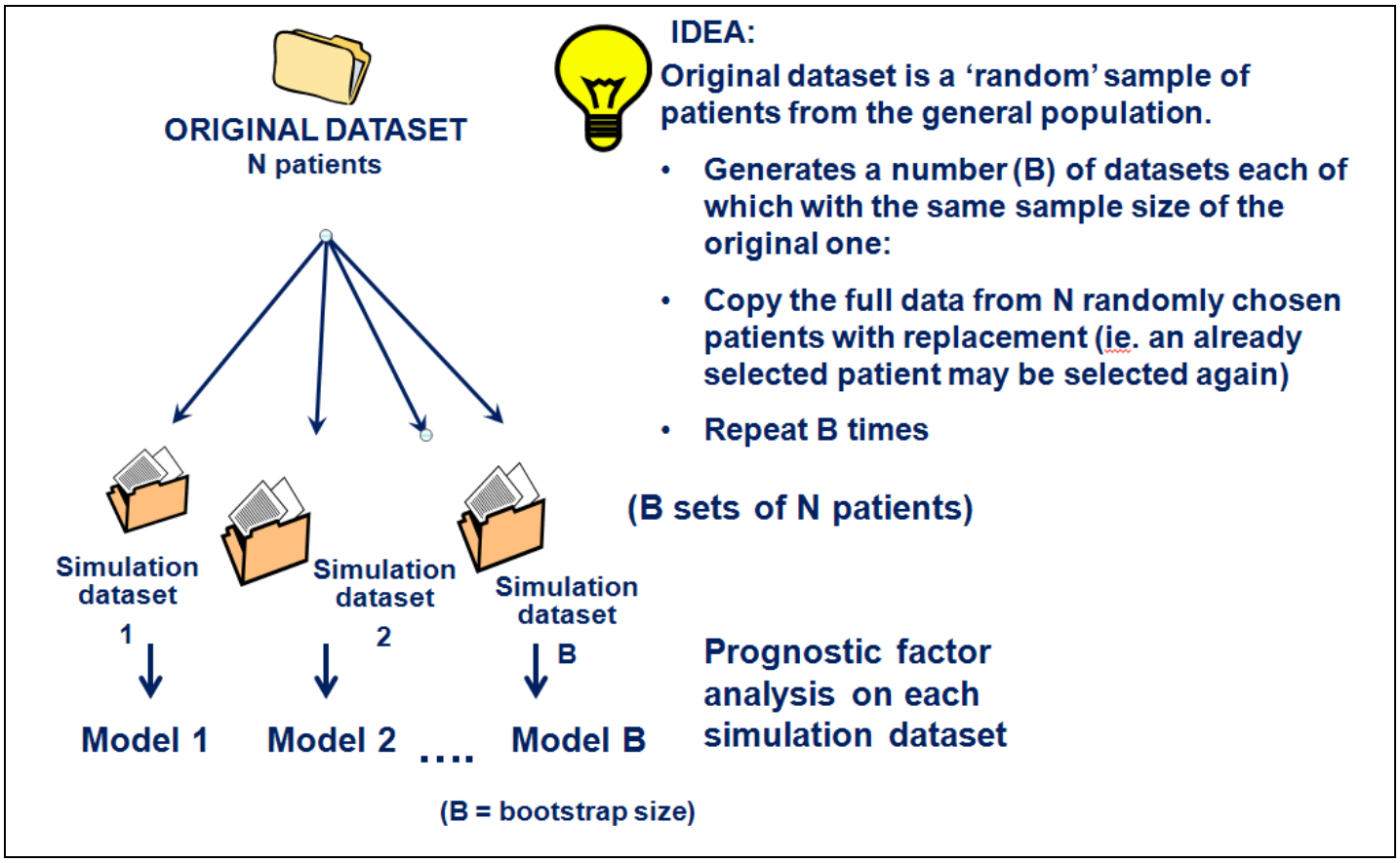
This technique also allows to test the functional form of a factor e.g. if the relationship between the factor and the outcome is linear or if more sophisticated function should be used. In this thesis, linearity was assessed by visual inspection of the Kaplan-Meier curves and the log(-log) plots.

1.7.2 Factor selection and use of bootstrap resampling technique for model building

Being 'parsimonious', i.e. selecting only factors which significantly influence patient outcome is a desirable property of statistical prognostic models. Several techniques were developed to select factors. In this thesis, automated stepwise or backward elimination techniques were used. Backward elimination begins with a model in which all candidate variables have been included (the full model). At each step, the variable that is the least significant is removed. This process continues until no non-significant variables remain. Stepwise elimination starts from a model without factor. A new factor is entered until a prespecified critical significance level is reached. The factor with the largest p-value can be removed if the p-value exceeds the specified significance level. Though they often do, there is no guarantee that backward and stepwise will produce the same final model. Getting different final models can be due to too small sample sizel and/or factors strongly correlated. Alternatively, getting the same model does not imply that the "best" model was identified. An explanation for these differences might be that none of these automatic procedures do preserve the overall significance of the model. P-values provided for each factor are generally too optimistic (i.e. too small) because they don't account for the multiple tests which were performed to obtain the final model. As a consequence, some factors of poor importance might still remain in the model (false positives). Bootstrap technique can be used to assess the importance of a factor in the model. The bootstrap is a simulation technique first described by Bradley Efron.[40] The idea is that the original dataset is a random

sample of patients representative of a general population. Bootstrapping means generating a large number of datasets (e.g 1000) each of which with the same sample size as the original by resampling from it with replacement (ie. a patient may be selected multiple times, some patients might not be included). Cox model is fitted to each bootstrap sample. The percentage of the samples with the factor included in the model by stepwise or backward selection is a criterion for the prognostic importance of this factor. It can be interpreted in a Bayesian way as an estimate of the posterior probability that the regression coefficients of the Cox model are truly different from zero (Figure I.5). The inclusion of a factor at a selection level of 5% in the original data is equivalent to a cut-off value of 50% for the bootstrap inclusion fraction using a selection level of 5% in each replication. In this work, we used a higher but not too conservative cut-off of 60%, meaning that for a factor to be included in the final cox model, it needed to be selected in at least 60% of bootstrap resampling. [41]

Figure I.5: The concept of bootstrap.



1.7.3 Assessing model predictive accuracy

A strong statistical relationship between an outcome and a prognostic factor characterized by small p-values in a regression model or large odd or hazard ratios does not necessarily mean that the factor can accurately separates patients who are likely to have the event (e.g. have disease progression within 2 years) from those who are unlikely to have it (e.g. be free of disease progression at year 2). Pepe et al (2004) showed for a binary outcome that in order to obtain reasonable classification accuracy characterized by a False Positive Fraction (FPF) equal to 10% and a True Positive Fraction (TPF) equal to 80%, an odd ratio (OR) of 36.0 was necessary. [42] Even with an OR as large as 36.0, one can't conclude that a marker has a good accuracy as a many different values of (FPF, TPF) are consistent with it (see OR formula below). An unacceptably high FPF equal 50% and TPF equal 97.3 % also yields to 36.0. These results are indepentent from the study sample size.

$$OR = \left\{ \frac{TPF}{(1-TPF)} \right\} * \left\{ \frac{(1-FPF)}{FPF} \right\}$$

Appropriate methods and indicators must be used to assess biomarker or model performance.
Table I-1 below summarizes the relationship between an outcome (progression and/or survival status) and a binary biomarker (M+ vs M-). It is assumed that M+ predicts for patient who progressed or died.

Table I-1: Definition of accuracy terms

	Progressed or died	Free of progression and alive	
	Positive prediction condition	Negative prediction condition	Total
M+	True Positive Fraction (TPF)	False Positive Fraction (FPF) *Type I error=1-specificity*	Positive Predictive Value **PPV** =TPF/(TPF+FPF)
M-	False Negative Fraction (FNF) *Type II error=1-sensitivity*	True Negative Fraction (TNF)	Negative Predictive Value **NPV** = TNF/(FNF+TNF)
	Sensitivity=TPF/(TPF+FNF)	**Specificity**=TNF/(FPF+TNF)	

Note: M- :biomarker negative, M+ biomarker positive,

Sensitivity measures the fraction of true positives which are correctly identified as such (e.g. the percentage of patients who progressed or died who are correctly identified as having this condition). Specificity measures the fraction of true negatives which are correctly identified (e.g. the percentage of patients free of progression and alive who are correctly identified as having this condition). These two measures are closely related to the type I and type II errors. A test with a high sensitivity has a low type II error rate (high power). A test with a high specificity has a low type I error rate. The positive predictive value (PPV) is the fraction of patients with biomarker M+ that are true positives (progressed or died). The negative predictive value (NPV) is the fraction of patients with biomarker M- that are true negatives (free of progression or alive). A high PPV (e.g. 90%) indicates that many M+ are true positives. In this situation, the biomarker can precisely predict if a patient will progress or die (case). A high NPV indicates that many M- are true negatives. In this case, a biomarker can precisely predict if a patient will remain non progressive and alive. PPV/NPV are related to sensitivity/specificity by the prevalence of the outcome (total fraction of patients who progressed or died).

$$PPV = \left\{ \frac{(sensitivity * prevalence)}{(sensitivity * prevalence) + (1-specificity) * (1-prevalence)} \right\}$$

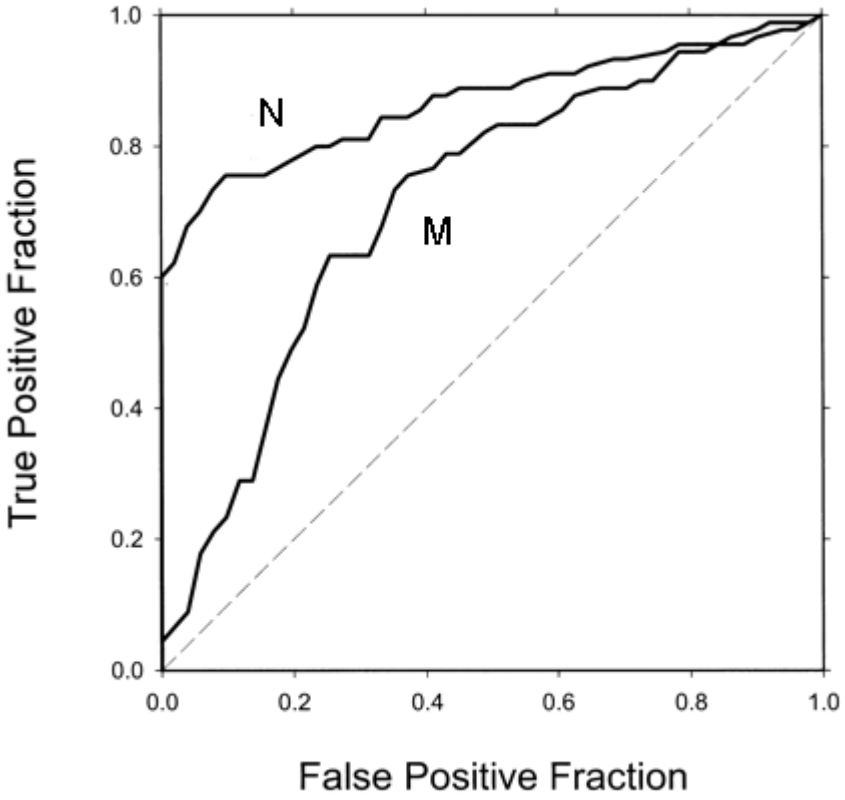
$$NPV = \left\{ \frac{(specificity) * (1-prevalence)}{(specificity) * (1-prevalence) + (1-sensitivity) * (prevalence)} \right\}$$

E.g. keeping sensitivity/specificity constant, a lower (higher) prevalence corresponds to a lower (higher) PPV.

For continuous biomarkers, Receiver operating characteristic (ROC) curve is a natural generalization of (FPF, TPF). In a ROC curve TPF (Sensitivity) is plotted in function of the FPF (1-Specificity) for different cut-off points "c" of a biomarker (M+ if ≥ c, M- if < c). Each point on the ROC curve represents a TPF/FPF pair corresponding to a particular cut-off. The closer the ROC curve is to the upper left corner, the higher the accuracy of the biomarker to predict the outcome. The area under the ROC curve (AUC) is the surface between the curve and the diagonal (TPF=FPF) plus 50%. It is a measure of how well a biomarker can distinguish between two outcome groups (progressive or dead/free of progression and alive). Unlike OR,

ROC curve or AUC do not depend on the units in which the biomarker was measured. They therefore provide natural common scales for comparing different biomarkers. In the example below biomarker N is better than M to discriminate between two outcome groups.

Figure I.6: ROC curves for two continuous biomarkers.

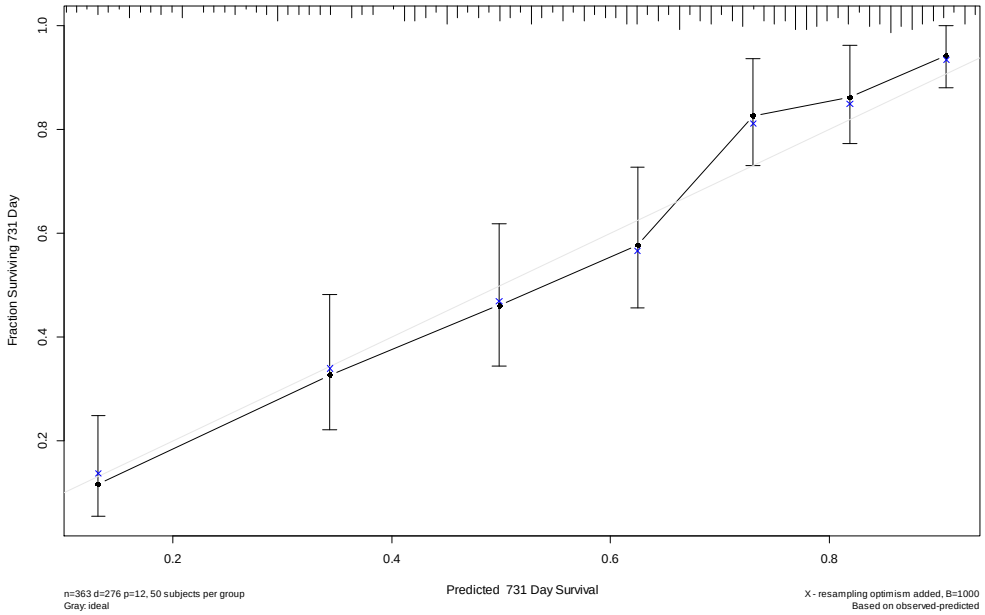


In survival analysis, the presence of censored times, makes the calculation of AUC more complex. The concordance index (C-index) introduced by Harrell (1996) is a natural extension of the ROC curve.[32] It is defined as the probability of concordance given that the pairs considered are usable in which at least one had an event. It can be interpreted as the probability that a subject from the event group has a higher predicted probability of having an event than a subject from the non-event (censored) group. C-index = 50% implies no predictive ability. C-index>0.5 implies some predictive ability, the higher the C-index the better the predictive ability. C-index= 100% implies a perfect predictive ability which is rarely observed. Bootstrap technique is also used for model internal validition in order to correct the C-index for "optimism". The procedure is straightforward. Using the same selection methods, Cox models are built on each bootstrap sample (training set) and evaluated in the full sample (test sets). The difference between the C-index in the bootstrap sample and the C-index in the full sample is computed. This process is repeated for each bootstrap sample (e.g 1000 times). The "optimism" is computed as the average of all the differences. A C-index corrected for overfitting is obtained by subtracting the optimism from the original C-index.

Both AUC and C-index are often described as measures to assess model "discrimination". Indeed, these measures allow classify or rank different individual patients based on their outcome. Another key statistics that describes model predictive accuracy is "calibration". Calibration refers to the extent of bias of a model. It is the degree of correspondence between the estimated probability produced by the model and the actual observed probability. If the average predicted probability of progression or death is 40% for a group of similar patients and the fraction of patients who actually progressed or died is 40% than the model is well calibrated for this group of patients. Poor calibration can occur in highly discriminating models when the output is transformed monotonically. Indeed, if all predictions were divided by 10 i.e. if someone with a 40% risk of progression or death was predicted with a 4% risk, the new model would have the same discrimination but would be poorly calibrated. Calibration can be corrected by using a shrinkage parameter

but no technique can correct for a lack of discrimination.[32] Calibration can be assessed by plotting the fraction of patients observed with the outcome (i.e. without the event) against the fraction of patients predicted with it (dots •). Bootstrap technique can also be used to obtain overfitting-corrected predictions (×). In a well calibrated model , all (×) should not be too far from the 45° line. This line should be included in the ninety five percent confidence intervals of the observed fraction for a particular predicted group of patients (e.g. those with 40% risk of progression or death) (Figure I.7).

Figure I.7: Prognostic model calibration plot.



Models with good discrimination and calibration can nevertheless provide survival probability estimates which can be highly variable between patients. The proportion of explained variation (PEV), is the amount of variation of the outcome variable that is attributable to one or more factors or a full prognostic model, relative to the total variation of the outcome variable. In linear regression model, R^2 is frequently used as a measure of explained variation. In Cox regression several methods have been proposed. One of them is the measure V by Schemper and Henderson (2000).[43]

Let's assume S(t) is the survival probability function at a time t. S(t|x) is this function conditioned to a factor x. f(t) and f(t|x) are the associated density functions i.e. the rates of death or failure events per unit time unconditional or conditional to factors x, obtained by computing the first derivative of the function 1-S(t) or 1-S(t|x)). The marginal and conditional mean absolute deviation are 2 S(t){1-S(t)} and 2 S(t|x){1-S(t|x)} respectively. They measure the average absolute distance between the true survival status and the survival probability. Taking (0,τ) as the global follow-up period, Schemper et al suggested D(τ) and D(τ|x) as measure of marginal and conditional predictive accuracy.

$$D(\tau) = \frac{2\int_0^\tau S(t)\{1-S(t)\}\, f(t)\, dt}{\int_0^\tau f(t)\, dt}$$

$$D_x(\tau) = \frac{2\int_0^\tau E_X\left[S(t/X)\{1-S(t/X)\}\right] f(t)\, dt}{\int_0^\tau f(t)\, dt}$$

marginal and conditional predictive accuracy can be compared to quantify the added value provided by the factors. The proportion of variation explained (PEV) by factors x is the ratio

$$V(\tau) = \frac{D(\tau) - D_x(\tau)}{D(\tau)}$$

For Heinze and Schemper (2000), A PEV of at least 20% is considered a minimum requirement for a model to provide "sufficiently precise individual survival predictions".[44]

1.7.4 Building nomograms and prognostic calculators

According to the Wikipedia definition, a nomogram is *a graphical calculating device, a two-dimensional diagram designed to allow the approximate graphical computation of a function* (sic).[45]
A nomogram is thus a graphical representation of a function or model but not the model by itself. See examples of nomograms for glioblastoma patients in chapter 5. In this thesis, the Cox models were used to compute for each patient a linear predictor (LP_j) obtained by summing up the products between the characteristic i of patient j (x_{ij}) and corresponding Cox coefficient (β_i):

$$LP_j = \sum_i \beta_i * x_{ij}$$

Baseline value LP_j equals 0 and corresponds to the best prognosis patients.
For each factor, prognostic scores can be computed with the following formula:

$$\text{Prognostic score for each value of characteristic i} = \frac{\beta_i * x_{ij}}{\text{B}} * 100$$

Where B is the Cox coefficient corresponding to the factor with the maximum product $\beta_{i*}x_{ij}$.
A prognostic score should not be interpreted as if to a high score should correspond a highly influential prognostic factors. Nomograms rank the importance of a factor only in the context of other factors. The total number of points for each patient is obtained by summing the prognostic scores for each of the individual factors included in the nomogram. Summary statistics like median or x-year probabilities are obtained by drawing a vertical line from the "total points" axis straight down to the outcome axes. With the advance of internet technology, it is now possible to obtain these summary statistics by entering patient individual characteristics in online prognostic calculators (see EORTC webpage at http://www.eortc.org/investigators-area/prognostic-calculators). An advantage of online calculators is that summary statistics can be presented with their 95% confidence intervals (CI). Ninety five percent CI are generally not available in statistical packages for building nomograms. A disadvantage is that it does not provide an indication of the relative importance of each factor. Neither nomograms nor calculators provide an evaluation of the predictive performances of prognostic models or the fraction of outcome variation explained by a factor. This information is obtained by estimating and interpreting parameters like C-index or PEV (see 1.7.3).

1.7.6 External validation of statistical prognostic models

Validating a statistical prognostic model means assessing its ability to predict the outcome of patients different from those used to develop the model. There are different reasons why statistical prognostic model might not perform well in other patients. Prognostic modeling unlike properly designed clinical trials are non-prespecified and data driven analyses. They generally provide overoptimistic estimation of their true predictive performance when it is assessed in the development dataset. As discussed earlier, internal validation partially fixes this problem by providing bias corrected model performance estimations. But the method fails when sample size of the development dataset is too small. [46,47] Simulation studies showed that

having 10 to 20 events (e.g. deaths) per factor screened could reduce the risks of both false negative and positive factor inclusion, i.e. selecting unimportant factors and failing to include important ones.[32] Finally, patients in the development dataset might not be completely representative of the population with the disease. Models are generally developed based on clinical trial data and in selected centers. Their predictions might be biased for community patients excluded from clinical studies (e.g. frail patients or presenting with comorbidities) or for patients from other centers with a different mix of individual and disease characteristics. One can never be sure that the model includes all important factors for all centers. All these issues strongly argue to perform an evaluation of model performance on a new series of patients in different hospitals, preferably in different locations. Altman and Royston (2000) provides guidelines on how to validate a prognostic model which were used in this thesis.[48]

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Chapter II. **Kros JM, Gorlia T, Kouwenhoven MC, Zheng PP, Collins VP, Figarella-Branger D, Giangaspero F, Giannini C, Mokhtari K, Mørk SJ, Paetau A, Reifenberger G, van den Bent MJ. Panel review of anaplastic oligodendroglioma from European Organization For Research and Treatment of Cancer Trial 26951: assessment of consensus in diagnosis, influence of 1p/19q loss, and correlations with outcome. J Neuropathol Exp Neurol. 2007 Jun;66(6):545-51.**

2.1 Abstract

The diagnosis of anaplastic oligodendroglioma (AOD) or anaplastic oligoastrocytoma (AOA) is subject to interobserver variation. The aim of this study was to estimate consensus in typing and grading of these tumors using tumor material collected in a large prospective randomized phase III study and to correlate the consensus diagnosis with the 1p/19q status of the tumors and the clinical outcome. The available pathology material of the first 150 patients, randomized into the European Organization for Research and Treatment of Cancer Trial 26951, was reviewed by an independent panel of 9 neuropathologists. The presence of deletions of 1p and 19q was assessed by fluorescence in situ hybridization with locus-specific probes. The panel reached consensus on the diagnosis of AOD in 52% of the tumors that had been diagnosed as AOD by the local pathologists, whereas only 8% of the local diagnosis of AOA was confirmed with consensus. The concordance on the panel diagnosis of AOD was high (intraclass correlation = 86%). The survival curves for AOD with 1p/19q loss, AOD without these losses, and AOA without 1p/19q loss ran separately in this order. The absence of necrosis and the presence of endothelial abnormalities were correlated with better outcomes. In multivariate analysis, patients' age, 1p/19q loss, and necrosis were identified as independent prognostic factors.

2.2 Introduction

Following the observation that anaplastic oligodendroglioma (AOD) and anaplastic oligoastrocytoma (AOA) are sensitive to chemotherapy with procarbazine, 1-(2-chloroethyl- 3-cyclohexyl-1-nitrosourea, and vincristine (PCV), in 1995 the European Organization for Research and Treatment of Cancer (EORTC) Brain Tumor Group initiated a prospective randomized trial in which the effects of the addition of 6 cycles of standard PCV chemotherapy to 59.4 Gy radiotherapy in newly diagnosed AOD and AOA were investigated. In this trial, in which 368 patients were included, it was shown that the addition of PCV chemotherapy increased the progression-free survival, but not the overall survival[1]. A central pathology review was part of the design of this trial. Patients were eligible for inclusion if their diagnosis of either AOD or AOA had been made in their local hospital. Because the diagnosis of AOD is subject to significant inter-observer variation with respect to assessment of tumor lineage and grade, we used this prospective trial to assess interobserver variation with regard to classification and grading of oligodendroglial tumors in a subset of patients from this trial. For this review, a panel of 9 independent and expert neuropathologists was installed, including the central reviewer of the pathologic material for EORTC Trial 26951. The 9 neuropathologists performed their review of the slides independent of each other and in ignorance of any clinical information. No selection of the available pathology material was made before the review; the slides were reviewed in just the state they had been received from the local pathologists. We investigated the consensus on the overall diagnosis and on individual histologic parameters. The consensus features and diagnoses were related to the 1p/19q status of the tumors and the clinical outcome of the patients.

2.3 Materials and methods

Design of European Organization for Research and Treatment of Cancer Trial 26951

For the design and approvals of EORTC Trial 26951 we refer the reader to Reference 1.

Pathology

For the central pathology review, the centers were required to submit hematoxylin and eosin (H&E)-stained slides and either tumor blocks or 10 to 15 unstained slides. Amendment 3 of the study (dated March 21, 2001) described the assessment of chromosomal loss of 1p and 19q within the study, with the objectives to assess the relation of 1p/19q loss with progression-free survival and with overall survival. For the assessment of 1p and 19q status, fluorescent in situ hybridization was applied as described previously [1,2].

Panel Review

The histopathologic diagnoses made by local pathologists and used to include patients in the trial were AOD and AOA. Paraffin-embedded and H&E-stained tumor sections of 114 patients were used for the panel review. For the pathology review, a panel of 8 expert neuropathologists (VPC, DF-B, FG, CG, KM, SJM, AP, and GR) was formed. The initial central review was done by JMK. The 9 neuropathologists performed their review of the slides independent of each other and in ignorance of any clinical information. Further, no selection of the slides was made before the review; the H&E-stained slides were reviewed in the state in which they had been received from the local pathologist. The original reports or diagnoses of the local pathologists were not communicated with the panelists. The reviewers were asked to classify the tumors according to the World Health Organization 2000 guidelines [3]. In addition, they were asked to grade the tumors by scoring 5 histologic features (i.e. nuclear pleomorphism, cell density, mitoses, endothelial abnormalities, and necrosis) in a simple yes-no fashion. As a result of the review, the diagnostic categories that emerged were low-grade glioma (LGG) (including low-grade astrocytoma [LA], low-grade oligodendroglioma [LOD], and low-grade mixed oligoastrocytoma [LOA]), anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AOD), anaplastic mixed oligoastrocytoma (AOA), glioblastoma (GBM), and a category of "other" diagnoses (for instance, ependymoma). Consensus diagnosis of the review panel was defined as 6 or more of the 9 reviewers agreeing on a diagnosis of a particular case, a majority diagnosis was defined as 4 or 5 reviewers agreeing on a diagnosis of a particular case, and cases were called indeterminate if only 3 or fewer reviewers made the same diagnosis.

Statistical Analysis

The distributions of diagnoses and scores of the panelists (including those of the central reviewer) were first assessed. Intraclass correlations (ICCs)[4] were used as chance corrected measures of agreement on the diagnosis (coded as AOD or not) and on each feature (coded as present or absent). ICCs and 95% confidence intervals were computed on the basis of the variance components of 2-way design general linear models, allowing for breaking the global variability into that between patients and between panelists. ICCs of 1p/19q loss were also computed separately in each group. ICC≥80 was considered as an excellent agreement. Exact confidence intervals were computed for the proportion of cases with 1p, 19q, and both 1p/19q loss in each diagnosis. Fisher`s exact test was used for comparison of proportions. The agreement between each reviewer (including the initial review made by the local pathologists) and the consensus was measured by a κ coefficient. A κ coefficient < 60 was considered a poor agreement. The Kaplan-Meier technique and logrank test were used to assess the prognostic value of the diagnoses and individual histologic features. The Cox proportional hazards model was fitted on the consensus diagnosis and features as well as available clinical factors to identify independent prognosticators of survival.

2.4 Results

Clinical Data

The clinical characteristics and survival outcome of the 114 patients for whom the pathology material was available for review were representative of the entire group of 368 patients. There were 60 male patients (53%) and 54 female patients (47%). Forty percent of tumors were located in the frontal, 25% in the parietal, and 18% in the temporal lobes. Other locations included occipital lobes (3%), basal ganglia and cerebellum (1 patient), and corpus callosum (2 patients). Of the patients, 16% had undergone a biopsy only. Enhancing tumors were more frequent in the subset of the present study, but tumor enhancement was not found to be of prognostic value [1] and was therefore not considered as a bias. The local pathologists had diagnosed AOD in 89 of the 114 cases (78%) and AOA in 25 cases (22%).

Results of Panel Review

Table II-1 shows the distribution of diagnoses made by the 9 reviewers and local pathologists. The percentage of AOD as diagnosed by the reviewers varied from 33% to 68% of all cases reviewed and the percentage of AOA varied from 7% to 34%. Diagnoses other than AOD or AOA made by the panelists included AA (0%-5%), GBM (1%-27%), LOD (1%-11%), LA (0%-2%), and LOA (0%-4%). Other diagnoses, such as ependymoma, were made in 1% to 17% of patients (Table II-1). The local pathologists had made the diagnosis AOD in 89 of the 114 cases. In 46 of these 89 cases (52%), the panel reached consensus and confirmed the diagnosis of AOD (Table II-2). In 9 cases (10%), the AOD diagnosis was confirmed with majority agreement. In 22 cases (35%), the reviewers reached a consensus or a majority diagnosis that was different from AOD, whereas in the other 12 cases (13%), no consensus or majority diagnosis was reached. Overall, consensus and majority diagnoses other than AOD included AOA in 8 cases, LGG (i.e. LOD, LA, and LOA) in 7 cases, and GBM in 5 cases, and in 2 cases other diagnoses (e.g. ependymoma) were made (Table II-2). In 25 of the 114 cases, the diagnosis of AOA was made by the local pathologists. In only 2 of these cases (8%), was there consensus among the panelists on this diagnosis (Table II-2). In 8 cases (32%), the panel reached a majority diagnosis of AOA. In 2 cases (8%), the diagnosis of AOA was mentioned, but only by 3 or fewer reviewers. Panel consensus or majority diagnoses other than AOA included AOD in 8 cases; LGG in 1 case, and GBM in 1 case (Table II-2). The agreement of each of the individual panelists with the 52 consensus diagnoses of AOD is shown in Table II-3. The κ coefficients ranged from 46.2 to 72.1. The ICC of the diagnosis AOD was 86.4 (95% confidence interval 80.8-89.0). Five reviewers (R2, R4, R6, R7, and R9) had poor agreement with consensus diagnoses (Table II-3). R2, R6, and R7 disagreed more often on the non-AOD diagnosis, whereas R9 more frequently disagreed on the AOD diagnosis. The κ coefficient between consensus and local diagnoses was as low as 18.1, showing excellent agreement on the AOD diagnosis but poor agreement on the non-AOD diagnosis (Table II-3).

Table II-1: Distribution of the diagnoses made by the 9 reviewers and the local pathologists.

	R1	R2	R3	R4	R5	R6	R7	R8	R9	Local Pathologist
AOD (%)	47	62	46	47	54	63	68	54	33	78
AOA (%)	25	18	7	17	24	15	9	34	18	22
AA (%)	5	0	3	4	2	2	0	0	11	0
GBM (%)	6	9	21	4	2	16	1	2	27	0
LOD (%)	5	6	8	11	5	1	8	3	1	0
LA (%)	0	0	1	1	2	0	0	1	1	0
LOA (%)	2	0	1	4	4	0	3	4	2	0
Other (%)	9	2	7	6	6	3	2	1	17	0

The diagnosis of AOD, which was made in 78% of cases by the local pathologists, was confirmed in percentages varying from 33% to 68%; the diagnosis of AOA made in 22% of cases by the local pathologists was confirmed in only 7% to 34% of cases. R5 is the central reviewer of European Organization for Research and Treatment of Cancer Trial 26951. R1YR4 and R6YR9 are the additional reviewers involved in this study.
AA, anaplastic astrocytoma; AOA, anaplastic mixed oligoastrocytoma; AOD, anaplastic oligodendroglioma; GBM, glioblastoma; LA, low-grade astrocytoma; LOA, low-grade mixed oligoastrocytoma; LOD, low-grade oligodendroglioma.

Table II-2: Results of Panel Review.

Diagnosis y local pathologist	Panel diagnosis	Consensus diagnosis	Majority diagnosis	Intermediate diagnosis	Total
AOD	AOD	46(52)	9(10)	1(1)	56(63)
	AOA	2(2)	6(7)	3(3)	11(12)
	LGG	1(1)	6(7)	2(2)	9(10)
	AA	0(0)	0(0)	0(0)	0(0)
	GBM	1(1)	4(4)	5(6)	10(11)
	Other	0(0)	2(2)	1(0)	3(3)
	Subtotal	50(56)	27(30)	12(16)	89(78)
AOA	AOD	6(24)	2(8)	0(0)	8(32)
	AOA	2(8)	8(32)	2(8)	12(48)
	LGG	0(0)	1(4)	1(4)	2(8)
	AA	0(0)	0(0)	0(0)	0(0)
	GBM	0(0)	1(4)	2(8)	3(12)
	Other	0(0)	0(0)	0(0)	0(0)
	Subtotal	8(32)	12(48)	5(20)	25(22)
Total		58(51)	39(34)	17(15)	114(100)

Data are n (%). Although appearing in diagnoses lists of individual panelists, the diagnoses “AA” and “other” were never made by consensus or the majority of panelists.
“Consensus Diagnosis” indicates that 6 or more of the 9 reviewers agreed on the diagnosis. “Majority Diagnosis” indicates that 4 or 5 reviewers of the 9 reviewers agreed on the diagnosis. “Indeterminate Diagnosis” indicates that 3 or fewer reviewers made the same diagnosis.
AA, anaplastic astrocytoma; AOA, anaplastic mixed oligoastrocytoma; AOD, anaplastic oligodendroglioma; GBM, glioblastoma; LGG, low-grade glioma (i.e. low-grade oligodendroglioma, low-grade mixed oligoastrocytoma, and low-grade astrocytoma).

Table II-3: Agreement of the 9 reviewers with the consensus diagnosis of AOD.

	Consensus vs Individual Diagnosis		
	Agreement on positive diagnosis of AOD	Agreement on negative diagnosis of AOD	κ Coefficient
	(n=52) (%)	(n=62) (%)	
R1	87	85	71.8
R2	92	63	53.6
R3	83	84	66.5
R4	77	77	54.2
R5*	92	77	68.7
R6	89	59	46.2
R7	94	55	47.2
R8	92	81	72.1
R9	65	94	59.7
Local	88	31	18.1

*, R5 is the central reviewer (JMK) of European Organization for Research and Treatment of Cancer Trial 26951. R1Y4 and R6Y9 are the additional reviewers for this study. Reviewers R2, R4, R6, R7, and R9 had poor agreement with consensus diagnoses.

Fluorescence In Situ Hybridization for 1p and 19q

Fluorescence in situ hybridization analysis was not possible in 6% of cases because of lack of available material or because the test results could not be interpreted. The frequencies for loss of 1p only, loss of 19q only, and combined loss of 1p/19q are listed in Table II-4. For AOD the consensus diagnosis was exclusively used, for AOA the consensus and majority diagnosis were used, and for GBM and LGG only majority diagnoses were available. Combined loss of 1p/19q was found in 35% of AOD, in 11% of AOA, in 0% of GBM, and in 50% of LGG. Loss of 1p only was never seen in GBM, whereas loss of 19q was seen in one third of these tumors. Loss of 1p and combined loss of 1p/19q was found significantly more often in AOD than in AOA or GBM ($p = 0.02$; $p = 0.04$). The difference was borderline non-significant for loss of 19q ($p = 0.07$) (Table II-4).

Table II-4: Distribution of losses of 1p,19q, combined 1p/19q in the consensus and majority diagnostic.

	AOD (n=50)*†		AOA (n=17)†‡		GBM (n=6)‡		LGG (n=8)§		
	n(%)	95% CI (%)‖	n(%)	95% CI (%)	n(%)	95% CI (%)	n(%)	95% CI (%)	Proportion in AOD vs in AOA or GBM (p)¶
1p loss (irrespective of 19q loss)	27 (52)	38-66	5(28)	10-53	0(0)	0-0	6(75)	35-97	0.02
19q loss (irrespective of 1p loss)	22(42)	29-57	2(11)	1-35	2(33)	4-78	4(50)	16-84	0.07
Combined 1p/19q loss	18(35)	22-49	2(11)	1-35	0(0)	0-0	4(50)	16-84	0.04

*, Consensus diagnosis.
†, Two of the 52 (consensus) AODs and 1 of the (consensus and majority) AOAs could not be processed for genotyping.
‡, Consensus and majority diagnosis.
§, Majority diagnosis.
¶, Fisher`s exact test.
‖, Exact 95% confidence interval of the proportion.
AOA, anaplastic mixed oligoastrocytoma; AOD, anaplastic oligodendroglioma; CI, confidence interval; GBM, glioblastoma; LGG, low-grade glioma.

Consensus on Individual Histologic Features

The ICCs and 95% confidence intervals for consensus on individual histologic features of AOD are summarized in Table II-5. Most reviewers scored the feature of nuclear abnormalities as being present in 100% of cases, and for that reason this feature was excluded from further analysis.
The ICC for the feature necrosis was highest, and this was the case for the tumors with and without loss of 1p/19q. The feature of mitotic count scored lowest, whereas cell density and endothelial abnormalities took intermediate positions in the ICC rankings. Except for the feature of endothelial abnormalities, the ICCs for all other features were slightly higher in the tumors with retention of 1p/19q, but differences were not statistically significant (Table II-5).

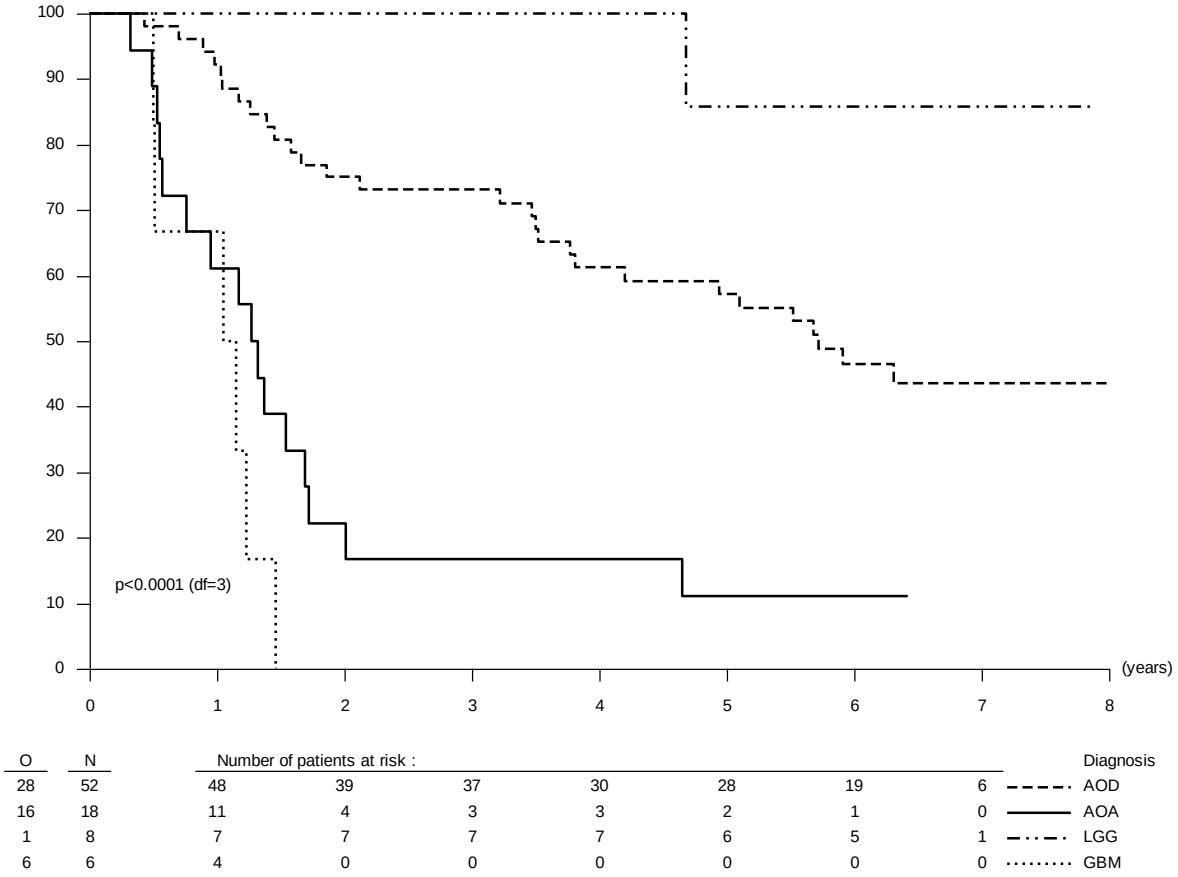
Table II-5: Intraclass Correlation for the histologic features in (consensus) AOD.

	Intraclass Correlation (95% Confidence Interval) (%)		
	All	1p/19q loss	1p/19q retained
Diagnosis of AOD	86.4(80.8-89.0)	83.4(70.4-91.3)	86(79.1-89.2)
Necrosis	92.4(89.6-94.0)	90.0(82.0-94.7)	93.3(90.4-95.0)
Endothelial abnormalities	86.7(79.8-88.4)	87.5(73.2-92.1)	86.7(79.2-89.2)
High cellularity	85.1(79.4-88.2)	79.7(60.1-88.3)	87.2(81.8-90.6)
Mitoses	84.4(78.2-87.5)	77.6(57.5-87.5)	85.1(78.4-88.8)

Prognostic Relevance of Tumor Typing, Tumor Grading, and Individual Histopathologic Features

The survival curves for the respective consensus and majority diagnoses, irrespective of 1p/19q status, are shown in Figure II.1. The curve of AOD is separated from the intertwined curves of the AOAs and the GBMs. A small group of low-grade gliomas (i.e. LOD, LA, and LOA) showed the best survival (Figure II.1). The Kaplan-Meier curves for AOD and AOA with and without 1p/19q loss (the single AOA with 1p/19q loss is left out) are shown in Figure II.2. The curve for AOD without 1p/19q was intermediate between that for AOD with loss of 1p/19q and AOA without loss of 1p/19 (Figure II.2). The p values for the differences between the respective survival curves according to the histologic features are listed in Table II-6. Only features on which consensus existed were used for this analysis. The features were tested irrespective of the diagnosis of the tumors. The 2 curves for the feature of cellularity of the tumors ran in the expected order but were not significantly different from each other ($p = 0.066$). The curves for endothelial abnormalities and necrosis ran in the expected order and differed significantly ($p = 0.028$ and $p = 0.015$, respectively) (Table II-6). The feature of nuclear abnormalities was almost univocally scored as present by all reviewers and was, therefore, not further analyzed. The curve for the few patients with tumors in which no mitoses were found ran steepest, but no significant differences between the curves were obtained. Consensus on the features of cell density, endothelial abnormalities, mitoses, and necrosis was reached for 79 tumors. These tumors were used in multivariate analysis to assess the prognostic value of the individual features and patient's age, extent of surgery, tumor location, and performance status. Patient's age, the presence of tumor necrosis, and 1p/19q status were identified as independent prognosticators (data no shown).

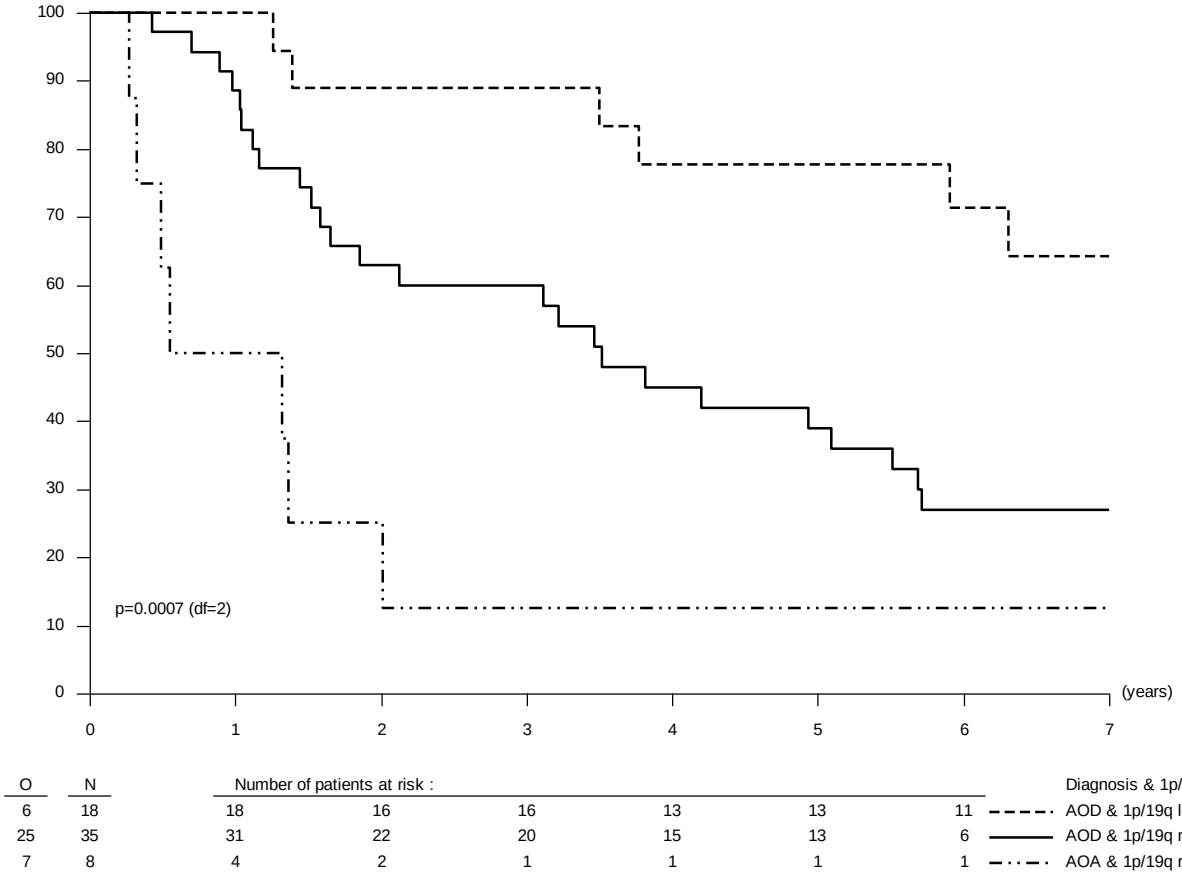
Figure II.1: OS curves for the respective diagnoses, irrespective of 1p/19q status.



O	N	Number of patients at risk :							Diagnosis
28	52	48	39	37	30	28	19	6	AOD
16	18	11	4	3	3	2	1	0	AOA
1	8	7	7	7	7	6	5	1	LGG
6	6	4	0	0	0	0	0	0	GBM

Note:All curves are based on agreement of 4 or more of the 9 reviewers. The curve of anaplastic oligodendroglioma (AOD) is separated from the intertwined curves of anaplastic oligoastrocytoma (AOA), glioblastoma (GBM), and anaplastic astrocytoma (AA). O, number of events; N, number of patients.

Figure II.2: OS curves according to 1p/19q genotypes for AOD and AOA.



Note: All curves are based on agreement of 4 or more reviewers. The patients with AOD with loss of 1p/19q do significantly better than those without these losses, and the patients with AOA do significantly worse than those with AOD with or without loss of 1p/19q. The majority of consensus AOAs are tumors without loss of 1p/19q. N, number of patients; O, number of events.

Table II-6: Significances of the differences of survival curves for the histologic features.

Histologic feature	p
Cellularity	0.066
Endothelial abnormalities (proliferation)	0.028
Necrosis	0.015
Nuclear abnormalities	-
Mitoses	n.s.

Only consensus features were used for the analysis.
n.s., not significant.

2.4 Discussion

Because of optional treatment with alkylating chemotherapy, making the diagnosis of oligodendroglioma became highly important[5]. The correlation between the histology of oligodendroglioma, the typical genetic signature of combined loss of 1p/19q, and the fair response to alkylating chemotherapy made this type of glioma the subject of many clinical and laboratory investigations [1,6]. In this study, the panelists confirmed the diagnosis of AOD made by the local pathologists in just over one half of the cases (range 33%-68%). The large discrepancy between the diagnosis made by local pathologists (used for inclusion of the patients into the trial) and the experts/panelists shows that central review of pathology material of trials on glial tumors is important. For making the diagnosis of oligodendroglioma, one may choose to apply either strict or relaxed criteria[7]. Obviously, allowing less strict criteria for making the diagnosis will lead to increased interindividual observer variability[8]. Despite the fact that the discriminating microscopic features for oligodendrogliomas as originally mentioned by Bailey and Bucy in 1929[9] are rather ill-defined and nonspecific and are thus prone to subjectivity, there was satisfactory concordance on the diagnosis of AOD made by the panelists (Table II-5). Because of the large histologic variability and the relatively poor definition of oligodendroglial tumors, many studies have focused on the correlation of the genetic signature and the histologic particularities of these neoplasms. Combined 1p/19q loss is an early genetic aberration that distinguishes AOD as a particular glioma subtype with a protracted clinical course and good response to both radiotherapy and chemotherapy[10]. The differences in concordance on the diagnosis of AOD of tumors with and without combined loss of 1p/19q were not significant, and the same was true for the scoring of the individual features. Taking advantage of the prospective randomized setup of EORTC Trial 26951 we were able to correlate the results of the panel review and the status of 1p/19q of the tumors with the parameters of outcome. The consensus diagnosis of AOD made by the review panel yielded a survival curve that was significantly different from those of consensus or majority diagnosis of AOA and GBM (Figure II.1), and it is concluded that there was good correlation of the consensus diagnoses AOD with the outcomes of the patients. In addition, AOD with the genetic signature of oligodendroglioma showed significantly better survival compared with the AOD without this genotype (Figure II.2), corroborating the notion that loss of 1p/19q is correlated with better outcome. The present results show that making the diagnosis of AOA is more difficult than that of pure AOD: the panel reached consensus in only 8% of cases diagnosed as AOA by the local pathologists, and in more than one half of cases the panel agreed that the locally made diagnosis was wrong. There was, however, a large range (7%-34%) in the diagnoses of AOA within the panel of expert reviewers (Table II-1). Recognition of the classic histology of oligodendroglioma may not be difficult, but problems arise when features are only present to some extent. Mixtures of oligodendroglia-like cells with astrocytic (tumor) cells may suggest the diagnosis of mixed oligoastrocytoma; there are, however, no sensible cutoff percentages for cells with features of the respective lineages in the definition of mixed oligoastrocytoma[3]. Proposals for cutoff cell fractions have been invalidated by tumor heterogeneity and difficulties in the distinction between reactive and neoplastic glial cells [3,11,12]. At present, the term "mixed oligoastrocytoma" is applicable to a variety of situations. For instance, in up to one half of classic oligodendrogliomas gemistocytic cells are seen[13], and the presence of such cells may also trigger the

diagnosis of mixed oligoastrocytoma. Tumors composed of cells with features that are intermediate between those of neoplastic oligodendrocytes and astrocytes usually receive the diagnosis of mixed oligoastrocytoma as well. In this study, 11% of cases with consensus or majority diagnosis of AOA showed combined 1p/19q loss, and thus most consensus or majority AOAs do not show the typical genetic signature found in classic oligodendroglioma in the literature [14, 15].

This was in agreement with the survival curve of the patients who did significantly worse than the patients with AOD. These findings confirm the experience that 1p/19q status is a powerful prognosticator in glioma with oligodendroglial cells. It is questionable to what extent pathomorphology will remain the gold standard for classification of gliomas and how far molecular tests will substitute the microscope for identifying specific biologic aggressiveness of tumors or relevance to the choice of therapy. In this multivariate analysis the presence of necrosis had independent prognostic significance, and the concordance of the reviewers on this feature was high (Table II-5).

Tumor necrosis has been identified as a histologic feature with prognostic value in various retrospective studies of oligodendrogliomas and astrocytomas [16,17]. Tumor necrosis is usually seen in the context of other anaplastic features, but when these other features are absent, the impact of necrosis on the biologic behavior of oligodendrogliomas should be interpreted with caution [18,19]. The feature with the second best concordance was endothelial abnormalities. The high concordance found for this feature in this panel review indicates that it is fairly reproducible. Microvascular proliferation has often emerged from retrospective studies as a feature that correlates well with the clinical outcomes [12,20,21]. Generally, microvascular proliferation correlates well with the disruption of the blood-brain barrier, leading to brain edema and tumor enhancement on radiologic presentations. Some authors included contrast enhancement of the oligodendrogliomas in their grading scheme [22], but others argued that this feature is not specific for high-grade oligodendrogliomas [23]. Overall, the biologic link between proliferation of tumor cells, hypoxia-induced necrosis, and proliferation of blood vessels underscores the prognostic significance of these features individually [24].

In conclusion, the results show that review of trial related pathology material of glial tumors is an important correction of the locally made diagnosis. The concordance on the various histologic features and on the overall diagnosis of AOD by expert neuropathologists appeared to be satisfactory. Only one half of the tumors with a consensus diagnosis of AOD appeared to harbor loss of 1p/19q. The large discrepancy between local pathologists` diagnosis of AOA and the considerable variation in this diagnosis between reviewers illustrates the lack of delineators for this diagnosis. Further exploration of molecular abnormalities of gliomas with diagnostic, prognostic, or predictive value is indicated.

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Chapter III. **Hegi ME, Janzer RC, Lambiv WL, Gorlia T, Kouwenhoven MC, Hartmann C, von Deimling A, Martinet D, Besuchet Schmutz N, Diserens AC, Hamou MF, Bady P, Weller M, van den Bent MJ, Mason WP, Mirimanoff RO, Stupp R, Mokhtari K, Wesseling P; EORTC BTG and ROG; NCIC Clinical Trials Group. Presence of an oligodendroglioma-like component in newly diagnosed glioblastoma identifies a pathogenetically heterogeneous subgroup and lacks prognostic value: central pathology review of the EORTC 26981-22981/NCIC CE.3 trial. Acta Neuropathol. 2012 Jun;123(6):841-52. doi: 10.1007/s00401-011-0938-4.**

3.1 Abstract

Glioblastoma (GBM) is a morphologically heterogeneous tumor type with a median survival of only 15 months in clinical trial populations. However, survival varies greatly among patients. As part of a central pathology review, we addressed the question if patients with GBM displaying distinct morphologic features respond differently to combined chemo-radiotherapy with temozolomide. Morphologic features were systematically recorded for 360 cases with particular focus on the presence of an oligodendroglioma-like component and respective correlations with outcome and relevant molecular markers. GBM with an oligodendroglioma-like component (GBM-O) represented 15% of all confirmed GBM (52/339) and was not associated with a more favorable outcome. GBM-O encompassed a pathogenetically heterogeneous group, significantly enriched for IDH1 mutations (19 vs. 3%, p = 0.003) and EGFR amplifications (71 vs. 48%, p = 0.04) compared with other GBM, while co-deletion of 1p/19q was found in only one case and the MGMT methylation frequency was alike (47 vs. 46%). Expression profiles classified most of the GBM-O into two subtypes, 36% (5/14 evaluable) as proneural and 43% as classical GBM. The detection of pseudo-palisading necrosis (PPN) was associated with benefit from chemotherapy (p = 0.0002), while no such effect was present in the absence of PPN (p = 0.86). In the adjusted interaction model including clinical prognostic factors and MGMT status, PPN was borderline non-significant (p = 0.063). Taken together, recognition of an oligodendroglioma-like component in an otherwise classic GBM identifies a pathogenetically mixed group without prognostic significance. However, the presence of PPN may indicate biological features of clinical relevance for further improvement of therapy.

3.2 Introduction

The introduction of combined chemo-radiotherapy adding temozolomide concomitantly and adjuvant to radiotherapy has modestly increased outcome of patients with newly diagnosed glioblastoma (GBM) [32], in particular in patients whose tumors contain an epigenetically inactivated MGMT gene [11]. However, outcome varies dramatically even in a homogenously treated patient population with a median survival of 15 months: 2- and 5-year survival rates of 27 and 11%, respectively [7, 19, 21, 32]. Histopathologically, GBM is a heterogeneous tumor type and distinct morphologic subtypes may benefit differently from combined chemo-radiotherapy. Furthermore, unequivocal separation of GBM and anaplastic astrocytomas from anaplastic oligo-astrocytic neoplasms is difficult. Previous reports suggested that distinct morphologic features present in GBM may have prognostic value, such as the presence of an oligodendroglioma-like component that was associated with better outcome in some studies, while the presence of necrosis has been reported as a negative prognostic factor [9, 12, 16, 18, 37]. Here, we addressed the question whether particular morphologic features in GBM can identify clinically meaningful subgroups in this patient cohort treated homogenously with combined radio-chemotherapy that has become the standard of care. A specific goal was to investigate the clinical relevance of recognition of an oligodendroglioma-like component in GBM in tumors that had been diagnosed as GBM (all subtypes) by the initial local pathology assessment. The histopathological study was carried out as part of the central review performed in the phase III EORTC 26981/22981-NCIC/CE.3 trial for newly diagnosed GBM [32, 33]. The results of this detailed histopathological review were correlated with outcome and benefit from the new concomitant chemo-radiotherapy and in a subset of cases associated

with genetic information including the MGMT methylation status, copy number aberrations (CNAs) of EGFR, CDK4 and MDM2, combined loss of chromosomes 1p and 19q, and mutations of IDH1.

3.3 Patients and methods

Patients

Patients were enrolled in the phase III EORTC 26981/22981-NCIC/CE.3 trial [33] (ClinicalTrials.gov, number NCT00006353) between August 2000 and March 2002. Eligibility criteria have been detailed elsewhere [32] and comprised age between 18 and 70 years, histologically proven newly diagnosed GBM (WHO grade IV) and a WHO performance status of 0–2. Patients were randomized to either standard focal radiotherapy (RT) with a total dose of 60 Gy or concomitant chemotherapy of oral temozolomide (TMZ) at a daily dose of 75 mg/m2 given 7 days per week during radiotherapy, followed by up to six cycles of adjuvant TMZ (150–200 mg/m2) for 5 days every 28 days. All patients had given written informed consent prior to entering the study, including for molecular analysis of their tumors. The study was approved by the local ethics committees.

Pathology review

Central review was performed jointly by three experienced neuropathologists (RCJ, KM, PW) according to WHO 2000 criteria [15] using a multiheaded microscope. H&E-stained full sections were used for the evaluation. In most cases GFAP-, MIB-1 and a reticulin silver stain were available (collectively performed in Lausanne). Morphologic features were systematically recorded in a semiquantitative manner and comprised cellular differentiation patterns, types of necrosis (large ischemic type vs. pseudopalisading necrosis), microvascular proliferation and MIB-1 labeling index (see evaluation form, Figure III.1, Supplementary Figure S1). In line with the WHO classification, pseudopalisading necrosis (PPN) was defined as irregular, often serpiginous foci of necrosis surrounded by densely packed, radially oriented tumor cells. The agreement between the three pathologists was recorded. For this study, GBM with an oligodendroglioma-like component (GBM-O) was defined according to the following histopathological criteria: presence of at least one of two ‘‘major criteria’’— ‘diffuse highly cellular and monotonous growth at low power magnification’, ‘monomorphous cell population’; and at least two of three ‘‘minor criteria’’— ‘perinuclear halo formation in tumor cells’, ‘rounded tumor cell nuclei with dense chromatin pattern’, ‘chickenwire architecture of tumor microvasculature’. The extent of these features in the viable tumor tissue was recorded (<25, 25–75,>75%). GBM with >25% of the tumor tissue showing oligodendroglioma-like component was subclassified as GBM-O (see Figure III.2, for some examples).

Table III-1: Pathology review form.

EORTC Brain Tumor & Radiotherapy Group : Trial 26981/22981
PATHOLOGY REVIEW

Sequid	Initials	DOB	Center no.	slides ○0 ○1
NP_No		date received		

Diagnosis — Exclusion ○0 ○1

Consensus ☐3:0 ☐2:1 ☐no consensus — Date Review — Material

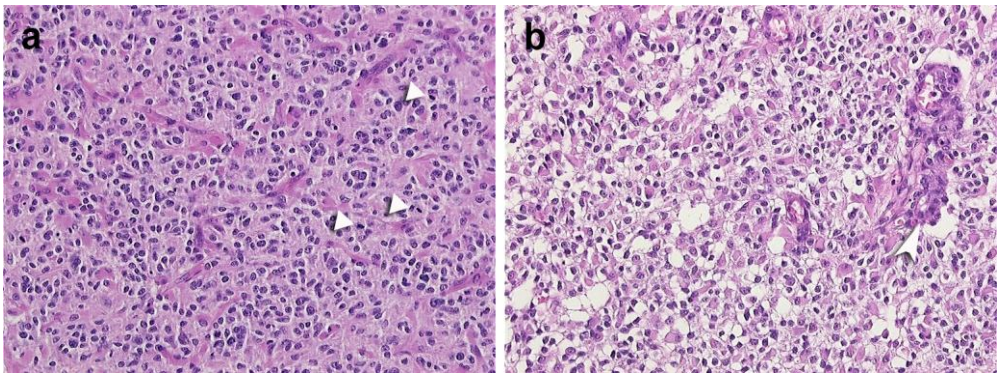
ConsSubtype ☐3:0 ☐2:1 ☐no consensus

CRITERIA	Over all		Semiquantitative on 10 HPF				Definition
Increased cellularity	○0	○1	ICsq	☐1	☐2	☐3	No cells/mm2: 1, <=500; 2, <=1000; 3, >1000
Nuclear pleomorphism	○0	○1	NPsq	☐1	☐2	☐3	No of nuclear phenotypes: 1, 1 -2; 2, 3-5; 3, >5
Mitotic activity	○0	○1	MAsq	☐1	☐2	☐3	No per HPF: 1, 1-5; 2, 6-20; 3, >20
MIB1 available	○0	○1	MIB1 (%)		SD		
Necrosis							
Large ischaemic type	○0	○1	LITsq	☐1	☐2	☐3	% surface: 1, 1 -5; 2, 6-15; 3, >15
Thrombosed vessels	○0	○1	TVsq	☐1	☐2	☐3	No per HPF: 1, 1-2; 2, 3-5; 3, >5
Pseudopalisading	○0	○1	PPsq	☐1	☐2	☐3	No per HPF: 1, 1-2; 2, 3-5; 3, >5
Microvascular Prolif	○0	○1	MPsq	☐1	☐2	☐3	No microvessels with multi-layered wall per 10 HPF: 1, 1-2; 2, 3-5; 3, >5
Perivascular Lymph	○0	○1	PLsq	☐1	☐2	☐3	No of foci per 10 HPF: 1, 1-2; 2, 3-5; 3, >5
Cellular differentiation pattern							
Oligodendroglial Comp	○0	○1	OCsq	☐1	☐2	☐3	No of HPF with presence of OC differentiation 1, 1-2; 2, 3-5; 3, >5
Sarcomatous Comp	○0	○1	SCsq	☐1	☐2	☐3	No of HPF with presence of SC 1, 1-2; 2, 3-5; 3, >5
Multinucleated Giant Cells	○0	○1	MGCsq	☐1	☐2	☐3	No of HPF with presence of > 20% MGC 1, 1-2; 2, 3-5; 3, >5
Gemistocytic cells	○0	○1	GCsq	☐1	☐2	☐3	No of HPF with presence of > 20% GC 1, 1-2; 2, 3-5; 3, >5
Other prominent patterns	○0	○1	Patterns				

Comments

Reviewers ☐Janzer ☐Wesseling ☐Mokthari ☐other

Figure III.1: Examples of histology in two tumors diagnosed as GBM with oligo-like component.



a Area showing diffuse highly cellular and monotonous growth of tumor cells with a dense chromatin pattern, perinuclear halo formation and chickenwire architecture of the microvasculature. **b** Highly cellular area showing rounded tumor cell nuclei with dense chromatin pattern and perinuclear halo formation. See ''Patients and methods'' for the definitions used in the present study for recognition of oligodendroglioma-like component in glioblastoma. Arrowheads in **a** mitotic figures, *arrowhead* in **b** florid microvascular proliferation. **a,b** Hematoxylin and Eosin staining, original magnification x200.

Tissue microarray, immunohistochemistry and molecular analysis.

Immunohistochemistry for GFAP and MIB-1, and histochemical reticulin staining were performed according to standard procedures on whole sections. A tissue microarray (TMA) was constructed comprising 130 patient samples where tumor blocks with sufficient tissue were available as reported previously[21]. The TMA was used to screen for the most common IDH1 mutation (R132H) using the specific antibody mIDH1R132H (clone H14) [4] and for copy number aberrations (CNAs) of selected genes by FISH. FISH for EGFR was performed as described [34]. Two-color FISH assay was performed using a mixed 1p36/1q25 and 19p13/19q13 dual color probe set (Cat. No 32-231004, Vysis, Inc., Applied Biosystems, Downers Grove, IL, USA) as described [31]. Samples showing sufficient FISH efficiency (~90% nuclei with signals) were evaluated. If possible, signals were scored in at least 200 non-overlapping, intact nuclei. Deletions of 1p and 19q were scored when at least 50% of tumor nuclei contained one signal. The following probes were used for CDK4 and MDM2: KBI-10725 CD4K/SE12 (12q14); KBI-10717 MDM2/SE12 (12q15) (Kreatech Diagnostics, Amsterdam; The Netherlands). The MGMT methylation status has been determined and reported previously [11, 32]. Expression of the EGFRvIII mutant, array comparative genomic hybridization (aCGH) data and gene expression data were available for a subgroup of patients [17, 21]. Additional EGFR amplification data were obtained by quantitative PCR as described [10]. Mutation analysis for IDH1 and IDH2 encompassing codon 132 and 172, respectively, was performed by direct Sanger sequencing.

Statistics

The Fisher's exact test (for binary or nominal categorical data) and the Wilcoxon rank-sum test (for continuous or ordinal categorical data) were used in the comparisons of patient and disease characteristics between subgroups. Survival analyses were performed with Kaplan–Meier technique with log-rank statistics. The Cox regression was used for multivariate analyses. All Cox models were fit with age (≤50, 51–60,>60), extent of surgery (total, partial, biopsy only), performance status (0, 1, 2), Mini Mental Score Examination (<27, 27–30) and MGMT methylation status (unmethylated, methylated). Pathological features significant at a 5% level in univariate analyses were included in the multivariate model. A treatment effect was assessed using Peto's heterogeneity test (predictive value). No adjustment for multiple testing was performed in these exploratory analyses. SAS version 9.2 was used for statistical analyses.

3.4 Results

Histological diagnosis and subclassification.

Central review comprised histological analysis of 360 of 573 patients enrolled (central review of Canadian patients was performed independently). Baseline characteristics have been published previously [32] and sub-cohort patient characteristics are summarized in Table III-1 (Supplemental Table S1). Overall, the patient characteristics of this subset did not differ significantly from the overall study population, other than molecular markers that could be determined in patients who had undergone tumor resection in contrast to biopsy only. From the total of 360 cases reviewed, 6 were considered undiagnosable due to insufficient tissue or quality of the sections. Fifteen (4.2%) tumors did not fulfill the criteria for GBM and comprised 4 anaplastic astrocytomas (WHO grade III; AA), 4 anaplastic oligoastrocytomas (WHO grade III, AOA), 1 anaplastic oligodendroglioma (WHO grade III; AO), 2 anaplastic ependymomas (WHO grade III), 2 pilocytic astrocytomas with malignant changes, 1 low grade glioma (WHO grade II) and 1 meningioma. Of the non-GBM tumors, 6 were in the RT and 9 in the RT/TMZ arm. The remaining 339 were diagnosed as GBM, of which 3 were subtyped as gliosarcoma and 6 as giant cell GBM. There was a 95% (338/354) consensus with regard to diagnosis of GBM versus non-GBM among the three neuropathologists. The median age of patients with confirmed GBM was 56 years of age (range 19–79) (Table III-2, Supplemental Table S2).

Table III-2: Baseline characteristics RT vs TMZ/RT in confirmed GBM.

Subsample Characteristics	**Treatment RT (N=173) N (%)**	**TMZ/RT (N=166) N (%)**	**Total (N=339) N (%)**	**P-value Fisher**
CLINICAL CHARACTERISTICS				
Sex				
Male	106 (61.3)	110 (66.3)	216 (63.7)	0.37
Female	67 (38.7)	56 (33.7)	123 (36.3)	
Mini mental state evaluation (class)				
≥27	102 (59.0)	103 (62.0)	205 (60.5)	0.64
<27	61 (35.3)	55 (33.1)	116 (34.2)	
Missing	10 (5.8)	8 (4.8)	18 (5.3)	
mini mental state evaluation				
Median	28.0	28.0	28.0	0.64 [a]
Range	9.0 - 30.0	7.0 - 30.0	7.0 - 30.0	
N obs	163	158	321	
corticosteroids at entry				
No	53 (30.6)	62 (37.3)	115 (33.9)	0.21
Yes	120 (69.4)	104 (62.7)	224 (66.1)	
Age (class)				
≤50 yrs	14 (8.1)	17 (10.2)	31 (9.1)	0.58 [a]
>50 & ≤60 yrs	104 (60.1)	99 (59.6)	203 (59.9)	
>60 yrs	55 (31.8)	50 (30.1)	105 (31.0)	
Age				
Median	56.0	55.0	56.0	0.62 [a]
Mean (SD)	54.72 (9.48)	53.94 (10.28)	54.34 (9.87)	
Range	25.0 - 70.0	19.0 - 70.0	19.0 - 70.0	
Extent of surgery				
Complete resection	66 (38.2)	68 (41.0)	134 (39.5)	0.52
Partial resection	84 (48.6)	71 (42.8)	155 (45.7)	
Biopsy	23 (13.3)	27 (16.3)	50 (14.7)	
Tumor location (all)				
Frontal	50 (28.9)	50 (30.1)	100 (29.5)	0.11
Temporal	37 (21.4)	49 (29.5)	86 (25.4)	
Parietal	37 (21.4)	25 (15.1)	62 (18.3)	
Occipital	12 (6.9)	10 (6.0)	22 (6.5)	
Central	5 (2.9)	10 (6.0)	15 (4.4)	
Multifocal	28 (16.2)	22 (13.3)	50 (14.7)	
Other	4 (2.3)	0 (0.0)	4 (1.2)	
MIB1				

Subsample Characteristics	Treatment RT (N=173) N (%)	TMZ/RT (N=166) N (%)	Total (N=339) N (%)	P-value Fisher
Median	30.0	30.0	30.0	0.08
Mean (SD)	32.72 (17.81)	36.40 (18.26)	34.53 (18.10)	
Range	5.0 - 80.0	5.0 - 90.0	5.0 - 90.0	
N obs	156	150	306	
TUMOR GENETICS				
MGMT				
Unmethylated	44 (25.4)	45 (27.1)	89 (26.3)	1.00
Methylated	38 (22.0)	38 (22.9)	76 (22.4)	
Missing	91 (52.6)	83 (50.0)	174 (51.3)	
IDH1				
Not mutated	54 (31.2)	67 (40.4)	121 (35.7)	0.73
Mutated	5 (2.9)	4 (2.4)	9 (2.7)	
Missing	114 (65.9)	95 (57.2)	209 (61.7)	
EGFR				
Normal	27 (15.6)	34 (20.5)	61 (18.0)	0.73
Amplified	34 (19.7)	36 (21.7)	70 (20.6)	
Missing	112 (64.7)	96 (57.8)	208 (61.4)	
1p/19q				
No co-deletion	65 (37.6)	70 (42.2)	135 (39.8)	0.24
Co-deletion	2 (1.2)	0 (0.0)	2 (0.6)	
Missing	106 (61.3)	96 (57.8)	202 (59.6)	
MDM2				
Normal	55 (31.8)	63 (38.0)	118 (34.8)	0.39
Amplified	8 (4.6)	5 (3.0)	13 (3.8)	
Missing	110 (63.6)	98 (59.0)	208 (61.4)	
CDK4				
Normal	49 (28.3)	58 (34.9)	107 (31.6)	0.37
Amplified	14 (8.1)	10 (6.0)	24 (7.1)	
Missing	110 (63.6)	98 (59.0)	208 (61.4)	

Note: [a] Wilcoxon rank sum test

Table III-3: Baseline characteristics GBM vs GBM-O.

Subsample Characteristics	Confirmed GBM GBM (N=287) N (%)	GBM-O (N=52) N (%)	Total (N=339) N (%)	P-value
CLINICAL CHARACTERISTICS				
Sex				
Male	186 (64.8)	30 (57.7)	216 (63.7)	0.35
Female	101 (35.2)	22 (42.3)	123 (36.3)	
Mini mental state evaluation (class)				
>=27	171 (59.6)	34 (65.4)	205 (60.5)	0.42
<27	101 (35.2)	15 (28.8)	116 (34.2)	
Missing	15 (5.2)	3 (5.8)	18 (5.3)	
mini mental state evaluation				
Median	28.0	29.0	28.0	0.19[a]
Range	7.0 - 30.0	10.0 - 30.0	7.0 - 30.0	
N obs	272	49	321	
Corticosteroids at entry				
No	102 (35.5)	13 (25.0)	115 (33.9)	0.15
Yes	185 (64.5)	39 (75.0)	224 (66.1)	
Age (class)				
<=50 yrs	23 (8.0)	8 (15.4)	31 (9.1)	**0.04** [a]
>50 & <=60 yrs	170 (59.2)	33 (63.5)	203 (59.9)	
>60 yrs	94 (32.8)	11 (21.2)	105 (31.0)	
Age				
Median	56.0	53.0	56.0	**0.02**[a]
Range	19.0 - 70.0	25.0 - 69.0	19.0 - 70.0	
Extent of surgery B/PR/CR				
Complete resection	111 (38.7)	23 (44.2)	134 (39.5)	0.16[a]
Partial resection	129 (44.9)	26 (50.0)	155 (45.7)	
Biopsy	47 (16.4)	3 (5.8)	50 (14.7)	
Tumor location (all)				
Frontal	84 (29.3)	16 (30.8)	100 (29.5)	0.22
Temporal	69 (24.0)	17 (32.7)	86 (25.4)	
Parietal	54 (18.8)	8 (15.4)	62 (18.3)	
Occipital	22 (7.7)	0 (0.0)	22 (6.5)	
Central	14 (4.9)	1 (1.9)	15 (4.4)	
Multifocal	40 (13.9)	10 (19.2)	50 (14.7)	
Other	4 (1.4)	0 (0.0)	4 (1.2)	
MIB1				
Median	30.0	30.0	30.0	0.24[a]
Mean (SD)	34.00 (18.05)	37.16 (18.31)	34.53 (18.10)	
Range	5.0 - 90.0	5.0 - 80.0	5.0 - 90.0	
N obs	255	51	306	

Note: [a] Wilcoxon rank sum test

Frequency of GBM with an oligodendroglioma-like component.

The criteria for GBM-O were met in 52 (15%) samples, at an expected frequency [12, 29, 37]. Subtyping of centrally confirmed GBM, including GBM-O, resulted in a 2:1 agreement for 24 cases, of which 16 overlapped with the debated cases for GBM versus non-GBM. In the group classified as GBM-O, two of five were considered as AOA and two as AO, by one of the neuropathologists. The median age of patients with GBM-O was lower than that of the other GBM patients (53 vs. 56 years, p=0.02) (Table III-2 ,Supplemental Table S2).

GBM-O encompass a pathogenetically heterogeneous group.

Evaluation of important prognostic molecular markers revealed the same frequency of MGMT methylation in GBM-O (47%, 16/34) versus the remaining GBM (46%, 60/131) (Table III-3). Furthermore, combined loss of 1p/19q, a hallmark of oligodendroglial tumors and associated with better prognosis in anaplastic glioma [35], was a rare event, observed in a single GBM-O, confirmed by aCGH, and one GBM (Figure III.3; Table III-3).

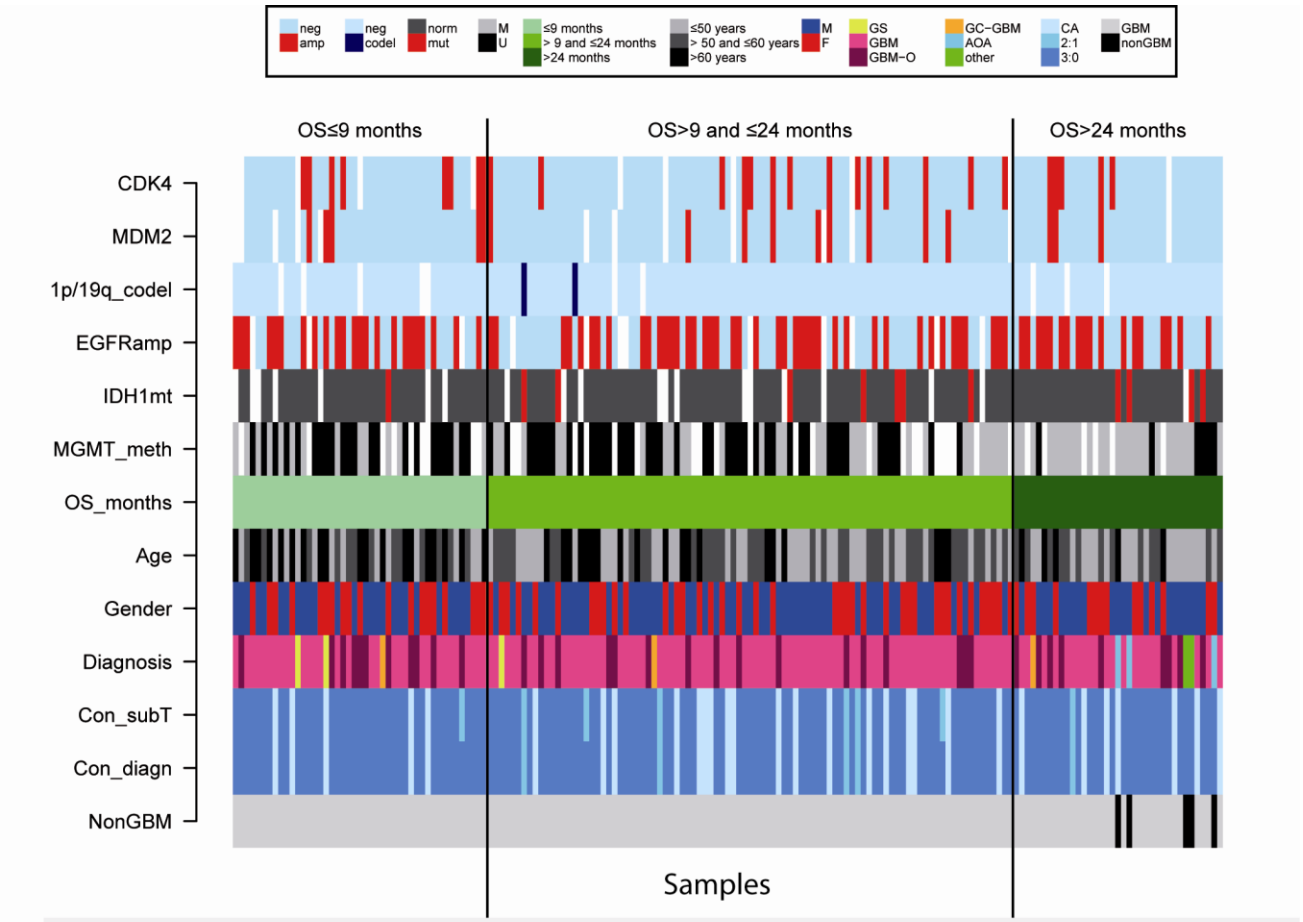
Table III-4: Tumor genetics of GBM-O versus GBM.

Overall cases in central review	Confirmed GBM (%)	GBM-O (%)	GBM (%)	P value [a]
Alterations				
meth*MGMT*	76/165 (46)	16/34 (47)	60/131 (46)	1.00
IDH1 mut	9/130 (7)	**6/32 (19)**	3/98 (3)	**0.007**
Co-del 1p/19q	2/137 (1)	1/31 (3)	1/106 (1)	0.4
EGFR amp	70/131 (53)	**22/31 (71)**	48/100 (48)	**0.038**
CDK4 amp	24/131 (18)	7/29 (24)	17/102 (17)	0.42
MDM2 amp	13/131 (10)	4/30 (13)	9/101 (9)	0.49

Note: Statistically significant values are given in bold. [a] Fisher exact test

Next, we investigated if GBM-O exhibit a particular pathogenetic makeup. Mutations of the IDH1 gene that are associated with better outcome in GBM [40] were significantly enriched in GBM-O (6/32, 19%) as compared to the remaining GBM (3/98, 3%; p=0.002). Similarly, EGFR amplification that has been associated with older age and potentially worse outcome was present in 71% of GBM-O (22/31) and 48% of the remaining GBM (48/100) (p=0.03). IDH1 and EGFR alterations were mutually exclusive as reported before [40]. Intriguingly, of 31 GBMO for which this genetic information was available, 6 carried the IDH1R132H mutation, 22 displayed an EGFR amplification, and only three had neither alteration. The presence of an IDH1 or IDH2 hot-spot mutation other than IDH1R132H was excluded by direct sequencing in these three cases. The notion that the GBM-O phenotype identifies at least two pathogenetically distinct subgroups is further supported by classification according to the four gene expression-based subtypes proposed by Verhaak et al. [36]. Of 14 evaluable GBM-O, 5 grouped with the proneural, 6 with the classical, 2 with the mesenchymal and 1 with the neural GBM subtypes. In accordance with the reported mutation pattern of the four subgroups, all GBMs with an IDH1 mutation were in the proneural group, while most EGFR-amplified and EGFRvIII-positive GBMs were in the classical subgroup (Figure III.4).

Figure III.2: Patterns of genetic alterations, diagnosis and outcome.



All Diagnosable Tumors in Central Review LN (n=354; including non-GBM)			
GBM-O (52/354, 15%)	14/101 (14%)	29/189 (15%)	9/64 (14%)
nonGBM (15/354, 4%)	1/101 (1%)	5/189 (3%)	9/64 (14%)
O-comp (94/345, 27%)	20/101 (20%)	53/185 (29%)	21/59 (36%)
PPN (215/349, 62%)	60/102 (59%)	115/208 (55%)	39/62 (63%)
Genetics, all Info Available (including non-GBM)			
IDH1 mt (12/160, 8%)	1/38 (3%)	7/84 (8%)	4/38 (3 nonGBM)
Codel 1p/19q (2/168, 1%)	0/39	2/94 (2%)	0/94
EGFR amp (83/166, 50%)	22/40 (55%)	43/89 (48%)	18/37 (49%)
*MGMT*_m (103/226, 46%)	20/50 (40%)	47/129 (36%)	36/47 (77%)
CDK4 amp (27/164, 16%)	6/38 (16%)	16/90 (18%)	5/36 (14%)
MDM2 amp (17/162, 11%)	3/38 (8%)	11/88 (13%)	3/26 (8%)
EGFRvIII (18/56, 30%)	7/18 (39%)	6/30 (17%)	5/13 (38%)

Patient data for 175 cases with 3/6 genetic tests available were ordered according to overall survival (OS). Many features are rare, such as GBM subtypes, or genetic alterations like IDH1 mutations. The visualization allows identification of patterns of genetic or clinical features that are enriched in either the short survival group or the long term survival group. Gene amplification is represented in *red* (CDK4,MDM2, EGFR) and deletions in *dark blue* (co-deletion of 1p/19q). Mutation of IDH1 is represented in *red*, MGMT methylated in *gray* and unmethylated in *black*. OS in months: *light green* short survival group (≤9 months); *green* intermediate survival group (>9 and <24 months); *dark green* long-term survival group (≥24 months). Age<50 years is represented in *gray*, 50–60 years in *dark gray*, and >60 years in *black*. *Red* female, *blue* male. *Pink* diagnosis as GBM; *purple* GBM-O; *yellow* Gliosarcoma; *orange* giant cell GBM (GC-GBM); *blue* AOA; *green* other non-GBM diagnosis. Concordance of reviewers 3:0 for subtype (Con_subT) or diagnosis (Con_diagn) in *dark blue*; *blue* concordance 2:1; *light blue* diagnosis by Canadian central review. Diagnosis of non-GBM is indicated in *black*. *White* No information for all criteria. The associated table below shows the respective numbers. *EGFRvIII* information was available for only 56 cases and is not included in the *upper panel*

Survival of patients with GBM-O is not different from those with GBM.

Patients with non-GBM pathology (15/354, 4%) were enriched in the patient group with overall survival (OS) exceeding 24 months (9/64, 14%), as compared to the short survival group (≤9 months, 1/101), and the intermediate group (5/189, 3%) ($p<0.001$, Chi-square-test) (Figure III.2). Subsequently, only patients with confirmed GBM (N = 339) are included for further analysis of morphologic features and outcome. There was no difference in OS between GBM and GBM-O (logrank test, p=0.48). Stratification by age (≤50, 51–60 or >60) (p = 0.55) or MGMT methylation status (p=0.27) did not differentiate survival in the two subgroups. When analyzing the GBM-O separately per randomized treatment arm, survival was not different for GBM-O in either arm (TMZ/RT → TMZ arm, p =0.81; RT-only arm, p=0.14) (Figure III.5). The respective values for progression-free survival were similar (p=0.97, TMZ/RT → TMZ; p=0.2, RT). Likewise, using less strict criteria, just the presence of any oligodendroglioma-like component did not show any association with outcome in either of the two treatment arms (Table III-3). The apparent enrichment of patients with the presence of any oligodendroglioma-like component in the long survivor group as visualized in Figure III.4 was due to inclusion of patients where GBM was not confirmed.

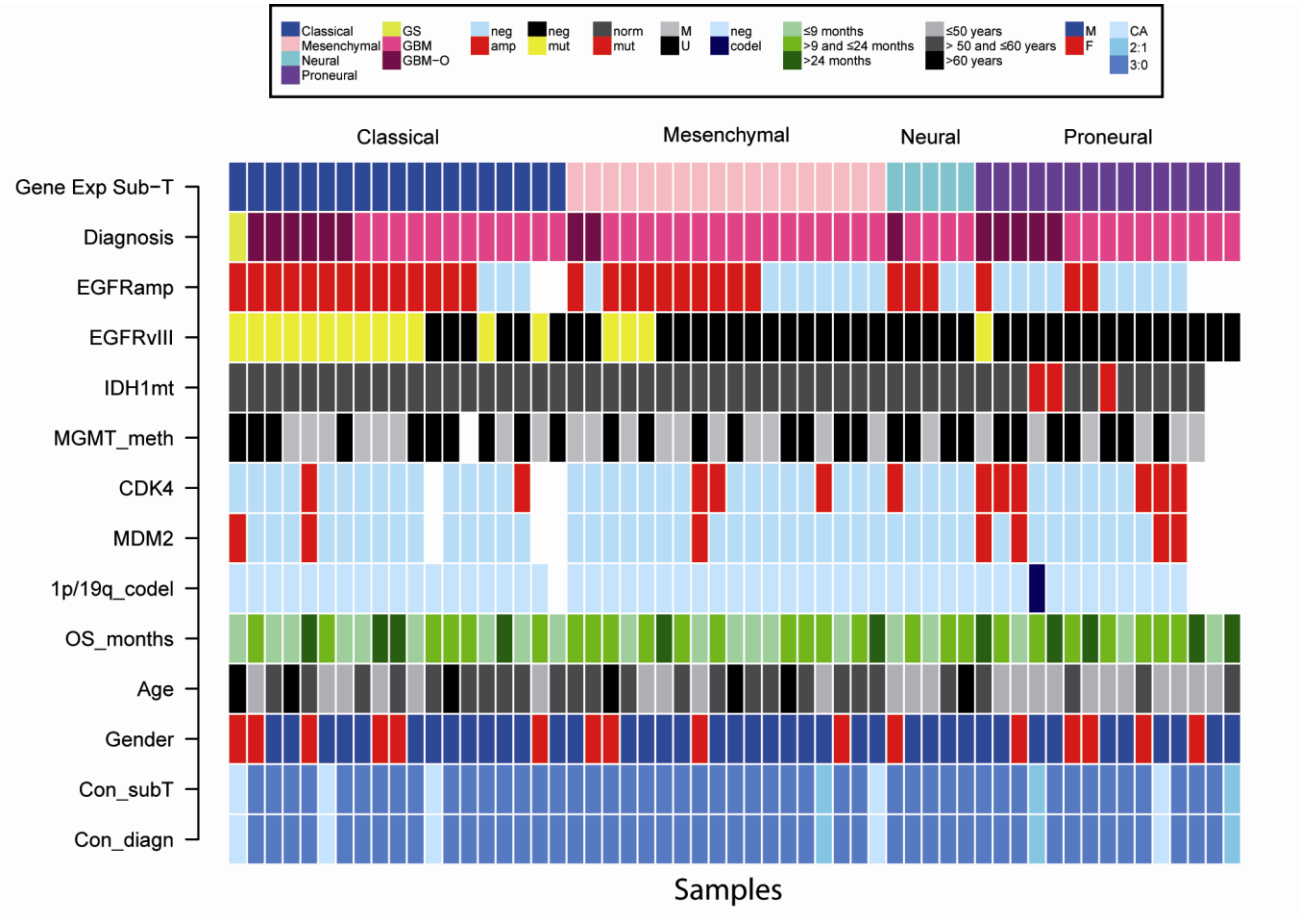
Associations of histopathological features with tumor genetics.

The MIB-1 labeling index was significantly higher in MGMT methylated GBM with a mean index of 38% (N = 69) as compared to 30% in MGMT unmethylated tumors (N = 84) (p=0.0015). A trend for a higher MIB-1 labeling index was associated with IDH1 mutations and EGFR amplifications (p=0.07, p=0.09). No significant association was observed between any morphologic feature and the MGMT methylation status.

Associations of tumor genetics and outcome

None of the genetic alterations investigated here was associated with a prognostic or a predictive value with the exception of MGMT methylation as previously reported (Table III-4, Supplementary Table S5)[32,38]. Mutations of IDH1 were rare in confirmed GBM (9/130; 7%, for which this information was available) as expected [25] and similarly distributed between the treatment arms (5, RT; 4, RT&TMZ), with five of eight assessable cases being MGMT methylated. These small numbers do not allow appropriate assessment of the prognostic value of IDH1 mutations (p=0.7, Figure III.6, Supplementary Fig. S2). The patterns of genetic alterations and outcome are displayed in Figure III.3.

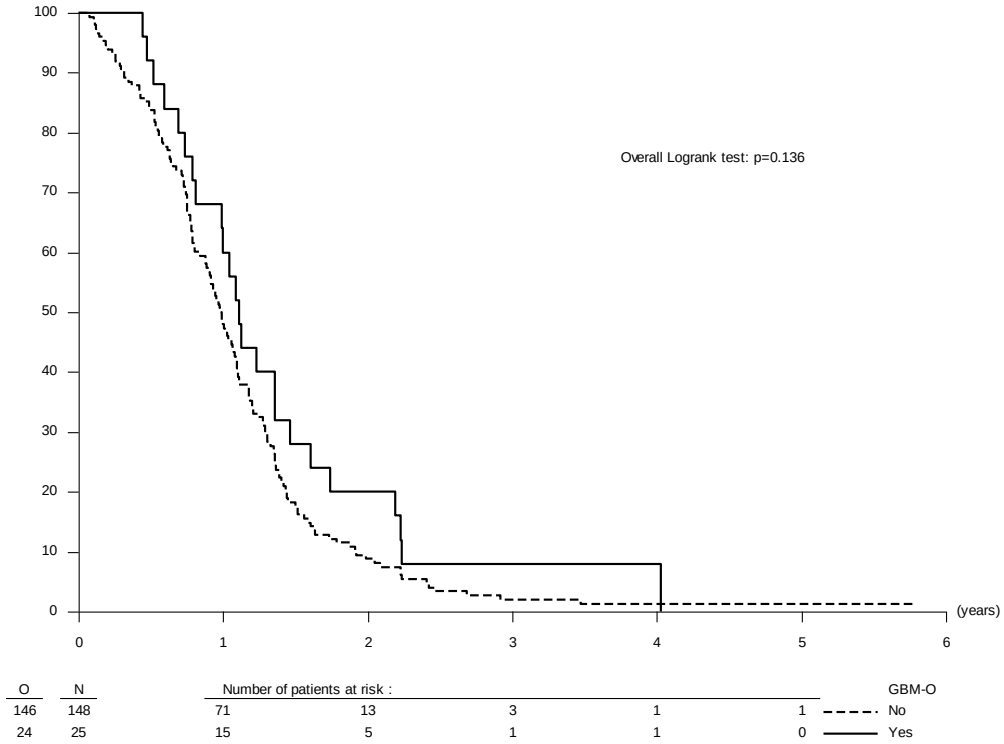
Figure III.3: Gene expression-based classification and GBM subtype.



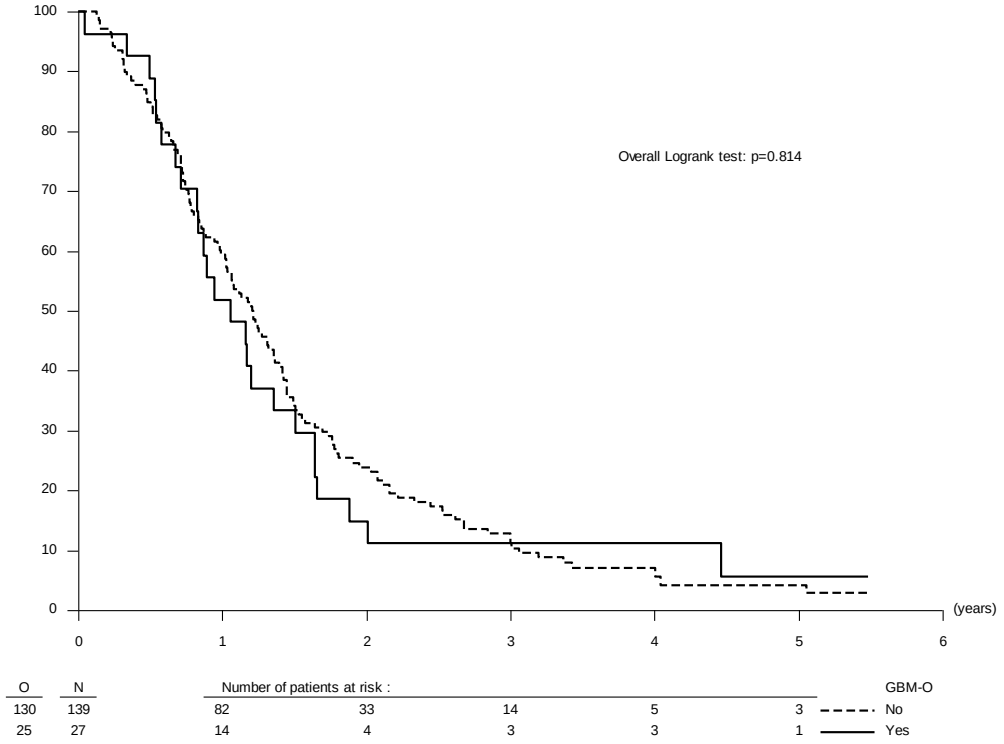
57 patients gene expression data, including for EGFRvIII, was available from frozen tumor tissue [21]. The tumor samples were classified according to the algorithm proposed by Verhaak et al. [36] into classic, mesenchymal, neural and proneural GBM. The samples are ordered by the gene expression-based classification, followed by diagnostic subtype, gliosarcoma (GS), GBM-O and GBM. The respective pathogenetic information and clinical information is the same as in Figure III.3. The enrichment of specific pathogenetic alterations, such as IDH1 mutations in the proneural and EGFR amplification and EGFRvIII expression in the classical subtype, is in accordance with the report by Verhaak et al. [36] . Gene amplification is represented in red (CDK4, MDM2, EGFR) and deletions in dark blue (co-deletion of 1p/19q). EGFRvIII expression determined by qRT-PCR is depicted in yellow. Mutation of IDH1 is represented red, MGMT methylated in gray and unmethylated in black. OS in months: light green short survival group (≤9 months); green intermediate survival group (>9 and <24 months); dark green long-term survival group (≥24 months). Age<50 is represented in gray, 50–60 in dark gray, and >60 years in black. Red female, blue male. Pink diagnosis as GBM; purple GBM-O; yellow gliosarcoma (GS). Concordance of reviewers 3:0 for subtype (Con_subT) or diagnosis (Con_diagn) in dark blue; concordance 2:1 in blue; diagnosis by Canadian central review in light blue. White no information for all criteria.

Figure III.4: Prognostic value of GBM-O for Overall Survival in RT and TMZ/RT→TMZ.

a. in RT



b. in TMZ/RT→TMZ



Note: GBM-O did not have better prognosis than all other GBMs. Kaplan–Meier curves show the OS of GBM versus GBM-O in the RT arm (log-rank test p = 0.136) (a) and the TMZ/RT → TMZ arm (p = 0.814) (b)

Table III-5: Molecular features of confirmed GBM and OS.

Subsample Characteristics	**Treatment RT (N=229) N (%)**	**TMZ/RT (N=221) N (%)**	**Total (N=450) N (%)**	P-value Fisher	Prognostic value for Overal Survival P-value Pooled	Prognostic value for Overal Survival P-value RT	Prognostic value for Overal Survival P-value TMZ/RT
MGMT							
Unmethylated	55 (24.0)	63 (28.5)	118 (26.2)	0.59	<0.0001	0.0003	0.002
Methylated	50 (21.8)	46 (20.8)	96 (21.3)				
Missing	124 (54.1)	112 (50.7)	236 (52.4)				
IDH1							
Not mutated	68 (29.7)	77 (34.8)	145 (32.2)	0.55	0.70	0.997	0.50
Mutated	5 (2.2)	4 (1.8)	9 (2.0)				
Missing	156 (68.1)	140 (63.3)	296 (65.8)				
1p/19q							
No loss	79 (34.5)	80 (36.2)	159 (35.3)	0.50	0.16	0.22	N/A
Loss	2 (0.9)	0 (0.0)	2 (0.4)				
Missing	148 (64.6)	141 (63.8)	289 (64.2)				
EGFR							
Normal	36 (15.7)	41 (18.6)	77 (17.1)	0.88	0.91	0.69	0.92
Amplified	41 (17.9)	41 (18.6)	82 (18.2)				
Missing	152 (66.4)	139 (62.9)	291 (64.7)				
CDK4							
Normal	63 (27.5)	68 (30.8)	131 (29.1)	0.30	0.83	0.09	0.06
Amplified	15 (6.6)	11 (5.0)	26 (5.8)				
Missing	151 (65.9)	142 (64.3)	293 (65.1)				
MDM2							
Normal	66 (28.8)	73 (33.0)	139 (30.9)	0.08	0.73	0.35	0.20
Amplified	11 (4.8)	5 (2.3)	16 (3.6)				
Missing	152 (66.4)	143 (64.7)	295 (65.6)				

Presence of pseudopalisading necroses (PPN) is associated with a treatment effect of TMZ.

Correlation of the distinct morphologic features assessed, such as type of necrosis, vascular pattern and cell differentiation, and including the MIB-1 (Ki67) labeling index (Figure III.1, Supplementary Fig. S1), identified PPN as the only morphologic feature associated with outcome (Table III-3). PPN was present in 63% of all GBM (212/339) and associated with a treatment effect (Figure III.7; Table III-3). Addition of TMZ to RT was beneficial in the patient cohort exhibiting PPN ($p = 0.0002$), while no such effect was present in the absence of PPN ($p = 0.86$; Figure III.7a). Peto's interaction test was significant ($p = 0.026$; Figure III.7b) and borderline nonsignificant in a Cox interaction model adjusted for known clinical prognostic factors ($p = 0.087$, Table III-5, Supplemental Table S3) not accounting for MGMT that was available only for a subset of 165 mostly resected tumors (Table III-1,Supplementary Table S1). This suggests that indeed PPN may identify a subgroup of chemo-sensitive GBM. The incidence of PPN was lower in patients with biopsy only (46 vs. 65.4%, $p = 0.01$), while no association with age was observed ($p = 0.15$). To exclude a bias of potential underestimation of PPN in stereotactic biopsies resulting from the small sample size, and the fact that biopsy only by itself is an unfavorable prognostic factor, the analyses were repeated in patients who underwent a tumor resection. Peto's test was significant ($p = 0.040$, Supplementary Fig. S3) and the adjusted Cox interaction model including MGMT was borderline nonsignificant ($p = 0.063$, Table III-5, Supplementary Table S4). A similar treatment effect of PPN was observed for PFS ($p<0.0001$) in the TMZ arm, while there was a trend in the RT arm ($p = 0.078$).

Figure III.5: Prognostic value of IDH1 for OS.

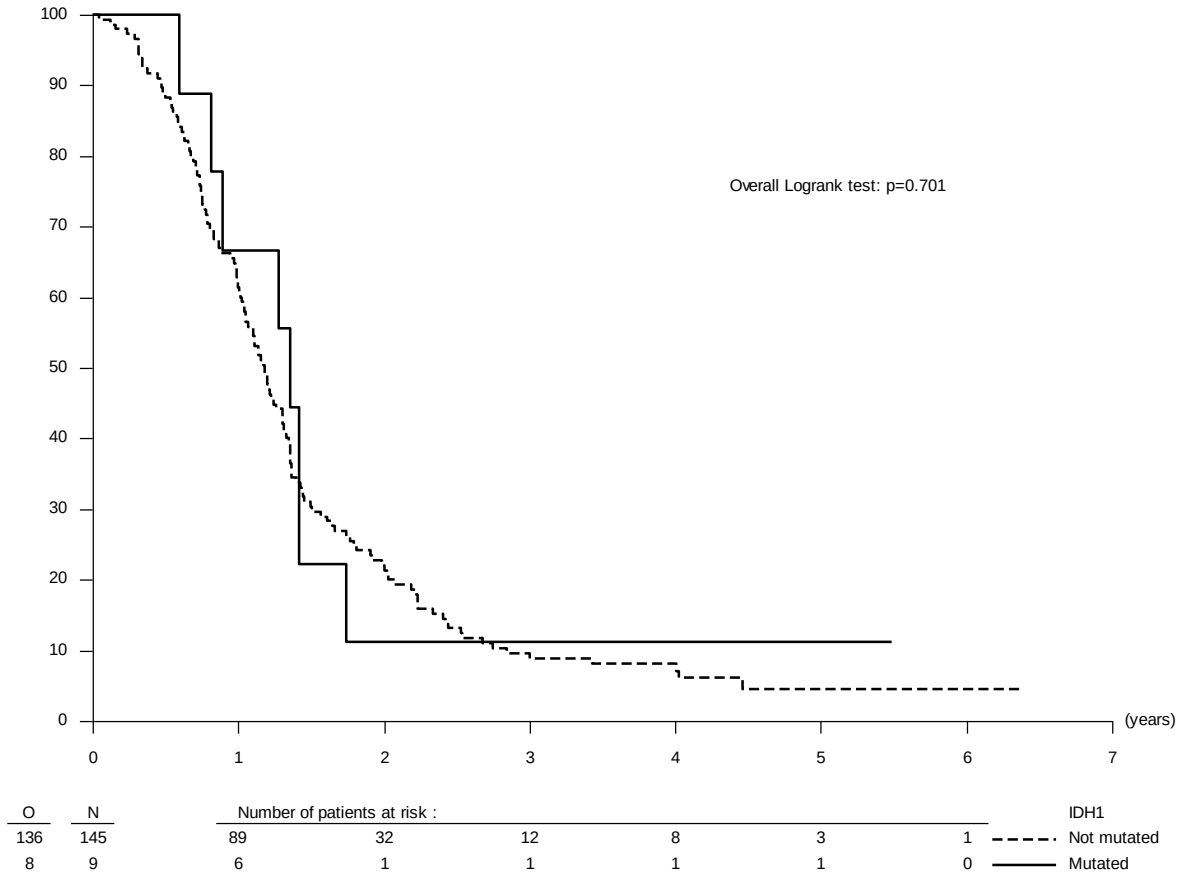


Table III-6: Predictive value of PPN for TMZ/RT effect on OS – all confirmed GBM.

Summary of the Number of Event and Censored Values			
Total	**Event**	**Censored**	**Percent Censored**
321	307	14	4.36

Parameter	**DF**	**Parameter Estimate**	**Standard Error**	**Chi-Square**	**p**	**Hazard Ratio**
Treatment	1	0.16014	0.18903	0.7177	0.3969	.
PPN	1	-0.33989	0.17527	3.7606	0.0525	.
Treatment*PPN	1	0.41465	0.24238	2.9267	**0.0871**	.
Age	1	0.19407	0.09932	3.8182	0.0507	1.214
Extent of surgery	1	0.37024	0.08471	19.1035	<.0001	1.448
MMSE	1	0.24354	0.13187	3.4107	0.0648	1.276
WHO PS	1	0.24945	0.08816	8.0068	0.0047	1.283

Note: in all confirmed GBM. Cox interaction model with adjustment excluding MGMT status

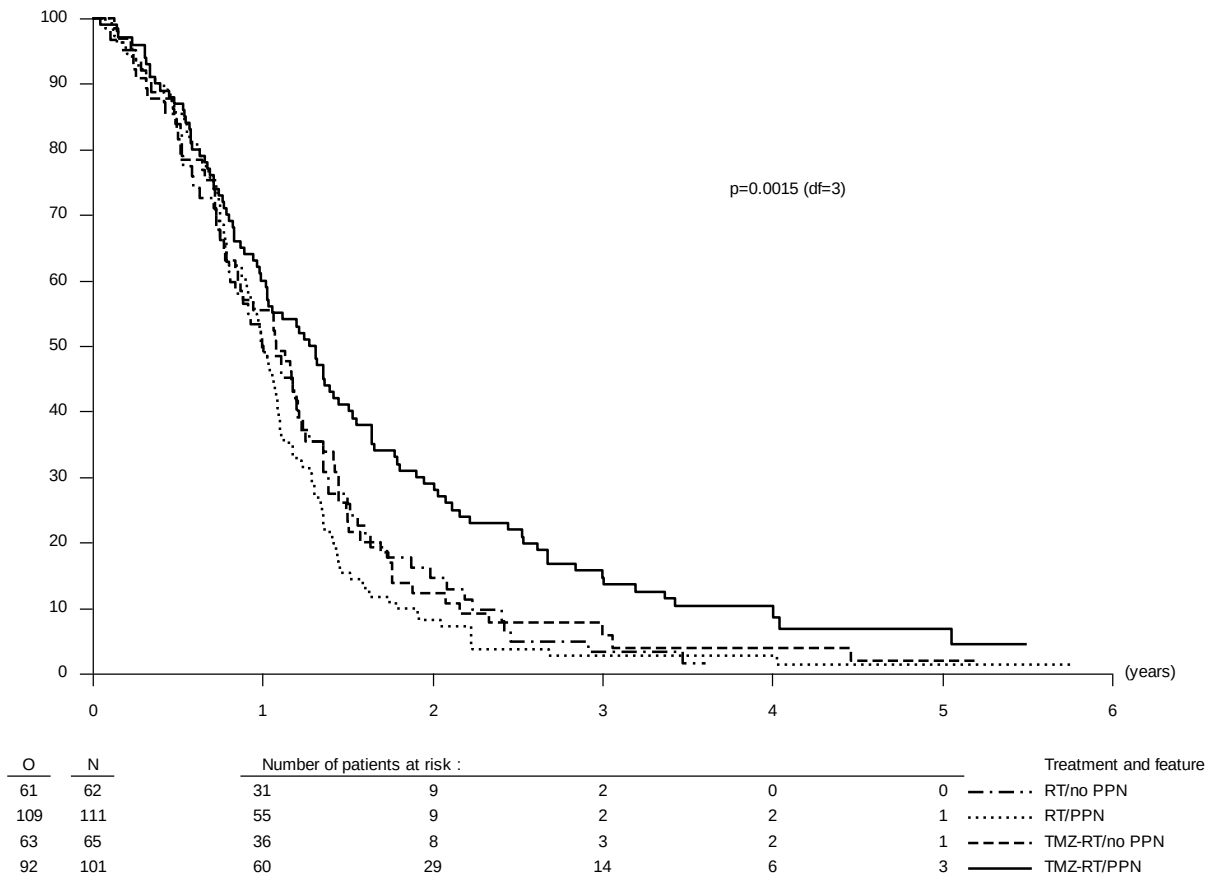
Table III-7: Predictive value of PPN for TMZ/RT effect on OS – resected GBM.

Summary of the Number of Event and Censored Values			
Total	**Event**	**Censored**	**Percent Censored**
153	144	9	5.88

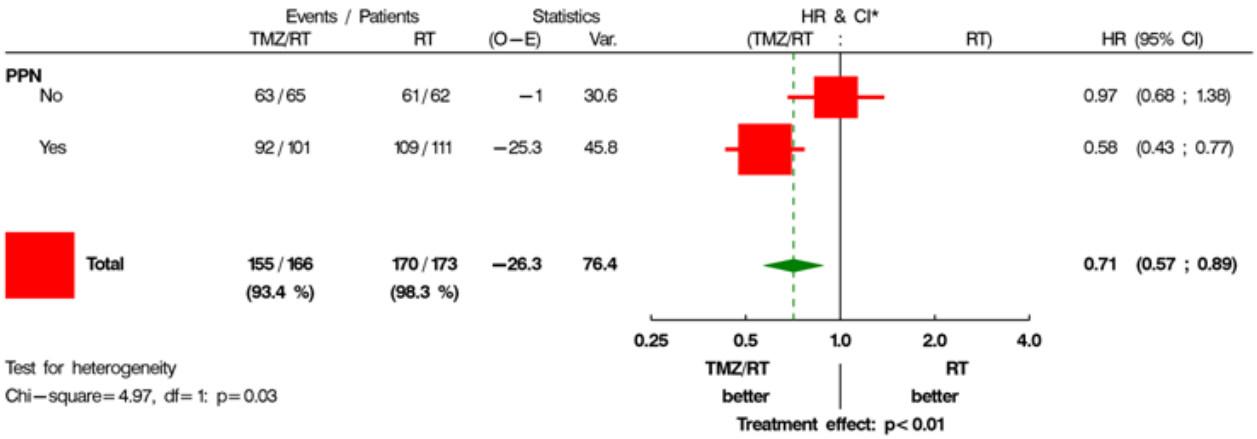
Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	p	Hazard Ratio
Treatment	1	-0.12946	0.33802	0.1467	0.7017	.
PPN	1	-0.38704	0.27127	2.0357	0.1536	.
Treatment*PPN	1	0.72863	0.39195	3.4559	**0.0630**	**.**
Age	1	0.20497	0.16359	1.5699	0.2102	1.227
Extent of surgery	1	0.16113	0.17634	0.8349	0.3609	1.175
MMSE	1	0.35953	0.20542	3.0631	0.0801	1.433
WHO PS	1	0.24902	0.14123	3.1090	0.0779	1.283
MGMT	1	-0.82561	0.18569	19.7682	<.0001	0.438

Figure III.6: Predictive value of PPN for TMZ/RT Effect on Overall Survival – all confirmed GBM

a Kaplan Meier Curve



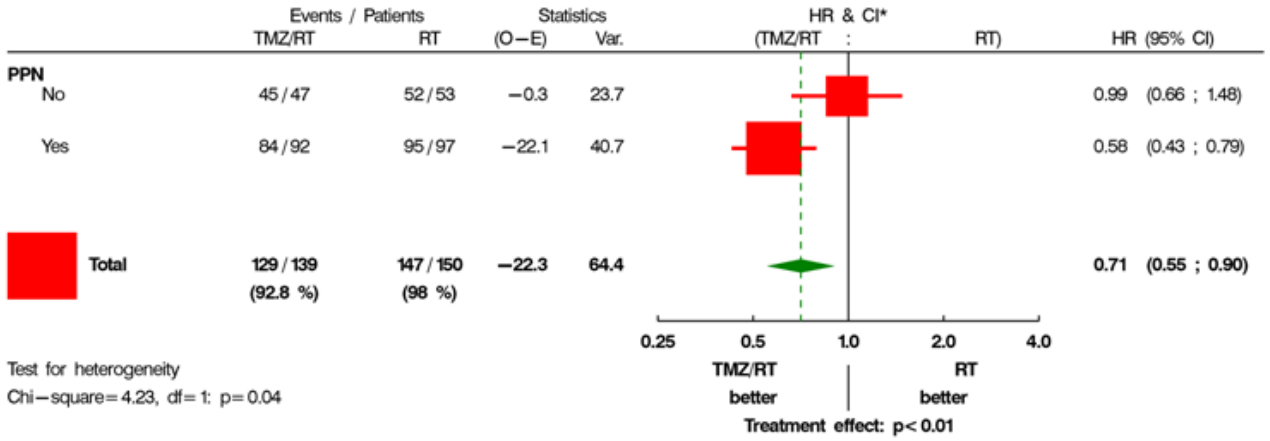
b Forest Plot



*95% CI everywhere

Note:The presence of Pseudopalisading necrosis is associated with a treatment effect. a The Kaplan–Meier curves visualize the overall outcome of the patients in the presence or absence of pseudo-palisading necrosis (PPN). In the presence of PPN, there is a treatment effect (RT vs. TMZ/RT → TMZ p = 0.002), while in the absence of PPN, no such difference is observed (RT vs. TMZ/RT → TMZ, p = 0.86). b Forest Plot and Peto's test of interaction between PPN and treatment for OS in all confirmed GBMs. Peto's test was significant (p = 0.03) indicating that reatment effects differ significantly as a function of PPN.

Figure III.7: Predictive value of PPN for TMZ/RT Effect on Overall Survival – resected GBM



*95% CI everywhere

3.5 Discussion

The present study was performed to assess prognostic significance of morphological features of GBM in the registration trial for temozolomide, with a focus on GBMO. Classification of GBM was in high concordance (>95%) between the 59 centers and central review. Expectedly, reclassification as a non-GBM histology was significantly enriched among long-term survivors (Figure III.3). The trial analyses and respective reports were on an intention-to-treat basis [32], and hence include patients with non-GBM histology.

Identification of unambiguous morphologic features with a prognostic or predictive value within GBM would be clinically valuable, as such markers could be easily implemented in routine histopathologic diagnostics. The recognized phenotypical GBM variants, giant cell GBM and gliosarcoma are rare (6,<2% and 3,<1% in this study)[15], precluding reliable assessment of a potential prognostic significance when patients are treated with the current standard of care. Evaluation of the prognostic value of an oligodendroglioma-like component in an otherwise classic GBM revealed no association with a more favorable disease course in either of the two treatment arms, in contrast to previous studies on GBM-O [9, 12, 16, 18, 29]. This discrepancy might be explained by the fact that most studies were performed in the pre-TMZ chemotherapy era. GBM-O, as defined in this report, seems to benefit similarly from chemoradiotherapy, in line with the identical MGMT methylation frequency compared to other GBMs that differ from frequencies reported for AO and AOA of over 70% [5, 20, 27, 39]. Further, the delineation of ‘‘pure’’ GBM versus GBM-O, and AOA and AO is difficult, as reflected in variable frequencies of reported 1p/19q codeletions in these studies ranging from 0 to over 20% for the GBM-O subgroup [9, 12, 13, 16, 18, 29]. This study uncovered that GBM-O encompasses at least two distinct pathogenetic subgroups, characterized either by EGFR amplifications or IDH1 mutations, and further supported by respective expression-based classification (Figure III.3). GBM-O, as defined here, may in part overlap with the small cell variant of GBM with high cellularity, diffuse more or less monotonous growth and relatively small, partly rounded nuclei that is known for increased EGFR amplification frequencies [12,18]. Conversely, GBM with IDH mutations are now recognized as a distinct subtype with a different pathogenetic/epigenetic origin, evolving from lower grade glioma with high frequencies of IDH mutations, characteristic of secondary GBM [1,23,40]. Interestingly, IDH mutant gliomas are associated with a DNA hypermethylation phenotype [24]. This association has recently also been reported in leukemia, identifying a new prognostic subtype, and mechanistically linking aberrant metabolism (onco-metabolite) with epigenetic deregulation [6,26]. Our finding that recognition of an oligodendroglioma-like phenotype in otherwise classic GBM associates two completely different genetic/epigenetic GBM subtypes is a surprise and questions the clinical utility of morphologic identification of GBM-O. The introduction in the 2007 WHO classification of high-grade malignant oligoastrocytic tumors with necrosis as GBM-O [14] has led to substantial controversy among pathologists [30] and will certainly have to be re-visited given the recently discovered distinct pathogenetic/epigenetic evolution. Determination of oncogenetic events such as IDH status and 1p/19q co-deletions provide a more promising tool for robust and reproducible (sub) classification of malignant gliomas [8]. Evaluation of distinct morphologic features in this homogenously treated patient population identified PPN as potentially associated with benefit from combined chemoradiotherapy. Presence of PPN may reflect the tumor milieu including the tumor vascularization type, which may have an effect on drug perfusion and thereby on response to chemotherapy. Pseudopalisades are enriched for hypoxic and apoptotic tumor cells, with a lower relative proliferation index, and are frequently associated with a central degenerating or thrombosed vascular lumen [2, 28]. Tumor-associated vascular injury has been associated with factors released from glioma cells after genetic alterations such as EGFR amplifications or cellular stress conditions such as hypoxia [3, 28]. Based on a comprehensive analysis of PPN in human GBM and experimental models, it has been hypothesized that pseudopalisades comprise hypoxic tumor cells migrating away from dysfunctional vessels [2, 28]. However, the presence of PPN does not directly correlate with hypoxia as suggested by gene expression profiles available for 50 patients of this cohort [21, 22]. No

correlation was observed with the previously identified hypoxia-induced gene expression signature, while the EGFR expression signature (G25) was significantly associated with the presence of PPN ($p = 0.02$). Evaluation of associations of PPN with previously identified expression signatures in appropriately powered studies may indicate underlying molecular mechanisms that merit further analysis for improvement of therapy. The respective hypotheses may be tested in the database of 'The Cancer Genome Atlas' (TCGA) once the morphologic information is publicly available [36]. In contrast to our study, Homma et al. [12] reported an association of the presence of any type of necrosis with worse outcome. This discrepancy may be explained by the fact that all these patients were treated before the TMZ era (before 1994) and likely received RT alone. This study has shown that systematic combined morphologic and molecular characterization of tumor samples of patients enrolled in clinical trials is instrumental for validating and identifying new prognostic and predictive factors that will have an impact on clinical practice. This was an exploratory study requiring validation in an independent data set of a homogenous patient population treated with combined chemo-radiotherapy. The limited numbers of samples available for molecular analyses unfortunately reduced the power of the study, once more emphasizing the importance of collecting sufficient tissues for all patients enrolled in clinical trials.

3.6 References

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Chapter IV. **Kouwenhoven MC, Gorlia T, Kros JM, Ibdaih A, Brandes AA, Bromberg JE, Mokhtari K, van Duinen SG, Teepen JL, Wesseling P, Vandenbos F, Grisold W, Sipos L, Mirimanoff R, Vecht CJ, Allgeier A, Lacombe D, van den Bent MJ. Molecular analysis of anaplastic oligodendroglial tumors in a prospective randomized study: A report from EORTC study 26951. Neuro Oncol. 2009 Dec;11(6):737-46. doi: 10.1215/15228517-2009-011.**

4.1 Abstract

Recent studies have shown that the clinical outcome of anaplastic oligodendroglial tumors is variable, but also that the histological diagnosis is subject to interobserver variation. We investigated whether the assessment of 1p/19q codeletion, polysomy of chromosome 7, epidermal growth factor receptor (EGFR) gene amplification ($EGFR^{amp}$), and loss of chromosome 10 or 10q offers additional prognostic information to the histological diagnosis and would allow molecular subtyping. For this study, we used the clinical data and tumor samples of the patients included in multicenter prospective phase III European Organisation for Research and Treatment of Cancer (EORTC) study 26951 on the effects of adjuvant procarbazine, chloroethyl cyclohexylnitrosourea (lomustine), and vincristine chemotherapy in anaplastic oligodendroglial tumors. Fluorescence in situ hybridization was used to assess copy number aberrations of chromosome 1p, 19q, 7, 10, and 10q and *EGFR*. Three different analyses were performed: on all included patients based on local pathology diagnosis, on the patients with confirmed anaplastic oligodendroglial tumors on central pathology review, and on this latter group but after excluding anaplastic oligoastrocytoma (AOA) with necrosis. As a reference set for glioblastoma multiforme (GBM), patients from the prospective randomized phase III study on GBM (EORTC 26981) were used as a benchmark. In 257 of 368 patients, central pathology review confirmed the presence of an anaplastic oligodendroglial tumor. Tumors with combined 1p and 19q loss ($1p^{loss}19q^{loss}$) were histopathologically diagnosed as anaplastic oligodendroglioma, were more frequently located in the frontal lobe, and had a better outcome. Anaplastic oligodendroglial tumors with $EGFR^{amp}$ were more frequently AOA, were more often localized outside the frontal lobe, and had a survival similar to that for GBM. Survival of patients with AOA harboring necrosis was in a similar range as for GBM, while patients with AOA with only endothelial proliferation had better overall survival. In univariate analyses, all molecular factors except loss of 10q were of prognostic significance, but on multivariate analysis a histopathological diagnosis of AOA, necrosis, and $1p^{loss}19q^{loss}$ remained independent prognostic factors. AOA tumors with necrosis are to be considered WHO grade IV tumors (GBM). Of all molecular markers analyzed in this study, especially loss of 1p/19q carried prognostic significance, while the others contributed little prognostic value to classical histology.

4.2 Introduction

Over the last 15 years, oligodendroglial tumors have been recognized as treatment-sensitive tumors with a favorable survival.[1,2] Molecular studies have shown that this is of particular concern in the subgroup with an unbalanced translocation of 19p to 1q, der(1;19)(p10;q10), resulting in the 1p/19q codeletion. [3–7] The favorable outcome even after radiotherapy (RT) only has recently been further confirmed by two randomized prospective studies on (neo)adjuvant procarbazine, chloroethyl cyclohexylnitrosourea (CCNU; lomustine), and vincristine (PCV) chemotherapy in anaplastic oligodendroglial tumors, which showed a more favorable survival in tumors with combined $1p^{loss}19q^{loss}$.[8,9] However, not all tumors with an oligodendroglial phenotype have such favorable outcome: up to 20% of patients died within 1 year after diagnosis, an outcome that is more consistent with glioblastoma multiforme (GBM). European Organisation for Research and Treatment of Cancer (EORTC) study 26951 investigated the benefit of six cycles of adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors.[8] We used this study to

investigate the correlation between clinical outcome and specific histological and molecular features. We were particularly interested to learn whether necrosis and endothelial proliferation have similar prognostic significance in mixed anaplastic oligoastrocytoma (AOA) and pure anaplastic oligodendroglioma (AOD), and whether the assessment of specific molecular aberrations usually associated with GBM (epidermal growth factor receptor [EGFR] gene amplification, loss of chromosome 10 or of 10q) contributes to histological diagnosis and clinical prognosis. While this research was ongoing, in 2007 a revised WHO classification for glioma was published.[10] Because this WHO 2007 classification of brain tumors classifies AOA with necrosis (previously considered grade III) as grade IV GBM, in a further analysis of prognostically important factors AOA with necrosis as diagnosed by the central review pathologist were left out. The first level of this analysis considered only the factors directly related to the analysis of the tissue samples; in the subsequent level of analysis, clinical information was introduced to explore factors with independent prognostic significance.

4.3 Materials and Methods

Patients were eligible for EORTC study 26951 if they had been diagnosed by the local pathologist with AOD or AOA with at least 25% oligodendroglial elements according to the 1994 edition of the WHO classification of brain tumors,11 had at least three of five anaplastic characteristics (high cellularity, mitoses, nuclear abnormalities, endothelial proliferation, and necrosis), were between 16 and 70 years of age, had an Eastern Cooperative Oncology Group performance status (PS) of 0 to 2, and had not undergone prior chemotherapy or RT to the skull. The clinical details of these studies have been published elsewhere.[12] Since no statistically significant differences in overall survival were observed between the patients assigned to RT and those assigned to RT followed by six cycles of adjuvant PCV chemotherapy, the patients in both arms were studied together. After inclusion and randomization, central pathology review took place (J.M.K.). Patients were then regrouped in three data sets: (1) all patients as diagnosed by the local pathologist using the local diagnosis of both histology and anaplastic features ("local diagnosis"), (2) centrally confirmed AOD and AOA according to the WHO 1994 classification ("WHO 1994"), and (3) all centrally confirmed AOD and only the centrally confirmed AOA without necrosis ("WHO 2007"). In groups 2 and 3, the central review diagnosis of anaplastic features was used. For comparison, a group of patients with GBM obtained from EORTC 26981 was used.[13]

Fluorescence In Situ Hybridization

Probes to 1p36 (D1S32), centromere 1 (pUC1.77), 19p (equivalent amounts of bacterial artificial chromosome [BAC] RPCI 11-959O6, 11-957I1, and 11-153P24), 19q (BAC 426G3), PTEN (PAC 190P6), CEP10 (CEP10; D10Z1), EGFR (BAC RPCI 11-148p17, a kind gift of Dr. A. Perry), centromere 7 (CEP7; P7t1), and CEP12 (CEP12; Pa12H8) were labeled with biotin-16-dUTP (pUC1.77; Roche Diagnostics, Mannheim, Germany), digoxigenin-16-dUTP (D1S32, 959O6, 957I1, 153P24, 190P6, 148p17; Roche Diagnostics), Spectrum Green (P7t1; Vysis Inc., Downers Grove, IL, USA), Spectrum Orange (D10Z1; Vysis Inc.), or Cy5 (Amersham Biosciences, Piscataway, NJ, USA). Tumor sections were deparaffinized, dehydrated, and microwave treated in citrate buffer (pH 6.0) and then digested in 0.4% pepsin solution (Sigma-Aldrich, St. Louis, MO, USA) in 0.9% NaCl (pH 1.5–2.0), as previously described.[14] Subsequently, slides were dehydrated, and probe solutions were applied. Tumor sections and probes were codenaturated on a slide moat preheated to 80°C for 5 min, and then cooled on ice and incubated at 37°C for 48 h in a moistened chamber. After incubation, slides were washed in 1.5 M urea saline–sodium citrate (SSC) at 45°C for 30 min and rinsed in 2xSSC. Probes were detected using antirhodamine-conjugated digoxigenin (426G3, 148p17; Roche Diagnostics, Mannheim, Germany), antidigoxigenin-conjugated with fluorescein isothiocyanate (D1S32, 959O6, 957I1, 153P24, 190P6; Roche Diagnostics), or Cy3-conjugated avidin (pUC1.77; Brunschwig Chemie, Amsterdam, the Netherlands) antibodies at a concentration of 4 and 15 μg/ml diluted in phosphate-buffered saline. Nuclei

were counterstained with diamidinophenylindole in antifade solution (Vector Laboratories, Burlingame, CA, USA). Locus-specific fluorescence in situ hybridization (FISH) probes were enumerated in 60 nonoverlapping nuclei per hybridization utilizing a Leica DM-RXA fluorescence microscope (Leica, Wetzlar, Germany). Images were captured using a COHU 4910 series monochrome CCD camera (Cohu Inc., San Diego, CA, USA) attached to the fluorescence microscope equipped with a PL Fluotar 3100, numerical aperture 1.30–0.60 objective, I3 and N2.1 filters (Leica), and Leica QFISH software (Leica Imaging Systems, Cambridge, UK). Ratios were calculated for 1p versus CEP1, 19q versus 19p, or 10q versus CEP10 (10qloss) by dividing the number of signals of the marker by the number of signals of the reference; a ratio of less than 0.80 was considered allelic loss. If a borderline ratio was obtained (0.75–0.90), spots in 200 nuclei were counted. For ratios of *EGFR* versus CEP7, CEP7 versus CEP12, or CEP10 versus CEP12, different cutoff levels were used. A ratio of *EGFR*/CEP7.2 was considered *EGFR* amplification (*EGFR*^{amp}), a ratio of CEP7/CEP12 > 1.1 displayed polysomy of chromosome 7 (7^{poly}), and a ratio < 0.85 for CEP10/CEP12 was indicative of monosomy 10 (10^{loss}). These cutoff values were determined in a set of nontumoral controls that displayed <10% nuclei with more than two signals for the investigated marker/nucleus.

Statistical Analysis

The prognostic significance of the tissue variables (diagnosis, anaplastic features, and molecular features) were first analyzed without taking any nontissue (clinical) factors into account. For the multivariate analysis, the following major prognostic clinical variables were used: WHO PS (0, 1, 2), age (,50, >50), type of surgery (biopsy or resection), and type of adjuvant treatment (none or PCV). For the histological factors, the diagnosis (AOD or AOA) and the five anaplastic features were used, diagnosed either by the local pathologist for the local diagnosis or by the central review pathologist for centrally confirmed tumors. For the molecular factors, 1p^{loss}, 19q^{loss}, 1p^{loss}19q^{loss}, *EGFR*^{amp}, 7^{poly}, 10^{loss}, and 10q^{loss} were used. Association between factors was assessed by the Spearman correlation coefficient (SCC); Fisher's exact test was used for inference. Survival analyses in the three populations were performed with the log-rank test and the Cox regression analysis stratified by the treatment, with and without backward selection. Internal validation was performed by bootstrap resampling technique (5% confidence) to assess the generalizability of the models. Factors with a probability of inclusion (PI) in regression models of less than 60% based on 1,000 bootstrap samples were considered not confirmed as independent prognostic factors. Patients with missing values in at least one factor were removed from the analyses. No formal adjustment for multiple testing was performed; nevertheless, a conservative significance level of 1% was considered for all comparisons.

4.4 Results

In this study, 368 patients were randomized; 265 had been diagnosed by the local pathologist with an AOD and 100 with an AOA (three missing). At central pathology review, in 257 patients the diagnosis of an anaplastic oligodendroglial tumor was confirmed (175 AOD, 82 AOA). Other frequent diagnoses at central review were low-grade tumors (39 patients) and high-grade astrocytic tumors (anaplastic astrocytoma or GBM, 39 patients); for 22 patients no material was received for review. Table IV-1 specifies the pathology findings, presence of necrosis, and endothelial proliferation in each of the three data sets.

Table IV-1: Main pathological findings.

Finding	Locally diagnosed AOD or AOA	Diagnosis at Central Pathology Review	WHO Definition 2007
N	368	346	202
Histology			
AOD	265	175	175
AOA	100	82	27
Missing	3	22	
HGA		39	
LGG		39	
Other		11	
Presence of necrosis			
AOD:total	148:265	119:175	119:175
AOA:total	51:100	55:82	0:27 (definition)
Endothelial proliferation			
AOD:total	211:265	166:175	166:175
AOA:total	69:100	80:82	25:27

Abbreviations: AOD, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; HGA, high-grade astrocytoma; LGG, low-grade glioma.

Molecular Alterations

Table IV-2 shows the distribution of molecular characteristics and Table IV-3 the correlations between the molecular characteristics. $1p^{loss}$ and $19q^{loss}$ were highly correlated with each other (SCC = 0.51). $EGFR^{amp}$ was correlated with 7^{poly} (SCC = 0.40) and with 10^{loss} (SCC = 0.48). $EGFR^{amp}$ and $1p^{loss}19q^{loss}$ were poorly anticorrelated (SCC = –0.24): of the 227 patients with both measures, 59 patients had $1p^{loss}19q^{loss}$, 50 had $EGFR^{amp}$, 3 had both, and 121 had neither (p = 0.0001).

Table IV-2: Presence or absence of chromosomal findings and numbers without test results.

	1p loss	19q loss	Combined 1p and 19q loss	EGFR Amplification	7 Polysomy	10q Loss	10 Loss
Absent	186 (51%)	198 (54%)	217 (59%)	182 (50%)	162 (44%)	211 (57%)	199 (54%)
Present	131 (36%)	98 (27%)	76 (21%)	51 (14%)	68 (19%)	37 (10%)	39 (11%)
Missing	51 (14%)	72 (20%)	75 (20%)	135 (37%)	138 (38%)	120 (37%)	130 (35%)

Table IV-3: Spearman correlation coefficients between the various molecular parameters.

	Spearman Correlation Coefficient (p Value) and Number of Patients				
Molecular Parameter	**1p and 19q loss**	***EGFR* Amplification**	**7 Polysomy**	**10q Loss**	**10 Loss**
1p and 19q loss	1	-0.24 (0.0002)	-0.10 (0.15)	-0.15 (0.02)	-0.19 (0.003)
	293	227	224	241	231
EGFR amplification		1	0.40 (<0.0001)	0.26 (0.0001)	0.48 (<0.0001)
		233	230	213	207
7 polysomy			1	0.07 (0.31)	0.26 (0.0001)
			230	211	206
10q loss				1	-0.18 (0.006)
				248	238
10 loss					1
					238

Necrosis but not endothelial proliferation discriminates a subgroup of AOA with similar survival profile as GBM.

Of the 82 AOA tumors, 55 (67%) showed necrosis. Endothelial proliferation was present in almost all AOA tumors (80 of 82) and in all 55 AOA tumors with necrosis. Table IV-4 shows the survival of patients with AOD and AOA with or without necrosis, and the reference group of GBM patients treated with RT only, and Figure IV.1 shows the survival curves of these patients in the RT arms of both studies. After correction for extent of resection, PS, and age, in the patients treated with RT alone survival for AOA with necrosis was in a similar range as the survival for GBM (hazard ratio [HR] = 1.53; 95% confidence interval [CI], 1.02–2.31; p = 0.042). Survival in the 25 patients with AOA showing endothelial proliferation but no necrosis was better than in patients with GBM (p = 0.007). The outcome of AOD patients without tumor necrosis was better than for those without tumor necrosis, but in the latter category survival was still much more favorable than for patients with GBM or with AOA without necrosis (Figure IV.1).

Table IV-4: Two-year OS rates in confirmed AOD and AOA in relation to the presence of necrosis.

Diagnosis	Median Survival [Range (months)]	Two-Year Survival Rate
AOA with necrosis	15.9 [12.7-18.0]	27.3 [16.4-39.4]
AOA without necrosis[a]	21.9 [16.0-33.5]	48.0 [27.8-65.6]
AOD with necrosis	34.7 [24.2-59.4]	59.32 [49.9-67.6]
AOD without necrosis	NR [NR-NR]	82.1 [69.4-90.0]
Glioblastoma EORTC 26981	12.1 [11.2-13.0]	10.4 [6.8-14.1]

Abbreviations: AOA, anaplastic astrocytoma; AOD, anaplastic oligodendroglioma; NR, not reached; EORTC, European Organisation for Research and Treatment of Cancer

[a]AOA with endothelial proliferation but no necrosis; two patients with AOA lacking both necrosis and endothelial proliferation have been left out.

Molecular alterations are distinct in subgroups of oligodendroglial tumors.

Table IV-5 shows the molecular findings in the centrally confirmed AOA without necrosis, AOD, and AOA with necrosis. Combined $1p^{loss}19q^{loss}$ was more frequent in AOD or AOA without necrosis; AOA with necrosis more often had $EGFR^{amp}$, 7^{poly}, and 10^{loss}. Despite the observed differences in frequencies, none of the items clearly separated both subgroups. Frontal tumors and previous resection for a low-grade tumor were more frequently observed in tumors with combined $1p^{loss}19q^{loss}$ (p = 0.0021 and p = 0.0087) and less frequent in tumors with $EGFR^{amp}$ (p = 0.0004 and p = 0.0045). No other clinical factor, including age, was related to any of the molecular factors (data not shown).

Table IV-5: Molecular findings in tumors with central review diagnosis of AOD and AOA.

Molecular Parameter	AOD	All AOA	AOA with Necrosis	AOA without Necrosis
1p and 19q loss	53/145 (37%)	5/69 (7%)	5/46 (11%)	0/23 (0%)
EGFR amplification	20/113 (18%)	25/59 (42%)	17/39 (44%)	8/20 (40%)
7 polysomy	28/110 (25%)	22/59 (37%)	18/39 (46%)	4/20 (25%)
10q loss	16/130 (12%)	14/56 (25%)	10/38 (26%)	4/18 (22%)
10 loss	17/124 (14%)	16/53 (30%)	12/35 (34%)	4/18 (22%)

Prognostic significance of tissue characteristics only (including molecular characteristics).

Table IV-6 shows the univariate analysis of all histological and molecular factors in all patients. All molecular factors except for loss of 10q were correlated with outcome ($p < 0.01$). Outcome for AOA with $EGFR^{amp}$ was similar to that for AOD with $EGFR^{amp}$ ($p = 0.354$). Except for combined $1p^{loss}19q^{loss}$, in all three data sets, multivariate analysis using tissue (molecular and histological) factors showed none of the other molecular factors to be of prognostic significance. The presence of necrosis was of significance, when assessed by the local pathologist or by the central reviewing pathologist. Furthermore, the central review histopathological diagnosis (AOD, AOA with or without necrosis) was of significance.

Table IV-6: Univariate survival analysis of all histological and molecular factors in all patients.

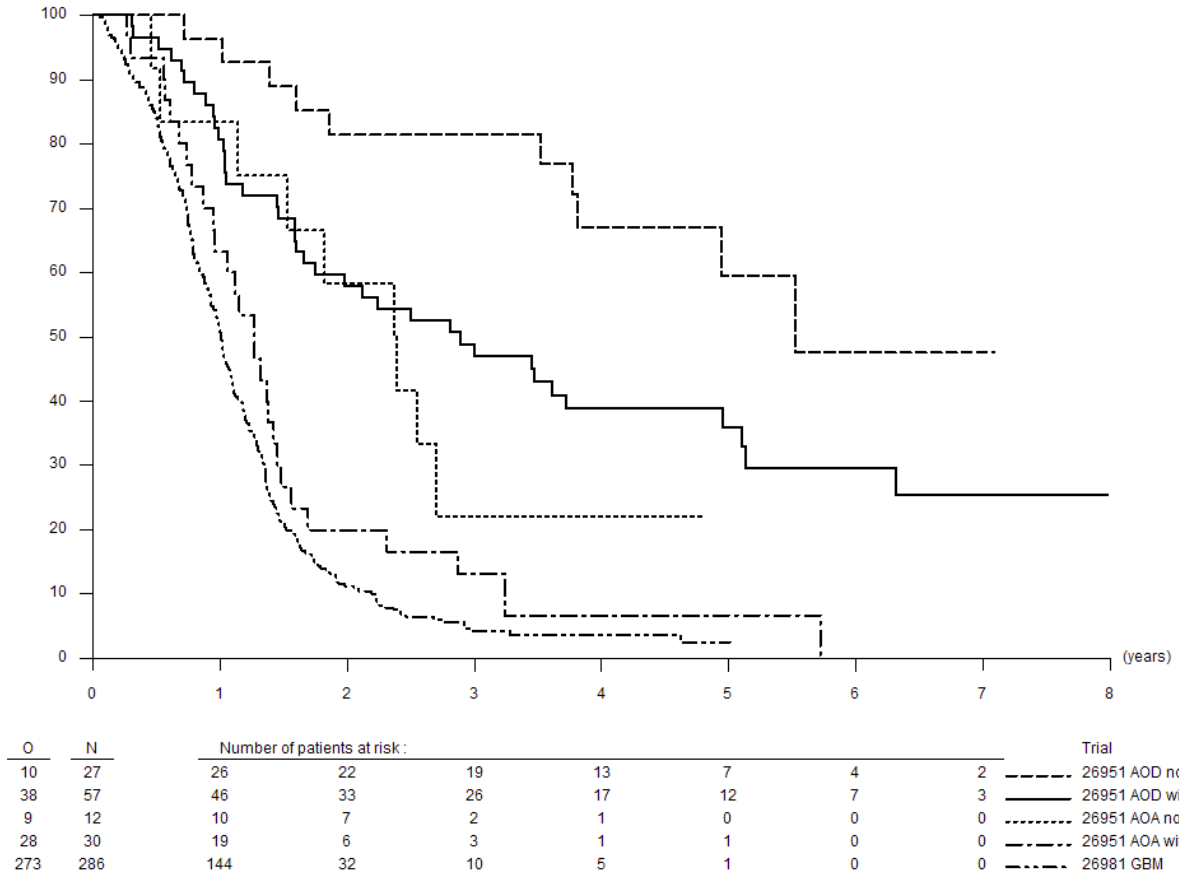
Factor	***p***	**Hazard Ratio (95% Confidence Interval)**
High cellularity	<0.0001	2.82 (1.81-4.40)
Nuclear abnormalities	0.0009	4.49 (1.85-10.91)
Mitoses	0.0001	2.20 (1.47-3.31)
Endothelial abnormalities	<0.0001	3.44 (2.03-5.83)
Necrosis	<0.0001	2.66 (1.94-3.64)
1p loss	<0.0001	0.27 (0.19-0.38)
19q loss	<0.0001	0.31 (0.21-0.45)
Combined 1p and 19 loss	<0.0001	0.25 (0.16-0.40)
EGFR amplification	<0.0001	2.68 (1.86-3.86)
7 polysomy	0.0002	1.94 (1.37-2.75)
10q loss	0.1	1.43 (0.93-2.19)
10 loss	<0.0001	2.47 (1.67-3.65)

Local diagnosis—all patients.
Selected were $1p^{loss}$, combined $1p^{loss}19q^{loss}$, necrosis, 7^{poly}, and 10^{loss}. With bootstrap resampling, not confirmed were 7^{poly} (PI = 52%) and 10^{loss} (PI = 49%). $1p^{loss}$ was borderline not confirmed (PI = 58%).

Centrally confirmed AOD and AOA (WHO 1994).
Selected were $1p^{loss}$, combined $1p^{loss}19q^{loss}$, AOA, and necrosis. With bootstrap resampling, $1p^{loss}$ was not confirmed (PI = 52%).

Central diagnosis—confirmed AOD and AOA but not AOA with necrosis (WHO 2007).
Selected were $1p^{loss}$ and combined $1p^{loss}19q^{loss}$; both were confirmed with bootstrap resampling.

Figure IV.1: OS curves of patients split by diagnosis and presence of necrosis compared to GBM.



Note: Only patients randomized to the radiotherapy control arm of European Organisation for Research and Treatment of Cancer trials 26951 and 26981 are represented.

Prognostic significance of tissue characteristics adjusted for main clinical factors.

Local diagnosis—all patients.
Selected were PS, age, $1p^{loss}$, $1p^{loss}19q^{loss}$, necrosis, 7^{poly}, and 10^{loss} (Table IV-7). With bootstrap resampling, $1p^{loss}$ (PI = 56%) and 10^{loss} (PI = 38%) were not confirmed. The PI of 7^{poly} was of borderline significance (59.7%); the other factors were confirmed.

Centrally confirmed AOD and AOA (WHO 1994).
Selected were PS, age, $1p^{loss}19q^{loss}$, AOD diagnosis, and necrosis (Table IV-7). All factors were confirmed by bootstrap resampling.

Central diagnosis—confirmed AOD and AOA but not AOA with necrosis (WHO 2007).
Selected were PS, age, $1p^{loss}$, $1p^{loss}19q^{loss}$, and 10^{loss} (Table IV-7). With bootstrap resampling, PS and $1p^{loss}19q^{loss}$ were confirmed. The other factors had PIs<60%.

Table IV-7: Multivariate analyses in confirmed AOD or AOA including or excluding AOA with necrosis.

	Locally diagnosed AOD or AOA		**Centrally confirmed AOD or AOA, WHO 1994**		**Centrally confirmed AOD or AOA (Excluding AOA with Necrosis), WHO 2007**	
Factor	**HR (95% CI)**	**p (% inclusion)**	**HR (95% CI)**	**p (% inclusion)**	**HR (95% CI)**	**p (% inclusion)**
Extent of surgery						
Partial/total resection	1.00	NS (53)	1.00	NS (23)	1.00	NS (16)
Biopsy	1.80 (0.99-3.27)		1.25 (0.62-2.53)		0.85 (0.35-2.07)	
WHO performance status[a]						
0						
1	1.45 (1.09-1.92	0.01 (66)	1.84 (1.29-2.64)	0.0009 (83)	1.90 (1.25-2.89)	0.003 (84)
2						
Age						
<50	1.00	0.002 (84)	1.00	0.0080 (72)	1.00	NS (20)
≥50	1.85 (1.27-2.71)		1.78 (1.16-2.73)		1.73 (1.04-2.88)	0.04 (57)
1p loss only						
No	1.00	0.04 (56)	1.00	NS (39)	1.00	0.04 (52)
Yes	0.57 (0.33-0.97)		0.59 (0.30-1.17)		0.49 (0.25-0.96)	
19q loss only						
No	1.00	NS (24)	1.00	NS (33)	1.00	NS (20)
Yes	0.63 (0.30-1.29)		0.47 (0.18-1.25)		0.57 (0.18-1.77)	
Combined 1p/19q loss						
No	1.00	<0.0001 (100)	1.00	<0.0001 (100)	1.00	<0.0001 (100)
Yes	0.12 (0.06-0.25)		0.16 (0.08-0.33)		0.10 (0.04)-0.22)	
AOD						
No	1.00	NS (9)	1.00	0.0003 (66)	1.00	NS (18)
Yes	1.06 (0.67-1.68)		0.43 (0.27-0.68)		0.80 (0.29-2.17)	
High cellularity						
No	1.00	NS (14)	1.00	NS (23)	1.00	NS (18)
Yes	1.48 (0.66-3.30)		0.72 (0.26-2.0)		1.08 (0.32-3.69)	
Nuclear abnormalities						
No	1.00	NS (17)	1.00	NS (3)	1.00	NS (4)
Yes	1.03 (0.46-2.30)		1.27 (0.12-13.39)		0.58 (0.05-7.50)	
Mitoses						
No	1.00	NS (18)	1.00	NS (30)	1.00	NS (38)
Yes	1.51 (0.70-3.24)		0.67 (0.34-1.32)		0.48 (0.21-1.09)	
Endothelial abnormalities						
No	1.00		1.00	NS (10)	1.00	NS (12)
Yes	1.49 (0.87-2.57)	NS (26)	1.48 (0.42-5.24)		1.49 (0.40-5.55)	
Necrosis						
No	1.00	0.0004 (92)	1.00	0.0006 (80)	1.00	NS (41)
Yes	2.00 (1.36-2.94)		2.34 (1.44-3.81)		1.77 (0.81-3.86)	
EGFR amplification						
No	1.00	NS (29)	1.00	NS (36)	1.00	NS (29)
Yes	1.24 (0.68-2.26)		1.36 (0.72-2.58)		1.24 (0.50-3.08)	
7^{poly}						
No	1.00	0.02 (59)	1.00	NS (30)	1.00	NS (35)
Yes	1.65 (1.09-2.49)		1.29 (0.74-2.24)		1.48 (0.73-2.98)	
10^{loss}						
No	1.00	0.03 (38)	1.00	NS (27)	1.00	0.04 (31)[b]
Yes	1.68 (1.06-2.68)		1.17 (0.63-2.19)		1.93 (1.02-3.65)	
$10q^{loss}$						
No	1.00	NS (24)	1.00	NS (28)	1.00	NS (16)
Yes	0.73 (0.38-1.38)		0.65 (0.33-1.32)		0.79 (0.35-1.79)	

Abbreviations: HR, hazard ratio; CI, confidence interval; NS, not significant. [a] For ordered categorical factors, the first value is the reference. HR=1 X x means the risk of death is increased by x% between patients belonging to adjacent groups. [b] Selected by backward selection but not kept in the final model because the percentage of inclusion in bootstrap simulations was less than 60%

4.5 Discussion

In the present study, losses of 1p (41%) and/or 19q (33%) were the most common genomic alterations. In addition, no less than 22% of cases displayed *EGFR*^{amp}, which was inversely related to the 1p/19q codeletion. Loss of 10q and/or copy number aberrations of chromosomes 7 (gain) and 10 (loss) were also observed in a substantial number of anaplastic oligodendroglial tumors, predominantly in tumors with *EGFR*^{amp}. The molecular, histological, and clinical properties identified two subgroups of tumors with distinct prognostic characteristics: (1) oligodendroglial tumors with 1p^{loss}19q^{loss}, mainly located in the frontal lobe, with a predominant AOD histology and a favorable prognosis, and (2) tumors with *EGFR*^{amp}, often with copy number alterations of chromosomes 7 and/or 10, located outside the frontal region, with a mixed oligoastrocytoma phenotype and with a less favorable prognosis. The subgroup with *EGFR*^{amp} and loss of chromosome 10 resembles the previously described "GBM with oligodendroglial phenotype." [15–21] The same genetic lesions (and often also with *EGFRvIII* mutations) have been described in the small-cell GBM variant, which is characterized by monomorphous, deceptively bland nuclei and is often misdiagnosed as AOD.[22,23] Together with *PTEN* (phosphatase and tensin homolog) mutations and deletions, *EGFR*^{amp}, 7 ^{poly}, and 10^{loss} are the most common genotypic alterations in GBM.[24] As expected, after correction for prognostic variables, comparison of this subset of patients with tumors with *EGFR*^{amp} to a group of GBM patients from EORTC study 26981 shows no statistically significant difference in survival.[25] Clearly, despite the intent of the EORTC study 26951 to include chemosensitive oligodendroglioma, this analysis shows that a large number of less sensitive GBM with some oligodendroglial features was entered into this clinical study. The EORTC study on anaplastic oligodendroglial tumors was initiated because studies on recurrent disease showed anaplastic oligodendroglial tumors as opposed to GBM to be sensitive to chemotherapy. Over the past years, it has become clear that the diagnosis of WHO grade III, including classical AOD, is subject to a considerable interobserver disagreement, and the exact delineation of AOD and AOA from GBM with oligodendroglial features is unclear.[26,27] During the conduct of the clinical study, the strong relationship between 1p^{loss}19q^{loss} and response to chemotherapy was discovered, limiting the subset of chemotherapy-sensitive tumors to the 1p19q codeleted tumors. The poor clinical outcome observed in some of the patients made us hypothesize that the use of molecular diagnostics aiming at genetic abnormalities associated with GBM could identify tumors with some oligodendroglial morphology but with an outcome similar to GBM (and to be treated like GBM). Our results show that molecular diagnostics can indeed serve this purpose, although the added benefit is less than anticipated. Simultaneously, retrospective studies have shown that survival of AOD differs from the survival of AOA, and that the presence of necrosis identifies a subgroup of AOA with unfavorable outcome.[28,29] Because of the latter finding, by simply leaving out the word "necrosis" in the section on AOA, the 2007 edition of the WHO classification of brain tumors considers the presence of necrosis no longer consistent with the diagnosis of AOA.[30,31] In the present prospective setting, the outcome of AOA with necrosis is in a similar range and clinically more or less equivalent to the outcome of GBM with a risk-adjusted *p*-value that did not meet preset levels of statistical significance ($p = 0.04$). The GBM-like nature of "AOA with necrosis" is further corroborated by the presence of *EGFR*^{amp} in 44% and 10^{loss} in 35% of the tumors, which is similar to the incidence of these genetic aberrations in studies of GBM. On the other hand, AOA patients with only endothelial abnormalities have a somewhat better outcome, justifying the inclusion of this population in the present WHO definition of AOA. Similarly, although for AOD necrosis had a prognostic impact, the outcome was much better than for GBM. In another review series, "grade IV" AOA (the diagnosis of which required necrosis) had a better survival than GBM, but median survival in the AOA grade IV group was 15.6 months, compared to 10.9 months in the GBM group.[32] Our findings (see Table IV-4) are also consistent with that conclusion, and the limited number of AOA with necrosis (55 patients) may have affected the power to reach statistically significant differences with the GBM group. In two previous studies, necrosis did affect outcome of AOA (or non classic oligodendroglioma) but not that of AOD (or classical

oligodendroglioma).[27,33] In the pathology review of Radiation Therapy Oncology Group study 94-02, age, multifocal disease, histology (classical vs. non classical), and 1p/19q status were independent prognostic factors but not necrosis. In our analysis, necrosis was of prognostic significance in locally diagnosed tumors and also in the WHO 1994 data set and in centrally confirmed AOD. In multivariate analysis of the entire study population, necrosis remained an independent prognostic factor (data not shown). With changing views on the diagnosis of oligodendroglial tumors, it will be interesting to repeat this analysis based on a repeated pathology review with stricter criteria for oligodendroglioma.

Our findings show that $1p^{loss}19q^{loss}$ is the most powerful molecular predictor of outcome. Although highly correlated to outcome in univariate analyses, the other molecular factors have little additional value when considered together with all other available information, in particular, the histological diagnosis and the histological features (the presence of necrosis). Both clinically and molecularly, the WHO 1994 "AOA with necrosis" classification indeed equals GBM, and previous studies have shown that in GBM the presence of *EGFR* amplification has no additional prognostic significance.[34] Of note, in the local diagnosis polysomy of chromosome 7 and loss of chromosome 10 were selected in multivariate analysis but not confirmed. This suggests that molecular studies may be more relevant when the pathological diagnosis is less certain (e.g., in a setting where the diagnosis is not made by an experienced neuropathologist or when only small biopsies are available for histopathological diagnosis). Retrospectively, the inclusion criteria of the EORTC phase III study on adjuvant PCV chemotherapy failed to reach its objective: to enter a subset of chemotherapysensitive tumors into the study. The inclusion of many GBM-like tumors is likely to have affected the power of this study, and thus the outcome of the study. Of note, the analysis of the pathology review within the similar North American Intergroup Radiation Therapy Oncology Group Trial 9402 suggests that adjuvant PCV treatment may be beneficial in the so-called classical AOD as diagnosed by microscopy.[27] In that study, it was suggested that inclusion based on central review is pivotal to keep the study population homogeneous. It is of note, however, that in that study the interobserver agreement was also moderate, and it remains unclear how a study population based on inclusion by central review reflects the patients locally diagnosed (and treated) in everyday clinical practice with such a tumor.

There are a number of potential shortcomings of the present study. First, FISH has a limited sensitivity and specificity. In particular, GBM may have partial deletions of 1p, which is picked up by FISH for 1p36.6.[35–37] This may explain why we observed 1p/19q codeletions together with $EGFR^{amp}$ in some patients. Also, FISH for 19q can be troublesome due to the often weak fluorescence signals that are obtained. Second, limitations in available material may have caused sample bias. More advanced techniques using better conserved tissue samples are likely to yield better results. In a comparable project including 60 patients also treated within EORTC study 26951 and from whom frozen tumor samples were available, BAC array-based comparative genomic hybridization (aCHG) was performed (Ibdaih et al., unpublished observations). This analysis revealed four genomic subgroups with prognostic information (combined $1p/19q^{loss}$, $EGFR^{amp}$, loss of chromosome 21, and neither of these). Multivariate analysis with all relevant prognostic factors identified age ($p = 0.0002$) and genomic profile ($p <, 0.0001$) as independent prognostic factors. The interobserver variation between the aCHG data from that study and the FISH data used in the present study was considered good for both $EGFR^{amp}$ (κ=0,796) and 1p/19q codeletion (κ=0,612). Still, a few samples were classified differently by these techniques. The two studies suggest that, in addition to pathological features (notably necrosis), molecular data are of interest for anaplastic oligodendroglial tumors. Both studies confirmed the 1p/19q codeletion as a strong biomarker in anaplastic oligodendroglial tumors and identified additional biomarkers requiring further investigations as candidates in the non-1p/19q-codeleted anaplastic oligodendroglial tumors.

Methylation status of the *MGMT* (O^6-methylguanine–DNA methyltransferase) promoter gene was no part of this analysis; with the limited amount of tissue available (usually slides only) and stored for many years, this was not yet possible at the time of this study, although it is currently being studied in a subset of patients. This is likely to be an additional prognostic or predictive factor, especially in patients treated with chemotherapy (although the recent German NOA4 study suggests it may also be of prognostic value in patients managed with RT only).[38,39] In conclusion, this study shows that, at the molecular level, EORTC study 26951 of anaplastic oligodendroglial tumors included a heterogeneous group of tumors, with almost 25% of tumors more resembling GBM, which should not have entered the study. Particularly, combined $1p^{loss}19q^{loss}$ contained additional prognostic significance, while most of the additional prognostic information of the other investigated molecular characteristics was already covered by the histopathology. Our study confirms that AOA with necrosis should indeed be considered GBM (WHO grade IV). The clinical outcome of patients with *EGFR* amplification was similar to that of a control group with GBM.

4.6 References

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Chapter V. **Gorlia T, van den Bent MJ, Hegi ME, Mirimanoff RO, Weller M, Cairncross JG, Eisenhauer E, Belanger K, Brandes AA, Allgeier A, Lacombe D, Stupp R. Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981-22981/CE.3. Lancet Oncol. 2008 Jan;9(1):29-38.**

5.1 Abstract

Background

A randomised trial published by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) Clinical Trials Group (trial 26981-22981/CE.3) showed that addition of temozolomide to radiotherapy in the treatment of patients with newly diagnosed glioblastoma significantly improved survival. We aimed to undertake an exploratory subanalysis of the EORTC and NCIC data to confirm or identify new prognostic factors for survival in adult patients with glioblastoma, derive nomograms that predict an individual patient's prognosis, and suggest stratification factors for future trials.

Methods

Data from 573 patients with newly diagnosed glioblastoma who were randomly assigned to radiotherapy alone or to the same radiotherapy plus temozolomide in the EORTC and NCIC trial were included in this subanalysis. Survival modeling was done in three patient populations: intention-to-treat population of all randomised patients (population 1); patients assigned temozolomide and radiotherapy (population 2, n=287); and patients assigned temozolomide and radiotherapy who had assessment of *MGMT* promoter methylation status and who had undergone tumour resection (population 3, n=103). Cox proportional hazards models were fitted with and without O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status. Nomograms were developed to predict an individual patient's median and 2-year survival probabilities. No nomogram was developed in the radiotherapy-alone group because combined treatment is now the new standard of care.

Findings

Independent of the *MGMT* promoter methylation status, analysis in all randomised patients (population 1) identified combined treatment with temozolomide, more extensive tumour resection, younger age, Mini-Mental State Examination (MMSE) score of 27 or higher, and no corticosteroid treatment at baseline as independent prognostic factors correlated with improved survival outcome. In patients assigned temozolomide and radiotherapy (population 2), younger age, better performance status, more extensive tumour resection, and MMSE score of 27 or higher were associated with better survival. In patients who had tumours resected, who were assigned temozolomide and radiotherapy, and who had available *MGMT* promoter methylation status (population 3), methylated *MGMT*, better performance status, and MMSE score of 27 or higher were associated with improved survival. Nomograms were developed and are available at http://www.eortc.be/tools/gbm calculator.

Interpretation

MGMT promoter methylation status, age, performance status, extent of resection, and MMSE are suggested as eligibility or stratification factors for future trials in patients with newly diagnosed glioblastoma. Stratifying by *MGMT* promoter methylation status should be mandatory in all glioblastoma trials that use alkylating chemotherapy. Nomograms can be used to predict an individual patient's prognosis, and they integrate pertinent molecular information that is consistent with a paradigm shift towards individualised patient management.

5.2 Introduction

A randomised trial published by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) Clinical Trials Group (trial 26981-22981/CE.3) showed that addition of temozolomide to radiotherapy for the treatment of patients with newly diagnosed glioblastoma significantly improved survival.[1] Radiotherapy plus concomitant and adjuvant temozolomide has rapidly become the new standard of care in Europe and North America. New strategies are now being developed that build on this treatment of glioblastoma. Despite this progress, the overall outcome of patients with glioblastoma remains unsatisfactory, and prognosis is highly variable in various categories of patients. Previous studies have identified several clinical factors that help to explain the variability of outcome in patients with glioblastoma. Age, performance status, and extent of surgical resection are the most consistently reported prognostic factors.[2–7] In particular, a recursive partitioning analysis (RPA) undertaken by the Radiation Therapy Oncology Group (RTOG) has identified four risk classes for glioblastoma (classes III, IV, V, and VI) based on patients' ages, Karnofsky performance status, neurological function, mental status, and extent of surgery.[2,3] Additionally, the effect of tumour location has been described in several published studies, and includes, in particular, the unfavourable effect of midline cranial shift involvement and of deep seated tumours, and the possible favourable prognosis of a frontal location.[4–10] The unfavourable prognostic effect of an abnormal mental status was first reported by Curran and colleagues[2] in the original report of the RPA classification, although a formal definition of abnormal mental status was not provided. In a study of prognosis in high-grade gliomas, the Folstein Mini-Mental State Examination (MMSE) was retained in an RPA together with age and grade.[11] Similarly, Brown and co-workers[12] identified MMSE as a prognostic factor in patients with low-grade and high grade gliomas. In another study,[13] the group also suggested increased fatigue as an independent predictor of poorer survival. Decreased expression of the O^6-methylguanine-DNA methyltransferase (*MGMT*) repair enzyme makes tumours sensitive to alkylating chemotherapy. Molecular analysis of the tumour tissue of a large subgroup of patients showed that the benefit of temozolomide chemotherapy might be restricted to patients who have a silenced *MGMT* gene by promoter methylation.[14,15] The main aim of this study was to confirm or identify new prognostic factors for survival in patients with glioblastoma and to derive nomograms, i.e., graphical representations of statistical models that predict patient prognosis. Nomograms have been used for other cancer sites, especially urological cancers, but so far have not been applied to neuro-oncology.

5.3 Methods

Patients and procedures

Five hundred seventy three patients with newly diagnosed glioblastoma (WHO astrocytoma grade IV) were randomly assigned treatment in the EORTC and NCIC trial.[1] Eligibility criteria were: age 18 to 70 years; WHO performance status less than or equal to 2; no more than 6 weeks since diagnostic surgery or biopsy; adequate haematological, renal, and hepatic function (absolute neutrophil count of $\geq 1500\times10^6$ cells per L, platelet count of $\geq 100\times10^9$ cells per L, serum creatinine concentration $\leq$1.5 times the upper limit of normal (ULN) in the laboratory where it was measured, total serum bilirubin concentration $\leq$1.5 times the ULN, and liver-function values <3 times the ULN for the laboratory); and patients who were receiving corticosteroids had to receive a stable or decreasing dose for at least 14 days before randomisation. Patients were assigned standard radiotherapy alone or the same radiotherapy plus daily temozolomide followed by up to six cycles of adjuvant temozolomide. Patients were stratified by centre, age, performance status, and extent of surgical resection. Other available baseline clinical factors were sex, tumour location, ongoing corticosteroid treatment, MMSE score, and haemoglobin concentration. Age was categorised into three groups of almost equal size ($\leq$50 years, 51–60 years, and >60 years). The cut-off for MMSE, i.e., normal (27–30) versus impaired (<27), was used as previously reported.[11]

Haemoglobin concentrations of 120 g/L or higher in women and 130 g/L or higher in men were deemed normal. Assessment of tumour characteristics was based on local interpretation of preoperative MRI images. Extent of surgical resection was ascertained perioperatively by the neurosurgeon (macroscopically complete *vs* partial *vs* biopsy only). Survival was calculated as time from randomisation to death from cancer or any other cause, or censored at the date of last follow-up. All patients provided written informed consent and the study was approved by the ethics committees of the participating centres.

Statistical analyses

Compared with previous publications that used data up to May, 2004, [1,14,16] this study is based on survival data updated in September, 2006. Univariate screening was done by use of Kaplan-Meier curves,[17] log-rank test for binary variables, and log-rank trend test for ordered categories. To identify subgroups of patients with potentially different survival if assigned temozolomide and radiotherapy compared with those assigned radiotherapy alone, treatment by factor interaction tests were computed. From these tests, p values less than an arbitrarily chosen significance level of 10% were candidates for the multivariate analyses. Since many factors were ordinal, the association between them was estimated by the Spearman rank correlation coefficient (rho).[18] A coefficient less than 0.30 was deemed a poor correlation. The Cox proportional hazards model was used with forward stepwise model selection with a significance level of 5%.[19] The probability of inclusion of a factor in the multivariate model, a criterion for the prognostic importance of the factor, was estimated by use of the bootstrap resampling technique (see webappendix).[20] Variables with a probability of inclusion higher than 60% based on 1000 bootstrap samples were included in the final model. Methylation status of the *MGMT* promoter was ascertained retrospectively in a representative subset of 206 (36%) patients for whom sufficient tumour material was available.[14] The subgroup of patients in which the *MGMT* promoter methylation status was assessed was not different from the group of patients without *MGMT* promoter methylation status assessment with respect to known prognostic factors, except for extent of resection. *MGMT* promoter methylation status could not usually be assessed in patients whose tumours were only biopsied because of absence of sufficient tumour tissue. Survival modelling was done in three patient populations: the intention-to-treat population of all randomised patients (n=573); a subgroup of patients assigned temozolomide and radiotherapy (n=287); and a subgroup of patients who underwent partial or complete resection and were assigned temozolomide and radiotherapy in the presence of an *MGMT* promoter methylation assessment (n=103). The reasons for doing the analyses in three different populations were the following: to identify the main clinical prognostic factors taking into account that the treatment was important; the strength and importance of some prognostic factors might differ according to the treatment assigned, especially in patients assigned temozolomide and radiotherapy; and the effect of *MGMT* promoter methylation status on the prognosis of patients assigned temozolomide and radiotherapy needed to be evaluated further. For the three populations, the R "Design" package was used to develop nomograms that predict median survival and probability of survival at 2 years taking into account patients' characteristics. The accuracy of predictions was assessed by estimating the models' calibration and discrimination measured by the Concordance index corrected for optimism (C-index). The C-index is the probability that for two patients chosen at random, the patient who had the event first had a higher probability of having the event according to the model. C-index=0.50 represents agreement by chance; C-index=1.0 represents perfect discrimination.[21]
Ideally, the accuracy of a model should be assessed in an independent dataset. However, an independent dataset was not available, therefore, the C-index needed to be corrected for "optimism". In this analysis, the bootstrap technique was used to estimate this correction (webappendix).
Calibration and discrimination of Cox models based on the RPA classification were also assessed and compared with those of our models. These prognostic factor analyses were exploratory. Their findings were therefore restricted by their small sample sizes, low power, and possible selection biases.

Table V-1: Patient demographics and baseline characteristics.

	Population 1 * n (%) (n=573)	Radiotherapy alone, n (%) (n=286)	Population 2 ‡ n (%) (n=287)	Population 3 ‡ n (%) (n=103)	Patients not in population 3 ¶ n(%) (n=470)
Extent of surgery					
Biopsy	93 (16.2)	45 (15.7)	48 (16.7)	0 (0.0)	93 (19.8)
Partial	254 (44.3)	128 (44.8)	126 (43.9)	56 (54.4)	198 (42.1)
Complete	226 (39.4)	113 (39.5)	113 (39.4)	47 (45.6)	179 (38.1)
Age					
≤50 years	183 (31.9)	88 (30.8)	95 (33.1)	44 (42.7)	139 (29.6)
51-60 years	220 (38.4)	111 (38.8)	109 (38.0)	40 (38.8)	180 (38.3)
>60 years	170 (29.7)	87 (30.4)	83 (28.9)	19 (18.4)	151 (32.1)
WHO performance status					
0	223(38.9)	110 (38.5)	113 (39.4)	42 (40.8)	181 (38.5)
1	277 (48.3)	141 (49.3)	136 (47.4)	49 (47.6)	228 (48.5)
2	73 (12.7)	35 (12.2)	38 (13.2)	12 (11.7)	61 (13.0)
Sex					
Male	360 (62.8)	175(61.2)	185 (64.5)	65 (63.1)	295 (62.8)
Female	212 (37.0)	110 (38.5)	102 (35.5)	38 (36.9)	174 (37.0)
Not recorded	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
Corticosteroids at randomization					
No	164 (28.6)	70 (24.5)	94 (32.8)	31 (30.1)	133 (28.3)
Yes	408 (71.2)	215 (75.2)	193 (67.2)	72 (69.9)	336 (71.5)
Missing	1 (0.2)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.2)
MMSE					
27-30	167 (29.1)	86 (30.1)	81 (28.2)	24 (23.3)	143 (30.4)
<27	384 (67.0)	188 (65.7)	196 (68.3)	75 (72.8)	309 (65.7)
Missing	22 (3.8)	12 (4.2)	10 (3.5)	4 (3.9)	18 (3.8)
Lobe					
Frontal	169 (29.5)	82 (28.7)	87 (30.3)	42 (40.8)	127 (27.0)
Temporal	160 (27.9)	79 (27.6)	81 (28.2)	30 (29.1)	130 (27.7)
Parietal	101 (17.6)	54 (18.9)	47 (16.4)	10 (9.7)	91 (19.4)
Occipital	37 (6.5)	17 (5.9)	20 (7.0)	8 (7.8)	29 (6.2)
Central	20 (3.5)	7 (2.4)	13 (4.5)	1 (1.0)	19 (4.0)
Multifocal	79 (13.8)	40 (14.0)	39 (13.6)	12 (11.7)	67 (14.3)
Other	4 (0.7)	4 (1.4)	0 (0.0)	0 (0.0)	4 (0.9)
Missing	3 (0.5)	3 (1.0)	0 (0.0)	0 (0.0)	3 (0.5)
Hemisphere					
Right	297 (51.8)	146 (51.0)	151 (52.6)	57 (55.3)	240 (51.1)
Left	269 (46.9)	135 (47.2)	134 (46.7)	46 (44.7)	223 (47.4)
Both	5 (0.9)	3 (1.0)	2 (0.7)	0 (0.0)	5 (1.1)
Missing	2 (0.3)	2 (0.7)	0 (0.0)	0 (0.0)	2 (0.4)
Hemoglobin level					
Anemia	140 (24.4)	72 (25.2)	68 (23.7)	24 (23.3)	116 (24.7)
Normal	429 (74.9)	214 (74.8)	215 (74.9)	77 (74.8)	352 (74.9)
Missing	4 (0.7)	0 (0.0)	4 (1.4)	2 (1.9)	2 (0.4)
***MGMT* promoter methylation status**					
Methylated	92 (16.1)	46 (16.1)	46 (16.0)	45 (43.7)	47 (10.0)
Unmethylated	114 (19.9)	54 (18.9)	60 (20.9)	58 (56.3)	56 (11.9)
Unknown	367 (64.0)	186 (65.0)	181 (63.1)	0 (0.0)	367 (78.1)

Abbreviations: MMSE=Mini-Mental Examination. MGMT=O^6-methylguanine-DNA methyltransferase. * All randomized patients (intent-to-treat population). † All patients assigned temozolomide and radiotherapy. ‡ Patients who underwent partial or complete resection and were assigned temozolomide and radiotherapy who had MGMT promoter methylation status available. ¶ Patients who were assigned to radiotherapy alone, or radiotherapy and temozolomide who did not have assessment of MGMT promoter methylation status or who underwent biopsy. Percentages might not add up to 100% because of rounding.

5.4 Results

Data from 573 patients with newly diagnosed glioblastoma who were randomised in the EORTC and NCIC trial were included in this subanalysis. Table V-1 shows the characteristics of the patients. In the population of all randomised patients (population 1), overall, MMSE was missing in 22 patients (4%). Except for the *MGMT* promoter methylation status, fewer than six patients (1%) had data missing for the other factors. The dataset was found to be representative of the population of patients with glioblastoma, therefore, no imputation technique was used and patients with missing data were excluded from analysis. Table V-2 summarises the univariate survival analyses of each factor by presenting the log-rank test p value. Apart from hemisphere (left or right), all factors passed the 10% statistical significance criterion. Patients with anaemia showed a better outcome compared with patients with normal haemoglobin concentrations (p=0.04). Nonetheless, the haemoglobin concentration by treatment interaction test was not significant (p=0.11). Absence of treatment with corticosteroids at baseline and more extensive surgery were correlated positively with survival. We did not note significant survival difference between frontal, temporal, occipital, and parietal locations (data not shown). Tumours with central location or that were multifocal (i.e., present on more than one lobe) had worse prognoses than unilobar tumours; patients with such tumours also underwent complete resection less often (25 of 103 [24%] *vs* 201 of 470 [43%] patients), and more often had impaired MMSE (42 of 96 [44%] *vs* 125 of 455 [27%] patients). The last column of table V-2 shows p values of the treatment by factor interaction tests. Treatment by WHO performance status was the only interaction test that passed the 10% statistical significance criterion (p=0.06). Kaplan-Meier survival estimates for the most important factors are available at http://www.eortc.be/tools/gbmcalculator/kmcurves.htm. Significant but poor correlations (rho<0.30) between various factors were noted. More extensive resection was positively correlated with: better performance status (rho=0.15); absence of treatment with corticosteroids (rho=0.24); monofocal location (rho=0.17); and normal mental status (rho=0.16). Younger age was positively associated with better performance status (rho=0.17) and normal mental status (rho=0.25). Better performance status was positively correlated with absence of corticosteroids treatment (rho=0.13) and normal mental status (rho=0.25). A negative correlation was recorded between normal MMSE and multifocal or central tumour location (rho=–0.13). Anaemic patients received corticosteroids at randomisation less frequently than did patients with normal haemoglobin concentrations (81 of 140 [58%] patients *vs* 323 of 429 [75%] patients), and these patients more often underwent complete resection (69 of 140 [49%] patients *vs* 154 of 429 [36%] patients). Therefore, in these patients, anaemia might be due, in part, to the preceding surgery. Table V-3 shows findings of the multivariate Cox proportional hazards analyses. Factors selected in the final model for population 1 were treatment, age, extent of surgical resection, MMSE score, and use of corticosteroids at baseline. Probabilities of inclusion (ie, of being selected in the Cox model) were 99.6% for treatment, 82% for age, 96% for extent of surgical resection, 98% for MMSE score, and 85% for use of corticosteroids at baseline. The C-index corrected for optimism was 65%. Accuracy was not improved when age and MMSE score were entered as continuous factors (C-index corrected for optimism equals 65%). Performance status was not selected and its probability of inclusion was 48%. However, performance status was selected in the absence of MMSE score in the Cox model (data not shown). Figure V.1 shows the nomogram for all randomized patients (population 1). Median survival and probability of survival at 2 years are obtained by drawing a vertical line from the "total points" axis straight down to the outcome axes. The total number of points for each patient is obtained by summing the points for each of the individual factors in the nomogram. Alternatively, prognosis can be obtained by summing the points for each factor in table V-4 and reading the median survival and probability of survival at 2 years from figure V.2. For example, a patient in population 1 who is treated with radio therapy alone, disregarding *MGMT* promoter methylation and performance status, who is aged 40 years, with a partially resected tumour, an MMSE score of 30, and who did not receive corticosteroids at baseline has a total prognostic score of 132 and is predicted to have a 15-month median survival and 24% probability of surviving 2 years. Baseline characteristics of patients assigned temozolomide and radiotherapy (population 2) were

similar to those assigned radiotherapy alone (table V-1). Table V-2 summarises the univariate analyses of each factor by showing the medians with 95% CI, p values, and hazard ratios (HR) with 95% CI in each of the two treatment groups. For example, in population 2, patients with an MMSE less than 27 and who were assigned temozolomide and radiotherapy had a risk of death of 1.87 times that of patients with an MMSE in the range of 27–30. Apart from hemisphere (left or right) and sex, all factors passed the 10% statistical significance criterion. Also, patients with anaemia who were assigned temozolomide and radiotherapy showed a better outcome compared with patients with normal haemoglobin concentrations (p=0.023). Table V-3 summarises the multivariate analyses. For patients assigned temozolomide and radiotherapy (population 2), factors that were selected and included in the final model were age (p=0.008, probability of inclusion 80%), performance status (p=0.006, 78%), extent of surgery (p=0.0004, 75%), and MMSE score (p=0.0009, 79%). In this subset, corticosteroids were not selected (p>0.05, 33%). Sex was selected, but had a percentage of inclusion in bootstrap simulations of less than 60% (p=0.03, 55%) and, therefore, was excluded from the final model. The C-index corrected for optimism was 63%. Since the performance status by treatment interaction test was significant and this factor was selected for the patients assigned temozolomide and radiotherapy (population 2), but not for the group of all randomized patients (population 1), a Cox model was also fitted for patients assigned radiotherapy alone. In this subset, extent of surgery (p=0.007, 80%), MMSE score (p<0.0001, 89%), and corticosteroid treatment at baseline (p=0.005, 81%) were selected, but not age (p>0.05, 29%) or performance status (p>0.05, 8%). In this subgroup, patients with anaemia did not show better outcomes compared with patients with normal haemoglobin concentrations (p>0.05). Figure V.3 shows the nomogram for patients who were assigned temozolomide and radiotherapy (population 2). Prognosis can also be obtained from table V-4 and figure V.4. For example, if the same patient used in the example for population 1 had a performance status of 0 and a total prognostic score of 50, their predicted median survival would be 21 months and probability of survival at 2 years would be 43%**.** Patients in population 3 (those assigned temozolomide and radiotherapy, and who had resected tumours and known *MGMT* promoter methylation status) were younger than those not in this subgroup (median age 53 years [range 19–70] *vs* 56 years [range 18–70]), and had more frontal tumours (42 of 103 [41%] patients) than those not in this subgroup (127 of 470 [27%] patients; table V-1). *MGMT* promoter methylation status was missing in 64% of patients. With such a high percentage of missing data, no substantial benefit was expected from imputation techniques and, therefore, analyses were done only for the dataset with complete data. *MGMT* promoter methylation status was not correlated with age or with any of the other prognostic factors tested. Due to the small sample size and low power of the analyses in population 3, factors selected in the univariate analyses of patients assigned temozolomide and radiotherapy were also considered for the multivariate analysis in this population. The final multivariate Cox model shown in table V-3 included *MGMT* promoter methylation status (p<0.0001, probability of inclusion 92%), performance status (p=0.003, 82%), and MMSE score (p=0.008, 81%). The C-index corrected for optimism was 66%. Figure V.5 shows the resulting nomogram for population 3. Prognosis can also be obtained from table V-4 and figure V.6. For example, the patient mentioned in the previous populations would have a median survival of 48.0 months compared with 16.9 months and a probability of survival at 2 years of 66% compared with 32.5%, in *MGMT* promoter methylated versus unmethylated tumours, respectively. The nomogram for the population of all randomized patients (population 1) was well calibrated but could not make an accurate prediction for patients with a probability of survival at 2 years better than 40%. The nomograms in the two other populations could predict patients with a survival probability at 2 years greater than 40% but were less well calibrated and predictions were less accurate. We show in the webtable that predictions of our nomograms are more accurate than those of models based on the RPA classification. C-index of RPA-based models is 58% in population 1, 59% in population 2, and 56% in population 3 (webtable). Accuracy was especially low for RPA classes III and V.

Table V-2: Univariate analyses of potential survival prognostic factors.

	RT alone patients (n=286)			**Population 2 * (n=287)**			**Population 1 † (n=573)**		**Treatment Interaction test**
	Median, mo (95% CI)	HR (95%CI)	p	Median, mo (95% CI)	HR-95%CI	p	HR (95%CI)	p	p
Treatment assignment									
Temozolomide and radiotherapy	NA	NA		NA	NA		1.00	<0.0001	NA
Radiotherapy	NA	NA		NA	NA		1.57 (1.32-1.87)		
Extent of surgery‡									
Complete resection	14.2 (13.0-16.2)		<0.0001	18.8 (16.4-22.9)		<0.0001		<0.0001	0.41
Partial resection	11.7 (9.7-13.1)	1.45 (1.22-1.73)		13.5 (11.9-16.4)	1.46 (1.22-1.74)		1.44 (1.27-1.63)		
Biopsy	7.9 (6.4-10.6)			9.4 (7.5-13.6)					
Age (years) ‡									
≤50 years	13.6 (11.6-15.6)		0.054	17.4 (15.3-21.5)		0.0004		<0.0001	0.17
51-60 years	12.0 (10.0-14.2)	1.16 (1.00-1.36)		14.6 (13.6-17.9)	1.34 (1.14-1.57)		1.26 (1.13-1.41)		
>60 years	11.8 (10.5-12.8)			11.3 (9.4-15.1)					
WHO performance status ‡									
0	13.3 (11.8-15.7)		0.050	17.4 (15.7-21.2)		0.0001		<0.0001	0.06
1	11.9 (10.0-13.2)	1.19 (1.00-1.42)		14.1 (12.5-17.0)	1.47 (1.21-1.79)		1.33 (1.17-1.51)		
2	10.5 (8.5-13.0)			9.9 (6.9-12.1)					
Sex									
Female	12.6 (11.9-16.1)	1.00	0.08	16.3 (13.4-20.4)	1.00	0.26	1.00	0.09	0.87
Male	11.4 (10.5-12.9)	1.24 (0.97-1.59)		14.4 (12.4-16.4)	1.16 (0.89-1.51)		1.17 (0.98-1.40)		
Corticosteroids at randomization									
No	16.3 (14.4-17.3)	1.70 (1.29-2.25)	0.0002	19.7 (16.4-24.9)	1.00	0.005	1.00	<0.0001	0.92
Yes	11.0 (9.7-12.1)			13.6 (11.9-14.9)	1.47 (1.12-1.94)		1.60 (1.32-1.95)		
MMSE score									
27-30	13.3 (12.2-14.8)	1.00	<0.0001	17.1 (15.3-19.1)	1.00	<0.0001	1.00	<0.0001	0.41
<27	9.3 (7.9-11.7)	1.78 (1.37-2.31)		10.3 (8.6-12.9)	1.87 (1.43-2.46)		1.81 (1.50-2.19)		
Tumour location									
Unilobal	12.5 (12.0-14.1)	1.00	0.03	16.3 (14.4-18.3)	1.00	0.0004		<0.0001	0.20
Central and multilobal	9.5 (7.5-11.7)	1.40 (1.02-1.91)		11.3 (9.2-14.0)	1.76 (1.28-2.42)		1.58 (1.26-1.96)		
Hemisphere									
Right	13.0 (11.9-14.4)	1.00	0.28	15.7 (13.9-18.1)	1.00	0.92	1.00	0.62	0.89
Left	11.4 (10.0-12.3)	1.07 (0.84-1.36)		14.4 (12.4-17.0)	1.00 (0.80-1.30)		1.05 (0.88-1.24)		
Hemoglobin level									
Low (anaemia)	11.4 (10.0-13.3)	1.00	0.71	18.6 (15.7-25.9)	1.00	0.023	1.00	0.04	0.11
Normal	12.2 (11.4-13.5)	1.05 (0.80-1.39)		13.5 (12.2-15.5)	1.41 (1.05-1.89)		1.24 (1.01-1.52)		
***MGMT* promoter methylation status**									
Methylated	15.3 (13.0-20.9)	1.00	0.0001	21.7 (18.6-N)	1.00	0.0003	1.00	<0.0001	0.31
Unmethylated	11.8 (10.0-14.4)	2.40 (1.53-3.78)		12.4 (11.6-14.4)	2.24 (1.43-3.51)		2.10 (1.54-2.85)		

NA=not available. MMSE=Mini-Mental State Examination. N=not enough events to calculate upper 95% CI boundary. MGMT=O^6-methylguanine-DNA methyltransferase. Age, performance status, and extent of surgery are treated as ordinal variables. *All patients assigned temozolomide and radiotherapy. †All randomised patients (intention-to-treat population). ‡For ordered categorical factors, the fi rst value is the reference. HR=1·x means hat the risk of death is increased by x% between patients belonging to adjacent groups—e.g., for the category of age in population 1, HR=1.16 indicates the risk of death increases by 16% between age ≤50 years and 51–60 years and by the same increase between groups 51–60 years and >60 years.

Table V-3: Cox proportional hazards analyses of survival prognostic factors.

	Population 1 * (n=573, 547used=547, 498 deaths)		**RT alone patients (n=286, 274 used, 263 deaths)**		**Population 2 † (n=287, 273 used, 235 deaths)**		**Population 3 ‡ (n=103,97 used=97, 77 deaths)**	
	HR (95% CI)	p (% inclusion)	HR (95% CI)	p (% inclusion)	HR (95% CI)	p (% inclusion)	HR (95% CI)	p (% inclusion)
Treatment assignment								
Temozolomide and Radiotherapy	1		NI	NI	NI	NI	NI	NI
Radiotherapy	1.60 (1.34-1.91)	<0.0001 (99.6)	NI	NI	NI	NI	NI	NI
***MGMT* promoter methylation status**								
Methylated	NI	NI	NI	NI	NI	NI	1.00	<0.0001 (92)
Unmethylated	NI	NI	NI	NI	NI	NI	2.75 (1.68-4.49)	
Age (years) §								
≤50 years		0.003 (82)		NS (29)		0.008(80)		NS(37)
51-60 years	1.19 (1.06-1.34)		1.12 (0.95-1.32)		1.26 (1.06-1.48)		1.32 (0.95-1.84)	
>60 years								
WHO Performance status §								
0		NS(48)		NS (8)		0.006(78)		0.003(82)
1	1.12 (0.98-1.28)		0.98 (0.82-1.19)		1.32 (1.08-1.60)		1.76 (1.21-2.55)	
2								
Interaction term between performance status and treatment	0.99 (0.82-1.19)	NS(40)	NI	NI	NI	NI	NI	NI
Extent of surgery §								
Complete resection		<0.0001(96)		0.007 (80)		<0.001(75)		NS(7) ¶
Partial resection	1.33 (1.17-1.52)		1.29 (1.07-1.55)		1.37 (1.14-1.63)		1.03 [0.64-1.64]	
Biopsy								
Tumor location								
Unilobal	1.00	NS(30)		NS (13)		NS(52)		NS(41)
Central and multilobal	1.17 (0.92-1.50)		0.94 (0.66-1.33)		1.40 (0.99-1.97)		1.62 (0.80-3.29)	
MMSE score								
27-30		<0.0001(98)		<0.0001 (89)		<0.001 (79)		0.008(81)
<27	1.63 (1.34-1.98)		1.71 (1.31-2.24)		1.66 (1.25-2.19)		1.98 (1.20-3.28)	
Corticosteroids at randomisation								
No	1.00	0.003(85)	1.00	0.005 (81)		NS(33)		NS(12)
Yes	1.36 (1.11-1.67)		1.52 (1.13-2.03)		1.19 (0.89-1.59)		1.17 (0.70-1.97)	
Sex								
Female	1.00	NS(51)		NS (22)		0.03‖ (55)		NS(10)
Male	1.16 (0.97-1.40)		1.13 (0.88-1.46)		1.30 (0.99-1.70)		1.10 (0.69-1.77)	
Hemoglobin								
Low (anaemia)	1.0	NS(9)		NS (9)		NS(36)		NS(21)
Normal	1.06 (0.86-1.31)		0.96 (0.72-1.28)		1.33 (0.98-1.81)		1.44 (0.85-2.46)	
C-Index corrected for optimism	65%		NI		63%		65.5%	

Note: MGMT=O^6-methylguanine-DNA methyltransferase. NI=not included in Cox model. NS=not significant. MMSE=Mini-Mental State Examination. Percentages might not add up to 100% because of rounding. *All randomised patients (intention-to-treat population). †All patients assigned temozolomide and radiotherapy. ‡Patients who underwent partial or complete resection and were assigned temozolomide and radiotherapy who had MGMT promoter methylation status available. §For ordered categorical factors, the first value is the reference. HR=1·x means that the risk of death is increased by x% between patients belonging to adjacent groups - e.g., for the category of age in population 1, HR=1.19 indicates the risk of death increases by 19% between age ≤50 years and 51–60 years and by the same increase between groups 51–60 years and >60 years. ¶ Partial vs complete resection. ‖ Sex was selected by stepwise selection but was not kept in the final model because the percentage of inclusion in bootstrap simulations was below 60%.

Table V-4: Prognostic scores of each factor in the three nomograms.

	Population 1* (n=573)	Population 2† (n=287)	Population 3‡ (n=103)
Treatment assignment			
Temozolomide and radiotherapy	0	NA	NA
Radiotherapy	82	NA	NA
***MGMT* promoter methylation status**			
Methylated	NI	NI	0
Unmethylated	NI	NI	90
Age, years			
≤50	0	0	NI
51-60	31	35	NI
>60	61	71	NI
Extent of surgery			
Total	0	0	NI
Partial	50	50	NI
Biopsy	100	100	NI
WHO performance status			
0	NI	0	0
1	NI	41	50
2	NI	82	100
MMSE score			
27-30	0	0	0
<27	85	78	61
Corticosteroids at randomisation			
No	0	NI	NI
Yes	54	NI	NI

NA=not applicable. MGMT=O6-methylguanine-DNA methyltransferase. NI=not included in fi nal model. MMSE=Mini-Mental State Examination. Points were summed to obtain a total prognostic score. Patients with good a prognosis have a low total prognostic score. *All randomised patients (intention-to-treat population). †All patients assigned temozolomide and radiotherapy. ‡Patients who underwent partial or complete resection and assigned temozolomide and radiotherapy who had MGMT promoter methylation status available.

Figure V.1: Nomogram for predicting survival in all randomised patients (population 1)

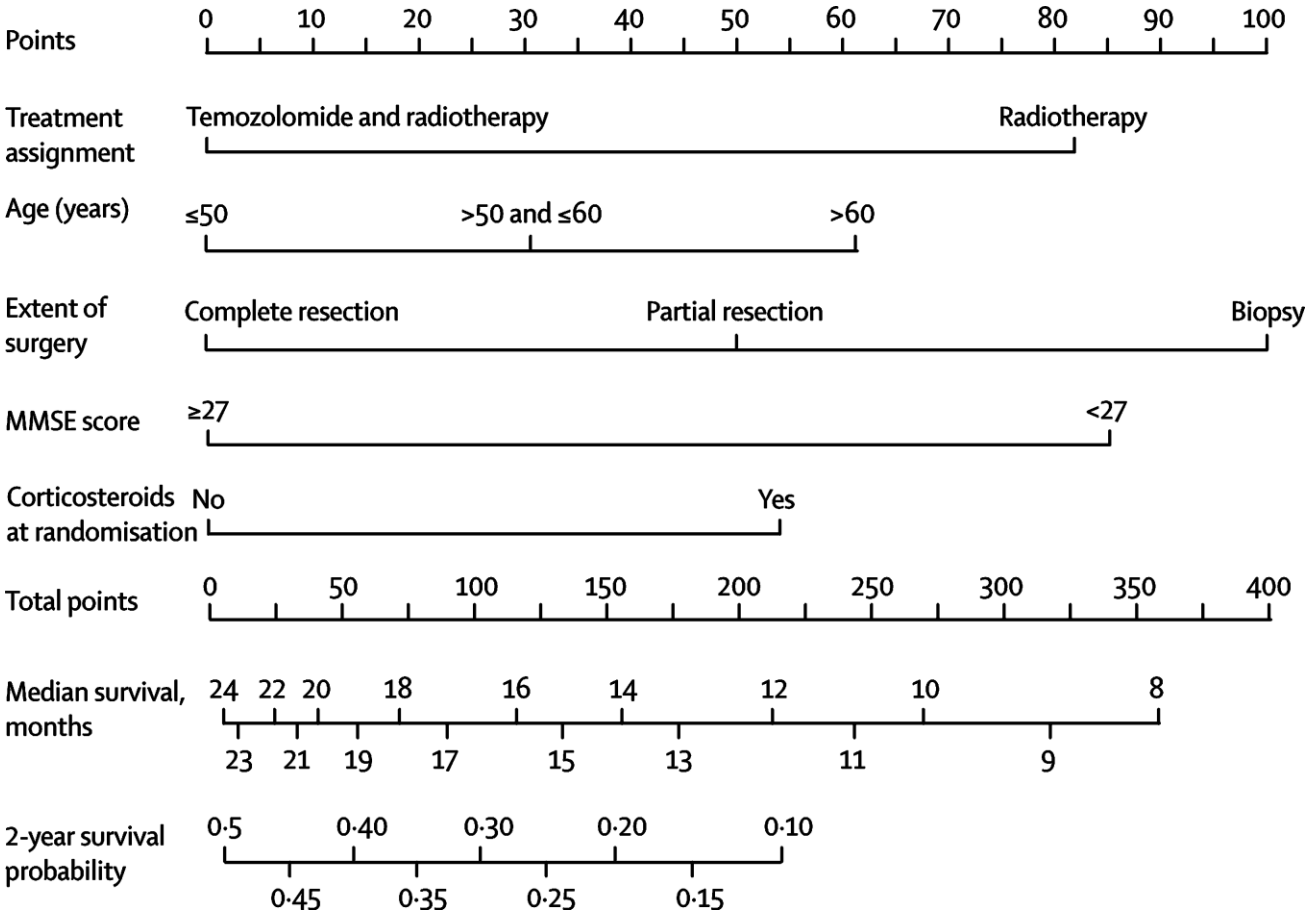
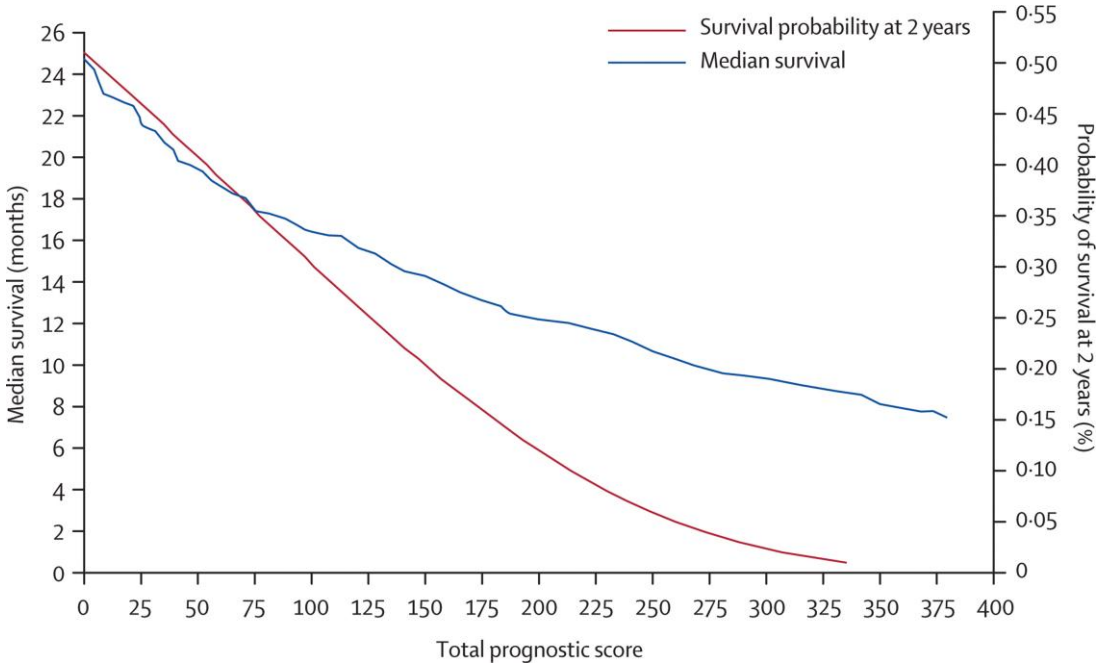


Figure V.2: Prognostic plots in all randomised patients (population 1)



Note: Median survival in months (blue line, left y-axis) and probability of survival at 2 years (red line, right y-axis) are plotted as a function of the total prognostic score.

Figure V.3: Nomograms for predicting survival in patients assigned TMZ/RT (population 2)

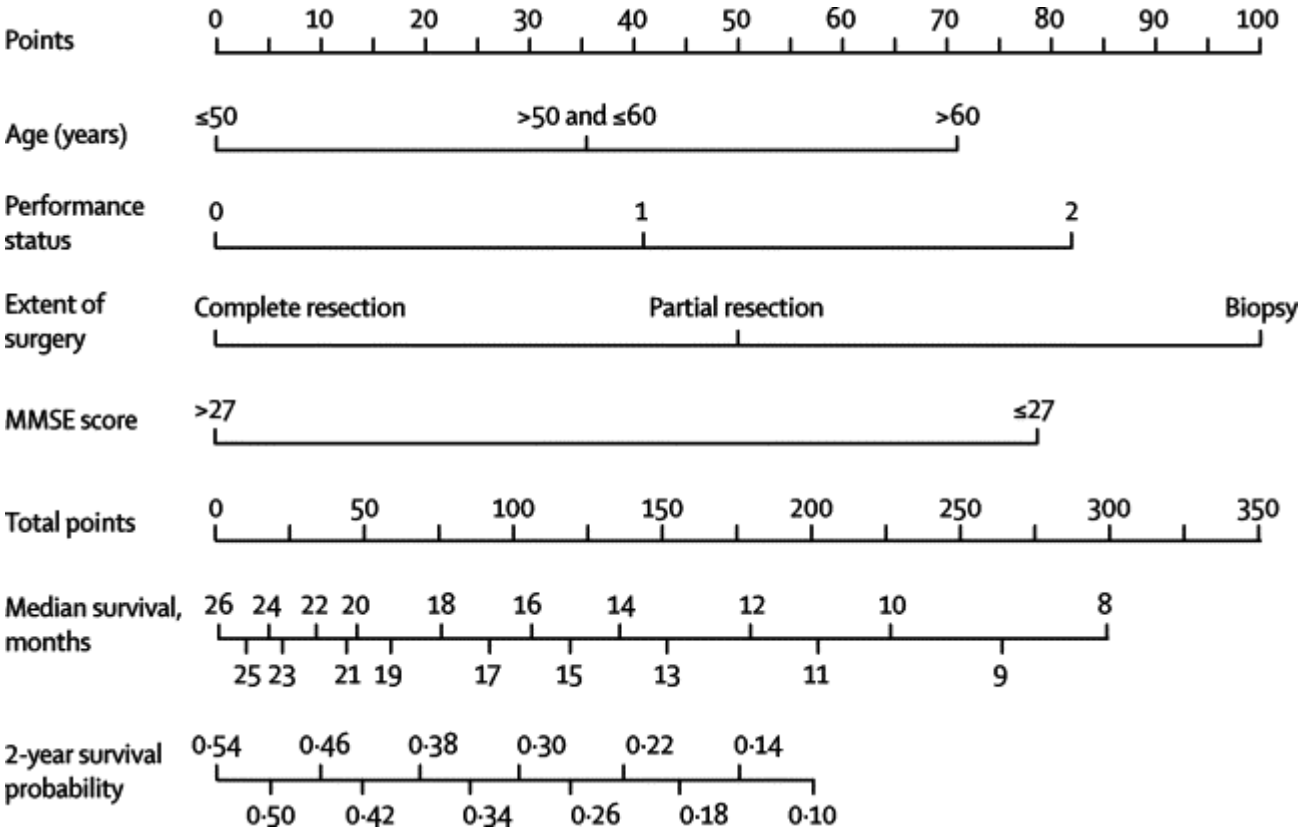
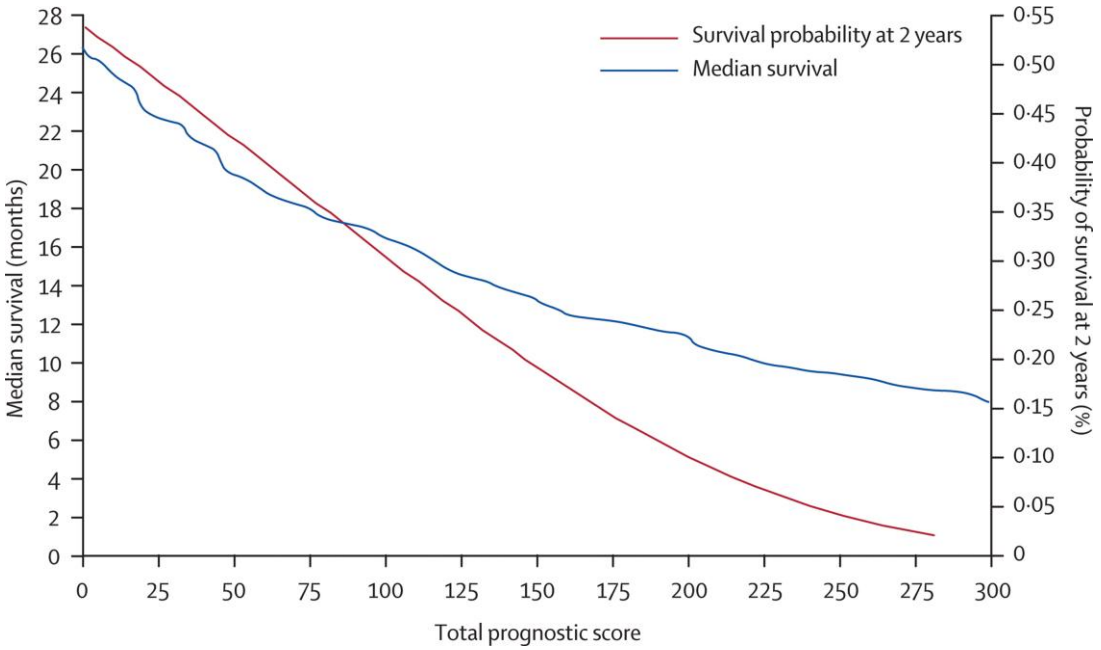


Figure V.4: Prognostic plots in patients assigned TMZ/RT (population 2).



Note: Median survival in months (blue line, left y-axis) and probability of survival at 2 years (red line, right y-axis) are plotted as a function of the total prognostic score.

Figure V.5: Nomogram in patients resected, assigned TMZ/RT and MGMT assessed (population 3)

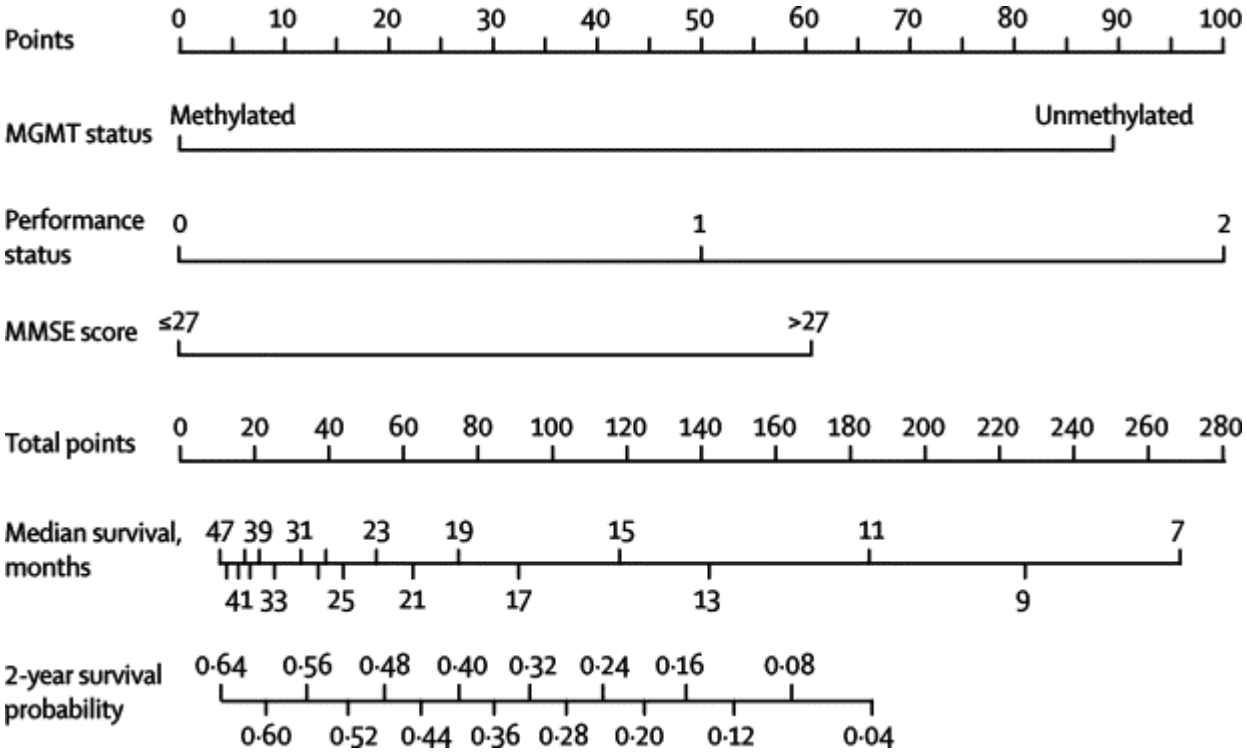
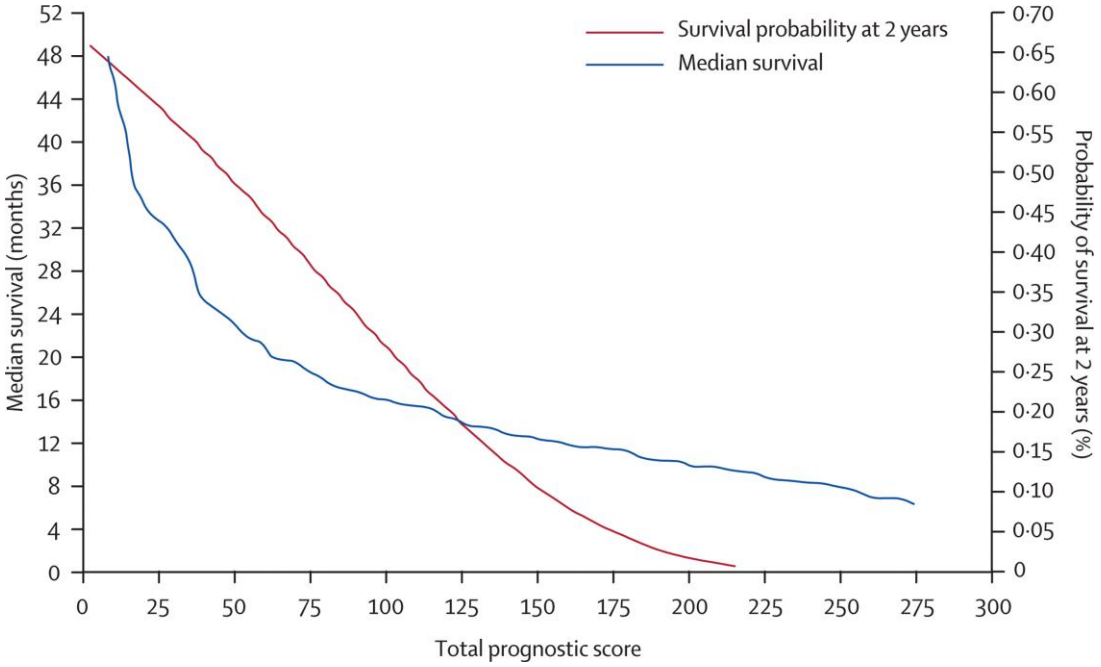


Figure V.6: Prognostic plots in patients resected, assigned TMZ/RT and MGMT assessed (population 3)



Note: Median survival in months (blue line, left y-axis) and probability of survival at 2 years (red line, right y-axis) are plotted as a function of the total prognostic score.

5.5 Discussion

A proper understanding of prognostic factors is important for the counselling of individual patients, to select patients for specific treatments, and for the design and interpretation of clinical trials. The EORTC and NCIC trial 26981-22981/CE.3 showed that treatment with concurrent and adjuvant temozolomide in addition to radiotherapy improved the overall outcome compared with treatment with radiotherapy alone.[1] The companion prognostic factor analysis reported here has identified treatment with radiotherapy, and concomitant and adjuvant treatment with temozolomide, age, extent of surgical resection, *MGMT* promoter methylation status, WHO performance status, neurological function expressed by the MMSE, and the need for corticosteroids administration after surgery or biopsy as the most relevant independent prognostic factors for the outcome of patients with glioblastoma. Although the prognostic significance of some of these factors has been discussed before, our finding in this study that the presence of corticosteroid treatment at baseline in patients assigned radiotherapy alone, but not in those assigned temozolomide and radiotherapy, has a substantial negative prognostic effect, deserves further investigation. Addition ally, our findings that WHO performance status and age are significantly correlated with survival in patients assigned temozolomide and radiotherapy, but not those assigned radiotherapy alone need further study. Corticosteroid use was identified as a poor prognostic factor in a small study published in 1989,[22] which had a heterogeneous patient population, but has not been assessed in many more recent trials. Use of corticosteroids might identify patients with more severe clinical signs and symptoms before surgery, or those with larger tumours or tumours that were amenable to biopsy only. Also, efficacy of corticosteroid treatment seems to be enhanced in patients with a good performance status. For both situations, confirmations in future trials are needed. The findings from our trial confirm the presence of a strong correlation between better outcome and completeness of tumour resection; however, interpretation of the findings is restricted by the fact that the extent of resection was based on perioperative assessment by a neurosurgeon, without a mandatory postoperative radiological confirmation. This restricts the reliability of the distinction between partially and completely resected patients. Patients in the study were not stratified according to the extent of surgery and we could not identify the relevance of the extent of resection in the subset of patients with known *MGMT* promoter methylation status. Therefore, the relative contribution from attempting maximum resection cannot be assessed. Consequently, whether more extensive resection improves outcome remains unclear from these data. However, in view of the better outcome of resected patients in many studies, all patients with glioblastoma should undergo resections that are as extensive as safely possible.[1] Our findings show that combined and adjuvant temozolomide treatment improves outcome, and they suggest that—although not reaching statistical significance—patients with a methylated *MGMT* promoter benefit especially from the addition of temozolomide to their treatment. Even patients who were not treated with concomitant and adjuvant chemotherapy presented with a better outcome in the presence of a methylated *MGMT* promoter, probably due to a greater efficacy of salvage chemotherapy with alkylating drugs administered at the time of recurrence.[14] Earlier studies in which all patients received adjuvant carmustine in addition to radiotherapy also noted that alkyltransferase expression was of prognostic importance.[23,24] We have discussed elsewhere the effect of stratifying by this molecular marker in the development of new therapeutic strategies for patients with glioblastoma.[15] The current analysis did not show any correlation between age and *MGMT* promoter methylation status, which implies that the poor prognosis of elderly patients cannot be explained by a lower frequency of *MGMT* promoter methylation. By contrast, in the presence of information on *MGMT* promoter methylation, age was no longer retained in the model and the nomogram. This observation suggests that older patients with methylated *MGMT* promoter might benefit from the new combination treatment, despite their age. However, the reason for this might be the absence of power in this subgroup analysis. Additional data will be necessary to assess the effect of temozolomide and radiotherapy in this subgroup. In an RPA, RTOG identified six prognostic classes of anaplastic gliomas based on clinical factors, in which classes III to VI are applicable to glioblastomas. However, this system,

developed and validated in the early 1990s, is based on data collected from 1974 to 1994. During the three decades since 1974, not only have diagnostic methods, radiotherapy planning, and treatment techniques dramatically changed, but also histopathological classification systems have been revised, and molecular factors relevant for outcome have been identified. Within the current clinical context, we have previously shown that RPA prognostic classes still separate prognostic groups after combined chemo radiotherapy with temozolomide in patients with newly diagnosed glioblastoma.[16,25] Our analysis adds a new dimension to the previous studies, in that it approaches prognosis from the individual patient's perspective: the nomogram offers a more tailored approach for individual patients taking into account their individual prognostic factors. Investigators might want to use their prediction in groups of patients from small phase II trials that assess innovative adjuvant treatment strategies to assess whether improved outcome is not a consequence of patient selection. Since formal comparisons are not possible in phase II trials, this use of nomograms should be limited to guide further research only. We claim that nomogram predictions are more accurate than those based on the RPA classification and, therefore, are better adapted to study tailored therapeutic options for individual patients. This study is exploratory and a limitation of these nomograms is that no validation is possible yet in a large independent set of patients.[3,26] Currently, no other large datasets are available on patients treated with radiotherapy with concurrent and adjuvant temozolomide chemotherapy, who also have MMSE scores and *MGMT* promoter methylation status. Some analyses have been undertaken in subgroups of patients, especially in those with sufficient biological material for assessment of *MGMT* promoter methylation. Validity and application of these nomograms need to be assessed in prospectively acquired data. In future trials of patients with newly diagnosed glioblastoma, *MGMT* promoter methylation status, age, performance status, extent of resection, and MMSE score should be considered as eligibility criteria or stratification factors, or both. Stratifying by *MGMT* promoter methylation status should be mandatory in adjuvant and recurrent glioblastoma trials that include the administration of alkylating drugs. When *MGMT* promoter methylation status cannot be assessed before randomisation, it should be ascertained after inclusion of patients and used as a correction factor in the survival analyses.

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Chapter VI. **Gorlia T, Stupp R, Brandes AA, Rampling RR, Fumoleau P, Dittrich C, Campone MM, Twelves CC, Raymond E, Hegi ME, Lacombe D, van den Bent MJ. New prognostic factors and calculators for outcome prediction in patients with recurrent glioblastoma: a pooled analysis of EORTC Brain Tumour Group phase I and II clinical trials. Eur J Cancer. 2012 May;48(8):1176-84. doi: 10.1016/j.ejca.2012.02.004.**

6.1 Abstract

Background:
Prognostic models have been developed to predict survival of patients with newly diagnosed glioblastoma (GBM). To improve predictions, models should be updated with information at the recurrence. We performed a pooled analysis of European Organization for Research and Treatment of Cancer (EORTC) trials on recurrent glioblastoma to validate existing clinical prognostic factors, identify new markers, and derive new predictions for overall survival (OS) and progression free survival (PFS).

Methods:
Data from 300 patients with recurrent GBM recruited in eight phase I or II trials conducted by the EORTC Brain Tumour Group were used to evaluate patient's age, sex, World Health Organisation (WHO) performance status (PS), presence of neurological deficits, disease history, use of steroids or anti-epileptics and disease characteristics to predict PFS and OS. Prognostic calculators were developed in patients initially treated by chemoradiation with temozolomide.

Results:
Poor PS and more than one target lesion had a significant negative prognostic impact for both PFS and OS. Patients with large tumours measured by the maximum diameter of the largest lesion (≥42 mm) and treated with steroids at baseline had shorter OS. Tumours with predominant frontal location had better survival. Age and sex did not show independent prognostic values for PFS or OS.

Conclusions:
This analysis confirms performance status but not age as a major prognostic factor for PFS and OS in recurrent GBM. Patients with multiple and large lesions have an increased risk of death. With these data prognostic calculators with confidence intervals for both medians and fixed time probabilities of survival were derived.

6.2 Introduction

The prognosis of patients with glioblastoma (GBM) remains dismal despite substantial therapeutic improvement provided by chemoradiation with temozolomide (TMZ) at the initial diagnosis.[1] As yet, there is still no universally accepted standard treatment at the first recurrence, many patients being treated with nitrosoureas (e.g. lomustine [CCNU]) or with bevacizumab or considered for experimental therapy within clinical trials.[2] Clinical trials of new treatments or novel approaches aiming at improving outcome after disease recurrence are urgently needed. In order to identify a real sign of activity of investigational treatments, reliable end-points for phase II trials are required. Probabilities of progression-free survival at 6 months (PFS6) and of overall survival at 1 year (OS12) are both recognised end-points for clinical trials to assess the outcome of patients with recurrent GBM.[3] The identification of accurate prognostic factors is an important issue to guide therapeutic decisions and patient management.[4] In a previous report, we reviewed the prognostic importance of clinicobiological factors for predicting survival in newly diagnosed GBM. We showed that combined and concomitant radio and TMZ chemotherapy (TMZ/Radiotherapy (RT) → TMZ), O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, extent of primary surgery, age, World Health Organization (WHO) performance status (PS), Mini-Mental State Examination (MMSE) and administration of corticosteroids at the baseline strongly impacted on patient's survival.[5] At the time of tumour progression other prognostic factors may be relevant. Patients commonly have an altered performance status, and will require more frequent corticosteroids administration. Furthermore, treatment specific molecular alterations may be selected for in the recurrent tumour, such as inactivation of mismatch repair pathway constituents in TMZ treated patients.[6] The New Approaches to Brain Tumor Therapy Central Nervous System (NABTT CNS) Consortium performed a Recursive Partitioning Analysis (RPA) for overall survival in recurrent high-grade gliomas. They identified histology, age, Karnofsky's index (KPS), tumour localisation and corticosteroids at the baseline as important prognostic factors.[7] Joint North Central Cancer Treatment Group (NCCTG) and North America Brain Tumor Coalition (NABTC) analyses found grade, age, PS, baseline steroids and time since initial diagnosis (Wu et al., 2010) as most influential factors for survival.[8] Dempsey et al. showed that a large tumour by volumetric measurement had a detrimental effect on survival in a group of malignant gliomas. They also identified older age and male sex as risk factors for survival.[9] Age was not identified as an independent prognostic factor for survival in two previous reports.[10,11] We have pooled the data from phase I and phase II clinical trials on recurrent GBM conducted by the European Organization for Research and Treatment of Cancer (EORTC) Brain Tumour Group (BTG) in order to further assess prognostic factors for clinical outcome and to develop prognostic models. We have derived prognostic calculators providing estimates with confidence intervals for both medians and fixed time probabilities of survival.

6.3 Patients and methods

Patient selection

Between 1999 and 2010, the EORTC has conducted eight prospective multicentre phase 1 and phase 2 clinical trials investigating safety and activity of novel therapeutic agents in recurrent malignant glioma.[12–19] Agents under study in dose finding phase I trials were SCH66336 (lonafarnib) and LY317615 (enzastaurin). The phase II trials involved XR5000 (DACA, Xenova_), D19575 (glufosfamide), RFS 2000, STI571 (imatinib, Glivec_), OSI 774 (erlotinib, Tarceva_) and ZK219477 (sagopilone). Table VI-1 (Supplemental Table 1) presents a description of trial characteristics. None of the experimental agents showed clinically relevant activity. In all studies, eligibility criteria were similar. Patients were at least 18 years of age, with WHO PS 0-2 or KPS 70–100%, adequate haematological, renal and hepatic functions. Corticosteroid doses, if applicable, were to be stable or decreasing for at least 1 week. In three studies, newly diagnosed patients with multifocal disease not amenable to radiotherapy were allowed. In the two

phase I trials, measurable disease was not mandatory but at least one bi-dimensional lesion was recommended. In phase II trials, prior radiotherapy had to be completed more than 3 months before registration in order to reduce the chance of treating a pseudoprogression. Three of the trials included patients who had received prior chemotherapy for a disease recurrence or progression. In two trials patients using enzyme inducing anti epileptic drugs (EIAED) were excluded. In one trial a higher dose of the investigational drug (erlotinib) in patients under EIAED was to be prescribed. Each trial was approved by the EORTC Protocol Review Committee as well as by the participating institutions local ethical committee and the respective national regulatory authorities. Written informed consent was obtained prior to enrolment into the trial. In order to determine whether our pooled patient population was representative of a standard patient population, the survival of the patients in the pooled dataset was compared to the survival from the date of first disease progression of patients recruited in the EORTC 26981/22981/National Cancer Institute of Canada (NCIC) CE.3 trial who were treated with TMZ/RT → TMZ and subsequently received another line of chemotherapy after first progression.[1]

Table VI-1: Description of trials characteristics and main eligibility criteria.

Trial characteristics	**16991G**	**16994G**	**16996G**	**16011**	**16027**	**26034**	**26061**	**26054**
ClinicalTrials.gov Identifier	NCT00004937	NCT00014300	NCT00005826	NCT00039364	NCT00083096	NCT00086879	NCT00424060	NCT00516607
Agents	XR5000 (DACA, Xenova ®)	D19575 (glufosfamide)	RFS 2000	STI571 (imatinib, Glivec ®)	SCH66336 (lonafarnib)	OSI 774 (erlotinib, Tarceva ®)	ZK219477 (sagopilone)	LY317615 (enzastaurin)
Phase	II	II	II	II	I	II	II	I
Activation year	1999	2001	2000	2002	2004	2004	2006	2007
Sample size	16	32	17	51	19	110	38	17
Primary endpoint	Objective Response	Objective Response	Objective Response	Objective Response + PFS 6 months	DLT & MTD	PFS 6 months	Objective Response + PFS 6 months	DLT& MTD
Publication	Twelves et al, Ann Oncol. 2002	van den Bent et al, Ann Oncol. 2003	Raymond et al, Eur J Cancer. 2002	Raymond et al, J Clin Oncol. 2008	Stupp et al,2011	van den Bent et al, J Clin Oncol. 2009	Stupp R et al. Ann. Oncol. 2011	Rampling et al. In press.
Eligibility criteria								
Histology	GBM	GBM	GBM	Any primary gliomas	Any primary glioma	GBM or GBM-O (<25% oligo component)	GBM or GBM-O (<25% oligo component)	Grade 3 or 4 primary glioma
Recurrent disease documented by MRI	Yes	CT or MRI	CT or MRI	CT or MRI	CT or MRI	MRI	MRI	CT or MRI
Patients not amenable to radiotherapy can enter the trial	Not specified	Not specified	Not specified	Not specified	Yes	Not specified	Yes	Yes
Measurable disease, at least one bidimensionally measurable target lesion	Yes	Yes	Yes	Yes	Not mandatory, but recommended	Yes	Yes	Not mandatory, but recommended
PS≤2 or KPS>70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age ≥18 yrs	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
On stable or decreasing dose of corticosteroids for 2 weeks prior to registration	Yes	Yes, for 1 week prior to start of treatment	Yes	Yes	Yes	Yes, for 2 weeks prior to start of treatment	Yes, for 1 week prior to registration	Yes, for 1 week prior to registration
Initial surgery occurred within 3 months of registration	No	No	No	No	Allowed	No	No	Allowed
Surgery at recurrence	No	No	No	Yes, within 3 months measurable disease confirmed on a post operative imaging made within 72 hours from surgery	Not specified	Yes, within 3 months measurable disease confirmed on a post operative imaging made within 72 hours from surgery	Yes, within 3 months measurable disease confirmed on a post operative imaging made within 72 hours from surgery	Not specified
Last dose of radiotherapy administered within 3 months of registration	No	No	No	No	Not specified	No	No	Not specified
One line of prior chemotherapy completed within 6 weeks	Yes, adjuvant only	Yes, adjuvant or for the recurrence	Yes, adjuvant only	Yes, adjuvant or for the recurrence completed within 4 weeks (6 for nitrosoureas)	Yes, adjuvant or for the recurrence completed within 4 weeks (6 for nitrosoureas)	Yes, adjuvant only completed within 4 weeks (6 for nitrosoureas)*	Yes, adjuvant only completed within 4 weeks (6 for nitrosoureas)*	Yes, adjuvant or for the recurrence completed within 4 weeks (6 for nitrosoureas)**
Administration	Not specified	Not specified	Not specified	Switched to	Switched to	Both EIAED	EIAED	EIAED

of Anti-epileptic				non EIAED recommended .	non EIAED recommended .	and non EIAED allowed. Erlotinib dose was increased in case of EIAED.	switched to non EIAED after a wash-out period of at least one month prior to start of treatment.	switched to non EIAED after a wash-out period of at 2 weeks prior to start of treatment

Note: MTD: Maximum Tolerated Dose. DLT: Dose Limiting Toxicities. MacDonald Criteria were used to assess response and progression in all trials. PS= WHO Performance Status, KPS, Karnofski Performance Scale, *: Patients who received concomitant/adjuvant Temozolomide at first presentation were eligible and randomized to Tarceva or BCNU, **: Patients previously exposed to temozolomide were allowed (except patients progressing during temozolomide treatment or progressing within 6 weeks of temozolomide treatment completion).

Candidate prognostic factors

Factors screened for their prognostic value were sex, age, WHO PS, time from initial surgery or biopsy, prior chemotherapy, time since last dose of chemotherapy, time since last day of irradiation, surgery for recurrent disease, use of corticoids, administration of anti-epileptic drugs, United Kingdom Medical Research Council (MRC) neurological evaluation score (available in six studies), tumour load as assessed by the number of target lesions (defined as MRI contrast enhancing lesions with the largest diameter of at least 2 cm) and tumour size measured by the maximum diameter of the largest lesion. The effect of the presence of non-targeted lesions was also evaluated. Tumour localisation could be retrieved in five trials. The effect on prognosis of any concomitant chronic disease was also assessed. Table VI-2 (Online Supplemental Table 2) lists the factors screened and the coding conventions.

Table VI-2: List of factors screened and coding conventions.

	Coding convention
Screened prognostic factors	
Prior chemotherapy	no/without/with temozolomide
Prior surgery for recurrence	no/yes
Age	Per quartile **or** <median/>=median
WHO performance status	0/1/2 **or** 0/>0
Sex	male/female
Neurological deficit (MRC score)	no/some/moderate or major **or** no/yes
Baseline steroids administration	no/yes
Baseline anti-epileptic therapy	no/yes **or** no/EIAED/Non EIAED only
Number of target lesions	0-1/>1
Presence of non target lesions	no/yes
Largest lesion area (mm^2)	Per quartile **or** <median/>=median
Largest lesion diameter (mm)	
Time since last chemotherapy	
Time since last radiotherapy	
Time since initial surgery	
Time since initial histological diagnosis	
Frontal location	no/yes
Presence of chronic disease	no/yes

Patient outcome measurements

In all trials, Macdonald's criteria were used to assess tumour response.[20] Follow-up assessments were obtained every 8 weeks until disease progression. Progression free survival was computed as the time between the date of registration or randomization and the date of progression or death, whichever occurred first. Patients alive without evidence of progression were censored at the date of the last visit. Overall survival was calculated from the date of registration or randomization until date of death for any cause. Surviving patients were censored at date of last visit. In the EORTC/NCIC 26981/22981 trial residual survival was computed from the date of start of a new chemotherapy for recurrence after chemoradiation with TMZ until date of death. Patients alive at the date of last visit were censored.

Statistical considerations

Categorical data were tabulated with frequencies and percentages. Medians and ranges (minimum–maximum) were used to summarise continuous variables. The significance of the association between categorical factors was assessed by the Fisher Exact test (nominal) or the Jonckheere-Terpstra test (ordinal). Between continuous variables, significance was computed based on a specific student's statistic for testing the null hypothesis of no association. For the association between continuous and categorical (nominal) factors, the Wilcoxon rank sum test (two levels) or the Kruskall Wallis test (more than two levels) was used. In all analyses, p-values lower than 1% ($p < 0.01$) have been reported. Survival analyses were carried out in two patient populations: all GBM patients and the subset of patients who were treated for first progression after chemoradiation with TMZ. Kaplan Meier curves and logrank tests were computed stratified by category of treatment for the recurrence (an experimental agent, a cytotoxic agent, and a combination of both). Multivariate Cox models were fit of a significant difference between the different categories of treatment, non-stratified multivariate Cox models were fitted. Factors with a p-value less than 10% in univariate analysis were considered for Cox multivariate analyses. Proportional hazards (PH) assumptions were tested with the Supremum Test and by graphical method (LLS plot). PH assumptions were considered strongly violated if the p-values were less than 1%. The stepwise forward method was used for factors selection. The model's internal validity was assessed by the bootstrap method. Factors with an importance (PI: posterior probabilities that the regression coefficients are different from zero) lower than 60% were excluded from the final models. A significance level of 5% was applied to all multivariate analyses. Model's discrimination was assessed by the Harrel's C-index corrected for optimism by the bootstrap technique.[21,22] The model's goodness of fit was assessed by the Schemper's percentage of explained variation (PEV).[23] A PEV of at least 20% is considered a minimum requirement for a model to provide sufficiently precise individual survival predictions.[24] Prognostic calculators were developed for each final model in the population pre-treated with TMZ/RT → TMZ and the model calibration was assessed. Predictions for median PFS, OS, 6-month PFS (PFS6) and 1-year OS (OS12) were derived. SAS version 9.2 (SAS Institute Inc., Cary, NC, United States of America (USA)) was used for all statistical analyses except the computation of the C-index and calibration plots which were obtained from the R 'Design' and 'Hmisc' Packages. The percentage of explained variation was computed using the SAS macro RELIMPCR (Comparing the importance of prognostic factors in Cox regression using SAS).[24] The reflected method was used to estimate median survival with 95% confidence interval.[25] The loglog transformation was used for the 95% confidence intervals of PFS6 and OS12.

6.4 Results

Patients characteristics and correlation analyses

Four hundred eleven patients were recruited in the eight trials, 300 had a local histopathological diagnosis of GBM (astrocytoma grade IV according to WHO). Central pathology review was available for 155 patients (52%), in 149 patients (96%) GBM was confirmed. Baseline characteristics are summarised in Table VI-3. One hundred thirty eight patients had received TMZ/RT → TMZ as first-line therapy. One hundred fifty eight patients received standard fractionated RT to the equivalent of approximately 60 Gy alone or with another chemotherapy, four were treated without previous radiotherapy. Patients who received TMZ/RT → TMZ were significantly less often under baseline steroids (57% versus 73%, $p = 0.004$). At progression, patients in this subgroup received Carmustine (BCNU) (11%) or TMZ (7%) or various other therapies (36%, including Procarbazine, Lomustine, and Vincristine (PCV), Lomustine (CCNU), Irinotecan (CPT11), Etoposide (VP16), Natulan). No patient received Bevacizumab after protocol treatment. Eight percent of the patients were re-operated at the time of progression. Table VI-4 (Supplemental Table 3) shows the results of correlation analyses.

Table VI-3: Characteristics of all GBM patients and of patients (non) pre-treated by TMZ/RT→TMZ.

Patient and disease characteristics				***p*-value**
	Prior administration of TMZ/RT→TMZ			
	No(N=162)	**Yes (N=138)**	**Total (N=300)**	
	N (%)	**N (%)**	**N (%)**	
Central review				
No	76 (46.9)	69 (50.0)	145 (48.3)	0.64
Yes	86 (53.1)	69 (50.0)	155 (51.7)	
Central Diagnosis				
GBM	85 (52.5)	64 (46.4)	149 (49.7)	0.10
OA	0 (0.0)	2 (1.4)	2 (0.7)	
AA	1 (0.6)	1 (0.7)	2 (0.7)	
Other	0 (0.0)	2 (1.4)	2 (0.7)	
Missing	76 (46.9)	69 (50.0)	145 (48.3)	
Disease Status				
First progression	142 (87.7)	138 (100.0)	280 (93.3)	N/A
Second progression	20 (12.3)	0 (0.0)	20 (6.7)	
Presence of measurable disease				
No	2 (1.2)	5 (3.6)	7 (2.3)	0.25
Yes	160 (98.8)	133 (96.4)	293 (97.7)	
Sex				
Male	101 (62.3)	95 (68.8)	196 (65.3)	0.27
Female	61 (37.7)	43 (31.2)	104 (34.7)	
WHO Performance Status				
0	44 (27.2)	40 (29.0)	84 (28.0)	0.20
1	88 (54.3)	84 (60.9)	172 (57.3)	
2	30 (18.5)	14 (10.1)	44 (14.7)	
Neurological deficit				
No	50 (30.9)	50 (36.2)	100 (33.3)	0.09
Some	47 (29.0)	54 (39.1)	101 (33.7)	
Moderate	38 (23.5)	20 (14.5)	58 (19.3)	
Major	5 (3.1)	2 (1.4)	7 (2.3)	
Missing	22 (13.6)	12 (8.7)	34 (11.3)	
Age (years)				
Median	53.5	53.5	53.5	0.72
Range	19.0 - 75.0	18.0 - 78.0	18.0 - 78.0	
N	162	138	300	
Associated chronic disease				
No	110 (67.9)	73 (52.9)	183 (61.0)	0.78
Yes	49 (30.2)	30 (21.7)	79 (26.3)	
Missing	3 (1.9)	35 (25.4)	38 (12.7)	
Time since initial diagnosis (weeks)				
Median	42.3	47.1	44.1	0.16
Range	2.0 - 319.6	6.4 - 393.1	2.0 - 393.1	
N	162	138	300	
Extent of initial surgery				
Biopsy	6 (3.7)	4 (2.9)	10 (3.3)	1.00
Resection	57 (35.2)	36 (26.1)	93 (31.0)	
Missing	99 (61.1)	98 (71.0)	197 (65.7)	
Time since initial surgery (weeks)				
Median	40.1	45.0	41.9	0.06
Range	2.0 - 222.4	11.7 - 393.1	2.0 - 393.1	
N	157	131	288	
Prior radiotherapy				
No	4 (2.5)	0 (0.0)	4 (1.3)	0.13
Yes	158 (97.5)	138 (100.0)	296 (98.7)	
Time since last dose of irradiation (weeks)				

Patient and disease characteristics				***p*-value**
	Prior administration of TMZ/RT→TMZ			
	No(N=162)	**Yes (N=138)**	**Total (N=300)**	
	N (%)	**N (%)**	**N (%)**	
Median	30.1	35.7	31.9	0.16
Range	4.9 - 308.6	13.0 - 221.6	4.9 - 308.6	
N	156	138	294	
Prior chemotherapy				
No	89 (54.9)	0 (0.0)	89 (29.7)	N/A
Yes, without temozolomide	55 (34.0)	0 (0.0)	55 (18.3)	
Yes, with temozolomide	18 (11.1) †	138 (100.0)	156 (52.0)	
Time since last chemotherapy (weeks)				
Median	12.1	8.1	9.6	0.02*
Range	3.4 - 128.6	3.9 - 171.3	3.4 - 171.3	
N	73	138	211	
Surgery for recurrence				
No	145 (89.5)	118 (85.5)	263 (87.7)	0.38
Yes	17 (10.5)	20 (14.5)	37 (12.3)	
Baseline steroids				
No	44 (27.2)	60 (43.5)	104 (34.7)	0.004**
Yes	118 (72.8)	78 (56.5)	196 (65.3)	
Baseline anti-epileptic				
No AED	56 (34.6)	51 (37.0)	107 (35.7)	0.72
EIAED	43 (26.5)	31 (22.5)	74 (24.7)	
Non EIAED only	63 (38.9)	56 (40.6)	119 (39.7)	
Number of target lesions				
0	2 (1.2)	5 (3.6)	7 (2.3)	0.22
1	132 (81.5)	114 (82.6)	246 (82.0)	
2	24 (14.8)	17 (12.3)	41 (13.7)	
3	3 (1.9)	2 (1.4)	5 (1.7)	
4	1 (0.6)	0 (0.0)	1 (0.3)	
Presence of non target lesions				
No	137 (84.6)	116 (84.1)	253 (84.3)	1.00
Yes	25 (15.4)	22 (15.9)	47 (15.7)	
Frontal location				
No	50 (30.9)	75 (54.3)	125 (41.7)	1.00
Yes	27 (16.7)	42 (30.4)	69 (23.0)	
Missing	85 (52.5)	21 (15.2)	106 (35.3)	
Largest lesion area (mm^2)				
Median	1483.5	1134.0	1289.0	0.02*
Range	125.0 - 8000.0	80.0 - 4950.0	80.0 - 8000.0	
N obs	160	132	292	
Largest lesion diameter (mm)				
Median	43.0	41.5	42.0	0.07
Range	19.0 - 100.0	10.0 - 94.0	10.0 - 100.0	
N obs	160	132	292	

† Temozolomide administered at first progression after radiotherapy. * $p<0.05$ ** $p<0.01$.

Table VI-4: Correlation analyses.

	PS	Age	Sex	Ster	AED	NeuroD	TarL	Tsize	InitD	Lrad	Psurg	Front
PS	NA											
Age	NS	NA										
Sex	NS	NS	NA									
Ster	P<0.0001	NS	NS	NA								
AED	P=0.008	NS	NS	P=0.002	NA							
NeuroD	P<0.0001	P=0.001	NS	P<0.0001	P=0.002	NA						
TarL	NS	NS	NS	P=0.001	NS	NS	NA					
Tsize	P=0.006	NS	P=0.009	P<0.0001	NS	P=0.005	NS	NA				
InitD	NS	NS	NS	P<0.0001	NS	NS	NS	NS	NA			
Lrad	NS	NS	NS	P<0.0001	NS	NS	NS	NS	NS	NA		
Psurg	NS	P=0.007	NS	NS	NS	NS	NS	NS	NS	P=0.008	NA	
Front	NS	NS	NS	NS	NS	P=0.006	NS	NS	NS	NS	NS	NA

Legend: NA: Not applicable, NS: p>1%, Patients under steroids (Ster) were more often under anti-epileptic drugs (AED, p=0.002), had a worse performance status (PS,p<0.0001) and more often neurological deficits (NeuroD p<0.0001). They had more often more than one target lesion (TarL,p=0.001). Times since their initial diagnosis (InitD,54 vs 40 weeks, p<0.0001) and last radiotherapy (Lrad,44 vs 29 weeks, p<0.0001) were shorter and they more often had larger tumors (Tsize,median 49 vs 31 mm, p<0.0001). Male patients had larger tumors (Sex,45 vs 38 mm for females, p=0.009). Patients with prior surgery for recurrence were younger (Psurg,median 48 vs 55 years, p=0.007) and had a longer time since radiotherapy (41 vs 30 weeks, p=0.008). Patients treated with AED more often had a poor performance status (p=0.008) and had more often and more severe neurological deficits (p=0.002). Patients with frontal involvement had less often or less severe neurological deficits (Front,p=0.006). Tumor area was larger in patients with a poorer performance status (p=0.006). They had more often or more severe neurological deficits (p<0.0001). Neurological deficits were also associated with older age (p=0.001) and a larger tumor (p=0.005). Older age was not significantly associated with a deteriorated performance status (p=0.053). The percentages of patients with PS 2 were 14.4% for age>54 and 15.2% for age ≤ 54.

Outcome description and prognostic factor analyses

Table VI-5 and VI-6 (Supplemental Table 4a & b) summarise the univariate analyses of PFS and OS for each candidate prognostic factor and each category of treatment at recurrence in the two populations by presenting median PFS, 6-month PFS rate (PFS6), median OS, 1-year OS rate (OS12), hazard ratios and p-values. Table VI-7 displays the results of the final multivariate Cox analyses. For both PFS and OS, the results of the proportional hazards assumptions analyses can be found at http://www.eortc.be/tools/recgbmcalculator/Sensitivity.aspx. No strong PH assumption violation was observed in all analyses.

Table VI-5: Univariate screening of prognostic factors for PFS

		GBM pre-treated with TMZ/RT→TMZ (n=138)					all GBM (n=300)			
	N	Median PFS (95% CI)	6-month PFS probability (95% CI)	HR (95% CI)	p	N	Median PFS (95% CI)	6-month PFS probability (95% CI)	HR (95% CI)	p
All patients	138	1.84 (1.74, 2.14)	18.25 (12.31,25.13)	N/A	N/A	300	1.81 (1.74, 1.91)	14.72 (10.98,18.98)	N/A	N/A
Treatment at the recurrence										
16011: Imatinib	15	1.71 (1.64, 5.82)	20.00 (4.89, 42.39)	1.00	0.12 df=7	51	1.81 (1.71, 2.43)	15.69 (7.34, 26.88)	1.00	<1E-4 df=9
16027: TMZ+Lonafarnib	15	2.04 (1.64, 5.32)	13.33 (2.19, 34.57)	1.01 (0.49, 2.08)		19	2.04 (1.77, 5.32)	15.79 (3.92, 34.94)	1.00 (0.59, 1.70)	
16991: XR5000	0	N/A	N/A	N/A		16	1.30 (1.38, 2.96)	0.00 (,)	3.02 (1.71, 5.36)	
16994G: Glufosfamide	3	1.77 (1.68, 9.79)	33.33 (0.90, 77.41)	0.83 (0.24, 2.88)		32	1.41 (1.35, 2.63)	3.13 (0.24, 13.72)	1.72 (1.10, 2.70)	
16996G: RFS 2000	2	1.36 (N, N)	0.00 (,)	5.01 (1.10, 22.8)		17	1.45 (1.38, 2.56)	5.88 (0.39, 23.50)	1.44 (0.83, 2.50)	
26054: TMZ+Enzastaurin	11	5.52 (5.09, 7.39)	36.36 (11.18, 62.68)	0.66 (0.30, 1.45)		17	5.52 (4.47, 8.77)	41.18 (18.58, 62.64)	0.59 (0.34, 1.03)	
26061: Sagopilone	35	1.58 (1.38, 2.04)	8.57 (2.20, 20.57)	1.48 (0.80, 2.73)		38	1.58 (1.38, 1.84)	7.89 (2.04, 19.10)	1.49 (0.97, 2.29)	
26034: TMZ	0	N/A	N/A	N/A		27	3.75 (2.30, 6.05)	29.63 (14.06, 47.03)	0.76 (0.47, 1.21)	
26034: BCNU	29	2.02 (1.84, 5.49)	21.43 (8.71, 37.83)	0.93 (0.50, 1.75)		29	2.02 (1.84, 5.49)	21.43 (8.71, 37.83)	0.92 (0.58, 1.46)	
26034: Erlotinib	28	1.92 (1.74, 5.13)	21.43 (8.71, 37.83)	0.91 (0.49, 1.71)		54	1.81 (1.71, 1.94)	12.96 (5.70, 23.30)	1.18 (0.80, 1.73)	
Category of treatment										
No cytotoxic (targeted agent only)	83	1.71 (1.61, 1.91)	15.66 (8.83, 24.26)	1.00	0.35 df=2	208	1.68 (1.54, 1.74)	9.62 (6.09, 14.08)	1.00	0.0005 df=2
Cytotoxic (BCNU or TMZ)	29	2.02 (1.74, 3.29)	21.43 (8.71, 37.83)	0.83 (0.54, 1.28)	0.40 df=1	56	2.37 (1.84, 3.75)	25.45 (14.89, 37.41)	0.63 (0.47, 0.86)	0.003 df=1
Combination (TMZ + targeted agent)	26	4.42 (1.94, 5.45)	23.08 (9.38, 40.31)	0.74 (0.47, 1.15)	0.18 df=1	36	3.68 (2.04, 5.45)	27.78 (14.48, 42.78)	0.58 (0.40, 0.82)	0.0025 df=1
Sex										
Male	95	1.74 (1.68, 2.10)	15.96 (9.41, 24.04)	1.00	0.66 df=1	196	1.77 (1.71, 1.87)	12.31 (8.17, 17.34)	1.00	0.41 df=1
Female	43	2.04 (1.77, 5.32)	23.26 (12.05, 36.60)	0.92 (0.64, 1.33)		104	1.84 (1.71, 2.07)	19.23 (12.32, 27.30)	0.90 (0.71, 1.15)	
Performance Status										
0	40	2.04 (1.71, 5.52)	30.00 (16.80, 44.37)	1.42 (1.06, 1.90)	0.02 df=1	84	2.04 (1.77, 3.75)	27.38 (18.36, 37.16)	1.41 (1.17, 1.69)	0.0003 df=1
1	84	1.84 (1.71, 2.14)	13.25 (7.04, 21.46)			172	1.77 (1.71, 1.94)	11.11 (6.96, 16.34)		
2	14	1.68 (1.02, 5.45)	14.29 (2.32, 36.55)			44	1.69 (1.41, 1.84)	4.55 (0.83, 13.61)		
0	40	2.04 (1.71, 5.52)	30.00 (16.80,44.37)	1.00	0.04 df=1	84	2.04 (1.77, 3.75)	27.38 (18.36, 37.16)	1.00	0.003 df=1
>0	98	1.81 (1.71, 2.07)	13.40 (7.54, 20.96)	1.50 (1.01, 2.22)		216	1.74 (1.71, 1.84)	9.77 (6.27, 14.18)	1.50 (1.15, 1.97)	
Neurological deficit										
No	50	1.77 (1.58, 2.86)	20.00 (10.32, 31.97)	1.06 (0.83, 1.36)	0.61 df=1	100	1.84 (1.74, 2.37)	20.00 (12.83, 28.32)	1.16 (0.99, 1.37)	0.06 df=1

Some	54	1.81 (1.71, 2.10)	9.43 (3.47, 19.05)			101	1.74 (1.68, 1.94)	8.00 (3.74, 14.35)		
Moderate/major	22	1.82 (1.64, 6.18)	27.27 (11.12, 46.37)			65	1.74 (1.51, 1.87)	13.85 (6.81, 23.34)		
Age (quartiles)	138	N/A	N/A	0.93 (0.80, 1.08)	0.36 df=1	300	N/A	N/A	0.99 (0.90, 1.09)	0.86 df=1
Age (median)										
< 54 yrs	69	1.81 (1.71, 2.14)	15.94 (8.49, 25.49)	1.00	0.54 df=1	150	1.74 (1.68, 1.94)	16.00 (10.66, 22.31)	1.00	0.60 df=1
≥ 54 yrs	69	1.99 (1.71, 3.75)	20.59 (11.96, 30.84)	0.90 (0.64, 1.27)		150	1.84 (1.74, 2.10)	13.42 (8.54, 19.41)	0.94 (0.75, 1.18)	
Associated chronic disease										
No	73	1.94 (1.74, 3.29)	23.29 (14.38, 33.45)	1.00	0.98 df=1	183	1.84 (1.77, 2.30)	16.39 (11.45, 22.11)	1.00	0.51 df=1
Yes	30	2.10 (1.91, 5.45)	17.24 (6.29, 32.73)	0.99 (0.64, 1.55)		79	1.87 (1.77, 2.60)	14.10 (7.50, 22.74)	0.91 (0.70, 1.20)	
Time since initial diagnosis (quartiles)	138	N/A	N/A	1.06 (0.91, 1.24)	0.46 df=1	300	N/A	N/A	0.95 (0.86, 1.05)	0.32 df=1
Time since initial surgery (quartiles)	131	N/A	N/A	1.04 (0.89, 1.21)	0.62 df=1	288	N/A	N/A	0.96 (0.86, 1.06)	0.38 df=1
Time since last irradiation (quartiles)	138	N/A	N/A	1.10 (0.94, 1.28)	0.23 df=1	294	N/A	N/A	0.96 (0.86, 1.06)	0.42 df=1
Prior chemotherapy										
No	0	N/A	N/A	N/A	N/A	89	1.84 (1.74, 2.14)	14.61 (8.23, 22.73)	1.00	0.54 df=2
Yes, without temozolomide	0	N/A	N/A	N/A	N/A	55	1.58 (1.38, 1.84)	9.09 (3.34, 18.41)	1.11 (0.78, 1.58)	0.57 df=1
Yes, with temozolomide	138	1.84 (1.74, 2.14)	18.25 (12.31, 25.13)	N/A	N/A	156	1.84 (1.74, 2.10)	16.77 (11.39, 23.06)	0.93 (0.71, 1.22)	0.60 df=1
Time since last chemotherapy (quartiles)	138	N/A	N/A	1.01 (0.85, 1.19)	0.95 df=1	211	N/A	N/A	1.00 (0.87, 1.13)	0.94 df=1
Surgery for recurrence										
No	118	1.81 (1.71, 2.10)	18.80 (12.33, 26.34)	1.00	0.43 df=1	263	1.81 (1.74, 1.94)	14.89 (10.89, 19.47)	1.00	0.30 df=1
Yes	20	1.91 (1.71, 3.29)	15.00 (3.73, 33.47)	1.21 (0.75, 1.97)		37	1.84 (1.71, 2.76)	13.51 (4.94, 26.40)	1.21 (0.85, 1.71)	
Baseline steroids										
No	60	1.77 (1.68, 2.76)	22.03 (12.52, 33.24)	1.00	0.82 df=1	104	1.84 (1.74, 2.79)	23.30 (15.68, 31.81)	1.00	0.08 df=1
Yes	78	1.87 (1.74, 2.56)	15.38 (8.44, 24.23)	0.96 (0.67, 1.38)		196	1.81 (1.71, 1.91)	10.20 (6.47, 14.92)	1.25 (0.97, 1.62)	
Baseline anti-epileptic										
No AED	51	2.04 (1.71, 4.04)	22.00 (11.80, 34.21)	1.00	0.17 df=2	107	1.86 (1.71, 2.30)	16.98 (10.56, 24.70)	1.00	0.39 df=2
EIAED	31	1.77 (1.68, 1.94)	6.45 (1.15, 18.62)	1.56 (0.97, 2.50)	0.06 df=1	74	1.71 (1.64, 1.94)	8.11 (3.31, 15.70)	1.22 (0.90, 1.65)	0.20 df=1
Non EIAED only	56	1.74 (1.71, 3.29)	21.43 (11.85, 32.88)	1.25 (0.84, 1.87)	0.27 df=1	119	1.81 (1.74, 2.10)	16.81 (10.74, 24.04)	1.03 (0.79, 1.34)	0.82 df=1
Number of target lesions										
0-1	119	1.94 (1.81, 2.76)	20.34 (13.63, 28.01)	1.00	0.02 df=1	253	1.84 (1.77, 2.10)	16.27 (12.02, 21.09)	1.00	0.007 df=1
>1	19	1.61 (1.45, 2.04)	5.26 (0.36, 21.43)	1.86 (1.12, 3.08)		47	1.58 (1.38, 1.81)	6.38 (1.66, 15.75)	1.55 (1.13, 2.13)	
Presence of non target lesions										
No	117	1.82 (1.74, 2.10)	17.24 (11.02, 24.63)	1.00	0.13 df=1	254	1.81 (1.74, 1.87)	13.83 (9.92, 18.39)	1.00	0.34 df=1
Yes	21	4.04 (1.48, 5.52)	23.81 (8.67, 43.08)	0.68 (0.42, 1.12)		46	1.68 (1.48, 4.04)	19.57 (9.67, 32.01)	0.85 (0.62, 1.18)	
Frontal location										
No	75	1.89 (1.74, 2.76)	16.22 (8.91, 25.45)	1.00	0.48 df=1	125	1.84 (1.74, 2.10)	16.13 (10.30, 23.12)	1.00	0.27 df=1
Yes	42	1.84 (1.58, 4.47)	21.43 (10.61, 34.72)	0.87 (0.58, 1.30)		69	1.94 (1.84, 3.75)	23.19 (14.08, 33.64)	0.84 (0.62, 1.15)	
Largest lesion area (quartiles)	132	N/A	N/A	1.06 (0.90, 1.23)	0.49 df=1	292	N/A	N/A	1.09 (0.99, 1.21)	0.09 df=1
Largest lesion	132	N/A	N/A	1.08	0.36	292	N/A	N/A	1.09	0.11

diameter (quartiles)				(0.92, 1.27)	df=1				(0.98, 1.21)	df=1
Largest lesion diameter (median)										
<=42 mm	66	1.77 (1.71, 2.37)	18.46 (10.17, 28.69)	1.00	0.77 df=1	147	1.79 (1.71, 1.94)	17.81 (12.10, 24.42)	1.00	0.13 df=1
>42 mm	66	1.84 (1.74, 2.76)	16.67 (8.88, 26.56)	1.05 (0.74, 1.50)		145	1.81 (1.71, 2.04)	10.34 (6.07, 15.93)	1.20 (0.95, 1.52)	

Note: df= degree of freedom of the statistical test. df=1 for binary factors, for continuous variables and scores (eg WHO performance status). df=9 is for the global homogeneity test among the 10 protocol treatment arms. Imatinib was used as the reference arm. Because sample sizes are small and treatments were not randomized, no pairwise comparisons were performed.

Table VI-6: Univariate screening of prognostic factors for OS

		GBM pre-treated with TMZ/RT→TMZ (n=138)					All GBM (n=300)			
	N	Median OS (95% CI)	1-year OS probability (95% CI)	HR (95% CI)	p	N	Median OS (95% CI)	1-year OS probability (95% CI)	HR (95% CI)	p
All patients	138	7.13 (6.21, 8.71)	26.64 (19.52, 34.27)	N/A	N/A	300	6.21 (5.68, 7.13)	22.07 (17.53, 26.95)	N/A	N/A
Treatment at the recurrence										
16011: Imatinib	15	5.75 (2.96, 14.39)	26.67 (8.26, 49.63)	1.00	0.37 df=7	51	6.14 (4.53, 8.97)	23.53 (13.04, 35.78)	1.00	0.0006 (df=9)
16027: TMZ+Lonafarnib	15	7.00 (5.32, 10.74)	6.67 (0.43, 26.03)	1.10 (0.53, 2.26)		19	6.37 (5.32, 9.92)	5.26 (0.36, 21.43)	1.26 (0.74, 2.14)	
16991: XR5000	0	N/A	N/A	N/A		16	3.19 (2.50, 5.06)	0.00 (,)	3.15 (1.74, 5.69)	
16994G: Glufosfamide	3	12.88 (11.01, 15.93)	66.67 (5.41, 94.52)	0.58 (0.17, 2.03)		32	4.83 (4.17, 9.26)	13.04 (4.12, 27.22)	1.20 (0.76, 1.90)	
16996G: RFS 2000	2	3.58 (3.58, N)	50.00 (0.60, 91.04)	0.23 (0.03, 1.74)		17	5.88 (4.47, 14.69)	29.41 (10.71, 51.15)	0.79 (0.45, 1.38)	
26054: TMZ+Enzastaurin	11	11.63 (7.39, 22.57)	36.36 (11.18, 62.68)	0.63 (0.28, 1.40)		17	11.70 (7.39, 22.57)	47.06 (22.96, 67.97)	0.52 (0.28, 0.96)	
26061: Sagopilone	35	7.72 (5.29, 12.32)	31.43 (17.09, 46.84)	0.79 (0.43, 1.47)		38	7.56 (5.29, 12.32)	31.58 (17.73, 46.39)	0.85 (0.55, 1.30)	
26034: TMZ	0	N/A	N/A	N/A		27	9.59 (8.11, 13.21)	33.33 (16.77, 50.86)	0.76 (0.48, 1.23)	
26034: BCNU	29	5.65 (4.70, 7.13)	14.29 (4.50, 29.50)	1.05 (0.55, 1.99)		29	5.65 (4.70, 7.13)	14.29 (4.50, 29.50)	1.11 (0.68, 1.79)	
26034: Erlotinib	28	8.38 (6.28, 15.24)	34.03 (17.26, 51.61)	0.63 (0.34, 1.20)		54	6.65 (5.22, 8.44)	19.23 (9.95, 30.80)	0.94 (0.64, 1.39)	
Category of treatment										
No cytotoxic (targeted agent only)	83		33.22 (23.32, 43.43)	1.00	0.29 df=2	208	5.75 (5.06, 6.80)	21.15 (15.85, 26.98)	1.00	0.42 df=2
Cytotoxic (BCNU or TMZ)	29		14.29 (4.50, 29.50)	1.43 (0.91, 2.25)	0.12 df=1	56	7.13 (5.49, 8.71)	23.64 (13.47, 35.43)	0.89 (0.66, 1.22)	0.48 df=1
Combination (TMZ + targeted agent)	26		19.23 (7.01, 35.97)	1.15 (0.73, 1.81)	0.55 df=1	36	7.57 (5.88, 10.94)	25.00 (12.43, 39.78)	0.79 (0.54, 1.15)	0.22 df=1
Sex										
Male	95	6.93 (5.59, 9.49)	27.66 (19.06, 36.92)	1.00	0.86 df=1	196	5.62 (4.83, 7.39)	21.66 (16.17, 27.69)	1.00	0.20 df=1
Female	43	7.75 (5.75, 10.94)	24.22 (12.61, 37.88)	0.97 (0.66, 1.41)		104	6.14 (5.19, 10.15)	22.80 (15.20, 31.33)	0.85 (0.66, 1.09)	
WHO Performance Status										
0	40	10.94 (9.49, 12.88)	35.00 (20.81, 49.55)	1.68 (1.22, 2.31)	2E-3 df=1	84	9.61 (8.41, 11.10)	33.33 (23.53, 43.42)	1.64 (1.35, 1.99)	<.1E-4 df=1
1	84	6.37 (5.75, 7.43)	24.73 (16.01, 34.45)			172	5.82 (5.19, 7.00)	19.85 (14.20, 26.21)		
2	14	3.07 (2.07, 9.76)	14.29 (2.32, 36.55)			44	3.68 (3.02, 5.19)	9.09 (2.90, 19.71)		
0	40	10.94 (9.49, 12.88)	35.00 (20.81, 49.55)	1.00	0.02 df=1	84	9.61 (8.41, 11.10)	33.33 (23.53, 43.42)	1.00	0.0001 df=1
>0	98	5.88 (5.49, 7.26)	23.22 (15.35, 32.06)	1.60 (1.07, 2.41)		216	5.29 (4.76, 5.88)	17.63 (12.83, 23.06)	1.70 (1.30, 2.24)	
Neurological deficit										

No	50	7.92 (6.21, 11.04)	32.00 (19.70, 44.97)	1.28 (0.99, 1.66)	0.06 df=1	100	8.18 (7.13, 9.89)	30.00 (21.36, 39.11)	1.37 (1.16, 1.62)	0.0002 df=1
Some	54	6.87 (5.75, 10.55)	25.47 (14.61, 37.82)			101	5.82 (5.22, 7.33)	19.68 (12.48, 28.09)		
Moderate/major	22	5.50 (3.12, 9.76)	13.64 (3.41, 30.87)			65	4.80 (3.22, 6.37)	12.31 (5.75, 21.50)		
Age (quartiles)	138		N/A	1.02 (0.87, 1.20)	0.81 df=1	300	N/A	N/A	1.07 (0.96, 1.18)	0.21 df=1
Age (median)										
< 54 yrs	69	7.00 (5.82, 10.15)	26.09 (16.43, 36.79)	1.00	0.68 df=1	150	7.13 (6.21, 8.41)	23.51 (17.06, 30.57)	1.00	0.30 df=1
≥ 54 yrs	69	7.26 (5.75, 10.38)	27.19 (17.19, 38.16)	1.08 (0.76, 1.53)		150	5.72 (4.93, 6.97)	20.64 (14.52, 27.52)	1.13 (0.90, 1.43)	
Associated chronic disease										
No	73	7.26 (5.82, 9.92)	23.29 (14.38, 33.45)	1.00	0.97 df=1	183	7.03 (5.82, 7.75)	19.38 (13.99, 25.43)	1.00	0.94 df=1
Yes	30	6.21 (5.49, 13.63)	29.89 (14.46, 47.03)	0.99 (0.63, 1.56)		79	5.08 (4.53, 6.21)	23.96 (15.14, 33.93)	1.01 (0.77, 1.33)	
Time since initial diagnosis (quartiles)	138	N/A	N/A	0.97 (0.83, 1.14)	0.73 df=1	300	N/A	N/A	0.90 (0.81, 1.00)	0.06 df=1
Time since initial surgery (quartiles)	131	N/A	N/A	1.00 (0.85, 1.17)	0.99 df=1	288	N/A	N/A	0.94 (0.84, 1.04)	0.23 df=1
Time since last irradiation (quartiles)	138	N/A	N/A	0.99 (0.85, 1.16)	0.91 df=1	294	N/A	N/A	0.94 (0.84, 1.04)	0.23 df=1
Prior chemotherapy										
No	0	N/A	N/A	N/A	N/A	89	6.14 (5.03, 7.79)	13.87 (7.61, 22.00)	1.00	0.096 df=2
Yes, without temozolomide	0	N/A	N/A	N/A	N/A	55	5.19 (4.34, 7.13)	25.45 (14.89, 37.41)	0.76 (0.53, 1.09)	0.14 df=1
Yes, with temozolomide	138	7.13 (6.21, 8.71)	26.64 (19.52, 34.27)	N/A	N/A	156	6.87 (5.82, 8.21)	25.49 (18.90, 32.57)	0.74 (0.56, 0.98)	0.03 df=1
Time since last chemotherapy (quartiles)	138		N/A	1.03 (0.87, 1.23)	0.70 df=1	211	N/A	N/A	0.99 (0.87, 1.13)	0.87 df=1
Surgery for recurrence										
No	118	7.13 (5.85, 9.92)	28.68 (20.76, 37.06)	1.00	.27 df=1	263	6.18 (5.62, 7.29)	22.53 (17.65, 27.79)	1.00	0.25 df=1
Yes	20	7.13 (4.44, 8.71)	15.00 (3.73, 33.47)	1.32 (0.81, 2.15)		37	6.87 (4.53, 8.41)	18.92 (8.33, 32.78)	1.23 (0.87, 1.74)	
Baseline steroids										
No	60	10.74 (9.76, 13.73)	39.85 (27.32, 52.08)	1.00	0.00 01 df=1	104	10.74 (9.23, 13.21)	42.31 (32.65, 51.64)	1.00	< 1E-4 df=1
Yes	78	5.40 (4.70, 6.28)	16.67 (9.41, 25.71)	2.05 (1.42, 2.98)		196	5.09 (4.37, 5.72)	11.37 (7.39, 16.30)	2.28 (1.75, 2.97)	
Baseline anti-epileptic										
No AED	51	7.26 (5.75, 10.94)	29.00 (17.12, 41.97)	1.00	0.28 df=2	107	6.28 (5.26, 7.79)	22.38 (14.92, 30.80)	1.00	0.69 df=2
EIAED	31	5.82 (5.19, 7.72)	16.13 (5.88, 30.88)	1.40 (0.87, 2.24)	0.16 df=1	74	5.83 (5.19, 7.43)	18.92 (10.97, 28.53)	1.12 (0.82, 1.52)	0.47 df=1
Non EIAED only	56	7.92 (6.21, 11.37)	30.36 (18.96, 42.54)	0.99 (0.66, 1.49)	0.98 df=1	119	7.03 (5.55, 8.21)	23.75 (16.53, 31.73)	0.99 (0.75, 1.30)	0.92 df=1
Number of target lesions										
0-1	119	7.75 (6.44, 10.38)	29.24 (21.29, 37.62)	1.00	1E-4 df=1	253	7.03 (5.88, 7.92)	25.03 (19.83, 30.55)	1.00	< 1E-4 df=1
>1	19	4.90 (4.37, 6.44)	10.53 (1.78, 28.43)	2.93 (1.73, 4.98)		47	4.70 (3.98, 5.85)	6.38 (1.66, 15.75)	1.98 (1.43, 2.74)	
Presence of non target lesions										
No	117	7.00 (5.85, 8.71)	24.76 (17.27, 32.97)	1.00	0.24 df=1	254	6.21 (5.55, 7.13)	21.01 (16.19, 26.27)	1.00	0.27 df=1
Yes	21	7.41 (5.72, 20.44)	36.36 (17.43, 55.67)	0.74 (0.45, 1.22)		46	6.26 (5.26, 9.13)	27.66 (15.86, 40.78)	0.83 (0.60, 1.16)	
Frontal location										
No	75	6.41 (5.82, 8.38)	24.31 (15.07, 34.74)	1.00	0.12 df=1	125	6.37 (5.75, 7.79)	22.96 (15.61, 31.18)	1.00	0.04 df=1

Yes	42	8.41 (6.97, 11.10)	28.57 (15.96, 42.52)	0.72 (0.48, 1.09)		69	8.41 (7.36, 10.78)	28.99 (18.85, 39.89)	0.71 (0.52, 0.98)	
Largest lesion area (quartiles)	132	N/A	N/A	1.53 (1.30, 1.80)	<1E-4 df=1	292	N/A	N/A	1.44 (1.29, 1.60)	<1E-4 df=1
Largest lesion diameter (quartiles)	132	N/A	N/A	1.56 (1.32, 1.85)	<1E-4 df=1	292	N/A	N/A	1.39 (1.24, 1.56)	<1E-4 df=1
Largest lesion diameter (median)										
<=42 mm	66	10.94 (8.41, 13.34)	39.36 (27.47, 51.02)	1.00	<1E-4 df=1	147	8.48 (7.10, 10.58)	32.57 (25.10, 40.24)	1.00	<1E-4 df=1
>42 mm	66	5.40 (4.47, 6.97)	12.12 (5.66, 21.20)	2.15 (1.49, 3.10)		145	4.96 (4.17, 5.72)	9.83 (5.65, 15.38)	1.97 (1.54, 2.52)	

Note: HR: Hazard Ratio. df= degree of freedom of the statistical test. df=1 for binary factors, for continuous variables and scores (eg WHO performance status). df=9 is for the global homogeneity test among the 10 protocol treatment arms. Imatinib was used as the reference arm. Because sample sizes are small and treatments were not randomized, no pairwise comparisons were performed.

Progression free survival

For all GBM patients, median PFS was 1.8 months (1.7–1.9) and PFS6 was 14.7% (11.0–19.0). Patients treated with experimental agents at the recurrence had a lower PFS6 (9.6%) compared to patients who received a combined therapy of an experimental agent and TMZ (27.8%, p = 0.003) or BCNU (25.5%, p = 0.003). Among the factors screened, a higher WHO PS (p = 0.0003), the presence of neurological deficits (p = 0.06), the administration of steroids (p = 0.08), multiple target lesions (p = 0.007), larger area of the target lesions (p = 0.09), were negatively associated to PFS in univariate analyses stratified by the category of treatment (p < 10%). The maximum diameter of the largest lesion was borderline not significant (p = 0.11). After stepwise selection and assessment of the model's internal validity by the bootstrap technique, two factors remained in the final prognostic model: WHO PS (code: 0/1/2, p = 0.0002, PI = 91%) and the number of target lesions (p = 0.004, PI = 84%). The C-index corrected for optimism was 0.62 and PEV was 3.4%. In the subset having received TMZ/RT → TMZ as first-line therapy (n = 138), median PFS was 1.84 months (1.74, 2.14) and PFS6 was 18.3% (12.3, 25.1). There was no significant difference of PFS between the three categories of treatment for the recurrence (p = 0.35). WHO PS (code: 0, >0) (p = 0.04), the number of target lesions (p = 0.02) were the only factors selected by univariate analysis. Both variables were selected by stepwise technique. Although performance status had a PI lower than 0%, it was maintained in the final model (PI = 58%) assuming that the reason was a lack of power in this subset. Discrimination and goodness of fit of this two factors model was low (C index = 0.56, PEV = 4.6%).

Overall survival

For all GBM patients, median OS was 6.2 months (5.7, 7.1), OS12 was 22.1% (17.5, 27.0). There was no significant difference of survival between the three categories of treatment (p = 0.42). WHO PS (p 6 0.0001), presence of neurological deficits (p = 0.0002), time since initial diagnosis (p = 0.06), baseline administration of steroids (p < 0.0001), number of target lesions (p < 0.0001), tumour size (largest tumour diameter, p < 0.0001), frontal tumour location (p = 0.02) and prior chemotherapy with TMZ (p = 0.096) were the factors which passed the 10% significance criterion (see http://www.eortc.be/tools/recgbmcalculator/Curves.aspx). Age was not related to survival outcome (p = 0.21) and undergoing a surgery for recurrence did not significantly impact on the survival (p = 0.25). After stepwise selection and assessment of factor importance by bootstrap, WHO PS (p = 0.008, PI = 69%), baseline steroids (p = 0.0001, PI = 91%), the number of target lesions (p = 0.003, PI = 80%), frontal location (p = 0.02,PI = 62%), tumour size (maximum diameter of the largest lesion, split by the median ie ≤42 mm versus >42 mm, p = 0.015, PI = 70%) were retained in the final multivariate model. The C-index was 0.68 and PEV = 15.7%. The C-index was not substantially increased when continuous measures for tumour size were considered (C-index = 0.69). Therefore, for ease of interpretation the model with binary

tumour size was considered. In the patient group having received TMZ/RT → TMZ as first-line therapy, median OS was 7.1 months (6.2, 8.7) and OS12 was equal to 26.6% (19.5, 34.3) not significantly different from the EORTC/NCIC phase III trial patient population (n = 125, median OS = 8.0 months (6.5,9.3), OS12 = 28.8% (21.1,37.0), p = 0.91, Figure VI.1). Our pooled dataset was considered representative of the recurrent GBM population receiving further chemotherapy at progression. There was no significant difference in survival between the three categories of treatment at recurrence (p = 0.29). In this pre-treated subgroup the same factors were selected in univariate analysis except the time since initial diagnosis and the frontal location. The final model included four factors: WHO PS (p = 0.009 PI = 79%), baseline steroids (p = 0.02, PI = 71%), number of target lesions (p < 0.0001, PI = 99%), maximum diameter of the largest lesion (binary, p = 0.0003, PI = 95%). The C-index was 0.70 and PEV was 19%.

Figure VI.1: OS in the pooled dataset compared to historical data.

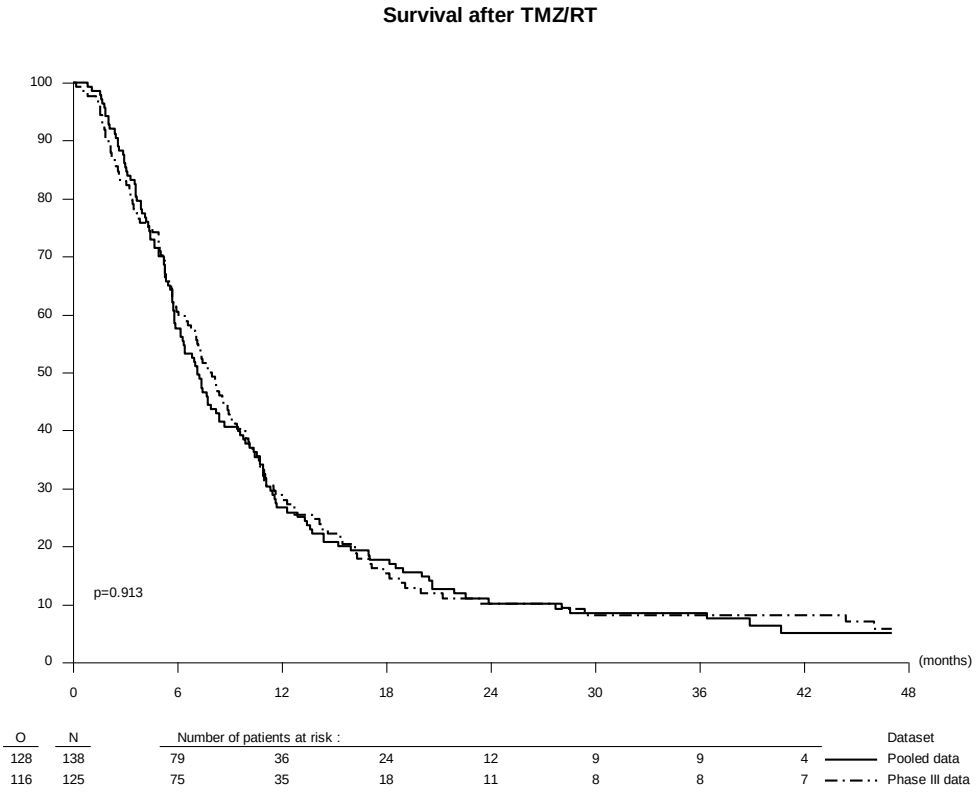

Table VI-7: Cox multivariate models for PFS and OS

	Final models for progression free survival				**Final models for overall survival**			
	GBM pretreated with TMZ/RT→TMZ (n=138, 138 used, 136 PFS events)		**All GBM (n=300, 300 used, 298 PFS events)**		**GBM pretreated with TMZ/RT→TMZ (n=138, 132 used, 122 deaths)**		**All GBM (n=300, 189 used, 176 deaths)**	
	Hazard Ratio (95% CI)	**p-value (Imp %)**	**Hazard Ratio (95% CI)**	**p-value (Imp %)**	**Hazard Ratio (95% CI)**	**p-value (Imp %)**	**Hazard Ratio (95% CI)**	**p-value (Imp %)**
Performance Status								
0	N/A	N/A	1.42 (1.18-1.71)	0.0002(91)	1.54 (1.11-2.13)	0.009 (79)	1.42 (1.10-1.83)	0.008 (69)
1								
2								
or								
0	1.56 (1.06-2.29)	0.02 (58)						
>0								
Neurological deficit	NI	NI	NS	NS (9)	NS	NS (11)	NS	NS (13)
No								
Some								
Moderate/major								
Prior chemotherapy	NI	NI	NI	NI	NI	NI	NS	NS (14)
No								
Yes, without temozolomide								
Yes, with temozolomide								
Baseline steroids	NI	NI	NS	NS (6)				
No					1.60 (1.09-2.36)	0.02 (71)	2.01 (1.40-2.88)	0.0001 (91)
Yes								
Number of target lesions								
0-1	2.14 (1.29-3.53)	0.003(83)	1.6 (1.16-2.19)	0.004 (84)	3.09 (1.82-5.27)	<0.0001 (100)	1.87 (1.24-2.82)	0.003 (80)
>1								
Frontal location	NI	NI	NI	NI	NI	NI		
No							0.69 (0.50-0.95)	0.02 (62)
Yes								
Largest lesion diameter (median)	NI	NI	NI	NI				
<=42 mm					2.01 (1.37-2.94)	0.0003 (95)	1.49 (1.08-2.05)	0.015 (70)
>42 mm								
C-index corrected for optimism	0.56		0.62		0.70		0.68	

Note: Only factors selected in the univariate analysis were included. NA: Not Applicable, NI: Not Included, factor was not selected in univariate analysis for the outcome in the subset, NS: Not Selected in multivariate model. Imp: Importance.

Development of prognostic calculators

The final multivariate models for PFS and OS in recurrent GBM patients having received TMZ/RT → TMZ as first line therapy were used to compute two prognostic calculators. They are available online at http://www.eortc.be/tools/recgbmcalculator/Default.aspx. Their calibration was satisfactory (see http://www.eortc.be/tools/recgbmcalculator/Calibration.aspx).

6.5 Discussion

In this report, baseline characteristics and outcome data were available for 300 patients diagnosed with GBM by the local pathologist. In all pooled phase II trials, the last dose of radiotherapy had to be administered more than 3 months from the time of recruitment thus making the chance of pseudoprogression less likely.[26,27] One hundred thirty eight had received TMZ/RT → TMZ at initial diagnosis. We have shown that tumour load measured by the maximum diameter of the largest target lesion and the number of target lesions have strong prognostic relevance for OS. In previous studies, WHO PS and baseline steroids were identified as major prognostic factors for OS. This report confirms these findings. Our patients tended to be older compared to previous report, nevertheless age did not show prognostic significance.[7] WHO PS and the number of target lesions were the two main factors selected in the PFS models. Patients with an initially large lesion and/or who were receiving steroids at baseline tended to progress more rapidly but the association was not statistically significant in this subset. The use of anti-angiogenic therapies might change the prognostic potential of some factors e.g. bevacizumab administration might reduce the detrimental effect of the need for steroids and of larger or multiple tumours, at least on PFS. Recently, Weller et al. assessed the prognostic value of 11 molecular markers in patients treated by TMZ/RT → TMZ.[28] The only factor of prognostic significance was MGMT gene promoter methylation.[29] It is however not clear if the status of molecular markers remains constant over time and how eventual changes might affect the markers prognostic value.[6,30] In our study, biological material was not systematically collected or analysed for molecular prognostic factors. Potentially, more accurate models for PFS and OS could be obtained by the addition of prognostic genomic signatures or biologically relevant biomarkers assessed at initial diagnosis and at recurrence, respectively, taking into account prior therapies.[31] The model's accuracy will also be improved once biomarkers predicting the activity of new active targeted agents are identified.[32] This study is exploratory and suffers some limitations: the heterogeneity of the treatments for recurrence, the small sample size, the lack of molecular data and the absence of validation of the prognostic models in a large independent dataset. This validation might be complicated because more and more patients will receive bevacizumab or other active treatments at different times of their disease, which may change their outcome. In the present study we developed prognostic calculators in the patients treated at initial diagnosis with TMZ/RT → TMZ. Four factors were retained for OS: WHOPS, baseline administration of steroids, tumour size (maximum diameter of the largest lesion; split by the median (≤ 42 mm versus >42 mm) and initial number of target lesions (1 versus >1). All four should be used as stratification factors in randomised trials when OS is the primary end-point. When PFS is the end-point stratifying by the WHO PS and the number of target lesions may suffice. The prognostic calculators provide outcome estimates with 95% confidence intervals. Our models and calculators can help physicians by providing objective information to patients and their families about their disease prognosis, discussing with them the best therapeutic strategy or the opportunity to participate to a clinical trial taking patient's individual characteristics into account.

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Chapter VII. **Thierry Gorlia, Jean-Yves Delattre, Alba A. Brandes, Johan M. Kros, Martin J.B. Taphoorn, Mathilde C.M. Kouwenhoven, H.J.J.A. Bernsen, Marc Frenay, Cees C. Tijssen, Denis Lacombe, Martin J. van den Bent .New clinical, pathological and molecular prognostic models and calculators in patients with locally diagnosed anaplastic oligodendroglioma or oligoastrocytoma. Submitted.**

7.1 Abstract

Background:
The prognosis of patients with oligodendrogliomas (AOD) and oligoastrocytomas (AOA) is variable. Biomarkers might be helpful to identify more homogeneous disease subtypes and improve therapeutic index. The aim of this study is to develop new clinical, pathological and molecular prognostic models for locally diagnosed anaplastic gliomas with oligodendroglial features (AOD or AOA).

Methods:
Data from 368 patients with AOD or AOA recruited in EORTC trial 26951 on adjuvant PCV chemotherapy in anaplastic oligodendroglial tumors were used to develop multifactor models to predict progression free survival (PFS) and overall survival (OS). Different models were compared by their percentage of explained variation (PEV). Prognostic calculators were derived from these new models.

Results:
Treatment (for PFS only), younger age, confirmed absence of residual tumor on imaging, frontal location, good WHO performance status, absence of endothelial abnormalities and/or necrosis, 1p/19q codeletion and IDH1 mutation were independent factors that predicted better PFS and OS.

Conclusions:
We identified important prognostic factors for AOD and AOA and showed that molecular markers added a major contribution to clinical and pathological factors in explaining PFS and OS. With a positive predictive value of 92% for PFS and 94% for OS, our models allow physicians to precisely identify high risk patients and aid in making therapeutic decisions.

7.2 Introduction

Anaplastic oligodendrogliomas (AOD), and anaplastic oligoastrocytomas (AOA) are classified as Grade III gliomas by the WHO classification and account for up to 20% of all newly diagnosed primary brain tumors. Recently, updated results of the EORTC 26951 trial have shown that the addition of 6 cycles of PCV (Procarbazine, CCNU, Vincristine) to 59.4 Gy radiotherapy significantly improves both progression free (PFS) and overall survival (OS) in patients with anaplastic oligodendroglial tumors. Patients with co-deletion of chromosomes 1p and 19q had a better prognosis and appear to benefit more from RT/PCV therapy. Similar results were obtained in a North American trial (RTOG 9402).[1,2]. In absence of more reproducible pathological criteria for typing and grading of gliomas, the management of anaplastic gliomas may remain sub-optimal.[3,4] Whether molecular diagnosis improves the situation is still a matter of debate, but accumulating data suggest these markers are indeed relevant for treatment decisions. In particular 1p/19q status, MGMT promoter methylation and IDH mutations are candidate markers to guide treatment decisions. Recent studies have shown a significant prognostic value for IDH1 mutations but whether it predicts benefit to PCV chemotherapy is still unclear.[5] MGMT promoter methylation had a predictive value for the efficacy of chemoradiation with temozolomide in glioblastoma but has prognostic value in anaplastic gliomas treated with either RT or RT/PCV.[6,7] This appears due to a relationship with CpG island hypermethylation in grade III gliomas, which may be induced by IDH mutation. [8,9]
In order to further evaluate the role of molecular factors for patient outcome prediction in this tumor type, we further analysed them in multivariate models together with clinical and pathological factors. Finally, prognostic calculators were derived from the regression models in order to provide clinicians with individualized outcome predictions.

7.3 Patients and methods

Patient selection

Between October 2nd 1996 and February 27th 2002, 368 patients with AOD or AOA (at least 25% oligodendroglioma components) diagnosed by local pathologists were randomized in EORTC trial 26951 to either receive radiotherapy alone or radiotherapy followed by 6 cycles of PCV regimen (Procarbazine, CCNU, Vincristine). The details of this study and the results have been published elsewhere.[1,10] All randomized patients (Intent-To-Treat) were used for prognostic model development.

Candidate prognostic factors

Twenty four available factors split into three categories (clinical, pathological, molecular) were screened for their prognostic value. Clinical factors analysed were: treatment, age, sex, time since first symptoms (< or ≥1 year), previous resection for low grade glioma, time since surgery (< or ≥ 42 days), tumor contrast enhancement on imaging, tumor location, extent of surgery (resection versus biopsy), presence of residual tumor on postoperative imaging (CT or MRI), postoperative Mini Mental State Examination (MMSE) score and WHO performance status. Pathological features as assessed by local pathologists were: tumor histological subtype (WHO classification 1993), presence of high cellularity, nuclear abnormalities, mitoses, endothelial abnormalities and necrosis. The analysed molecular markers were: 1p/19q codeletion, IDH1/2 mutation, MGMT methylation status, EGFR amplification, trisomy 7, loss of chromosome 10, loss of chromosome 10q (Table VII-1, see supplemental table 1). The methods with which these molecular abnormalities were assessed have been described elsewhere. [5, 6, 11]

Table VII-1: Clinically relevant factors for Grade III glioma patients with their coding conventions.

	Coding conventions
1) Clinical factors	
Treatment	0=RT,1=RT/PCV
Age	Continuous
Sex	0=Male,1=Female
Time since first symptoms (days)	0=≥1 year, 1=<1 year
Previous resection for low grade glioma	0=No,1=Yes
Time since surgery (days)	0=>42, 1=<=42 days
Tumor contrast enhancement on imaging	0=No,1=Yes
Frontal location	0=No/1=Yes
Extent of resection with confirmation	1=Biopsy **with** residual tumor on CT/MRI or status of residual tumor missing. 2=Partial Removal (PR) or Complete Removal (CR) **with** residual tumor on CT/MRI or status of residual tumor missing 3=Biopsy or PR or CR **without** residual tumor on CT/MRI.
Mini Mental State Examination (MMSE)	0=<27,1=[27-30]
WHO performance status	0=WHO 0,1=WHO 1,2=WHO 2
2) Pathological factors	
Tumor histological subtype	0=AOD/1=AOA>25% oligo compents
Presence of high cellularity	0=No/1=Yes
Presence of nuclear abnormalities	0=No/1=Yes
Presence of mitoses	0=No/1=Yes
Presence of endothelial abnormalities	0=No/1=Yes
Presence of necrosis	0=No/1=Yes
3) Molecular factors	
1p/19q	0=non codeleted/1=codeleted
MGMT	0=Unmethylated, 1=Methylated
IDH1	0=Normal, 1=Mutated
EGFR	0=Normal, 1=Amplified
Trisomy 7	0=Normal, 1=Amplified
Chromosome 10	0=No loss,1=Loss
Chromosome 10q	0=No loss,1=Loss

Patient outcome measurements

Macdonald's criteria were used to define progression.[12] Progression free survival (PFS) was computed as the time from randomization till signs of clinical or radiological progression or death whichever occurred first. Overall Survival (OS) was calculated as the time from randomization until death regardless of cause. In absence of events, PFS and OS were censored at the last follow-up date.

Statistical considerations

Categorical data were tabulated with frequencies and percentages. Medians and ranges (minimum-maximum) were used to summarize continuous variables. The Spearman rank correlation coefficient (SCC) was computed pairwise for all factors. SCC superior or equal to 0.40 are reported. For each factor, Kaplan Meier curves and log-rank tests were computed. Proportional Hazards (PH) assumptions were tested by examining the plot of the log of negative log estimates over the log survival time (LLS) and by assessing the Schoenfeld residual plots.[13] All clinically relevant factors independent of their significance in univariate analyses were considered for Cox multivariate analyses. For factors whose percentage of missing value was more than 5%, the missing value was replaced by a dummy category in the Cox analyses. Factors with SSC greater than +0.4 were at risk to generate multicolinearity problems in the models. They were pre-screened in separate Cox models. Factors who did not add to the model discrimination (see definition below) were not retained for further multivariate modeling. For the final multivariate models, the stepwise backward method was used for factor selection. Model internal validity was assessed by the bootstrap method. Factors with an importance (percentage of bootstrap samples with factor selected in multivariate analysis) lower than 60% were not included in the final models. [14] A significance level of 5% was used in multivariate analyses. Harrel's C-index corrected for optimism by bootstrap resampling was used to assess model's discrimination.[15] Calibration plots and Schemper's percentage of explained variation (PEV) were also computed.[16,17] A PEV of at least 20% was considered a minimum requirement for a model to provide sufficiently precise individual survival predictions. [18] From the final models, prognostic calculators were developed and predictions for median PFS, OS, 2-year PFS (PFS2y) and 5-year OS (OS5y) were derived. Individual prognostic scores were computed. Based on their scores, patients were classified into three distinct risk groups of equal size (low, intermediate, high). For all statistical analyses, SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.) was used except for the computation of the C-index and calibration plots which were obtained from the R "Design" and "Hmisc" Packages. The percentage of explained variation was computed using the SAS macro RELIMPCR. [17] The reflected method was used to estimate the 95% confidence interval (CI) for the median survival . [19] The loglog transformation was used for the 95% CI of PFS2y and OS5y.
Model sensitivity, specificity, positive and negative predictive value (PPV/NPV) were computed. In this study, PPV measures the ability of the model to correctly identify patients at high risk of progression or death within two years (PFS2y) or of death within five years (OS5y). The capacity of the model to correctly identify patients at low risk for these events is measured by the NPV (see table VII-6 for the other definitions). In absence of an independent dataset, no external validation could be realized.

7.4 Results

Correlation analysis

Table VII-2 displays patient and disease characteristics for all patients. SSC >=0.4 are presented in table VII-3 (supplemental table 2) (all p-values lower than 0.001). Time since first symptoms and previous resection for LGG had SSC=0.4. IDH1 mutation was positively correlated with 1p/19q codeletion (SSC=0.47) and MGMT methylation (SSC=0.51) and was negatively correlated with EGFR amplification (SSC=-0.40). Trisomy 7 (SSC=0.40) and chromosome 10 loss (SSC=0.49) were positively correlated with EGFR amplification.

Table VII-2: Patients and disease characteristics.

Baseline characteristics	All patients (N=368) N (%)
Treatment	
RT	183 (49.7)
RT/PCV	185 (50.3)
Age	
Median	49.5
Range	18.6 - 68.7
N obs	368
Sex	
Male	212 (57.6)
Female	156 (42.4)
Time since first symptoms	
≥1 year	90 (24.5)
<1 year	268 (72.8)
Missing	10 (2.7)
Previous resection for LGG	
No	313 (85.1)
Yes	52 (14.1)
Missing	3 (0.8)
Time since surgery (days)	
Median	27.0
Range	5.0 - 132.0
N obs	368
>42 days	38 (10.3)
<=42 days	330 (89.7)
Tumor contrast enhancement on imaging	
No	63 (17.1)
Yes	286 (77.7)
Missing	19 (5.2)
Frontal location	
No	190 (51.6)
Yes	178 (48.4)
Extent of resection with confirmation	
Biopsy	51 (13.9)
Not confirmed without residual tumor on imaging	208 (56.5)
Confirmed without residual tumor on imaging	109 (29.6)

Baseline characteristics	All patients (N=368) N (%)
MMSE	
<27	99 (26.9)
27-30	230 (62.5)
Missing	39 (10.6)
WHO performance status	
0	134 (36.4)
1	171 (46.5)
2	58 (15.8)
Missing	5 (1.4)
Local diagnosis	
AOD	266 (72.3)
AOA>25% oligo components	100 (27.2)
Missing	2 (0.5)
High cellularity	
No	28 (7.6)
Yes	338 (91.8)
Missing	2 (0.5)
Nuclear abnormalities	
No	33 (9.0)
Yes	333 (90.5)
Missing	2 (0.5)
Mitoses	
No	31 (8.4)
Yes	335 (91.0)
Missing	2 (0.5)
Endothelial Abnormalities	
No	86 (23.4)
Yes	280 (76.1)
Missing	2 (0.5)
Necrosis	
No	166 (45.1)
Yes	200 (54.3)
Missing	2 (0.5)
1p/19q	
Non codeleted	236 (64.1)
Codeleted	80 (21.7)
Missing	52 (14.1)
MGMT	
Unmethylated	45 (12.2)
Methylated	138 (37.5)
Missing	185 (50.3)
IDH1	
Normal	99 (26.9)
Mutated	83 (22.6)
Missing	186 (50.5)
EGFR	
Normal	193 (52.4)
Amplified	58 (15.8)
Missing	117 (31.8)
Trisomy 7	
No	168 (45.7)
Yes	73 (19.8)
Missing	127 (34.5)
Chromosome 10	

Baseline characteristics	
	All patients (N=368) N (%)
No loss	205 (55.7)
Loss	46 (12.5)
Missing	117 (31.8)
Chromosome 10q	
No	221 (60.1)
Yes	38 (10.3)
Missing	109 (29.6)

Table VII-3: Correlation analyses.

	Previous resection for LGG	Time since first symptoms	1p/19q	MGMT	IDH1	EGFR	Trisomy 7	10 loss	10q loss
Previous resection for LGG	1.00000								
	365								
Time since first symptoms	0.40	1.00000							
	<.0001								
	356	358							
1p/19q	0.21	0.08	1.00000						
	0.0002	0.16							
	313	307	316						
MGMT	0.16	0.16	0.28	1.00000					
	0.03	0.05	0.0002						
	181	178	179	183					
IDH1	0.28	0.29	0.47	0.51	1.00000				
	0.0002	0.0001	<.0001	<.0001					
	176	173	176	152	178				
EGFR	-0.17	-0.25	-0.28	-0.13	-0.40	1.00000			
	0.009	0.0001	<.0001	0.08	<.0001				
	248	244	249	166	158	251			
Trisomy 7	-0.08	-0.12	-0.11	-0.16	-0.28	0.40	1.00000		
	0.21	0.07	0.09	0.04	0.0004	<.0001			
	238	234	239	160	150	241	241		
10 loss	-0.13	-0.17	-0.24	-0.15	-0.33	0.49	0.30	1.00000	
	0.04	0.006	0.0002	0.06	<.0001	<.0001	<.0001		
	248	244	247	166	156	223	220	251	
10q loss	-0.07	-0.08	-0.12	-0.10	-0.09	0.22	0.07	-0.19	1.00000
	0.29	0.20	0.06	0.21	0.25	0.0007	0.31	0.002	
	256	251	255	167	158	229	224	251	259

Note: Factors with at least one correlation coefficient >=0.4. In each cell, the three lines corresponds to : Spearman Correlation Coefficients (SSC), P-value, number of observations.

Development of prognostic models

Table VII-5 (Supplemental table 3) displays the results of PFS and OS univariate analyses. MMSE and all molecular markers were missing in more than 5% of the patients, here a dummy category ("missing") was used . Molecular factors which passed the pre-screening process and were considered for further multivariate modeling are: 1p/19q codeletion, IDH1 mutation, EGFR amplification and Trisomy 7. MGMT did not add to discrimination in the model that includes 1p/19q and IDH1. Loss of chromosome 10 of loss or chromosome 10q did not add to models that include EGFR and trisomy 7. Extent of surgery and confirmed absence of residual tumor were combined into the factor named "confirmed extent of resection" (see definition in table VII-1). There was no strong violation of PH assumptions based on Schoenfeld residuals (data not shown). For PFS, 9 independent factors were included in the final Cox

model: treatment (p<0.0001), age (p<0.0001), confirmed extent of resection (p=0.0001), frontal location (p=0.0008), WHO performance status (p=0.00007), endothelial abnormalities (p=0.013), necrosis (p<0.0001), 1p/19q codeletion (p<0.0001), IDH1 mutation (p<0.0001). Discrimination of the model was good (C-index=0.725). The factors included could explain 26.7% of PFS variations. For OS, 8 factors were selected: age (p<0.0001), extent of resection with confirmation (p<0.0001), frontal location (p=0.02), WHO performance status (p=0.0008), endothelial abnormalities (p=0.028), necrosis (p<0.0001), 1p/19q codeletion (p<0.0001), IDH1/2 mutation (p<0.0001). Treatment was not selected (p>0.05). For OS, C-index was 0.737 and PEV 27.5%. For both PFS and OS, MMSE and tumor histological subtype had no independent prognostic value. Calibration plots indicated that PFS and OS models might provide slightly pessimistic predictions for low risk patients (data not shown). Final multivariate models are presented in Table VII-6. The added value of different combinations of factors was assessed by comparing the PEV of each model. For both PFS and OS, molecular factors significantly increased PEV when added to clinical factors alone (PFS: 22.5 vs 14.4 p<0.0001, OS:22.8 vs 13.1 p<0.0001) or clinical and pathological factors (PFS: 26.7 vs 18.9 p<0.0001, OS:27.5 vs 18.3 p<0.0001; see Tables VII-7 and VII-8). For final PFS and OS multivariate models, figures VII.1 and VII.2 show PFS and OS Kaplan Meier curves split by the three risk groups. Table VII-6 displays also PFS and OS estimates by risk group and models performance. Median OS was 127 months 95% CI (95 mo - not reached) in low risk patients, 42 months 95% CI (29-56) in intermediate risk patients and 14 months 95% CI (12 mo – 16 mo) in the high risk group. NPV was 77% for PFS and 74% for OS. PPV was 92% for PFS and 94% for OS. For both PFS and OS, sensitivity and specificity were below 70%.

Table VII-4: Univariate analyses of PFS and OS

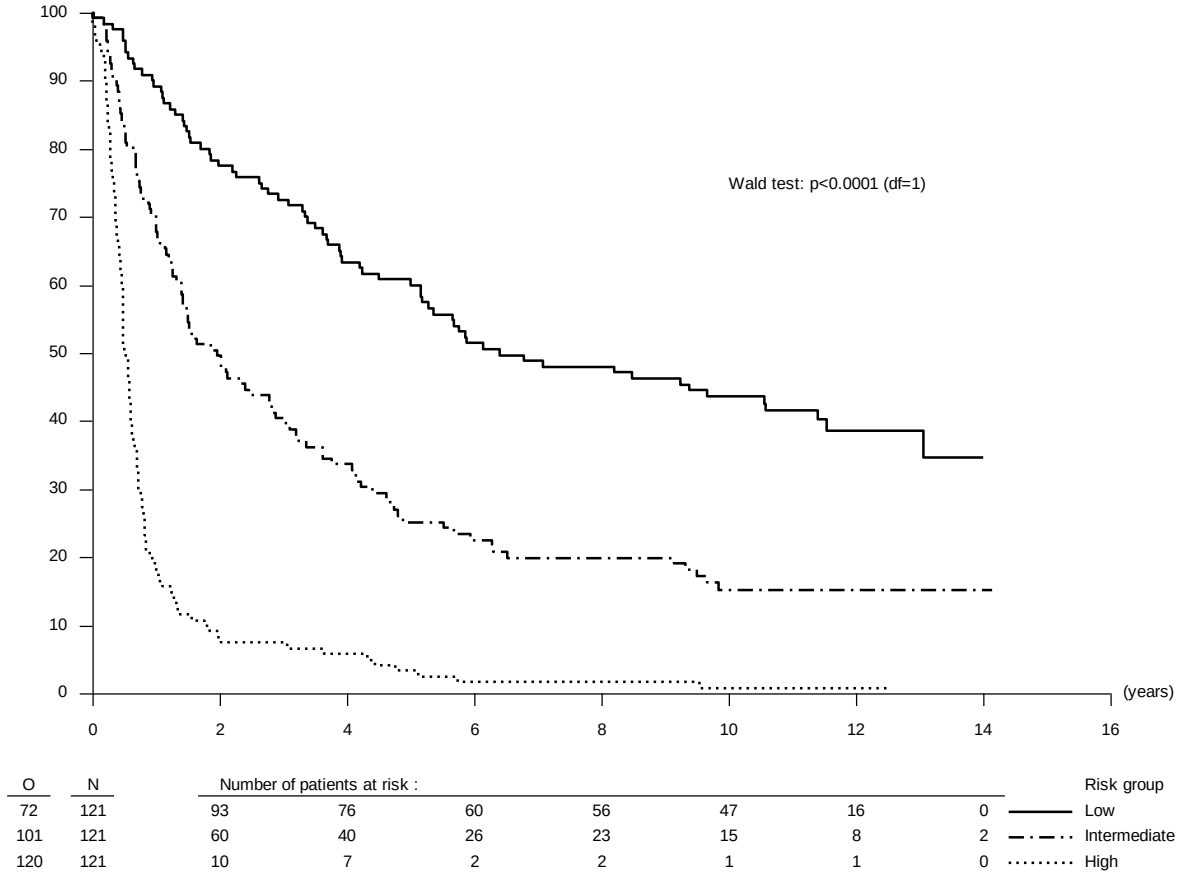
Progression Free Survival							Overall Survival				
	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 2 Years (95% CI)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 5 Years (95% CI)
Treatment											
RT	183	161	1.00	0.0003	13.21 (9.23, 17.91)	39.34 (32.26, 46.34)	153	1.00	0.0179	30.62 (21.45, 44.45)	36.98 (30.01, 43.95)
RT/PCV	185	137	0.66 (0.52, 0.83)		24.31 (17.38, 40.67)	50.00 (42.58, 56.97)	128	0.75 (0.60, 0.95)		42.33 (28.71, 62.03)	43.44 (36.20, 50.46)
Age (years)											
<41	92	64	1.00	0.0000 (df=3)	39.84 (19.38, 61.40)	57.61 (46.87, 66.94)	58	1.00	0.0000 (df=3)	75.73 (44.45, 103.75)	56.52 (45.79, 65.92)
[41-49.5)	92	68	1.19 (0.85, 1.68)	0.31 (df=1)	23.66 (15.05, 47.11)	49.45 (38.84, 59.20)	65	1.26 (0.88, 1.79)	0.21 (df=1)	59.30 (30.03, 79.80)	49.17 (38.52, 58.97)
[49.5-56)	92	81	1.64 (1.18, 2.28)	0.003 (df=1)	14.82 (9.79, 26.48)	41.30 (31.20, 51.11)	75	1.70 (1.20, 2.39)	0.0026 (df=1)	28.68 (19.91, 45.83)	34.78 (25.25, 44.47)
≥56	92	85	2.18 (1.57, 3.02)	<0.0001 (df=1)	8.30 (7.06, 13.96)	30.43 (21.39, 39.95)	83	2.46 (1.75, 3.44)	0.0000 (df=1)	18.79 (14.85, 26.61)	20.50 (12.93, 29.28)
Sex											
Male	212	174	1.00	0.35	16.08 (12.12, 22.11)	41.98 (35.29, 48.52)	167	1.00	0.2094	30.19 (22.54, 41.46)	37.58 (31.07, 44.07)
Female	156	124	0.90 (0.71, 1.13)		21.98 (13.27, 40.67)	48.39 (40.33, 55.98)	114	0.86 (0.68, 1.09)		45.83 (28.48, 66.23)	43.82 (35.90, 51.44)
Time since first sym ptoms											
<1 year	90	69	1.00	0.0009	47.80 (32.03, 57.03)	64.44 (53.63, 73.36)	64	1.00	0.0007	75.71 (59.30, 101.0)	60.00 (49.13, 69.27)
>=1 year	268	222	1.58 (1.20, 2.07)		12.12 (9.46, 17.15)	37.08 (31.31, 42.85)	211	1.61 (1.22, 2.14)		25.46 (20.34, 34.43)	32.79 (27.22, 38.46)
Previous resection for LGG											
No	313	258	1.00	0.003	14.82 (11.14, 17.91)	40.06 (34.61, 45.45)	243	1.00	0.0072	28.83 (22.14, 38.64)	36.53 (31.21, 41.86)
Yes	52	37	0.60 (0.42, 0.85)		54.08 (37.09, 75.43)	73.08 (58.82, 83.08)	35	0.62 (0.43, 0.88)		79.80 (66.33, 111.8)	64.88 (50.17, 76.23)
Time since surgery (days)											
>42	38	33	1.00	0.3028	15.64 (6.90, 32.03)	42.11 (26.42, 57.00)	31	1.00	0.2042	27.19 (16.59, 44.45)	30.56 (16.74, 45.55)
<=42	330	265	0.83 (0.58, 1.19)		17.91 (13.96, 24.25)	44.99 (39.54, 50.27)	250	0.79 (0.54, 1.14)		38.31 (28.71, 50.73)	41.30 (35.95, 46.57)
Tumor contrast enhancement on imaging											

Progression Free Survival							Overall Survival				
	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 2 Years (95% CI)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 5 Years (95% CI)
No	63	48	1.00	0.07	29.57 (16.26, 49.94)	53.97 (40.95, 65.30)	45	1.00	0.1518	61.17 (28.71, 94.95)	50.79 (37.92, 62.30)
Yes	286	236	1.33 (0.97, 1.81)		16.89 (11.89, 22.11)	42.81 (37.02, 48.47)	222	1.26 (0.92, 1.74)		34.43 (25.26, 44.35)	38.46 (32.80, 44.08)
Frontal location											
No	190	168	1.00	<0.0001	9.92 (8.25, 12.32)	31.58 (25.10, 38.24)	160	1.00	0.0000	21.13 (17.61, 28.48)	27.17 (21.03, 33.64)
Yes	178	130	0.54 (0.43, 0.68)		42.09 (28.71, 54.08)	58.76 (51.14, 65.60)	121	0.55 (0.43, 0.70)		70.93 (44.45, 87.95)	54.20 (46.57, 61.20)
Extent of resection with confirmation											
Biopsy	52	49	0.66 (0.55, 0.79)	<0.0001 (df=1)	6.87 (5.36, 9.95)	26.92 (15.79, 39.34)	49	0.64 (0.53, 0.77)	0.0000 (df=1)	16.15 (11.56, 24.18)	17.31 (8.53, 28.65)
Not confirmed without residual tumor on imaging	202	163			17.15 (12.78, 22.31)	42.29 (35.41, 49.00)	153			34.43 (26.61, 45.31)	38.59 (31.85, 45.28)
Confirmed without residual tumor on imaging	114	86			43.48 (19.12, 62.13)	57.02 (47.42, 65.51)	79			70.87 (44.45, 94.95)	53.49 (43.93, 62.14)
MMSE											
<27	99	85	1.00	0.31 (df=2)	13.96 (9.79, 23.66)	40.40 (30.73, 49.86)	81	1.00	0.4362 (df=2)	28.71 (20.80, 56.21)	38.28 (28.74, 47.73)
27-30	230	183	0.83 (0.65, 1.08)	0.17 (df=1)	18.27 (14.78, 27.76)	45.85 (39.30, 52.15)	172	0.86 (0.66, 1.12)	0.2515 (df=1)	38.64 (27.70, 51.94)	40.91 (34.50, 47.20)
Missing	39	30	0.78 (0.51, 1.18)	0.24 (df=1)	22.11 (8.74, 42.09)	48.72 (32.46, 63.16)	28	0.80 (0.52, 1.23)	0.3112 (df=1)	45.31 (16.89, 84.24)	41.03 (25.69, 55.76)
WHO performance status											
0	134	97	1.59 (1.34, 1.88)	<0.0001 (df=1)	40.25 (22.31, 52.90)	58.21 (49.39, 66.03)	89	1.59 (1.33, 1.89)	0.0000 (df=1)	66.33 (41.99, 89.40)	52.06 (43.27, 60.14)
1	171	143			18.23 (11.89, 25.17)	43.53 (35.99, 50.82)	135			37.60 (25.26, 55.56)	39.34 (31.99, 46.61)
2	58	53			6.72 (5.52, 11.89)	18.97 (10.13, 29.90)	52			17.18 (12.55, 21.65)	15.33 (7.46, 25.78)
Local diagnosis											
AOD	266	212	1.00	0.12	20.01 (14.82, 33.02)	47.37 (41.26, 53.22)	200	1.00	0.13	43.37 (30.03, 59.30)	43.47 (37.44, 49.34)
AOA>25% O	100	84	1.22 (0.95, 1.57)		14.62 (9.43, 19.38)	38.38 (28.86, 47.81)	79	1.22 (0.94, 1.58)		28.48 (18.00, 37.39)	32.32 (23.37, 41.58)
High cellularity											
No	28	23	1.00	0.16	6.57 (4.34, 11.89)	28.57 (13.54, 45.61)	21	1.00	0.96	28.68 (14.55, 101.72)	42.86 (24.57, 59.96)
Yes	338	273	0.74 (0.48, 1.13)		18.53 (15.38, 26.48)	46.29 (40.89, 51.51)	258	0.99 (0.63, 1.54)		38.31 (28.71, 45.77)	40.24 (34.97, 45.43)

Progression Free Survival							Overall Survival				
	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 2 Years (95% CI)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 5 Years (95% CI)
Nuclear abnormalities											
No	33	28	1.00	0.15	13.73 (5.06, 24.34)	36.36 (20.59, 52.34)	27	1.00	0.34	28.78 (16.69, 70.93)	35.42 (19.62, 51.61)
Yes	333	268	0.75 (0.51, 1.11)		18.33 (14.78, 25.49)	45.78 (40.35, 51.04)	252	0.82 (0.55, 1.22)		37.85 (28.71, 49.94)	40.94 (35.62, 46.17)
Mitoses											
No	31	25	1.00	0.87	22.31 (7.16, 49.48)	48.39 (30.18, 64.41)	22	1.00	0.67	33.54 (16.03, 101.06)	38.71 (22.01, 55.15)
Yes	335	271	1.04 (0.69, 1.56)		17.68 (13.73, 23.66)	44.61 (39.22, 49.85)	257	1.10 (0.71, 1.70)		36.90 (28.48, 45.31)	40.61 (35.31, 45.83)
Endothelial abnormalities											
No	86	61	1.00	0.001	38.80 (23.66, 68.96)	60.47 (49.33, 69.89)	58	1.00	0.002	79.80 (45.77, 114.07)	53.17 (42.06, 63.08)
Yes	280	235	1.58 (1.19, 2.10)		14.78 (10.87, 18.33)	40.14 (34.38, 45.84)	221	1.57 (1.17, 2.09)		28.78 (21.91, 37.39)	36.53 (30.90, 42.17)
Necrosis											
No	166	123	1.00	<0.0001	38.88 (24.25, 52.90)	58.43 (50.55, 65.50)	115	1.00	<0.0001	68.63 (50.46, 85.36)	54.79 (46.91, 62.00)
Yes	200	173	1.70 (1.35, 2.15)		11.01 (8.74, 15.38)	33.67 (27.19, 40.25)	164	1.74 (1.37, 2.21)		21.91 (17.87, 28.71)	28.43 (22.33, 34.83)
1p/19q											
Non codeleted	236	206	1.00	<0.0001 (df=2)	11.07 (8.74, 15.21)	33.05 (27.13, 39.08)	197	1.00	<0.0001 (df=2)	22.72 (18.89, 28.71)	28.21 (22.61, 34.06)
Codeleted	80	50	0.39 (0.28, 0.53)	<0.0001 (df=1)	75.70 (49.94, 136.77)	78.48 (67.69, 86.03)	44	0.36 (0.26, 0.50)	<0.0001 (df=1)	122.71 (94.95, N)	74.64 (63.50, 82.83)
Missing	52	42	0.76 (0.54, 1.06)	0.11 (df=1)	17.68 (10.87, 49.48)	46.15 (32.29, 58.92)	40	0.76 (0.54, 1.07)	0.12 (df=1)	35.06 (20.67, 84.24)	42.22 (28.72, 55.10)
MGMT											
Normal	97	91	1.00	<0.0001 (df=2)	8.38 (6.60, 9.63)	21.65 (14.09, 30.28)	42	1.00	<0.0001 (df=2)	15.90 (11.60, 19.02)	12.77 (5.18, 23.89)
Mutated	81	55	0.34 (0.24, 0.48)	<0.0001 (df=1)	53.01 (38.80, 71.20)	66.25 (54.77, 75.46)	93	0.39 (0.27, 0.57)	<0.0001 (df=1)	59.30 (36.90, 73.56)	48.89 (40.23, 56.99)
Missing	190	152	0.53 (0.41, 0.69)	<0.0001 (df=1)	18.99 (13.73, 33.02)	47.37 (40.12, 54.26)	146	0.52 (0.36, 0.73)	0.0002 (df=1)	38.87 (25.13, 51.94)	40.92 (33.78, 47.91)
IDH1											
Normal	97	90	1.00	<0.0001 (df=2)	16.46 (12.75, 19.55)	39.18 (29.49, 48.71)	90	1.00	<0.0001 (df=2)	16.46 (12.75, 19.55)	18.56 (11.56, 26.85)
Mutated	81	47	0.30 (0.21, 0.43)	<0.0001 (df=1)	101.13 (59.40, 140.09)	83.75 (73.67, 90.22)	47	0.30 (0.21, 0.43)	<0.0001 (df=1)	101.13 (59.40, 140.09)	61.18 (49.59, 70.87)
Missing	190	144	0.54 (0.41, 0.70)	<0.0001 (df=1)	38.64 (25.13, 56.18)	58.42 (51.07, 65.05)	144	0.54 (0.41, 0.70)	<0.0001 (df=1)	38.64 (25.13, 56.18)	42.49 (35.39, 49.40)
EGFR											

Progression Free Survival							Overall Survival				
	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 2 Years (95% CI)	Observed Events (O)	Hazard Ratio (95% CI)	p	Median (95% CI) (Months)	% at 5 Years (95% CI)
Normal	193	152	1.00	<0.0001 (df=2)	30.37 (19.65, 43.37)	53.13 (45.82, 59.89)	140	1.00	<0.0001 (df=2)	55.23 (40.34, 68.63)	47.40 (40.19, 54.25)
Amplified	58	55	2.54 (1.85, 3.47)	<0.0001 (df=1)	7.01 (5.62, 9.43)	13.79 (6.45, 23.89)	54	2.55 (1.85, 3.51)	<0.0001 (df=1)	16.51 (12.75, 19.09)	12.07 (5.30, 21.82)
Missing	117	91	1.09 (0.84, 1.42)	0.50 (df=1)	17.38 (11.89, 38.47)	46.15 (36.94, 54.87)	87	1.15 (0.88, 1.51)	0.29 (df=1)	34.07 (21.65, 71.36)	42.53 (33.46, 51.29)
Trisomy 7											
No	168	130	1.00	<0.0001 (df=2)	28.71 (18.23, 40.67)	52.69 (44.85, 59.93)	118	1.00	0.0001 (df=2)	51.42 (36.90, 68.24)	47.31 (39.57, 54.64)
Yes	73	69	1.84 (1.37, 2.47)	<0.0001 (df=1)	9.89 (7.13, 15.38)	26.03 (16.63, 36.43)	68	1.96 (1.45, 2.64)	<0.0001 (df=1)	19.09 (16.46, 30.00)	21.92 (13.28, 31.94)
Missing	127	99	1.11 (0.86, 1.45)	0.41 (df=1)	16.26 (10.87, 33.35)	44.88 (36.09, 53.27)	95	1.19 (0.91, 1.57)	0.20 (df=1)	34.07 (21.59, 56.18)	41.53 (32.88, 49.94)
10 loss											
No loss	205	164	1.00	<0.0001 (df=2)	27.24 (18.23, 38.80)	51.96 (44.90, 58.56)	150	1.00	0.0001 (df=2)	49.94 (38.87, 66.33)	45.92 (38.95, 52.60)
Loss	46	43	2.34 (1.67, 3.29)	<0.0001 (df=1)	6.31 (4.40, 8.74)	15.22 (6.69, 26.97)	42	2.17 (1.54, 3.07)	<0.0001 (df=1)	16.94 (12.55, 24.87)	15.22 (6.69, 26.97)
Missing	117	91	1.04 (0.80, 1.34)	0.76 (df=1)	16.26 (11.89, 31.38)	43.59 (34.50, 52.32)	89	1.16 (0.89, 1.51)	0.27 (df=1)	30.62 (20.80, 55.56)	40.15 (31.26, 48.87)
10q loss											
No	221	179	1.00	0.47 (df=2)	19.65 (13.37, 34.46)	46.82 (40.11, 53.24)	164	1.00	0.18 (df=2)	42.68 (30.19, 58.64)	42.57 (35.96, 49.00)
Yes	38	33	1.25 (0.86, 1.81)	0.24 (df=1)	15.64 (7.82, 24.31)	36.84 (21.98, 51.78)	33	1.41 (0.97, 2.06)	0.07 (df=1)	20.34 (16.46, 42.81)	28.95 (15.68, 43.63)
Missing	109	86	0.99 (0.77, 1.28)	0.95 (df=1)	15.38 (10.87, 31.38)	43.12 (33.72, 52.15)	84	1.12 (0.86, 1.46)	0.38 (df=1)	28.71 (20.34, 55.56)	39.43 (30.27, 48.45)

Figure VII.1: PFS curves split by risk group



O	N	Number of patients at risk :							Risk group
72	121	93	76	60	56	47	16	0	Low
101	121	60	40	26	23	15	8	2	Intermediate
120	121	10	7	2	2	1	1	0	High

Figure VII.2: OS curves split by risk group

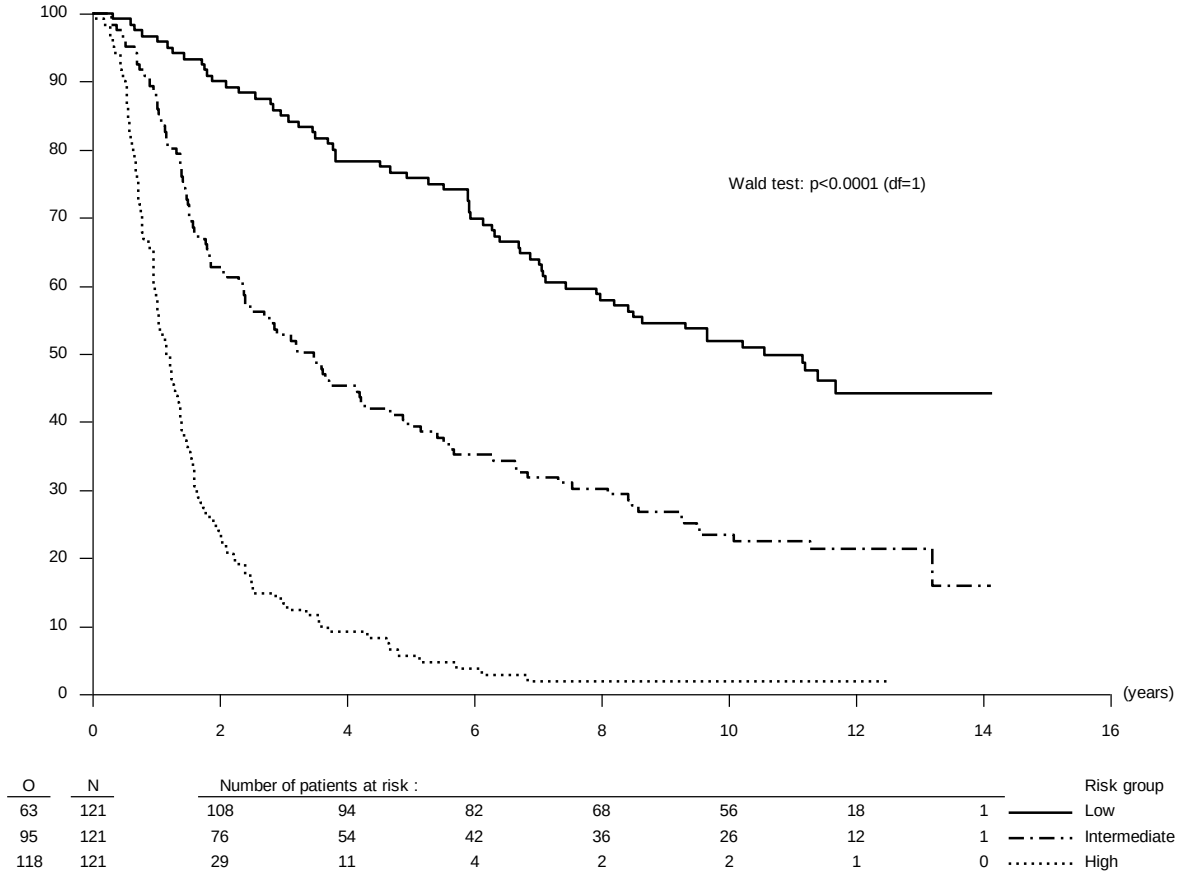


Table VII-5: Multivariate analyses of PFS and OS, performance, outcome estimates by risk group.

Multivariate analyses									
		Progression Free Survival N/E(363/293)				**Overall Survival N/E(363/276)**			
Parameter		**P**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**		**p**	**Hazard Ratio**	**95% Hazard Ratio Confidence Limits**	
				Low boundary	High boundary			Low boundary	High boundary
Treatment		0.0001	0.631	0.498	0.800	NS	NA	NA	NA
Age (quartiles)‡		<.0001 (df=1)	1.303	1.168	1.454	<.0001 (df=1)	1.383	1.232	1.553
Frontal location		0.0008	0.654	0.510	0.838	0.0197	0.738	0.572	0.953
Extent of resection with confirmation *	**Not confirmed without residual tumor on imaging**	0.0010	0.571†	0.410	0.797	0.0005	0.545†	0.387	0.769
	Confirmed without residual	<.0001	0.437†	0.298	0.639	<.0001	0.408†	0.274	0.607

	tumor on imaging								
WHO performance status		0.0007	1.361	1.140	1.625	0.0008	1.355	1.135	1.617
Endothelial abnormalities		0.0133	1.457	1.081	1.962	0.0281	1.409	1.038	1.914
Necrosis		<.0001	1.704	1.329	2.185	<.0001	1.794	1.379	2.335
1p/19q	**Missing (dummy)**	0.7076	0.930	0.638	1.356	0.3411	0.831	0.567	1.217
	Codeleted	<.0001	0.470	0.338	0.655	<.0001	0.429	0.303	0.607
IDH1	**Missing (dummy)**	<.0001	0.520	0.384	0.703	<.0001	0.551	0.410	0.741
	Mutated	<.0001	0.478	0.334	0.682	<.0001	0.422	0.291	0.610
Models performance									
Discrimination (C-index)		0.725				0.737			
Sensitivity (%)		57				62			
Specificity (%)		54				51			
Negative Predictive Value (%)		77				74			
Positive Predictive value (%)		92				94			
Outcome estimates									
		Median PFS		PFS 2 years		Median OS		OS 5 years	
Risk group									
Low		76.9 (61.9, 136.8)		77.5 (68.9, 84.0)		126.8 (95.0, N)		75.8 (67.1, 82.5)	
Intermediate		23.5 (16.9, 33.8)		49.6 (40.4, 58.1)		41.7 (28.7, 56.2)		39.4 (30.7, 48.0)	
High		6.3 (5.7, 7.2)		8.3 (4.2, 14.0)		14.0 (11.8, 16.5)		5.6 (2.4, 10.8)	

Note: N: Not reached. NS: not significant. NA: not applicable. N/E: Number of patient data used/ Number of events. * Global test: PFS: p=0.0001, OS: p<0.0001. † Biopsy is the reference value (Hazard Ratio=1) . ‡ Age quartiles are considered as scores (df=1).

Table VII-6: Definition of predictive accuracy parameters.

Criteria	Definitions for Progression Free Survival	Definitions for Overall Survival
Sensitivity	Percentage of patients alive without progressive disease at year 2, who were classified as low risk	Percentage of patients alive at year 5, who were classified as low risk.
Specificity	Percentage of patients who died or had progressive disease prior to year 2, who were classified as high risk	Percentage of patients who died prior to year 5, who were classified as high risk.
Negative Predictive Value (NPV)	Percentage of patients classified as low risk, who were alive without progressive disease at year 2.	Percentage of patients classified as low risk, who were alive at year 5.
Positive Predictive Value (PPV)	Percentage of patients classified as high risk who died or had progressive disease prior to year 2.	Percentage of patients classified as high risk who died prior to year 5.

Note: patients lost to follow-up before years 2 or 5 were considered as failures for PFS and OS respectively.

Table VII-7: Percentage of explained PFS variation between different prognostic models.

		Clinical+pathological	Clinical+molecular	Clinical+pathological+molecular
	PEV(%)	18.9	22.5	26.7
Clinical	14.4	0.0042	<0.0001	<0.0001
Pathological	4.6	<0.0001	<0.0001	<0.0001
Molecular	11.2	0.014	<0.0001	<0.0001
Clinical+pathological	18.9	NA	0.15	<0.0001

Table VII-8: Percentage of explained OS variation between different prognostic models.

		Clinical+pathological	Clinical+molecular	Clinical+pathological+molecular
	PEV(%)	18.3	22.8	27.5
Clinical	13.1	0.0026	<0.0001	<0.0001
Pathological	4.9	<0.0001	<0.0001	<0.0001
Molecular	12.4	0.067	<0.0001	<0.0001
Clinical+pathological	18.3	NA	0.080	<0.0001

Prognostic calculators

Prognostic calculators were developed based on final prognostic models. They provide with estimates for median PFS and OS and for PFS2y and OS5y based on individual patient characteristics and classified according to their risk group (low/intermediate/high). Prognostic calculators are available online at http://www.eortc.be/tools/GIIIcalculator/.

7.5 Discussion

In this prognostic factor analysis, we used baseline patient's characteristics and outcome data from prospective EORTC 26951 trial on RT vs RT/PCV in anaplastic oligodendroglial tumors. Patients were entered in this trial based on the diagnosis of the local pathologists. The analyses showed that younger age, a good WHO performance status, frontal location of the tumor, an extensive and confirmed resection without residual tumor on scan, absence of endothelial abnormalities and of necrosis, 1p/19q codeletion and IDH1 mutation had significant positive prognostic value both for PFS and OS. Thus, prognosis is related to a mixture of patient, tumor and treatment characteristics. In contrast to trials on glioblastoma and low grade tumors, in this study MMSE was not related to outcome [20,21]. Of the histological features, only endothelial abnormalities and necrosis had significant prognostic value, pointing to worse outcome in histologically more anaplastic tumors regardless of molecular features. Of note, the histological classification (AOD vs AOA) by the local pathologist did not impact outcome. The substantial contribution of the presence of 1p/19q codeletion and of IDH1 to prognostic models advocates for molecular studies in oligodendroglial tumors in a routine diagnostic setting. Extent of resection clearly matters, especially if at post-operative imaging prior to initiation of radiotherapy no residual tumor was described. Although this was not a randomized trial into the role of extent of surgery, our analysis supports the assumption that the optimal management of brain tumors starts with an extensive but safe resection, leaving the least possible amount of residual tumor. [22] RT/PCV therapy was selected for PFS but not for OS. An explanation might be the crossover, as many patients (75%) in the radiotherapy alone arm received PCV or Temozolomide at the progression. Recent reports showed that treatment effect might be confined patients with 1p/19q codeletion. This study was not powered for subgroup analyses.
In this study, models including clinical, pathological and molecular factors had PEV greater than 20% and significantly added to PEV compared to other models. A limitation of this prognostic study is the absence of external validation. Results might suffer from a lack of generalizability (calibration) when used outside the population of patients that do not meet the inclusion criteria for this clinical trial. Nevertheless, with PPV of 92% and 94% for PFS and OS respectively, our models are able to identify patients at high risk of progression and/or death and provide clinically useful information to physicians for treatment decision and discuss patients' prognosis.

7.6 References

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Chapter VIII. **Thierry Gorlia, Wenting Wu, Meihua Wang, Brigitta Baumert , Minesh Metha, Jan C.Buckner, Edward Shaw ,Paul Brown, Roger Stupp, Eva Galanis, Denis.Lacombe, Martin J. van den Bent. New validated prognostic models and calculators in patients with low grade gliomas diagnosed by central pathology review: a pooled analysis of EORTC/RTOG/NCCTG phase III clinical trials. Submitted.**

8.1 Abstract

Background:
In a previous study, the European Organisation for Research and Treatment of Cancer (EORTC) reported a scoring system to predict survival of patients with low-grade gliomas (LGG). This was recently validated by the North Central Cancer Treatment Group (NCCTG) using a dataset from a US LGG clinical trial. A major issue in the diagnosis of brain tumors is the lack of agreement between pathologists. New models in patients with low grade gliomas diagnosed by central pathology review are needed.

Methods:
Data from 339 EORTC patients with LGG diagnosed by central pathology review were used to develop new prognostic models utilizing clinical and histopathologic data, but not including molecular features for Progression Free Survival (PFS) and Overall Survival (OS). Data from 450 patients with centrally diagnosed LGG recruited into two large studies conducted by North American cooperative groups [Radiation Therapy Oncology Group (RTOG), and North Central Cancer Treatment Group (NCCTG)] were used to validate the models.

Results:
Both PFS and OS were negatively influenced by the presence of baseline neurological deficits assessed by the Medical Research Council (MRC) score, a shorter time since first symptoms (<30 weeks), an astrocytic tumor type, and tumors larger than 5 cm in diameter. As previously reported, early irradiation improved PFS but not OS. Three risk groups have been identified (low/intermediate/high) and validated.

Conclusions:
We have developed new prognostic models in a more homogenous LGG population diagnosed by central pathology review. This population better fits to modern practice where patients are enrolled in clinical trials based on central or panel pathology review. We could validate the models in a large, external and independent dataset. They can divide LGG patients into three risk groups and provide reliable individual survival predictions that can help physicians and their patients or families to discuss disease prognosis and therapeutic options. In the future, inclusion of other clinical (e.g. MMSE) and molecular factors might still improve models predictions.

8.2 Introduction

Low-grade glioma (LGG) is a heterogeneous group of primary, diffuse and slowly growing glial brain tumors. These tumors often remain clinically stable for many years, and patients are commonly only followed clinically without specific antitumor therapy. Based on retrospective studies suggesting an improved survival with early, extensive and maximal tumor resections, radical surgery is often advocated. Prospective controlled studies evaluating the role of surgery are lacking, and a large part of the benefit presumed from extensive resection may be due to patient selection. If tumor location makes the surgery difficult or even impossible, a biopsy is performed to ascertain the nature of the tumor and establish a pathological diagnosis.

Immediate (postoperative) radiotherapy has not been shown to offer an advantage in overall survival over deferred radiotherapy, although progression-free survival is lengthened, the optimal timing remains debatable. There is no apparent effect of dose; two randomized studies of the European Organisation for Research and Treatment of Cancer (EORTC) and of the North Central Cancer Treatment Group (NCCTG) - Radiation Therapy Oncology Group (RTOG) - Eastern Cooperative Oncology Group (ECOG) Intergroup showed no significant difference in survival when lower and higher irradiation doses (45 vs 59.4 Gy and 50.4 vs 64.8 Gy, respectively) were compared[1,2]. The role of postoperative chemotherapy alone or in combination with radiation therapy remains investigational.[3]

Individual prognosis of patients is highly variable. In order to choose the best strategy for a patient among the various treatment options, prognostic models and score can be useful. A major limitation in addressing prognostic models for LGG is the considerable inter-observer variability in both the grading and typing of these tumors.[4,5] The widely used EORTC prognostic scoring model for LGG was based on 2 prospective randomized clinical trials. However, patient inclusion into these trials relied upon a diagnosis made by the local pathologist, that was often not confirmed by central pathology review.[6] The external validity of the EORTC scoring system was recently evaluated in a dataset of LGG patients treated in a North American Intergroup trial (NCCTG 86-72-51).[7] In that dataset, the distinction between the low-risk and the high-risk group was predominantly determined by the prognostic impact of histology and tumor size; other factors like age or extent of surgery did not contribute significantly. As a major difference between the US and European trials was the mandatory central pathology review prior to inclusion in the American trials. Thus, we re-analysed the pooled data from the 2 EORTC studies restricting the analysis to patients with LGG whose histology had been confirmed upon central pathology review. Patients with histologies other than grade II glioma and patients where no tumor tissue was available for central review were excluded. We subsequently assessed the external validity of the EORTC studies with the individual patient data from large studies conducted by two US cooperative group (RTOG and NCCTG).[8,9] Based on this analysis, we developed prognostic calculators for progression free and overall survival that provide estimates for both median and fixed time probabilities of survival.

8.3 Patients and Methods

Patient selection

Three hundred seventy nine and 311 patients with LGG at first diagnosis were randomized in EORTC trials 22844 and 22845, respectively. Central pathology review was available for 428 patients, 182 (53%) in 22844 and 246 (81%) in 22845, out of 648 eligible patients [6].

Candidate prognostic factors

Factors screened for their prognostic value were: patient's age, gender, postoperative neurological signs and symptoms (history of seizures and/or headaches, presence of mental and/or motor disturbance), time since first tumor-associated symptoms, postoperative World Health Organization (WHO) performance status (PS) and Medical Research Council (MRC) neurological score, extent of resection assessed by the surgeon, time since surgery, baseline administration of steroids and/or anticonvulsants, histological type (astrocytic vs oligodendroglial), predominant tumor location, tumor crossing midline, and largest tumor diameter (details are provided in Table VIII-1, supplemental table 1).

Table VIII-1:Clinically relevant factors for LGG patients with their coding conventions.

Factors	**Coding conventions**
Patient's age (years)	Split by median 0=<median , 1= >=median or 0=<40 vs 1 >=40
Patient's sex	0=M vs 1= F
Time since first symptoms (weeks)	Split by median 0=<median , 1= ≥median
Post-op neurological signs and symptoms Seizures Headache Mental disturbance Motor disturbance Neurologic deficits	 0=no,1=yes 0=no,1=yes 0=no,1=yes 0=no,1=yes 0=no,1=yes
Post-op WHO PS	Binary: 0 vs >0 or score 0,1,≥2
Post-op MRC Neurological scale	0=no deficit,1=some deficit,2=moderate or major deficit
Extent of resection	0=biopsy only, 1=resection
Time since surgery	Split by median 0=<median , 1=>=median
Steroids intake	0=no, 1 =yes
AED intake	0=no, 1 =yes
Histology type	0=astrocytoma (AA), 1=oligoastrocytoma (OA), 2=oligodendroglioma (OD) or 0=OD or OA, 1=AA
Predominant tumor location Frontal Parietal Temporal Occipital	 0=no,1=yes 0=no,1=yes 0=no,1=yes

Other locations	0=no,1=yes
Tumor: Lateralization Left Right	 0=no,1=yes 0=no,1=yes
Tumor crossing the midline	0=no,1=yes
Tumor: Pre-op largest diameter (cm)	0= <5cm, 1= >=5cm

Patient outcome measurements

Computed tomography (CT) scans were used pre and post-operatively for diagnosis and for evaluation of disease progression. Progression free survival (PFS) was computed as the time from randomization till signs of clinical or radiological progression or death whichever occurred first. Overall Survival (OS) was calculated as the time from randomization until death regardless of cause. In the absence of events, PFS and OS were censored at the last follow-up date. For descriptive purpose, PFS and OS from the date of first LGG symptoms were also computed.

Statistical considerations

For model development

Categorical data were tabulated with frequencies and percentages. Medians and ranges (minimum-maximum) were used to summarize continuous variables. Spearman rank correlation coefficient (SCC) was computed pairwise for all factors. The significance of the association between categorical (nominal) factors was assessed by the Fisher Exact test. For the association between continuous covariates or scores and categorical (nominal) factors, the Wilcoxon rank sum test was used. P-values less than 1% and SCC superior or equal to 0.40 were reported. Immediate versus delayed irradiation (RT) was entered as a factor in PFS analyses. Since treatment effect did not impact OS, it was not entered in the OS models but used as a stratification factor. For each factor, Kaplan Meier curves and log-rank tests were computed. All factors were considered for Cox multivariate analyses, i.e. no systematic screening by univariate analysis was performed. The number of factors was lower than the number of PFS or OS events divided by 10 which is generally considered to provide sufficient power in multivariate analyses.[10] Proportional Hazards (PH) assumptions were tested by examining the plot of the log of negative log estimates over the log survival time (LLS) and by interpreting the Schoenfeld residual plots.[11] The stepwise backward method was used for factor selection. For factors whose missing value rate was more than 5%, the missing value was considered as a dummy category in the Cox analyses. Model internal validity was assessed by the bootstrap method. Factors with an importance (percentage of bootstrap samples with factor selected in multivariate analysis) lower than 60% were not included in the final models.[12] A significance level of 5% was used in multivariate analyses. Harrel's C-index corrected for optimism by bootstrap resampling was used to assess the model's discrimination.[13] Calibration plots and Schemper's percentage of explained variation (PEV) were also computed.[14,15] A PEV of at least 20% was considered a minimum requirement for a model to provide sufficiently precise individual survival predictions. [16] From the final models, prognostic calculators were developed and predictions for median PFS, OS, 3-year PFS (PFS3y) and 5-year OS (OS5y) were derived. Individual prognostic

scores were computed. Based on their scores, patients were classified into three distinct risk groups (low, intermediate, high). In absence of predefined cut-offs, groups were taken with equal size.
For all statistical analyses, SAS version 9.2 (SAS Institute Inc., Cary, NC, USA.) was used except for the computation of the C-index and calibration plots which were obtained from the R "Design" and "Hmisc" Packages. The percentage of explained variation was computed using the SAS macro RELIMPCR. [12] The reflected method was used to estimate median survival with 95% confidence interval (CI). [17] The loglog transformation was used for the 95% CI of PFS3y and OS5y. The model's sensitivity, specificity, positive and negative predictive value (PPV/NPV) were also computed. In this study, PPV measures the ability of the model to identify patients at high risk of progression or death at year 3 (PFS3y) or of death at year 5 (OS5y). The capacity of the model to identify patients at low risk for these events is measured by the NPV. See table VIII-7 for more definitions.

For model validation

Data from RTOG 98-02 and NCCTG 86-72-51 trials were pooled for model validation. PFS and OS curves in US data were split according to the EORTC risk groups and compared between EORTC and RTOG/NCCTG cooperative groups. EORTC Cox models were fit on US data to determine which factors kept their prognostic influence. Model sensitivity, specificity, NPV and PPV were computed on US data.

8.4 Results

Comparison of EORTC patient characteristics and outcomes

Among the 585 EORTC patients locally diagnosed with LGG, 390 were centrally reviewed and 308 (79%) were confirmed as LGG, sixty five patients (16.7%) being high grade gliomas (HGG, GIII or GIV). Six (1.5%) had grade I and another pathology was diagnosed in 11 patients (2.8%). The central pathologist identified 339 LGG (79%) and 69 HGG (16%). HGG patients were older (median 43 years vs 39 years, p=0.008), had a worse performance status (p=0.007), more often underwent resection (89.9% vs 64.6%, p<0.0001), had less frequent astrocytoma (50.7% vs 68.4%, p=0.02) and had worse PFS (p=0.01) and OS (p=0.03). Table VIII-2 displays tumor grade by local and central pathology review. Table VIII-3 compares patient and disease characteristics between various subgroups. There were no significant different characteristics and outcomes between patients with and without central pathological review (PFS, p=0.08, OS, p=0.92).

Table VIII-2: Comparison of tumor grade by local and central pathology review.

Central by local grade	**Tumor grade by local pathology review**			
	Missing (N=16) N (%)	**Grade I (N=47) N (%)**	**LGG (N=585) N (%)**	**Total (N=648) N (%)**
Tumor grade by central pathology review				
Missing	2 (12.5)	23 (48.9)	195 (33.3)	220 (34.0)
LGG	12 (75.0)	19 (40.4)	308 (52.6)	339 (52.3)
HGG	2 (12.5)	2 (4.3)	65 (11.1)	69 (10.6)
Grade I	0 (0.0)	3 (6.4)	6 (1.0)	9 (1.4)
Other pathology	0 (0.0)	0 (0.0)	11 (1.9)	11 (1.7)

Table VIII-3: Patients characteristics and outcomes: various subsets comparisons

	No central review (1) (N=220) N (%)	Central review (2) (N=428) N (%)	p (2)/(1)	EORTC centrally reviewed LGG (3) (N=339) N (%)	EORTC centrally reviewed HGG (4) (n=99) N (%)	p (4)/(3)	RTOG/NCCTG centrally reviewed LGG (5) (n=450) N (%)	p (5)/(3)
Age (years)								
Median	39.2	39.3	0.60	39.0	43.0	0.008	40.0	0.07
Range	16.0 - 66.4	17.2 - 65.7		17.2 - 65.3	18.1 - 65.7		18.0 - 82.0	
Gender								
Male	122 (55.5)	264 (61.7)	0.13	214 (63.1)	41 (59.4)	0.59	259 (57.6)	0.12
Female	98 (44.5)	164 (38.3)		125 (36.9)	28 (40.6)		191 (42.4)	
History of seizure								
No	143 (65.0)	304 (71.0)	0.10	235 (69.3)	54 (78.3)	0.19	101 (22.4)	<0.0001
Yes	77 (35.0)	121 (28.3)		101 (29.8)	15 (21.7)		98 (21.8)	
Missing	0 (0.0)	3 (0.7)		3 (0.9)	0 (0.0)		251 (55.8)	
Headache								
No	181 (82.3)	334 (78.0)	0.30	267 (78.8)	53 (76.8)	0.63	143 (31.8)	0.06
Yes	39 (17.7)	91 (21.3)		69 (20.4)	16 (23.2)		56 (12.4)	
Missing	0 (0.0)	3 (0.7)		3 (0.9)	0 (0.0)		251 (55.8)	
Mental disturbance								
No	184 (83.6)	347 (81.1)	0.51	277 (81.7)	52 (75.4)	0.18	178 (39.6)	0.03
Yes	35 (15.9)	78 (18.2)		59 (17.4)	17 (24.6)		21 (4.7)	
Missing	1 (0.5)	3 (0.7)		3 (0.9)	0 (0.0)		251 (55.8)	
Motor disturbance								
No	186 (84.5)	312 (72.9)	0.05	245 (72.3)	51 (73.9)	1.0	175 (38.9)	0.003
Yes	34 (15.5)	90 (21.0)		72 (21.2)	15 (21.7)		24 (5.3)	
Missing	0 (0.0)	26 (6.1)		22 (6.5)	3 (4.3)		251 (55.8)	
Performance Status								
0	81 (36.8)	149 (34.8)	0.81	127 (37.5)	15 (21.7)	0.007	109 (24.2)	0.40
1	98 (44.5)	216 (50.5)		164 (48.4)	42 (60.9)		154 (34.2)	
>1	41 (18.6)	62 (14.5)		47 (13.9)	12 (17.4)		25 (5.6)	
Missing	0 (0.0)	1 (0.2)		0(0.0)	0(0.0)		162 (36.0)	
MRC score								
No	139 (63.2)	246 (57.5)	0.33	194 (57.2)	42 (60.9)	0.10	201 (44.7)	0.009
Some	49 (22.3)	129 (30.1)		101 (29.8)	21 (30.4)		183 (40.7)	
Moderate/Major	32 (14.5)	53 (12.4)		44 (13.0)	6 (8.7)		56 (12.4)	
Missing	0 (0.0)	0 (0.0)		0(0.0)	0(0.0)		10 (2.2)	
Extent of resection by neuro-surgeon								
Biopsy	84 (38.2)	127 (29.7)	0.04	116 (34.2)	4 (5.8)	<0.0001	211 (46.9)	<0.0001
Resection	135 (61.4)	294 (68.7)		219 (64.6)	62 (89.9)		239 (53.1)	
Missing	1 (0.5)	7 (1.6)		4 (1.2)	3 (4.3)		0 (0.0)	
Baseline steroids								
No	102 (46.4)	201 (47.0)	0.003	160 (47.2)	30 (43.5)	1.0	219 (48.7)	<0.0001
Yes	86 (39.1)	94 (22.0)		77 (22.7)	14 (20.3)		228 (50.7)	
Missing	32 (14.5)	133 (31.1)		102 (30.1)	25 (36.2)		3 (0.7)	

New validated prognostic models and prognostic calculators in patients with low grade gliomas diagnosed by central pathology review: a pooled analysis of EORTC/RTOG/NCCTG phase III clinical trials.

	No central review (1) (N=220) N (%)	**Central review (2) (N=428) N (%)**	**p (2)/(1)**	**EORTC centrally reviewed LGG (3) (N=339) N (%)**	**EORTC centrally reviewed HGG (4) (n=99) N (%)**	**p (4)/(3)**	**RTOG/NCCTG centrally reviewed LGG (5) (n=450) N (%)**	**p (5)/(3)**
Baseline anticonvulsants								
No	23 (10.5)	50 (11.7)	0.15	36 (10.6)	13 (18.8)	0.03	51 (11.3)	0.12
Yes	165 (75.0)	236 (55.1)		193 (56.9)	29 (42.0)		396 (88.0)	
Missing	32 (14.5)	142 (33.2)		110 (32.4)	27 (39.1)		3 (0.7)	
Local diagnosis								
Astrocytoma	154 (70.0)	279 (65.2)	0.36	232 (68.4)	35 (50.7)	0.02	104 (23.1)	<0.0001
Mixed Oligoastrocytoma	21 (9.5)	42 (9.8)		31 (9.1)	11 (15.9)		133 (29.6)	
Oligodendroglioma	44 (20.0)	107 (25.0)		76 (22.4)	23 (33.3)		213 (47.3)	
Missing	1 (0.5)	0 (0.0)		0(0.0)	0(0.0)		0(0.0)	
Frontal location								
No	137 (62.3)	215 (50.2)	0.004	172 (50.7)	30 (43.5)	0.29	176 (39.1)	0.001
Yes	83 (37.7)	213 (49.8)		167 (49.3)	39 (56.5)		274 (60.9)	
Temporal location								
No	145 (65.9)	316 (73.8)	0.04	251 (74.0)	51 (73.9)	1.0	289 (64.2)	0.007
Yes	75 (34.1)	112 (26.2)		88 (26.0)	18 (26.1)		157 (34.9)	
Missing	0 (0.0)	0 (0.0)					4 (0.9)	
Parietal location								
No	181 (82.3)	356 (83.2)	0.82	277 (81.7)	63 (91.3)	0.05	296 (65.8)	<0.0001
Yes	39 (17.7)	72 (16.8)		62 (18.3)	6 (8.7)		151 (33.6)	
Missing	0 (0.0)	0 (0.0)					3 (0.7)	
Occipital location								
No	213 (96.8)	424 (99.1)	0.05	336 (99.1)	69 (100.0)	1.0	426 (94.7)	0.002
Yes	7 (3.2)	4 (0.9)		3 (0.9)	0 (0.0)		20 (4.4)	
Missing							4 (0.9)	
Other location								
No	204 (92.7)	401 (93.7)	0.62	320 (94.4)	63 (91.3)	0.41	227 (50.4)	0.08
Yes	16 (7.3)	27 (6.3)		19 (5.6)	6 (8.7)		24 (5.3)	
Missing							199 (44.2)	
Left Lobe								
No	114 (51.8)	237 (55.4)	0.45	188 (55.5)	39 (56.5)	0.90	221 (49.1)	0.08
Yes	105 (47.7)	191 (44.6)		151 (44.5)	30 (43.5)		229 (50.9)	
Missing	1 (0.5)	0 (0.0)					0 (0.0)	
Right Lobe								
No	114 (51.8)	201 (47.0)	0.24	158 (46.6)	31 (44.9)	0.89	252 (56.0)	0.01
Yes	105 (47.7)	227 (53.0)		181 (53.4)	38 (55.1)		198 (44.0)	
Missing	1 (0.5)	0 (0.0)					0(0.0)	
Midline crossing								
No	163 (74.1)	310 (72.4)	0.55	247 (72.9)	50 (72.5)	0.87	136 (30.2)	0.06
Yes	53 (24.1)	88 (20.6)		68 (20.1)	15 (21.7)		57 (12.7)	
Missing	4 (1.8)	30 (7.0)		24 (7.1)	4 (5.8)		257 (57.1)	
Tumor size (cm)								

New validated prognostic models and prognostic calculators in patients with low grade gliomas diagnosed by central pathology review: a pooled analysis of EORTC/RTOG/NCCTG phase III clinical trials.

	No central review (1) (N=220) N (%)	**Central review (2)** (N=428) N (%)	**p (2)/(1)**	**EORTC centrally reviewed LGG (3)** (N=339) N (%)	**EORTC centrally reviewed HGG (4)** (n=99) N (%)	**p (4)/(3)**	**RTOG/NCCTG centrally reviewed LGG (5)** (n=450) N (%)	**p (5)/(3)**
<5	84 (38.2)	157 (36.7)	0.47	124 (36.6)	24 (34.8)	0.67	218 (48.4)	0.07
>=5	102 (46.4)	220 (51.4)		173 (51.0)	38 (55.1)		231 (51.3)	
Missing	34 (15.5)	51 (11.9)		42 (12.4)	7 (10.1)		1 (0.2)	
Time since first LGG symptoms (weeks)								
Median	27.9	30.0	0.55	30.5	26.3	0.40	14.5	<0.0001
Range	2.9 - 1749.0	2.0 - 1542.4		2.0 - 1542.4	2.1 - 828.7		1.3 - 787.7	
N obs	220	426		338	68		426	
Time since surgery (weeks)								
Median	3.6	2.3	<0.0001	2.3	1.7	<0.0001	4.1	<0.0001
Range	0.0 - 31.7	0.3 - 157.6		0.3 - 157.6	0.4 - 9.7		0.3 - 214.6	
N obs	220	428		339	69		420	
Median PFS (months – 95% CI)	53 (44,76)	54 (47,60)	0.08	55 (49,63)	41 (27,55)	0.01	66 (55,75)	0.01
Median OS(months – 95% CI)	80 (61,111)	84 (77,95)	0.92	87 (79,99)	62 (44,89)	0.03	110(96,129)	0.02

Note: LGG: Low Grade Glioma. MRC: Medical Research Council. Fisher test was used for binary or categorical factors. Wilcoxon rank sum test was used for continuous variables and scores. Log-rank test used for outcome comparisons.

Development of prognostic models

Table VIII-4 (Supplemental Table 2) presents the factors with correlation coefficients greater than or equal to 0.40. Presence of mental (rho=0.44, p<0.0001) or motor disturbances (rho=0.59, p<0.0001) and WHO performance status (rho=0.46, p<0.0001) were correlated with the MRC neurological scale. In order to minimize the problems linked to multicolinearity, separate multivariate models with MRC score or WHO performance were fit and their performance was compared. Supplemental Table VIII-5 displays the results of PFS and OS univariate analyses. Use of steroids and anticonvulsants were collected during radiotherapy and not collected in the delayed RT arm. They did not show prognostic significance in univariate analyses in the RT arms and were not used in the multivariate analyses. Only tumor location involving the temporal lobe was significant for OS and thus considered for multivariate analyses. Information on tumor crossing midline was not systematically available in the US dataset. Models without and with this factor were fit for sensitivity. Midline crossing and tumor size were missing in 8 and 13% of the EORTC patients respectively. A dummy category ("missing") was used instead. For all LGG patients (n=339), median PFS was 55.3 months (95% CI 49.4, 63.4) and the 3-year progression-free survival rate (PFS3y) was 68.0% (62.6-72.7). Median OS was 86.5 months (95% CI 78.6-99.2) and 5-year Overall Survival (OS5y) was 65.9% (60.4-70.9). For both PFS and OS, models had all similar discrimination power (for PFS, C-index ranging 64-66% and PEV ranging 10-13%, for OS, C-index=67% for all models, PEV ranging 9-15%) irrespective of the combination of covariates. For all these models, Percentage of Explained Variation (PEV) was below the 20% threshold necessary to consider a model sufficiently precise for individual predictions. Extent of resection and age were not identified with significant prognostic value in any analysis. Among all tested EORTC models, final ones were selected taking into account availability of covariates and maximal sample size in the US validation datasets. Five factors were retained in the final PFS prognostic model: immediate irradiation (p=0.0008, HR=0.62 95% CI (0.47-0.82), time since first LGG symptoms (<30 weeks vs >=30 weeks, p=0.01, HR=0.70 95% CI (0.53-0.92)), presence of neurological deficit (p=0.0003, HR=1.64 95% CI (1.25-2.15)), independent confirmation of astrocytoma (p=<0.0001, HR=1.93 95% CI (1.47-2.54)), and tumor size (<5cm/>=5cm, p=0.004, HR=1.53 95% CI (1.15-2.03)). C-index was 0.64 and PEV was 10.1%. In the final OS model, time since first LGG symptoms (p=0.009, HR=0.67 95% CI (0.49-0.91)), MRC score (p=0.0001, HR=1.51 95% CI (1.22-1.86)), independent confirmation of astrocytoma (p<0.0001, HR=1.96 95% CI (1.43-2.69)), and tumor size (p=0.001, HR=1.74 95% CI (1.25-2.43) were identified as independent prognostic factors. C-index was 0.67 and PEV was 8.8%. Final multivariate models are presented in Table VIII-6. Figures VIII.1 and VIII.2 show PFS and OS Kaplan Meier curves by the three equally sized risk group in EORTC data. For PFS and OS, sensitivity, specificity, PPV were low (36-57%). NPV was 74% for PFS and 61% for OS. See Table VIII-6 for details. In both PFS and OS models, calibrations plots did not suggest large systematic differences (biases) between predicted and observed outcomes (data not shown). Variability was nevertheless high.

Table VIII-4: Correlation analyses.

Spearman Correlation Coefficients					
	Mental disturbance	Motor disturbance	Neurological deficit	MRC score	WHO performance Status
Mental disturbance (No/Yes)	1.0 N=336				
Motor disturbance (No/Yes)	0.18 p=1E-3 N=316	1.0 N=317			
Neurologic deficit (No/Yes)	0.41 p<1E-4 N=336	0.56 p<1E-4 N=317	1.0 N=339		
MRC score (No/Some/Moderate-Major)	0.44 p<1E-4 N=336	0.59 p<1E-4 N=317	0.97 p<1E-4 N=339	1.0 N=339	
Performance Status 0,1,>1	0.32 p<.1E-4 N=335	0.31 p<1E-4 N=317	0.46 p<1E-4 N=338	0.52 p<1E-4 N=338	1.0 N=338

Note: Factors with at least a spearman correlation equal or superior to 0.40.
In each cell, the three lines corresponds to : Spearman Correlation Coefficients (SSC), P-value, number of observations.

Validation in RTOG/NCCTG data

Baseline characteristics were different between EORTC and RTOG/NCCTG patients (Table VIII-3). In particular, tumor of EORTC and RTOG/NCCTG patients had different histological types. Oligodendrogliomas or mixed oligoastrocytoma were diagnosed in 76.9% of US patients compared to 31.5% of EORTC patients ($p<0.0001$). It is beyond the scope of this manuscript to interpret these differences. For both PFS and OS, MRC score or presence of neurological deficit, central pathology diagnosis (non astrocytic vs astrocytic tumor type) and tumor size but not time since first LGG symptoms had significant prognostic influence in US data. (Table VIII-6) An explanation for this could be that time since first LGG symptoms was significantly shorter in US data ($p<0.0001$, median 14 vs 30 weeks), which may reflect a more aggressive therapeutic approach. Compared to EORTC, the C-index and PEV were slightly lower in US data (Table VIII-6, PFS: C-index=0.61, PEV=5.5%; OS: C-index=0.62, PEV=7.1%). Table VIII-7 compares PFS and OS by risk groups between EORTC and US data. Overall, US patients had significantly different outcome compared to EORTC patients (PFS:, p=0.01, OS: p=0.03). Nevertheless, there was no significant difference in PFS and OS between EORTC and US patients within risk groups. There was no difference in both PFS and OS when they were computed from the time of first symptoms (PFS: p=0.95, OS: p=0.92, curves not shown). Figure VIII.2 shows PFS and OS Kaplan Meier curves by risk group in US data. Curves separated well between the three risk groups. Sensitivity, specificity and PPV were low (<70%). NPV was equal to 73% for PFS and 71% for OS. See Table VIII-3.

Table VIII-5: Univariate analyses of PFS and OS

	Progression Free Survival						Overall Survival					
	Patients (N)	**Observed Events (O)**	**Hazard Ratio (95% CI)**	**P**	**Median (95% CI) (Months)**	**% at 3 Year(s) (95% CI)**	**Patients (N)**	**Observed Events (O)**	**Hazard Ratio (95% CI)**	**P**	**Median (95% CI) (Months)**	**% at 5 Year(s) (95% CI)**
LGG by central review	339	235	N/A	N/A	55.33 (49.35, 63.38)	67.96 (62.60, 72.73)	339	183	N/A	N/A	86.54 (78.55, 99.19)	65.92 (60.36, 70.90)
Treatment												
Delayed RT	96	79	1.00	0.0002	41.33 (34.63, 53.26)	57.93 (47.34, 67.13)	96	50	1.00	0.13	96.13 (80.39, 126.42)	73.66 (63.28, 81.53)
Immediate RT	243	156	0.60 (0.46, 0.79)		62.13 (52.96, 70.60)	72.03 (65.77, 77.35)	243	133	1.29 (0.93, 1.79)		81.94 (70.70, 98.10)	62.78 (56.03, 68.79)
RT dose (Gy)												
45	80	52	0.95 (0.78, 1.15)	0.59 (df=1)	56.08 (42.81, 75.17)	70.07 (58.47, 79.00)	80	40	1.07 (0.86, 1.33)	0.56 (df=1)	85.55 (53.45, 179.61)	58.70 (46.41, 69.10)
54	85	59			63.08 (53.55, 74.84)	76.09 (65.42, 83.86)	85	52			84.73 (69.13, 105.49)	68.71 (57.23, 77.70)
59.4	78	45			64.76 (47.41, 80.95)	69.63 (57.57, 78.87)	78	41			74.28 (55.13, 101.72)	60.21 (47.74, 70.61)
Age (median)												
<=39 yrs	169	122	1.00	0.77	56.08 (46.42, 64.07)	72.93 (65.38, 79.09)	169	93	1.00	0.78	82.40 (75.27, 100.60)	67.58 (59.52, 74.38)
>39 yrs	170	113	0.96 (0.74, 1.24)		55.13 (44.48, 72.94)	63.05 (55.18, 69.92)	170	90	1.04 (0.78, 1.39)		89.76 (71.92, 105.49)	64.28 (56.27, 71.21)
Gender												
Male	214	153	1.00	0.23	53.26 (42.81, 59.79)	64.28 (57.33, 70.40)	214	121	1.00	0.17	80.07 (70.70, 91.24)	64.93 (57.76, 71.19)
Female	125	82	0.85 (0.65, 1.11)		64.07 (49.45, 73.86)	74.26 (65.45, 81.15)	125	62	0.80 (0.59, 1.09)		98.10 (81.94, 110.98)	67.57 (58.25, 75.25)
History of Seizure												
No	235	171	1.00	0.49	57.59 (50.20, 65.71)	69.60 (63.20, 75.11)	235	132	1.00	0.61	86.54 (78.55, 99.19)	66.56 (59.89, 72.38)
Yes	101	64	1.11 (0.83, 1.48)		47.41 (39.33, 64.07)	63.39 (52.85, 72.19)	101	51	1.09 (0.79, 1.51)		80.39 (62.85, 107.43)	63.62 (52.71, 72.66)
Headache												
No	267	185	1.00	>0.99	55.33 (49.18, 64.07)	68.72 (62.68, 73.99)	267	145	1.00	0.95	86.64 (77.34, 99.19)	65.22 (58.89, 70.83)
Yes	69	50	1.00 (0.73, 1.37)		51.78 (37.65, 70.51)	64.20 (51.49, 74.39)	69	38	1.01 (0.71, 1.45)		80.39 (68.01, 106.84)	67.66 (54.76, 77.60)
Mental disturbance												
No	277	186	1.00	0.003	61.60 (52.96, 68.90)	72.75 (66.99, 77.68)	277	144	1.00	0.02	89.76 (80.39, 103.82)	70.02 (63.98, 75.25)
Yes	59	49	1.63 (1.18, 2.24)		31.08 (18.83, 41.69)	44.57 (31.49, 56.84)	59	39	1.55 (1.08, 2.22)		45.17 (32.82, 89.30)	44.83 (31.25, 57.50)
Motor disturbance												
No	245	166	1.00	0.09	57.23	71.95	245	130	1.00	0.15	86.54	67.20

					(51.55, 65.77)	(65.72, 77.24)					(78.26, 102.14)	(60.62, 72.93)
Yes	72	54	1.31 (0.96, 1.78)		44.09 (29.77, 63.08)	56.48 (44.17, 67.07)	72	44	1.29 (0.91, 1.83)		84.53 (55.75, 104.11)	60.09 (47.30, 70.72)
Performance Status												
0	127	81	1.41 (1.15, 1.71)	0.0007 (df=1)	69.68 (57.13, 76.94)	77.19 (68.68, 83.65)	127	57	1.50 (1.20, 1.87)	0.0004 (df=1)	102.14 (84.73, 119.79)	77.04 (68.29, 83.66)
1	164	118			51.91 (43.43, 64.76)	67.37 (59.48, 74.06)	164	95			82.99 (71.26, 103.82)	64.16 (55.99, 71.21)
>1	47	35			35.09 (13.40, 50.20)	46.09 (31.05, 59.88)	47	30			47.34 (33.64, 88.15)	43.05 (27.82, 57.41)
MRC score (class)												
No	194	125	1.38 (1.16, 1.65)	0.0003 (df=1)	67.19 (57.13, 74.84)	77.46 (70.75, 82.82)	194	93	1.42 (1.16, 1.73)	0.0006 (df=1)	98.10 (82.99, 108.02)	73.53 (66.34, 79.42)
Some	101	75			41.59 (35.38, 56.94)	60.29 (49.88, 69.21)	101	60			80.39 (60.58, 104.11)	62.14 (51.54, 71.07)
Moderate/Major	44	35			31.31 (13.40, 52.96)	44.15 (29.02, 58.25)	44	30			40.02 (27.93, 91.24)	41.22 (25.94, 55.87)
Extent of resection by neuro-surgeon												
Biopsy	116	81	1.00	0.38	53.26 (38.77, 70.01)	61.48 (51.75, 69.82)	116	57	1.00	0.73	102.14 (81.94, 107.43)	65.50 (55.61, 73.71)
Resection	219	151	0.89 (0.68, 1.16)		56.48 (47.57, 64.95)	71.22 (64.61, 76.83)	219	123	1.06 (0.77, 1.45)		82.40 (74.28, 94.36)	66.00 (59.00, 72.10)
Baseline steroids												
No	160	105	1.00	0.08	65.45 (55.13, 74.84)	77.53 (70.12, 83.31)	160	91	1.00	0.16	85.29 (71.26, 99.19)	66.43 (58.29, 73.35)
Yes	77	48	1.35 (0.96, 1.91)		47.57 (35.09, 70.60)	59.46 (47.10, 69.83)	77	41	1.31 (0.90, 1.89)		62.85 (43.33, 110.26)	52.64 (39.88, 63.89)
Baseline anticonvulsants												
No	36	22	1.00	0.99	59.70 (37.09, 76.94)	68.51 (50.36, 81.18)	36	21	1.00	0.70	73.46 (40.02, 175.28)	58.63 (40.20, 73.13)
Yes	193	125	1.00 (0.63, 1.57)		63.08 (51.91, 71.00)	73.45 (66.43, 79.23)	193	105	0.91 (0.57, 1.46)		82.99 (70.67, 98.10)	63.37 (55.78, 70.02)
Central diagnosis												
Astrocytoma	200	155	1.00	<1E-4 (df=2)	45.34 (39.72, 52.96)	60.89 (53.64, 67.36)	200	124	1.00	0.0006 (df=2)	75.27 (62.69, 84.53)	58.82 (51.35, 65.53)
Oligoastrocytom	81	39	0.41 (0.29, 0.59)	<1E-4 (df=1)	93.11 (74.55, N)	81.14 (70.67, 88.18)	81	31	0.47 (0.31, 0.70)	0.0002 (df=1)	103.82 (95.31, N)	81.97 (71.43, 88.91)
Oligodendroglio	58	41	0.86 (0.61, 1.21)	0.38 (df=1)	55.13 (43.43, 72.31)	74.18 (60.29, 83.83)	58	28	0.72 (0.48, 1.10)	0.13 (df=1)	105.49 (62.85, 152.02)	68.21 (53.32, 79.23)
Frontal location												
No	172	116	1.00	0.83	53.55 (41.59, 65.48)	65.51 (57.78, 72.16)	172	96	1.00	0.20	80.95 (71.56, 101.72)	63.21 (55.26, 70.13)
Yes	167	119	0.97		56.94	70.55	167	87	0.82		90.71	68.84

			(0.75, 1.26)		(49.18, 65.71)	(62.79, 76.98)			(0.61, 1.11)		(78.26, 105.49)	(60.74, 75.60)
Temporal location												
No	251	171	1.00	0.21	57.59 (51.55, 68.27)	67.82 (61.53, 73.31)	251	125	1.00	0.008	95.31 (81.94, 106.97)	68.86 (62.42, 74.43)
Yes	88	64	1.20 (0.90, 1.60)		47.57 (41.00, 59.14)	68.47 (57.46, 77.19)	88	58	1.53 (1.12, 2.09)		71.56 (55.36, 84.73)	57.71 (46.29, 67.56)
Parietal location												
No	277	197	1.00	0.65	54.21 (47.34, 62.13)	69.27 (63.34, 74.44)	277	157	1.00	0.31	84.73 (75.27, 95.31)	65.13 (58.91, 70.66)
Yes	62	38	0.92 (0.65, 1.31)		64.95 (35.09, 77.67)	62.21 (48.80, 73.04)	62	26	0.81 (0.53, 1.22)		111.80 (73.69, N)	69.68 (56.20, 79.74)
Occipital location												
No	336	235	1.00	0.04	55.13 (49.18, 63.08)	67.67 (62.27, 72.47)	336	183	1.00	0.97	85.55 (78.26, 98.10)	65.60 (59.99, 70.61)
Yes	3	0	0.00 (0.00, 2.66)		Not reached	(,)	3	0	0.00 (0.00,)		Not reached	(,)
Other location												
No	320	221	1.00	0.98	56.08 (49.35, 63.38)	68.60 (63.09, 73.47)	320	171	1.00	0.69	86.54 (78.55, 98.10)	66.16 (60.42, 71.27)
Yes	19	14	1.01 (0.59, 1.73)		40.94 (22.77, 107.43)	57.89 (33.21, 76.26)	19	12	1.13 (0.63, 2.03)		79.44 (40.02, 126.42)	62.20 (36.44, 79.98)
Left Lobe												
No	188	132	1.00	0.90	56.94 (47.34, 67.19)	68.96 (61.64, 75.17)	188	103	1.00	0.81	88.67 (74.71, 102.14)	66.71 (59.14, 73.19)
Yes	151	103	1.02 (0.78, 1.32)		54.21 (41.69, 65.48)	66.76 (58.50, 73.74)	151	80	1.04 (0.77, 1.39)		84.53 (73.69, 106.84)	64.99 (56.39, 72.30)
Right Lobe												
No	158	109	1.00	0.61	53.55 (41.33, 63.08)	65.00 (56.89, 71.97)	158	85	1.00	0.55	82.40 (71.56, 99.19)	63.96 (55.57, 71.18)
Yes	181	126	0.94 (0.72, 1.21)		59.14 (49.35, 68.90)	70.60 (63.20, 76.79)	181	98	0.91 (0.68, 1.22)		89.30 (75.27, 103.82)	67.68 (59.98, 74.23)
Midline crossing												
No	247	164	1.00	0.0001	64.07 (53.06, 71.00)	72.83 (66.71, 78.02)	247	122	1.00	< 1E-4	98.10 (84.73, 108.02)	71.43 (65.03, 76.86)
Yes	68	55	1.83 (1.35, 2.50)		37.65 (23.98, 44.09)	51.56 (38.92, 62.81)	68	51	2.26 (1.62, 3.16)		45.04 (37.78, 70.67)	43.72 (31.33, 55.45)
Tumor size (cm)												
<5	124	77	1.00	0.008	69.68 (55.33, 76.88)	70.50 (61.38, 77.85)	124	51	1.00	0.0007	128.76 (85.55, N)	70.47 (60.99, 78.05)
>=5	173	132	1.46 (1.10, 1.93)		46.42 (41.20, 55.13)	64.88 (57.20, 71.52)	173	113	1.78 (1.28, 2.48)		75.27 (67.35, 84.53)	60.55 (52.60, 67.58)
Time since first LGG symptoms (weeks)												
<30	169	127	1.00	0.03	53.26 (44.48, 61.60)	66.67 (58.90, 73.30)	169	101	1.00	0.05	82.40 (70.67, 94.36)	63.89 (55.91, 70.82)
>=30	169	108	0.76 (0.58, 0.98)		56.94 (47.18, 74.55)	69.06 (61.30, 75.58)	169	82	0.74 (0.55, 1.00)		100.60 (78.55, 119.79)	67.82 (59.72, 74.64)

Time since surgery(weeks)												
<2.3	172	129	1.00	0.16	52.24 (43.43, 57.13)	69.81 (62.26, 76.13)	172	103	1.00	0.22	81.22 (69.13, 90.71)	63.21 (55.27, 70.12)
>=2.3	167	106	0.83 (0.64, 1.07)		65.45 (51.55, 75.17)	65.99 (58.02, 72.80)	167	80	0.83 (0.62, 1.12)		96.13 (77.34, 106.97)	68.98 (60.90, 75.73)

Note: RT: Ratiotherpapy. df=degree of freedom of the statistical test. df=1 for binary factors (not added to the table) and for scores (eg WHO performance status). df=2 is for the global homogeneity test among the 3 histological subtypes. Astrocytoma was used as reference subgroup.

Table VIII-6: Multivariate analyses of PFS and OS

	Progression Free Survival				Overall Survival			
	EORTC		RTOG/NCCTG		EORTC		RTOG/NCCTG	
	p	Hazard Ratio (95% CI)	p	Hazard Ratio (95% CI)	p	Hazard Ratio (95% CI)	p	Hazard Ratio (95% CI)
	N=338/E=235		N=418/E=293		N=338/E=183		N=418/E=239	
Treatment (delayed/immediate irradiation)*	0.0008	0.62 (0.47-0.82)	N/A†		N/A†*		N/A†	
Time since first symptoms (<30 weeks/>=30 weeks)	0.01	0.70 (0.53-0.92)	0.34	1.14 (0.87-1.47)	0.009	0.67 (0.49-0.91)	0.42	1.13 (0.85-1.50)
MRC score (no/some/moderate or major deficit)	NI	NI	NI	NI	0.0001	1.51 (1.22-1.86)	<.0001	1.46 (1.22-1.75)
MRC score (no/at least some deficit)	0.0003	1.64 (1.25-2.15)	0.01	1.36 (1.07-1.71)	NI	NI	NI	NI
Central histological type (OA or OD/AA)	<.0001	1.93 (1.47-2.54)	<0.0001	1.93 (1.49-2.52)	<0.0001	1.96 (1.43-2.69)	<.0001	2.08 (1.56-2.76)
Tumor size (cm) (<5/>=5)	0.004	1.53 (1.15-2.03)	<0.0001	1.78 (1.41-2.26)	0.001	1.74 (1.25-2.43)	0.0005	1.58 (1.22-2.05)
C-index♪	0.64		0.61		0.67		0.62	
PEV(%)‡	10.1		5.5		8.8		7.1	
Sensitivity (%)♫	36		65		38		61	
Specificity (%)♫	47		26		43		29	
NPV (%)♫	74		73		61		71	
PPV (%)♫	50		58		57		51	

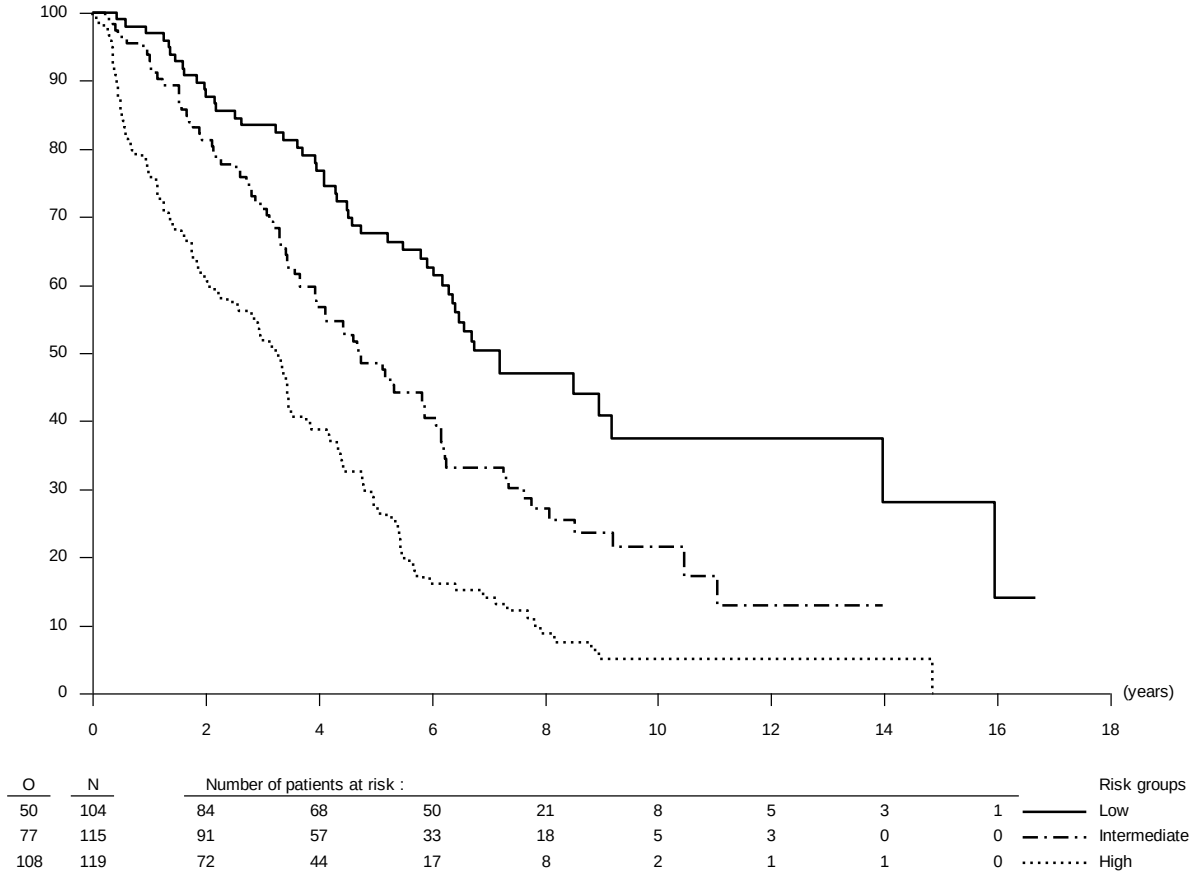
Note: N=sample size, E=Number of events. NI: Not included in this model. * In PFS analyses, treatment was considered a variable in the regression equation. In OS analyses it was used as a stratification factor in the Cox model. † All US patients were treated with immediate radiotherapy. ♪ C-index was corrected for optimism by bootstrap technique. ‡ A PEV of at least 20% is considered a minimum requirement for a model to provide sufficiently precise individual survival predictions. ♫, see table VIII-7 for definition.

Table VIII-7: Sensitivity, Specificity, NPV and PPV.

Criteria	Definitions for Progression Free Survival	Definitions for Overall Survival
Sensitivity	Percentage of patients alive without progressive disease at year 3, who were classified as low risk	Percentage of patients alive at year 5, who were classified as low risk
Specificity	Percentage of patients who died or had progressive disease prior to year 3, who were classified as high risk	Percentage of patients who died prior to year 5, who were classified as high risk
NPV	Percentage of patients classified as low risk, who were alive without progressive disease at year 3.	Percentage of patients classified as low risk, who were alive at year 5.
PPV	Percentage of patients classified as high risk who died or had progressive disease prior to year 3.	Percentage of patients classified as high risk who died prior to year 5.

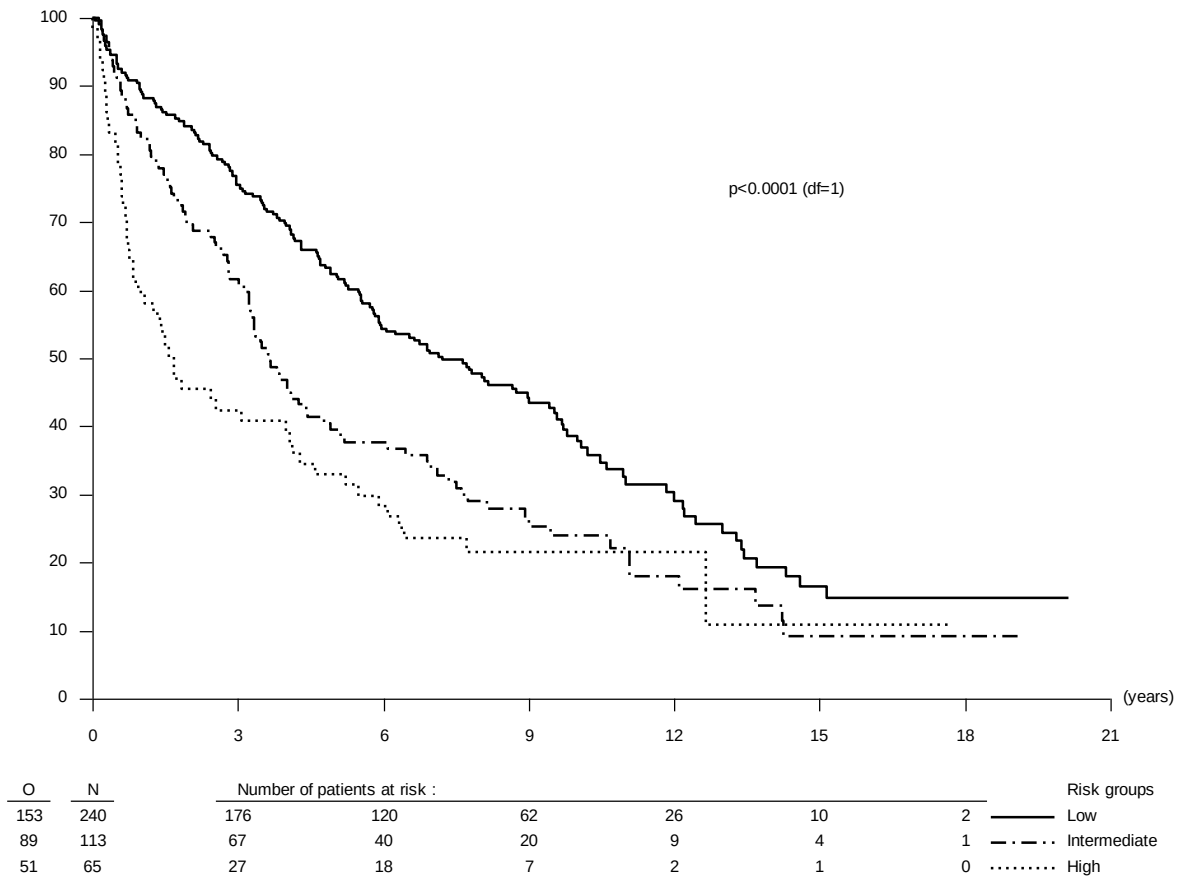
Note: patients lost to follow-up before years 3 or 5 were considered as failures for PFS and OS respectively.

Figure VIII.1: PFS curves split by risk group in the EORTC dataset.



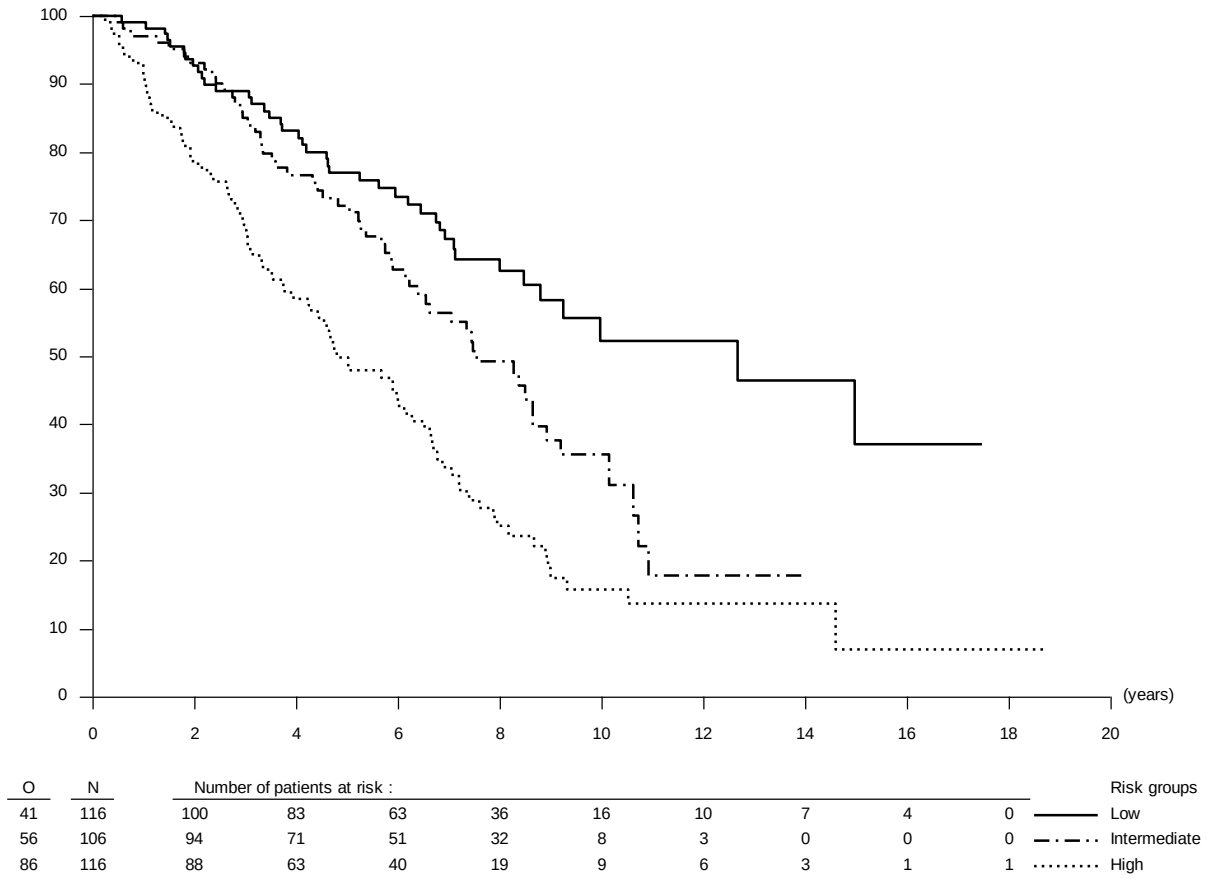
Note: Compared to low risk patients, patients with intermediate risk had PFS Hazard Ratio= 1.78 with 95% Confidence Interval (1.24, 2.55) and patients with high risk had PFS Hazard Ratio=3.32 with 95% Confidence Interval (2.36, 4.67).

Figure VIII.2: PFS curves split by prognostic risk group in the NCCTG/RTOG dataset.



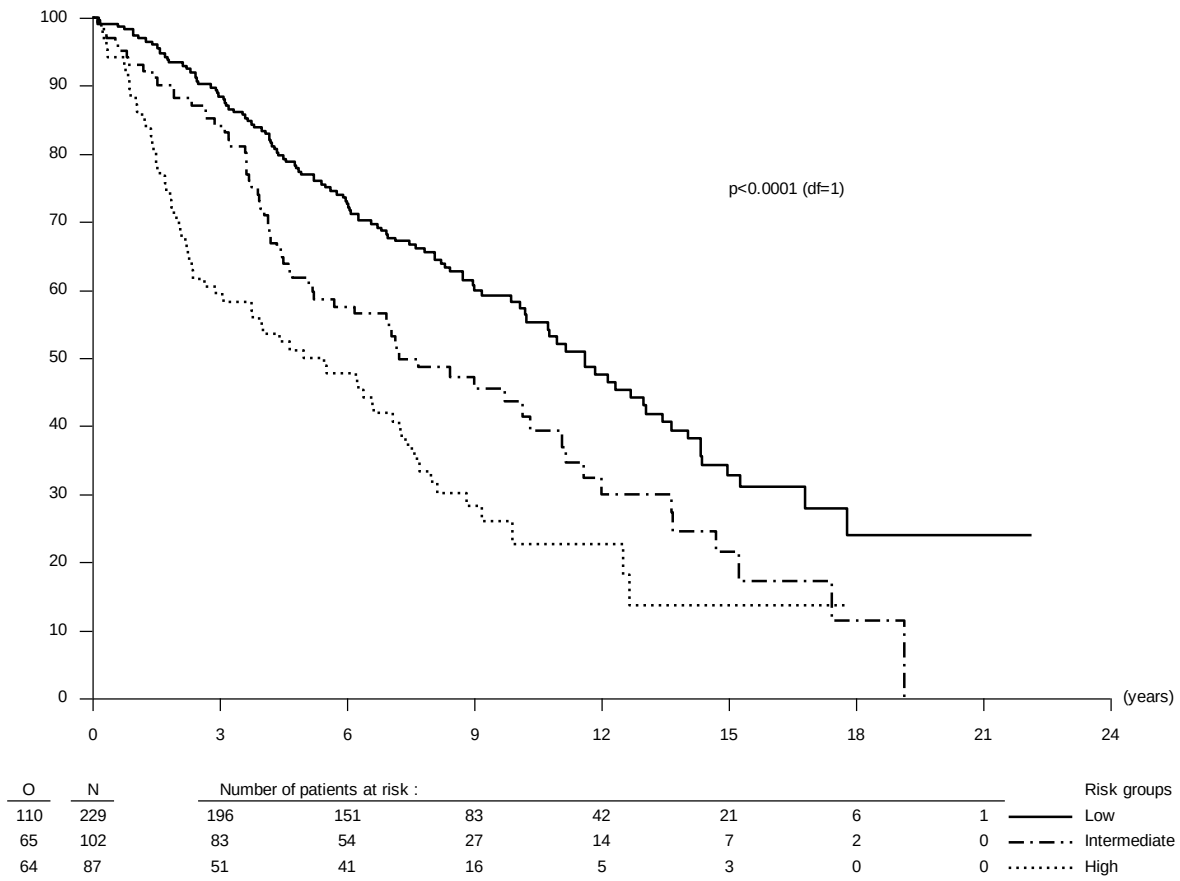
Note: PFS Hazard Ratios with 95% Confidence Intervals and p-values were 1.56 (1.20,2.03),p=0.0009 for intermediate risk patients and 2.17 (1.58,2.99),p<0.0001 for high risk compared to low risk patients.

Figure VIII.3: OS curves split by prognostic risk group in the EORTC dataset.



Note: Compared to low risk patients, patients with intermediate risk had OS Hazard Ratio= 1.67 with 95% Confidence Interval (1.12, 2.51)) and patients with high risk had OS Hazard Ratio=2.90 with 95% Confidence Interval (2.00, 4.22).

Figure VIII.4: OS curves split by prognostic risk group in the NCCTG/RTOG dataset.



Note: OS Hazard Ratios with 95% Confidence Intervals and p-values were 1.58(1.16,2.14), p=0.004 for intermediate risk patients and 2.47(1.81,3.38),p<0.0001 for high risk compared to low risk patients.

Prognostic calculators

Prognostic calculators based on new prognostic models have been developed. Like nomograms, these prognostic calculators provide patients with PFS3y and OS5y estimates based on their individual characteristics. Prognostic calculators are available online for physicians and patients at http://www.eortc.be/tools/lggcalculator. As a disclaimer, prognostic calculators must be used cautiously, individual precision and prediction of outcome is limited. A patient's prognosis may depend on other factors than those taken into account. Any decisions concerning patient care should not be based only on the use of these calculators, but should also take into account the patient's past history, other current patient and tumor characteristics, and new therapeutic development.

8.5 Discussion

This is a pooled prognostic factor analysis of two large EORTC trials of patients with LGG. Only patient with independently confirmed eligible histology were included, thus providing a more homogenous dataset and increasing the precision of the prediction. A total of 21% of cases reviewed had to be excluded due to differing central review pathology diagnosis, 17% were qualified as a high-grade glioma. Survival was substantially worse for the excluded patients retrospectively

considered as HGG. This inter-observer variation is a known factor in trials on LGG and HGG, and is related to the subjectivity of the criteria used.[5]
Both PFS and OS were negatively influenced by a worse baseline neurological status (i.e. presence of neurological deficits (PFS) or MRC score (OS)), a shorter time since first symptoms (<30 weeks), an astrocytic histology, and a tumor size of more than 5 cm in diameter. Of note, presence of neurological deficits and WHO performance status measure are interrelated. Treatment, namely immediate irradiation improved PFS, but not OS.[1] Contrary to earlier report, age no longer showed a prognostic importance in the now more homogenous dataset of histologically confirmed low-grade tumors.[6] Elderly high-grade patients were removed from this dataset. In this analysis, debulking surgery or complete tumor resection (as reported by the operating neurosurgeon without confirmation by imaging) did not significantly improve neither PFS nor OS (although tumor size was inversely correlated with extent of resection).[6] In everyday clinical practice, histological diagnosis is based on the skills and expertise of the local pathologist, independent and central expert review is rarely routine practice. Thus general applicability of our data may be confounded by a higher variation in histological subtypes and grades seen in clinical practice. Our models had moderate discrimination measured by C-index (max 0.67). Their percentage of explained variation in survival times was limited (PEV<20%) leading to large confidence intervals for outcome estimates. Sensitivity, specificity, PPV were low for both PFS and OS in all datasets as well as NPV for OS in EORTC data. Our models had moderate NPV in both EORTC and US dataset for PFS (~74%). They could nevertheless separate patients into three distinct risk groups (low/intermediate/high) in both EORTC (development) and US (validation) datasets. A major limitation of our study is the absence of molecular data in EORTC trials designed in the mid eighties, without tissue collection, as well as the estimation of tumor size based on CT as opposed to MR imaging. The prognostic value of new biomarkers relevant for gliomas could therefore not be assessed in our dataset. In particular, 1p/19q co-deletion was since identified as a favorable prognostic factor for oligodendroglial tumors associated with more indolent disease, prolonged natural history, and increased responsiveness to therapy.[18] Results of randomized trials must further distinguish between prognostic and predictive information related to 1p/19q status. Similarly, *IDH* mutations are of major prognostic significance in diffuse gliomas, although its value in grade II tumors is disputed.[19] Furthermore, not all trials collected the same clinical data. As an example, the Mini Mental State Examination (MMSE) score was not collected in the EORTC patients. Previous reports showed that presence of an abnormal baseline MMSE score was a strong predictor of poorer PFS and OS.[20] The addition of these factors to our prognostic models might significantly improve their predictive accuracy and precision.

Conclusions:

In our previous report, all patients diagnosed by local pathologists were used in the prognostic modeling.[6] In this study, patients were selected based on the LGG diagnosis of a central pathology reviewer. This population is more homogeneous because fewer patients with higher grade were included. It better fits to modern practice where patients are enrolled in clinical trials based on central or panel pathology review. With more similar patients, the new prognostic models provide more reliable and precise predictions. With their limitations correctly understood, they can help physicians to classify patients into three risk groups and propose them most adapted therapeutic strategy including their participation to clinical trials. They can be used to discuss disease prognosis with patients and families. Characteristics of patients and how they were managed were different between EORTC and RTOG/NCCTG patients but discrimination and predictive accuracy were comparable making these prognostic models useful for both European and American patients.

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Chapter IX. Summary and conclusions

Chapter 1 briefly presents brain cancer as a public health problem including the most recent incidence and epidemiological data. The history of the classification of brain tumor by the World Health Organization is summarized. The problem of using this classification in the clinical practice die to the large inter-observer variability is outlined. This thesis further characterizes this variability for the diagnosis of anaplastic oligodendrogliomas (AOD)/oligoastrocytomas (AOA) and glioblastomas. The importance of prognosis and prognostic factors in patient clinal management is reviewed. The methods used to build statistical prognostic models were explained to prepare the reading of chapters on prognostic modeling (Chapters 5-8).

Chapter 2 presents the result of a study which aim was to estimate consensus in typing and grading of AOD and AOA by a panel of 9 independent neuropathologists using tumor material collected in EORTC 26951 trial. Consensus diagnosis was correlated with the 1p/19q status of the tumors and the clinical outcome. Confirmation rate by the panel of the diagnosis of AOD (52%) and AOA (8%) made by local pathologists was low. Within the panel, the concordance on the diagnosis of AOD was high (86%). Survival curves split by consensus diagnosis (AOD/AOA) and 1p/19q status were significantly separated (AOD with 1p/19q loss vs AOD without these losses vs AOA without 1p/19q loss). In multivariate analysis, patients' age, 1p/19q loss, and necrosis were identified as independent prognostic factors.

In **Chapter 3** the results of a central pathology review of EORTC 26981/22981 NCIC CE.3 trial on GBM made by three independent reviewers are presented. Whether patients with GBM displaying distinct morphologic features had different response to combined chemo-radition was evaluated. GBM with an oligodendroglioma-like component (GBM-O) was found not associated with a more favorable outcome compared to classical GBM. GBM-O were found to be enriched for IDH1 mutations and EGFR amplifications. Co-deletion of 1p/19q was found in only one case and the MGMT methylation rate was found to be similar compared with other GBMs. Unexpectedly the presence of pseudo-palisading necrosis (PPN) displayed a trend for a higher benefit from chemotherapy. No treatment effect was significantly present in the absence of PPN. The presence of PPN should be systematically collected in new prospective trials to confirm its clinical interpretation.

In **Chapter 4**, the added value of 5 molecular markers (1p/19q codeletion, polysomy of chromosome 7, epidermal growth factor receptor (EGFR) gene amplification ($EGFR^{amp}$), and loss of chromosome 10 or 10q) to predict the outcome of patients with AOA or AOD treated with RT/PCV was assessed based on survival data from EORTC 26951 trial. In patient with AOD/AOA diagnosis confirmed by central pathology, codeleted 1p/19q only, AOA type and WHO performance status had independent prognostic value. We showed that AOA tumors with necrosis had similar survival when compared to GBM from EORTC 26981 trial and should also be considered WHO Grade IV tumors. However, patients with AOA with only endothelial abnormalities had better overall survival and should not be confounded with GBM.

In **Chapter 5**, data from patients with newly diagnosed GBM enrolled in EORTC 26981/22981 NCIC CE.3 trial to receive either radiotherapy alone or radiotherapy plus temozolomide where used for prognostic modeling in GBM. Prognostic factors with a strong independent prognostic impact were combined treatment with temozolomide, more extensive tumour resection, younger age, Mini-Mental State Examination (MMSE) score of 27 or higher, and no corticosteroid treatment at baseline

In patients who were assigned temozolomide and radiotherapy, methylated *MGMT* , better WHO performance status, and MMSE score of 27 or higher were associated with improved survival. These factors should be used as eligibility or stratification factors for future trials in patients with newly

diagnosed GBM. Stratifying by *MGMT* promoter methylation status should be mandatory in all glioblastoma trials that use alkylating chemotherapy. Implemented nomograms and online calculators can be useful tools which contribute to individualised patient management.

In **Chapter 6**, data from patients in eight EORTC phase I or II trials were pooled to update the prognosis information of GBM patients at the recurrence of the disease. Patients with poor WHO performance status, multiple lesions, large tumours measured by the maximum diameter of the largest lesion (≥42 mm) and treated with steroids at baseline had shorter OS. Tumours with predominant frontal location had better survival. Age and sex did not show independent prognostic influence.

In **Chapter 7**, the prognosis of patients with Grade III gliomas (oligodendrogliomas (AOD) and oligoastrocytomas (AOA)) was studied. In view of the high interobserver variability in the diagnosis of these tumors, identification of biomarkers with prognostic significance is helpful to identify more homogeneous disease subtypes and improve patient management. Data from patients with AOD or AOA recruited in EORTC trial 26951 on adjuvant PCV chemotherapy were used to develop multifactor prognostic models. Younger age, confirmed absence of residual tumor on imaging, frontal location, good WHO performance status, absence of endothelial abnormalities and/or necrosis, 1p/19q codeletion and IDH1 mutation were independent factors that predicted better Progression Free and Overall Survival, treatment was found to influence Progression Free Survival. The prognostic models have high positive predictive value (>90%). Patients at high risk of disease progression and death can be better identified which can help physician in making therapeutic decisions.

In **Chapter 8,** new prognostic models were developed for patients with at central pathology review confirmed low grade gliomas (LGG) based on data from two EORTC trials where newly diagnosed patients had received either no radiotherapy or immediate radiotherapy at different doses. Data from centrally reviewed LGG patients recruited in two studies conducted by North American cooperative groups [Radiation Therapy Oncology Group (RTOG), and North Central Cancer Treatment Group (NCCTG)] were used to validate the models. The presence of baseline neurological deficits assessed by the Medical Research Council (MRC) score, a shorter time since first symptoms (<30 weeks), an astrocytic tumor type, and tumors larger than 5 cm in diameter identified patients with poor outcomes. Prognostic models allow dividing LGG patients into three well discriminated risk groups which proved to be valid for both European and American patients. In the future, inclusion of other clinical (e.g. MMSE) and molecular factors might further improve the predictive value of prognostic models.

Conclusion. In this thesis on brain tumors in clinical trials, we contributed to a further characterization of the inter-observer variability for the diagnosis in grade III glioma, to clarify the role of biomarkers for both diagnosis and prognosis, to validate known prognostic factors and identify new factors for grade II – IV glioma, to develop (for the first time in neuro-oncology) nomograms and prognosis calculators for individualized outcome prediction. Low grade gliomas prognostic models could be validated in a large external dataset. With these results, this research helps to take treatment decisions in individual patients and in the design of new studies on diffuse glioma.

Chapter X. Samenvatting en conclusies

Hoofdstuk 1 geeft een kort overzicht van gliomen, de meest voorkomende primaire hersentumoren bij volwassenen, inclusief incidentie en epidemiologische data. De geschiedenis van de glioom classificatie door de World Health Organization wordt kort samengevat, en de problemen die in de dagelijkse praktijk ontstaan door interobserver variatie worden belicht. Dit proefschrift brengt de variabiliteit van de diagnose van anaplastische oligodendrogliomen (AOD) en anaplastische oligoastrocytomen (AOA) verder in kaart. Het belang van het vaststellen van de prognose en prognostische factorten voor de behandeling van patiënten wordt benadrukt. De methoden die gebruikt worden omstatische prognostische modellen te bouwen worden uitgelegd om het lezen van de hoofdstukken in dit proefschrift over het bouwen van prognostische modellen te vergemakkelijken

Hoofdstuk 2 presenteert de resultaten van een studie naar de consensus in typering en graderingvan AOD en AOA door een panel van 9 neuropathologen die gebruik maakten tumor monsters uit EORTC studie 26951. De consensus bleek gerelateerd aan de 1p/19q status van deze tumoren en de overleving. De bevestiging van de lokale gestelde diagnose door het panel was zowel voor AOD (bevestiging in 52% van de gevallen) als voor AOA (bevestiging in 9% van de gevallen) laag. Binnen het panel was de concordantie voor AOD hoog (86%). Overlevingscurves aan hand van consensus diagnose (AOD/AOA) en 1p/19q status lieten (AOD met 1p/19q verlies vs AOD zonder 1p/19q verlies vs AOA zoner 1p/19q verlies) een duidelijk verschil zien. In multivariate analyse bleken de leeftijd van de patient, 1p/19q verlies en de aanwezigheid van necrose in het histologische preparaat onafhankelijke prognostische factoren.

Hoofdstuk 3 presenteert de resultaten van de centrale pathologie revisie doro 3 onafhankelijke pathologen van EORTC studie 26981/22981 NCIC CE.3 naar gecombineerde radiochemotherapie met temozolomide bij glioblastomen. Hierin werd onderzocht of bepaalde histologische karakteristieken een verschil in behandeluitkomst met zich mee brachten. Glioblastoma met een oligodendrogliale component (GBM-O) bleek niet geassocieerd te zijn met een gunstigere uitkomst vergeleken met klassieke glioblastomen. GBM-O bleken een verhoogde incidentie IDH1 mutaties en EGFR amplificatie te vertonen. Co-deletie van 1p/19q werd maar in 1 geval vastgesteld, en MGMT romoter methylering bleek even frequent voor tekomen in in vergelijking met andere glioblastomen. Onverwacht bleek de aanwezigheid van necrose bij pseudopallisadering (PPN) een trend telaten zien voro meer winst van chemotherapie. In de afwezigheid vn PPN werd geen significant effect gezien van de toevoeging van temozolomide. In toekomstige studies dient de aanwezigheid van PPN systematisch moeten worden onderzocht om dit te bevestigen.

In **Hoofdstuk 4** wordt de toegevoegde waarde van 5 moleculaire markers (gecombineerd verlies van 1p/19q, polysomy van chromosoom 7, Epidermal Growth Factor Receptor amplificatie ($EGFR^{amp}$), en verlies van chromosoom 10 of 10q) onderzocht op de prognose van patienten met een AOD of AOA die behandeld werden in EORTC studie 26951 met radiotherapie (RT) of RT gevolgd door PCV chemotherapie . In patienten waarbij centrale pathologie revisie een AOD of AOA bevestigd had bleek gecombineerd verlies van1p/19q, type AOA en WHO performance status een onafhankelijke prognostische betekenis te hebben. We toonden aan dat AOA met necrose een overleving hadden die gelijk was aan die van glioblastomen behandeld in de EORTC studie 26981, en dat deze tumoren beschouwd moeten worden als WHO graad IV tumoren. Daarentegen hebben AOA met alleen endotheel proliferatie een betere overleving en moeten niet als glioblastoom worden beschouwd.

In **Hoofdstuk 5** worden data van patiënten met een nieuw gediagnosticeerd glioblastoom behandeld in EORTC 26951/22981 NCIC CE.3 studie met RT of RT met temozolomide gebruikt voor het opstellen van een prognostisch model. Gecombineerde behandeling met temozolomide, een meer uitgebreide tumor resectie, jongere leeftijd, MiniMental Status Examination (MMSE) score van 27 of hoger en geen behandeling corticosteroiden ten tijde van randomisatie en methylering van de *MGMT* promoter bleken onafhankelijke prognostische factoren voor een betere overleving. Het verdient aanbeveling deze factoren te gebruiken bij toekomstige studies naar glioblastoom studies waarbij alkylerende chemotherapie gebruikt wordt. Nomogramen en een web-based online prognose calculator kunenn zinvolle middelen zijn die kunnen bijdragen aan een meer geïndividualiseerde patiënten behandeling.

In **Hoofdstuk 6** worden de data van acht EORTC fase I of II studies naar recidief glioblastomen geanalyseerd voor prognostische informatie van patiënten met een recidief glioblastoom. Patienten met een minder goede WHO performance status, meerdere laesies, tumoren met een grotere maximale diameter (> 42 mm), en steroid gebruik bij inclusie in de studie bleken een slechtere overleving te hebben. Patienten met een overwegend frontale tumor lokalisatie hadden een betere overleving, leeftijd en geslacht hadden geen invloed op de overleving.

In **Hoofdstuk 7** wordt de prognose van AOD en AOA bestudeerd. Gezien de klinisch significante interobserver variatie bij het stellen van de diagnose AOD en AOA is het vaststellen van biomarkers met prognostische betekenis belangrijk, zowel voor het identificeren van homogene groepen patienten als voor de behandeling van individuele patiënten. De gegevens van patienten met een AOD of AOA behandeld in EORTC studie 26951 naar de betekenis van adjuvante PCV chemotherapie werden gebruikt voor het ontwikkelen van multifactoriële prognostische modellen. Jongere leeftijd, bij CT or MRI scan bevestigde totale resectie, frontale lokalisatie van de tumor, goede WHO performance status, afwezigheid van necrosis en/of endotheel proliferatie, gecombineerd 1p/19q verlies en de aanwezigheid van IDH1 mutaties bleken van onafhankelijke prognostische betekening voor OS en Progressie vrije overleving (PFS); de behandeling was ook van invloed op de PFS. De prognostische modeleln hebben een hoge positieve predictieve waarde. Patienten met een hoog risico op ziekte progressie en dood kunnen hiermee goed worden geïdentificeerd hetgeen hun behandelaren kan helpen bij het nemen van behandelingsbeslissingen.

In **Hoofdstuk 8** worden nieuwe prognostische modellen ontwikkeld voor laaggradige glioom (LGG) patienten waarbij de diagnose bevestigd was door centrale pathologie revisie. Deze werden ontwikkeld uit de gegevens van twee prospectieve EORTC studies naar de radiotherapeutische behandeling van het LGG. Deze modellen werden gevalideerd met data van patiënten met een eveneens bij centrale pathologie revisie bevestigde LGG uit twee Noord-Amerikaanse studies, van de Radiation Therapy and Oncology Group (RTOG) en North Central Cancer Treatment Group (NCCTG). De aanwezigheid van neurologische uitval zoals vastgesteld met de Medical Research Council (MRC) score, een kortere duur sinds het eerste symptoom, astrocytaire histologie, en tumoren met een diameter meer dan 5 cm identificeerden patiënten met een slechte prognose. De prognostische modellen maakten het mogelijk de patienten in drie verschillende groepen onder teverdelen, die zowel voor Europese als Noord-Amerikaanse patiënten valide bleken. Moleculaire facotren en andere klinische factoren als de MMSE zullen het wellicht in de toekomst mogelijk maken de prognose nog beter in te schatten.

Conclusie. Dit proefschrift over patienten met hersentumoren die zijn behandeld in klinische studies draagt bij aan het vaststellen van de interobserver variatie bij de histologische diagnose van graad III gliomen, heldert het de mogelijke betekenis op van moleculaire biomarkers voor zowel prognose als

predictie van behandelings resultaat bij graad III gliomen, valideert het bekende prognostische factoren en identificeert nieuwe prognostische factoren bij glioblastomen en graad II /III gliomen, en ontwikkelt nomogrammen en prognose calculators voor uitkomst en predictie in individuele patiënten. Daarnaast werden prognostische modellen voor LGG in grote externe datasets gevalideerd. Daarmee biedt dit proefschrift steun bij behandelingsbeslissingen bij individuele patiënten en bij het opzetten van nieuwe klinische studies.

Chapter XI. Future perspectives

Currently, the diffuse glioma are classified and graded based on their morphological appearance. This classification was originally built on the presumed cell of origin, and as a consequence tumors were named astrocytoma, oligodendroglioma and ependymoma. The sheer existence of the so-called mixed oligoastrocytoma's having elements of both is however already proof that in some cases no clear classification was possible. The current WHO classification of brain tumors grades the diffuse glioma into grade II (low grade), grade 3 (or anaplastic) and grade 4 (glioblastoma).[1] The importance of this classification is the clear prognostic information that is captured in the histological diagnosis, and the fact this is the pillar on which adjuvant therapies following surgery are based. The current treatment guidelines for diffuse glioma are based on data from large randomized phase III studies in which patient eligibility depended on meeting inclusion criteria which included the histopathological diagnosis according to the WHO classification. This underlines the clinical relevance of this classification. Historically, the histopathological WHO criteria have been compiled based on the results of retrospective studies or expert opinions, and indeed they are rarely based on prospective data related to patient outcome and/or response to therapy.[2-4] Nevertheless, in 2013 histopathology remains the gold standard for diagnosing and treating glioma patients and for inclusion into trials. However, it is also clear that within individual diagnostic categories outcome may vary significantly, and a more individualized, tailored approach to patients is desirable. A further development is the coming of age of molecular subdivisions of the diffuse gliomas, some of which are beginning to have therapeutic consequences.

For this thesis, analyses of data from EORTC Brain Tumor Group trials were used to further study the 'performance' of classical histology. Although this work confirmed that mixed anaplastic oligoastrocytomas with tumor necrosis had similar prognosis compared to glioblastomas, and that the prognosis of glioblastomas with oligodendroglial components were not prognostically different from other glioblastomas, it also showed major shortcomings of classical histologogical classification.[5-7] The amount of the variation in typing and grading of gliomas between neuropathologists was found to be unacceptably high in grade III gliomas, experiences have also been documented in trials on low grade glioma. In order to improve the functioning of the histopathological diagnosis of gliomas:

- Histopathological criteria must be made more objective and reproducible.
- They must better reflect the prognosis of the patients.
- They must be evaluated together with recently established molecular markers.

Review by panel of neuropathologists of materials collected in prospective trials from homogeneously treated patients can be used to select the most reproducible criteria. Non - reproducible phenotypic criteria, and those irrelevant to predict patient outcome must be eliminated. Selected histopathological criteria must be analysed together with molecular markers in multivariate prognostic models. The results of ongoing panel review of histological materials collected in EORTC trial 26951 (AOD/AOA) and 26882 (GBM/AA) is expected to contribute to establish a more reproducible and prognostically more meaningful glioma typing and grading for gliomas.
The two other major shortcomings of the current WHO classification are the variation in prognosis within one tumor class/grade, and the absence of molecular factors. The other element is that even in clearly defined subgroups of patients outcome may differ substantially. This has great clinical relevance, as in the outcome of poor prognostic factors intensive treatments may not be warranted, and a more conservative approach or less aggressive in some patients with very favorable outcome may be indicated. For this analysis of well-defined and homogeneously treated patients populations

is crucial. To make a more indidualized prognostication possible, even within defined histopathological subsets of glioma, as part of this thesis prognostic models were developed for all grades of gliomas including recurrent glioblastomas.[8] To have clinical relevance,

- The prognostic models must be validated in large independent datasets of the same population.
- Old factors must be challenged and/or replaced by new markers if shown to be clinically and/or biologically more relevant.
- Improvement in prognostication must be measured by assessing model's discrimination and predictive accuracy.

One rationale to leave out molecular factors in the current WHO classification is that this classification aimed at being suitable for use throughout the world – and molecular marker assessment is not. The current advances in molecular understanding of glioma make this 'leaving out' of molecular factors however untenable. Current data show that the strong predictive and prognostic factors which were identified in the recent years are overriding the prognostic information derived from the histopathological classification alone. For the assessment of the clinical relevance of these molecular factors several items are relevant:

- Their respective role and utility for the management of gliomas must be further investigated, preferably in prospective trials
- The difference must be made between factors which can predict patient outcome (prognostic value) and those which can identify patient responding to therapy (predictive value)

Such analysis were presented in this thesis for several molecular factors.[5,8] In addition, assays must be available that allow reliable assessment of these markers, according to clinical standards.[9-11] This requires quality control as part of the implementation process. Unfortunately, this principle is more often ignored than adhered to.[10] Despite the demonstrated clinical relevance of these molecular factors, the implementation of these factors does not imply that the histopathological classification has lost it's relevance. Molecular factors need to be understood in the histopathological context. Also, even the presence of molecular factors with demonstrated relevance, other clinical factors remain of independent prognostic value. For some factors, the relevance could no longer be demonstrated in the presence of clinical factors (although that may have been related to the limited group size of some projects).[5] It remains likely though, that prognostication will remain a process of several factors of different nature (clinical, molecular, and treatment related).

It is at present unclear which molecular techniques are required for an optimal classification of gliomas: single gene studies for IDH, MGMT status, and1p/19q determination, or more genome wide approaches like whole genome sequencing, expression analysis or genome wide methylation analysis; or any combination of these techniques. As all of these approaches have advantages and shortcomings, this matter remains at present unresolved. This is further complicated by the improvement on an almost daily basis of techniques to demonstrate DNA changes, RNA expression and epigenetic changes. More powerful techniques are rapidly approaching and will affect the day to day clinical practice in a few years. The challenge is how to implement these techniques in order to optimize patient treatment, or even to advance patient treatment. Now that whole genome approaches are becoming robust enough to be used on formalin fixed, paraffin embedded tumor samples these techniques are ready to be evaluated in clinical trials as well.[12,13]

With more targeted treatments approaching the clinical arena of brain tumors further a routinely conducted full molecular characterization of gliomas becomes of interest. Indeed, the holy grail of molecular diagnostic oncology is to identify predictive factors that are correlated to outcome, in particular those that are qualitative. This implies that in the presence of a factor a treatment will work

but not in its absence (or vice versa). These are the most relevant factors for patient treatment. Currently, within the field of neuro-oncology two of these predictive factors have been defined: MGMT promoter methylation and 1p/19q loss. These factors however predict responsiveness to classical chemotherapy, which is useful to avoid overtreatment in patients that will not respond. The implementation of these factors will therefore not improve overall outcome, for that novel treatments are needed. Since several other molecularly targeted therapies are actively being explored, and new molecularly defined targets – that may exist in only small subgroups of glioma patients- are likely to follow. Again, clinical studies are needed to fully elucidate the clinical relevance of these factors, and whether established targets in gliomas response to the same treatments as the patients with similar targets in other cancers. The concept of personalised medicin based on target identification as opposed treatment based on classical diagnostic procedures remains to be proven for gliomas. Such a proof of concept requires well considered studies and genome wide screening procedures of large groups of patients. The rarity of 'drug-able' chromosomal lesions in gliomas prohibit the classical phase II and phase III approaches. But targeted therapies in glioma patients should no longer be conducted in all comers, but only in molecularly relevant glioma subtypes. This is the challenge for the future of glioma treatment.

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Since 1999, I have got the great honor to work for the EORTC Headquarters and since 2001 for the EORTC Brain Tumour Group. At the start of my career at EORTC, I had a limited idea of what clinical cancer research really was and what was the role of a statistian in this area. Indeed, my initial backgrounds, Physics and Actuarial Sciences did not completely prepare me for this position. Most of my present knowledge has come from trainings I received at the EORTC HQ either, during clinical presentations or "Statsclub", but also from external seminars I was allowed to attend in different universities or congresses (Harvard Medical School, LUC, UCL, Lancaster University, ISCB, ASCO, ENASCO, SNO,…).

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Curriculum Vitae

Thierry Gorlia is born on March 27th 1967 in Ath (Aat), Belgium. After graduating from secondary education at the Athenée Royal in Ath (Belgium) he started studying Physics at the University of Mons (Hainaut, Belgium). He was graduated in 1989 with mention "Distinction". In 1990, he started the Master of Sciences in Actuarial Science at the Free University of Brussels (ULB). He was graduated in 1992 with mention "Grande Distinction". Between 1993 and 1995, he worked as research actuary at the Institute of Statistics (Department of Actuarial Science) at the ULB. Between 1996 and 1998 he worked for different pension fund companies as consulting actuary. In 1998 he worked as consulting statistician and IT specialist for the Statistical Software company SPSS Belgium.

In June 1999, he joined the European Organisation for Research and Treatment of Cancer (EORTC) Data Center. Up to 2001, he worked part time for the Health Economics Unit (HEU) and the Quality of Life Units. As from 2001, he worked for HEU and became the statistician of EORTC Brain Tumor Group. In 2003 HEU was closed and he took over the position of statistician of the EORTC Chronotherapy Group till it was also close in 2006. Since 2006, he is providing support to other group statisticians to develop statistical designs and analyses plan for Translational Research projects. He also worked in the field of Head and Neck cancer. He has made a number of contributions to Health Economics (ESMDM, SMDM) , Neuro-Oncology (SNO), Biostatistics (ISCB) conferences. He contributed to over 50 publications in major peer reviewed journals.

In 2009, after discussion with Dr. M.J. van den Bent, he registered as a PhD student at Rotterdam University for the present research. EORTC offered him the possibility to carry out this project in conjunction with his regular work at the organization.

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