LOWER-GRADE GLIOMAS: A SINGLE-CENTRE ANALYSIS OF 218 PATIENTS

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DECLARATION

I, Alberto A. Gabbai, confirm that the work presented in this dissertation is my own and has not been submitted to any previous degree. Where information has been derived from other sources, I confirm that this has been indicated in the dissertation.

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ABSTRACT

Lower-grade gliomas (LGG) are the most common tumours of the central nervous system in young adults. They were classified by the 2007 World Health Organization (WHO) as Grade II and III in astrocytomas, oligoastrocytomas and oligodendrogliomas, as part of a grading system (I, to IV) aiming to predict the biological behavior of a neoplasm (I benign – IV malignant). But due to the interobserver variability in the distinction between WHO grades II and III, and in the definition of the oligoastrocytomas, a new 2016 revised WHO Classification introduced an integrated phenotypic and genotypic classification that was aimed to minimize diagnostic variability. The two most important molecular markers for the diagnosis of LGG are the IDH- and the 1p/19q-molecular status.

Hence, our main purpose herein was to confirm or refute the extant evidence that patients with IDH-wildtype tumours do have a significantly lower overall and progression-free survivals compared with those with IDH-mutant tumours.

We analyzed a single-institution cohort of 218 patients with lower-grade gliomas diagnosed between 2009 -2015, according to the 2007 WHO Classification, but also with results of IDH and 1p/19q status, the two defining molecular markers for lower-grade gliomas set by the 2016 Classification. We present their demographics, pathologic data and overall (OS) and progression-free (PFS) survivals, associated with histologic class, WHO grade and molecular subtypes.

There were 118 males and 100 females; 94 astrocytomas, 45 oligoastrocytomas and 79 oligodendrogliomas. 154 were IDH-mutant (71, 1p/19q-codeleted and 81, 1p/19q-retained, and unknown in 2) and 64, IDH-wildtype.

The longest median OS (20.45 years) was found for oligoastrocytomas grade II, oligoastrocytomas IDH-mutant and LGG IDH mutant 1p/19q-codeleted grade II.

The shortest median OS was 1.24 years for LGG IDH wild-type grade III. Oligoastrocytomas IDH-wildtype, and astrocytomas IDH-wildtype had OS of 1.5 years.

The longest median PFS was 6.72 years for oligodendrogliomas IDH-mutant, and

the shortest was for LGG IDH-wildtype grade III (0.74 years).

We have found that in LGG, the IDH molecular marker is the most important predictor of outcome, over and above histologic class and tumor grade.

IMPACT STATEMENT

Lower-grade gliomas are the most common tumours of the central nervous system in young adults. They were classified by the 2007 World Health Organization (WHO) as Grade II and III in astrocytomas, oligoastrocytomas and oligodendrogliomas, as part of a grading system (I, to IV) aiming to predict the biological behavior of a neoplasm (I benign – IV malignant). Nonetheless due to the marked interobserver variability in the distinction between WHO grades II and III, and also in the definition of the oligoastrocytomas, a new 2016 revised WHO Classification introduced an integrated phenotypic and genotypic classification that was aimed to minimize diagnostic variability. That new 2016 Classification allowed for a better correlation with prognosis and hence capable of optimizing patients' therapies.

Notwithstanding, recent advances in the molecular diagnosis (targeted next-generation sequencing and epigenetic changes) have clearly improved the prognostic and predictive accuracy of lower-grade gliomas on top of the IDH and 1p/19q status, the two defining molecular markers for lower-grade gliomas set by the 2016 WHO Classification. But those advances have had a small impact; for example, DNA methylation-based classification (epigenetic changes) of central nervous system tumours have resulted in a change of diagnosis in only 12% of prospective cases.

It is obviously commendable for research to continue in order to find better treatments for those hitherto incurable and mostly fatal tumours.

Nonetheless from a practical point-of-view the question is that what are the minimal requirements for a molecular classification of lower-grade gliomas to guide a more precise oncologic treatment.

We think that our data, that are mostly consistent with previous studies on the subject, give some guidance: just the histologic class and grade, associated with the IDH status (IDH-mutant or IDH-wildtype) was sufficient to discern our longest and shortest medial overall and progression-free survivals.

Hence from a cost-benefit analysis, an experienced pathologist and a non-difficult immunohistochemistry technique to diagnose IDH-mutations appear to be enough to guide appropriate medical interventions.

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INTRODUCTION

The Gliomas

Glioma as a separate histopathological entity was first recounted by Virchow in his lectures from 1863-1865, when he distinguished them from “psammomas”, melanomas and “other sarcomas”, and even separated them into two groups that are now recognized as low- and high-grade gliomas (Virchow, 1863 - 1865) indeed, it was also he who in 1858 coined the term glia (neuro-glia) that was derived from the Greek word “glue”, for the cells that bind the central nervous tissue together (Chance, 1859).

In 1926 (63 years later) a new histological classification of brain tumours was published by Percival Bailey and Harvey Cushing (Bailey and Cushing, 1928) where for the first time a more robust correlation between histopathology and prognosis was made. In that classification the histopathological names were based upon the resemblance that the tumours had towards distinctive central nervous system cells, i.e., glia and neurons. That distinction had been made possible by the pioneering work of Santiago Ramón y Cajal and Pio del Rio Hortega, founders of the Spanish School of Neurohistologists, to whom Bailey and Cushing’s work was dedicated.

Due to the early days of tissue fixation, microtome construction (Bracegirdle, 1986) and staining techniques, it was not possible for Virchow to differentiate among astrocytes, oligodendrocytes and microglia (Koblenz, 2013).

Using a particular silver staining devised by Camilo Golgi in 1873, Santiago Ramón y Cajal and Pio del Rio Ortega were able to clearly differentiate neurons from the three types of glia, astrocytes, oligodendrocytes and microglia (de Castro and Merchan, 2016, Koblenz, 2013, Perez-Cerda et al., 2015, Shepherd, 2015).

Notwithstanding the fact that Heinrich Wilhelm Gottfried Waldeyer-Hartz coined the term neuron in 1891 (Winkelmann, 2007), it was Ramon y Cajal who, starting in 1888, walked all the way through to really demonstrate that the cell theory (Mazzarello, 1999) factually enunciated by Theodor Schwann in 1839 (Smith, 1857),

also belonged to the central nervous system, the “neuron doctrine” (Shepherd, 2015).

Based upon the first relevant classification of brain tumours in 1926 a plethora of publications followed but it took 53 more years until most of the world specialists agreed to a common brain tumor nomenclature (Zulch, 1986). In 1979 KJ Zülch managed to get through the first edition of that agreed classification via the World Health Organization (Zulch, 1979). The second edition, published in 1993 by Paul Kleihues, incorporated advances in immunohistochemistry (Kleihues, 1993). The third edition, published in 2000, also by P Kleihues, included succinct sections on epidemiology, clinical aspects, imaging, prognosis and predictive factors (Kleihues, 2000).

The fourth edition published in 2007, by DN Louis, listed several new entities and again it was accompanied by a concise commentary on clinicopathological characteristics of each tumor type (Louis et al., 2007).

In the fourth edition of the WHO classification of Central Nervous System tumours, gliomas were divided in astrocytomas (astrocytic tumours), oligodendrogliomas (oligodendroglial tumours) and oligoastrocytomas (oligoastrocytic tumours) and were graded I, II, III and IV (Louis et al., 2007).

Astrocytomas

Grade I astrocytomas, pilocytic astrocytomas (that occur mostly in children and young adults), pleomorphic xantoastrocytomas and subependymal giant cell astrocytomas are considered “circumscribed” in growth and behavior and will be only briefly considered here.

WHO grades II to IV were used as an important component to predict outcome. In astrocytic, oligodendroglial and oligoastrocytic tumours, grade II is defined as diffusely infiltrative tumours with cytologic atypia alone, grade III tumours show

anaplasia and mitotic activity, and grade IV show additionally microvascular proliferation and/or necrosis (Louis et al., 2007).

Grade II, III and IV are considered “diffuse” gliomas, have the ability to infiltrate the surrounding normal brain parenchyma and will almost always recur whatever the amount of “gross total resection” (Rees, 2016). Indeed, that has been known since 1928, when Walter E. Dandy resected the whole right hemisphere that harboured gliomas in four patients, only to see them eventually recur in the patients’ left hemisphere (Dandy, 1928).

Grade II (diffuse) and III (anaplastic) astrocytomas (Low Grade) are composed of neoplastic astrocytes with nuclear atypia within a loosely structured tumor matrix. Cellularity is moderately increased and in grade II mitotic figures are rare or absent. At the infiltrative edge of the tumor glioma cells are intermingled to varying degrees with reactive brain elements (entrapped neurons, reactive astrocytes) making it impossible to determine the true margin of the tumor. In fact, individual glioma cells often migrate several centimetres from the tumor to populate histologically normal appearing brain. There is no endothelial proliferation or necrosis. Most grade II astrocytomas and if not, all grade III will eventually progress to grade IV tumours (Louis et al., 2007).

Grade IV astrocytomas, glioblastoma multiforme (GBM), (Malignant Glioma). As the name “multiforme” implies, the histopathology of GBMs can be highly variable, even within the same tumor. While some regions show a high degree of nuclear pleomorphism with numerous multinucleated giant cells, other areas may be highly cellular but rather monotonous. The astrocytic nature of the neoplastic cells may be easily identifiable in some regions, but difficult to recognize in others due to the high degree of anaplasia. Thus, the diagnosis of GBM is usually based on the characteristic histological features rather than the identification of certain cell types. These include nuclear atypia, mitoses, and as essential features, marked endothelial proliferation and/or necrosis (Louis et al., 2007).

Oligodendrogliomas

Like astrocytomas, grade II (oligodendroglioma) and III (anaplastic oligodendroglioma) oligodendrogliomas are diffusely infiltrating gliomas. They are composed of sheets of cells with round regular nuclei and clear cytoplasm (giving them a fried egg appearance) and a delicate network of branching capillaries (chicken wire vasculature). Oligodendrogliomas are typically slow-growing tumours but most will also eventually progress to malignancy (glioblastoma, WHO grade IV), with endothelial proliferation and/or necrosis (Louis et al., 2007).

Oligoastrocytomas

In varying degrees those have the histological appearance of both astrocytomas and oligodendrogliomas and are also classified as Low Grade II (oligoastrocytoma) and III (anaplastic oligoastrocytoma) (Louis et al., 2007).

The updated 2016 World Health Organization Classification of Tumours of the Central Nervous System

In the update to the fourth edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS), published in 2016 (Louis et al., 2016), molecular parameters were incorporated complementing histology, to define the diffuse gliomas, ependymomas, medulloblastomas, and other embryonal tumours. In this “integrated” phenotypic and genotypic classification (Louis Haarlem), the “family tree” of the diffuse gliomas include now the WHO grade II and III astrocytic tumours, the grade II and III oligodendrogliomas and the grade IV glioblastomas. The astrocytomas that have a more circumscribed growth pattern, pilocytic astrocytoma, pleomorphic xanthoastrocytoma and the subependymal cell astrocytoma are now considered distinct from the diffuse gliomas. The diagnosis of oligoastrocytoma is strongly discouraged (Louis et al., 2016).

The most important molecular markers for the classification and prognostication of diffuse gliomas are now mutations in the isocitrate dehydrogenase 1/2 (IDH1/IDH2) genes and the presence of the allelic loss of chromosomes 1p and 19q (1p/19q codeletion). Lower-grade gliomas (Grades II and III) that are IDH-mutant and 19/19q-codeleted are termed oligodendrogliomas while those IDH-mutant lacking 1p/19q codeletion (and with a loss of expression of ATRX) are astrocytomas. Astrocytomas may be IDH-mutant or IDH-wildtype and the rare finding of oligodendrogliomas IDH-wildype are termed oligodendroglioma not otherwise specified (NOS) (Louis et al., 2016).

Gliomas Incidence and Survival Rates

Incidence

The incidence of new astrocytomas and oligodendrogliomas (Grades II and III) in Western countries is ~1/100,000. Glioblastomas have an incidence of ~3.5/100,000, and all new intrinsic primary tumours occur in ~5,5/100,000/year (Ohgaki and Kleihues, 2005a, Zada et al., 2012). In the USA (population ~320 million), according to the Center of Brain Tumor Registry of the Unites States (Ostrom et al., 2017) the incidence/100,000/year was 3.2 for glioblastomas, 0.48 for astrocytomas grade II, 0.40 for astrocytomas grade III, 0.24 for oligodendrogliomas grade II, 0.11 for oligodendrogliomas grade III, and 0.19 for oligoastrocytomas. The number of new cases/year in the years 2010 to 2014 was: 1,559 astrocytomas grade II, 1,307 astrocytomas grade III, 751 oligodendrogliomas grade II, 344 oligodendrogliomas grade III, 598 oligoastrocytomas (total of 4,559 Lower-grade Gliomas), and 11,284 glioblastomas (Ostrom et al., 2017). In the UK (population ~70 million) that would equate to ~900 new astrocytomas, oligoastrocytomas and oligodendrogliomas/year and ~2,200 new glioblastomas.

The incidence/year of pilocytic astrocytomas (0.3/100,000); pleomorphic xantoastrocytomas (0.2/100.00) and subependymal astrocytomas (0.1/100,000) is much lower, leading respectively to 960, 640 and 320 new cases/year in the USA, and 220, 140 and 70 cases/year in the UK (Ohgaki and Kleihues, 2005b).

Survival Rates

The best survival rates are for pilocytic astrocytomas – 96% are still alive after 10 years.

For the infiltrating gliomas, the prognosis is less favourable being the most dismal for glioblastomas (grade IV) with a median survival, without treatment, of 0.4 years (5 months). With chemoradiation 10% of patients may survive for 5 years (Stupp et al., 2009) and in younger patients, median survival may reach 4 years (Herrlinger et al., 2019); but the median survival for the elderly is still very low, no more than 10 months (Rusthoven et al., 2017).

Grade II astrocytomas were reported to have a median survival in the range of 6 – 8 years, influenced mainly by the time to malignant progression reported at 4 – 5 years (Ohgaki and Kleihues, 2005a, Okamoto et al., 2004). A multivariate analysis of two European Organization of Research and Treatment of Cancer (EORTC) trials showed that age ≥ 40 years, astrocytoma histology, tumours ≥ 6 cm, tumours crossing the midline and presence of neurologic deficit before surgery, were all unfavourable prognostic factors for survival (the “Pignatti risk score”) (Pignatti et al., 2002).

Grade III astrocytomas median survival has been reported in the range of 3 – 5 years. Older patients and the presence of neurologic deficit before surgery were also unfavourable prognostic factors, but a larger extend of resection seemed to prolong survival. Virtually all grade III astrocytomas will either recur or progress to glioblastoma (Wick et al., 2009).

Grade II oligodendrogliomas typically have relatively long median survivals reported at 11.6 years with a 10-year survival rate of 51% (Ohgaki and Kleihues, 2005b). The CBTRUS has documented 5-year survival rates of 79.5% (Ostrom et al., 2017).

However, there is a wide reported variation in the survival estimates. One single-institution has documented an even longer median survival time of 16.7 years (Olson et al., 2000) while another has documented a very short median survival of 3.3 years, but with an intriguing 84% 5-year survival rate (Dehghani et al., 1998).

Grade III oligodendrogliomas typically have a much shorter median survival, when compared to grade II. It is reported at 3.5 years, with a 5-year survival rate of 52.2% and a 10-year survival rate of 39.3% (Louis et al., 2016).

However, none of these data considered molecular status (IDH-mutation and 1p/19q-codeletion) which is robustly associated with much longer survival and response to adjuvant radiotherapy/chemotherapy (Cairncross et al., 2013, van den Bent et al., 2013).

An international retrospective study of 1013 patients with grade III oligodendrogliomas documented a median overall survival of 8.5 years in patients with 1p/19q-codeleted tumours versus 3.7 years in 1p/19q-retained tumours (Lassman et al., 2011).

There is scant separate data in the literature related to median survival of the mixed oligoastrocytomas. The CBTRUS has documented 5-year survival rates of 61.1% and 10-year of 46.9% (Ostrom et al., 2017).

Most of the epidemiological data described above, although accurate, have not been validated by molecular parameters and precede the more intensive recent chemoradiation protocols that have substantially improved the overall survival of lower-grade gliomas (Berger et al., 2016).

Recent data has shown that molecular analysis may be even more accurate for estimating survival than the “Pignatti risk score” (Etxaniz et al., 2017).

Treatment

Much of the variation in reported overall and progression-free survival estimates probably occur because of lead and length time biases, a lack of standard treatments and follow-up analyses, and the interobserver variation in diagnosis based on morphological criteria alone (van den Bent, 2010).

Before the three pivotal treatment trials in LGG that were published in 2013 and 2016, van den Bent et al (EORTC 22845 randomized trial) had shown in 2005 that for grade II gliomas, early radiotherapy after surgery extended the median progression-free time but did not affect overall survival (Sarmiento et al., 2015, van den Bent et al., 2005).

In 2013, Cairncross et al (RTOG 9402 treatment trial) randomized 291 patients with oligodendroglioma (AO) or oligoastrocytoma (AOA) grade III to either procarbazine, lomustine (also called CCNU), and vincristine (PCV), plus radiotherapy (RT) versus RT alone. Follow-up revealed that PCV plus RT did not prolong median overall survival (OS) time that was 4.6 years for one group and 4.7 for the other. Nonetheless, patients with 1p/19q-codeleted tumors lived much longer than all others (PCV plus RT: 14.7 v 2.6 years, hazard ratio (HR) = 0.36, 95% CI, 0.23, 0.57, P = 0.001; RT: 7.3 v 2.7 years, HR = 0.40, 95% CI, 0.27 to 0.60, P = 0.001), and their median progression-free survival (PFS) was also significantly longer. They did not have data on their patient's IDH-mutation status.

They concluded that in the subset of patients with 1p/19q-codeletd AO or AOA, PCV plus RT was an especially effective treatment (Cairncross et al., 2013).

Also, in 2013, van den Bent et al (EORTC study 26951) randomized 368 patients with oligodendroglioma grade III (AO) either to initial RT alone or RT followed by PCV. After a median follow-up of 140 months (11.6 years) median OS in the RT/PCV arm was significantly longer (42.3 (3.5 years) v 30.6 (2.5 years) months in the RT arm, HR, 0.75; 95% CI, 0.60 to 0.95). In the 80 patients with a 1p/19q codeletion, OS was increased, with a trend toward more benefit from adjuvant PCV (OS not reached in the RT/PCV group vs 112 months (9.3 years) in the RT group; HR, 0.56; 95% CI, 0.31 to 1.03). Median PFS was also significantly longer. IDH-mutational status was also of prognostic significance.

They concluded that the addition of six cycles of PCV after 59.4 Gy of RT increases both OS and PFS in anaplastic oligodendrogliomas. 1p/19q-codeleted tumors derive more benefit from adjuvant PCV compared with 1p/19q-retained (van den Bent et al., 2013).

In 2016, Buckner et al (ClinicalTrials.gov number, NCT00003375 - final result of the RTOG 9802 trial) studied 251 patients with grade II gliomas who were younger than 40 years of age and had undergone subtotal resection or biopsy or who were 40 years of age or older and had undergone biopsy or resection of any of the tumor. They were randomly assigned to RT alone or to RT followed by six cycles of PCV. After a median follow-up of 11.9 years, patients who received RT plus PCV had longer median OS than did those who received RT alone (13.3 vs. 7.8 years; HR for death, 0.59; P = 0.003). Also, the rate of progression-free survival at 10 years was 51% in the group that received RT plus PCV versus 21% in the group that received RT alone.

They concluded that the patients that received RT plus PCV had a much longer OS and PFS than those who received RT alone (Buckner et al., 2016).

More recently, in 2017, van den Bent et al published the interim results of the CATNON trial (EORTC study 26053-22054), another success of upfront chemotherapy in LGG.They studied 745 patients with gliomas grade III, 19/19q-retained. It was a randomized study with a 2x2 factorial design. Patients were assigned in equal numbers (1:1:1:1), to receive radiotherapy (59.4 Gy in 33 fractions of 1.8 Gy) alone or with adjuvant temozolomide (12 4-week cycles of 150–200 mg/m2 temozolomide given on days 1–5); or to receive radiotherapy with concurrent temozolomide 75 mg/m. per day, with or without adjuvant temozolomide. After a mean follow-up duration of only 27 months (2.25 years) the Data Monitoring Committee recommended early release of the results because the hazard ratio (HR) of OS in the two adjuvant temozolomide arms was 0.65 (99.145% CI, 0.45-0.93). They concluded that adjuvant temozolomide chemotherapy was associated with a significant survival benefit in patients with newly diagnosed 1p/19q-retained anaplastic glioma (van den Bent et al., 2017).

Hence it appears that upfront adjuvant chemotherapy after RT is better than RT alone in LGG (in patients 40 years-old and older or younger than 40 years-old with

less than total resection) but many questions remain regarding its optimal use. It is still not clear how many cycles of chemotherapy are ideal and what is the value of PCV versus temozolomide. Moreover, the hypermutated phenotype of LGG that results from treatment with temozolomide and leads to an alternative evolutionary path to high-grade (Grade IV) glioma will have to be sorted out (Johnson et al., 2014). The maturity of outcome data and the refinement of molecular subgroup analyses will probably answer those questions (Penas-Prado and de Groot, 2017).

Understanding Cancer - Historical Perspective

A long time had elapsed before our final understanding that cancers arise from the sequential acquisition of genetic alterations in specific genes (Nowell, 1976).

In 1888, Waldeyer named chromosomes the nuclear elements that were known to split longitudinally during mitosis (Winkelmann, 2007). These structures were conspicuously stained following the standard fixation and staining procedures during cytological preparation and hence the term, from “chroma”, that means coloured in Greek (Cremer and Cremer, 1988).

In 1890, David von Hansemann described aberrant mitotic figures in human carcinoma samples and postulated that those were responsible for the abnormal chromatin content found in cancer cells (Cancer Milestones, 2006).

The early 1900’s saw the “rediscovery” of the Mendelian laws, the nascent chromosome theory of heredity and cancer, and in 1906, William Bateson coined the term genetics (Bateson, 1906).

Theodor Boveri, intrigued by von Hansemann’s findings, worked from 1902 to 1914 on the fertilization of sea urchin eggs and devised a first chromosome theory of heredity, but most importantly also a theory of cancer based on chromosome abnormalities. Looking back, it is astonishing to appreciate his predictions on the

mechanisms of cancer and specially: the clonal origin of cancer, oncogenes, tumor-suppressor genes and genetic mosaicism (Balmain, 2001).

Parallel to the ground-breaking work of Theodor Boveri, starting in 1871 Miesher, Altmann and Kossel defined that there were nucleic acids and not proteins the core substances within cell nuclei and by 1910 Kossel was able to identify the fundamental building blocks of those substances (Dahm, 2008, Satzinger, 2008).

During the first half of the 20th century researchers systematically noted that tumor cells showed aberrant morphologies and those were putatively caused by the conspicuous aberrant chromosome numbers and mitoses. The descriptions concerned gross chromosomal abnormalities and marked deviant numbers, i.e., marked aneuploydia, mostly polyploydia (Koller, 1947).

At that time, the one-gene-one-enzyme hypothesis had already been demonstrated in Neurospora by GW Beadle and EL Tatum in 1941 (Beadle and Tatum, 1941) and in 1944, OT Avery, CM MacLeod and M McCarty had shown that the molecule inducing transformation in Pneumococcus was actually DNA, i.e., genes were made of DNA (Avery et al., 1944).

Finally, in 1953, Watson and Crick unveiled the double-helix molecular structure of nucleic acids (Watson and Crick, 1953).

Nonetheless, researchers were unable to be more precise about the observed chromosome alterations in cancer since nobody knew the exact normal number of chromosomes in humans.

Until the pivotal demonstration, in 1956, of Tjio and Levan (Tjio and Levan, 1956) that there are 46, it was still believed them to be 48 (Gartler, 2006).

Soon after that definition, P Nowell and D Hungerford, in 1960, described the first genetic defect to be associated with cancer. It was the presence of a “minute chromosome” that replaced one of the four smallest autosomes in chronic myelogenous leukaemia (CML) and was called the Philadelphia chromosome, from the city where the paper was presented (Sciences, 1960).

At that time, the human chromosomes were divided by groups according to size and had not been numbered yet. The precise numbering of human chromosomes occurred after the introduction of staining techniques (quinacrine fluorescence and Giemsa banding) capable of discerning characteristic banding patterns. Only after the meeting of the “Paris Nomenclature”, in1971, is that everybody agreed on the precise numbering of the human chromosomes (Paris, 1973).

Peyton Rous in 1911 could not imagine that he had discovered an oncogene. He showed that cell-free filtrates from a spindle-cell sarcoma originated in a Plymouth Rock hen induced tumor growth when inoculated into the breast and peritoneum cavity of other healthy hens (Rous, 1983).

Fifty years later, with the discovery of the retroviruses in the 1960’s (Baltimore, 1970) that biological agent in cell-free filtrates capable of inducing sarcomas in hens was shown to be exactly that, an RNA-virus, and named RSV (Rous Sarcoma Virus) (Martin, 1970).

In a series of experiments during the 1970’s, Varmus and Bishop subsequently demonstrated that the oncogene (v-src - src is short for sarcoma) responsible for the transforming properties of the RSV existed in a normal physiologic form (proto-oncogene) in the genomes of healthy vertebrates. What were the functions of those proto-oncogenes and how were they mutated, rearranged, translocated or amplified (overexpressed) leading or contributing to malignancy remained unclear. Nonetheless Varmus and Bishop’s work paved the way for the discovery of all the oncogenes presently known (Bishop, 1989, Varmus, 1989).

In 1973, using quinacrine fluorescence, Janet Rowley discovered that the Philadelphia chromosome was due to a translocation between the long arm of chromosome 22 and the long arm of 9 (Rowley, 1973).

In 1982, Annelis de Klein and colleagues demonstrated that the proto-oncogene ABL1 (9q34.12) (c-ABL), the cellular human homologue of the transforming sequence of Abelson murine leukaemia retrovirus was translocated from chromosome 9 to chromosome 22 in chronic myelogenous leukaemia cells. Thus, c-ABL had a role in the generation of CML – an oncogene (de Klein et al., 1982).

When Alfred Knudson in 1971 proposed that retinoblastomas were caused by two mutations (double hit or two-hit hypothesis), he planted the seed for the discovery of tumor-suppressor genes. Using a mathematical model, he showed that cancer can arise in as few as two steps and each step can occur at a rate that is compatible with accepted values for mutation rates. In the dominantly inherited form, one mutation is inherited via the germinal cells and the second occurs in somatic cells. In the nonhereditary form, both mutations occur in somatic cells. This explains why patients with the nonhereditary form have single retinoblastomas and those with the hereditary form have multiple retinoblastomas, usually in both eyes (Knudson, 1971).

In 1983, Cavenee and colleagues localized the retinoblastoma gene (RB1) to 13q14.2 and showed that tumorigenesis may result from the development of homozygosity for the mutant allele at the RB1 locus (Cavenee et al., 1983). That phenomenon has since then been known as “loss of heterozygosity” (LOH). Finally, in 1986, Friend and colleagues sequenced the RB1 gene, the first tumor-suppressor gene discovered (Friend et al., 1986).

TP53 (tumor protein 53) (17p13.1) that was once thought to be an oncogene, was the second tumor-suppressor gene discovered. Subsequent studies demonstrated that TP53 is the most frequently mutated gene in all human cancers (Lane, 1992).

Understanding Gliomas – Historical Perspective

In 1962, AI Spriggs and MM Boddington showed that a primary tumor (glioma) of the temporal lobe carried 81 chromosomes (Spriggs et al., 1962).

In 1965, HA Lubs and JH Salmon described “markedly different types of abnormal karyotypes in three glial tumours; one medulloblastoma in an 8 year-old girl, one oligodendroglioma in a 40 year-old man, and one glioblastoma multiforme in a 43 year-old man (Lubs and Salmon, 1965).

In 1970, CB Wilson and Lili Kaufman described aneuploidy in 11 glioblastomas; 2 had polyploidy and 9 had 45 chromosomes (Wilson et al., 1970).

In 1970, J Mark and Granberg described double-minutes (chromosomes) in three gliomas, but they were not sure about the significance of this finding (Mark and Granberg, 1970).

In 1971, J Mark described chromosomal alterations in 50 astrocytic gliomas: ¾ had diploid-near-diploid stem lines (one peak at 47 and the other at 44-45) and the others were near-triploids and near-tetraploids. Of note he described, again, double-minutes in four gliomas and also in nine additional ones (Mark, 1971).

Still the significance of the double minutes was unknown.

In 1977, relying also in previous observations of their own, J Mark and colleagues described the banding patterns in four glioma cell lines. They found an increased proportion of chromosomes 7 and 19 and a reduction of chromosomes 4, 22, 10 and 12, along with a series of “recurrent marker chromosomes” (Mark et al., 1977).

In 1979, Cuatico using DNA probes showed that malignant gliomas contained tumor-specific DNA sequences that were not found in normal brain tissue (Cuatico and Cho, 1979).

In 1984, Sandra Bigner studying chromosomal banding in 12 human gliomas established clearly that simple numerical changes (aneuploidy) were the primary deviation in these tumours. Nonetheless she also pointed out three important findings: gain of chromosome 7, with concomitant loss of chromosome 10; loss of chromosome 22; and loss of gonosome (Bigner et al., 1984).

Overexpression of epidermal growth factor receptors (EGFR) in glial tumours was first described by Liberman et al in1984 (Libermann et al., 1984) and one year later the same group demonstrated that the overexpression of EGFR was due to the amplification of the EGFR gene (7p11.2) (Libermann et al., 1985).

Soon afterwards, in 1987, Bigner showed evidence that the double minutes frequently found in gliomas were related to amplified genes, mostly EGFR amplification (Bigner et al., 1987).

Finally, in 2004, Vogt et al demonstrated the molecular structure of the double-minute chromosomes bearing amplified copies of the EGFR gene in gliomas (Vogt et al., 2004).

In 1988, Bigner confirmed in 54 human gliomas that besides the frequent findings of marked aneuploidy and the loss of gonosomes, statistically significant numerical deviations in the near-diploid group of 32 tumours were gains of chromosome 7, and losses of chromosome 10. They also confirmed the findings of double minutes in 18 of the 32 tumours. Of note, they also found structural abnormalities in 9p and 19q (Bigner et al., 1988).

James el al in 1989, described mitotic recombination of chromosome 17 in astrocytomas, suggesting that the somatic attainment of homozygosity for loci on chromosome 17p is associated with the oncogenesis of these tumours (James et al., 1989). Interestingly they did not mention the possibility of LOH in TP53 that was later shown to be the case (Bigner and Vogelstein, 1990).

In 1992, von Deimling et al presented evidence of a tumor suppressor gene on chromosome 19q. In 122 gliomas from 116 patients they found that 29 tumours had loss of constitutional heterozygosity of 19q, and four had partial deletions of 19q. These abnormalities were found in grade III and IV astrocytomas, grade II and III oligodendrogliomas, and grade II and III mixed oligoastrocytomas. Those findings were the first to demonstrate genetic similarities between astrocytomas, oligodendrogliomas and mixed glial tumours (von Deimling et al., 1992).

Despite the fact of the genetic similarities among glial tumours, in 1994, Reifenberger et al demonstrated for the first time the preferential genetic marker in oligodendrogliomas: loss of genetic information from 1p and 19q and the rarity of TP53 mutations (Reifenberger et al., 1994).

In 1994, Cairncross et al showed that anaplastic oligodendroglioma are also distinct in respect to treatment, i.e., in contrast to unmanageable astrocytomas, they responded to chemotherapy. Eighteen of 24 patients responded to a combination of procarbazine, lomustine (CCNU) and vincristine; a combination that is still used today and known as PCV (Cairncross et al., 1994).

By the middle of the 2000's, the molecular signature of gliomas was thought to be established, and it was possible to differentiate between "de novo" glioblastomas (more frequent ~ 95%, and in older patients) and secondary glioblastomas (in younger patients). "De novo", means that they start as glioblastomas and do not arise through a progression from lower-grade gliomas.

CDKN2A (cyclic-dependent kinase inhibitor 2A) (9p21.3) deletions, LOH in PTEN (phosphate and tensin homolog) (10q23.21) and EGFR amplification were more prevalent in de novo glioblastomas, whereas LOH in *TP53* were more common in secondary glioblastomas. LOH in chromosome 10 was seen in both. Oligodendrogliomas exhibited loss of 1p and 19q alleles, and oligoastrocytomas with 1p/19q deletions were related to oligodendrogliomas and those with TP53 mutations were related to astrocytomas (Ohgaki et al., 2004, Rasheed et al., 1999).

In 2001, the quest to understand the nature and content of genetic information reached another quantum-leap with the first ever sequencing and analysis of the human genome (Lander et al., 2001).

In 2008, Parsons and col discovered a breakthrough in the molecular diagnosis of gliomas. Using next-generation sequencing technologies in 22 human tumor samples they found recurrent mutations in the active site of NADP+ dependent isocitrate dehydrogenase 1 gene (*IDH1*) (2q34) in 12% of glioblastoma patients. Most importantly, mutations in *IDH1* occurred in a large fraction of young patients and in most patients with secondary glioblastomas and were associated with an increase in overall survival. All mutations were heterozygous (no LOH) point mutations, a change of a guanine to an adenosine at position 395 of the *IDH1* transcript (G395A), leading to the replacement of an arginine with a histidine at amino acid residue 132 (R132H) of the protein (Parsons et al., 2008).

Soon afterwards, in 2009, Yan et al, found mutations that affected amino acid 132 of IDH1 in more than 70% of grade II and III astrocytomas, oligodendrogliomas and secondary glioblastomas. Importantly they found that tumours without a mutation in IDH1 often had a mutation affecting the analogous amino acid (R172) of the IDH2 (15q26.1) gene. Tumours with IDH1 or IDH2 mutations had the same distinctive

genetic characteristics and a better outcome than those with wildtype IDH genes. Of note, they found IDH mutations in only one “circumscribed” glioma: 1 in 7 pleomorphic xantoastrocytomas, and 0 in 21 pilocytic astrocytomas (Yan et al., 2009).

Those finding provided a new insight into the understanding and molecular classification of human gliomas.

Nonetheless, IDH mutations are also found in acute myeloid leukaemia, cholangiocarcinoma, and are particularly frequent in cartilaginous tumours. Patients with genetic syndromes that lead to enchondromatosis carry somatic mosaic mutations in IDH1 or IDH2 and are also prone to develop gliomas (Bonnet et al., 2016, Golub et al., 2019).

Gliomas do not occur only in humans but also in other vertebrates: they are found in dogs (Schiffman and Breen, 2015), and a glioblastoma was even detected at autopsy in a dolphin (Diaz-Delgado et al., 2015).

In 1995, the ATRX (alpha thalassemia/mental retardation X-linked) (Xq21.1) gene mutation was shown to cause a disease called, X-linked mental retardation with alpha-thalassemia syndrome (Gibbons et al., 1995). Since the ATRX protein (and its binding partner DAXX – death associated protein 6) was known to have a role in regulating chromatin remodelling, nucleosome assembly and telomere maintenance, Heaphy et al in 2011(Heaphy et al., 2011) found frequent ATRX mutation in pancreatic neuroendocrine tumours. ATRX loss is a diagnostic feature of IDH-mutant astrocytomas and glioblastomas, but not of oligodendrogliomas. It is also consistently lost in H3 G34R/V mutant gliomas and in a proportion of H3 K27M mutant glioma (Louis et al., 2016).

In 2012, Kannan et al found that ATRX mutation was another molecular determinant in lower-grade human gliomas: using whole exome sequencing in four low grade gliomas, followed by focused sequencing in an additional 28, they found a high incidence of mutations in the ATRX gene. ATRX mutation was restricted to IDH-mutant tumours, closely correlated with TP53 mutation and astrocytic differentiation, and mutually exclusive with 1p/19q codeletion (Kannan et al., 2012).

TERT (telomerase reverse transcriptase) is an RNA-dependent DNA polymerase lengthening telomeric DNA. Most human somatic cells lack telomerase activity leading to progressive telomere shortening during replication. Eventually telomers shorten to a critical size leading to physiologic senescence. In contrast, TERT expression is found in the majority of human malignancies, including gliomas, conceivably providing them with infinite cell proliferation (Liu et al., 2016, Vinagre et al., 2013). The overexpression of TERT depends on mutations on its promoter, a DNA sequence that is part of the TERT gene (5p15.33). The two mutations that have been described are the cause of the maintenance of telomerase activity that is so frequently seen in cancer (Leu et al., 2016).

Aberrant cancer promoted methylation (epigenetic) of the 0(6)-methylguanine-DNA methyl transferase (MGMT) gene (10q26.3) (MGMT promoter methylation) does not have diagnostic value. In the 1990's it was shown that the expression of MGMT, was a resistant factor for the chemotherapeutic treatment of gliomas. In 2000 it was demonstrated that inactivation of the MGMT, via its methylation, conferred a better response to BCNU (carmustine) and in 2005, Hegi demonstrated that glioblastoma patients with the MGMT promoter methylated fare better when treated with temozolomide (Hegi et al., 2005, Hegi and Stupp, 2015).

Analysing data up to the end of 2012, Zou et al performed the first meta-analysis on the role of IDH1/IDH2 mutations in the prognosis and molecular profiles of patients with glioma. In 10 articles that described 2,190 total patients, IDH mutations were present in 59% of lower-grade (grades II and III) gliomas, 63% of secondary glioblastomas and 7% of primary glioblastomas. IDH mutations were associated with MGMT-promoter methylation, 1p/19q codeletions and TP53 gene alterations, but mutually exclusive with EGFR amplification. They also showed a better survival in patients with IDH mutations compared to those with wildtype IDH. Indeed, in a sub-group analysis, patients with grade III tumours also had a better prognosis (Zou et al., 2013).

Two years later, considering the still debatable role regarding the status of the IDH1/IDH2 genes in the prognosis of patients with lower-grade (grades II and III) gliomas, Xia et al gathered literature data up to the end of 2014 and performed a

second meta-analysis on the subject. That analysis included 55 studies that combined the data of 9,487 adult patients from North America, Europe and Asia. They found that irrespective of place of origin, patients with gliomas carrying the IDH mutations had a significant advantage in overall and progression-free survival. Moreover, they found that survival advantage persisted in the subgroup analysis of patients with lower-grade tumours (Xia et al., 2015).

In 2015, immediately prior to the 2016, revised 4th edition WHO classification of brain tumours, two very important data sets on the molecular profiling and prognosis of human gliomas were published.

In a concerted effort, the Cancer Genome Atlas Research Network (TCGA) performed an integrated genomewide analysis of 293 lower-grade gliomas in adults. They incorporated exome sequencing, DNA copy-number profiling, messenger RNA (mRNA) sequencing, microRNA sequencing, DNA methylation profiling, TERT promoter sequencing and reverse-phase protein lysate array (RPPA) profiling. Additionally, whole-genome sequencing was performed in 21 samples and low-pass whole genome sequencing in 52. They identified three cohesive tumor classes that had distinct clinical behavior and were concordant with IDH, 1p/19q and TP53 status to a greater extent than with histologic class. Patients who had lower-grade gliomas with IDH mutation and 1p/19q codeletions had the most favourable clinical outcomes. Nearly all lower-grade gliomas with IDH mutation and no 1p/19q co-deletions had mutations in TP53 and ATRX inactivation. The large majority of lower-grade gliomas without IDH mutations had genomic aberrations and behavior similar to those found in primary glioblastoma. Comparisons were made using previously published Cancer Genome Atlas data on glioblastomas (Cancer Genome Atlas Research et al., 2015).

A team from the Mayo Clinic and the University of California, San Francisco (UCSF) concentrated their approach for the presence of IDH 1/IDH2 mutations, 1p/19q codeletions and TERT promoter mutations in 1087 diffuse gliomas of grades II, III and IV (i.e., glioblastomas included). They also searched for germline polymorphisms that could be associated with specific types of glioma and particularly with the presence or absence of tumor-specific mutations. In their analysis, 97% of

all tumours were placed into one of five molecular subgroups defined on the basis of those three markers: triple positive (mutations in both IDH and TERT, plus 1p/19q co-deletion), mutation in both IDH and TERT, mutation in IDH only, triple negative and mutation in TERT only. Only 28 (2.6%) of the gliomas could not be assigned to one of these five groups: 21 of these (75%) were oligodendrogliomas or mixed oligoastrocytomas with IDH mutations and 1p/19q codeletions but no TERT mutation. Eighty two percent (82%) of all 615 grade II or III gliomas were IDH-mutant and 91% of glioblastomas were IDH-wildtype. The molecular groups were independently associated with overall survival among patients with grade II and III tumours, but not among patients with glioblastomas. Particularly, the best overall survival among patients with grade II and III gliomas were those who had IDH and TERT mutations, followed by those with IDH-only mutations, the worst were those with only TERT mutations; that last group behaved like glioblastomas. Finally, they found association of molecular groups with specific germline variants, particularly the CCDC26 single nucleotide polymorphism (SNP) (rs55705857) was associated with a greatly increased risk of the development of any glioma with IDH mutation (Eckel-Passow et al., 2015).

Most of all present molecular data on gliomas, point to a reliable association between genetic status and survival (Ellison, 2015); particularly IDH-mutant lower-grade gliomas having a good prognosis (Cancer Genome Atlas Research et al., 2015, Houillier et al., 2010) and IDH-wildtype a poor prognosis (Cancer Genome Atlas Research et al., 2015, Metellus et al., 2010).

Neomorphic mutations in isocitrate dehydrogenase (IDH) genes 1 and 2 lead to global changes in the epigenome, weaken DNA damage repair (DDR), and drive tumorigenesis (Takiar et al., 2017). IDH1/2 are key metabolic enzymes that generate reduced nicotinamide adenine dinucleotide phosphate (NADPH) to maintain a pool of reduced glutathione and peroxiredoxin and produce alpha-ketoglutarate (alpha-KG), a co-factor of numerous enzymes. The mutants IDH1/2, in addition to losing their normal catalytic activity, gain the function of producing D-(R)-2-hydroxyglutarate (2-HG). Overproduction of 2-HG in cancer cells interferes with cellular metabolism and inhibits histone and DNA demethylases, which results in histone and DNA

hypermethylation and the blockade of cellular differentiation. IDH1 mainly occurs in the cytoplasm and peroxisomes, while IDH2 is found in the mitochondrial matrix. IDH1 and IDH2 participate in protection from oxidative stress by producing the molecules NADPH and alpha-KG which have strong reductive properties and protect against DNA damage via their interactions with glutathione- and thioredoxin-producing systems. The reaction driven by IDH1 is the main source of NADPH in the human brain, producing as much as 65% of the brain's NADPH (Tommasini-Ghelfi et al., 2019).

All IDH mutations are monoallelic. Mutations in IDH1 and IDH2 genes are mostly missense variants leading to a single amino-acid substitution of arginine residues at codon 132 in exon 4 of the IDH1 gene or codons 140 or 172 of the IDH2 gene (IDH1-R132, IDH2-R140, or IDH2-R172). IDH1-R132 mutants have dominant-negative, inhibitory effects on IDH1-wildtype in vitro (Kaminska et al., 2019).

The 2-HG compound has properties of an oncometabolite and its accumulation in the cells contributes to cancerogenesis. The 2-HG compound adopts almost identical location to alpha-KG at the catalytic sites of DNA hydroxylases and enzymes containing histone dimethylases. Changes in histone methylation profiles are associated with IDH mutations and result in inhibition of cell differentiation. Furthermore, 2-HG directly inhibits homologous recombination, thus weakening DDR and potentially improving the outcome from DNA damaging agents in patients receiving standard-of-care cytotoxic therapies (Kaminska et al., 2019, Tommasini-Ghelfi et al., 2019).

Hence it seems so far that IDH mutation is a central and defining event in the development and progression of gliomas and are now a key target for promising future therapies (Turkalp et al., 2014, Venteicher et al., 2017).

Two new inhibitors targeting IDH-mutated proteins, Enasidenib (AG-221), and Ivosidenib (AG-120), have already been licensed for IDH-mutant relapsed or refractory acute myeloid leukemia based on phase 1 safety and efficacy data and continue to be studied in trials in hematologic malignancies, as well as in glioma, cholangiocarcinoma, and chondrosarcoma (Golub et al., 2019, Huang et al., 2019, Kaminska et al., 2019).

OBJECTIVES

To present a retrospective analysis of a single-institution cohort of lower-grade gliomas (LGG) (astrocytomas, oligoastrocytomas and oligodendrogliomas, WHO grades II and III) diagnosed between 2009 and 2015 with focus on the overall and progression-free survivals of the IDH-wildtype tumours. Our main purpose was to confirm or refute the extant evidence that patients with IDH-wildtype tumours do have a significantly lower overall and progression-free survivals than those with IDH-mutant tumours.

METHODS

Pathology and Clinical data

Pathology data were retrieved from the Neuropathology Laboratory Information Management System (LIMS), COPath v6.2 that held records of all patients operated at the National Hospital for Neurology and Neurosurgery (QSH), London, UK. Retrieval period - 01 Jan 2009 to 31 Dec 2015. The retrieval data set comprised the histological diagnosis, based on the 2007 WHO Classification (Louis et al., 2007) of a lower-grade glioma (astrocytoma, oligoastrocytoma or oligodendroglioma, WHO grades II or III) and with IDH-molecular status (IDH-mutant or IDH-wildtype). All IDH-1 and 2 molecular status were detected by Sanger sequencing (UCL Queen Square, 2019).

For the purpose of this study, the 1p/19q-molecular status (1p/19q-codeletd or 1p/19q-retained), detected by qPCR (UCL Queen Square, 2019) was also collected. When available, the PTEN and ATRX molecular status, the presence or absence of EGFR amplification and the MGMT methylation status were also collected.

Paediatric low-grade gliomas were excluded as were pilocytic astrocytoma, pleomorphic xanthoastrocytoma and subependymal giant cell astrocytoma, and tumours located in the spinal cord, brainstem or thalamus. None of the few tumours that were tested carried any BRAF or H3F3A K27M mutations or MYB amplifications or rearrangements.

All diagnoses were established by the consultant neuropathologists in the Division of Pathology.

Appropriate consent form from the patients was obtained from QSH.

The clinical data mining period from the patient notes spanned from 23 Oct 2017 to 31 Aug 2018 and consisted of: patient sex, date of birth and date of diagnosis based on the first MRI (or CT) scan that showed a tumor, date of first surgery and of recurrence or progression, and date of death or last follow up. Information on

symptoms, tumor location and whether the patient has ever had radiotherapy or chemotherapy was also collected.

Statistical Analysis

We used Fisher's exact test (FET) for association of categorical variables: histologic type, grade, and IDH- and 1p/19q-status. And one-way analysis of variance (ANOVA) for association with continuous outcomes: differences in age ((Rowe, 2015). P values<0.05 were considered statistically significant.

Since the main outcomes are time-to-event variables, we chose survival analysis models to analyse overall and progression-free survivals (Fletcher et al., 1988). Survival curves were estimated and plotted using the Kaplan-Meier method (Kaplan and Meier, 1958) and log-rank tests were used to compare curves between groups (Rowe, 2015). We also calculated the Cox proportional-hazards regression model for single and multiple predictor models of survival (Cox, 1972).

In Cox regression models (a multiple regression model), each independent variable has a coefficient corresponding to its effect on survival. Because of the proportional hazard assumption, these effects can be expressed by a hazard ratio (HR) and 95% confidence intervals (CI). All variables included in the models are categorical, the comparison and reference categories are explicit in the tables (Katz and Hauck, 1993, LaMorte 2016).

In multiple regression models, each estimated coefficient is adjusted by the other variables. It means that the effect of each variable is controlled for the effects of the other variables in the model. The correction for multiple variables is implicit in the estimation procedure where all estimates are derived simultaneously (Rowe, 2015).

Survival Modelling

We considered the date of the first image related to the diagnosis, not the first operation, as the date of the initial diagnosis.

Overall survival (OS) was defined as the time from initial diagnosis until death. Cases still alive at the time of this study had overall survival time censored at the time of the last follow-up. Progression-free survival (PFS) was defined as the years from initial diagnosis until either the time of the first new tumor event (progression or recurrence) or death. That first new tumor event may have happened before the first surgery. Patients may have been diagnosed at another institution, followed up for some time and then operated at our institution upon progression or recurrence. Persons who were alive and without progression or recurrence at the time of the study had progression-free survival time censored at the time of last follow-up.

As mentioned, survival curves were estimated and plotted using the Kaplan-Meier method, a non-parametric model, i.e., the model structure is not specified a priori, but is instead determined from the data (Rowe, 2015). The Kaplan-Meier is a frequently used statistical method to estimate the survival probability of persons living for a certain period after a determined occurrence. In this study, the determined occurrence, the time start point, was the initial image diagnosis (CT or MRI).The plotted curves move down as each drop in the curve represents one event (death in OS; and recurrence or death in PFS) and each tick mark represents censoring (patients who dropped from the study without the event, or at the end of the study). For each specified point in time, survival probability is estimated as the number of participants surviving divided by the number of persons at risk. Participants who died or have been censored are not more counted as at risk and will not be included in the denominator.

We have also included the numbers of participants at risk along the time of the study. That is relevant because estimates of survival toward the end of the follow-up period are not exact and are markedly affected by what occurs to the relatively few patients at risk left. Moreover, due to the few patients left, the confidence intervals increase markedly, and the curves frequently overlap.

Another important caveat regarding the median overall survival in the Kaplan-Meier method is that it does not represent the time when the absolute number of patients dropped to a half, but the estimated time when 50% of the patients will still be alive. If there were no censored patients, i.e., if all patients during the study period had been followed until death, then the median overall survival would be the time when exactly half of the patients would have died.

Log-rank tests were used to compare curves between groups. P values<0.05 were considered statistically significant. The Log-rank test is necessary because the Kaplan Meier plots frequently give a visual false impression of difference, i.e., an exaggeration of difference between groups. The log-rank test does not provide an estimate of the size of the difference between groups; it simply indicates whether there are statistical differences between curves (Etikan et al., 2016, Rich et al., 2010).

Box plots were included once as an illustration to show the distributions of years from diagnosis in two groups (Fletcher et al., 1988).

Also as already mentioned, we calculated the Cox proportional-hazards regression for single and multiple predictor models of survival (the hazard ratio = HR, and 95% confidence intervals = CI). We used Cox models, that are semiparametric (since there are no assumptions about the shape of the baseline hazard function), with fixed (non-time-dependent) covariates. The model assumes that the HR does not change over time (proportional hazard assumption). Consequently, the estimated effect of each independent variable can be expressed directly by the HR. If the HR for a predictor is 1 or its 95% confidence interval (CI) crosses 1, then that predictor does not affect survival (Rowe, 2015).

We planned to include in the multiple predictor models the variables (covariates) that were clinically relevant and those whose log-rank test had P values<0.05 (Storer et al., 2008). For the Cox results, P values<0.05 were also considered statistically significant.

SPSS® IBM® version 25 was used for the calculations and R [A language and environment for statistical computing (version 3.6.1 (2019-07-05)] was used for the Kaplan-Meier plots.

RESULTS

Demographics and Pathologic Data

We analysed 218 LGG patients and the IDH status was known for all. Their characteristics, pathologic data and treatment summary are described in Table 1.

Tumours with a mutation in either IDH1 or IDH2 were further subdivided by whether there was a codeletion of 1p/19q (1p/19q-codeletd) or not (1p/19q-retained). IDH and 1p/19q status was known for 211 patients. The Table in the Appendix describes their characteristics, pathologic data and treatment summary.

There was a strong correlation between the presence of an IDH mutation and 1p/19q codeletion and the oligodendroglioma histologic class (59 of 71 tumours). Lower-grade gliomas IDH-mutant and 1p/19q-retained (81 tumours, 38.4% of the cohort) represented mostly astrocytomas and oligoastrocytomas (72 tumours). IDH-wildtype tumours were mostly astrocytomas (42 of 64 tumours) and grade III gliomas (40 of 64 tumours). Overall, classification based on IDH- 1p/19q status correlated strongly with oligodendroglioma and astrocytoma (Clinical Data, Summary of Results and Table 1 in the Appendix).

Patients with lower-grade gliomas IDH-wildtype were older than those who had mutated IDH. Regarding the anatomical location of the tumours, lower-grade gliomas IDH-mutant arose in the frontal lobes more often than did those IDH-wildtype and the latter arose more often in the temporal lobes ($P<0.001$). The surgical approach also differed; IDH-wildtype patients were more likely to have a biopsy (49%) than the IDH-mutant, 1p/19q-codeleted (29%) ($P=0.01$). (Table 1, and Clinical Data, Summary of Results in the Appendix).

Outcomes Associated with Histologic Class and Molecular Subtypes

Clinical follow-up showed that 171 (78.8%) of 217 patients had a tumor recurrence and 85 (39%) of the 218 were deceased at the time of the analysis (Survival Modelling, Summary of Results in the Appendix). Patients who had IDH-wildtype

lower grade gliomas had a substantially shorter overall median survival (1.9 years) than did those with mutated IDH (15.8 years) (P<0.0001) (Figure OS 6). The longest overall survival time (20.45 years) occurred in patients with oligoastrocytoma WHO grade II, and in the lower-grade gliomas IDH-mutant (Figures OS 5 and OS 7). Our oligodendrogliomas WHO grade II did not reach an overall median survival time; their 15-year survival rate was 82% (Figure OS 5). The shortest overall median survival time (1.24 years) occurred in patients with IDH-wildtype lower grade gliomas WHO grade III (Figure OS 10). Persons with lower-grade gliomas IDH-mutant, 1p/19q-codeleted had a median overall survival of 17.6 years and those with IDH-mutant, 1p/19q-retained, 10.58 years, but that difference was not statistically significant (hazard ratio for death, 1.44; 95% confidence interval 0.74 to 2.8, P=0.28) (Figure OS 9).

The longest median progression-free survival time occurred in persons with oligodendrogliomas IDH-mutant (6.72 years) (Figure PFS 7), and the shortest [0.74 years (8.8 months)] was in persons with IDH-wildtype lower-grade gliomas WHO grade III (Figure PFS 9). Patients who underwent a total resection had a much longer estimated median progression-free survival time (5.92 years) than those who had a biopsy (1.55 years) (P<0.001) (Figure PFS 2). Interestingly, there were two, out of 24 (8.3%) IDH-wildtype WHO grade II patients who had a recurrence more than 6 years after diagnosis (Appendix, Box-Plot 2).

Incidental Findings

There were 10 persons (4.6%) out of the entire cohort (218 patients) whose tumor discovery was an incidental finding.

Their estimated median overall median survival time was not reached, and their 15-year survival rate was 70%. (P=0.3 by log-rank test, not shown).

Their estimated progression-free survival time was 5.54 years (P=0.43 by log-rank test, not shown).

Eight were grade II (2 astrocytomas, 5 oligodendrogliomas and one oligoastrocytoma) and 2 grade III (oligoastrocytomas). Only one was IDH-wildtype (oligoastrocytoma). Five had a partial resection, 2 a biopsy and 3 a complete resection. The 2 deceased patients (deaths at 3.7 and 6.7 years) had a partial resection and were IDH-mutant.

Tables Figures and Subgroup Analyses

Table 1. Clinical Characteristics of the Sample Set According to IDH Mutation Status.[a]			
Characteristic	**Total (N = 218)**	**IDH-mutant (N = 154)**	**IDH-wildtype (N = 64)**
Histologic Type[b] and Grade[b] no. (%)			
Oligodendroglioma			
Grade II	40 (18.3%)	36 (23.4%)	4 (6.2%)
Grade III	39 (17.9%)	33 (21.3%)	6 (9.4%)
Oligoastrocytoma			
Grade II	26 (11.9%)	22 (14.3%)	4 (6.2%)
Grade III	19 (8.7%)	11 (7.2%)	8 (12.5%)
Astrocytoma			
Grade II	55 (25.2%)	39 (25.3%)	16 (25%)
Grade III	39 (17.9%)	13 (8.6%)	26 (40.6%)
Age at diagnosis – yr[b]			
Mean/Med (SD)	41.7/38.1 (14.0)	37.6/35.9 (10.7)	51.0/54.3 (16.2)
Range	15.8 – 77.5	15.8 – 64.4	21.4 – 77.5
Male/Fem sex - no. (Male %)	118/100 (54.1)	78/76 (50.6)	40/24 (62.5)
Year of diagnosis - no. (%)			
Before 2009	58 (26.6)	55 (35.7)	3 (4.7)
2009-2015	160 (73.4)	99 (64.3)	61 (95.3)
Extent of resection - no. (%)			
Biopsy	74 (39.9)	40 (26.3)	34 (51.5)
Less than total resection	119 (54.6)	96 (62.3)	23 (36)
Total resection	25 (11.5)	18 (11.8)	7 (10.6)
Tumor location - no. (%)[b]			
Frontal lobe	114 (52.3)	91 (59)	23 (36)
Parietal lobe	33 (15.1)	27 (17.5)	6 (9.4)
Temporal lobe	54 (24.8)	32 (20.7)	22 (34.4)
Other	17 (7.8)	4 (2.8)	13 (20.3)
Laterality - no. (%)			
Left	109 (49.9)	81 (52.0)	28 (44.6)
Midline	10 (4.6)	2 (1.3)	8 (12.3)
Right	99 (45.5)	71 (46.7)	28 (43.1)
First presenting symptom - no. (%)			
Seizures	157 (72.0)	118 (76.6)	39 (61.0)
Headache	26 (11.9)	18 (11.5)	8 (12.5)
Mental change	8 (3.7)	4 (2.6)	4 (6.2)
Motor/Movement	8 (3.7)	3 (2.0)	5 (7.8)
Speech	7 (3.2)	2 (1.3)	5 (7.8)
Visual	2 (0.9)	0 (0)	2 (3.0)
Incidental	10 (4.6)	9 (4.1)	1 (1.5)
Radiotherapy (N = 188) (%)			
Radiotherapy before 2009	14 (7.4)	14 (10.3)	0 (0)
Radiotherapy after 2009	123 (65.4)	85 (62.5)	38 (73.1)
No Radiotherapy	51 (27.1)	37 (27.2)	14 (26.9)
Chemotherapy (N = 174) (%)			
Yes	87 (50.0)	61 (49.2)	26 (52.0)
No	87 (50.0)	63 (50.8)	24 (48.0)

[a] Categorical distributions were compared with the use of Fisher's exact. Analysis of variance was used to compare between age groups.

[b] P<0.01 for the difference among the molecular subtypes.

Tables 2 and 3 present a summary of our findings where we compared the results with both histologic classification and grade, and classification based on molecular markers (IDH and 1p/19q status) and grade. A detailed analysis of each result is further described below.

Table 2. Lower-grade Gliomas: Median Overall Survival and Median Progression-free Survival According to Histologic Class and WHO Grade.

	Astrocytoma		Oligoastrocytoma		Oligodendroglioma	
	OS (years)	PFS (years)	OS (years)	PFS (years)	OS (years)	PFS (years)
Grade II	8.67	2.91	20.45	3.52	Not reached	4.45
Grade III	3.13	1.13	3.71	2.0	14.61	5.17
	P=0.001	**P=0.004**	**P=0.006**	**P=0.04**	**P=0.038**	P=0.88

In bold P is significant (P<0.05) by log-rank test. OS, overall survival; PFS, progression-free survival. The log-rank test is calculated in the vertical columns.

Table 3. Lower-grade Gliomas: Median Overall Survival and Median Progression-free Survival According to Molecular Status and WHO Grade.

	IDH-mutant 1p/19q-codeletd		IDH-mutant 1p/19q-retained		IDH-wildtype	
	OS (years)	PFS (years)	OS (years)	PFS (years)	OS (years)	PFS (years)
Grade II	20.45	4.31	10.58	3.58	2.55	1.88
Grade III	14.61	5.54	Not reached	4.26	1.24	0.74
	P=0.17	P=0.89	P=0.49	P=0.76	**P=0.015**	**P=0.04**

In bold P is significant (P<0.05) by log-rank test. OS, overall survival; PFS, progression-free survival. The log-rank test is calculated in the vertical columns.

Overall Survival

Kaplan-Meier plots of estimated overall survival (OS) with patient numbers at risk are presented in Figures OS1 to OS10.

Their purposes are described before each plot and their findings are detailed after each plot.

We are aware that estimates of survival toward the end of our long follow-up period are not exact and are markedly affected by what occurs to the relatively few patients at risk left. Notwithstanding we have decided to always depict the whole curves.

The pertinent Cox proportional-hazards (HR) subgroup single-predictor model calculations, with 95% confidence intervals (CI), are presented after each Figure.

OS 1: Patients ≥40years old are usually considered to have a shorter median overall survival.

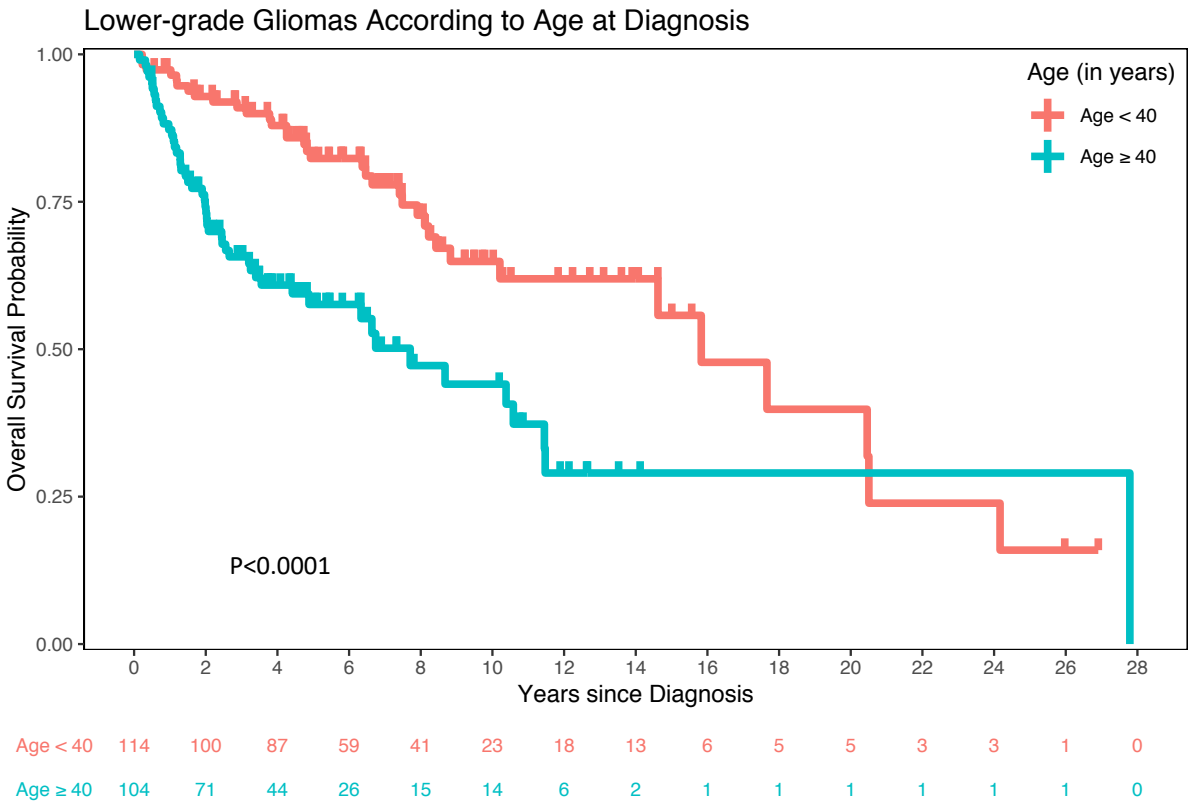


Figure OS 1.Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to age at diagnosis.

The 104 patients (48%) aged ≥40 years at diagnosis had a shorter estimated overall median survival time, 7.7 years, than did the 114 (52%) aged <40 years, 15.8 years. (P<0.0001 by log-rank test)

OS 2: Patients with complete resection usually have a longer median overall survival.

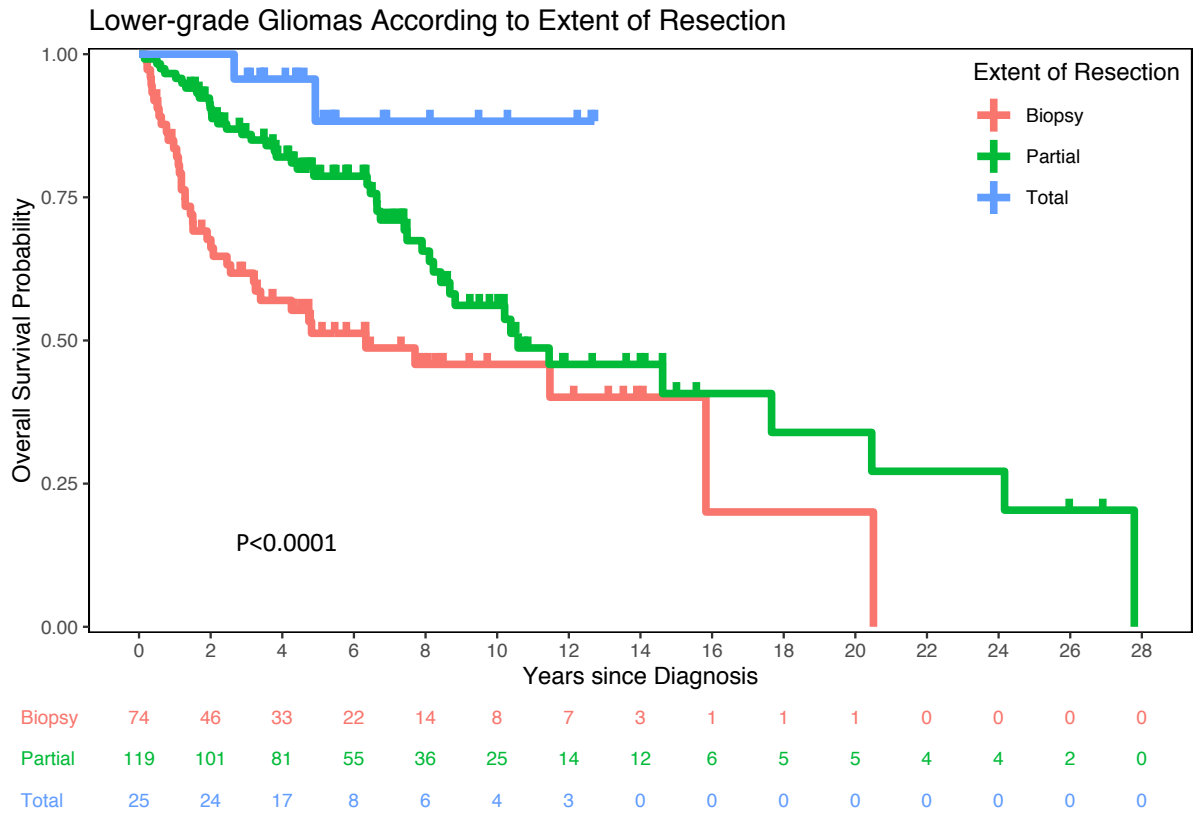


Figure OS 2. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to extent of resection.

The estimated overall median survival time differed according to the extent of resection: it was 6.3 years for the 74 patients (34%) who had a biopsy compared to 10.5 years for the 119 (54%) who had a partial resection. The 25 (12%) patients with total resection that was defined as no evidence of residual tumor and no enhancement on the post-operative MRI did not reach estimated median overall survival, with a 10-year survival rate of 88%. *(P<0.0001 by log-rank test).*

The difference between the OS of those who had a biopsy compared to those with a partial resection was also significant: P=0.001 by log-rank test (not shown).

OS 3: Patients with astrocytomas are expected to have a shorter median overall survival.

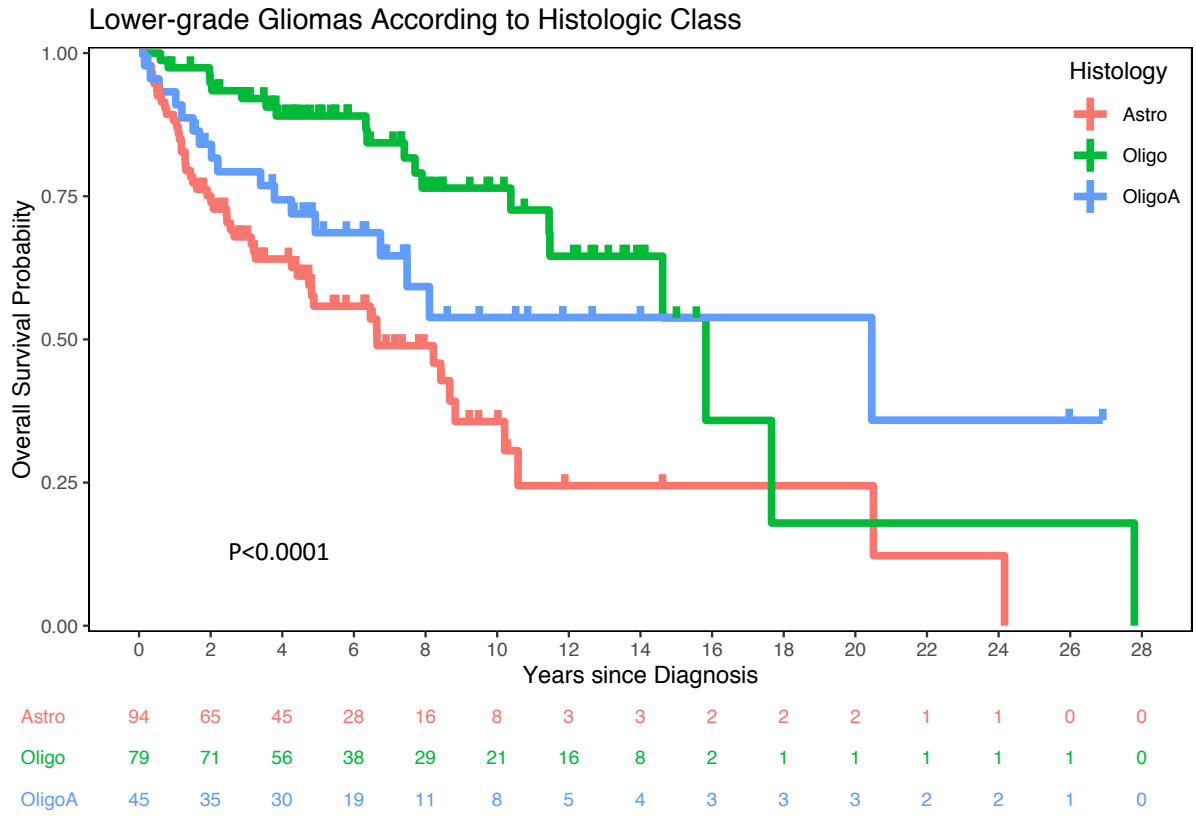


Figure OS 3. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class.

Overall estimated median survival times for patients according to histologic class. Estimated median survival for the 94 astrocytomas (44%) was 6.6 years, for the 45 oligoastrocytomas (20%) was 20.4 years, and for the 79 oligodendrogliomas (36%) was 15.8 years. (P<0.0001 by log-rank test). Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma.

OS 4: Patients with LGG, WHO grade III are expected to have a shorter median overall survival.

/

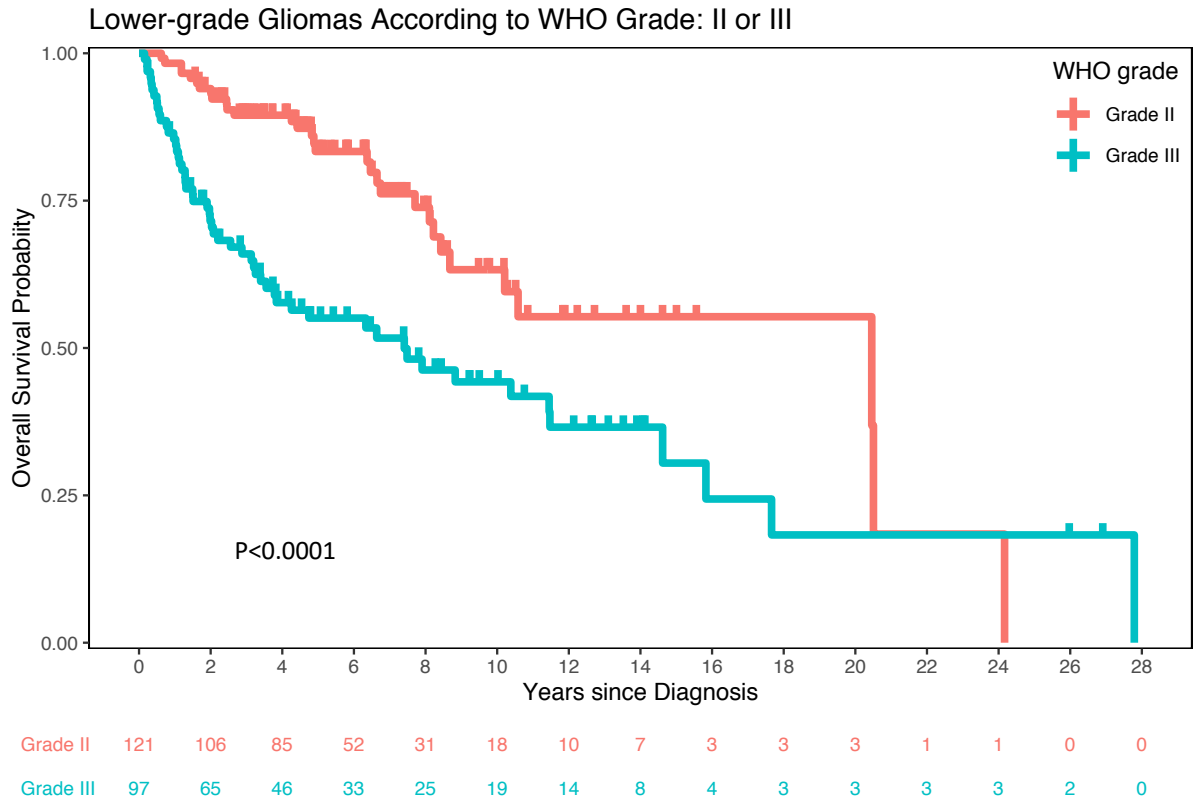


Figure OS 4. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to WHO grade: II or III.

Overall estimated median survival times for patients according to WHO grade. Median survival for the 121 (55.5%) grade II was 20.45 years and for the 97 (44.5%) grade III was 7.40 years (P<0.0001 by log-rank test).

OS 5: Patients with astrocytomas grade III are expected to have the shortest median overall survival when compared to other LLG according to histologic class and WHO grade.

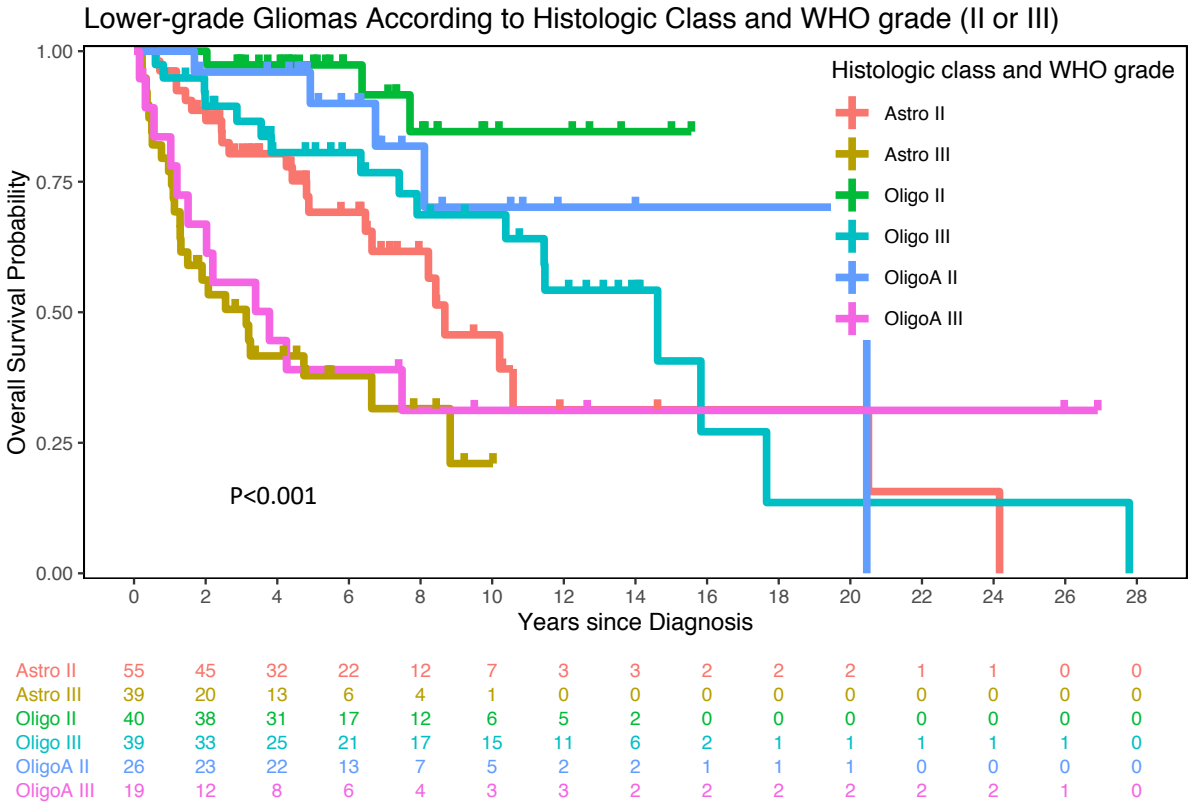


Figure OS 5. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class and WHO grade (II or III).

Overall estimated median survival times for patients according to histologic class and grade. Median survival for the 55 (25%) astrocytoma II was 8.67 years, for the 39 (18%) astrocytoma III was 3.13 years, for the 26 (12%) oligoastrocytoma II was 20.45 years, for the 19 (9%) oligoastrocytoma III was 3.71 years, for the 40 (18%) oligodendroglioma II - did not reach (the 15-year survival rate was 82%), and for the 39 (18%) oligodendroglioma III was 14.61 years. (P<0.001 by log-rank test). Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma

The hazard ratio (HR) for each pair of histologic class and grade showed a statistical significance of the risk of death, in Figure OS 5, for all 3 of them:

HR 2.75 (1.50, 5.01) for astrocytoma grade III compared to grade II. (P=0.001).

HR 4.38 (1.52, 12.57) for oligoastrocytoma grade III compared to grade II. (P=0.006).

HR 3.80 (1.07, 13.38) for oligodendroglioma grade III compared to grade II. (P=0.038).

LGG grade II showed a significant risk of death when compared by histologic class only with astrocytoma:

Oligodendroglioma HR 1 (ref).

Astrocytoma HR 5.92 (1.76, 19.93, P=0.004)

Oligoastrocytoma HR 2.40 (0.57, 10.09, P=0.23).

LGG grade III showed a significant risk of death when compared by histologic class only with astrocytoma:

Oligodendroglioma HR 1 (ref).

Astrocytoma HR 3.27 (1.68, 6.37, P<0.001)

Oligoastrocytoma HR 2.10 (0.98, 4.49, P=0.06).

OS 6: Patients with IDH-wildtype LGG are expected to have a shorter median overall survival when compared to IDH-mutant

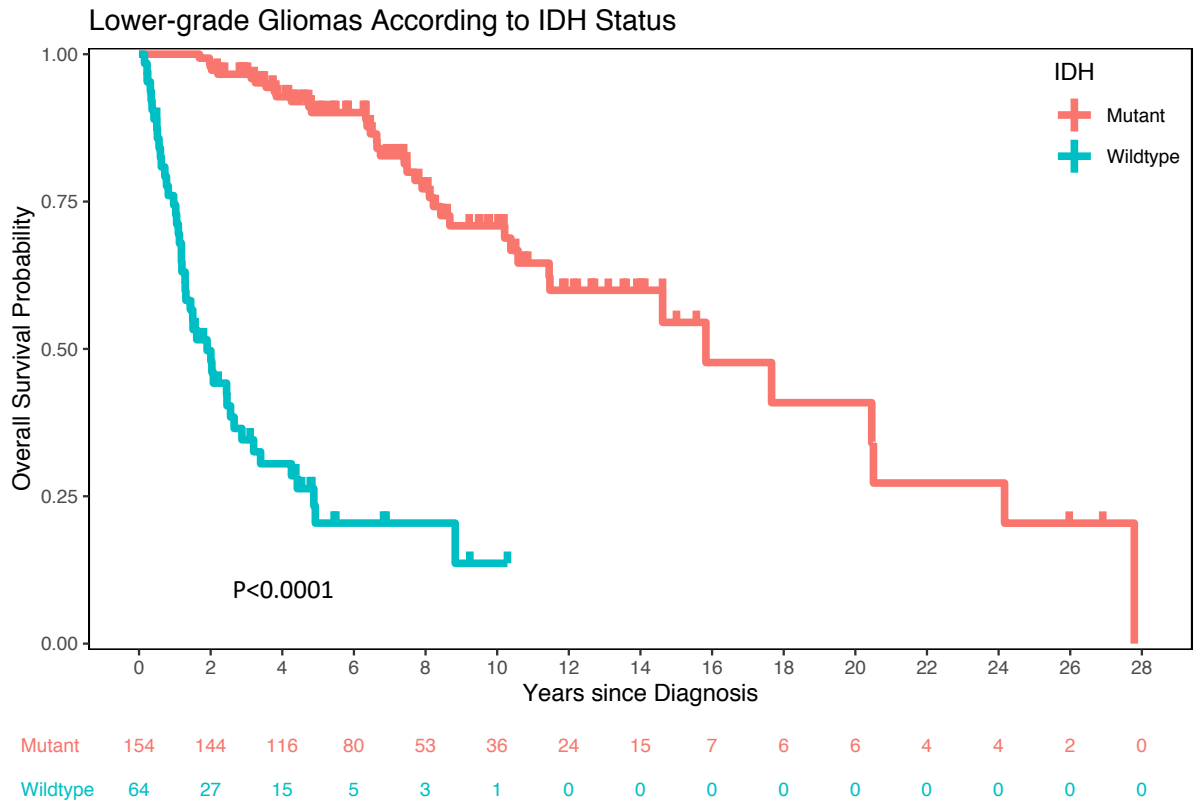


Figure OS 6. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to IDH status.

The 64 patients (30%) with IDH-wildtype tumours had a substantially shorter estimated overall median survival time (1.9 years) than did the 154 (70%) with mutated IDH (15.8 years) (P<0.0001 by log-rank test)

IDH-wildtype tumours showed an increased risk of death compared to IDH-mutant independent of the age at diagnosis:

Age <40 years: HR 5.53 (2.59, 11.83, P<0.0001).

Age ≥40 years: HR 16.75 (7.43, 37.75, P<0.0001).

In patients with IDH-wildtype tumours, age ≥40 years further increases the risk of death when compared with age <40 years. HR 2.45 (1.17, 4.96, P=0.017).

But in patients with IDH-mutant tumours, age ≥ 40 years did not affect the risk of death when compared to age <40 years, HR 1.33 (0.68, 2.61, P=0.39).

All LGG grade III showed a significantly greater risk of death when comparing IDH-wildtype to IDH-mutant tumours:

HR 8.49 (4.48, 16.09, P<0.001).

Particularly, the risk of death was also significantly higher for oligodendrogliomas grade III IDH-wildtype when compared to IDH-mutant:

HR 5.65 (1.40, 22.77, P=0.015).

All LGG grade II showed a significantly greater risk of death when comparing IDH-wildtype to IDH-mutant tumours:

HR 12.12 (5.47, 26.85, P<0.0001).

Particularly the risk of death was also significantly higher for astrocytomas grade II IDH-wildtype when compared to IDH-mutant:

HR 9.24 (3.60, 27.74, P<0.0001).

OS 7: Patients with astrocytomas IDH-wildtype are expected to have the shortest median overall survival when compared to all LGG according to histologic class and IDH-status.

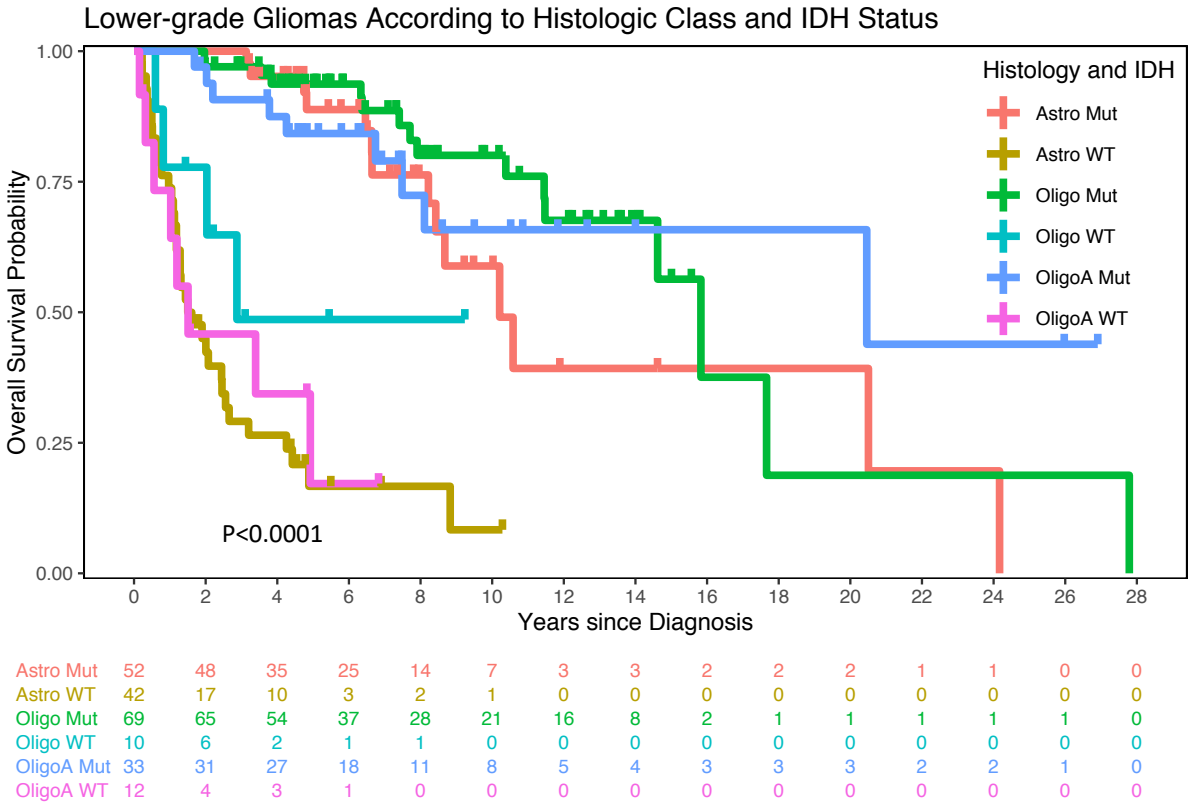


Figure OS 7. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class and IDH status.

The estimated overall median survival time was substantially different when comparing tumours according to histologic class and molecular IDH status. Median survival time for the 52 (24%) astrocytoma IDH-mutant was 10.20 years, for the 42 (19%) astrocytoma IDH-wildtype, 1.50 years, for the 33 (15%) oligoastrocytoma IDH-mutant, (20.45 years, for the 12 (5.5%) oligoastrocytoma IDH-wildtype, 1.50 years, for the 69 (32%) oligodendroglioma IDH-mutant, 15.82 years, and for the 10 (4.5%) oligodendroglioma IDH-wildtype, 3.83 years. (P<0.0001 by log-rank test) Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma. Mut, IDH-mutant, WT, IDH-wildtype.

Particularly the overall median survival time was substantially different when comparing the 55 astrocytomas grade II according to their IDH- molecular status (plots not shown): median survival time for the 39 astrocytomas grade II, IDH-mutant was 10.58 years, and 2.44 years for the 16 astrocytomas grade II, IDH-wildtype (P<0.0001 by log-rank test).

We calculated the hazard ratio (HR) for each pair of histologic class and IDH-status and verified that the statistical significance of the risk of death, in Figure OS 7, holds true for all 3 of them.

HR 9.08 (4.51, 18.29) for astrocytoma IDH-wildtype compared to IDH-mutant. (P<0.001).

HR 8.58 (2.89, 25.44) for oligoastrocytoma IDH-wildtype compared to IDH-mutant. (P<0.001).

HR 7.96 (2.35, 26.96) for oligodendroglioma IDH-wildtype compared to IDH-mutant. (P=0.001).

But the HR for the risk of death was not statistically significant for IDH-wildtype tumours according to histologic class (astrocytoma, oligoastrocytoma or oligodendroglioma):

Oligodendroglioma: HR 1 (ref).

Astrocytoma: HR 2.35 (0.83, 6.63, P=0.10)

Oligoastrocytoma: HR 2.10 (0.62, 7.00, P=0.22).

Nor was a statistically significant the risk of death for IDH-mutant tumours according to histologic class (astrocytoma, oligoastrocytoma or oligodendroglioma):

Oligodendroglioma: HR 1 (ref).

Astrocytoma: HR 1.64 (0.78, 3.44, P=0.18).

Oligoastrocytoma: HR 1.15 (0.49, 2.68, P=0.73).

Interestingly, there was no difference in the risk of death when comparing all IDH-mutant histologic class tumours, grade III and grade II.

IDH mutant Astrocytoma: HR 2.15 (0.60, 7.67) comparing grade III to grade II (P=0.24).

IDH mutant Oligoastrocytoma: HR 2.06 (0.54, 7.87) comparing grade III to grade II (p=0.29).

IDH mutant Oligodendroglioma: HR 4.14 (0.90, 18.98) comparing grade III to grade II (P=0.67).

OS 8: Patients with LGG IDH-mutant 1p/19q-codeleted are expected to have a longer median overall survival compared to those IDH-mutant 1p/19q-retained.

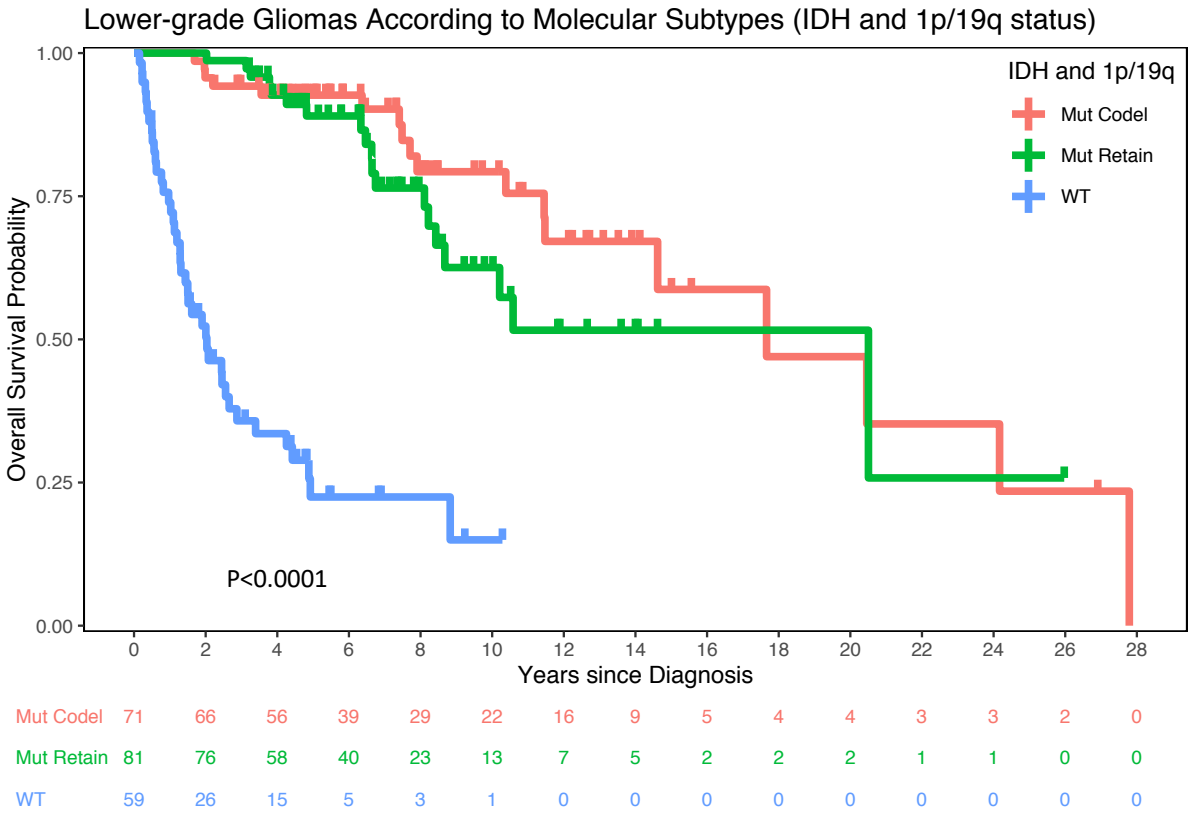


Figure OS 8. Kaplan Meier estimates with patient numbers at risk of lower-grade gliomas classified according to IDH and 1p/19q status.

The 71 (33.5%) IDH-mutant, 1p/19q-codeleted patients had an estimated overall median survival of 17.66 years, the 81 (38.5%) IDH-mutant, 1p/19q-retained, 10.58 years, both significantly longer than the 59 (28%) IDH-wildtype group with 2.0 years (P<0.0001 by log-rank test). Mut Codel, IDH-mutant, 1p/19q-codeleted; Mut Retain, IDH-mutant, 1p/19q-retained; WT, IDH-wildtype.

But there was no statistically significant difference in risk of death when comparing patients with IDH-mutant, 1p/19q-codeleted lower-grade gliomas and the and IDH-mutant, 1p/19q-retained (Figure OS 9).

OS 9: A detailed sub-group Cox regression analysis plot depicting patients with LGG IDH-mutant 1p/19q-codeleted and those IDH-mutant 1p/19q-retained.

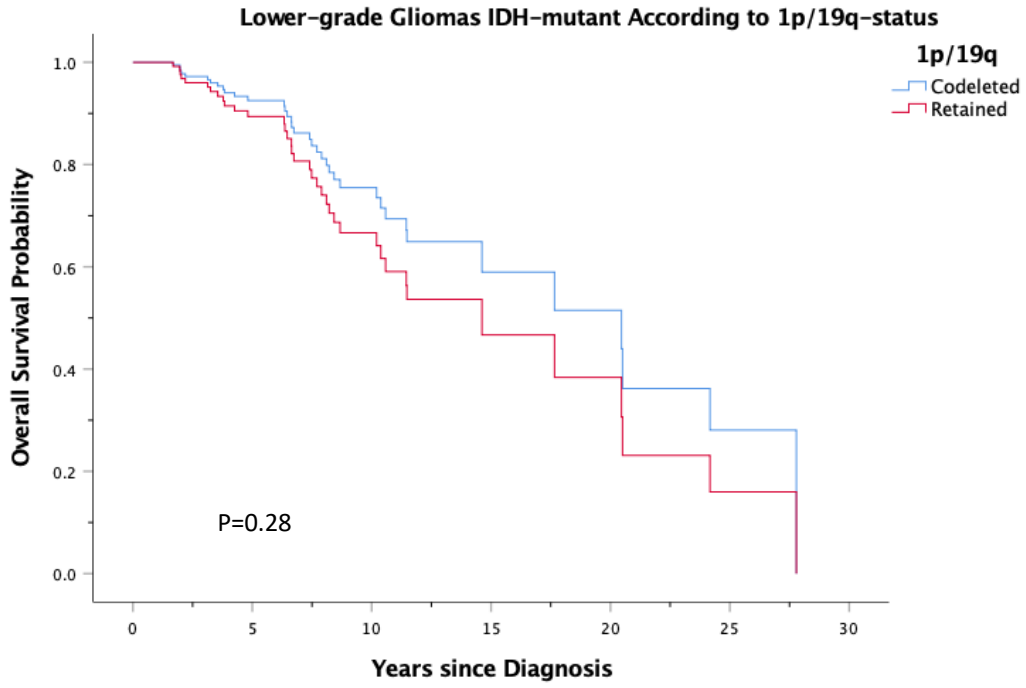


Figure OS 9. Cox regression analysis plot of lower-grade gliomas, IDH-mutant, classified according to 1p/19q status.

152 IDH-mutant patients: there was no difference in the HR risk of death between the 71 (46.7%) 1p/19q-codeleted and the 81 (53.3%) 1p/19q-retained. HR 1.44 (0.74, 2.8). (P=0.28)

There was no statistically significant difference in the risk of death for IDH-mutant, 1p/19q-codeleted, and IDH-mutant, 1p/19q-retained according to histologic class.

IDH-mutant, 1p/19q-codeleted:

Oligodendroglioma: HR 1 (ref).

Astrocytoma: HR 0.81 (0.09, 7.18, P=0.85)

Oligoastrocytoma: HR 1.29 (0.39, 4.34, P=0.67).

IDH-mutant, 1p/19q-retained:

Oligodendroglioma: HR 1 (ref).

Astrocytoma: HR 1.26 (0.28, 5.7, P=0.76)

Oligoastrocytoma: HR 0.85 (0.16, 4.46, P=0.85).

And there was no difference in the risk of death for LGG grade III tumours when comparing IDH-mutant, 1p/19q-retained and the IDH-mutant, 1p/19q-codeleted.

Grade III: HR 1.22 (0.49, 3.06, P=0.66)

And no significant risk of death for LGG grade II tumours when comparing IDH-mutant, 1p/19q-retained and the mutant, codeleted.

Grade II: HR 2.28 (0.72, 7.25, P=0.16)

We also calculated the hazard ratio (HR) for each pair of molecular subtypes (IDH- and 1p/19q-status) and grade II or III and verified that there was a greater risk of death only for IDH-wildtype grade III compared to grade II.

HR 2.08 (0.72, 5.99) IDH-mutant, 1p/19q-codeleted grade III compared to grade II (P=0.18).

HR 1.47 (0.57, 3.78) for IDH-mutant, 1p/19q-retained grade III compared to grade II (P=0.42).

HR 2.12 (1.13, 3.92) for IDH-wildtype grade III compared to grade II (P=0.018).

OS 10: Since the HR for death of the IDH-wildtype grade III tumours was statistically greater when compared to the LGG IDH-wildtype II, we present the survival plots of both.

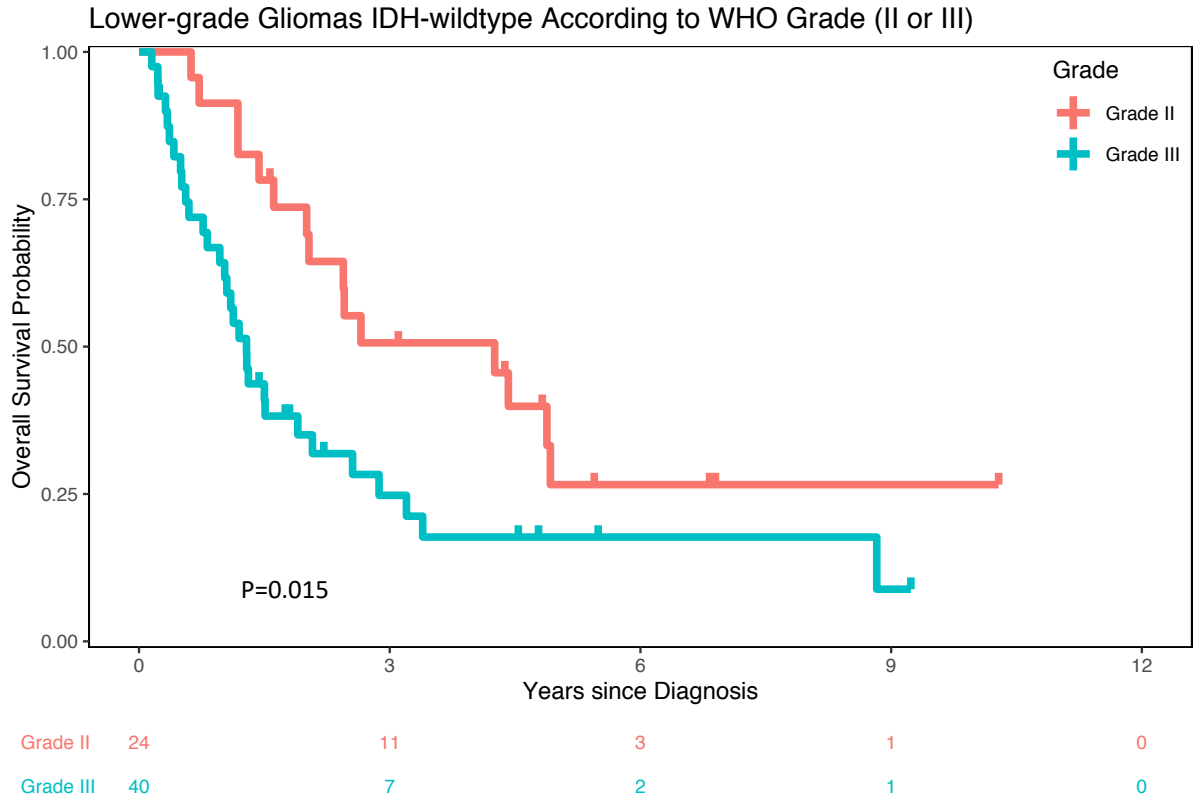


Figure OS 10. Kaplan Meier estimates with numbers at risk of IDH-wildtype, lower-grade gliomas classified according to WHO grade (II or III).

The estimated overall median survival times of the 64 (30%) patients with IDH-wildtype tumours differed significantly when comparing grades II, 2.55 years and III, 1.24 years. The 5-year survival rate was 25% for those grade II and 18% for grade III. (P=0.015 by log-rank test)

The outliers are better depicted in Box Plot 1 in the Appendix 1, that also shows an overlap of the median survivals. Box Plots show the distribution of individual values and it can be noted in Box Plot 1 that in the grade III group, most patients (around 75%) had observed times up to 2 years, whereas in the grade II group, around 75% of patients had observed times up to 5 years..

Of the 24 IDH-wildtype grade II patients, all 15 (62.5%) died and five were censored less than five years after diagnosis, and the other four were censored between five years and 10 years.

Of the 40 IDH-wildtype grade III patients, 36 (90%) died; 35 (87.5%) less than four years after diagnosis, 1 (2.5%) lived for 8.8 years, and four were censored between four and 9.3 years.

IDH-wildtype Long Survivors

Since patients with IDH-wildtype lower-grade gliomas are supposed to have short survivals, similar to glioblastomas (Hartmann et al., 2010, Metellus et al., 2010), and since long-term survivors in glioblastomas are considered to be patients who live for more than 3 years (Lu et al., 2016), we separated, for further analysis, our seven (11%), out of 64 IDH-wildtype lower-grade glioma patients who have lived more than 5 years since diagnosis. None had a midline location. They are presented in Table 4 and include all (but one) outliers depicted in the Appendix, Box-Plot 1.

Table 4. IDH-wildtype long survivors

Age yrs/Sex	Resection	Histology	1p/19q	EGFR	PTEN	MGMT	Survival (years)	Censored
23.5/M	Partial	Astro II	Codeleted	No	No	Yes	10.29	Yes
32.5/F	Partial	Oligo III	Codeleted	No	No	Yes	9.24	Yes
25.6/M	Biopsy	Astro III	Retained	No	No	Yes	8.82	No
64.9/M	Partial	Astro II	Retained	No	No	Yes	6.89	Yes
23.6/F	Total	Astro II	Retained	No	No	No	6.82	Yes
28.4/M	Total	Astro III	Retained	Yes	No	No	5.49	Yes
54.3/M	Partial	Oligo II	Codeleted	No	No	No	5.44	Yes

Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma.

IDH-mutant Short Survivors

Moreover, since patients with IDH-mutant lower-grade gliomas are supposed to have longer survivals (Cancer Genome Atlas Research et al., 2015, Houillier et al., 2010), we separated, for further analysis, our 10 (6.5%) out of 154 patients with IDH-mutant lower-grade gliomas who have lived less than four years since diagnosis. None had a midline location. They are presented in Table 5.

Table 5. IDH-mutant short survivors

Age yrs/Sex	Resection	Histology	1p/19q	EGFR	PTEN	MGMT	Survival (years)
26.7/F	Partial	OligoA II	Codeleted	Yes	Yes	Unknown	1.68
59.6/F	Partial	Oligo III	Codeleted	No	No	Yes	1.96
57.8/F	Partial	Oligo III	Codeleted	No	No	Yes	1.98
44.8/M	Biopsy	OligoA III	Retained	Yes	No	Yes	2.02
34.3/F	Partial	OligoA III	Codeleted	No	No	Yes	2.20
25.9/F	Partial	Astro III	Retained	No	No	No	3.13
45.7/F	Biopsy	Astro III	Retained	Yes	No	Yes	3.25
58.2/F	Partial	Oligo III	Codeleted	Yes	Yes	Yes	3.55
32.8/M	Partial	OligoA III	Retained	Yes	No	Yes	3.78
32.5/F	Partial	Oligo III	Retained	No	No	No	3.83

Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma.

Cox Regression Models

Given that age at diagnosis, extent of resection, histologic type, WHO grade, and IDH and 1p/19q status were all significantly associated (by log-rank test) with overall survival (shown in all the Kaplan Meier plots), we examined two multivariate Cox regression models.

We thought these two models to be appropriate since Model I reflects the diagnoses made in our cohort according to the fourth edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS), published in 2007 (WHO 2007) (Louis et al., 2007), and Model II adds the two important molecular status (IDH- and 1p/19q-) for LGG diagnoses, incorporated in the updated fourth edition of the WHO Classification of Tumours of the CNS (WHO 2016) (Louis et al., 2016).

Each hazard ratio (HR) indicates the risk of dying at any point in time holding all other variables constant. The confidence interval (CI) provides the precision of the estimates and indicates a range (with a 95% probability) of possible values of the true parameter. If the CI overlaps the value of 1 then the HR result is not statistically significant, and the P value will exceed 0.05. If the CI keeps out the value of 1 (on either side) then the HR is statistically significant.

For example, Table 6 shows in Model I that the risk of a patient with an astrocytoma dying at any point in time is 4.79-fold that of a patient with an oligodendroglioma holding all other variables (age, extent of resection, and grade) constant.

Table 6 also shows that the most substantial predictor of death is IDH-wildtype molecular status ($P<0.0001$): a patient with an IDH-wildtype LGG had a 9.32-fold risk of dying at any point in time during this study (compared to a patient with an IDH-mutant LGG), irrespective of their age, extent of resection, histologic type, or grade. If we were to repeat this study n times, there would be only a 0.001% probability of error of rejecting the null hypothesis (HR=1).

Table 6 presents all the estimates from the two multiple predictor models (Models I and II).

In Model I, adjusting for age (< 40 and ≥40 years) maintains the relative increased risk of death (HR 2.54). Astrocytoma (HR 4.79) and oligoastrocytoma (HR 2.54) also show increased risk relative to oligodendroglioma type. Less-than-complete resection has a marginal non-statistically significant (P=0.058) increased risk compared to complete resection. The effect a WHO grade III classification relative to grade II is retained (HR 2.60).

In Model II, age≥40 (HR 1.97), a grade III diagnosis (HR 1.93), less than total resection (HR 5.75), astrocytoma (HR 2.14) and oligoastrocytoma (HR 2.12) and an IDH-wildtype molecular status (HR 9.32) remain statistically significant predictors of increased risk of death. Astrocytoma and oligoastrocytoma show increased risk of death when compared to oligodendroglioma, but with a marginal statistical significance (P=0.046 and P=0.039 respectively). 1p/19q status is not a significant predictor. As mentioned, the most substantial predictor, with a major prognostic significance, is IDH-wildtype molecular status (P<0.0001).

Table 6. Overall Survival Models.

		Model I	Model II
Predictor	Levels	HR (95% CI)	HR (95% CI)
Age at Diagnosis	<40 years	1.0 (ref)	1.0 (ref)
	≥40 years	**2.54 (1.60, 4.04) (P<0.0001)**	**1.97 (1.19, 3.26) (P=0.008)**
Extent of Resection	Complete	1.0 (ref)	1.0 (ref)
	< Complete	3.91 (0.95, 16.04) (P=0.058)	**5.75 (1.36, 24.22) (P=0.017)**
Histologic Type	Oligodendroglioma	1.0 (ref)	1.0 (ref)
	Oligoastrocytoma	**2.56 (1.30, 5.04) (P=0.007)**	**2.12 (1.03, 4.34) (P=0.039)**
	Astrocytoma	**4.79 (2.71, 8.44) (P<0.0001)**	**2.14 (1.01, 4.53) (P=0.046)**
WHO Grade	II	1.0 (ref)	1.0 (ref)
	III	**2.60 (1.64, 4.12) (P<0.0001)**	**1.93 (1.21, 3.24) (P=0.007)**
IDH, 1p/19q group	IDH-mutant, 1p/19q-codeleted		1.0 (ref)
	IDH-mutant, 1p/19q-retained		1.08 (0.49, 2.14) (P=0.83)
	IDH-wildtype		**9.32 (4.19, 20.74) (P<0.0001)**

Overall Survival Models. Cox regression models of overall survival considering age at diagnosis, extent of resection, histologic class, WHO grade, and IDH, 1p/19q status in combination in two multiple-predictor models. Bold denotes hazard ratios (HR) significantly different from 1 (no difference).

Progression Free Survival

Kaplan-Meier plots of estimated progression-free survival (PFS), with numbers at risk, are presented in Figures PFS 1 to PFS 9. The pertinent Cox proportional-hazards (HR) subgroup single-predictor model calculations, with 95% confidence intervals (CI), are presented after each Figure.

Except for OS 9 whose equivalent is not depicted in the progression-free survival plots, the purpose of each plot follows the same as described in the Overall Survivals. Their findings are detailed below each plot.

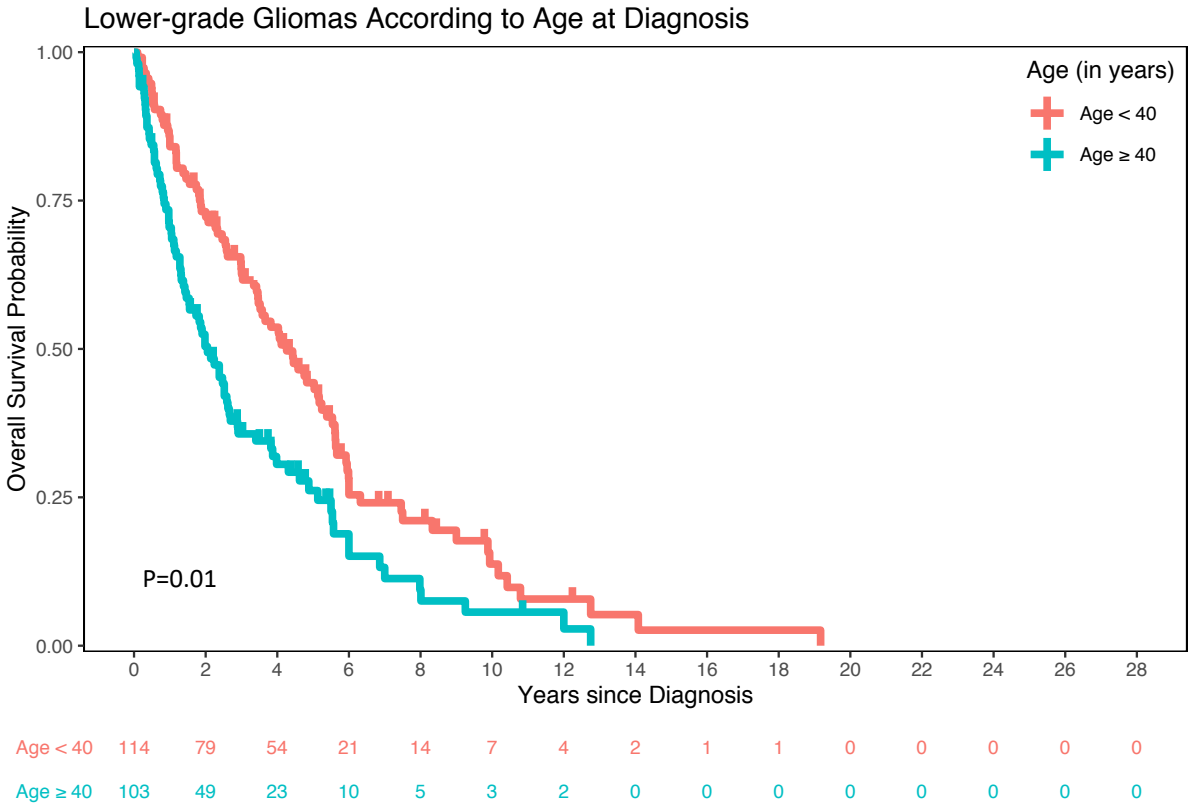


Figure PFS 1. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to age at diagnosis.

Patients aged ≥40 years at diagnosis had a shorter estimated median progression-free survival time, 2.05 years than did those <40 years, 4.26 years. (P=0.01 by log-rank test).

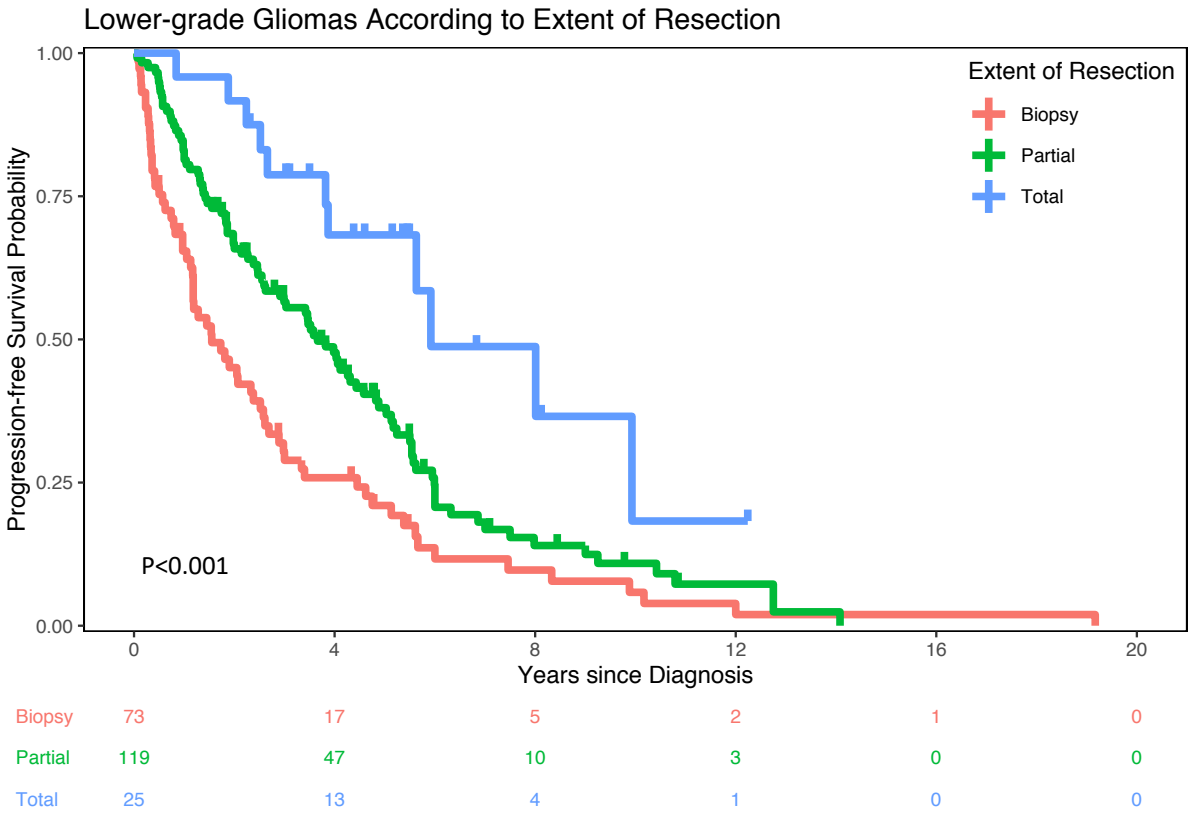


Figure PFS 2. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to extent of resection.

Estimated median progression-free survival time differed according to the extent of resection. It was 1.55 years for patients who had a biopsy compared to 3.66 years for a partial resection and 5.92 years for patients with total resection that was defined as no evidence of residual tumor and no enhancement on the post-operative MRI. (P<0.001 by log-rank test).

The difference between the PFS of those who had a biopsy compared to those with a partial resection was also significant: P=0.003 by log-rank test (not shown).

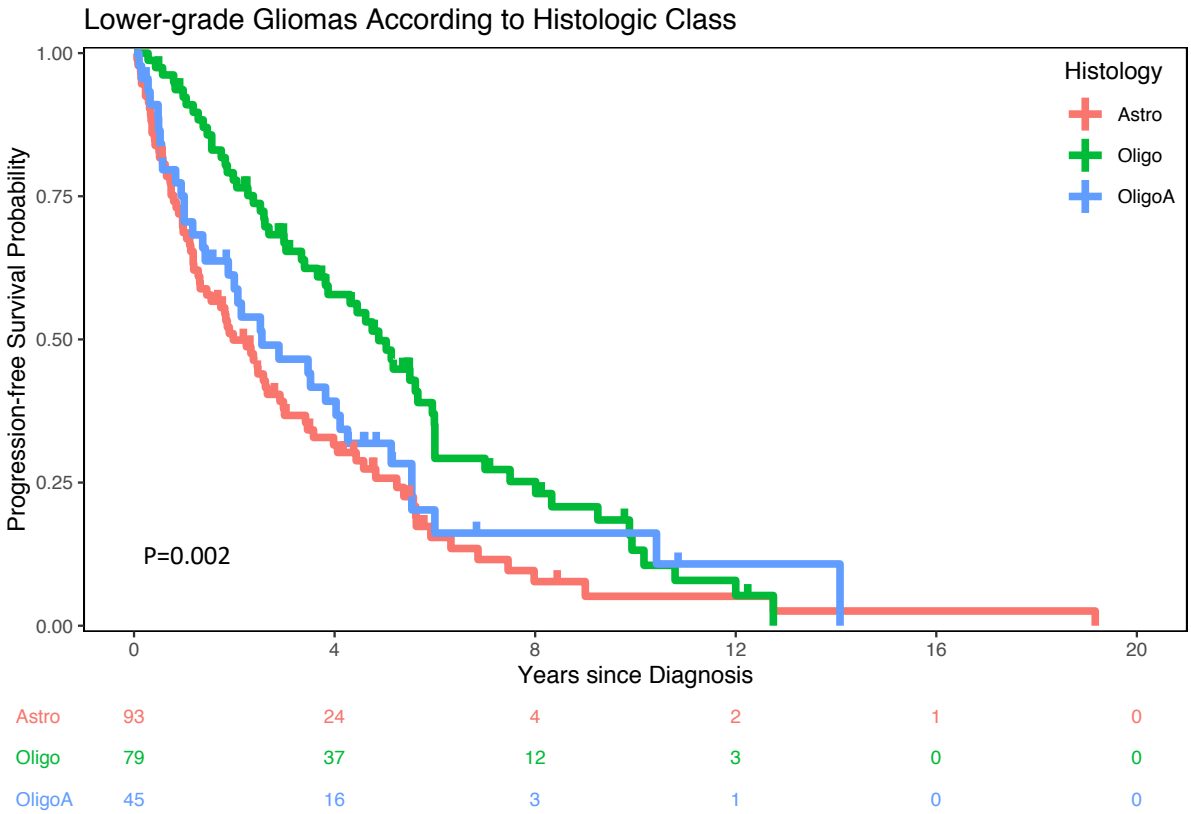


Figure PFS 3. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class.

Estimated progression-free survival was 1.98 years for astrocytomas, 2.55 years for oligoastrocytomas and 4.88 years for oligodendrogliomas (P= 0.002 by log rank test). Astro, astrocytoma, Oligo, oligodendroglioma, OligoA, oligoastrocytoma.

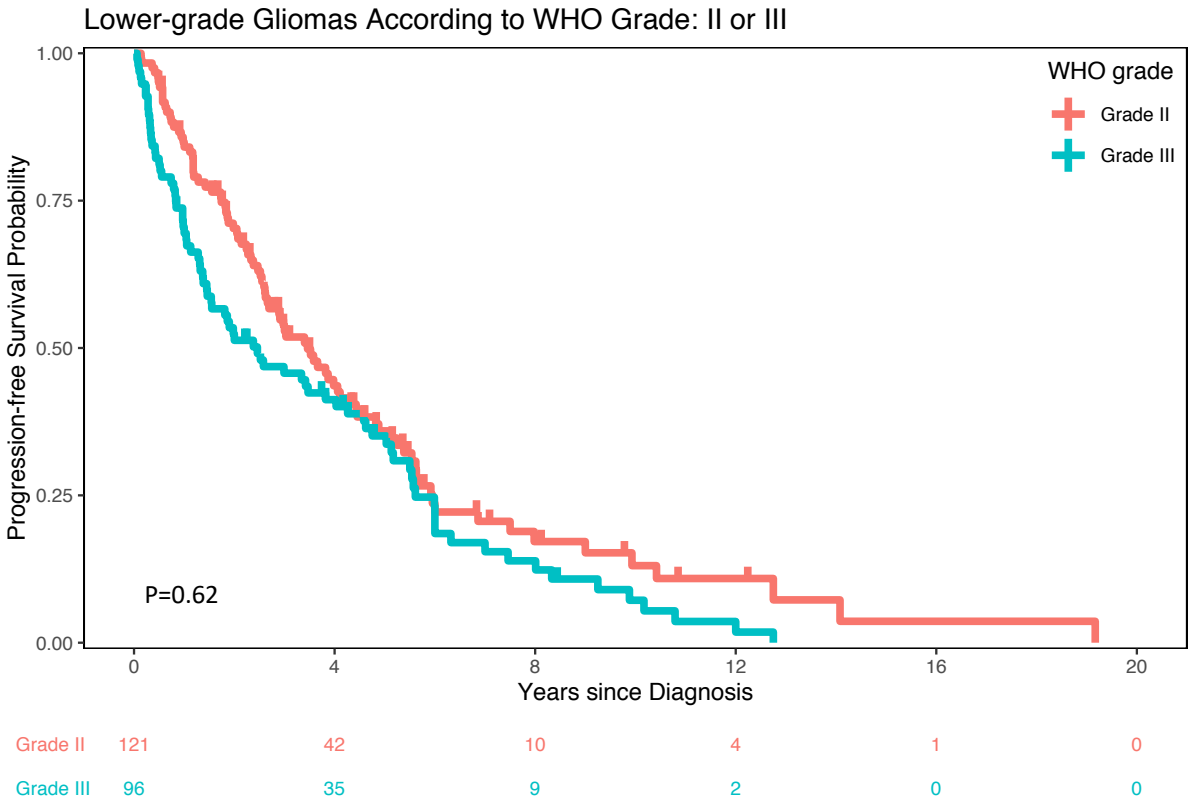


Figure PFS 4. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to WHO grade: II or III.

Estimated progression-free survival times for patients according to WHO grade II or III was non-significant. Median PFS survival estimates for grade II, 3.46 years, grade III, 2.46 years (P=0.62 by log-rank test).

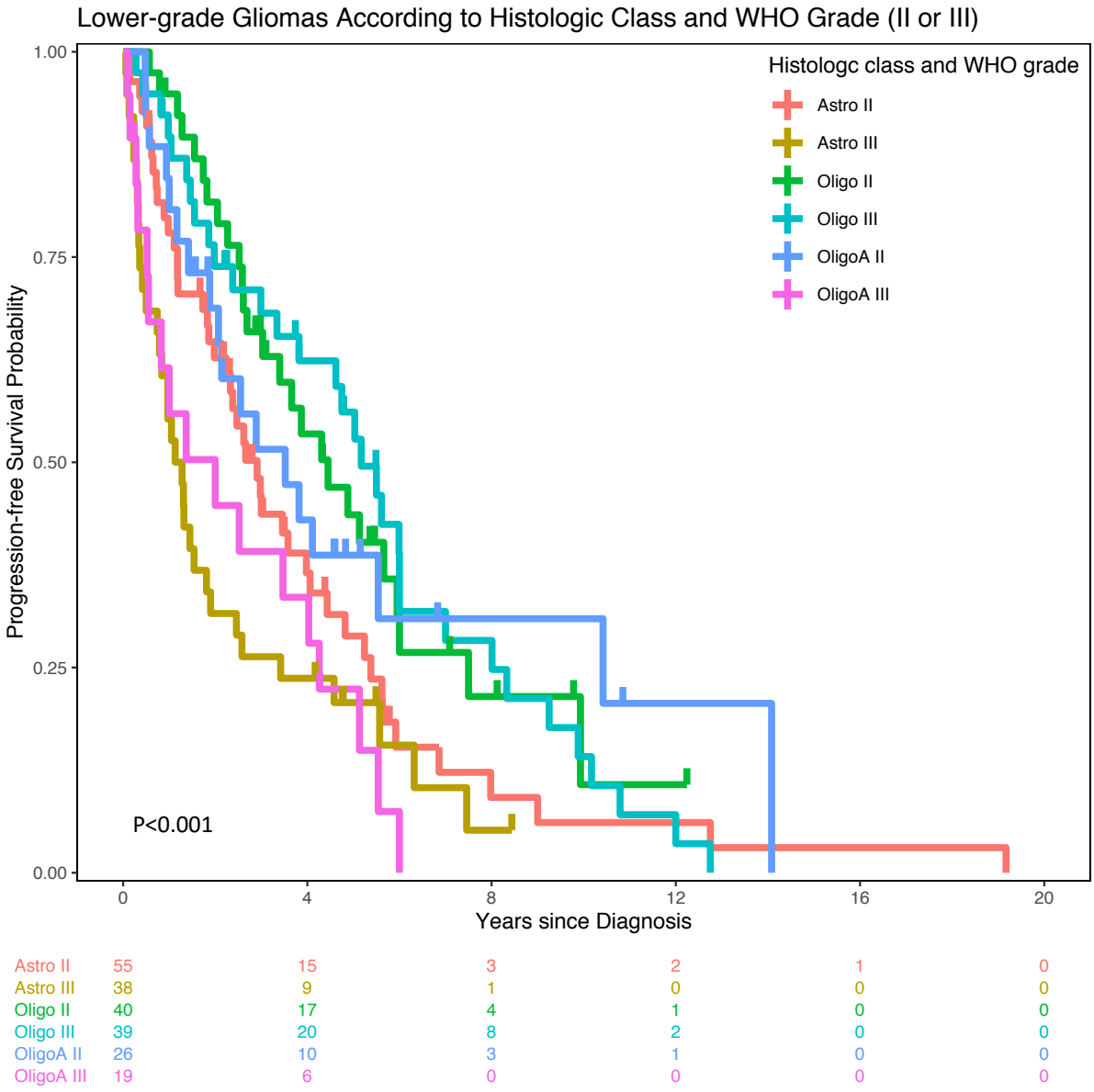


Figure PFS 5. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class and WHO grade (II or III).

The difference was statistically significant with the following median progression-free survival times (in years): astrocytoma II (2.91), astrocytoma III (1.13), oligoastrocytoma II (3.52), oligoastrocytoma III (2.0), oligodendroglioma II (4.45), oligodendroglioma III (5.17). (P<0.001 by log-rank test). Astro, astrocytoma; Oligo, oligodendroglioma; OligoA, oligoastrocytoma.

We also calculated the hazard ratio (HR) for each pair of histologic class and grade and verified that the statistical significance of the risk of progression, in Figure PFS 5, holds true for 2 of them: there was no statistical difference when comparing oligodendrogliomas grade II and III.

HR 1.59 (1.00, 2.51) for astrocytoma grade III compared to grade II. (P=0.046).

HR 2.05 (1.03, 4.07) for oligoastrocytoma grade III compared to grade II. (P=0.040).

HR 0.96 (0.56, 1.63) for oligodendroglioma grade II compared to grade III. (P=0.88).

LGG grade III showed a statistically significant risk of progression when compared by histologic class:

Oligodendroglioma HR 1 (ref).

Astrocytoma HR 2.67 (1.58, 4.52, P<0.001)

Oligoastrocytoma HR 2.75 (1.46, 5.17, P=0.002).

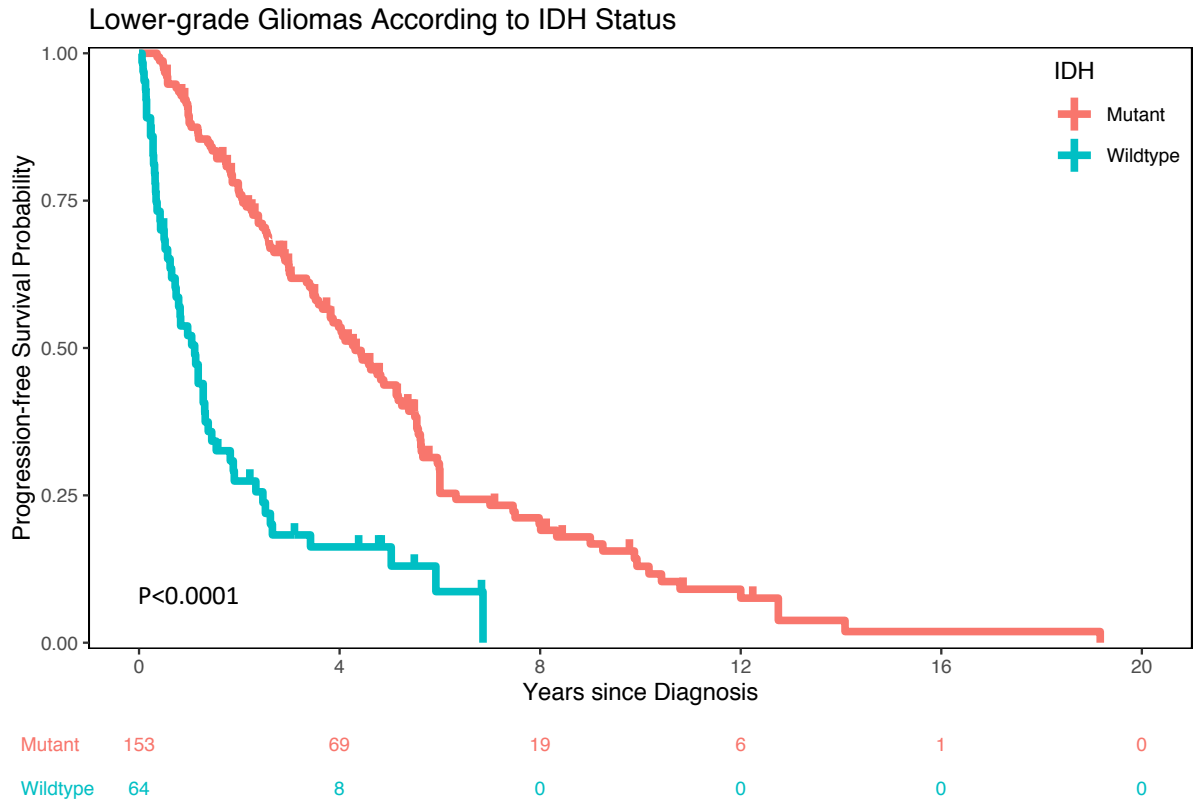


Figure PFS 6. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to IDH status.

IDH-wildtype tumours had substantially shorter median progression-free survival time, 1.13 years than did those with mutated IDH, 4.43 years. (P<0.0001 by log-rank test)

LGG grade III showed a greater statistically significant risk of progression when comparing IDH-wildtype to IDH-mutant:

HR 5.83 (3.43, 9.89, P<0.001)

Particularly, the risk of progression was also significantly higher for Oligodendrogliomas grade III IDH-wildtype when compared to IDH-mutant:

HR 5.51 (1.90, 15.98, P=0.002).

LGG grade II showed a statistically significant risk of progression when comparing IDH-wildtype to IDH-mutant:

HR 1.79 (1.06, 3.04, P=0.029)

Particularly the risk of progression was also significantly higher for astrocytomas grade II IDH-wildtype when compared to IDH-mutant:

HR 2.22 (1.11, 4.01, P=0.02).

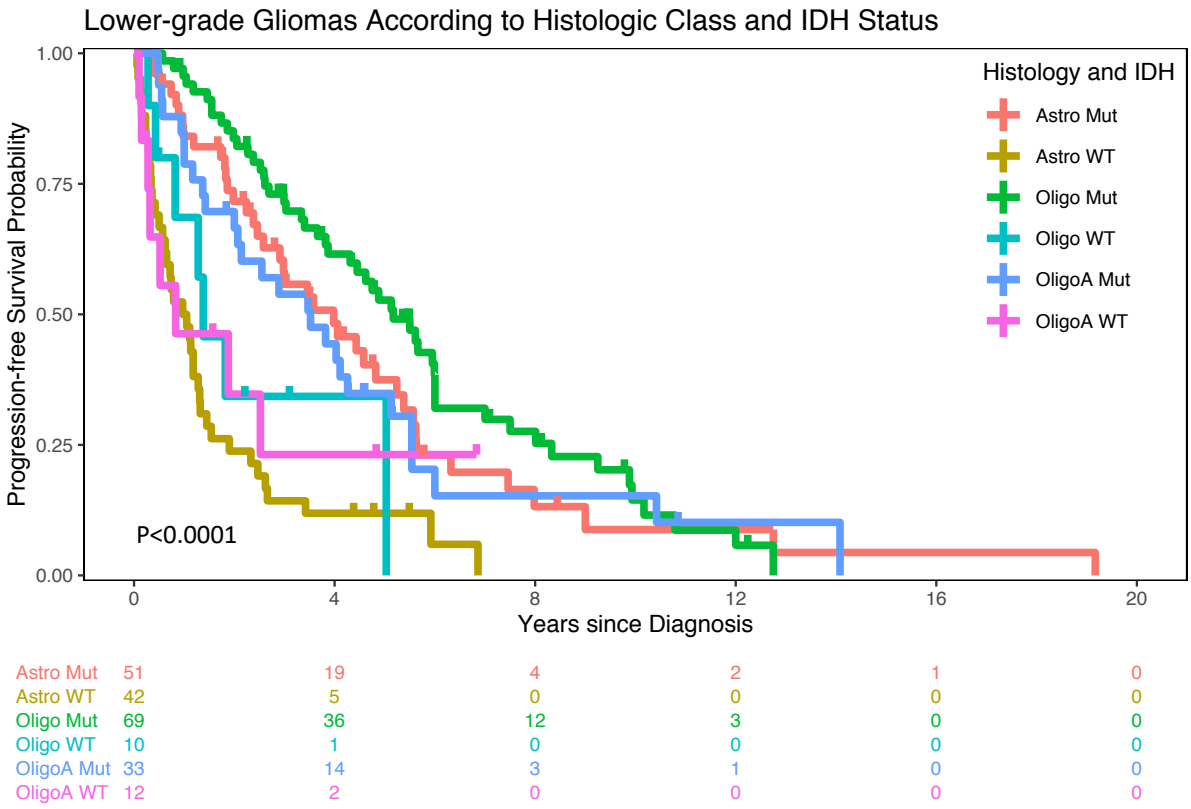


Figure PFS 7. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to histologic class and IDH status.

Median progression-free survival time (in years) for astrocytoma IDH-mutant (3.98), astrocytoma IDH-wildtype (0.97), oligoastrocytoma IDH-mutant (3.52), oligoastrocytoma IDH-wildtype (0.83), oligodendroglioma IDH-mutant (6.72) and oligodendroglioma IDH-wildtype (1.38). (P<0.0001 by log rank-test). Astro, astrocytoma; Oligo, oligodendroglioma; OligoA, oligoastrocytoma. Mut, IDH-mutant; WT=IDH-wildtype.

Particularly the median progression-free survival time was substantially different when comparing the 55 astrocytomas grade II according to their IDH- molecular status (plots not shown): median progression-free survival time for the 39 astrocytomas grade II, IDH-mutant was 3.58 years, and 1.18 years for the 16 astrocytomas grade II, IDH-wildtype (P=0.019 by log-rank test).

We also calculated the hazard ratio (HR) for each pair of histologic class and IDH-status and verified that the statistical significance of the risk of progression, in Figure PFS 7, holds true for 2 of them: there was no statistical significance when comparing oligoastrocytomas IDH-wildtype and IDH-mutant.

HR 4.03 (1.89, 4.85) for astrocytoma IDH-wildtype compared to IDH-mutant. (P<0.0001).

HR 1.76 (0.78, 3.92) for oligoastrocytoma IDH-wildtype compared to IDH-mutant. (P=0.16).

HR 4.21 (1.81, 9.76) for oligodendroglioma IDH-wildtype compared to IDH-mutant. (P=0.001).

And the HR for the risk of progression was not statistically significant for IDH-wildtype tumours according to histologic class (astrocytoma, oligoastrocytoma or oligodendroglioma):

Oligodendroglioma HR 1 (ref).

Astrocytoma HR 1.58 (0.67, 3.38, P=0.31)

Oligoastrocytoma HR 1.11 (0.40, 3.09, P=0.82).

Particularly, there was no difference in the risk of progression when comparing Astrocytoma IDH-mutant, grade III and grade II.

HR 1.21 (0.57, 2.60, P=0.60).

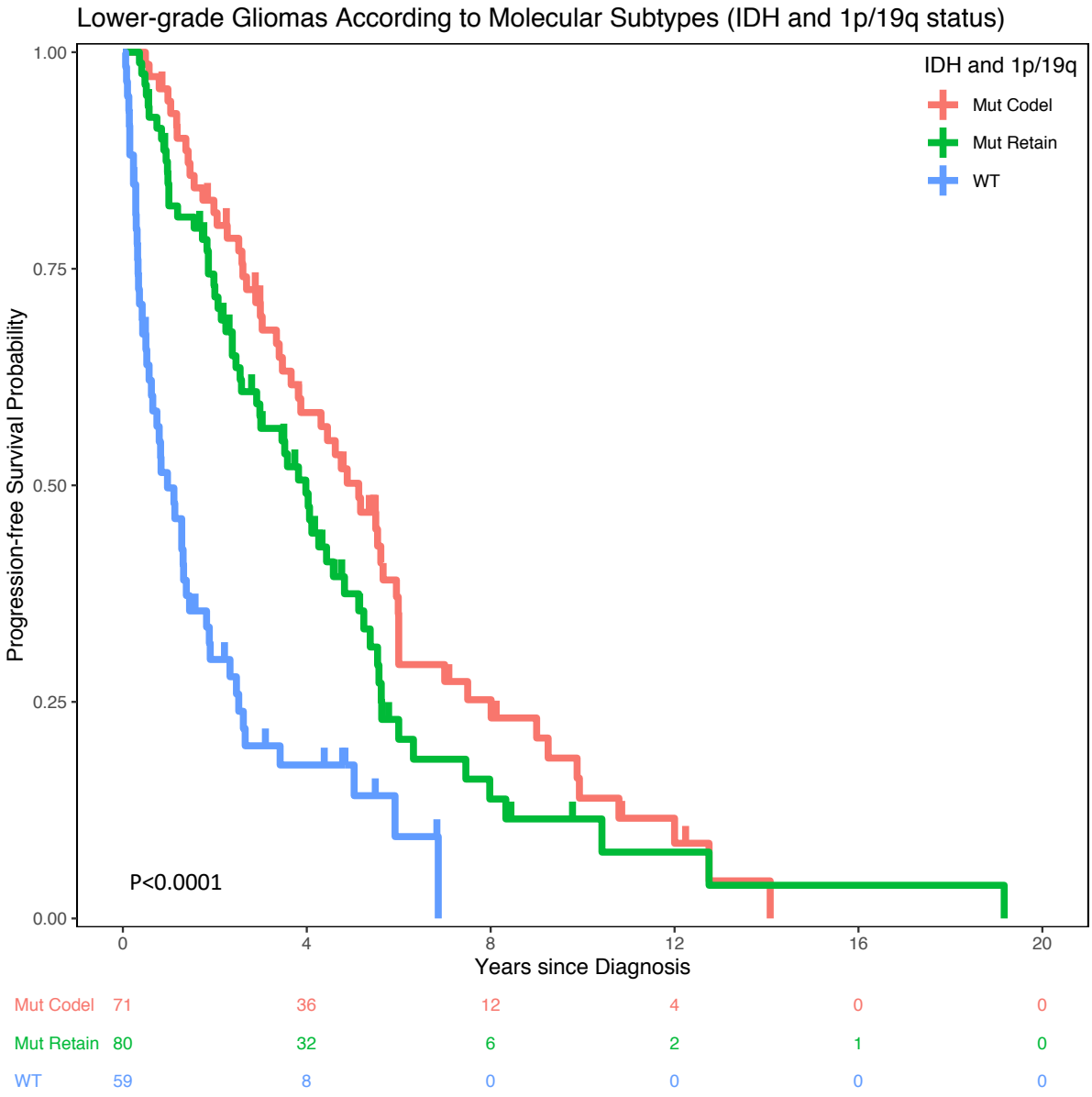


Figure PFS 8. Kaplan-Meier estimates with patient numbers at risk of lower-grade gliomas classified according to molecular subtypes (IDH and 1p/19q status).

IDH-mutant, 1p/19q-codeleted patients had an estimated median progression-free survival of 5.13 years compared to 4.03 years for the IDH-mutant, 1p/19q-retained, both significantly longer than the IDH-wildtype group with 1.11 years. (P<0.0001 by log-rank test). Mut Codel, IDH-mutant, 1p/19q-codeleted; Mut Retain, IDH-mutant, 1p/19q-retained; WT, IDH-wildtype.

When we compared the 151 IDH-mutant patients, there was no difference in the estimated risk of progression between the 71 (47.0%) 1p/19q-codeleted and the 80 (53.0%) 1p/19q-retained.

HR 1.37 (0.93, 1.95) when comparing IDH-mutant, 1p/19q-retained and IDH-mutant, 1p/19q-codeleted. (P=0.11)

And there was no difference in the risk of progression of either grade II or grade III tumours when comparing IDH-mutant, 1p/19q-retained and the IDH-mutant, 1p/19q-codeleted.

Grade II: HR 1.25 (0.80, 1.95, P=0.30)

Grade III: HR 1.60 (0.85, 3.03, P=0.14)

We also calculated the hazard ratio (HR) for each pair of molecular subtypes (IDH- and 1p/19q-status) and grade II or III and verified that there was a greater risk of progression only for IDH-wildtype grade III compared to grade II.

HR 0.96 (0.56, 1.64) IDH-mutant, 1p/19q-codeleted grade III compared to grade II. (P=0.89).

HR 0.91 (0.51, 1.61) for IDH-mutant, 1p/19q-retained grade III compared to grade II. (P=0.75).

HR 2.32 (1.28, 4.22) for IDH-wildtype grade III compared to grade II. (P=0.006).

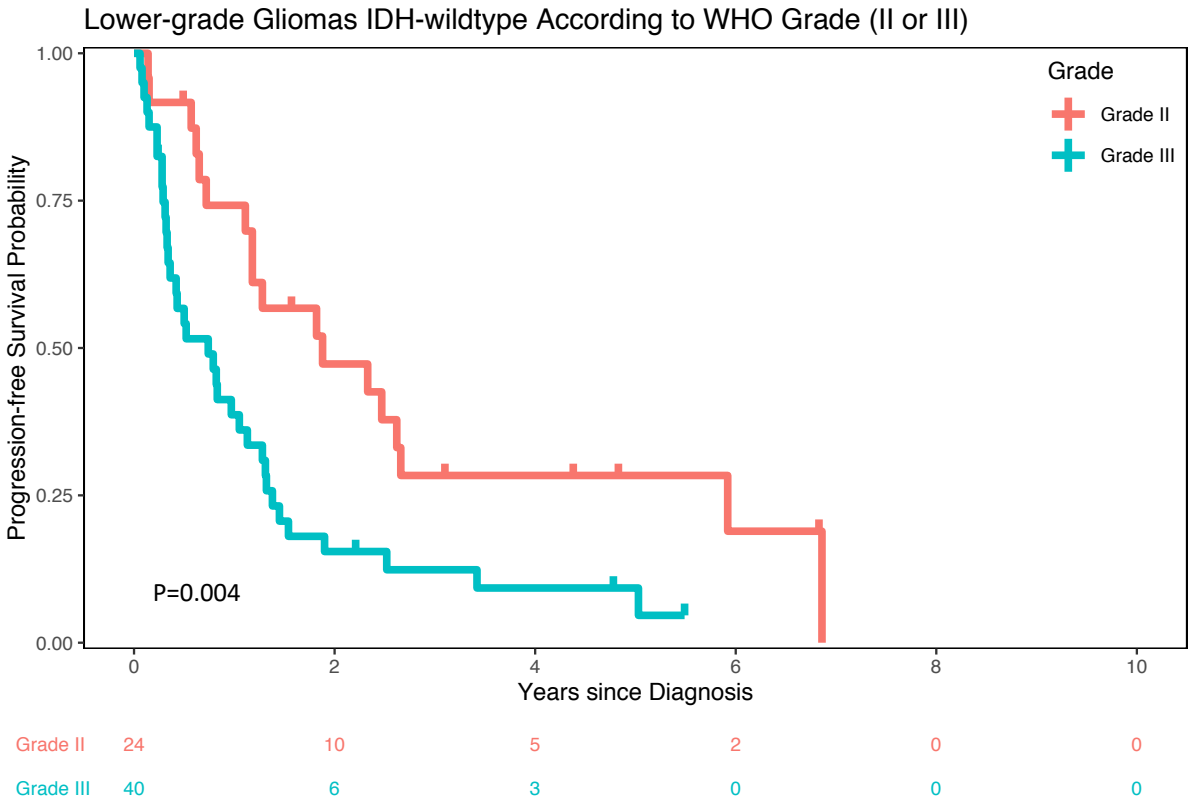


Figure PFS 9. Kaplan-Meier estimates with patient numbers at risk of IDH-wildtype lower-grade gliomas classified according to WHO grade (II or III).

There was a statistical difference in the estimated median progression free survival of IDH-wildtype LGG grade II (1.88 years) compared to grade III (0.74 years). (P=0.004 by log rank test).

The outliers are better depicted in Box Plot 2 in the Appendix 2.

Cox Regression Models

Given that age at diagnosis, extent of resection, histologic type, and IDH and 1p/19q status were all significantly associated (by log-rank test) with progression-free survival, we examined two multivariate Cox regression models: Model I accounting for all described variables except the molecular status and Model II accounting for all variables. We have included WHO grade in order to be in line with the OS Cox regression models, even knowing that WHO grade was not significantly associated with progression by the log-rank test.

Table 7 presents the estimates of two multiple predictor models (Models I and II) of progression free survival.

In Multivariate Model I, adjusting for age ≥40 years increases the risk of progression compared to <40 years. Astrocytoma (HR 2.08, P<0.001), and oligoastrocytoma (HR 1.74, P=0.013) have increased risk of progression relative to oligodendroglioma. Less than complete resection (HR 2.3) compared to complete resection also increases the risk (P=0.001). The effect of WHO grade III (HR 1.36) relative of grade II is not statistically significant (P=0.9).

In Model II, age≥40 years (HR 1.39, P=0.05) does not seem to increase the risk of progression compared to age <40 years. Less than complete resection (HR 3.08) compared to complete resection retains a risk (P=0.001) but a histological diagnosis of astrocytoma (HR 1.4) compared to oligodendroglioma does not seem to increase the risk (P=0.16). The effects of WHO grade III (HR 1.17) relative of grade II is also not statistically significant (P=0.30). IDH-wildtype molecular class (HR 3.45, P<0.0001) retains a highly significant risk of an event; but not a diagnosis of IDH-mutant, 1p/19q-retained compared to IDH-mutant, 1p/19q-codeleted (P=0.57). Hence, the most substantial predictor of progression or recurrence, with a major prognostic significance, is IDH-wildtype molecular status (P<0.0001).

Table 7. Progression-free Survival Models.

		Model I	Model II
Predictor	Levels	HR (95% CI)	HR (95% CI)
Age at Diagnosis	<40 years	1.0 (ref)	1.0 (ref)
	≥40 years	**1.74 (1.27, 2.37) (P<0.0001)**	1.39 (1.00, 1.95) (P=0.05)
Extent of Resection			
	Complete	1.0 (ref)	1.0 (ref)
	< Complete	**2.3 (1.23, 4.2) (P=0.009)**	**3.27 (1.73, 5.84) (P=0.001)**
Histologic Type	Oligodendroglioma	1.0 (ref)	1.0 (ref)
	Oligoastrocytoma	**1.74 (1.12, 2.69) (P=0.013)**	1.31 (0.81, 2.1) (P=0.71)
	Astrocytoma	**2.08 (1.45, 2.98) (P<0.0001)**	1.4 (0.87, 2.24) (P=0.36)
WHO Grade	II	1.0 (ref)	1.0 (ref)
	III	1.36 (0.95, 1.81) (P=0.09)	1.17 (0.84, 1.62) (P=0.30)
IDH, 1p/19q group	IDH-mutant, 1p/19q-codeleted		1.0 (ref)
	IDH-mutant, 1p/1pq-retained		1.15 (0.7, 1.87) (P=0.42)
	IDH-wildtype		**3.45 (2.08, 5.74) (P<0.0001)**

Progression Free Survival Models. Cox regression models of progression-free survival considering age at diagnosis, extent of resection, histologic class, WHO grade, and IDH, 1p/19q groups in combination in two multiple-predictor models. Bold denotes hazard ratios (HR) significantly different from 1 (no difference).

DISCUSSION AND CONCLUSIONS

In this study we have shown that patients with IDH-wildtype lower-grade gliomas (LGG), grades II and III have a highly significant shorter median overall and progression-free survivals when compared to patients with IDH-mutant LGG (Figs OS 6, PFS 6 and Tables 6 and 7).

Hence, we confirm that the IDH-status is a very important prognostic biomarker for LGG. (Ma et al., 2013, Brandner and von Deimling, 2015, van den Bent et al., 2010). The National Institutes of Health (NIH) Biomarkers Definitions Working Group defines biomarkers as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, or pharmacologic responses to a therapeutic intervention” (Strimbu and Tavel, 2010, Biomarker Definitions, 2001, FDA-NIH Biomarkers, 2016).

In our cohort of 218 patients its demographics were all consistent with published data (Louis et al., 2007, Ohgaki and Kleihues, 2005a, Ostrom et al., 2014).

The median age at diagnosis was 41.5 years and those harbouring IDH-wildtype tumours (median age 51 years) were significantly older than those with IDH-mutant (median age 37.6 years) (Table 1). Like others, (Soffietti et al., 2010) we found that in the entire cohort, and when divided by histologic class and grade, patients 40 years and older presented a highly statistically significant ($P<0.0001$) shorter estimated overall median survival compared to younger ones (Figure OS 1). In fact, patients with lower-grade gliomas 40 years and older have been considered high-risk and hence included to receive upfront chemotherapy and radiation therapy in treatment trials (Baumert et al., 2016, Buckner et al., 2016). Nonetheless it is possible that the shorter overall survival found in those older patients be due to their IDH-status, since as it has been already pointed out (Hartmann et al., 2010, Olar et al., 2015, Reuss et al., 2015b) we have also found that the prognostic impact of higher age was found only in patients with IDH-wildtype lower-grade gliomas [hazard ratio (HR) for death, 2.45; 95% confidence interval (CI), 1.17 to 4.96, $P=0.017$] and not in the IDH-mutant (HR 1.33; CI 0.68, 2.61, $P=0.39$). Confirming a recent published finding (Hartmann et al., 2010, Olar et al., 2015, Reuss et al., 2015b) we

also did not find a statistically difference in age when comparing IDH-mutant astrocytomas grade III (mean age at presentation 37.4 years) and grade II (mean age 35.9 years) (P=0.64 by ANOVA).

Our cohort was diagnosed between 2009 and 2015, according to the WHO 2007 classification (Louis et al., 2007), i.e., phenotypic only, before the integrated phenotypic (histologic class and grade) and genotypic (molecular markers) classification criteria that provided an increased level of objectivity (Louis et al., 2016). Nonetheless, since the prognostic role of the 1p/19q and IDH molecular markers was discovered before 2009, all our patients had the IDH marker evaluated and the 1p/19q status was determined in 211 (Table Appendix). A too small number of our patients had ATRX molecular data (Kannan et al., 2012) for our analysis to have any statistical significance.

The histologic class and grade diagnosis of LGG is known to suffer from a significant interobserver variation and hence lacks precision to inform clinicians the better course of action (Coons et al., 1997, van den Bent, 2010). This interobserver variation that involves the classification and grade of all lower-grade gliomas has been particularly prominent in the diagnosis of the now practically defunct oligoastrocytomas (Louis et al., 2016), still present in 45 (20.5%) of our patients (Table 1).

Since the discovery of the 1p/19q codeletion as a predictor of better outcome in oligodendrogliomas in 1988 (Cairncross et al., 1994), but specially after the discovery of IDH mutations in 2008 (Brandner and von Deimling, 2015) that has fundamentally changed the approach of glioma diagnosis, researchers have continuously analysed data accumulated in the literature in order to reach an integrated approach to diagnosis that would deliver more reflective disease subtypes than histologic class and grade alone (Cancer Genome Atlas Research et al., 2015, Eckel-Passow et al., 2015, Hartmann et al., 2009, Metellus et al., 2010, Sahm et al., 2014, Theeler et al., 2012). That goal was eventually achieved with the Haarlem

consensus guidelines and the ensuing 4th revised 2016 WHO Classification (Louis et al., 2014).

Other than eliminating the difficult management problem of the oligoastrocytomas, the new 2016 classification brought better clarity and prognostic value to all lower-grade gliomas diagnoses.

Oligoastrocytomas are now diagnosed either as astrocytomas, IDH-mutant, 1p/19q-retained (supported by ATRX mutation) or oligodendrogliomas, IDH-mutant, 1p/19q-codeleted (ATRX retained) (Louis et al., 2016).

Hence, as expected with the new integrated diagnosis our oligoastrocytomas had molecular markers equally distributed between astrocytomas and oligodendrogliomas (Table Appendix) (Sahm et al., 2014). When considering those molecular markers, our oligoastrocytomas also behaved similarly in terms of survival to the equivalent astrocytomas and oligodendrogliomas.

Like others (Cancer Genome Atlas Research et al., 2015) our oligoastrocytomas IDH-wildtype had the same very short median overall survival (1.5 years) as our astrocytomas IDH-wildtype (1.5 years) (Figure OS 7), but our oligoastrocytomas grade II (Figure OS 5) and oligoastrocytomas IDH-mutant (Figure OS 7) had a very long median overall survival (20.45 years), indeed the longest in our cohort, even longer than our oligodendrogliomas IDH-mutant (15.82 years) (Figure OS 7) most likely because, as already pointed out (Tabouret et al., 2016), we also found our oligoastrocytomas IDH-mutant to be enriched with canonical oligodendrogliomas [i.e., 11 (33%) of our 33 oligoastrocytomas IDH-mutant were also 1p/19q-codeleted] (Table Appendix). Of note our oligodendrogliomas grade II did not reach an estimated median overall survival time and had a very high 15-year survival rate (82%) (Figure OS 5), in accordance with others (Killela et al., 2014, Olson et al., 2000).

The only other group that did not reach an estimated overall survival was the LGG IDH-mutant, 1p/19q-retained, surprisingly grade III (Table 3) (with a 15-year survival rate of 60%) (not shown), but that was not statistically significant when compared to

both the LGG IDH-mutant, 1p/19q-retained grade II (P=0.49) (Table 3), and the LGG IDH-mutant, 1p/19q-codeleted grade III (HR 1.22; CI 0.49, 3.06, P=0.66).

Also our data strongly corroborated the fact that both IDH-mutant, 1p/19q-codeleted and IDH-mutant, 1p/19q-retained groups had a substantially longer median overall and progression-free survivals than did persons who had IDH-wildtype tumours (P<0.0001) (Figures OS 6 and PFS 6) (Cancer Genome Atlas Research et al., 2015, Eckel-Passow et al., 2015, Leeper et al., 2015, Picca et al., 2018)s Research et al. Interestingly our HR for death (9.32) in Cox Model II for patients with IDH-wildtype LGG in comparison with those with IDH-mutant LGG was almost the same (9.22) (Cancer Genome Atlas Research et al., 2015).

Like others (Wang et al., 2014), our patients with IDH-mutant, 1p/19q-codeleted tumours had a much longer median overall survival than those with IDH-mutant, 1p/19q-retained tumours (Figure OS 8). But confirming a large study (Cancer Genome Atlas Research et al., 2015) we also did not find that difference to be statistically significant (Figure OS 9).

Therefore, our data did not confirm (statistically) the widely acknowledged fact (Cairncross et al., 1998, Chamberlain and Born, 2015, Jenkins et al., 2001)of 1p/19q co-deletion as being a prognostic, and a predictive of chemosensitivity biomarker, leading to increased survival times.

That is probably due to the variation in nature and timing of treatment of our patients. This heterogeneity in treatment needs to ignore the potential benefits of therapy when we compared survival times. Also, we may have had 1p/19q falsely co-deleted tumours (Clark et al., 2013, Horbinski et al., 2012) or a particularly more aggressive sub-group of 1p/19q-codeleted as has recently been described in an “Integrated multi-omics analysis of oligodendrogliomas” (Kamoun et al., 2016).

Also as expected (Cancer Genome Atlas Research et al., 2015), our astrocytomas were mostly IDH-mutant, 1p/19q-retained and our oligodendrogliomas were mostly IDH-mutant, 1p/19q-codeleted and, again, the oligoastrocytomas not having a

preponderant molecular marker (Figure Appendix and Clinical Data: Summary of Results in the Appendix).

As others, we also had 10 tumours classified as oligodendrogliomas IDH-wildtype (Frenel et al., 2013). Their overall and progression-free survivals were 3.83 years (Figure OS 7) and 1.38 years respectively (Figure PFS 7). The majority of these tumours probably do not represent true (i.e., molecular entity – 1p/19q-codeleted) oligodendrogliomas and will probably fall in other categories that could be discerned with new molecular analyses (Capper et al., 2018).

WHO Grade II and III

In our patients, grade was shown to be a statistically significant prognostic marker when comparing the median overall (Figure OS 5) and progression-free survivals (Figure PFS 5) of the histologic classes (astrocytomas, oligoastrocytomas and oligodendrogliomas), and also the IDH-wildtype (Figure OS 10 and Figure PFS 9) (Aibaidula et al., 2017, Louis et al., 2007).

Like others (Reuss et al., 2015b), we found that grade was not significant when comparing median overall (Grade II: HR 2.28; CI 0.72, 7.25, P=0.16. Grade III: HR 1.22; CI 0.49, 3, 3.06, P=0.66) and progression-free survivals (Grade II: HR 1.25; CI 0.80, 1.95, P=0.30. Grade III, HR, 1.60; CI 0.85, 3.03, P=0.14) of the IDH-mutant, 1p/19q-codeleted and IDH-mutant, 1p/19q-retained groups.

Grade III conferred a worse prognosis (our shortest overall median survival, of 1.24 years, and progression-free survival, of 0.74 years) (Figure OS 10 and Figure PFS 9, respectively) on top of the already low overall median survival (2.0 years) and progression-free survival (1.11 years) to IDH-wildtype tumours (Figure OS 8 and Figure PFS 8, respectively) (Dubbink et al., 2016, Frenel et al., 2013, Reuss et al., 2015a). IDH-wildtype lower-grade gliomas WHO grade III have been shown to have the same molecular characteristics as IDH-wildtype glioblastomas and hence may be considered as such (WHO grade IV); indeed, those tumours have a worse prognosis than glioblastomas IDH-mutant (also WHO grade IV) (Hartmann et al., 2010).

Extent of resection

It is known that LGG are infiltrative tumours that cannot be surgically cured. Nonetheless, despite the lack of prospective clinical trials addressing the issue of surgery in LGG (Jiang et al., 2017), there is now a concurrent understanding that surgery, the earliest possible and with the maximum safest tumor removal is the best approach both in terms of OS and PFS (Hayhurst, 2017, Jakola et al., 2017).

Since we found that there was no standard in the definition of gross total resection and partial resections in the more than 20 studies that addressed the issue (Berger et al., 2016), and because most reported a mean survival benefit of "maximal resection" we divided our patients, for the purpose of the Cox analyses, as subjected either to complete resection (no evidence of residual tumor and no enhancement on the post-operative MRI) or less-than-complete (that included biopsies).

Extensive tumours and those that involve more than one lobe are not amenable to complete resection and in those cases large tumor volume and extension, prior to surgery, are highly predictors of poor outcome (Mariani et al., 2004).

In our cohort, complete resection was achieved in 25 (12%) patients and like with others (Eseonu et al., 2017) they did not reach a median overall survival (our 10-year survival rate was 88%) and had a median progression-free survival of 5.9 years (Figure OS 2 and Figure PFS 2, respectively). Our low percentage of complete resection is in line with the RTOG 9802 (Buckner et al., 2016) trial that had 10% of completed resected tumours and the EORTC 22033-26033 (Baumert et al., 2016) with 17%. Although we had more (18 patients) IDH-mutant complete resection patients than IDH-wildtype (7 patients), that was not statistically significant (Clinical Data: Summary of Results in the Appendix). Notwithstanding, others found that IDH-mutant tumours were more amenable to complete surgical resection (Beiko et al., 2014) most probably because as recently shown IDH-wildtype tumours tend to be more diffuse (Hyare et al., 2019).

Like others, we found a statistically better overall survival (P=0.001) and progression-free survival (P=0.003) for those undergoing a partial resection compared to biopsy (Berger et al., 2016).

Moreover, our Cox analyses showed that less-than-total resection had a higher risk of death in Model II of overall survival (Table 6), that included IDH-status, and in both progression-free survival models (Table 7), confirming the present understanding of the important role of resection in lower-grade gliomas (Hayhurst, 2017).

Incidental Tumours

In 10 of our 218 patients (4.5%) the tumor diagnosis was incidental. Their median overall survival was not reached (15-year survival rate of 70%), and their median progression-free survival was 5.54 years, but that was not statistically significant (P=0.30 and P=0.43 respectively). Also, not surprisingly due to the early incipient and clinically silent nature of those tumours (Rees, 2016), nine were IDH-mutant and eight were grade II. The two deceased patients were IDH-mutant and had a partial resection. Also, in line with the most probable benefit of early resection, we think that a maximal possible excision would be beneficial for all incidental tumours (Lima and Duffau, 2015, Yordanova and Duffau, 2017).

Cox models

In our Cox regression model I, according to WHO 2007 (Louis et al., 2007), without the IDH- and 1p/19q- molecular status, we confirmed three of the parameters that have for long been considered as unfavourable prognostic factors (Pignatti et al., 2002): age ≥40 years, WHO grade III, and astrocytoma histology showed a highly significant increase in the risk of death (Table 6) (P<0.0001). We found a trend of increased risk of death in patients who had a less-than-complete resection compared to complete, but that was not statistically significant (P=0.58).

Age ≥40 years, astrocytoma histology (P<0.0001), and less-than-complete resection (P=009) showed an increased risk for progression, but not WHO grade III compared to grade II (P=0.09) (Table 7).

In our model II, according to WHO 2016 (Louis et al., 2016), including IDH- and 1p/19q- IDH molecular status, we confirmed (Cancer Genome Atlas Research et al.,

2015, Etxaniz et al., 2017, Leeper et al., 2015) that the highest predictor of an increased risk of death (P<0.0001) (Table 6) and progression (Table 7) (P<0.0001) was the IDH-wildtype status.

Also in our overall survival model II we found a statistical significance of increased risk of death (but not of progression) for WHO grade III (P=0.007) and a statistical significance of increased risk of death for astrocytoma histologic class (P=0.046) and oligoastrocytoma (P=0.039), compared to oligodendroglioma (Table 6).

Hence our data confirm that, at the present time, classification of lower-grade gliomas should not proceed on the basis of genotype alone (i.e., without histology) (Louis et al., 2016) - the diagnosis of oligodendroglioma still confers a chance of better survival.

The prognostic relevance of IDH-wildtype lower-grade gliomas

Controversy still exists regarding the poor prognosis of IDH-wildtype tumours, in particular those grade II. Authors have described patients with IDH-wildtype tumours that lived longer than 5 years, hence the need for reappraisal (Aibaidula et al., 2017).

Although with a smaller number of patients (55 WHO grade II astrocytomas) our results markedly differed from a widely cited study where the authors did not find a prognostic value of IDH mutations in a series of 100 WHO grade II astrocytomas. Their 79 IDH-mutant patients had a median overall survival of 81.4 months (6.8 years) compared to 80.2 months (6.7 years) for the IDH-wildtype (P=0.12) and their median progression-free survival was 44.6 months (3.7 years) and 67.4 months (5.6 years) respectively (P=0.46) (Ahmadi et al., 2012). Our IDH-mutant patients had a median overall survival of 10.58 years compared to 2.44 years for the IDH-wildtype (P<0.0001), and our median progression-free survival was 3.8 years and 1.18 years respectively (P=0.019). Moreover, our HR of death for the IDH-wildtype was 9.24 (3.60, 27.74, P<0.0001) when compared to the IDH-mutant. Our data shows that the IDH-status is an important prognostic factor also in WHO grade II astrocytomas.

As described above, we did not support what was described as a “Janus head like phenomenon”, i.e., an unfavourable prognostic influence in progression-free survival that turns into favourable on post recurrence survival in grade II astrocytomas (Thon et al., 2012). The median progression-free survival of our patients with IDH-mutant grade II astrocytomas was longer when compared to those IDH-wildtype (P=0.019).

A recent review and meta-analysis on the outcomes of LGG grade II, IDH-mutant and IDH-wildtype presented exactly the same demographics as ours: 23% of IDH-wildtype among LGG grade II, we had 20%; mean age of patients with IDH-wildtype was higher than those with IDH-mutant; a significant higher number of IDH-wildtype tumours had a temporal lobe location compared to IDH-mutant; most studies were based on the 2007 WHO classification; most of IDH-wildtype were astrocytomas; a significant higher number of IDH-mutant were oligodendrogliomas; and the oligoastrocytomas were divided between IDH-mutant and IDH-wildtype (Di Carlo et al., 2018).

We differed in relation to the overall survival: they present a mean of 10 years for IDH-mutant and our median for IDH-mutant tumours was 15.8 years (Figure OS 6), but both of us showed a significant difference with IDH-wildtype. Their mean progression-free survival for IDH-mutant tumours was 4.5 years and for IDH-wildtype, 3 years (P=0.023) (Di Carlo et al., 2018), ours was 4.3 years, and 1.13 years respectively (Figure PFS 6) (P<0.0001).

The point they make is that their meta-analysis of patients with lower-grade gliomas, IDH-wildtype, WHO grade II showed a significant variation in the mean overall survival corroborated by a report that described 31 patients with lower-grade gliomas IDH-wildtype, WHO grade II where 21 (70%) were still alive 5 years after diagnosis (Poulen et al., 2018). They did not find any significant difference in the clinical, radiologic or molecular characteristics relative to those patients who died. Our data had much less variation, we found that only 4 (16.6%) of our 24 IDH-wildtype, WHO grade II patients were still alive 5 years after diagnosis (Table 4).

Our IDH-wildtype Long Survivors

Lower-grade gliomas IDH-wildtype have an overall median survival of less than 2 years (Picca et al., 2018). We found seven (11%) out of 64 IDH-wildtype patients that survived at least 5 years (Table 4). Due to their small number we could not find any significant statistical correlation among them, but importantly, none had a PTEN mutation and only one had an EGFR amplification (Table 4), two known glioblastoma molecular markers (Brandner and v(Brandner and von Deimling, 2015). Below are the clinical and pathologic characteristics of the three longest survivors (From Table 4) and the possible reason for their long survival.

The longest IDH-wildtype patient censored at 10.29 years was a 23.5-year-old man diagnosed with a partial resection in the left trigone area in 2006 that showed an astrocytoma grade II. He progressed and underwent a complete resection in 2012 with the same astrocytoma grade II diagnosis (now shown to be 1p/19q co-deleted). His other molecular markers showed no EGFR amplification, no loss of PTEN, no presence of BRAF fusion genes, and presence of MGMT methylation That could be a real oligodendroglioma with a failed IDH mutation detection (Wesseling et al., 2015).

The second longest follow-up IDH-wildtype patient was censored at 9.24 years: a 32.5-year-old woman had a head MRI in 2008 that showed a low-grade glioma in the left frontal lobe. She was followed up until progression in 2013 when a partial resection showed an oligodendroglioma grade III (1p/19q-codeleted). Her other molecular markers showed no EGFR amplification, no loss of PTEN and presence of MGMT methylation. That also could be a real oligodendroglioma with a failed IDH mutation detection (Wesseling et al., 2015).

The third longest IDH-wildtype patient survived 8.8 years: a 25.5-year-old man diagnosed with a biopsy in 2005 that showed an astrocytoma grade II in the left insula. He was followed up for 5 years before being re-operated (partial resection) and then diagnosed with an astrocytoma grade III. His other molecular markers showed no EGFR amplification, no loss of PTEN and positive MGMT methylation.

That could be a real IDH-wildtype long-survivor, lacking a better prognostic evaluation.

Our IDH-mutant short survivors

IDH-mutant patients are a priori assumed to live longer (Picca et al., 2018). Notwithstanding, we found 10 (6.5%) of our 154 IDH-mutant patients who had a short survival, i.e., died less than 4 years after diagnosis (Table 5).

Except for the fact that there were 9 (90%) grade III and none had a total resection, both unfavourable prognostic factors (Aibaidula et al., 2017) we could not find any other significant statistical correlation among them, but we can speculate regarding the shortest survivor (1.68 years), and the one who survived for 3.55 years (Table 5).

The shortest survivor was 26.7-year-old woman, the only one in the entire cohort that presented with an acute intracranial hypertension syndrome (headache and papilledema). She had a partial resection of an oligoastrocytoma grade II, 1p/19q-codeleted, but had EGFR amplification and loss of PTEN (two glioblastoma markers) (Brandner and von Deimling, 2015).

The one who survived 3.55 years was a 52.8-year-old woman who soon after a syncope underwent a partial resection of an oligodendroglioma grade III that was also 1p/19q-codeleted and had EGFR amplification, loss of PTEN (two glioblastoma markers) (Brandner and von Deimling, 2015) and a positive MGMT methylation.

Both were probably “de novo” glioblastomas IDH-mutant, not detected in the partial resection sample (Brandner and Jaunmuktane, 2019).

Epigenetic changes, complementary to the genetic changes we presented (IDH- and 1p/19q-), may explain the tumour behaviours of our IDH-wildtype long survivors and IDH-mutant short survivors – our outliers.

Epigenetic was originally defined by C.H. Waddington in 1942, when studying embryonic development, as “the causal interaction between genes and their products, which brings the phenotype into being”. The current definition is “the study

of heritable changes in gene expression that occur independent of changes in the primary DNA sequence" (Sharma et al., 2010).
Several epigenetic mechanisms may operate in synchrony to modify the packaging of the genome being DNA methylation the most extensively studied epigenetic mechanism implicated in carcinogenesis (Malta et al., 2018).
A study of 1.122 diffuse gliomas (WHO grades II to IV) from the Cancer Genome Atlas recapitulated the glioma classification based on IDH- and 19/19q-status and through analysis of DNA methylation profiles identified clinically relevant molecular subsets. A subtype of IDH-mutant glioma was associated with DNA demethylation and poor outcome, and a group of IDH-wildtype diffuse glioma showed molecular similarity to pilocytic astrocytoma and relatively favorable survival (Ceccarelli et al., 2016).
Our not presently explained outliers may have fallen into either of those categories.

Much had to be elucidated in order to better define the prognostic and predictive value of the markers we had at hand during this study. We did not want to overtreat our patients and hence incur in unnecessary morbidity, but we also did not wish to undertreat and shorten their survival (Louis et al., 2016).

It is possible that new algorithms like the one being developed here, in this same institution, will help solve this conundrum (Jaunmuktane et al., 2019).

Moreover, a fundamental work is also afoot in order to refine the prognostic and predictive molecular markers of brain tumours. cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) has been established to provide a forum to evaluate and recommend proposed changes to future CNS tumor classifications and hence improve the difficult management of our patients (Louis et al., 2019).

Finally, as a further exploration of our cohort, a follow-up of our IDH-wildtype outliers associated with the DNA methylation signatures of both outliers (as part of our well described combined histology and molecular tumour classification) would probably define their real diagnosis and add to the constantly changing knowledge of LGG. (Capper et al., 2018).

Limitations of the Study

Our study has several limitations.

Ours is a single-institution retrospective cohort analysis and our conclusions cannot be extended to the whole population.

Although our cohort had a sizable number of subjects and events, we were unable to fit a Cox regression model with interaction terms. Our calculations were based on subgroup analyses.

Since molecular testing was performed between 2009 and 2015 and not repeated, some mutations may have been missed at the time, and others, particularly the 1p/19q status may have yielded false-positive results (Clark et al., 2013, Horbinski et al., 2012). Those facts may explain why we found eight LGG IDH-wildtype, 1p/19q-codeleted (Clinical Data: Summary of Results in the Appendix).

Being a retrospective cohort analysis, we were unable to acquire reliable data on the presence of neurologic deficit prior to the first surgery and treatment was heterogeneous.

Finally, due to the observational nature of our study, only association and not causation can be inferred from our results.

Conclusions

Our single institution cohort of 218 patients confirmed that in Lower-grade Gliomas, the IDH molecular marker is the most important predictor of outcome. Patients with IDH-wildtype tumours had a much shorter median overall and progression-free survivals than patients with IDH-mutant tumours.

Nonetheless, we were unable to find a significant difference in median overall and progression-free survivals between patients carrying IDH-mutant, 1p/19q-codeleted tumours versus IDH-mutant, 1p/19q-retained.

Grade had an impact only on IDH-wildtype survival: patients with IDH-wildtype grade III tumours had the shortest median overall and progression-free survivals of the

entire cohort. On the other hand, IDH-mutation showed to be an important marker that trumped the importance of grade: IDH-mutant grade II and III tumours had no significant difference in median overall and progression-free survivals.

We also showed that there were a few (11%) patients carrying IDH-wildtype tumours who survived more than 5 years; and we also demonstrated that there were a few (6.5%) IDH-mutant patients that died less than 4 years after diagnosis.

New research is needed in order to envisage better prognostic markers and hence deliver appropriate treatment to our patients.

APPENDIX

Table Appendix. Clinical Characteristics of the Sample Set According to IDH Mutation and 1p/19q Status.[a]				
Characteristic	**Total**	**IDH-mutant 1p/19q-codeletd**	**IDH-mutant 1p/19q-retained**	**IDH – WT**
	(N = 211)	(N = 71)	(N = 81)	(N = 59)
Histologic Type[b] and Grade[b] no. (%)				
Oligodendroglioma				
Grade II	40 (19.0)	32 (45.1)	4 (4.9)	4 (6.8)
Grade III	38 (18.0)	27 (38.0)	5 (6.2)	6 (10.2)
Oligoastrocytoma				
Grade II	26 (12.3)	7 (9.9)	15 (18.5)	4 (6.5)
Grade III	19 (9.0)	4 (5.6)	7 (8.6)	8 (13.1)
Astrocytoma				
Grade II	52 (24.6)	1 (1.4)	38 (46.9)	13 (21.3)
Grade III	36 (17.1)	0 (0)	12 (14.8)	24 (39.3)
Age at diagnosis – yr [b]				
Mean/Median (SD)	41.5/38.0 (14.0)	39.9/38.0 (11.1)	35.7/33.5 (10.1)	51.0/54.4 (16.4)
Range	15.8 – 77.5	16.9 – 63.1	15.8 – 64.4	21.4 – 77.5
Male/Female sex - no. (Male %)	113/98 (53.5)	32/39 (45.1)	44/37 (55.7)	37/22 (60.7)
Year of diagnosis - no. (%)				
Before 2009	57 (27.0)	29 (40.8)	25 (31.6)	3 (4.9)
2009-2015	154 (73.0)	42 (59.2)	56 (68.4)	56 (95.1)
Extent of resection - no. (%)				
Biopsy	68 (32.2)	21 (29.6)	17 (21.5)	30 (49.2)
Less than total resection	118 (55.9)	42 (59.2)	54 (65.8)	22 (39.3)
Total resection	25 (11.8)	8 (11.3)	10 (12.7)	7 (11.5)
Tumor location[b] - no. (%)				
Frontal lobe	113 (53.6)	44 (62.0)	46 (55.7)	23 (41.0)
Parietal lobe	31 (14.7)	15 (21.1)	12 (15.2)	4 (6.6)
Temporal lobe	54 (25.6)	11 (15.5)	21 (26.6)	22 (36.1)
Other	13 (6.2)	1 (1.4)	2 (2.5)	10 (16.4)
Laterality no. (%)				
Left	107 (50.6)	38 (53.5)	42 (51.9)	27 (45.0)
Midline	6 (2.9)	0 (0)	1 (1.3)	5 (8.3)
Right	98 (46.5)	33 (46.5)	38 (46.8)	27 (46.7)
First presenting symptom - no. (%)				
Seizures	153 (72.5)	53 (74.6)	63 (79.7)	37 (60.7)
Headache	26 (12.3)	9 (12.7)	9 (8.9)	8 (16.4)
Mental change	7 (3.3)	2 (2.8)	2 (2.5)	3 (4.9)
Motor/Movement	8 (3.8)	2 (2.8)	1 (1.3)	5 (8.2)
Speech	6 (2.8)	1 (1.4)	1 (1.3)	4 (6.6)
Visual	1 (0.5)	0 (0)	0 (0)	1 (1.6)
Incidental	10 (4.7)	4 (5.6)	4 (6.3)	1 (1.6)
Radiotherapy (N = 182) no. (%)				
Radiotherapy before 2009	13 (7.1)	9 (13.4)	4 (6.2)	0 (0)
Radiotherapy after 2009	120 (65.9)	40 (59.7)	44 (64.6)	36 (76.0)
No Radiotherapy	49 (26.9)	18 (26.9)	19 (29.2)	12 (24.0)
Chemotherapy (N = 168) no. (%)				
Yes	82 (48.8)	33 (53.2)	26 (41.1)	23 (52.1)
No	86 (51.2)	29 (46.8)	34 (58.6)	23 (47.9)

[a] Categorical distributions were compared with the use of Fisher's exact. Analysis of variance was used to compare between age groups.

[b] P<0.01 for the difference among the molecular subtypes

Clinical Data: Summary of Results

There were 218 LGG patients in the database. One patient from abroad who flew back home a fortnight after the operation, was removed. Only one patient, seen regularly at Dr. Jeremy H. Rees's Clinic, not in the database, was added.

There were 79 oligodendrogliomas, 45 oligoastrocytomas and 94 astrocytomas.

IDH status was known for all 218 patients, and Table 1 describes their clinical characteristics.

There were 154 IDH-mutant (70.6%) and 64 (29.4%) IDH-wildtype patients.

There were 118 males (54.1%) with a mean age at diagnosis of 41.7 ±14 years and 100 females (45.9%) with a mean age at diagnosis of 40.1 ±13 years.

Fifty-eight patients (26.6%) had their diagnosis before 2009 and 160 (73.4%) from 2009 to 2015.

Tumours with a mutation in either IDH1 or IDH2 were further subdivided by whether there was a codeletion of 1p/19q (1p/19q-codeletd) or not (1p/19q-retained).

IDH and 1p/19q status was known for 211 patients. Of the seven unknown 1p/19q status, two were IDH-mutant and five IDH-wildtype.

IDH-mutant, 1p/19q-codeleted status was detected in 71 (33.6%), IDH-mutant, 1p/19q-retained in 79 (37.4%), and IDH-wildtype (30%) in 59. Out of the 59 IDH-wildtype LGG, eight were also 1p/19q-codeleted, but we did not analyze them separately. The eight IDH-wildtype, 1p/19q-codeleted patients had exactly the same estimated overall (2.0 years) and progression-free (1.1 years) survivals as the 51 IDH-wildtype, 1p/19q-retained (not shown). (Table Appendix).

Histologic type was associated with IDH and 1p/19q status ($P<0.0001$) with 83% of IDH-mutant, 1p/19q-codeleted cases classified as oligodendroglioma and 60% of IDH-wildtype cases classified as astrocytoma.

The IDH-wildtype group was predominantly WHO grade III at diagnosis (64%) compared to the IDH-mutant group (36%) ($P<0.0001$).

Age at diagnosis differed significantly between the IDH-1p/19q molecular groups used in the comparison of outcomes (ANOVA $P<0.01$). The IDH-wildtype group is

the oldest (51 ± 16.2 years) and the IDH-mutant, 1p/19q-retained group the youngest (37.5 ± 10.14 years) (P<0.001); the IDH-mutant, 1p/19q-codeleted group has intermediate age, but still significantly older (39.9 ± 11.14 years) than the IDH-mutant, 1p/19q-retained (P<0.01).

IDH-mutant tumours were present predominantly in the frontal lobe (59%) compared to IDH-wildtype tumours (36%). IDH-wildtype tumours presented more frequently in the temporal lobe (34% versus 21%) (P<0.001).

IDH-wildtype patients were more likely to undergo a biopsy (49%) than IDH-mutant, 1p/19q-codeleted (29%) (P=0.01) but were just as likely to undergo total resection, 7 (10.6%) in 64 as IDH-mutant patients 18 (11.8%) in 154 (P=0.54).

There was no evidence of differences in sex or first presenting symptom. IDH-wildtype patients were not more likely to receive radiotherapy (76%) than the IDH-mutant, 1p/19q-codeleted (73%) (P=0.06) and there was no evidence of difference in chemotherapy receipt by IDH, 1p/19q groups (P=0.30).

Regarding first presenting symptom, overall 72% of LGGs presented first with seizure, whereas 12% presented first with headache (P=0.05). No other categorical variable distribution was statistically associated with histologic type and WHO grade.

Age at diagnosis was significantly associated with grade, with grade III presenting in persons four years older than grade II on average (P=0.02).

The grade III LGG glioma cases were more likely to report receipt of radiotherapy (82.9%) compared to grade II cases (52%) (P<0.0001) and also chemotherapy (73% versus 33%, P<0.0001).

Survival Modelling: Summary of Results

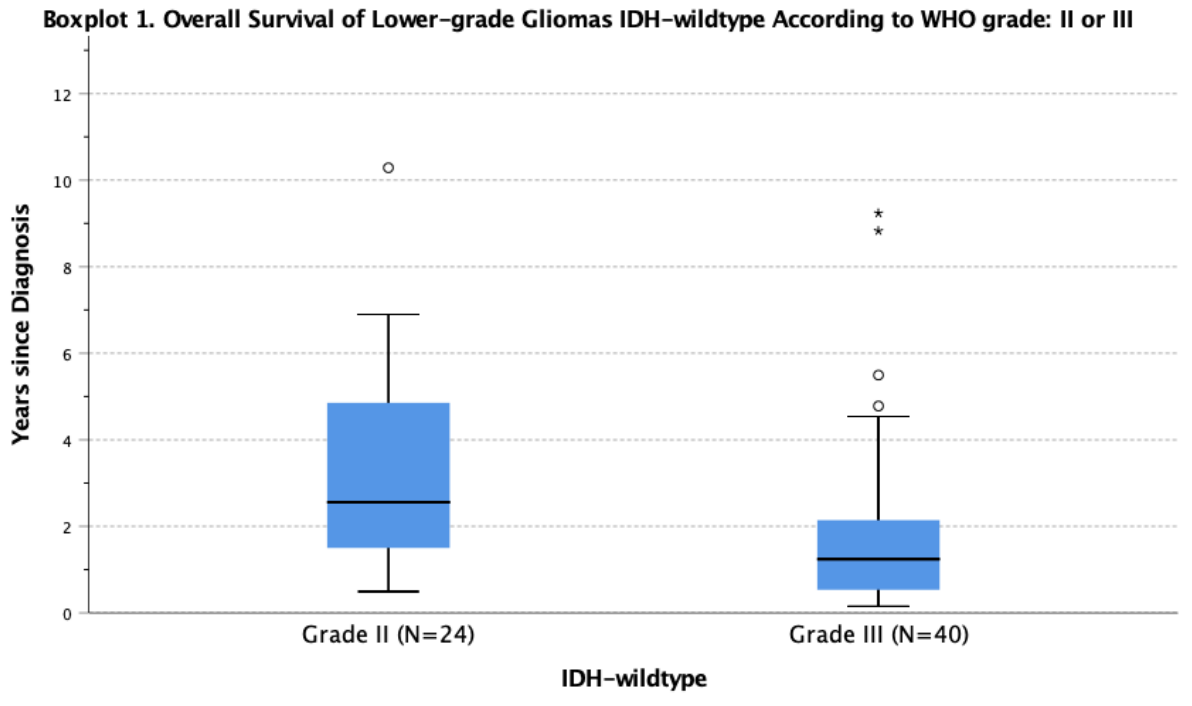
There were 218 observations included in this study. Among these, 85 cases (39%) where deceased at the time of analysis with a median overall survival (OS) estimated at 11.45 years (95% CI 6.98, 15.92). The median follow-up time is estimated at 6.82 years (95% CI 5.68, 7.96) with 16% of cases with follow-up of at least 10 years. Additionally, 171 patients (78.8%) experienced at least one

progression or recurrence (PFS, n=217). The median time to progression is estimated at 3.0 years (95% CI 2.27, 3.72).

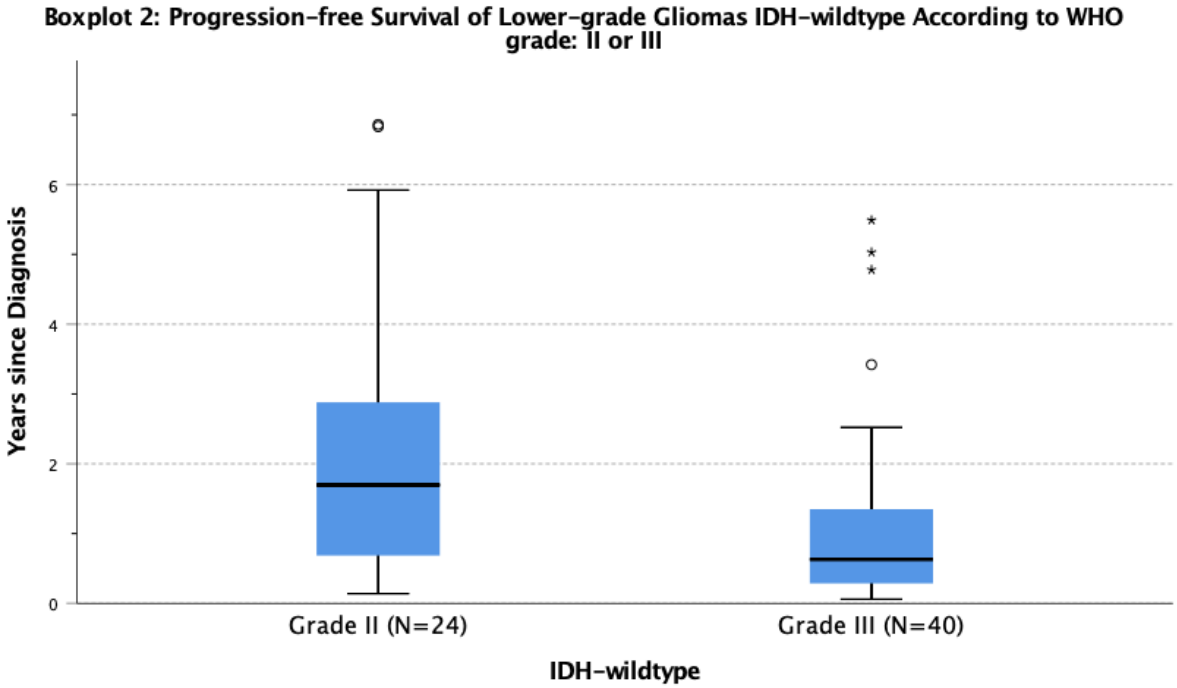
Extent of resection was known for all cases.

Survival models including extent of resection are based on 218 observations with 85 deaths for OS and 217 observations with 85 deaths for PFS.

1p/19q status was unknown for seven cases. Models including IDH and 1p/19q status are based on 211 observations with 78 deaths (40%) for OS, and 210 observations and 164 events (78%) for PFS. Models including both extent of resection and 1p/19q status are based on 210 observations with 78 deaths (37%) and 164 (78%) PFS events.



*Appendix 1. Box Plot 1 depicts the estimated median overall survival time from diagnosis until censored or death, the 50% and 75% higher and 25% lower percentiles, and the outliers (1.5x the interquartile range) and extreme outliers (3x the interquartile range) of the 24 IDH-wildtype, grade II and the 40 IDH-wildtype, grade III patients. There was one grade II and two grade III outliers (1.5x the interquartile range) and 2 grade III extreme outliers (3x the interquartile range). ◦=outliers; *=extreme outliers.*



*Appendix 2. Box Plot 2 depicts the estimated median progression-free survival time from diagnosis until censored or death, the 50% and 75% higher and 25% lower percentiles, and the outliers (1.5x the interquartile range) and extreme outliers (3x the interquartile range) of the 24 IDH-wildtype, grade II and the 40 IDH-wildtype, grade III patients. There were two grade II and one grade III outliers (1.5x the interquartile range) and 3 grade III extreme outliers (3x the interquartile range). ◦=outliers; *=extreme outliers.*

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