Peer-reviewed version available at *Brain Sci.* **2020**, *10*, 91; doi:10.3390/brainsci10020091

Article

Retrospective and Randomized Analysis of Influence and Correlation of Molecular and Clinical Prognostic Factors in a Mono-Operative Series of 122 Patients with Glioblastoma Treated with STR or GTR

Maurizio Salvati PhD[1], Placido Bruzzaniti MD[1], Michela Rilucenti PhD[3], Mariagrazia Nizzola MD[1], Pietro Familiari PhD[1], Santi Galletta MD[6], Marco Giugliano MD[1], Anthony Kevin Scafa MD[1], Xiaobo Li MD[5], Rui Chen MD[5], Claudio Barbaranelli PhD[3], Alessandro Frati MD[2,] Antonio Santoro MD[1],

[1] Department of Neurological Sciences, Neurosurgey, "La Sapienza" University of Rome, Rome : maurizio.salvati@uniroma1.it; placido.bruzzaniti@uniroma1.it; grazia.nizzola@gmail.com; pietro.familiri@uniroma1.it; marco.giugliano@uniroma1.it; ak.scafa@gmail.com; antonio.santoro@uniroma1.it;

[2] Department of Neuroscience, Mental Health and Sense Organs NESMOS, "La Sapienza" University of Rome, Rome : alessandro.frati@uniroma1.it;

[3] Department of Anatomical, Histological, Forensic Medicine and Orthopedic Science, "La Sapienza" University of Rome, Rome: michela.rilucenti@uniroma1.it;

[4] Department of Psychology, Faculty of Medicine and Psychology "La Sapienza" University of Rome, Rome: claudio.barbaranelli@uniroma1.it;

[5] Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Dingjiaqiao 87, Nanjing, 210009: xiaobo.li@njit.edu.cn; chenrui@njit.edu.cn;

[6] UOSD of Neurophysiopathology and DISMOV, AOU G Martino, Department of Clinical and Experimental Medicine, University of Messina, Italy, Messina, Italy: santi.galletta@polime.it;

* Correspondence: placido.bruzzaniti@uniroma1.it ; Tel.:+393349753520(F.L.)

Abstract: Glioblastoma is a solid, infiltrating and the most frequent highly malignant primary brain tumor. Our aim was to find the prognostic value of mutations of IDH1, MGMT, EGFR, p53, ATRX, Ki67 genes and the correlation between sex, age, presenting with seizures, number of interventions, extent of resection with Overall Survival (OS), Progression Free Survival (PFS) and Karnofsky performance status (KPS) score. A randomized retrospective analysis of 122 patients treated by a single operator at Sapienza University of Rome, was carried out. After surgery patients followed standard treatment Stupp protocol [6]. Exclusion criteria were: patients with primitive brainstem and spinal cord gliomas and patients who underwent partial resections (resection < 90%) and cases of brain biopsy exclusively for diagnostic purposes. Statistical analysis with a simultaneous regression model was carried on by SPSS 25 ® (IBM) program. Results showed statistically significant survival increase in four groups: 1) patients treated with gross total resection ($p< 0.03$); 2) patients with methylated MGMT promoter ($p<0.005$); 3) patients with non

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

EGFR amplification or EGFRvIII mutation (p<0.035); 4) mutated IDH1/IDH2 (p<0.0161). Higher survival rates (but not statistically significant) were observed also in patients with: age < 75 years, Ki 67 <10%, lesions in non eloquent areas, ATRX gene mutation and presentation with seizures.

Keywords: glioblastoma multiforme; MGMT; IDH1; EGFR; P53; ATRX; Ki67; neurosurgery; oncology; epilepsy

1. Introduction

Glioblastoma (GBM) is a solid, infiltrating, highly malignant tumor (grade IV glioma according to the 2016 World Health Organization classification) [1]. It is believed that GBM is derived from a small population of cancer cells known as glioma stem cells (GSCs) and that these derive from the uncontrolled proliferation of neuronal stem cells (NSCs) residing in restricted germinal areas: ventricular subependymal zone of the temporal horn of the lateral ventricle (SVZ), the sub granular zone of the dentate gyrus of the hippocampus (SGZ) and of the white subcortical substance [2] and is the most frequent malignant primary brain tumor (16% of all primitives of the CNS and 54% of glial tumors). [3][4]. The average survival from diagnosis is less than 15 months, with survival rates at between 26-33% at 2 years and 3-10% at 5 years [5][6]. The development of GBM involves different molecular pathways between the primary and secondary lesions [8, 9]. In the literature the prognostic factors for the survival of patients with GBM are: patient's age[10][11], extent of surgical resection [10, 12, 13], performance status [10, 14], recursive partitioning analysis (RPA) class[10, 15], adjuvant treatment with chemotherapy and/or radiotherapy [6, 12, 14]. Sex influences survival only when combined with the methylation state of the MGMT promoter: women with a methylated phenotype have a higher OS than men with the same phenotype [15]. Standard treatment provides for maximum surgical resection followed by conformational radiotherapy (~60 Gy/30 fractions) for up to 6 weeks concomitant with temozolomide (75 mg/m2/day) and then maintenance therapy with standard temozolomide schedule (150-200 mg/m2 x 5 days, every 28, for 12 cycles) [6]. We used Levetiracetam as a preventive treatment for seizures, instead of valproate, as described by other authors [7]. The objective of this retrospective study is to clarify the influence and correlation of prognostic molecular and clinical factors on the survival and quality of life of GBM in a mono-operative series. The current survey continues and expands the work carried out in our department by Salvati et al. on YKL-40, an independent prognostic factor more specifically related to OS than MGMT promoter [17]. This study was carried out by avoiding biases like multi-operator casuistry and the lack of homogeneity of adjuvant treatments. The study also specifically investigated the independence or correlation of the variables examined by means of a multivariate analysis performed with a simultaneous regression model.

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

2. Results

The average OS and PFS of the population studied were 23.7 and 9.93 months respectively. Patients under 50 years of age had an OS of 22.76 months (STD 13.59), a PFS of 9.61 months (STD 9.83), a preoperative KPS of 83.07 (STD 23.93) and a postoperative KPS of 82.06 (STD 17.02); patients aged 51 to 75 years had an OS of 26.19 months (STD 21.74), a PFS of 11.11 (STD 13.71), a preoperative KPS of 84.4 (STD 11.48) and an average postoperative KPS of 78.26 (STD 17.6); patients over 75 years of age had an OS of 13.8 months (STD 6.41), a PFS of 4.4 months (STD 1.81), a preoperative KPS of 78 (STD 8.36) and a postoperative KPS of 84 (STD 5.47).

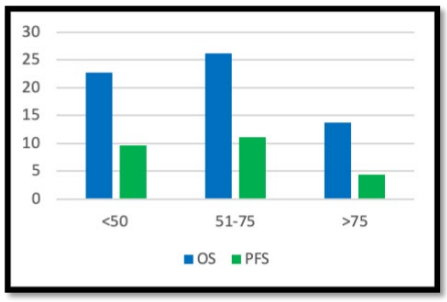

Figure 1. OS and PFS in age-related months

Patients with preoperative KPS greater than or equal to 80 points had an OS of 26.44 months (STD 20.31) and a PFS of 11.52 (STD 13.04); patients with preoperative KPS between 50 and 80 points had an OS of 13.25 months (STD 4.06) and a PFS of 4.62 months (STD 2.92); patients with preoperative KPS less than 50 points (only 1.32%) had an average OS of 15.66 months (STD 7.63) and a PFS of 4 months. Patients undergoing GTR had an average OS of 27.61 months (STD 20.38) and a PFS of 11.87 (STD 13.52). The control group underwent a STR with an OS of 14.38 months (STD 8.57) and a PFS of 5.3 months (STD 3.77). The Student T-test survey showed a statistically significant difference between the OS of the two groups (27.61 months vs. 14.38 months, p-value=0.03), while the difference in PFS was remarkable but not significant (11.87 months vs. 5.3 months, p-value=0.09).

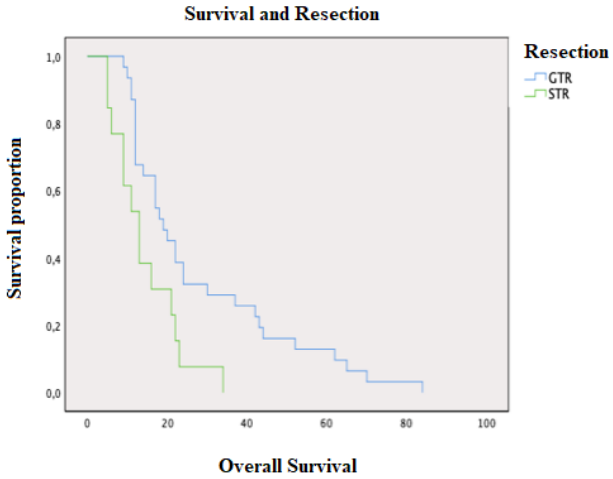

Figure 2. Survival of patients treated with GTR (in blue) and STR (in green).

Patients with MGMT methylation had an OS of 31.95 months (STD 5.19) while for patients without this methylation it was 16.83 months (STD 2.006), p-value=0.06. The mean preoperative KPS in patients with promoter methylation was 87.75 (STD 11.75), whereas in patients without methylation it was 79.78 (DS 18.12), p-value=0.10. The mean postoperative KPS in patients in the methylation

group was 77.5 (STD 19.15) whereas in the methylation-free group it was 82.29 (DS 13.98) p-value=0.34.

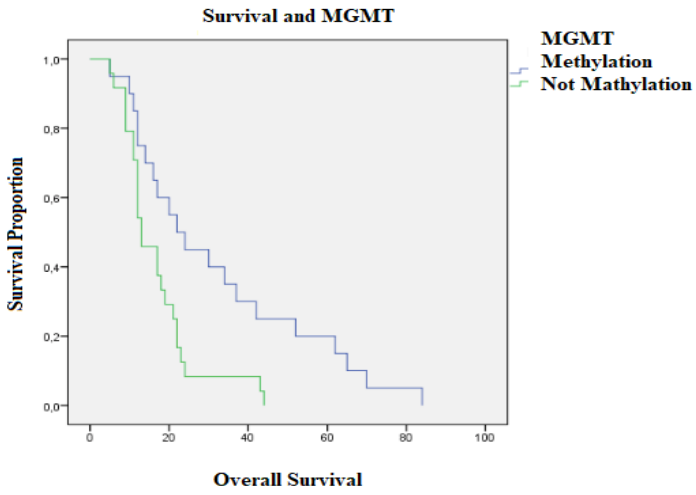

Figure 3. Survival of patients with methylation of the MGMT promoter (in blue) and patients without methylation (in green).

Patients with IDH1 wild type gliomas had an OS of 22.63 months (DS 17.67) while for the IDH1 mutated group it was 38.33 (STD 29.73), p-value=0.0161. PFS in the wild type group was 10.07 months (STD 12.25) vs 8 months in the mutated group (STD 4.0), p-value=0.77. Preoperative KPS for the wild type group was 83.5 (STD 16.06) while for the IDH1 mutated group it was 83.33 (STD 15.27). Postoperative KPS for the wild type group was 70 (STD 10), p-value=0.10. The sample with Ki-67≤10% had an average OS of 31.69 months (STD 19.17), an average PFS of 13.15 months (STD 13.52), preoperative KPS of 90 (STD 11.28) and post-operative KPS of 76.92 (STD 22.13); in patients with 10%<Ki-67≤20% an average OS of 28.46 months (STD 24.68), an average PFS of 10.93 months (STD 13.02), preoperative KPS 79 (STD 21.23) and postoperative KPS 75.66 (STD 24.70); the population with a Ki-67>20% average OS of 16.33 months (STD 11.89), a PFS 7.26 months (STD 8.95), preoperative KPS 84.33 (STD 12.08) and postoperative KPS 81.33 (STD 9.90). Comparing the group with Ki-67≤10% and the group with Ki-67>20% we observed an OS of 31.69 vs 16.33 months respectively with p-value=0.021.

Patients with EGFR amplification/EGFRvIII mutation had a mean OS of 19.83 months (STD 16.31) vs 32 months (STD 21.15) of the control group with p-value=0.035. The mean PFS in the EGFR amplification/EGFRvIII mutation group was of 7.4 months (STD 7.84) whereas in the control group it is of 15.75 (STD 16.79) p-value=0.055. Preoperative KPS was 81.5 (STD 17.27) in the EGFR amplification/EGFRvIII mutation group vs 88.07 (STD 11.08) while average postoperative KPS was 80.5 (STD 12.94) vs 77.14 (STD 22.97).

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

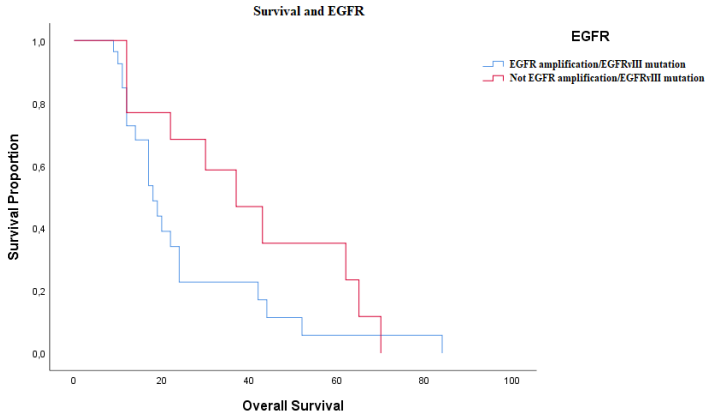

Figure 4. Survival of patients with EGFR amplification/EGFRvIII mutation (in blue) and patients without EGFR amplification/EGFRvIII mutation (in red).

Patients with ATRX loss had an OS of 30.75 months (STD 29.62) while patients without mutation had an OS of 22.13 months (STD 15.42) p-value=0.24. The PFS of the group with the ATRX loss was 14.12 (STD 17.51) while in the control group it was 9 (STD 10.33) p-value=0.27. The group of patients with ATRX loss had a preoperative KPS of 82.5 (STD 11.64) vs a KPS of 83.71 (STD 16.77) in the normal type, p=0.84. The ATRX loss group had a postoperative KPS of 76.25 (STD 16.85) vs a KPS of 80.97 (STD 16.55) of the wild type ATRX group.

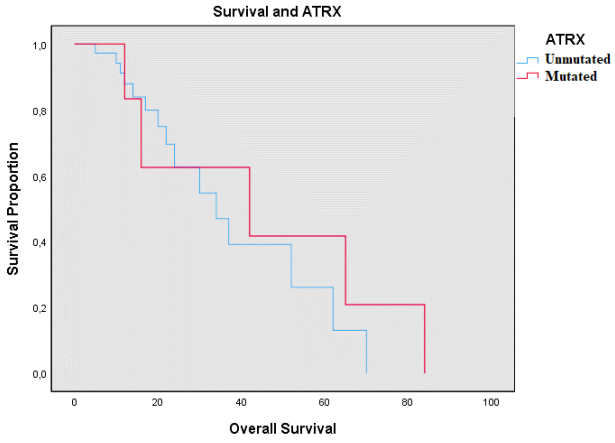

Figure 7. Survival of patients with ATRX loss (in blue) and patients without ATRX loss (in red).

Half of our population started with epileptic seizures. The mean age of patients who started with seizures was 52.4 years (STD 12.58), OS 26.36 months (STD 22.05) vs 21.04 (STD 14.52) with p-value=0.35 and a mean PFS of 8.86 months (STD 11.56) vs 11 (STD 12.33) in the control group. Preoperative KPS in patients with seizures was 81.13 (STD 19.87) vs 85.95 (STD 9.95) of the controls with a p-value=0.32. Postoperative KPS in patients with seizures was 80.22 (STD 18.15) vs 80 (STD 15.11) of the controls and a p-value=0.96.

The multivariate analysis showed that more than 50% of the OS of our population depended on the variables examined (R2=0.496, F(9,34) = 3.723, p=.002). The number of months between the first procedure and the recurrence of disease was significantly associated with OS (B = .313, t(42)= 3.213, p=.003). The percentage of Ki-67 showed an association with OS tending to statistical significance (B = -.025, t(42)= -1.816, p=.078). The independent variables examined as a whole also statistically significantly correlated for about 50% with PFS (R2=0.496, F(9,32) = 3.493, p=0.004).

3. Discussion

We examined, in a consecutive series of 122 GBM patients, surgically treated from 2013 to 2017 at Sapienza University of Rome, the prognostic value and the existing correlation between sex, age, preoperative KPS, extent of resection, presentation with seizures and number of interventions with OS, PFS and postoperative KPS.

Our results showed that patients' sex does not affect prognosis. OS and PFS were instead significantly higher in the group of patients younger than 75 years of age. Age ≥ 75 years was an independent negative prognostic factor. The group of patients with preoperative high KPS (KPS ≥ 80) showed a significantly better prognosis. The GTR group had significantly higher OS and PFS than the STR group, in line with current literature [27]. Patients with lesions in eloquent areas were treated with STR in order to avoid neurological deficits resulting in a postoperative KPS and quality of life worsening (figure 8). In our series, none of the patients in the STR group exceeded 36 months of OS, while in the group of patients treated with GTR there were survival rates of up to 85 months (none of the patients was treated with GTR in re-intervention after being treated with STR, as reported by Block O et al. [28]). When STR was performed, residual disease volume seemed to be the most important factor influencing OS and PFS [30], being - in our series - an independent prognostic factor. We did not find any difference in terms of survival in patients with residual tumor when the extent of resection exceeds 90%.

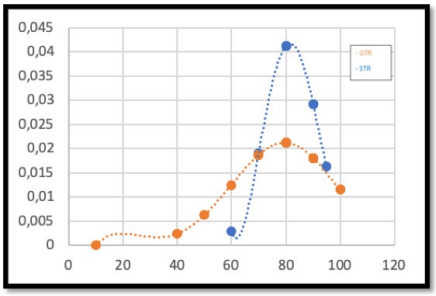

Figure 8. Distribution, in relation to KPS, of patients treated with STR (in blue) and patients treated with GTR (in orange).

Another factor influencing OS and PFS appeared to be the presence of seizures. OS and PFS were, in fact, higher in the group with seizures than in the group without seizures.[32-33] This finding might be related to the possibility of an early diagnosis, being more frequent in cortical lesions and mutated IDH1 gliomas. The mutation of IDH1 leads, as is well known, to the formation of 2-HG (2-hydroxyglutarate) which has a molecular structure similar to glutamate and is able to bind and activate N-methyl-D-aspartate receptors (NMDA) thus resulting in a possible reduction in seizure threshold (this mutation is associated with about 70-88% of low-grade gliomas and these are therefore more epileptyc than high-grade gliomas). Antiepileptic therapy could have a sensitizing role in chemotherapy treatment, Vecth et al [32]. Recently, it has been found that combining valproic acid (VPA) with TMZ leads to improved survival of patients with GBM as well as children with brain tumors. This could possibly be explained by the chemotherapy-sensitizing properties of VPA, including the inhibition of histone deacetylase, leading to improved survival.[32]

We also evaluated the prognostic value of MGMT gene promoter methylation, ATRX gene mutation, IDH1/ID2 gene mutations and EGFR amplification.
MGMT promoter mutation revealed to be an independent positive prognostic factor for OS and PFS, but not a predictive factor for postoperative KPS: it is the molecular marker that correlated the most with survival. The same phenotype could also be induced by ATRX loss that, in our study,

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

was associated with an increase in survival but without statistical significance.
Mutation of the IDH1/IDH2 gene was found in less than 10 % of the sample and is associated with a significant increase in OS and PFS but does not statistically significantly affect postoperative KPS. This finding is in agreement with the Hai Yan report [34].
EGFR amplification/EGFRvIII mutation was - in our population - a negative independent prognostic factor for survival, presenting a trend towards statistical significance for PFS too (this did not correlate, however, with postoperative KPS). Nonetheless, no improvement in survival was found with the EGFRvIII Rindopepimut® vaccine [21] while the addition of Nimotuzumab® to the standard therapy yielded results only in a post hoc analysis where it revealed an improvement in survival in patients with residual tumor and unmethylated GMT (PFS 6.2 vs 4 months; OS 19 vs 13.8 months). This could be due to receptor interference and associations with multiple transduction pathways and proteins of invasion and angiogenesis regulation and the development of resistance mechanisms. Therefore, new therapies are focused on a combination of targeted gene therapy against EGFR and EGFRvIII and transduction pathways and proteins related to this pathway [22]. Recently new EGFR-targeted therapies have been proposed (e.g. depatuximab, mafodotin, depatuximab), which completed Phase I in a study with recurrent GBM patients with EGFR amplification and entered Phase III in the RTOG 3508 trial [23] as an adjunct therapy to standard therapy. In addition, a phase I study with T cells activated with a chimerical antigen against EGFRvIII shows good treatment tolerance and encouraging results [23, 24].

The number of months between tumour removal and disease recurrence was, however, the independent variable most specifically related to OS (B = .313, t(42)= 3,213, p=.003) and was also related to post-operative KPS. This parameter summarizes the validity of the treatment and can be influenced by the presence of tumor residue [31] and resistance to treatment with temozolomide [20].

4. Materials and methods

A randomized retrospective analysis was performed on 122 patients with histological diagnosis of supra-tentorial GBM, treated from January 2013 to December 2017 at the Department of Neurosurgery of Sapienza University of Rome. Preoperative study included an objective neurological examination with determination of the KPS score and a radiological study performed with MRI 3T after administration of gadolinium with the integration of sequences in DWI, PWI and spectroscopy. In case of patients with a lesion in eloquent area, a functional MRI was performed. The extension of resection was determined by comparing the MRI images with contrast agent, acquired within 24 hours of surgical treatment, with the preoperative ones, and calculated with the ABC/2 technique. All patients performed an antiepileptic prophylaxis: during the induction was administered Levetiracetam 1000 mg subsequently treated with maintenance therapy with 500mg twice daily for 6 months.

Patients characteristics

Characteristics of patient population are summarised in Table 1.
The age was between 31 and 82 years with an average of 56.3 years. Our population consisted of 76 males and 46 females. 77.27% of the population had a preoperative KPS greater than or equal to 80 points; this group had an average age of 55.61 years, 18.18% of the population had a preoperative KPS less than 80 points and greater than or equal to 50 with an average age of 59.37 years, only 1.32% of patients had a preoperative KPS less than 50 points with an average age of 64.66 years. Half of our population presented with

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

seizures, treated with antiepileptic therapy. The average age of the group with epileptic seizures was 52.4 years.

Table 1. Patients' characteristics.

Variable		N(%)
Age (years)		
	≤50	29,54
	51-75	59,1
	>75	11,36
Sex		
	Male	59,01
	Female	40,99
Preoperatory KPS		
	≤50	1,32
	50-79	18,18
	>80	77,27

Characteristics of the tumor

59.09% of the tumors were located in the left hemisphere with the following sites in order of frequency: frontal lobe (45.45%), temporal lobe (25.03%), parieto-occipital (15.9%) and parieto-insular-occipital (2.27%). 34.09% of patients presented the involvement of eloquent areas.
The molecular characteristics are shown in table 2. The analysis of DNA methylation was performed by PCR or Southern Blotting, whereas the status of IDH and ATRX and the expression of EGFR, p53 and Ki-67 were evaluated with immunohistochemical technique. 45.44% of patients presented methylation of MGMT promoter. 91.18% of the sample had a wild type IDH1 status. In 59.08% of the cases there was EGFR over-expression. P53 was not expressed in 36.36% of the cases, over-expressed in 27.2% and focally expressed in 13.6%. In 36% of patients there was ATRX loss.

Treatment characteristics

Surgery was performed by the same first operator with the aid of the following: neuronavigation system, intraoperative ultrasound, ultrasound aspirator (CUSA-CAVITRON), thulium laser and intraoperative neurophysiological monitoring. 19.40% of the procedures was conducted in awake surgery so as to monitor, real-time, the functions of the patient during surgery in eloquent areas. GTR was performed in 85.95% of cases, while STR in the residual 14.05%. Partial resections (resection < 90%) and biopsies were not included. Our follow-up consisted of radiological evaluation through brain MRI

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

with gadolinium 20 days after surgery and subsequent clinical re-evaluation. Cases of recurrence were also treated in our department: 66.54% of patients underwent a second procedure, and 24.95% underwent three.

Table 2. Molecular characteristics

Variable		%
MGMT methylated		45,44
MGMT not methylated		54,56
IDH wilde type		91,18
IDH not changed		8,82
EGFR Over-expressed		59,08
P53 not expressed		36,36
P53 Hyper-expressed		27,2
P53 expressed focally		13,6
ATRX mutated		36,01
Ki-67		
	0-10	29,54
	10-20	34,1
	>20	36,36

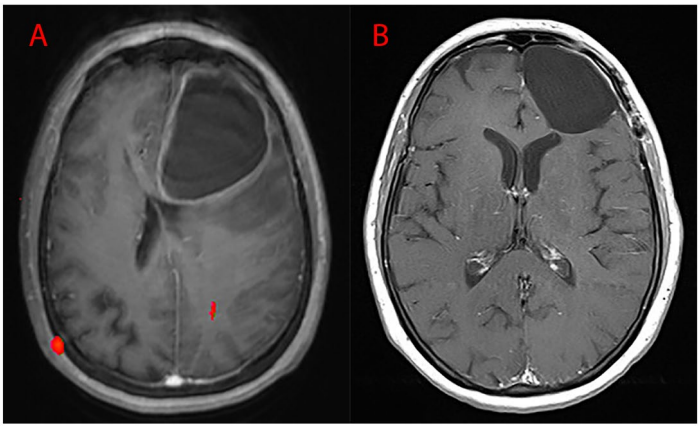

Figure 9. MRI T1WI axial section. (**A**) preoperative left parieto-occipital Glioblastoma (**B**) post-operative images (absence of residual disease-GTR).

Peer-reviewed version available at Brain Sci. 2020, 10, 91; doi:10.3390/brainsci10020091

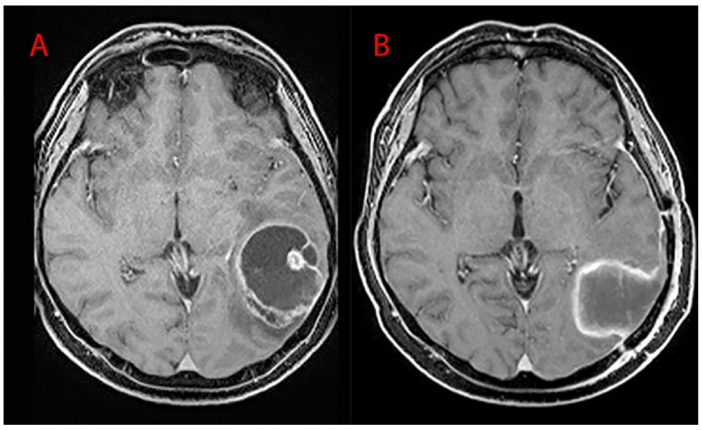

Figure 10. MRI T1WI axial section. (**A**) preoperative right frontal Glioblastoma (**B**) post operative images (extent resection 90-100%, STR).

Statistical analysis

Univariate analysis for KPS, MGMT, IDH1, EGFR, p53, Ki67, GTR/STR, eloquent/non-eloquent areas, presence of group crises, sex and age compared to OS, was conducted with Kaplan Meier curves and compared with log rank tests, with X2 or t-student tests depending on the variables taken into account. Multivariate statistical analysis was carried out with bootstrap regression, conducting different regression analyses on the data. The independent variables used are: Age, preoperative KPS, resection (GTR or STR), EGFR over-expression, months between surgery I and II, sex, p53 mutation, methylation of the MGMT gene promoter and percentage of Ki-67. The dependent variables are: OS, PFS and postoperative KPS. The so-called "enter" method has been used in all regressions: it corresponds to a simultaneous regression model in which all the independent variables are simultaneously introduced into the regression equation [18]. The latter was conducted using the IBM® SPSS 25 statistics software.

5. Conclusions

According to our results, Gross Total Resection is the only treatmen which can allow more than 36 months survival in patients with GBM. GTR is sometimes associated, however, with post-operative KPS < 60. MGMT gene promoter methylation is an independent positive prognostic factor for OS and PFS. IDH1 mutation, present in less than 10% of the population studied, is a positive prognostic factor for OS and PFS. Patients with Ki-67 > 20% have a lower OS than the rest of the population. EGFR amplification/EGFRvIII mutation is a negative prognostic factor for OS but the clinical trials performed so far have not been significantly effective. Multivariate data analysis showed that more than 50% of OS and PSF in the population depend on the variables examined, while there is less correlation with post-operative KPS. The interval in months between removal and recurrence of disease summarizes the efficacy of treatment and is the parameter that most clearly correlates with OS and KPS. Further prospective studies and clinical trials are necessary to evaluate, especially in patients without methylation of the MGMT promoter, the efficacy of therapies against EGFR mutations and their combination to stop this pathway at different levels. Treatment of glioblastoma should be targeted according to molecular features.

Author Contributions: Conceptualization, M.S., P.B. and A.S.; Methodology, P.B., A.F. and M.G.; Software, C.B. and P.B.; Validation, C.B., M.S. and A.K.S.; Formal Analysis, M.R.; Investigation, M.N. and P.B.; Resources, M.N.; Data Curation, C.B.; Writing – Original Draft Preparation, M.N. and A.F.; Writing – Review & Editing, M.N., A.K.S. and S.G.; Visualization, X.L. and R.C.; Supervision, M.S., A.S. and P.F.; Project Administration, M.G., S.G., X.L. and R.C.;.",

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ACD	Apparent diffusion coefficient
ATRX	Adult thalassemia mental retardation x-linked
CBV	Cerebral blood volume
CSC	Cancer stem cell
DCS	Direct cortical stimulation
DWI	diffusion weighted imaging
EGFR	Epidermal growth factor receptor
GF	Growth factor
GTR	Gross total resection
ICP	Intra cranic pressure
IDH	Isocitrate dehydrogenases
ITH	Intra-tumor heterogeneity
KPS	Karnofsky performance status
MGMT	O-6-methylguanine DNA methyltransferase
MTT	Mean Transit Time
NSC	neural stem cell
OS	Overall Survival
PFS	Progression Free Survival
PWI	perfusion-weighted imaging
RTK	receptor tyrosine kinases
STD	Standard error
STR	Sub-total resection

VPA	valproic acid
TMZ	Temozolomide

References

1. Perry, A. & Wesseling, P. Histologic classification of gliomas. Handbook of Clinical Neurology vol. 134 (Elsevier B.V., 2016).

2. Ryskalin, L. et al. The autophagy status of cancer stem cells in gliobastoma multiforme: From cancer promotion to therapeutic strategies. Int. J. Mol. Sci. 20, (2019).

3. Louis, D.N., et al., The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol, 2016. 131(6): p. 803-20

4. Ostrom, Q.T., et al., CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011. Neuro Oncol, 2014. 16 Suppl 4: p. iv1-63.

5. Gilbert, M.R., et al., Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol, 2013. 31(32): p. 4085-91.

6. Stupp, R., et al., Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med, 2005. 352(10): p. 987-96.

7. Kerkhof, M. et al. Effect of valproic acid on seizure control and on survival in patients with glioblastoma multiforme. Neuro. Oncol. 15, 961–967 (2013).

8. Ohgaki, H. and P. Kleihues, Genetic pathways to primary and secondary glioblastoma. Am J Pathol, 2007. 170(5): p. 1445-53.

9. Mansouri, A., J. Karamchandani, and S. Das, Molecular Genetics of Secondary Glioblastoma, in Glioblastoma, S. De Vleeschouwer, Editor. 2017: Brisbane (AU).

10. Lamborn, K.R., S.M. Chang, and M.D. Prados, Prognostic factors for survival of patients with glioblastoma: recursive partitioning analysis. Neuro Oncol, 2004. 6(3): p. 227-35.

11. Crocetti, E., et al., Epidemiology of glial and non-glial brain tumours in Europe. Eur J Cancer, 2012. 48(10): p. 1532-42.

12. Marina, O., et al., Treatment outcomes for patients with glioblastoma multiforme and a low Karnofsky Performance Scale score on presentation to a tertiary care institution. Clinical article. J Neurosurg, 2011. 115(2): p. 220-9.

13. Lacroix, M., et al., A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J Neurosurg, 2001. 95(2): p. 190-8.

14. Gorlia, T., et al., Nomograms for predicting survival of patients with newly diagnosed glioblastoma: prognostic factor analysis of EORTC and NCIC trial 26981-22981/CE.3. Lancet Oncol, 2008. 9(1): p. 29-38.

15. Hegi, M.E., et al., MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med, 2005. 352(10): p. 997-1003.

16. Franceschi, E., et al., The Prognostic Roles of Gender and O6-Methylguanine-DNA Methyltransferase Methylation Status in Glioblastoma Patients: The Female Power. World Neurosurg, 2018. 112: p. e342-e347.

17. Salvati, M., et al., Extent of tumor removal and molecular markers in cerebral glioblastoma: a combined prognostic factors study in a surgical series of 105 patients. J Neurosurg, 2012. 117(2): p. 204-11.

18. Tabachnick, B.G. and L.S. Fidell, Using multivariate statistics. 6th ed. 2013, Boston: Pearson Education. xxxi, 983 p.

19. Yong, R.L., et al., Residual tumor volume and patient survival following reoperation for recurrent glioblastoma. J Neurosurg, 2014. 121(4): p. 802-9.

20. Jiapaer, S., et al., Potential Strategies Overcoming the Temozolomide Resistance for Glioblastoma. Neurol Med Chir (Tokyo), 2018. 58(10): p. 405-421.

21. Weller, M., et al., Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol, 2017. 18(10): p. 1373-1385.

22. Keller, S. and M.H.H. Schmidt, EGFR and EGFRvIII Promote Angiogenesis and Cell Invasion in Glioblastoma: Combination Therapies for an Effective Treatment. Int J Mol Sci, 2017. 18(6).

23. Gan, H.K., et al., Safety, pharmacokinetics, and antitumor response of depatuxizumab mafodotin as monotherapy or in combination with temozolomide in patients with glioblastoma. Neuro Oncol, 2018. 20(6): p. 838-847.

24. Reardon, D.A., et al., Efficacy and safety results of ABT-414 in combination with radiation and temozolomide in newly diagnosed glioblastoma. Neuro Oncol, 2017. 19(7): p. 965-975.

25. Morgan, R.A., et al., Recognition of glioma stem cells by genetically modified T cells targeting EGFRvIII and development of adoptive cell therapy for glioma. Hum Gene Ther, 2012. 23(10): p. 1043-53.

26. Badhiwala, J., et al., Clinical trials in cellular immunotherapy for brain/CNS tumors. Expert Rev Neurother, 2013. 13(4): p. 405-24.

27. Brown, T. J. et al. Association of the extent of resection with survival in glioblastoma: a systematic review and meta-Analysis. JAMA Oncol. 2, 1460–1469 (2016).

28. Bloch, O. et al. Impact of extent of resection for recurrent glioblastoma on overall survival: Clinical article. J. Neurosurg. 117, 1032–1038 (2012).

29. Kos, I. et al. Mutations in Gliomas (2009) N Engl J Med 2009;360:765-73.

30. Li, Y. M., Suki, D., Hess, K. & Sawaya, R. The influence of maximum safe resection of glioblastoma on survival in 1229 patients: Can we do better than gross-total resection? J. Neurosurg. 124, 977–988 (2016).

31. Yong, R. L. et al. Residual tumor volume and patient survival following reoperation for recurrent glioblastoma. J. Neurosurg. 121, 802–809 (2014).

32. Vecht, C. J., Kerkhof, M. & Duran-Pena, A. Seizure Prognosis in Brain Tumors: New Insights and Evidence-Based Management. Oncologist 19, 751–759 (2014).

33. Hague, T. Seizure Prognosis in Brain Tumors : New Insights and Evidence-Based Management. 751–759 (2014).

34. Waitkus, M. S., et al. (2018). "Biological Role and Therapeutic Potential of IDH Mutations in Cancer." Cancer Cell **34**(2): 186-195.