A concurrent ultra-fractionated radiation therapy and temozolomide treatment; a promising therapy for newly diagnosed, inoperable glioblastoma

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A CONCURRENT ULTRA-FRACTIONATED RADIATION THERAPY AND TEMOZOLOMIDE TREATMENT; A PROMISING THERAPY FOR NEWLY DIAGNOSED, INOPERABLE GLIOBLASTOMA.

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Running Head: Ultra fractionated radiotherapy plus temozolomide for de novo Glioblastoma unresectable.

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We report on a phase II clinical trial to determine the effect of a concurrent ultra-fractionated radiotherapy and temozolomide treatment in inoperable glioblastoma patients. A phase II study opened; patients over 18 years of age who were able to give informed consent and had histologically proven, newly diagnosed inoperable diagnosed and supratentorial glioblastoma were eligible. Three doses of 0.75 Gy spaced apart by at least four hours were delivered daily, five days a week for six consecutive weeks for a total of 67.5Gy. Chemotherapy was administered during the same period, which consisted of temozolomide given at a dose of 75 mg/m2, for seven days a week. After a four-week break, chemotherapy was resumed for up to six cycles of adjuvant temozolomide treatment, given every 28 days, according to the standard five-day regimen. Tolerance and toxicity were the primary endpoints; survival and progression-free survival were the secondary endpoints. In total 40 patients were enrolled in this study, 31 men and 9 women. The median age was 58 years, and the median Karnofsky performance status was 80. The concomitant ultra fractionated radiotherapy and temozolomide treatment was well tolerated. Complete responses were seen in four patients, and partial responses were reported in seven patients. The median survival from the initial diagnosis was 16 months. Several long-term survivors were noted. Concurrent ultra-fractionated radiation therapy and temozolomide treatment is well accepted by the patients. The results showed encouraging survival rates for these unfavorable patients.

KEY WORDS : Glioblastoma - Inoperable,
Low doses - Radiation therapy, Ultrafractionated regimen.

INTRODUCTION

Glioblastoma (GBM) is the most common primary brain tumor in adults and is characterized by a high rate of local recurrences because of its intrinsic radio resistance[1-4]. Indeed, GBM is considered one of the most radio resistant tumors[1-4]. After maximal surgical tumor resection as safe as possible, the current standard of care is based on a phase III randomized trial from the EORTC / NCIC[1-5]. This treatment comprises a concurrent combination of conformational brain radiotherapy (RT) and chemotherapy using temozolomide (TMZ), followed by a four-week break and adjuvant chemotherapy with TMZ for up to six cycles[1-5]. Despite the improvement in outcome with the new standard regimen, the median overall survival (OS) does not exceed 15 months; therefore, new therapeutic strategies are needed[1-5].

Conformational RT remains the backbone of care for GBM. Although RT is not a curative treatment for GBM, it results in a longer survival rate and optimized quality of life[6]. It is unclear whether clinical radio resistance in GBM is a result of intrinsic resistance at the cellular level. The mechanisms involved in radiation resistance in mammalian cells are more complex than once believed[7]. *In vitro* studies have shown that some human tumor cell lines are sensitive to low radiation doses of <1 Gy, a phenomenon that has been termed low-dose hypersensitivity (HRS)[8-17]. Strikingly, this "radio-sensitivity" is more apparent in radio resistant cell lines, such as glioma cells[8-17]. We demonstrated this phenomenon in a number of various human malignant glioma cell lines using a common clinical device for

irradiation[7,17]. Daily repeated irradiation of cells with low doses compared with irradiation with a single biologically equivalent dose resulted in significantly higher cell death (using a clonogenic assay)[7,17]. Experiments conducted on glioma xenografts revealed that repeated irradiation with low doses (0.8 Gy, three times a day) is more effective than a single dose (2 or 2.4 Gy, once a day) in inhibiting tumor growth[7,17].

Consequently, in 2003 we began a phase II study testing an ultra fractionated RT for inoperable *de novo* GBM[18]. The results were promising, and are comparable with the results using the TMZ/RT treatment from the EORTC/NCIC trial. However, in the ultra-fractionated RT trial, there were only a few long-term survivors that was unexpected since these types of patients have an unfavorable prognosis (a survival expected at least 10 months), the rate of two-years survival was 15.48%[18]. These data suggested that the combination of ultra fractionated radiation therapy and concomitant and adjuvant TMZ chemotherapy (combination radiotherapy and chemotherapy is the standard) should be more efficient**.** Here, we report the results of a second phase II trial that tested a concurrent combination of ultra fractionated brain irradiation (three-daily doses – five times a week for six consecutive weeks) and TMZ treatment followed by adjuvant TMZ therapy, in *de novo* inoperable GBM patients.

MATERIAL AND METHODS

PATIENTS: This phase II study was conducted in eight French centers. Patients were eligible for the study if they were at least 18 years old and had newly diagnosed, inoperable supratentorial GBM (based on neurosurgical criteria such as Rolando and/or callosum corpus or deep locations of tumor) that was histologically confirmed (astrocytoma grade IV according to the WHO classification). Additional inclusion criteria were a WHO performance status of 0–2; adequate hematologic; hepatic, and renal function; acceptable blood coagulation levels; and ability to give informed consent. Patients who had undergone a partial or complete tumor resection were not eligible.

TREATMENT: The RT regimen consisted of ultra-fractionated focal irradiation, with three daily doses of 0.75 Gy delivered at least four hours apart. Irradiation of the tumors was performed five days a week (Monday through Friday), for six consecutive weeks, resulting in 90 fractions and a total of 67.5 Gy of radiation. Irradiation was delivered to the gross tumor volume with a 2.5 cm margin for the clinical target volume. RT was planned with dedicated computed tomography or magnetic resonance imaging (MRI) and three-dimensional planning systems; conformal ultra-fractionated RT was delivered with linear accelerators with a nominal energy $\geq$ 6 MeV. The patients were treated with thermo-plastic immobilization masks to ensure adequate immobilization and reproducibility. Chemotherapy consisted of TMZ treatment at a dose of 75 mg/m^2/day, given seven days a week during the ultra-fractionated RT. After a four-week break, TMZ chemotherapy was resumed at 150-200 mg/m^2/day, for up to six cycles every 28 days, in accordance with the EORTC trial.

PATIENT EVALUATION: Patients were assessed weekly for tolerance and toxicity during the RT. The baseline examination included a cranial MRI (with and without contrast), physical and neurologic examinations, Mini-Mental-Status score (MMS) and a quality of life questionnaire (EORTC—QLQ-C30, Brain Cancer Module BN-20). A baseline examination was performed at the end of the RT regimen (within the first 10 days after completion of the ultra-fractionated irradiation) and then every two months until death. The first MRI (at the end of RT) was the baseline imaging used to evaluate the tumor response, keeping in mind that RT artifacts could be present and should be considered in the interpretation of the MRI. Tumor progression was defined according to the modified WHO criteria (Macdonald criteria) as a 25 % increase in tumor size (size of the product of the largest perpendicular diameters of the contrast-enhanced tumor), the appearance of new lesions, or an increased need for corticosteroids[19]. When tumor progression was found, patients were treated at the investigator's discretion, and the type of subsequent therapy (usually chemotherapy) was recorded.

MGMT ANALYSIS: DNA was extracted from FFPE samples, directly followed by bisulfite conversion using the EpiTect Fast Bisulfite Conversion Kit. Pyrosequencing was performed with the PyroMark Q96 MGMT kit on a PSQTM96 MA system, as previously described[20]. All the reagents were from Qiagen; Courtaboeuf, France. For data analysis, the average percentage of the five CpGs was determined and the cutoff set à 8% [20]

STATISTICAL METHODS: The primary end points of the study were to document the treatment-related toxicity and tolerance of all patients treated with this novel regimen. The secondary end points were the progression-free survival (PFS) and OS reported as an intent-to-treat analysis on all 40 patients included. Survival times were calculated from the date of the initial diagnosis (date of stereotactic biopsy) to the date of death, progression, or last follow-up. The Kaplan–Meier technique was used to compute the estimates for PFS and OS parameters and their 95% confidence intervals (CI). SPSS statistical software (SPSS, Inc.) was used for the primary analyses. SAS v 9.1.3 (SAS Institute, Inc.) was the statistical software used by the EORTC for the survival analyses. To estimate the efficacy of the ultra-fractionationed therapy (TEMOFRAC) on patients, we compared our results with the subgroup of patients that underwent only a biopsy and who were treated within the EORTC/NCIC 26981-22981/CE.3 trial. This randomized trial established the combination of standard RT and concomitant treatment and maintenance with temozolomide chemotherapy (TMZ/RT) compared with once daily fractionated RT alone. A Kaplan-Meier curve, log-rank test, and Cox regression were used at an exploratory 5% significance to assess the effects of TEMOFRAC compared to RT or TMZ/RT, with and without adjustment for possible confounding effects. Available factors were age and WHO performance status. MMSE scores were collected in only about half of the patients and were not included. MGMT data were missing in 91% of the cases for each arm of EORTC/NCIC trial. Adjusted hazard ratios (HR) were computed with 95% CI. Survival analyses were performed in the intent-to-treat population. P-values in figures are from unadjusted analyses, and adjusted values are given in the text.

RESULTS

PATIENT CHARACTERISTICS: From July 2008 until July 2011, 40 patients were enrolled in this phase II study; there were 29 males and 11 females. Five patients were diagnosed with multifocal GBM. Three sudden deaths (probably due to pulmonary embolism or myocardial infarction) and three deaths unrelated to GBM (two pulmonary infections and one grade 4 hematological toxicity with severe sepsis) were reported in our series, so, thirty-four patients were finally included for the analysis (Table 1). The median age of the population was 59 years old, and ranged from 29.1 - 73.5, 14 patients were aged from > 50 to ≤ 60, 18 were > 60, and 10 were ≥ 70 years old. Twenty-six patients had a performance status of ≤ 1, and 14 patients had a performance status of 2.

TREATMENT DELIVERY SAFETY AND TOLERABILITY: All of the 40 patients underwent and completed the ultra-fractionated irradiation and TMZ treatment. No disruptions in the concomitant chemotherapy were reported. The treatment was delivered on an inpatient basis, five days of hospitalization per week for six consecutive weeks. Although this ultra-fractionated irradiation could have cause side effects, this regimen was well tolerated by the patients. The most common adverse event was fatigue, which is usually noted in standard cranial RT. The main adverse effects reported were:

- Fatigue, grade II in 30 patients,
- Alopecia, grade II in 20 patients,
- Skin reaction, grade I in 10 patients,

- Headache, grade I in 6 patients,
- Nausea and seizures were not reported

TMZ was administered concomitantly in all of the patients, and adjuvant chemotherapy (six cycles of TMZ) was completed in 26 patients (76.4%). Two patients developed a pulmonary infection and one patient presented grade 4 hematological toxicity; these were all fatal (Table 2**)**.

MGMT STATUS: The MGMT promoter analysis was only performed in 23 patients; the data were missing in the remaining 11 cases. The MGMT promoter was found methylated in 12 of the patients (52.2%), and unmethylated in the last 11 patients (47.8%). Due to some data missing (32 %), no statistical analysis was allowed. In the case of the EORTC/NCIC trial, MGMT data were documented in less than 10% of the cases. However, we noted a high rate of longer OS within the patients with a methylation of MGMT promoter (8 patients of 12 with MGMT methylated had a OS $\geq$ 19 months).

EFFICACY OUTCOMES: The median follow-up of this trial was four years. Three of the patients are currently still alive (8.8%), and 31 patients are dead (91,2%). The median OS was 16 months (15.92; 95% CI 9.7-22.6) in the analyzed population; the two-year survival rate was 32.4% (95% CI 17.6%-48.0%), the three-year 17.2% (95% CI 6.7%-31.6%) and the four-year rate was 9.2 % (95% CI 2.0%-23.3%). The Median PFS was 9.6 months (95 % CI 7.2-12.12), and the PFS rate at six months was 76.5% (95% CI 2.0%-23.3%). The tumor response was analyzed; four complete responses were reported, and seven partial responses were noted. Three patients progressed during the irradiation schedule. The quality of life questionnaire (EORTC—

QLQ-C30, Brain Cancer Module BN-20) was completed by only a minority of the patients; therefore, we did not pursue any further analysis. When tumor progression was observed, the patients were treated at the physician's discretion. The response to salvage therapy was not recorded, but an association, including bevacizumab agent, was diffusely used.

COMPARISON WITH THE EORTC/NCIC TRIAL: We compared our results with those obtained during the EORTC/NCIC trial on patients who only had a biopsy. The RT arm included 45 patients, the TMZ/RT arm had 48 patients and the TEMOFRAC group from this study included 34 patients. The median and two-year OS for the RT arm and the RT/TMZ arm of the EORTC/NCIC trial were 8.7 months (95% CI 6.3-11.0) and 4.6% (95% CI 0.8%-13.7%) and 10.2 months (95% CI 7.3-14.1) and 10.4% (95% CI 3.8%-20.9%), respectively (Table 3 and 4**).** The median PFS in the RT group and RT/TMZ group was 5.0 (95% 3.2-5.9) and 6.0 (95% CI 5.0%-8.8%) months respectively.

- TEMOFRAC versus EORTC/NCIC RT: In the PFS and OS analyses, TEMOFRAC showed a significant difference for an improved outcome over EORTC/NCIC RT (adjusted PFS: $p < .0001$, HR 0.46 (95% CI 0.34 – 0.61) and adjusted OS: $p = 0.0002$ HR 0.62 (95% CI 0.48–0.80) (Fig.1).

- TEMOFRAC versus EORTC/NCIC RT/TMZ: Again, an improvement in the outcome for PFS and OS was reported for TEMOFRAC versus RT/TMZ (adjusted PFS: $p = 0.047$, HR 0.62 (95% CI 0.39–0.99) and adjusted OS $p = 0.0184$, HR 0.57 (95% CI 0.35–0.91) (Fig. 2).

DISCUSSION

RT remains the standard of care for GBM and has an undisputed major benefit on survival[5,21-23]. Currently, concomitant and adjuvant TMZ chemotherapy during RT is the standard of care for adult GBM patients aged up to 70 years old and in good general and neurological condition; however, the OS for unresected GBM (biopsy) remains low, approximately 10 months[5]. Despite their high inherent radio-resistance, and survival fraction at 2 Gy, GBM tumors receive the same dose per fraction, similar total dose and equivalent overall duration of RT as others tumors considered less radiosensitive, such as breast tumors[21-23]. In the past decade, many drugs have been developed to improve the outcome of GBM patients, but novel approaches to the RT regimen have been ignored, except for the development of the ballistic and intensity-modulation radiation therapy techniques (IMRT)[21-24]. In the past, alternative regimens of radiotherapy utilizing fractionation were proposed based on the hypothesis that radiation therapy could be improved by increasing total dose or decreasing overall time of treatment[25-30]. These regimens are called “hyper fractionation” (the dose per fraction is decreased, the number of fractions increased, the total dose is increased, and the total treatment time remains similar to conventional therapy time) or “accelerated fractionation schedules” (the total dose and dose per fraction remain unchanged, but the number of fractions per day is increased and thus the overall treatment time is reduced and treatment intensity increased). Hyper fractionation exploits the difference in fractionation sensitivity between tumors and normal tissues manifesting late morbidity. In contrast, accelerated fractionations attempt to reduce tumor proliferation as a major cause of radiotherapy failure[25-30]. A few hyper

fractionated or accelerated regimens of RT were tested on GBM patients, but all of the studies failed to demonstrate any improvement in the OS rate, and moreover, some neurological toxicity was reported[25-30].

Our previous studies, especially the *in vitro* ones showed that daily repeated low-dose irradiation of cells, compared to a single biologically equivalent dose, resulted in significantly higher cell death[7,17]. Experiments conducted on glioma xenografts demonstrated that repeated low-dose irradiation was more effective for inhibiting tumor growth than a single large dose[7,17]. The exact mechanisms underlying HRS are not clear. The demonstration of marked HRS in some human radio resistant tumors suggests that inducible repair might be an important component of the radio resistance that is apparent in these tumors at high doses. Radio resistance may only occur when there is enough initial damage or accumulated damage to trigger DNA repair mechanisms, which are more efficient than the constitutive DNA maintenance functions. Therefore, so-called induced radio resistance may occur only after relatively large doses but not at doses below a certain threshold.

This low-dose hypersensitivity (HRS) phenomenon seemed to provide a new promising and effective treatment for GBM patients; clinical trials were performed to confirm its benefit[7,17]. Our first clinical study (ULTRA-RT), which tested ultra-fractionated RT in *de novo*, inoperable GBM patients showed that this regimen was safe and well tolerated[18]. However, the OS was only 9.53 months, which is comparable with the survival rate reported in the literature for these unresectable GBM[18]. Interestingly, an increased number

of long survivors were reported (two-years survival was 15.48 %)[18]. TEMOFRAC is the first trial to explore the effects of a combined fractionated low-dose radiation therapy and TMZ as a first-line treatment for inoperable, *de novo* GBM patients. The expected low-dose hypersensitivity was observed after the ultra-fractionated RT and TMZ treatments, and an additive effect was suggested. The TEMOFRAC clinical trial confirmed that fractionated low-dose RT is feasible, can be performed daily and is well accepted by patients. However, this ultra fractionated regimen could be lived by the patient as more binding that the standard treatment, for a little gain of survival.

It is noteworthy that four complete responses and seven partial responses were reported in our series; to the best of our knowledge, this type of response has never been reported with RT for GBM patients[1-6,21-23]. Moreover, TMZ used in conjunction with RT and as an adjuvant regimen did not show a similar range of responses[5,24]. Therefore the combination of ultra-fractionated RT and TMZ could explain this unusual high rate of response rate in our study. Unfortunately, the toxicity in our trial was higher than expected and similar to that reported with concomitant RT and TMZ treatment in the literature; two fatal pulmonary infections and a grade 4 hematological toxicity with a major sepsis, also fatal. At the beginning of the study, prophylactic treatment for pneumocystis lung infection was not recommended; the absence of prophylaxis could explain the two fatal pulmonary infections. Although, the hematological toxicity from the TMZ was considered moderate, it can be severe[5,24]. The unusual toxicity reported in our series suggested that ultra fraction regimen plus TMZ is not as safe as expected for this type of combination of therapies[5,24]. The dose per fraction is correlated to the tolerance to RT as reported in our previous ULTRA-RT

clinical trial ULTRA-RT, neurological symptomatology evoking a post-RT leukoencephalopathy was not recorded.

The results obtained for this group of GBM patients with unfavorable prognoses (biopsy only, class RPA V, some patients > 70 years old) are both surprising and promising. Taking into account our trial was a phase II study with only 34 patients and did not have a predictive factor such as MGMT status, our results displayed one of the longer OS rates reported for inoperable GBM patients. Moreover, they are better than those noted in EORTC/NCIC trial for unresected GBM patients[5]. The high rate of long-term survivors reported in the TEMOFRAC (32,4 % two-year survival and 17,6 % three-year survival) confirms the efficacy of this new regimen of RT[5,24]. GBM is a highly vascularized tumor that overexpresses vascular endothelial growth factor A (VEGF-A), a key regulator of tumor-associated angiogenesis[23]. Previous results from clinical trials support a role for the anti-VEGF-A molecule bevacizumab in recurrent and newly diagnosed GBM[31]. Two large phase III studies have recently been published that evaluated bevacizumab treatment in conjunction with RT and concomitant and adjuvant TMZ treatment as the first-line treatment for GBM – (AVAGLIO and RTOG 0825)[32,33]. Both trials showed a 3-4 month prolongation of the PFS with bevacizumab but without significant effects on the OS (AVAGLIO OS was 16.8 months in the bevacizumab arm and 16.7 months in the control arm; RTOG 0825 OS was 15.7 months in the bevacizumab group and 16.1 months in the control group). It is noteworthy that only 10 % of patients underwent a stereotactic biopsy in the AVAGLIO trial; at least 3 % underwent one in the RTOG study[32,33]. TEMAVIR, a French phase II randomized trial, was conducted to evaluate bevacizumab and irinotecan as neo-adjuvant and adjuvant treatments combined with TMZ chemo-radiation

for unresectable GBM; there were no differences found in the two arms for survival (OS was 11.1 months)[31]. Our results are significantly better than those from the TEMAVIR trial, and are comparable with those obtained from the AVAGLIO and RTOG 0825 trials[31-33].

In conclusion, this trial reported one of the longer OS rates for unresectable GBM, and the regimen is feasible for routine clinical practice, and well accepted by the patients. The combination of ultra-fractionated RT and TMZ given concomitantly and in an adjuvant schedule merits further evaluation especially in resected GBM patients.

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TABLE 1

Patient's characteristics and survival status

	Treatment		
	RT (N=45)	**TMZ/RT (N=48)**	**TEMOFRAC (N=34)**
	N (%%)	**N (%%)**	**N (%%)**
Extent of surgery: Biopsy	45 (100.0)	48 (100.0)	**34 (100)**
Sex			
Female	12 (26.7)	19 (39.6)	**10 (29.4)**
Male	33 (73.3)	29 (60.4)	**24 (70.6)**
MGMT			
unmethylated	2 (4.4)	3 (6.3)	**11 (32.4)**
methylated	2 (4.4)	1 (2.1)	**12 (35.3)**
Missing	41 (91.1)	44 (91.7)	**11 (32.4)**
Performance status			
0	14 (31.1)	17 (35.4)	**4 (11.8)**
1	24 (53.3)	22 (45.8)	**18 (52.9)**
2	7 (15.6)	9 (18.8)	**12 (35.3)**
Age			
<=50 yrs	0 (0.0)	2 (4.2)	**1 (2.9)**
>50 & <=60 yrs	32 (71.1)	24 (50.0)	**18 (52.9)**
>60 yrs	13 (28.9)	22 (45.8)	**15 (44.1)**
Median	56.0	59.0	**59.0**
Range	41.0 - 69.0	30.0 - 70.0	**29.1 – 73.5**
Tumor location			
One lobe	29 (64.8)	34 (70.9)	**29 (85.3)**
Multilobal	12 (26.7)	14 (29.2)	**5 (14.7)**
Other/Missing	4 (8.8)	0 (0.0)	**0 (0.0)**
PFS event			
No	0 (0.0)	2 (4.2)	**3 (8.8)**
Yes	45 (100.0)	46 (95.8)	**31 (91.2)**
Survival status			
Alive	2 (4.4)	2 (4.2)	**3 (8.8)**
Dead	43 (95.6)	46 (95.8)	**31 (91.2)**

TABLE 2

Toxicities reported during the phase II Trial

Type of Toxicity	Number of patients	Percentage
Fatigue gde II	30	88 %
Alopecia gde II	20	58 %
Skin reaction gde I	10	29 %
Headaches gde I	6	17 %
Pulmonary infection gde IV	2	5 %
Hematological toxicity gde IV	1	2 %

TABLE 3

Survival Time

Treatment	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	P-Value (Log-Rank)	Median (95% CI) (Months)	% at 2 Year(s) (95% CI)
RT	45	43	1.00	0.0007	8.67 (6.31, 10.97)	4.60 (0.84, 13.74)
TEMOFRAC	34	30	0.44 (0.27, 0.72)		15.92 (9.69, 22.60)	32.35 (17.62, 48.02)

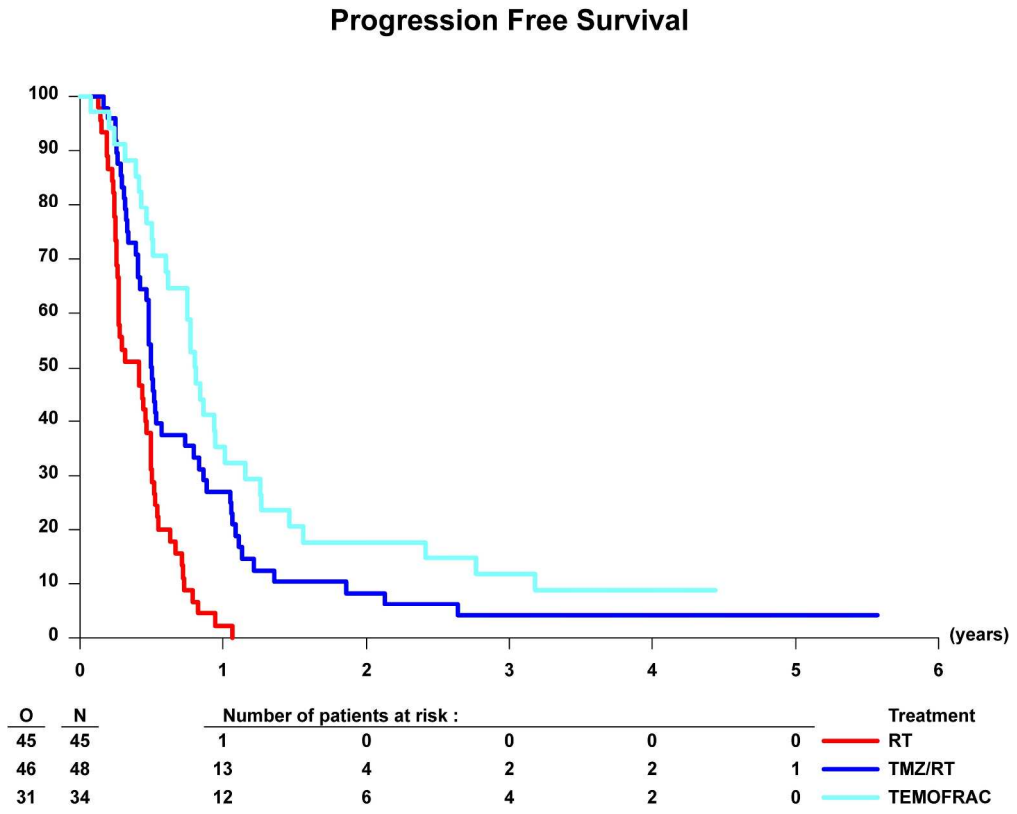
Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Treatment	1	-0.47940	0.13000	13.5997	**0.0002**	**0.619**	**0.480**	**0.799**
WHO PS	1	0.18214	0.18955	0.9234	0.3366	1.200	0.827	1.740
AGE	1	0.02304	0.01360	2.8709	0.0902	1.023	0.996	1.051

TABLE 4

Survival Time

Treatment	Patients (N)	Observed Events (O)	Hazard Ratio (95% CI)	P-Value (Log-Rank)	Median (95% CI) (Months)	% at 2 Year(s) (95% CI)
RT	45	43	1.00	0.0007	8.67 (6.31, 10.97)	4.60 (0.84, 13.74)
TEMOFRAC	34	30	0.44 (0.27, 0.72)		15.92 (9.69, 22.60)	32.35 (17.62, 48.02)

Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
Treatment	1	-0.47940	0.13000	13.5997	**0.0002**	**0.619**	**0.480**	**0.799**
WHO PS	1	0.18214	0.18955	0.9234	0.3366	1.200	0.827	1.740
AGE	1	0.02304	0.01360	2.8709	0.0902	1.023	0.996	1.051



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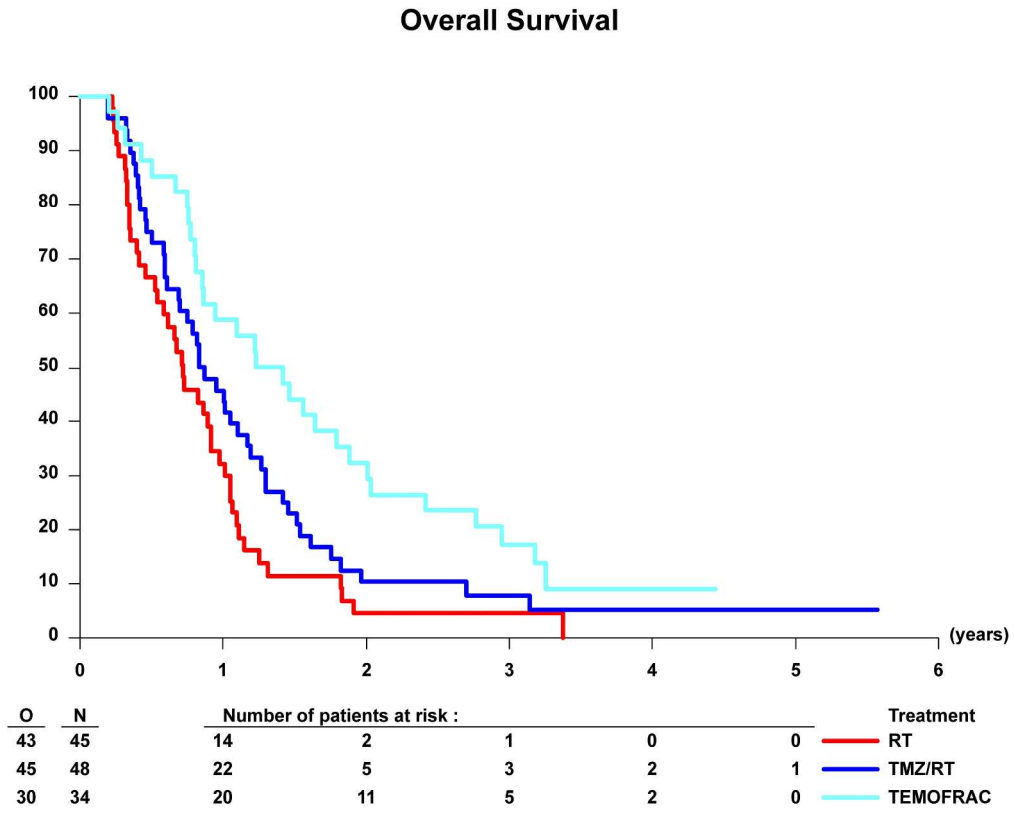

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