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ORIGINAL ARTICLE

Postoperative neoadjuvant temozolomide before radiotherapy versus standard radiotherapy in patients 60 years or younger with anaplastic astrocytoma or glioblastoma: a randomized trial

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ABSTRACT

Introduction: A pilot study of temozolomide (TMZ) given before radiotherapy (RT) for anaplastic astrocytoma (AA) and glioblastoma (GBM) resulted in prolonged survival compared to historical controls receiving RT alone. We therefore investigated neoadjuvant TMZ (NeoTMZ) in a randomized trial. During enrollment, concomitant and adjuvant radio-chemotherapy with TMZ became standard treatment. The trial was amended to include concurrent TMZ.

Patients and methods: Patients, after surgery for GBM or AA, age ≤60 years and performance status (PS) 0–2, were randomized to either 2–3 cycles of TMZ, 200 mg/m^2 days 1–5 every 28 days, followed by RT 60 Gy in 30 fractions or RT only. Patients without progressive disease after two TMZ cycles, received the third cycle. From March 2005, TMZ 75 mg/m^2 was administered daily concomitant with RT. TMZ was recommended first-line treatment at progression. Primary endpoint was overall survival and secondary safety.

Results: The study closed prematurely after enrolling 144 patients, 103 with GBM and 41 with AA. Median age was 53 years (range 24–60) and 89 (62%) were male. PS was 0–1 for 133 (92%) patients, 53 (37%) had complete surgical resection and 18 (12%) biopsy. Ninety-two (64%) received TMZ concomitant with RT. Seventy-two (50%) were randomized to neoadjuvant treatment. For the overall study population survival was 20.3 months for RT and 17.7 months for NeoTMZ ($p = .76$), this not reaching the primary objective. For the preplanned subgroup analysis, we found that NeoTMZ AA patients had a median survival of 95.1 months compared to 35.2 months for RT ($p = .022$). For patients with GBM, no difference in survival was observed ($p = .10$). MGMT and IDH status affected outcome.

Conclusions: No advantage of NeoTMZ was noted for the overall study population or subgroup of GBM, while NeoTMZ resulted in 5 years longer median survival for patients diagnosed as AA.

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Introduction

For glioblastoma (GBM) patients concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) is standard treatment [1]. For anaplastic astrocytoma, RT or chemotherapy, either TMZ or PCV have been shown to result in similar outcomes [2]. Few studies have examined the benefit of postoperative TMZ before RT, which is safe and feasible [3,4]. Neoadjuvant TMZ can lead to radiosensitization of tumor cells [5,6], can be initiated faster than RT, diminishing the risk of clinical deterioration due to tumor regrowth [7,8], which might thereby reduce the volume to be irradiated. In a pilot study of neoadjuvant TMZ followed by RT for grade 3–4

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Supplemental data for this article can be accessed here.

astrocytoma, median survival exceeded 27 months (Skovgaard, unpublished data, Supplement S1), which was superior to historical controls receiving RT only. Therefore, the Nordic Clinical Brain Tumor Study Group initiated this randomized trial comparing neoadjuvant TMZ followed by RT to RT alone.

Patients and methods

Patients with newly diagnosed, histologically confirmed GBM or AA according to the local pathology department, 18–60 years old, were recruited from 13 oncology departments in Sweden, Norway, Denmark and Finland, if they had a performance status (PS) WHO 0–2, a life expectancy of >3 months and normal organ function defined as platelets $\geq 100 \times 10^9$/L, hemoglobin >90 g/L, neutrophils $\geq 1.5 \times 10^3/mm^3$ or leucocyte count $\geq 3.0 \times 10^9$/L, serum creatinine and bilirubin <1.5x upper limit of normal (ULN) and AST/ALT $\leq 3 \times$ ULN. Men and women of child bearing potential had to use adequate contraception. Exclusion criteria were prior RT or chemotherapy for glioma, previous other malignancy within 5 years of inclusion except radically treated basal or squamous cell carcinoma of the skin or carcinoma *in situ*, pregnancy or breastfeeding and any condition that would prevent adequate treatment and follow-up. Patients with prior surgery for grade 2 glioma recurring as grade 3 or 4 were eligible.

The study protocol was approved by Ethics Committees of all participating countries. All patients signed written informed consent.

The study was registered ISRCTN45209900.

Randomization

Patients were stratified for center and randomized 1:1 to standard RT (SRT) or TMZ followed by RT (NeoTMZ), according to a computer-generated code which was available in sealed envelopes. Randomization forms were faxed to the Oncology Centre, Umeå University Hospital, Umeå, Sweden and treatment allocation was returned by fax to the trial center.

Study treatment

Study treatment should start within four weeks after surgery. Neoadjuvant TMZ was dosed 200 mg/m^2, days 1–5, every 28 days. A radiologic evaluation was performed after TMZ cycle 2 and the third cycle was administered to patients without progressive disease. Treatment was also discontinued for unacceptable toxicity or patient's wish.

The recommended RT-schedule was 60 Gy in 30 fractions (five fractions á 2 Gy per week), but alternative fractionations, representing standard at the participating center, were accepted.

Planning target volumes were defined from CT or MRI scans, including the tumor volume from the preoperative examination or after NeoTMZ cycle 2, as appropriate. Multiple field technique for optimal dose distribution was used. (RT description: S2)

After March 2005, all patients received a daily dose of TMZ 75 mg/m^2 concurrent with RT (concTMZ) [1].

No adjuvant TMZ was planned, but recommended at first recurrence.

Monitoring and follow-up

Evaluation before inclusion and each cycle of TMZ included physical examination, assessment of PS, adverse events (AEs) and blood tests. For NeoTMZ patients, a baseline radiologic examination was performed within 10 days of treatment start. During every TMZ cycle, blood counts including neutrophils were performed days 21–22 and within 72 h of the next cycle. During concTMZ, patients were monitored according to local practice.

Patients were reassessed one and three months after finalizing RT and thereafter followed for progression and survival according to local routine. At first relapse, TMZ was recommended (200 mg/m^2 days 1–5 every four weeks). Relapse treatment for patients who had progressed during NeoTMZ was provided at the discretion of the treating physician.

AEs were assessed according to the WHO grading system of toxicity [9], except for nausea and vomiting where the National Cancer Institute Common Toxicity Criteria version 2.0 was used. Only grade 3–5 toxicity was reported.

Study data were monitored at each center and collected by an independent company.

Statistical analyses

To detect a 16% difference in two-year survival (from 18% to 34%) with a power of 80% and a two-sided significance level of 5%, 145 evaluable patients were required in each treatment arm. We estimated a drop-out rate of maximum 10% and aimed at enrolling 322 patients.

The primary endpoint was overall survival from the date of randomization. The secondary endpoint was safety. An interim analysis of two-year survival was planned after inclusion of 145 patients. Analyses were conducted according to Intention-to-treat. Statistical calculations were done with SPSS version 22.0.

According to protocol, survival analyses included the complete study cohort, subgroups GBM and AA and treatment without or with concTMZ (early vs. late inclusion).

Survival was estimated by the Kaplan–Meier method and a two-sided log-rank test. Hazard ratios (HRs) were calculated by Cox regression and for pairwise comparisons by log-rank tests. Multivariate analysis for all patients included diagnosis (GBM vs. AA), age (≥50 vs. <50 years), gender (men vs. women), WHO performance score (2 vs. 0–1), surgery (complete vs. partial vs. biopsy), baseline steroids (yes vs. no) and treatment arm. An additional multivariate analysis included MGMT promotor methylation status (MGMT) (methylated vs. unmethylated tumor) and IDH status (mutated vs. wildtype). For comparison between groups, Fischer's exact test was used. The significance level was $p < .05$.

Methods for molecular markers

According to 2016 WHO criteria, molecular markers were included for reclassification of tumors [10]. Representative

tumor areas were chosen for TMA construction and DNA extraction (S3). MGMT was assessed by pyrosequencing [11]. Samples with a mean methylation <9% were considered unmethylated and ≥9% as methylated (Table S1).

IDH1, p53 and ATRX were investigated by immunohistochemistry and IDH1/IDH2 mutations (R132H and R172K, respectively) also by pyro- and dideoxynucleotide termination sequencing [12,13]. 1p/19q codeletions were assessed both by FISH and droplet digital PCR (ddPCR) (Table S2).

Results

Between 13 January 2003 and 21 May 2008, 145 patients were randomized, after which inclusion was stopped due to slow enrollment. Patients only screened were not registered. One patient, rediagnosed with anaplastic meningioma, was excluded from the intention to treat analysis. For five patients, previous surgery for low-grade glioma was reported. Median age was 53 years. Median follow-up was 20 months. At the date of last follow-up, 1 March 2014, 124 subjects (86%) had died. Patient characteristics were well balanced between treatment arms (Table 1).

Planned study treatment was completed by 74% of all patients ($n = 107$, SRT = 69 and NeoTMZ = 38) (Consort diagram: Figure 1). All patients that completed RT, received 54–64 Gy, apart from 12 patients from one center where 52 Gy (36 Gy whole brain plus 16 Gy tumor boost) initially represented their standard and one patient had palliative RT, 34 Gy in 10 fractions, administered.

AA patients had a median age of 46 years. All randomized to NeoTMZ completed two cycles and 90% ($n = 19$) three cycles of TMZ. All patients completed RT, whereof 76% ($n = 16$) concTMZ. SRT was completed by 90% ($n = 18$) and 65% ($n = 13$) received concTMZ (Figure 1). The difference between treatment arms, regarding concTMZ or not, was not significant ($p = .43$).

Median age was 55 years for GBM patients. Of those randomized to NeoTMZ, 96% ($n = 49$) completed one cycle of TMZ, 92% ($n = 47$) two cycles and 39% ($n = 20$) all three. Eighty percent ($n = 41$) completed RT, being concTMZ for 51% ($n = 26$). For two patients, treatment was not reported. SRT patients started and completed radiotherapy according to schedule in 98% of cases ($n = 51$), which for 69% ($n = 36$) was concTMZ (Figure 1). Administration of concTMZ or not per allocated treatment did not reach significance ($p = .09$).

TMZ after completion of radiotherapy was administered to 88 patients (61%) (AA $n = 22$, GBM $n = 66$), for four adjuvant and 84 at progression. AA patients randomized to SRT received in median six cycles of TMZ compared to four for NeoTMZ. For those with GBM, the corresponding numbers were six versus three cycles.

Molecular characteristics

Tumor tissue was available for 112 patients, 109 for TMA and 107 for DNA, both available in 104 cases and were analyzed for MGMT, IDH1/2 mutations and 1p/19q codeletions. To obtain maximal molecular information for key markers, IDH1 expression and mutations were analyzed on TMA and DNA and 1p/19q codeletions where complemented by ddPCR analyses where IHC failed, TMA was missing and for confirmation. All patients included as AA and IDH1 mutated GBM were analyzed for ATRX and p53 ($n = 34$) (Table 2).

IDH1, but no IDH2, mutations (IDHmut) were detected in 20 patients, four of these with previous low-grade glioma. Seventeen of 31 AA patients harbored IDH1mut and three

Table 1. Patient characteristics and molecular markers per diagnosis and randomization.

	Anaplastic astrocytoma (AA)		Glioblastoma (GBM)	
Variable	Radiotherapy $N = 20$	Neoadjuvant TMZ $N = 21$	Radiotherapy $N = 52$	Neoadjuvant TMZ $N = 51$
Concomitant TMZ	13 (65.0)	16 (76.2)	36 (69.2)	27 (52.9)
Age years, median (range)	47.5 (27–60)	45 (28–57)	53 (25–60)	56 (24–60)
Gender, *n* (%)				
Male	15 (75.0)	11 (52.4)	33 (63.5)	30 (58.8)
Female	5 (25.0)	10 (47.6)	19 (36.5)	21 (41.2)
WHO performance status *n* (%)				
0–1	19 (95.0)	21 (100)	47 (90.4)	46 (90.2)
2	1 (5.0)	0 (0)	5 (9.6)	5 (9.8)
Surgery type, *n* (%)				
Complete	5 (25.0)	6 (28.6)	18 (34.6)	24 (47.1)
Partial	10 (50.0)	11 (52.4)	28 (53.9)	21 (41.2)
Biopsy	5 (25.0)	4 (19.0)	5 (9.6)	4 (7.8)
Not reported	0 (0.0)	0 (0.0)	1 (1.9)	2 (3.9)
Baseline steroids, *n* (%)				
Yes	7 (35.0)	9 (42.9)	17 (32.7)	15 (29.4)
No	13 (65.0)	12 (57.1)	35 (67.3)	34 (66.7)
Not reported	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.9)
Molecular markers, *n* (%)	$N = 15$	$N = 16$	$N = 44$	$N = 37$
Concomitant TMZ	11 (73.3)	13 (81.2)	32 (72.7)	20 (54.1)
IDH1 mut/wt	6/9 (40.0/60.0)	11/5 (68.7/31.3)	3/41 (6.8/93.2)	0/37 (0.0/100.0)
1p/19q codeletion/noncodel	1/13 (6.7/86.6) 1 m[b] (6.7)	0/15 (0.0/93.7) 1 m[b] (6.3)	1/42 (2.3/95.4) 1 m[b] (2.3)	0/36 (0/97.3) 1 m[b] (2.7)
MGMT promotor[a] methylated/unmethylated	10/3(66.7/20.0) 2 m[b] (13.3)	14/2 (87.5/12.5)	24/19(54.5/43.2) 1 m[b] (2.3)	24/11(64.9/29.7) 2 m[b] (5.4)

One patient with AA and one patient with GBM, randomized to radiotherapy, were both IDH1 mutated and 1p/19q codeleted.
[a]Cutoff for MGMT methylation ≥9%.
[b]*m*= missing.

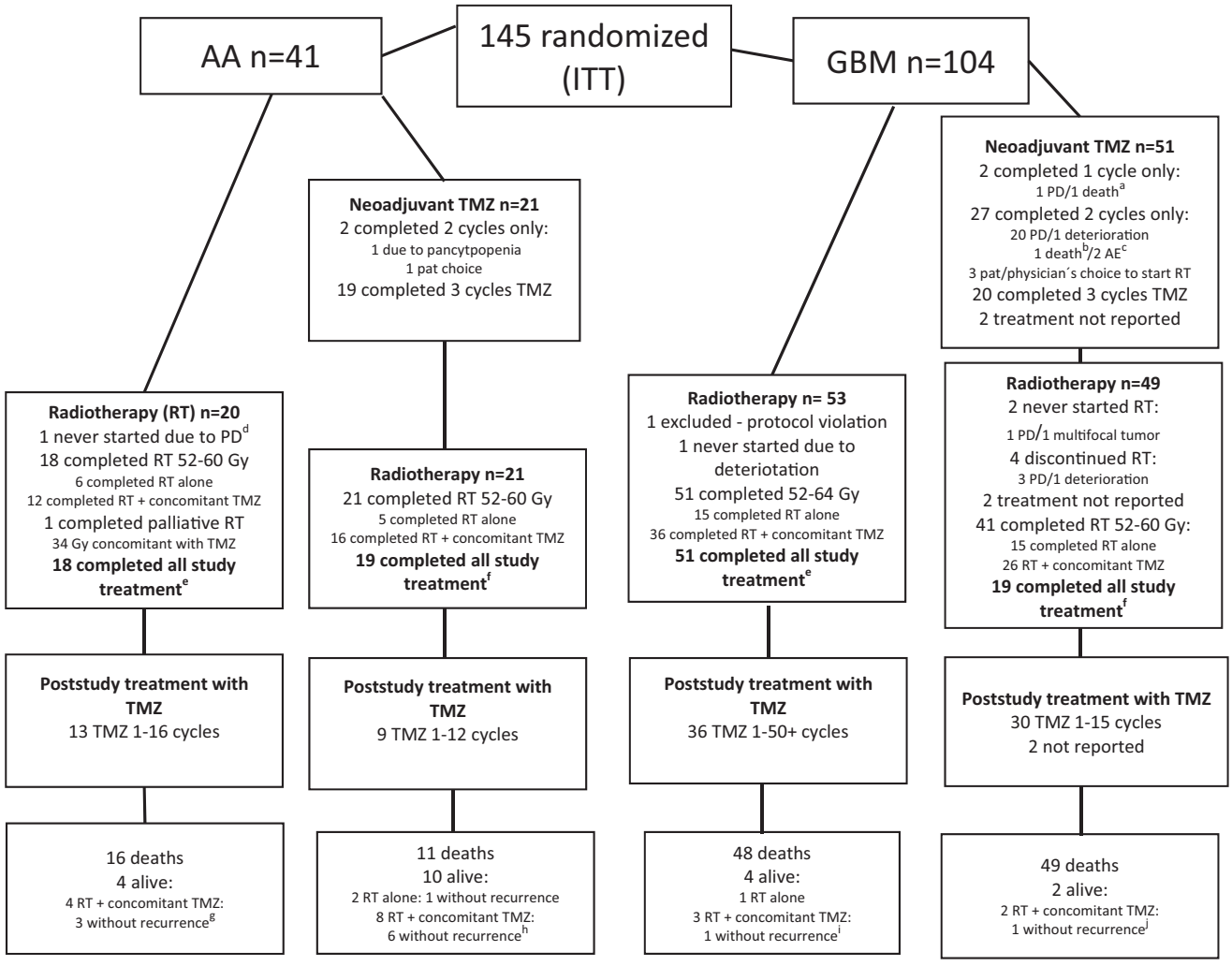

Figure 1. Consort diagram for all randomized patients.
[a]death due to intracerebral bleeding after one cycle of TMZ.
[b]1 death after two cycles of TMZ.
[c]1 rash and 1 infection.
[d]No RT, but received two cycles of TMZ.
[e]For treatment arm SRT: full dos RT.
[f]For NeoTMZ: three cycles of TMZ followed by full dose RT.
Without recurrence since randomization at last follow-up 1 March 2014:
[g]77, 87 and 98 months.
[h]90, 95, 96, 96, 103 and 119 months.
[i]102 months.
[j]102 months.

patients with GBM had IDHmut tumor, probably representing secondary GBM. Two patients with IDH1mut tumor were also 1p/19q codeleted. IDH mutations for AA were evenly distributed between treatment arms ($p = .074$). Fifteen tumors were positive for p53 immunostaining, indicating a p53-mutated tumor, and 10 had loss of ATRX.

Survival

Median overall survival (MOS) was 17.7 months (95% CI: 13.2–22.3) for NeoTMZ and 20.3 months (95% CI: 15.7–25.0) for SRT ($p = .76$, NeoTMZ vs. SRT HR = 0.95; 95% CI: 0.66–1.35). Survival for all patients at 24 months ($n = 144$) was 38.9% (95% CI: 0.28–0.50) for NeoTMZ and 41.7% (95% CI: 0.30–0.053) for SRT with overlapping confidence intervals (Figure 2(a)).

In multivariate analysis ($n = 142$), diagnosis and surgery were significant prognostic factors, while baseline steroids was borderline significant and treatment arm nonsignificant (Table 3). For those analyzed for MGMT and IDH mutation ($n = 107$), MGMT- and IDH status together with baseline steroids were significant for survival, while histological diagnosis was of borderline significance (Table 4). We found no correlation or interaction between MGMT and IDH status. We found a significant survival benefit of adding concomitant TMZ in the overall population ($p = .010$) (Figure S1).

Subgroup analyses

Among patients randomized as AA ($n = 41$), MOS was 95.1 months (95% CI: 52.2–138.0) for NeoTMZ and 35.2 months

Table 2. Diagnosis, prognostic factors, molecular markers and survival.

Randomization	Diagnosis	Age	Surgery	IDH1	1p/19q	ATRX[a]	P53[a]	MGMT promotor	Survival months
NeoTMZ	AA	32	biop	mut	–	loss	pos[b]	meth	33.9
NeoTMZ	AA	34	comp	mut	–	loss	pos	meth	95.1
SRT	AA	27	part	mut	uc	loss	pos	N/A[e]	35.2
NeoTMZ	AA	35	part	mut	–	loss	pos	meth	[f]103.4
SRT	AA	29	comp	mut	–	loss	pos	N/A[e]	[f]97.6
NeoTMZ	AA	28	part	mut	–	loss	–	meth	51.5
NeoTMZ	AA	44	part	mut	–	+	pos	meth	[f]96.1
NeoTMZ	AA	37	part	mut	–	N/A[d]	N/A[d]	meth	[f]95.8
NeoTMZ	AA	38	comp	mut	uc	+	pos	meth	[f]95.1
SRT	AA	32	comp	mut	–	+	pos	meth	23.4
NeoTMZ	AA	51	part	mut	–	loss	–	unmeth	[f]93.2
SRT	AA	60	comp	mut	codel	+	–	meth	48.8
NeoTMZ	AA	49	part	mut	–	+	pos	meth	[f]90.4
NeoTMZ[c]	AA	46	part	mut	–	loss	pos	meth	[f]89.6
SRT[c]	AA	39	part	mut	–	loss	pos	meth	[f]86.7
SRT[c]	AA	38	part	mut	–	+	pos	meth	[f]77.0
NeoTMZ[c]	AA	35	part	mut	–	loss	pos	meth	44.7
NeoTMZ	AA	57	biop	wt	–	+	pos	meth	20.6
SRT	AA	32	part	wt	–	loss	pos	meth	82.0
SRT	AA	56	part	wt	–	+	–	unmeth	16.1
SRT	AA	47	part	wt	–	+	pos	unmeth	4.5
NeoTMZ	AA	51	comp	wt	–	loss	–	meth	20.6
SRT	AA	56	comp	wt	–	loss	pos	meth	44.5
NeoTMZ	AA	51	part	wt	–	loss	pos	meth	81.2
SRT	AA	38	part	wt	–	+	pos	unmeth	19.3
SRT	AA	41	part	wt	–	+	–	meth	17.3
SRT	AA	51	biop	wt	–	loss	pos	meth	42.8
NeoTMZ	AA	35	comp	wt	–	loss	pos	unmeth	70.7
SRT	AA	40	comp	wt	–	+	–	meth	[f]81.1
NeoTMZ	AA	45	part	wt	–	+	pos	meth	57.0
SRT	AA	55	part	wt	–	+	–	meth	22.7
SRT	GBM	52	biop	mut	codel	+	–	meth	30.5
SRT	GBM	41	comp	mut	–	+	pos	meth	[f]102.4
SRT	GBM	56	comp	mut	–	+	pos	meth	46.2
NeoTMZ	GBM	57	part	wt	–	–	–	meth	17.7
NeoTMZ	GBM	39	comp	wt	–	–	–	meth	20.4
SRT	GBM	48	part	wt	–	–	–	meth	[f]129.3
NeoTMZ	GBM	59	comp	wt	–	–	–	meth	21.1
NeoTMZ	GBM	53	comp	wt	–	–	–	unmeth	9.0
SRT	GBM	59	part	wt	–	–	–	meth	20.3
SRT	GBM	59	part	wt	–	–	–	meth	3.7
NeoTMZ	GBM	45	comp	wt	–	–	–	meth	6.5
SRT	GBM	53	comp	wt	–	–	–	meth	107.7
NeoTMZ	GBM	58	part	wt	–	–	–	unmeth	8.6
SRT	GBM	41	part	wt	–	–	–	unmeth	11.1
SRT	GBM	59	part	wt	–	–	–	meth	38.4
NeoTMZ	GBM	58	part	wt	–	–	–	unmeth	11.0
NeoTMZ	GBM	30	comp	wt	–	–	–	meth	11.1
SRT	GBM	56	comp	wt	–	–	–	meth	22.9
NeoTMZ	GBM	36	comp	wt	–	–	–	meth	41.3
SRT	GBM	59	part	wt	–	–	–	unmeth	0.5
SRT	GBM	59	comp	wt	–	–	–	meth	24.1
NeoTMZ	GBM	56	comp	wt	–	–	–	meth	7.0
SRT	GBM	41	part	wt	–	–	–	unmeth	8.7
NeoTMZ	GBM	58	part	wt	–	–	–	meth	1.9
SRT	GBM	54	part	wt	–	–	–	unmeth	5.6
NeoTMZ	GBM	54	part	wt	–	–	–	unmeth	17.2
SRT	GBM	58	part	wt	–	–	–	unmeth	19.6
NeoTMZ	GBM	54	part	wt	–	–	–	unmeth	1.7
SRT	GBM	45	part	wt	–	–	–	unmeth	13.6
NeoTMZ	GBM	56	part	wt	–	–	–	meth	12.0
NeoTMZ	GBM	54	comp	wt	–	–	–	meth	20.2
SRT	GBM	59	comp	wt	–	–	–	meth	48.3
NeoTMZ	GBM	55	comp	wt	–	–	–	meth	9.1
SRT	GBM	58	comp	wt	–	–	–	unmeth	9.3
NeoTMZ	GBM	51	comp	wt	–	–	–	unmeth	13.7
SRT	GBM	60	comp	wt	–	–	–	meth	39.6
NeoTMZ	GBM	59	comp	wt	–	–	–	meth	50.9
SRT	GBM	40	part	wt	–	–	–	meth	12.5
NeoTMZ	GBM	58	comp	wt	–	–	–	meth	[f]102.4
SRT	GBM	47	part	wt	–	–	–	meth	17.3
SRT	GBM	48	part	wt	–	–	–	meth	28.8
NeoTMZ	GBM	57	part	wt	–	–	–	meth	20.0
NeoTMZ	GBM	54	comp	wt	–	–	–	meth	53.3

(*continued*)

Table 2. Continued

Randomization	Diagnosis	Age	Surgery	IDH1	1p/19q	ATRX[a]	P53[a]	MGMT promotor	Survival months
SRT	GBM	58	part	wt	–	–	–	unmeth	18.1
SRT	GBM	58	comp	wt	–	–	–	meth	16.7
SRT	GBM	25	part	wt	–	–	–	meth	92.9
NeoTMZ	GBM	60	comp	wt	N/A	–	–	N/A[e]	15.3
NeoTMZ	GBM	24	biop	wt	–	–	–	N/A[e]	0.5
SRT	GBM	48	part	wt	–	–	–	unmeth	22.1
NeoTMZ	GBM	57	comp	wt	–	–	–	unmeth	10.0
SRT	GBM	60	comp	wt	–	–	–	unmeth	25.6
NeoTMZ	GBM	56	comp	wt	–	–	–	unmeth	20.8
SRT	GBM	58	comp	wt	–	–	–	unmeth	27.6
SRT	GBM	51	comp	wt	–	–	–	unmeth	50.0
NeoTMZ	GBM	56	comp	wt	–	–	–	meth	4.8
NeoTMZ	GBM	53	comp	wt	–	–	–	unmeth	8.0
SRT	GBM	52	part	wt	–	–	–	meth	21.3
NeoTMZ	GBM	55	comp	wt	–	–	–	unmeth	8.3
SRT	GBM	52	part	wt	–	–	–	unmeth	14.7
SRT	GBM	56	part	wt	–	–	–	meth	42.9
NeoTMZ	GBM	47	part	wt	–	–	–	meth	14.6
SRT	GBM	37	part	wt	–	–	–	meth	[f]87.3
SRT	GBM	50	part	wt	–	–	–	unmeth	11.7
NeoTMZ	GBM	55	part	wt	–	–	–	meth	15.7
SRT	GBM	59	comp	wt	–	–	–	unmeth	20.3
SRT	GBM	52	biop	wt	uc	–	–	N/A[e]	10.0
SRT	GBM	58	part	wt	–	–	–	unmeth	11.1
NeoTMZ	GBM	58	part	wt	–	–	–	meth	16.0
NeoTMZ	GBM	49	part	wt	–	–	–	meth	20.8
SRT	GBM	53	biop	wt	–	–	–	unmeth	18.2
SRT	GBM	44	part	wt	–	–	–	meth	6.5
NeoTMZ	GBM	60	part	wt	–	–	–	unmeth	17.7
NeoTMZ	GBM	45	comp	wt	–	–	–	meth	12.2
SRT	GBM	50	part	wt	–	–	–	meth	15.6
NeoTMZ	GBM	53	part	wt	–	–	–	meth	16.2
SRT	GBM	59	part	wt	–	–	–	unmeth	4.3
NeoTMZ	GBM	59	part	wt	–	–	–	meth	35.0
SRT	GBM	50	comp	wt	–	–	–	unmeth	[f]72.4
NeoTMZ	GBM	59	part	wt	–	–	–	meth	26.1
SRT	GBM	38	comp	wt	–	–	–	meth	16.1
SRT	GBM	52	part	wt	–	–	–	meth	14.3

NeoTMZ: Neoadjuvant treatment.
SRT: Standard radiotherapy.
[a]p53 and ATRX immunostaining reported for those included as AA- and IDH1-mutated GBM.
[b]pos = immunostaining indicating p53 mutation.
[c]Previous low-grade glioma.
[d]No TMA available.
[e]No DNA available.
[f]alive at follow-up 1st March 2014.
N/A: no tissue available.
Age reported in years.
Surgery: comp: complete; part: partial; biop: biopsy.
uc: uncountable.

(95% CI: 0–77.7) for SRT ($p = .022$, NeoTMZ vs. SRT HR = 0.41; 95% CI: 0.19–0.90) (Figure 2(b)).

For GBM ($n = 103$), there was no significant difference in survival related to treatment arm. MOS was 15.3 months (95% CI: 11.6–19.0) for NeoTMZ versus 18.1 months (95% CI: 13.9–22.3) for SRT ($p = .10$, NeoTMZ vs SRT HR = 1.40; 95% CI: 0.93–2.09) (Figure 2(c)).

For GBM patients randomized to SRT, harboring methylated tumor MGMT (mMGMT), survival was superior compared to the other three arms ($p < .001$) ($n = 78$) (Figure 2(d)).

Patients with IDH1-mutated tumor ($n = 20$), receiving NeoTMZ, seemed to have longer survival (MOS not reached at 100 months (95% CI cannot be computed) vs. SRT 48.8 months (95% CI: 41.3–56.3)), but this was not statistically significant ($p = .27$, NeoTMZ vs. SRT HR 0.48; 95% CI: 0.13–1.81) (Figure 2(e)).

For 92 patients with IDH1/2 wildtype (wt) glioma, there was no difference in survival according to treatment arm, with MOS for NeoTMZ versus SRT being 16.0 months (95% CI: 12.7–19.2) and 19.3 months (95% CI: 15.9–22.8), respectively, ($p = .10$, NeoTMZ vs. SRT HR 1.42; 95% CI: 0.93–2.17) (Figure 2(f)).

For those with IDH1/2wt tumor and MGMT status determined ($n = 89$), survival was superior for SRT patients with mMGMT compared to SRT and unmethylated MGMT promotor (uMGMT) ($p = .0040$), NeoTMZ and uMGMT ($p < .001$) and of borderline significance for NeoTMZ with mMGMT ($p = .059$) (overall $p = .0026$) (Figure S2).

Safety

All but two patients with AEs ≥3 were treated with TMZ. One patient suffered fatal cerebral hemorrhage 11 days after initiating TMZ, two patients died of MDS/aplastic anemia and four patients developed thromboembolic disease, one being fatal. One patient died of undiagnosed breathing problems.

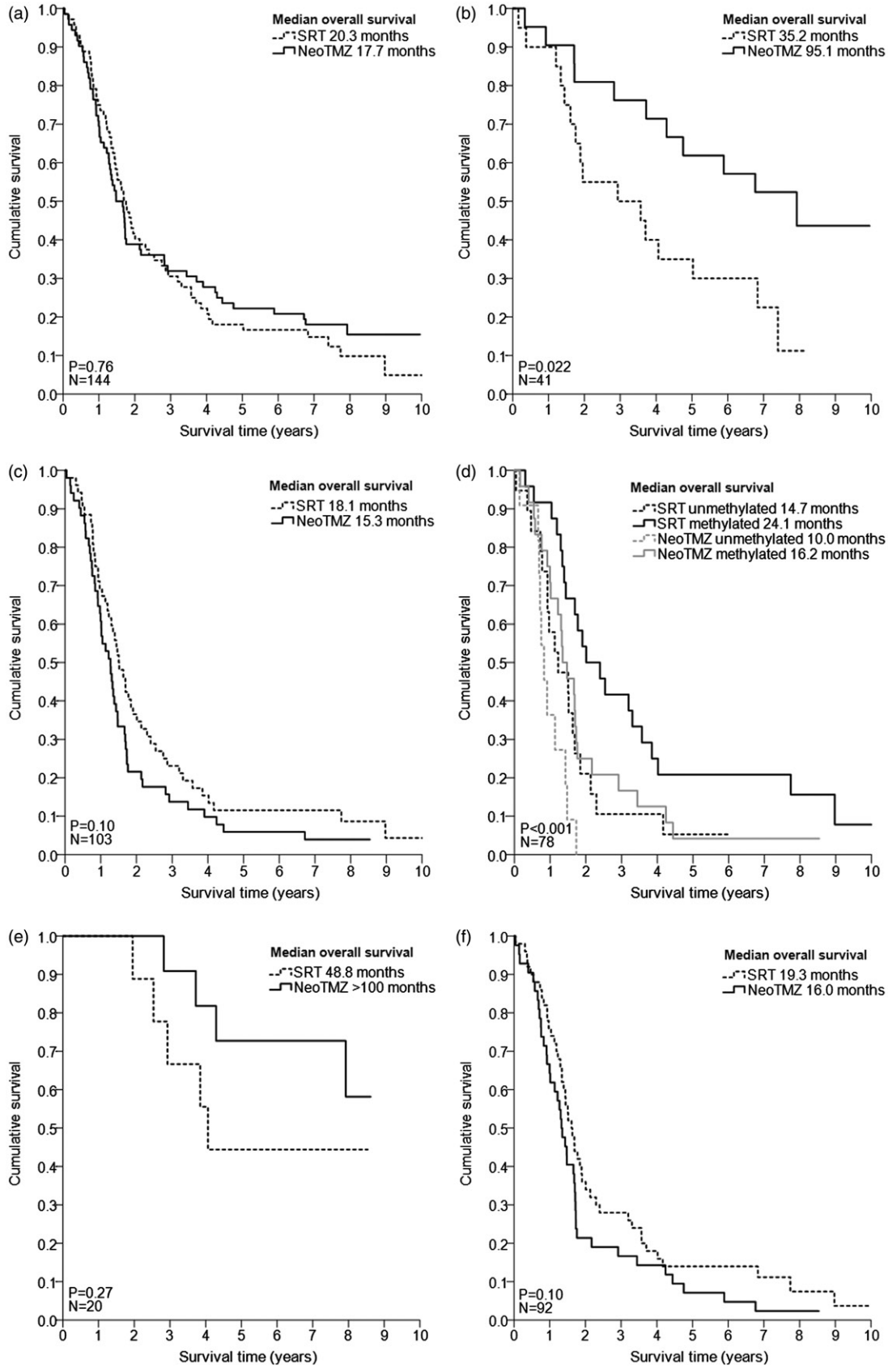

Figure 2. (a–f) Overall Survival by Kaplan–Meier: standard radiotherapy (SRT) and neoadjuvant temozolomide (NeoTMZ). (a) All patients: SRT ($n = 72$) versus neoTMZ ($n = 72$). (b) Anaplastic astrocytoma (AA): SRT ($n = 20$) versus neoTMZ ($n = 21$). (c) Glioblastoma (GBM): SRT ($n = 52$) versus neoTMZ ($n = 51$). (d) GBM: SRT unmethylated ($n = 19$) versus SRT methylated ($n = 24$) versus neoTMZ unmethylated ($n = 11$) versus neoTMZ methylated ($n = 24$). All patients received TMZ, apart from three in the SRT arm with unmethylated MGMT. (e) IDHmut glioma: SRT[a] ($n = 9$) versus neoTMZ ($n = 11$). [a]2 patients 1p/19q codeleted. (f) IDHwt glioma: SRT ($n = 50$) versus neoTMZ ($n = 42$).

Table 3. Multivariate cox regression analysis for baseline patient characteristics and treatment arm ($n = 142$[a]).

Variable	Number of patients	Number of events (%)	Hazard ratio	95% Confidence interval	*p* value
Age					
<50 years	51	36 (70.6)	1.00	–	–
≥50 years	91	86 (94.5)	1.29	0.81–2.05	.28
Gender					
Women	54	44 (81.5)	1.00	–	–
Men	88	78 (88.6)	1.42	0.96–2.10	.077
WHO performance status					
0–1	132	112 (84.8)	1.00	–	–
2	10	10 (100.0)	1.61	0.80–3.24	.18
Type of surgery					
Biopsy	18	18 (100.0)	1.00	–	–
Partial resection	70	58 (82.9)	0.46	0.24–0.88	.019
Complete resection	53	45 (84.9)	0.34	0.17–0.67	.0018
Resection unknown	1	1 (100.0)	2.00	0.25–16.28	.52
Steroids at baseline[a]					
No	94	78 (83.0)	1.00	–	–
Yes	48	44 (91.7)	1.50	0.98–2.30	.059
Diagnosis at inclusion					
Anaplastic astrocytoma	41	27 (65.9)	1.00	–	–
Glioblastoma	101	95 (94.1)	3.35	2.00–5.62	<.001
Treatment arm					
Radiotherapy	72	64 (88.9)	1.00	–	–
Neoadjuvant TMZ	70	58 (82.9)	1.17	0.81–1.70	.40

[a]For 2 patients steroids at baseline is not reported and these patients are excluded from the analysis.

Table 4. Multivariate cox regression analysis of patients with MGMT and IDH status determined ($n = 107$).

Variable	Number of patients	Number of events (%)	Hazard ratio	95% Confidence interval	*p* value
Age					
<50 years	40	28 (70.0)	1.00	–	–
≥50 years	67	64 (95.5)	1.09	0.64–1.87	.75
Gender					
Women	42	35 (83.3)	1.00	–	–
Men	65	57 (87.7)	1.21	0.78–1.87	.40
WHO performance status					
0–1	100	85 (85.0)	1.00	–	–
2	7	7 (100.0)	1.42	0.59–3.41	.44
Type of surgery					
Biopsy	5	5 (100.0)	1.00	–	–
Partial resection	60	50 (83.3)	0.88	0.30–2.56	.81
Complete resection	42	37 (88.1)	0.59	0.19–1.81	.36
Steroids at baseline					
No	76	64 (84.2)	1.00	–	–
Yes	31	28 (90.3)	1.74	1.02–2.96	.041
Diagnosis at inclusion					
Anaplastic astrocytoma	29	19 (65.5)	1.00	–	–
Glioblastoma	78	73 (93.6)	1.92	1.00–3.70	.050
Treatment arm					
Radiotherapy	56	49 (87.5)	1.00	–	–
Neoadjuvant TMZ	51	43 (84.3)	1.54	0.98–2.43	.063
MGMT methylated					
No (<9%)	35	33 (94.3)	1.00	–	–
Yes (≥9%)	72	59 (81.9)	0.52	0.33–0.83	.0054
IDH mutated					
No	89	84 (94.4)	1.00	–	–
Yes	18	8 (44.4)	0.23	0.10–0.56	.0012

The AEs unrelated to TMZ were seizures, and breast cancer diagnosed 4 years after SRT (Table 5).

Discussion

We found no survival advantage for NeoTMZ for the whole study group or the subgroup with GBM. The neoadjuvant treatment was generally well tolerated and did not cause unexpected toxicity. In the overall cohort, concTMZ prolonged survival compared to RT alone.

In contrast to GBM, for those included as AA patients, neoadjuvant temozolomide achieved 5 years longer median survival. Also, for these patients, TMZ at recurrence and/or concurrent with RT did not appear to compensate for not administering TMZ neoadjuvant, as thirteen of 20 patients in the SRT arm received concTMZ. This might suggest a larger benefit of more extensive upfront TMZ treatment, although these findings need to be interpreted with caution, as the cohort of AA patients included both those with IDHmut and IDHwt tumor and also numbers are small.

For GBM patients, the neoadjuvant treatment did not confer any advantage over SRT, the subgroup of SRT patients with mMGMT having the longest survival. Our data indicate that in GBM patients, start of RT should not be delayed 2-3

Table 5. Adverse events all patients: Patient may have had several AE:s of the same type, highest grade per patient reported.

Grade 3–5 adverse events				
	Grade 3	Grade 4	Grade 5	Serious
Seizures[a]	6[b]	0	0	3
Rash/Urticaria	2	0	0	0
Infection/Fever	5	0	0	1
Abdominal pain	1	0	0	1
Syncope	1	0	0	0
Breathing problems	0	0	1[c]	1
Psychosis[d]	1	0	0	0
Thromboembolic event	3	1	1	4
Hydrocephalus	1	0	0	1
Intracranial hemorrhage	0	0	1	1
Breast cancer[a]	0	0	1	1
Elevated ALT[e]	2	0	0	0
Elevated GT	1	0	0	0
Pancytopenia	0	1	0	1
Myelodysplastic Syndrome/Aplastic Anemia	0	0	2	2

[a]All patients, except for 1 with seizures and 1 with breast cancer, received TMZ either neoadjuvant and/or concomitant with radiotherapy.
[b]For 1 patient seizures grade 2 is rated serious due to hospitalization, for 1 patient grade of seizures is not reported.
[c]Patient progressed early and received TMZ instead of allocated treatment, which was standard RT.
[d]Steroid induced.
[e]For 1 patient unrelated to study treatment.

months for treatment with TMZ, not even for those with mMGMT. When analyzing the role of MGMT methylation in patients with IDHwt tumors, the same pattern emerged.

In a phase 2 trial, 50 patients with GBM underwent 2 weeks of daily TMZ followed by concomitant hypofractionated accelerated radiotherapy to 60 Gy, followed by adjuvant TMZ. This resulted in a median survival of 22.3 months for the whole study group and 53.8 months for those with mMGMT versus 16.2 months for uMGMT [14]. We are only aware of one randomized study of neoadjuvant TMZ before concurrent TMZ and RT. In this study, where 99 GBM patients were enrolled, a survival benefit for neoadjuvant TMZ was demonstrated (17.6 vs. 13.2 months; $p = .021$). Also, in this trial, TMZ was administered daily only during 14 days before chemo-radiotherapy [15], this being an important difference compared to our study. These trials indicate that the duration of TMZ before start of RT could be important.

In grade 3 glioma, several studies have demonstrated that the addition of chemotherapy improves survival when compared to RT alone. In the CATNON trial (NCT00626990) for anaplastic glioma without 1p/19q co-deletions, patients were randomized between RT with or without TMZ concomitant and/or adjuvant. Preliminary results from ASCO 2016 demonstrated a benefit for the addition of TMZ [16]. The molecular background related to this outcome is not yet determined, but it is probable that this trial included both IDHmut and IDHwt patients, as our subgroup of AA patients. Interestingly, CATNON randomized patients to 12 cycles of adjuvant TMZ. As we found a survival benefit for three cycles of neoadjuvant TMZ, this could imply that a shorter course of TMZ might be sufficient.

Two large randomized studies (RTOG 9402, EORTC 26951) have evaluated PCV chemotherapy for anaplastic oligodendroglioma, which was shown to confer a major survival benefit compared to RT alone, these studies exploring neoadjuvant (pre-RT) and adjuvant (post-RT) therapy, respectively [17,18]. Both trials reported IDH mutational status being of importance for the effect of PCV [19]. In our trial, the number of patients with IDH-mutated tumor is small but results seem to be in line with previous findings, with a trend towards longer survival for those receiving NeoTMZ treatment.

Our trial has some shortcomings. We did not include the planned number of patients, due to slow accrual. The cohort of AA patients is small, including both IDHmut and wildtype astrocytomas and some potential imbalances between treatment characteristics can be noted, such as IDHmut/wt and concTMZ or not, although none reaching statistical significance, a contribution of these to the survival differences cannot be ruled out. The need for incorporating concomitant TMZ to RT was not foreseen at the time of study initiation in 2003. At this time, molecular differences between subgroups of high-grade tumors where not well defined and was not incorporated into the study protocol. Because of the inherent difficulties in glioma grading, we chose to analyze outcome both according to the original histological diagnosis and to include molecular markers, in accordance with the latest classification system for high-grade glioma [10], the analyses of molecular markers being done *post hoc*. The small subgroup of patients with IDH-mutated tumor does not allow for a statistically solid conclusion.

Our data indicate that neoadjuvant temozolomide for 2–3 cycles before radiotherapy cannot be recommended to high-grade glioma in general or the subgroup of GBM, while for those included as AA a substantial survival benefit was noted. If this is caused by IDH mutation may be clarified by the molecular outcome of the CATNON trial.

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Disclosure statement

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