Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated *MGMT* promoter (CeTeG/NOA–09): a randomised, open-label, phase 3 trial

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Summary

Background There is an urgent need for more effective therapies for glioblastoma. Data from a previous unrandomised phase 2 trial suggested that lomustine-temozolomide plus radiotherapy might be superior to temozolomide chemoradiotherapy in newly diagnosed glioblastoma with methylation of the *MGMT* promoter. In the CeTeG/NOA-09 trial, we aimed to further investigate the effect of lomustine-temozolomide therapy in the setting of a randomised phase 3 trial.

Methods In this open-label, randomised, phase 3 trial, we enrolled patients from 17 German university hospitals who were aged 18–70 years, with newly diagnosed glioblastoma with methylated *MGMT* promoter, and a Karnofsky Performance Score of 70% and higher. Patients were randomly assigned (1:1) with a predefined SAS-generated randomisation list to standard temozolomide chemoradiotherapy (75 mg/m² per day concomitant to radiotherapy [59–60 Gy] followed by six courses of temozolomide 150–200 mg/m² per day on the first 5 days of the 4-week course) or to up to six courses of lomustine (100 mg/m² on day 1) plus temozolomide (100–200 mg/m² per day on days 2–6 of the 6-week course) in addition to radiotherapy (59–60 Gy). Because of the different schedules, patients and physicians were not masked to treatment groups. The primary endpoint was overall survival in the modified intention-to-treat population, comprising all randomly assigned patients who started their allocated chemotherapy. The prespecified test for overall survival differences was a log-rank test stratified for centre and recursive partitioning analysis class. The trial is registered with ClinicalTrials.gov, number NCT01149109.

Findings Between June 17, 2011, and April 8, 2014, 141 patients were randomly assigned to the treatment groups; 129 patients (63 in the temozolomide and 66 in the lomustine-temozolomide group) constituted the modified intention-to-treat population. Median overall survival was improved from 31·4 months (95% CI 27·7–47·1) with temozolomide to 48·1 months (32·6 months–not assessable) with lomustine-temozolomide (hazard ratio [HR] 0·60, 95% CI 0·35–1·03; p=0·0492 for log-rank analysis). A significant overall survival difference between groups was also found in a secondary analysis of the intention-to-treat population (n=141, HR 0·60, 95% CI 0·35–1·03; p=0·0432 for log-rank analysis). Adverse events of grade 3 or higher were observed in 32 (51%) of 63 patients in the temozolomide group and 39 (59%) of 66 patients in the lomustine-temozolomide group. There were no treatment-related deaths.

Interpretation Our results suggest that lomustine-temozolomide chemotherapy might improve survival compared with temozolomide standard therapy in patients with newly diagnosed glioblastoma with methylated *MGMT* promoter. The findings should be interpreted with caution, owing to the small size of the trial.

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Introduction

Chemotherapy for patients with newly diagnosed glioblastoma has not been substantially improved since the registration trial for temozolomide.[1] The addition of tumour-treating fields to temozolomide was associated with moderate improvements in survival.[2] Randomised trials with dose-intensified temozolomide regimens[3] or combinations of temozolomide with other drugs[4–7] did not prolong overall survival. Nevertheless, the low toxicity of temozolomide suggests that a more intense alkylating

Research in context

Evidence before this study

The standard therapy for patients with newly diagnosed glioblastoma is radiotherapy (59–60 Gy) with concomitant daily low-dose (75 mg/m²) temozolomide chemotherapy, followed by six courses of adjuvant temozolomide therapy (150–200 mg/m² per day on days 1–5 of the 4-week course). The *MGMT* promoter methylation status is a predictor for the benefit of temozolomide therapy: patients with methylated *MGMT* promoter have a particularly high survival benefit from temozolomide therapy. A single-arm, phase 2 trial (UKT-03) assessing lomustine-temozolomide combination therapy to patients with newly diagnosed glioblastoma found a signal of improvement in overall survival for patients with glioblastoma with methylated *MGMT* promoter. The CeTeG/NOA–09 trial aimed to further analyse the value of lomustine-temozolomide combination chemotherapy in such patients in a randomised phase 3 setting.

Added value of this study

The predefined final analysis of the primary endpoint showed that combined lomustine-temozolomide chemotherapy plus radiotherapy could improve overall survival compared with standard temozolomide chemotherapy plus radiotherapy.

Implications of all the available evidence

Lomustine-temozolomide combination chemotherapy prolonged overall survival in a selected group of patients with glioblastoma with *MGMT* promoter methylation in this small randomised trial. These encouraging results require further confirmation and, if confirmed, this regimen has the potential to become a standard-of-care option. Ongoing research aims to further investigate the molecular determinants of response to lomustine-temozolomide and the cellular changes induced by this combination.

combination therapy might be feasible and should be further investigated.

Nitrosourea compounds are well established in glioma therapy,[8–12] and they are capable of penetrating the brain through an intact blood–brain barrier. The combination of nitrosoureas with temozolomide would not be simply a dose escalation of alkylating therapy, but might combine different qualities of DNA damage with the potential for additive or even synergistic effects. By contrast with temozolomide, which exerts its therapeutic effect preferably through alkylation of guanine, lomustine has effects beyond DNA alkylation: it acts as an bifunctional agent introducing interstrand crosslinks[13] and leads to carbamoylation of amino acids, thus interfering with transcriptional, translational, and post-transcriptional processes.[14] By contrast with the alkylating mechanism of action shared by temozolomide and nitrosoureas, the non-alkyating mechanisms of action might not depend on the methylation status of the O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter and on the MGMT enzyme activity, which counteracts guanine alkylation. Therefore, it was not surprising that combined nitrosourea and temozolomide therapy showed enhanced activity in high-grade glioma xenograft models.[15] Additionally, a single-arm trial with carmustine (BCNU) and unescalated temozolomide provided promising results in patients with inoperable glioblastoma.[16] These concepts and experimental results provide a clear rationale to assess the efficacy of combined lomustine-temozolomide therapy in glioblastoma patients.

The single–arm phase 2 UKT-03 trial[17,18] included 31 patients and explored the value of combined lomustine-temozolomide chemotherapy in patients with newly diagnosed glioblastoma. In line with previous trials that used nitrosoureas,[8,9] which defined the standard in 2002, when UKT-03 began, the first course of chemotherapy started during radiotherapy. With lomustine-temozolomide combination therapy, the findings from UKT-03 suggested an improved overall survival with a median of 23 months, as opposed to 15–17 months in contemporary historical controls. However, improved overall survival was exclusively seen in patients with glioblastoma with methylated *MGMT* promoter. The median overall survival of these patients was 34·5 months, comparing favourably with 23·4 months in the temozolomide registration trial,[19] whereas median overall survival remained at 12·5 months in patients with unmethylated *MGMT* promoter.[18] Favourable overall survival was also seen in a non–randomised trial in which lomustine-temozolomide combination therapy was received by children with high-grade glioma.[20] The encouraging data of the UKT-03 trial led to the CeTeG/NOA-09 trial, which assessed whether lomustine-temozolomide therapy is superior to temozolomide standard therapy in a randomised phase 3 setting. For this, we implemented the same lomustine-temozolomide treatment regimen as the UKT-03 trial (including omission of radiotherapy-concomitant daily chemotherapy) and we restricted enrolment to patients with glioblastoma with methylated *MGMT* promoter on the basis of the previous UKT-03 trial subgroup analyses.

Methods

Study design and participants

CeTeG/NOA–09 was a randomised, open-label, phase 3 trial. Patients were recruited in 17 German university hospitals on the basis of the following inclusion criteria: no previous chemotherapy or radiotherapy, age 18–70 years, newly diagnosed glioblastoma or gliosarcoma (resection or biopsy) centrally confirmed (by TP; appendix), methylated *MGMT* promoter according to central testing (MDXHealth, Herstal, Belgium), Karnofsky performance score (KPS) of 70% or higher, stable or decreasing corticosteroids within 5 days before

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See Online for appendix

For the **trial protocol** see https://neurologie.uni-bonn.de/sektionen/klinische-neuroonkologie/therapiestudien.htm

randomisation, and adequate haematological, hepatic, renal, and coagulation function. Exclusion criteria included previous malignancy treated less than 5 years before this study; previous medical treatment for any cancer; other severe psychological, cognitive, familial, sociological, or geographical conditions that could interfere with compliance with the study protocol, and any other antitumour therapy not described in the protocol.

The study was approved by the ethics committees of all 17 participating centres. All patients gave written informed consent. All trial procedures adhered to the Declaration of Helsinki and the guidelines for Good Clinical Practice. An independent Data Monitoring and Safety Board reviewed all safety–relevant information every 6 months. The trial protocol is available online.

Randomisation and masking

Patients were randomly assigned (1:1) to either lomustine-temozolomide combination therapy or temozolomide standard therapy according to a predefined SAS-generated randomisation list (fax response from the Clinical Study Core Unit of the Study Centre Bonn, appendix). All procedures associated with randomisation, data management, and monitoring were done at the Clinical Study Core Unit of the Study Centre Bonn and the Centre for Clinical Studies Cologne, and the investigators could not interfere with these processes. Because of the different schedules for application of lomustine-temozolomide (6-week courses) and standard temozolomide (4-week courses), masking of patients and of the treating physician was not possible. The only part of the trial that was masked was the final analysis of MRIs for the determination of progression.

Procedures

Before randomisation, tumour specimens were analysed centrally for *MGMT* promoter methylation with methylation-specific real-time PCR.[21] Tumours were classified as having methylated *MGMT* promoter if the ratio of *MGMT* to the β-actin reference gene (*ACTB*), calculated as (methylated *MGMT*/*ACTB*)×1000, was greater than 2.[19,22,23] Responding to changes implemented by the WHO classification of brain tumours in 2016,[24] all available tumour tissue of patients in the trial was retrospectively reclassified. We did analyses for ATRX chromatin remodeler (*ATRX*) loss (immunohistochemistry with monoclonal antibody clone CL0537, Sigma, St Louis, MO, USA) and isocitrate dehydrogenase (*IDH*) mutation (with an Arg132His-specific antibody).[25] Tumours of patients younger than 55 years classified as Arg132His-immunonegative underwent *IDH1* and *IDH2* pyrosequencing and, in *IDH* mutated cases, 1p/19q co-deletion analysis by the multiplex ligation-dependent probe amplification method (SALSA probe mix P088, MRC Holland, Amsterdam, Netherlands).

Patients started involved-field radiotherapy (59–60 Gy in 30–33 single day fractions) 22–35 days after surgery. In the temozolomide therapy group, patients additionally had daily concomitant temozolomide orally (75 mg/m²) followed by six courses of temozolomide (150–200 mg/m² per day for 5 days every 4 weeks).[1] In the lomustine-temozolomide therapy group, patients received up to six 6–week courses of lomustine-temozolomide orally (lomustine 100 mg/m² on the first day and temozolomide 100 mg/m² [in the first course] on days 2–6). The first course started after radiotherapy for the temozolomide group and started in the first week of radiotherapy for the lomustine-temozolomide group. No daily concomitant temozolomide therapy was given to patients in the lomustine-temozolomide group (figure 1). If the nadir (white blood count <1500 cells per µl or platelets <50 000 per µl) occurred after day 25, lomustine was reduced by one dose level, with the levels being 100%, 75%, and 50% of the initial dose. In patients with white blood count lower than 1500 cells per µl or platelet count lower than 50 000 per µl at the dose level of 50%, lomustine was permanently discontinued. Depending on the nadirs during the first 25 days of the preceding course, temozolomide was decreased to the lower dose levels of 75 mg/m² or 50 mg/m² or increased stepwise to the higher dose levels of 120 mg/m², 150 mg/m², and 200 mg/m², according to the following schedule: reduction by one dose level if white blood count was lower than 1500 cells per µl or platelet count lower than 50 000 per µl; reduction by two dose levels if white blood count was lower than 1000 cells per µl or platelets lower than 25 000 per µl; and increase by one dose level if radiotherapy was completed, white blood count was higher than 2500 cells per µl, and platelets higher than 100 000 per µl. Temozolomide was permanently discontinued for patients who had white blood count lower than 1500 cells per µl or platelet count lower than 50 000 per µl at the lowest temozolomide dose level of 50 mg/m². If any non-haematological grade 3–4 adverse

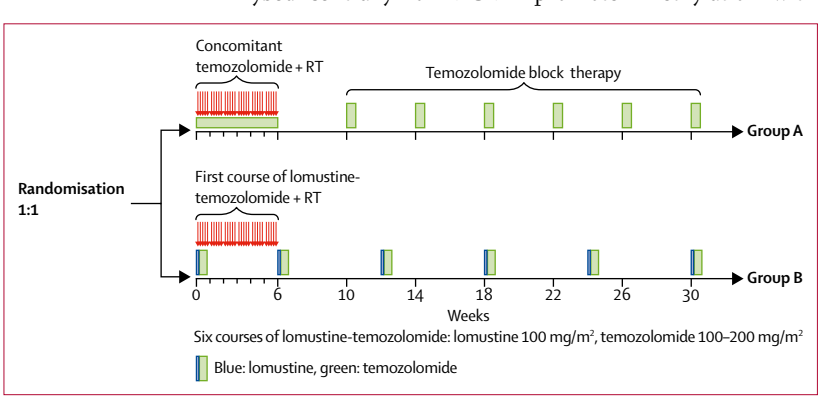

Figure 1: **Schematic overview of the CeTeG/NOA-09 trial**
Group A comprises the standard chemotherapy with temozolomide. This included daily radiotherapy (RT) with concomitant temozolomide therapy. 4 weeks after the end of RT, the first of six adjuvant temozolomide courses started. According to the standard, and if no toxicity ensued, temozolomide was escalated to 200 mg/m² per day in further courses. In group B, lomustine-temozolomide therapy was given in six 6-week courses. The first course started on the first day of radiotherapy. Therefore, there was no extended daily concomitant chemotherapy in the lomustine-temozolomide group. If no toxicity ensued, temozolomide was escalated stepwise to a maximum daily dose of 200 mg/m² per day in further courses.

event occurred (according to the Common Terminology Criteria for Adverse Events, version 4.0), the substance causing the toxicity was withheld in further treatment courses and therapy within the trial could continue with the substance that was not causing the toxicity. The choice of oral or intravenous post-progression therapy was the responsibility of the treating physician and had to be documented in all visits.

Patients were assessed by neurological examination and KPS at baseline, at the beginning of each course, and every 12 weeks after completion of chemotherapy until the end of the trial or death of the patient. Contrast-enhanced MRI was done every 12 weeks until death of the patient. We assessed progression on the basis of the Response Assessment in Neuro-Oncology (RANO) criteria,[26] with the following modifications: up to 12 weeks after completion of radiotherapy, disease progression was considered only for new enhancing lesions outside the radiation field (ie, beyond the 80% isodose) or unequivocal histological demonstration of viable tumour; according to previous experience with late pseudoprogression,[27] disease progression 12 to 24 weeks after completion of radiotherapy could only be diagnosed if it was confirmed 4–6 weeks afterwards by another MRI showing further progression. Progressive disease was confirmed by central reference neuroradiology by investigators masked to the protocol (HU, EH). Adverse events were recorded until at least 30 days after the end of study therapy.

Outcomes

The primary endpoint was overall survival measured from the day that patients were randomly assigned to death or last observation. Secondary endpoints included progression-free survival, best response as determined by modified RANO criteria in patients with incomplete tumour resection and documented postoperative residual disease, frequency of delay of the next chemotherapy course by more than 2 weeks, and acute toxicity. Additional secondary endpoints included assessment of quality of life, as determined by the European Organisation for Research and Treatment of Cancer (EORTC) quality of life questionnaire core-30 (QLQ-C30) and the EORTC brain cancer module (BN20); assessment of neurotoxicity by neurocognitive testing with the Mini Mental State Examination; and a neurocognitive test battery, including Trial-Making Test A and B, digit span forward and backwards, and a controlled word association test for semantic word fluency (naming animals and food items under a time limit, in separate tests) and lexical word fluency. Further exploratory analyses included pseudoprogression rates and application of postprogression therapy.

Statistical analysis

We calculated sample size with the PS power and sample size program.[21] The sample size was based on the assumption that lomustine-temozolomide could increase 2-year overall survival from 48·9%[18] to 70·0% (overall survival in the UKT-03 trial was 75%).[18] We assumed exponentially distributed survival times (event or death rate 0·356 in the temozolomide group and 0·176 in the lomustine-temozolomide group per patient year, hazard ratio [HR] 0·50) and a constant recruitment of 64 eligible patients per treatment group (plus four early dropouts per group) over 2 years with a follow-up of at least 2 years, resulting in a power of 80% for the intended two-sided log-rank test (significance level 0·05). The CeTeG/NOA-09 trial had to recruit 128 eligible patients. The recruitment period was initially planned for 24 months (from April, 2011, to April, 2013), but had to be prolonged until April, 2014 (last patient enrolled) because the prevalence of tumours with methylated *MGMT* promoter (36%) was lower than had been

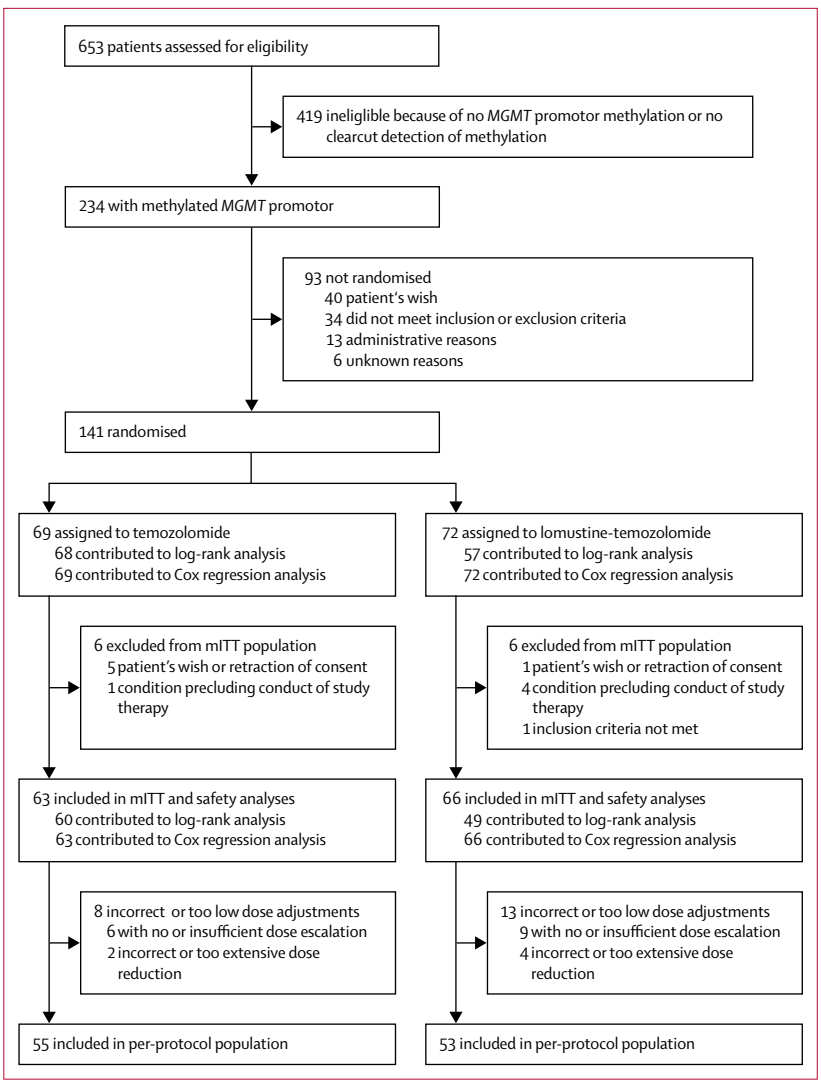

Figure 2: **Trial profile**
mITT=modified intention-to-treat.

previously reported (45%).[19] The planned follow-up time of 24 months after the last patient was enrolled had to be prolonged to 36 months (to April, 2017, the closure of the trial as planned) because a treatment arm-blinded analysis of overall survival 14 months after the last patient was enrolled showed a low overall mean risk of death of 0·1994 per patient year. Prolonging the follow-up time to 36 months allowed us to retain the power of 80% despite the lower event rates.

All statistical analyses were done by an experienced statistician who is one of the co-authors (RF). According to the protocol, the primary analysis was done in the modified intention-to-treat population, including all randomly assigned patients who received their first dose of study chemotherapy (figure 2). Secondary analyses were done on a standard intention-to-treat population, a subpopulation of patients with *IDH1* or *IDH2* wildtype tumours, and the per-protocol population. Safety analyses were done on the safety population, which was identical to the modified intention-to-treat population. The pre-specified primary analysis was done with a log-rank test with stratification by centre and recursive partitioning analysis (RPA) class.[28] RPA 3 class includes participants younger than 50 years with KPS of 90–100%; RPA 4 includes participants younger than 50 years with KPS of 70–80% or older than 50 years with at least partial resection and a Mini Mental State Examination score of 27 points or higher; and RPA 5 includes participants older than 50 years with a Mini Mental State Examination score lower than 27 points or older than 50 years with biopsy alone. All centres with less than three randomly assigned patients per group were taken together as one centre so that the log-rank analysis had 11 categories for the feature termed centre. Prespecified overall survival analyses in the modified intention-to-treat population also included Cox regression analyses, yielding estimated HRs with 95% CI. Survival was plotted according to the Kaplan-Meier method. In line with the log-rank test stratified for centre and RPA class, the graphs included only those patients with control counterparts in their respective centre and RPA class strata, thus enabling a balanced analysis and visualisation of survival. Progression-free survival was a secondary endpoint and analysed with the same methods previously described. Statistical analyses were done using SAS, version 9.14. The trial had a data monitoring and safety board that reviewed all safety-relevant information every 6 months.

This study is registered with ClinicalTrials.gov, number NCT01149109, and with the European Clinical Trials Database, number 2009-011252-22.

	Total (n=129)	Temozolomide (n=63)	Lomustine-temozolomide (n=66)
Sex			
Men	77 (60%)	30 (48%)	47 (71%)
Women	52 (40%)	33 (52%)	19 (29%)
Median age (IQR)	58 (50–63)	59 (51–65)	56 (49–61)
<50 years	29 (22%)	11 (17%)	18 (27%)
≥50 years	100 (78%)	52 (83%)	48 (73%)
Initial KPS			
90–100%	106 (82%)	49 (78%)	57 (86%)
70–80%	23 (18%)	14 (22%)	9 (14%)
Extent of resection*			
Stereotactic biopsy	4 (3%)	1 (2%)	3 (5%)
Partial resection	46 (36%)	22 (35%)	24 (36%)
Complete resection	79 (61%)	40 (63%)	39 (59%)
MMSE			
<27	19 (15%)	8 (13%)	11 (17%)
≥27	108 (84%)	55 (87%)	53 (80%)
Missing	2 (2%)	0 (0%)	2 (3%)
Molecular subgroup			
Glioblastoma with wild-type *IDH*	103 (80%)	52 (83%)	51 (77%)
Glioblastoma with mutated *IDH*	8 (6%)	5 (8%)	3 (5%)
GBM-O	6 (5%)	3 (5%)	3 (5%)
Not assessable	12 (9%)	3 (5%)	9 (14%)
RPA class†			
3	25 (19%)	9 (14%)	16 (24%)
4	88 (68%)	47 (75%)	41 (62%)
5	16 (12%)	7 (11%)	9 (14%)

Data are n (%), unless otherwise specified. KPS=Karnofsky performance score. MMSE=mini mental state examination. *IDH*=isocitrate dehydrogenase. GBM-O=glioblastoma with oligodendroglial component. *As determined by early (≤72 h) post-operative contrast-enhanced MRI. †Recursive partitioning analysis (RPA) class according to the modified European Organisation for Research and Treatment of Cancer classification.[28]

Table 1: **Patient characteristics in the modified intent-to-treat population**

Role of the funding source

The German Federal Ministry of Education and Research is a non-commercial funder and had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study after data bank closure and had final responsibility for the decision to submit for publication.

Results

Between May 4, 2011, and April 8, 2014, 657 patients were screened in 17 study centres. We processed submitted tumour tissue from these patients and, in four cases, obligatory central reference neuropathology did not confirm glioblastoma histology (three anaplastic astrocytomas and one pilocytic astrocytoma); these patients were not considered for any further steps in the trial. Therefore, 653 patients with confirmed glioblastoma were assessed for *MGMT* promoter methylation, and 141 patients with glioblastoma with methylated *MGMT* promoter were randomly assigned to the treatment groups (figure 2). The modified intention-to-treat population comprised 129 patients (63 assigned to temozolomide and 66 to lomustine-temozolomide). Table 1 shows that the trial predominantly included patients with a high KPS and that the majority of patients had complete resections. The distribution of sex was

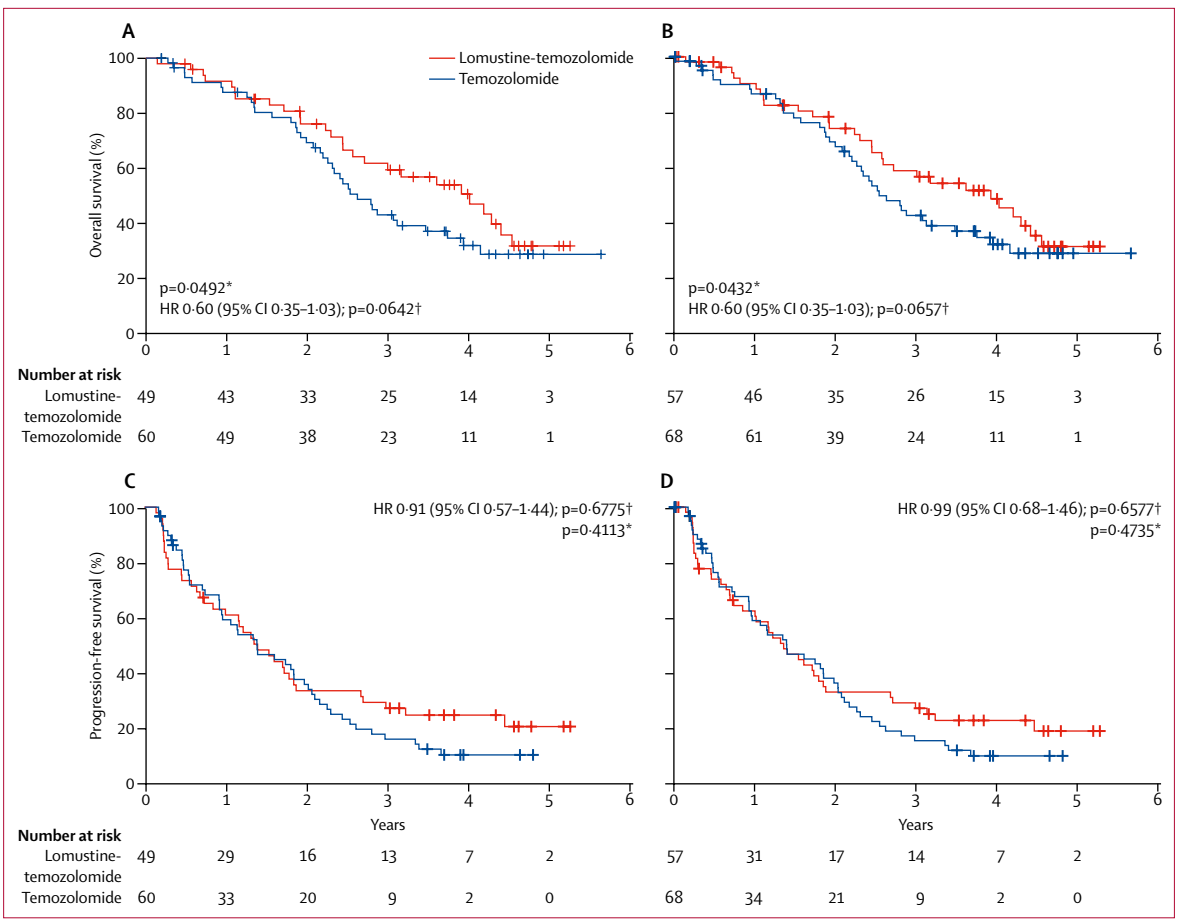

Figure 3: **Kaplan-Meier plots of overall survival and progression-free survival**
Kaplan-Meier plots of patients in both groups matched by respective centre and RPA class strata. Overall survival (A) in the modified intention-to-treat population (n=109; stratified log-rank test) and (B) in the intention-to-treat population (n=125; stratified log-rank test). Progression-free survival in the modified intention-to-treat population (C) and the intention-to-treat population (D). HR=hazard ratio. *Stratified log-rank test (primary analysis). †Multivariate Cox regression analysis.

imbalanced between groups but was not relevant for overall survival in the modified intention-to-treat population (HR 0·99, 95% CI 0·63–1·57, p=0·98) and in the treatment arms (data not shown). There was an imbalance of RPA class distribution in three large trial centres (comprising 40 of 129 patients): in these centres, all 17 patients with RPA 3 or 5 were randomly assigned to lomustine-temozolomide whereas the temozolomide group had only patients with RPA 4 assigned to it. In accordance with the 2007 WHO classification of CNS tumours,[29] applicable throughout the recruiting and treatment phase, our study included six patients with a glioblastoma with oligodendroglial component (GBM-O). All six GBM-O had *IDH* mutation and 1p/19q co-deletion and were thus retrospectively reclassified as anaplastic oligodendroglioma (table 1).

59 (94%) of 63 patients in the temozolomide group and 60 (91%) of 66 in the lomustine-temozolomide group completed radiotherapy as required, with a total dose of 59–60 Gy. 60% of patients with temozolomide and 39% with lomustine-temozolomide had all six chemotherapy courses. The median number of courses was six in the temozolomide group and five in the lomustine-temozolomide group. 42 (67%) of 63 patients in the temozolomide group and 25 (38%) of 66 in the lomustine-temozolomide group received the maximum temozolomide dose level of 200 mg/m². Dose reductions below 100 mg/m² per day occurred only with lomustine-temozolomide treatment (temozolomide reduction in eight [12%] of 66 patients, lomustine reduction in 17 [26%]). Further details on dose adjustments and mean cumulative daily chemotherapy doses are provided in the appendix. The median length of courses was 28 days (range 26–111 days) with temozolomide and 42 days (range 36–84 days) with lomustine-temozolomide. During courses four to six, the percentage of patients with courses substantially delayed for 2–6 weeks was higher with lomustine-temozolomide (eg, 40% of patients in course five) than with temozolomide (17% in course five; appendix).

In the primary analysis (log-rank test stratified for centre and RPA class in the modified intention-to-treat

	Temozolomide (n=63)		Lomustine-temozolomide (n=66)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4
Haematological events				
Leukopenia	10 (16%)	8 (13%)	24 (36%)	10 (15%)
Neutropenia	7 (11%)	4 (6%)	12 (18%)	8 (12%)
Thrombocytopenia	19 (30%)	15 (24%)	40 (61%)	19 (29%)
Lymphopenia	4 (6%)	4 (6%)	6 (9%)	3 (5%)
Anaemia	3 (5%)	3 (5%)	5 (8%)	1 (2%)
Infections				
Upper airways	7 (11%)	..	9 (14%)	..
Lung	4 (6%)	1 (2%)	2 (3%)	2 (3%)
Gastrointestinal	1 (2%)	..	3 (5%)	1 (2%)
Wound, other than CNS	1 (2%)	1 (2%)	3 (5%)	3 (5%)
Fatigue	14 (22%)	..	17 (26%)	..
Gastrointestinal				
Nausea	12 (19%)	..	20 (30%)	..
Vomiting	8 (13%)	..	6 (9%)	..
Diarrhoea	4 (6%)	..	2 (3%)	..
Constipation	12 (19%)	..	15 (23%)	..
Anorexia	2 (3%)	..	4 (6%)	..
Weight loss	1 (2%)	..	2 (3%)	..
Stomatitis	1 (2%)	..	4 (6%)	..
Liver or pancreas				
Elevated transaminases	4 (6%)	..	3 (5%)	..
Elevated GGT	..	..	4 (6%)	4 (6%)
Elevated bilirubine	1 (2%)	..	..	..
Elevated lipase	2 (3%)	1 (2%)	..	..
Cardiac or vascular				
Arrhythmia	2 (3%)	..	..	..
Haemorrhage	..	..	2 (3%)	..
Hypertension	..	..	1 (2%)	..
Venous thrombosis	2 (3%)	2 (3%)	3 (5%)	1 (2%)
Pulmonary embolism	..	..	..	..
Neurological or psychiatrical				
Seizures	16 (25%)	4 (6%)	17 (26%)	6 (9%)
Ischaemic stroke	..	..	1 (2%)	1 (2%)
CNS bleeding	..	..	2 (3%)	..
Brain oedema	2 (3%)	1 (2%)	9 (14%)	2 (3%)
Memory impairment	1 (2%)	..	2 (3%)	..
Motor dysfunction	10 (16%)	2 (3%)	8 (12%)	2 (3%)
Sensory dysfuntion	1 (2%)	..	7 (11%)	1 (2%)
Speech impairment	4 (6%)	..	9 (14%)	3 (5%)
Cognitive disturbance	2 (3%)	..	5 (8%)	..
Personality change	2 (3%)	1 (2%)	5 (8%)	..
Anxiety	..	..	3 (5%)	..
Sleeping problems	4 (6%)	..	5 (8%)	..
Incontinence	1 (2%)	..	..	..
Hearing impairment	2 (3%)	..	1 (2%)	..
Dysgeusia	..	..	5 (8%)	..
Dizziness	6 (10%)	..	5 (8%)	..
Wound problems in CNS or skull	3 (5%)	2 (3%)	2 (3%)	1 (2%)

(Table 2 continues on next page)

population), overall survival was significantly improved in the lomustine-temozolomide group compared with that of the temozolomide group (p=0·0492). Median overall survival was 31·4 months (95% CI 27·7–47·1) with temozolomide versus 48·1 months (32·6–not assessable) with lomustine-temozolomide (figure 3A). A multivariable Cox regression analysis with centre and RPA class as covariates in the modified intention-to-treat population yielded a non-significant HR of 0·60 (95% CI 0·35–1·03; p=0·0641). A significant difference between treatment groups in overall survival was also found in the intention-to-treat population (termed as-randomised population in the protocol; n=141; figure 3B). In this analysis, median overall survival was 30·4 months (95% CI 27·0–44·9) with temozolomide versus 46·9 months (31·0–not assessable) with lomustine-temozolomide (HR 0·60, 95% CI 0·35–1·03; stratified log-rank test p=0·0432). Additionally, in the per-protocol population (n=108), a significant difference in overall survival was found: median overall survival was 30·4 months (95% CI 25·9–47·2) with temozolomide versus 40·3 months (26·8–51·4) with lomustine-temozolomide (HR 0·53, 95% CI 0·29–0·99; multivariable Cox regression analysis p=0·0453).

In post-hoc sensitivity analyses, a univariate Cox regression analysis for overall survival in the modified intention-to-treat population showed an HR of 0·90 (95% CI 0·58–1·41). Median unstratified overall survival in the modified intention-to-treat population was 31·4 months with temozolomide (95% CI 27·0–44·8) and 37·9 months with lomustine-temozolomide (29·2–51·4; p=0·6579; appendix). Additional exploratory post-hoc overall survival analyses taking into account the imbalance of RPA class distribution in some centres showed a separation of overall survival curves that seemed to favour the lomustine-temozolomide group: the appendix shows a Kaplan-Meier graph with inverse probability weights and inclusion of all 129 patients in the modified intention-to-treat population into the analysis, and a Kaplan-Meier graph (89 patients) excluding the three centres where the temozolomide group contained only patients with RPA 4, but none with RPA 3 or RPA 5.

An additional post-hoc analysis showed that, in the modified intention-to-treat subpopulation of patients with *IDH*-wildtype glioblastoma (n=103), overall survival was improved (p=0·0374, stratified log-rank test; HR 0·57, 95% CI 0·30–1·05). Therefore, the inclusion of six patients with GBM-O, nowadays reclassified as anaplastic oligodendroglioma, and eight patients with glioblastoma with *IDH* mutations had no influence on the findings for the primary endpoint.

Best response according to RANO criteria was assessed in 50 patients of the modified intention-to-treat population who had a less than complete resection before enrolment (23 patients in the temozolomide group and 27 in the lomustine-temozolomide group; table 1). Three (13%) of 23 patients in the temozolomide group had a partial response and four (15%) of 27 patients

in the lomustine-temozolomide group had at least a partial response, with three (11%) having a complete response. Progression-free survival did not differ between the treatment groups in the modified intention-to-treat population (p=0·4113, stratified log-rank test) or in the intention-to-treat population (p=0·4735; figure 3C, D). In the modified intention-to-treat population, median progression-free survival was 16·7 months (95% CI 11·4–24·2) with temozolomide and 16·7 months (12·0–32·0) with lomustine-temozolomide (figure 3). Pseudoprogression, confirmed by central reference neuroradiology (according to RANO criteria) or histology (predominance of therapy-induced changes), was found in five patients (8%) with temozolomide and in seven (11%) with lomustine-temozolomide. Six of the seven patients with pseudoprogression in the lomustine-temozolomide group had a re-resection due to suspected progression, which yielded a histological assessment compatible with pseudoprogression (in the temozolomide group, two of five patients had pseudoprogressions confirmed histologically).

The median number of further lines of therapy was two (range two to four) with temozolomide and one (range one to four) with lomustine-temozolomide (appendix). Although the overall frequency of reoperations was higher after lomustine-temozolomide treatment (15 [24%] of 63 patients) than after temozolomide (20 [30%] of 66), complete resections at progression were done with similar frequency (5 [8%] of 63 with temozolomide *vs* 6 [9%] of 66 with lomustine-temozolomide); however, repeat biopsies were exclusively done in the lomustine-temozolomide group (3 [5%] of 66 patients). Re-radiotherapy was applied with similar frequency (15 [24%] of 63 with temozolomide *vs* 12 [18%] of 66 lomustine-temozolomide). The frequency of patients receiving any form of systemic antitumour therapy was higher after temozolomide treatment (39 [62%] of 63) than after lomustine-temozolomide (33 [50%] of 66; appendix). Bevacizumab was applied with similar frequency (18 [29%] of 63 patients with temozolomide *vs* 21 [32%] of 66 with lomustine-temozolomide).

Table 2 summarises adverse events observed until 30 days after the end of study therapy. There were no treatment-related deaths. The prevalence of patients with adverse events of grade 3 or 4 was higher with lomustine-temozolomide (39 [59%] of 66 patients had any adverse event, 24 [36%] of 66 had haematological adverse events) than with temozolomide (32 [51%] of 63 patients had any adverse event, 18 [29%] of 63 had haematological adverse events). Infectious complications were not increased with lomustine-temozolomide therapy. Regarding CNS adverse events, brain oedema was reported more frequently with lomustine-temozolomide therapy than with temozolomide alone. The prevalence of some CNS symptoms, such as speech impairment and sensory dysfunction, were moderately increased in the lomustine-temozolomide group. The prevalence of nausea was higher with lomustine-temozolomide than with temozolomide, without an increase of vomiting. Low-grade alopecia was more frequent with lomustine-temozolomide treatment than with temozolomide alone. There was no excess of other non-haematological, non-CNS organ toxicity in the lomustine-temozolomide group compared with that of the temozolomide group, including no additional liver toxicity (table 2). All patients were also assessed for adverse events reported during a minimum of 2 years after they were randomly assigned (appendix), far beyond the end of study treatment. The extended observation period, potentially confounded by further lines of therapy, provided data on infrequent (3–6% of patients) vascular events in the lomustine-temozolomide group: four patients with pulmonary embolism occurring at least 4 months after completion of study therapy, although the prevalence of deep venous thromboses was not substantially different between groups; two patients with CNS haemorrhage (one subdural and one epidural haematoma) had this adverse event during lomustine-temozolomide therapy, whereas another patient had a tumour haemorrhage later during bevacizumab therapy; and three patients had an ischaemic stroke, one of whom during lomustine-temozolomide therapy (table 2), the other two afterwards and after having received bevacizumab therapy. The longitudinal analysis of quality of life and neurocognitive testing did not reveal systematic differences between the treatment groups. Detailed results will be presented in a separate publication.

	Temozolomide (n=63)		Lomustine-temozolomide (n=66)	
	All grades	Grade 3 or 4	All grades	Grade 3 or 4
(Continued from previous page)				
Skin				
Alopecia	10 (16%)	1 (2%)	18 (27%)	1 (2%)
Erythema	2 (3%)	1 (2%)	6 (9%)	..
Exanthema or rash	9 (14%)	..	7 (11%)	..
Pain				
Headache	12 (19%)	..	12 (18%)	..
Radicular or peripheral nerve	2 (3%)	..	1 (2%)	..
Muscle	1 (2%)	..	2 (3%)	..
Joints	2 (3%)	..	4 (6%)	..

Data are n (%). There were no recorded grade 5 adverse events. GGT=γ-glutamyl transferase.

Table 2: **Adverse events until 30 days after end of study treatment**

Discussion

The CeTeG/NOA-09 trial results provide evidence that, in patients with newly diagnosed glioblastoma with methylated *MGMT* promoter, lomustine-temozolomide therapy might be better than standard temozolomide therapy, in the context of well tolerated toxicity. These results could be a first step towards improving drug therapy of glioblastoma beyond temozolomide monotherapy and

separating drug therapy for patients with glioblastomas with or without methylated *MGMT* promoter.

The small size of the CeTeG/NOA-09 trial, compared with that of previous phase 3 trials, was a limitation.[1–3,5–8] Results from a small number of patients were behind the effect leading to significant survival differences between the treatment groups, meaning that the findings were more susceptible to confounding factors. For this reason, we tried to anticipate potential imbalances of prognostic factors during the planning of the trial and to minimise their influence by using a test stratified for centre and RPA class. An analysis stratified for RPA class accounts for known strong prognostic factors (KPS, age, and extent of resection) because they constitute the compound parameter that is RPA class. Nevertheless, the prespecified stratified log-rank test leads to small strata, so that imbalances might have substantial influence on the results. For example, the definition of the stratified log-rank test implies that patients from strata without matching patients in the other treatment group do not contribute to the log-rank analysis. This is a very straightforward way to enable valid comparisons; however, this characteristic of the stratified log-rank test led to the exclusion of 20 patients from the analysis. Under these circumstances, the Cox regression model, another prespecified analysis of the primary endpoint, becomes important. The multivariable Cox regression model supported, but not significantly so, the finding of the stratified log-rank analysis. Importantly, the stratified Cox regression model did not exclude any patients from the analysis and took into account the problems with the covariates centre and RPA class, brought in by the small sample size. The treatment effect was further supported by exploratory analyses (appendix). Nevertheless, the results favouring lomustine-temozolomide therapy should be interpreted with caution and conclusions have to consider the mentioned limitations of the stratified analysis and the results of the unstratified analysis (appendix).

A further limitation was the discrepancy between the improvement in overall survival and the absence of an effect on progression-free survival. This discrepancy was not based on differences in further lines of therapy: reirradiations and further chemotherapies were more frequent after temozolomide treatment and complete reresections and antiangiogenic treatments were similarly applied in both groups. An increased prevalence of late and prolonged pseudoprogressions after lomustine-temozolomide therapy might have had a major role in the discrepancy between overall survival and progression-free survival. Late and prolonged pseudoprogressions that, by definition, would have remained undetected by RANO criteria have already been described after lomustine-temozolomide therapy.[27] The observation that most pseudoprogressions with lomustine-temozolomide (six of seven with lomustine-temozolomide *vs* two of five with temozolomide) were defined only by histology would be in line with this hypothesis. Another hypothesis would be that undetected pseudoprogression was particularly prevalent in the first 2 years after the start of therapy, thus providing an explanation for the late separation of the progression-free survival curves, after 2 years (figure 3). Future studies will have to analyse this occurrence prospectively. Another influencing factor for the absence of a clear progression-free survival signal would be the small size of the trial, which made the detection of small differences in progression-free survival less likely. We could speculate whether the observed overall survival improvement might be partly due to long-term effects of lomustine, as already described for lower-grade tumours.[30] Finally, in our study, the progression-free survival curves of temozolomide and lomustine-temozolomide separated late, about 2 years after randomisation. A similar late separation occurred in the overall survival curves, which was not observed in the temozolomide registration trial[1] and the EF14 trial (with tumour-treating fields),[2] but is well known from trials in patients with anaplastic oligodendroglioma with 1p/19q co-deletion.[10,11] These results could suggest that there might be two populations, one with and one without an additional benefit brought by combined lomustine-temozolomide therapy. It would be interesting to see whether there are molecular differences between the tumours of the patients in these two groups.

Median overall survival in the temozolomide group of our trial was greater than that of comparable historical groups of patients with tumours with methylated *MGMT* promoter (CENTRIC trial[6] 26·4 months, 95% CI 23·9–34·7; temozolomide registration trial[19] 21·7 months, 95% CI not supplied). Additionally, the 2-year survival rate was higher in the temozolomide group of our study than in the CENTRIC trial[6] (56%, 95% CI 49–62). These modest differences between CeTeG/NOA-09 and CENTRIC—another trial for patients with newly diagnosed glioblastoma, with recruitment restricted to patients who had tumours with methylated *MGMT* promoter (same *MGMT* test and identical cutoff values to CeTeG/NOA-09 trial)—might not be accounted for by improvements in further lines of therapy; the portfolio of available therapies did not change between CENTRIC and CeTeG/NOA-09, except for the availability of tumour-treating fields, which had not been applied to any patient in our study. Part of the differences might be explained by the high rate of patients with complete resection and patients with high performance score in our study. Additionally, age was restricted to less than 70 years in CeTeG/NOA-09, but not in CENTRIC. These features indicate that the results of our trial cannot be readily extrapolated and generalised to an unselected patient population. It would be straightforward to apply these results to patients with an at least partially resected tumour or a very high KPS, although the subgroups of patients who had biopsy alone and lower KPS were too small to allow a meaningful subgroup analysis. Because of limitations of the methylation-specific PCR,[31] many neurooncological centres nowadays use

pyrosequencing[32,33] for the determination of the methylation status of the *MGMT* promoter. Nevertheless, the quantitative methylation-specific PCR[22] applied here was the method of choice for many large randomised glioma trials[2,6,7,23,34] and was the only certified method for *MGMT* promoter methylation analysis at the time when CeTeG/NOA-09 started.

Toxicity of lomustine-temozolomide was acceptable, increasing moderately only in a few domains (eg, haematological adverse events), compared with that of temozolomide. Classic lomustine-associated organ toxic effects, such as hepatopathy, were not observed. The vascular adverse events that occurred predominantly late and with a long interval after termination of first-line therapy (ischaemic stroke and pulmonary embolism) have to be noted, and future cohorts of patients treated with lomustine-temozolomide should be systematically and prospectively analysed for such events. Such an analysis should consider the potential relationship with supportive therapy such as steroids and further lines of antitumour therapy, especially bevacizumab, both known to increase the rate of vascular adverse events.

In conclusion, the data of the CeTeG/NOA-09 trial showed an overall survival benefit of lomustine-temozolomide versus temozolomide alone in the context of moderate toxicity. This conclusion might be somewhat restricted because of the limitations of the trial, most prominently its small size and, by association, its susceptibility to confounding factors. Nevertheless, lomustine-temozolomide might be a promising therapeutic option for patients younger than 70 years who have glioblastoma with methylated *MGMT* promoter. The CeTeG/NOA-09 trial, with its positive results on overall survival, provides an example of molecular subgroup-specific therapy for glioblastoma and further optimisation of combination chemotherapy for those with methylated *MGMT* promoter.

Contributors

UH, MSi, MC, RF, CC, and MG were responsible for the design of the trial. UH, TT, FM, JPS, US, MSa, PH, R-DK, DK, OG, RG, OS, OB, MU, CSe, GT, TK, FR, FS-G, BS, SB, AW, MR, LB, NG, PV, MM, HV, MSt, NS, SK, JW, CSc, WS, JCT, MSi, and MG contributed to patient recruitment, treatment, and data collection. MC and CC provided administrative support, TP did the reference neuropathology, and VCK, MN, HU, and EH did the reference neuroradiology. UH, TT, NS, JW, WW, MW, RF, MSc, CC, and MG did the data analysis and writing of the first drafts. All authors approved the final version of the manuscript.

Declaration of interests

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