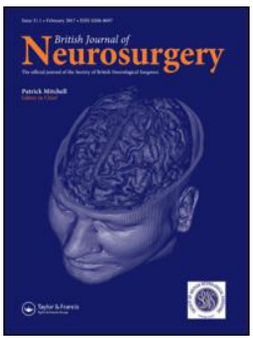

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ORIGINAL ARTICLE

The addition of chloroquine and bevacizumab to standard radiochemotherapy for recurrent glioblastoma multiforme

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ABSTRACT

Introduction: Hypoxia-induced autophagy leads to an increase in vasculogenic-mimicry (VM) and the development of resistance of glioblastoma-cells to bevacizumab (BEV). Chloroquine (HCQ) inhibits autophagy, reduces VM and can thus produce a synergistic effect in anti-angiogenic-therapy by delaying the development of resistance to BEV.

Purpose: We retrospectively compared the combined addition of HCQ+BEV and adjuvant-radiochemotherapy (aRCT) to aRCT alone for recurrent-glioblastoma (rGBM) in regards of overall survival (OS).

Methods: Between 2006 and 2016, 134 patients underwent neurosurgery for rGBM at our institution. Forty-two patients (Karnofsky-Performance-Score>60%) with primary-glioblastoma underwent repeat-surgery and aRCT for recurrence. Four patients (9.5%) received aRCT+HCQ+BEV. Five patients received aRCT+BEV.

Results: In rGBM-patients who were treated with aRCT+HCQ+BEV, median OS was 36.57 months and median post-recurrence-survival (PRS) was 23.92 months while median PRS in the control-group was 9.63 months (p=0.022). In patients who received aRCT+BEV, OS and PRS were 26.83 and 12.97 months, respectively.

Conclusions: Although this study was performed on a small number of highly selected patients, it demonstrates a synergistic effect of HCQ+BEV in the treatment of rGBM which previously could be demonstrated based on experimental data. A significant increase of OS in patients who receive aRCT+HCQ+BEV cannot be ruled out and should be further investigated in randomised-controlled-trials.

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Glioblastoma multiforme; chloroquine; bevacizumab; radiochemotherapy; autophagy

Introduction

Glioblastoma multiforme (GBM) accounts for 12–15% of all brain tumours[1] and is associated with an extremely poor diagnosis despite extensive efforts to improve treatment options. Median survival is 14.6 months in patients receiving current standard treatment.[2] GBM has an incidence rate of 2 to 3 new cases per 100,000 population per year. People aged between 50 and 70 years account for the majority of new cases.[3]

Current standard treatment for patients younger than 65 years consists of a combination of radiotherapy at a total radiation dose of 54–60 Gy and chemotherapy with temozolomide in accordance with the Stupp protocol. Since the publication of a study by Perry *et al.*, a combination of radiotherapy and chemotherapy has considerably gained in importance in the management of elderly patients with methylated O6-methylguanine-DNA methyltransferase (MGMT) status.[4] Prior to the study by Perry *et al.*, elderly patients with GBM were commonly treated with radiotherapy alone. Hypermethylation of MGMT was found to have a favourable influence on prognosis.[5,6] Treatment with tumour-treating fields, which involves the use of electromagnetic fields, was reported to increase overall survival (OS) time from 16 months to 20.9 months.[7]

Novel targeted therapies, which have been successfully established in the management of other tumour entities, have thus far not led to a significant improvement in the survival of GBM patients. Especially anti-angiogenic treatment with bevacizumab, a monoclonal antibody to vascular endothelial growth factor (VEGF), did not notably increase OS (16.8 versus 16.7 months; $p = 0.10$) in a phase III trial. It was only associated with significantly longer progression-free survival (PFS: 8.4 versus 4.3 months; $p < 0.001$). For this reason, bevacizumab was not approved in Europe as a treatment for GBM.[8,9] Bevacizumab, however, was found to reduce oedema associated with radiation necrosis.[10]

In an experimental study, Reyes *et al.* investigated an entirely different treatment approach and were able to show that quinacrine, which, like chloroquine, is a quinolone derivative, is effective against GBM cells.[11] Reyes and colleagues implanted C6 rat glioma cells into rats that then underwent chemotherapy with carmustine (bis-chloroethylnitrosourea; BCNU). They found that the addition of a quinolone derivative substantially increased the antineoplastic effect of carmustine.

This finding was confirmed by Briceño *et al.* and Sotelo *et al.* in two prospective randomised studies on GBM patients who

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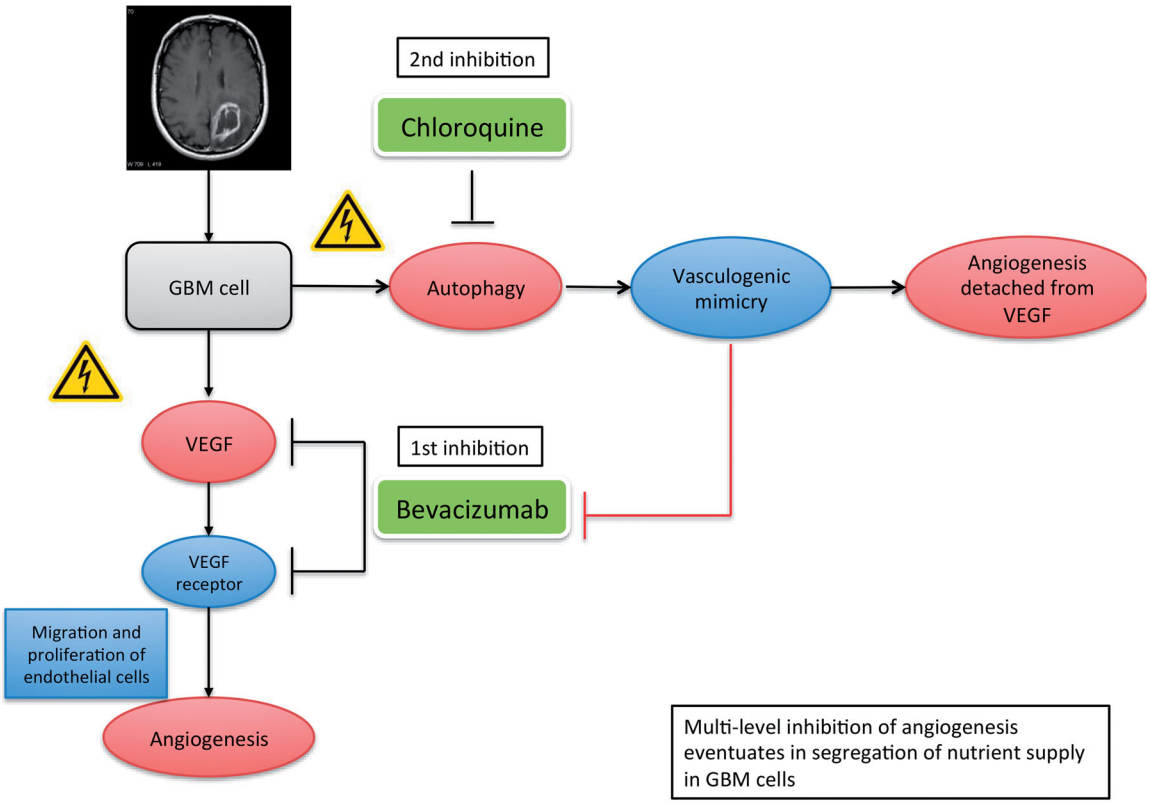

Figure 1. Using the P13K/mTOR signalling pathway shown in this figure, glioblastoma cells produce growth factors such as VEGF. VEGF binds to receptors on endothelial cells and stimulates endothelial cell migration and proliferation. This mechanism enables glioblastoma cells to induce tumour angiogenesis and thus to increase nutrient supply. Bevacizumab is a monoclonal IgG1 antibody to VEGF and binds to both free VEGF molecules and VEGF receptors on endothelial cells. As a result, bevacizumab can block tumour angiogenesis and restrict nutrient supply to the tumour. Glioblastoma cells, however, are able to make bevacizumab ineffective by inducing vasculogenic mimicry (VM). VM can be regarded as a resistance mechanism that is induced by autophagy processes, which in turn can be inhibited by chloroquine.

received combined radiochemotherapy with carmustine and in addition adjuvant chloroquine (150 mg/day) (Briceño *et al.*[12]: OS 33 versus 11 months; Sotelo *et al.*[13]: OS 24 versus 11 months). The study by Sotelo *et al.* was a randomised, placebo-controlled and double-blind trial.

In August 2017, Wu *et al.* conducted an experimental study and offered a possible explanation as to why the addition of bevacizumab failed to improve OS in the AVAglio study by Chinot *et al.*[8] Wu *et al.* found that GBM stem cells can change the angiogenesis of tumour vessels in such a way that angiogenesis is independent of VEGF – a process referred to as vasculogenic mimicry.[14] As a result, bevacizumab becomes ineffective. Vasculogenic mimicry can be regarded as a mechanism of resistance of GBM stem cells to bevacizumab and is caused by hypoxia-induced autophagy. At the same time, inhibition of hypoxia-induced GBM stem cell autophagy was found to delay or even inhibit vasculogenic mimicry. In an experimental setting, autophagy was inhibited genetically by the knockout of autophagy-related 5 (ATG5) or ATG9A or pharmacologically by the administration of chloroquine.[14,15]

Mechanisms of autophagy help cells regulate their lifespan in an energetically efficient manner by maintaining a balance between the production and degradation of cell components. Dysregulation of autophagy is involved in carcinogenesis. 'Programming errors' are therefore a potential target for tumour therapies. In experimental studies, the inhibition of autophagy by chloroquine was reported to improve the efficacy of temozolomide and radiation therapy. In addition, chloroquine alone was found to prolong GBM stem cell survival.

These studies also demonstrated a synergistic effect of combined treatment with chloroquine and bevacizumab. In 2012, Hu and colleagues were the first to explore the hypothesis that autophagy inhibition has a synergistic effect on anti-angiogenic therapy.[16] This effect is graphically displayed in Figure 1.

In a phase I/II trial, Rosenfeld *et al.* confirmed that chloroquine was able to augment the DNA-damaging effects of temozolomide in GBM patients and showed that 600 mg/day was the maximum tolerated dose (MTD).[17] Experience with chloroquine, which has been used for the past 60 years in the management of malaria and rheumatoid disorders, has shown that chloroquine and its derivative hydroxychloroquine (HCQ), which can cross the blood–brain barrier, are able to induce the inhibition of cellular autophagy.[18] Rosenfeld *et al.* used electron microscopy to demonstrate a significant accumulation of intracellular vacuoles in patients who received HCQ in addition to standard therapy over a period of 9 weeks. The authors interpreted this finding as evidence of an augmentation of autophagy inhibition. This was a dose-dependent effect, which was not achieved at the maximum tolerated dose of 600 mg of HCQ per day. For this reason, the American Brain Tumor Consortium (ABTC) 0603 phase I/II study by Rosenfeld *et al.* did not show a significant improvement in survival for patients who received HCQ in addition to radiotherapy and chemotherapy when compared to patients who underwent radiotherapy in combination with temozolomide in a European Organisation for Research and Treatment of Cancer (EORTC) phase III study.[2,17]

In the present study, we report our first clinical experience with the treatment of patients with recurrent GBM using a

combination of bevacizumab and HCQ in addition to standard therapy on the basis of the Stupp protocol. The objective of this study was to underline the experimentally observed synergistic effects of this augmentative pharmaceutical combination due to clinical setting and to provide a basis for prospective randomised controlled trials.

Materials and methods

In this single-centre retrospective study, we investigated three groups of patients who were treated for recurrent glioblastoma at the Department of Neurosurgery of the University Hospital of Schleswig–Holstein in Luebeck between 2006 and 2016. The objective of treatment was to prolong survival and to preserve the patients' quality of life (Karnofsky Performance Score >60).[19] GBM patients received standard treatment plus bevacizumab alone or in combination with HCQ for indications other than those for which the medications are approved. Since this treatment approach was considered appropriate as a salvage therapy in these individual cases, it was both ethically and legally justified. The patients or their legally authorised representatives gave their written informed consent to this form of treatment.

Endpoints

In this study, the primary endpoint was post-recurrence survival (PRS). The second endpoint was OS of patients undergoing treatment for recurrent GBM with or without additional augmentative treatment with bevacizumab alone or in combination with HCQ.

Patients

A total of 134 adult GBM patients (over the age of 18 years) were screened for inclusion in the study. Of these, 52 patients underwent repeat surgery for recurrence. Ten patients with secondary GBM were excluded. The remaining 42 patients presented with primary GBM. Further exclusion criteria were a history of hepatic or renal dysfunction, haematopoietic disorders, previous other malignancies, pregnancy, retinopathy, cataract, a Karnofsky Performance Score <60 or refractory disease concomitant with the missing response to first-line adjuvant radiochemotherapy. The inclusion period (2006 – 2016) has been selected as a result of the implementation of the Stupp protocol as the standard of care in 2006. Moreover, the opportunity for an additional treatment modification was provided since 2006 as well.

At the occurrence of relapse, the option of the additive administration of bevacizumab and/or HCQ in an adjuvant setting was discussed with the patients and their relatives based on findings from recent publications if salvage treatment was appropriate. Detailed elucidation referring to this individual salvage treatment approach has been performed in this context. Therefore, only patients who met the abovementioned inclusion criteria and those who consented to the offered proceeding were eligible for inclusion into the first or the second subgroup in the current study. The control group was composed of GBM patients at relapse who also met the abovementioned inclusion criteria but denied further treatment modifications.

The first group of patients ($n = 4$) was treated with a combination of bevacizumab and HCQ in addition to radiochemotherapy. The second group of patients ($n = 5$) received only bevacizumab in addition to the Stupp protocol for radiochemotherapy. The control group consisted of 33 patients who were treated with standard adjuvant temozolomide and radiation therapy for recurrent GBM (Figure 2).

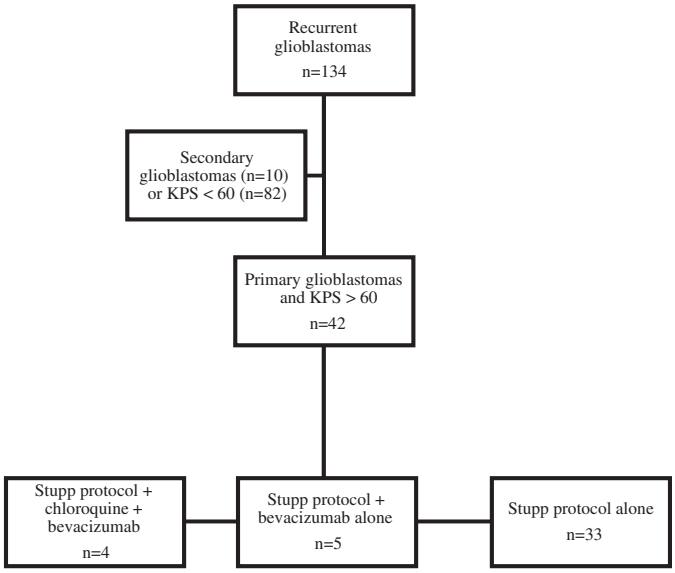

Figure 2. Ninety-two of 134 patients were excluded from the study because they had a Karnofsky Performance Score of <60 or secondary glioblastomas. The remaining 42 patients with recurrent GBM were included in the study. Of these, four patients were treated with a combination of bevacizumab and HCQ and five patients received only bevacizumab in addition to standard therapy according to the Stupp protocol. The control group consisted of 33 patients who were treated with standard adjuvant therapy on the basis of the Stupp protocol.

Patient characteristics were collected and included age, sex and comorbidities (i.e. arterial hypertension, type 2 diabetes mellitus, and thyroid dysfunction). According to the 2016 World Health Organization (WHO) classification of brain tumours, tumour-related patient characteristics are O^6-methylguanine-DNA methyl-transferase (MGMT) status, isocitrate-dehydrogenase 1/2 (IDH1/2) status, the presence or absence of 1p/19q codeletion, and p53 status. The intracranial location of GBM and the extent of surgical resection were documented as well.[20]

Treatment and response to treatment

Patients received intravenous bevacizumab at a dose of 10 mg/kg body weight at 3-week intervals. HCQ was administered orally in tablet form (Resochin) at a dose of 250 mg/day.[21] Patients received bevacizumab alone ($n = 5$) or in combination with HCQ ($n = 4$) at the aforementioned doses in addition to radiochemotherapy for recurrent GBM according to the Stupp protocol.

Treatment was discontinued for 1 week in patients with grade 2 cytopenia as defined by the National Cancer Institute Common Toxicity Criteria (NCI CTC), version 2.0. Likewise, treatment was temporarily discontinued in patients with CTC grade 2 or grade 3 non-haematologic toxicities.[22] In these cases, treatment was resumed once toxicity-related symptoms were consistent with CTC grade 1 or 0.

Positive treatment response was defined as the persistent absence of GBM progression under adjuvant therapy. The recommended treatment approach is maximal tumour debulking followed by adjuvant therapy that has the objective to maintain the benefits of surgical resection. Depending on the extent of surgical tissue removal, patients have a biopsy only, or a partial, subtotal or complete resection.

Patients underwent routine cranial magnetic resonance imaging (cMRI) every 3 months at follow-up examinations. When

patients showed worsening signs and symptoms, radiological assessments were performed earlier. Clinical follow-up examinations were performed at 2-week intervals or at shorter intervals when patients presented with therapy-associated toxicities or tumour progression.

Statistical analysis

PRS was calculated from the date of treatment initiation and OS from the time of initial diagnosis. Survival was estimated using the Kaplan–Meier method. The log-rank test was used for comparing groups. All statistical analyses were conducted with GraphPad Prism 6 and SPSS. We performed a separate subgroup analysis of patients receiving bevacizumab (alone or in combination with HCQ) as a salvage treatment that was considered appropriate in these individual cases.

Patient characteristics

Of the 42 GBM patients who were included in the study, nine (21.43%; three males and six females, median age: 49 years, range: 32–61 years) received bevacizumab in addition to combined radiotherapy and chemotherapy for recurrent GBM. Four of these nine patients were treated with a combination of bevacizumab and HCQ in addition to radiochemotherapy. The other five patients received additional treatment with bevacizumab alone.

None of the patients who received additional treatment had type 2 diabetes mellitus or thyroid disease. Three of the four patients who were treated with a combination of bevacizumab and HCQ and one patient who received standard therapy and bevacizumab alone had arterial hypertension. In the control group of patients ($n = 33$) who underwent radiochemotherapy alone, there were 13 patients (39.4%) with arterial hypertension, 6 patients (18.2%) with type II diabetes mellitus, and 1 patient (3.0%) with thyroid disease. The presence of comorbidity did not have a significant influence on prognosis.

Complete tumour resection was possible in six of the nine patients who were treated additionally to radiochemotherapy. Partial resection was achieved in two patients and subtotal resection in one patient. In the control group, 23 of the 33 GBMs were completely resected. Subtotal resection was achieved in seven patients and partial resection in a further three patients. A stereotactic biopsy was not performed in the control group (Table 1).

Five of the nine GBMs that were treated with bevacizumab involved the parietal lobe, three GBMs the frontal lobe, and one GBM the occipital lobe. In the control group, eleven of the 33 GBMs were located at parietal sites, nine at frontal sites, seven at occipital sites, and six at temporal sites.

All nine patients received the additional medications at first recurrence. Since GBM is associated with a dismal prognosis and rapid worsening, we thoroughly informed the patients and their families about the use of medications in addition to evidence-based treatment on the basis of the Stupp protocol and discussed the risks and potential benefits on the basis of available data. We then decided together whether salvage treatment was appropriate and ethically and legally acceptable in each individual case.

Histopathology

The histopathological examination focused on MGMT status, p53 mutations and the presence or absence of 1p/19q codeletion. All patients who received bevacizumab in addition to standard therapy for recurrent GBM were MGMT-negative. In the control group ($n = 33$), seven patients were positive for MGMT

Table 1. Patient characteristics.

	Chloroquine and bevacizumab (Avastin) ($n = 4$)	Bevacizumab alone ($n = 5$)	Control group ($n = 33$)
Median age and range	56 (44–61)	47 (32–60)	61 (27–70)
Sex			
Female	2 (50.0%)	4 (80.0%)	14 (42.4%)
Male	2 (50.0%)	1 (20.0%)	19 (57.6%)
MGMT status			
Methylated	0 (0.0%)	0 (0.0%)	5 (15.2%)
Unmethylated	4 (100.0%)	5 (100.0%)	28 (84.8%)
1p/19q codeletion			
Yes	0 (0.0%)	0 (0.0%)	0 (0.0%)
No	4 (100.0%)	5 (100.0%)	33 (100.0%)
p53 status			
Positive	2 (50.0%)	3 (60.0%)	8 (24.2%)
Negative	2 (50.0%)	2 (40.0%)	25 (75.8%)
IDH 1/2 mutation			
Yes	0 (0.0%)	1 (20.0%)	4 (12.1%)
No	4 (100.0%)	4 (80.0%)	29 (87.9%)
Tumour location			
Frontal	1 (25.0%)	2 (40.0%)	4 (12.1%)
Temporal	0 (0.0%)	0 (0.0%)	10 (30.3%)
Parietal	2 (50.0%)	3 (60.0%)	5 (15.2%)
Occipital	1 (25.0%)	0 (0.0%)	3 (9.1%)
Extent of resection			
Complete	3 (75.0%)	3 (60.0%)	23 (69.7%)
Subtotal	0 (0.0%)	1 (20.0%)	7 (21.2%)
Partial	1 (25.0%)	1 (20.0%)	3 (9.1%)
Biopsy only	0 (0.0%)	0 (0.0%)	0 (0.0%)
Comorbidities			
Arterial hypertension	3 (75.0%)	1 (20.0%)	13 (39.4%)
Type 2 diabetes mellitus	0 (0.0%)	0 (0.0%)	6 (18.2%)
Hypothyroidism	0 (0.0%)	0 (0.0%)	1 (3.0%)

Abbreviations. MGMT: methylated O6-methylguanine-DNA methyltransferase; IDH: isocitrate dehydrogenase.

methylation and thus had a better prognosis and response to treatment with temozolomide. Since the study included only primary GBM, none of the patients demonstrated prognostically favourable 1p/19q codeletion, which suggests the presence of a secondary GBM of oligodendroglial origin. P53 mutations were detected in five of nine patients (55.5%) who received bevacizumab alone or in combination with HCQ in addition to standard treatment. In the control group, p53 mutations were observed in 8 of 33 patients (24.2%). IDH1/2 mutations were observed in four patients of the control group (12.1%) and in one patient that has been treated with a bevacizumab-based regimen (11.1%). Table 1 provides an overview of histopathological findings.

Treatment and toxicity

Treatment with bevacizumab alone or in combination with HCQ in addition to standard therapy was generally well tolerated. The main side effects were haematological in nature. One patient who was treated with bevacizumab developed a neutropenic fever. Treatment was continued uninterrupted. In one patient, the combined use of bevacizumab and HCQ led to bicytopenia (thrombocytopenia and neutropenia) and required discontinuation of HCQ for a week. There were no cases of retinopathy, which is a commonly known complication of treatment with HCQ. In the control group, three patients who received treatment with temozolomide developed lymphocytopenia with opportunistic infections. Prolonged NCI grade 3 thrombocytopenia was observed in one patient from the control group. This patient required repeated transfusions.

Outcome and effectiveness

All patients included in the study eventually died from GBM. Median follow-up was 18.50 months (mean: 23.62 months, range: 6.30–73.86 months). Median follow-up was 31.82 months (range: 9.80–52.86 months) in the group of patients who received only bevacizumab in addition to standard therapy and 39.07 months (range: 15.13–63.76 months) in the group of patients who underwent treatment with bevacizumab and HCQ.

There was no significant difference between the patient groups in PFS or, in other words, before treatment with bevacizumab alone or in combination with HCQ was initiated. Median PFS was 10.70 months for patients who received standard therapy plus bevacizumab and HCQ for recurrent GBM, 20.61 months for patients who were treated with standard therapy and bevacizumab alone for recurrent GBM ($p = 0.109$), and 10.25 months for the control group ($p = 0.733$).

Median PRS was 12.97 months for patients who received bevacizumab alone and 23.92 months for patients who were treated with bevacizumab plus HCQ. Median PRS in the control group was 9.63 months. Median OS was 26.83 months in patients who underwent standard therapy and bevacizumab and 36.57 months in patients who received bevacizumab and HCQ in addition to standard therapy. Median OS for the control group was 17.52 months (Figure 3).

In spite of the small number of patients, post-recurrence survival was significantly longer in patients who received bevacizumab and HCQ in addition to standard treatment when compared to patients in the control group ($p = 0.022$). In addition, there was a trend towards longer PRS in GBM patients who received a combination of bevacizumab and HCQ when compared to patients who received only bevacizumab in addition to standard therapy. This difference, however, did not reach statistical significance ($p = 0.1126$). Median PRS was 9.70 months in patients with secondary and 10.10 months in primary GBM (HR: 2.956, 95% CI: 1.123–5.431; $p = 0.0391$). The median OS of GBM patients who did not undergo surgery for recurrence was 9.63 months.

Discussion

The management of glioblastoma is a particular interdisciplinary challenge in the field of neuro-oncology since no major improvements in the prognosis for GBM patients have thus far been achieved despite extensive research work.

A variety of treatment options have been tested in phase II trials but did not have the hoped-for success in terms of a significant and marked prolongation of survival. Available studies reported significant results but provided only marginal improvements in prognosis. In particular, there is a lack of evidence-based alternative treatments for patients with recurrent GBM.

Novel targeted therapies, which have proven useful for example in the management of haematologic neoplasms, have also been effective in treating a variety of solid tumours. We believe that, despite the complexity of GBMs, this form of treatment will be an important element of future therapeutic approaches for this entity. A combination of medications and other therapeutic agents may effectively address the ability of GBM to adapt to treatment. Currently, available studies suggest that targeted therapy alone does not appear to be an adequate treatment option for GBM patients. Lessons learned in oncology have repeatedly demonstrated that aggressive tumours often necessitate equally aggressive treatment. Research efforts in (neuro-) oncology and related disciplines must focus on developing treatment regimens that can achieve cytoreduction and at the same time are associated with side effects that are acceptable to patients.

As mentioned before, a median OS of 14.6 months is reported for GBM patients in the literature.[2] In the group of patients who had a Karnofsky Performance Score (KPS) >60, the prognosis was much better since these patients were suitable for repeat surgical resection. We were able to reproduce this finding using our data (KPS > 60, median OS: 17.5 months; KPS < 60, median OS: 6.73 months; $p < 0.0001$). In the present study, we investigated patients with a KPS >60, which is associated with a better prognosis than a KPS <60, in order to assess whether the addition of bevacizumab (Avastin) alone or bevacizumab plus hydroxychloroquine to combined radiochemotherapy with temozolomide can significantly prolong PRS.

As a result of the retrospective study design and the small number of patients, we applied strict inclusion criteria in order to ensure the best possible comparability of the different groups of patients. The small sample size can be explained by the use of a salvage therapy that is ethically acceptable in individual cases. There are only a small number of patients who meet the criteria for this type of treatment and at the same time, there are a large number of patients who refuse this treatment because of the dismal prognosis and the absence of evidence in support of these forms of treatment.

There were no significant differences in median PFS between the groups. The potential comparability of the groups was demonstrated by the fact that the investigated patients, who presented with recurrent GBM, had a KPS >60 and similar median PFS and, in addition, did not show significant differences in other characteristics (age, arterial hypertension, diabetes mellitus, thyroid diseases).

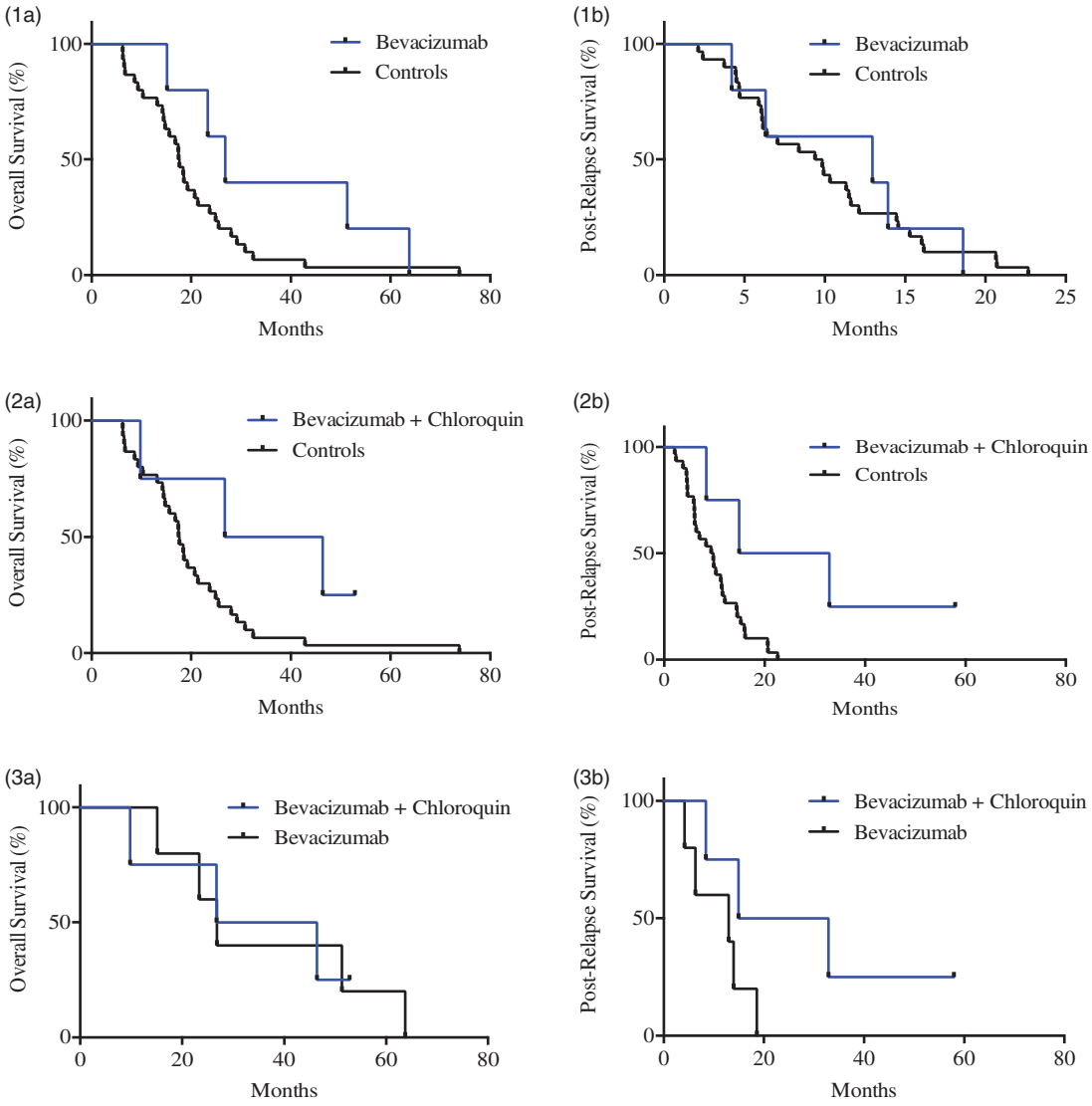

Figure 3. Kaplan–Meier survival curves. (1a) Cumulative overall survival (OS). (1b) Cumulative post-recurrence survival (PRS) in patients who received standard therapy plus bevacizumab and in patients who received standard therapy alone (Stupp protocol). (2a) Cumulative overall survival (OS). (2b) Cumulative post-recurrence survival (PRS) in patients who received standard therapy plus a combination of bevacizumab and HCQ and in patients who received standard therapy alone (Stupp protocol). (3a) Cumulative overall survival (OS). (3b) Cumulative post-recurrence survival (PRS) in patients who received radiochemotherapy with temozolomide plus a combination of bevacizumab and HCQ and in patients who were treated with standard therapy and bevacizumab alone.

The difference in median OS was not significant between the control group (17.52 months) and the two groups of patients who received additional treatment. There was, however, a trend towards longer survival in patients receiving additional treatment (HCQ and bevacizumab: 36.57 months, $p = 0.0811$; hazard ratio: 0.38, 95% confidence interval: 0.196–1.054; bevacizumab alone: 26.83 months, $p = 0.134$, hazard ratio: 0.51, 95% confidence interval: 0.593–3.937). Larger sample sizes are required for differences to reach significance. However, it appears unlikely that the level of significance can be reached if a larger group of GBM patients who receive additional treatment with bevacizumab alone is investigated as previous studies could demonstrate.[8] Until recurrence, the three subgroups of patients had shown similar results, such as median PFS.

Treatment with bevacizumab and HCQ in addition to standard therapy would most likely be expected to result in a possible increase in median OS if it were initiated at the time of diagnosis or, in a larger patient sample, even at recurrence.

An analysis of PRS showed that additional treatment with bevacizumab and HCQ led to a significant increase in survival when compared with PRS in the control group (23.92 versus 9.63 months; $p = 0.021$). Additional treatment with bevacizumab alone did not result in a significant increase in median PRS (12.97 versus 9.63 months; $p = 0.841$). A direct comparison of the two additional treatment options showed that the combined use of bevacizumab and HCQ led to a longer median PRS than bevacizumab alone. This difference, however, did not reach statistical significance ($p = 0.113$). Our study thus nearly reproduced the results reported in the AVAglio study by Chinot *et al.* for additional treatment with bevacizumab in patients with recurrent GBM.[8,9] Additional treatment with bevacizumab alone thus can be recommended neither for patients with newly diagnosed GBM nor for patients with recurrent GBM.

Recent molecular experimental approaches to explain why bevacizumab, as a VEGF inhibitor, is effective only for a limited time appear to be correct. Autophagy inhibition with HCQ appears to prolong anti-angiogenic treatment since it suppresses resistance mechanisms, that is, vasculogenic mimicry that allows GBM cells to form vessels independently of VEGF.[14,15,17] The study by Rosenfeld *et al.* suggests that 800 mg of HCQ is the

minimum daily dose required to achieve adequate autophagy inhibition in GBM cells. This dose level is higher than the maximum tolerated dose (600 mg/day). A higher degree of autophagy could be achievable if the HCQ dose were 600mg/day during the period of radiochemotherapy and then dose-escalated in adjuvant temozolomide setting.[17] The clinical data presented in this study appear to confirm the augmented effects of HCQ in combination with bevacizumab which were reported for the first time in 2012 in an experimental setting.[16]

This study demonstrated trends suggesting that the combined use of bevacizumab and HCQ in addition to standard therapy prolongs survival when compared to a control group that underwent standard radiochemotherapy. Although these trends, with the exception of PRS, did not reach significance, they may be expected to become significant when larger patient samples are investigated. Our study showed that HCQ is an effective and well-tolerated substance that can be combined with other chemotherapeutic drugs or targeted therapies in the treatment of GBM. It can delay the development of the resistance of GBM cells to cytoreductive treatment and can thus prolong the effectiveness of therapeutic agents.

Conclusions

In spite of the highly selected patient sample and the small number of patients, our results emphasise the need for a prospective evaluation of treatment with a combination of HCQ and bevacizumab in addition to standard therapy on the basis of the Stupp protocol. In addition, the optimal intensity of treatment must be determined. Available data strongly suggest that the augmented effects of this type of additional treatment can be expected to significantly prolong survival. For this reason, GBM patients will likely benefit from further clinical studies involving larger sample sizes and a prospective randomised study design.

Ethical approval and consent to participate

This retrospective study was approved by the ethics committee of the University of Luebeck (reference no. 19-067A) and conducted in accordance with the declaration of Helsinki. Patients had given written informed consent regarding the routine diagnostic and academic assessment of their biopsy specimen at the Department of Pathology and transfer of their clinical data.

Author contributions

Study conception and drafting of the manuscript: HW and JL. Data collection and analysis, revision of the manuscript: HW, AR, KS, NG, and JL. Initial drafting of manuscript: HW. Revision and final approval of manuscript: all authors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

Data supporting the conclusions of this article are included in the article.

References

1. Hess KR, Broglio KR, Bondy ML. Adult glioma incidence trends in the United States, 1977-2000. *Cancer* 2004;101:2293–9.
2. Stupp R, Mason WP, van den Bent MJ, *et al.* Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
3. Ohgaki H, Kleihues P. Epidemiology and etiology of gliomas. *Acta Neuropathol* 2005;109:93–108.
4. Perry JR, Laperriere N, O'Callaghan CJ, *et al.* Short-course radiation plus temozolomide in elderly patients with glioblastoma. *N Engl J Med* 2017;376:1027–37.
5. Hegi ME, Diserens A-C, Gorlia T, *et al.* MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352:997–1003.
6. Hermisson M, Klumpp A, Wick W, *et al.* O6-methylguanine DNA methyltransferase and p53 status predict temozolomide sensitivity in human malignant glioma cells. *J Neurochem* 2006;96:766–76.
7. Stupp R, Taillibert S, Kanner A, *et al.* Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA* 2017;318:2306–16.
8. Chinot OL, de La Motte Rouge T, Moore N, *et al.* AVAglio: phase 3 trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme. *Adv Ther* 2011;28:334–40.
9. Chinot OL, Wick W, Mason W, *et al.* Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *N Engl J Med* 2014;370:709–22.
10. Levin VA, Bidaut L, Hou P, *et al.* Randomized double-blind placebo-controlled trial of bevacizumab therapy for radiation necrosis of the central nervous system. *Int J Radiat Oncol Biol Phys* 2011;79:1487–95.
11. Reyes S, Herrera LA, Ostrosky P, Sotelo J. Quinacrine enhances carmustine therapy of experimental rat glioma. *Neurosurgery* 2001;49:969–73.
12. Briceno E, Reyes S, Sotelo J. Therapy of glioblastoma multiforme improved by the antimutagenic chloroquine. *FOC* 2003;14:e3–6.
13. Sotelo J, Briceno E, Lopez-Gonzalez MA. Adding chloroquine to conventional treatment for glioblastoma multiforme: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2006;144:337–43.
14. Wu H-B, Yang S, Weng H-Y, *et al.* Autophagy-induced KDR/VEGFR-2 activation promotes the formation of vasculogenic mimicry by glioma stem cells. *Autophagy* 2017;13:1528–42.
15. Abdul Rahim SA, Dirkse A, Oudin A, *et al.* Regulation of hypoxia-induced autophagy in glioblastoma involves ATG9A. *Br J Cancer* 2017;117:813–25.
16. Hu Y-L, Jahangiri A, De Lay M, *et al.* Hypoxia-induced tumor cell autophagy mediates resistance to anti-angiogenic therapy. *Autophagy* 2012;8:979–81.
17. Rosenfeld MR, Ye X, Supko JG, *et al.* A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy* 2014;10:1359–68.
18. LactMed. Chloroquine. In: *Drugs and Lactation Database (LactMed)*. Bethesda (MD): LactMed; 2006.
19. Karnofsky DA, Burchenal JH. Present status of clinical cancer chemotherapy. *Am J Med* 1950;8:767–88.
20. Louis DN, Perry A, Reifenberger G, *et al.* The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol* 2016;131:803–20.
21. LactMed. Bevacizumab. In: *Drugs and Lactation Database (LactMed)*. Bethesda (MD): LactMed; 2006.
22. Trotti A, Byhardt R, Stetz J, *et al.* Common toxicity criteria: version 2.0. an improved reference for grading the acute effects of cancer treatment: impact on radiotherapy. *Int J Radiat Oncol Biol Phys* 2000;47:13–47.