Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial

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Lancet Oncol 2006; 7: 392–401
Published Online April 13, 2006
DOI:10.1016/S1470-2045(06)70665-9

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Summary

Background **5-aminolevulinic acid is a non-fluorescent prodrug that leads to intracellular accumulation of fluorescent porphyrins in malignant gliomas—a finding that is under investigation for intraoperative identification and resection of these tumours. We aimed to assess the effect of fluorescence-guided resection with 5-aminolevulinic acid on surgical radicality, progression-free survival, overall survival, and morbidity.**

Methods **322 patients aged 23–73 years with suspected malignant glioma amenable to complete resection of contrast-enhancing tumour were randomly assigned to 20 mg/kg bodyweight 5-aminolevulinic acid for fluorescence-guided resection (n=161) or to conventional microsurgery with white light (n=161). The primary endpoints were the number of patients without contrast-enhancing tumour on early MRI (ie, that obtained within 72 h after surgery) and 6-month progression-free survival as assessed by MRI. Secondary endpoints were volume of residual tumour on postoperative MRI, overall survival, neurological deficit, and toxic effects. We report the results of an interim analysis with 270 patients in the full-analysis population (139 assigned 5-aminolevulinic acid, 131 assigned white light), which excluded patients with ineligible histological and radiological findings as assessed by central reviewers who were masked as to treatment allocation; the interim analysis resulted in termination of the study as defined by the protocol. Primary and secondary endpoints were analysed by intention to treat in the full-analysis population. The study is registered at http://www.clinicaltrials.gov as NCT00241670.**

Findings **Median follow-up was 35·4 months (95% CI 1·0–56·7). Contrast-enhancing tumour was resected completely in 90 (65%) of 139 patients assigned 5-aminolevulinic acid compared with 47 (36%) of 131 assigned white light (difference between groups 29% [95% CI 17–40], p<0·0001). Patients allocated 5-aminolevulinic acid had higher 6-month progression free survival than did those allocated white light (41·0% [32·8–49·2] *vs* 21·1% [14·0–28·2]; difference between groups 19·9% [9·1–30·7], p=0·0003, *Z* test). Groups did not differ in the frequency of severe adverse events or adverse events in any organ system class reported within 7 days after surgery.**

Interpretation **Tumour fluorescence derived from 5-aminolevulinic acid enables more complete resections of contrast-enhancing tumour, leading to improved progression-free survival in patients with malignant glioma.**

Introduction

Malignant gliomas are locally invasive tumours that have poor prognosis despite treatment with a combination of surgery, radiotherapy, and chemotherapy. A trial by the European Organisation for Research and Treatment of Cancer (EORTC)[1] showed that overall survival was 2 months higher with concomitant adjuvant radiochemotherapy with temozolomide followed by adjuvant temozolomide than with radiotherapy alone. Further analyses[2] showed that patients who had previously had complete resection derived the most benefit from the temozolomide regimen compared with those who had had incomplete resection (4·1 months *vs* 1·8 months overall survival). Thus, in addition to the survival benefit associated with maximum cytoreductive surgery,[3–7] such surgery seems essential for the efficacy of modern adjuvant treatment. However, past surgical studies[4–9] have noted that complete resection of contrast-enhancing tumour is achieved in fewer than 20% of patients, suggesting difficulty in defining marginal, enhancing tumour intraoperatively.[4]

5-aminolevulinic acid is a natural biochemical precursor of haemoglobin that elicits synthesis and accumulation of fluorescent porphyrins in various epithelia and cancerous tissue;[10] it also results in accumulation of porphyrins within malignant glioma tissue. Porphyrin fluorescence can be visualised by use of a modified neurosurgical microscope, and has been investigated for identification of residual malignant glioma intra-operatively, with the aim of improving surgery.[11,12] Because of its intratumoral synthesis, 5-aminolevulinic acid differs from other fluorescing agents that have been investigated for tumour discrimination such as fluorescein,[13] which penetrates malignant gliomas via the defective blood–brain barrier.

We aimed to do a randomised controlled trial to assess the use of porphyrin fluorescence in malignant glioma after administration of 5-aminolevulinic acid for

improving resection as defined by postoperative MRI, and to analyse the effect of resection on progression-free survival, neurological morbidity, and type and frequency of treatment after progression.

To allow premature termination of the study, a confirmatory interim analysis was scheduled after 270 of 350 planned patients had been recruited in the full-analysis population. This report gives the results of the interim analysis, which resulted in termination of the study as defined in the protocol.

Methods

Patients

Individuals aged 18–72 years with suspected (as assessed by study surgeon), newly diagnosed, untreated malignant glioma and who were eligible for surgery acording to the study surgeon were eligible for trial participation. The protocol stipulated that patients had tumours with a distinct ring-like pattern of contrast-enhancement with thick irregular walls on MRI, and a core area of reduced signal suggestive of tumour necrosis as assessed at the study centre by the study surgeon.

Exclusion criteria, which were assessed before randomisation by study surgeons at every study centre, were: tumours of the midline, basal ganglia, cerebellum, or brain stem as assessed by MRI; more than one contrast-enhancing lesion; substantial, non-contrast-enhancing tumour areas suggesting low-grade glioma with malignant transformation; medical reasons precluding MRI (eg, pacemaker); inability to give consent because of dysphasia or language barrier; tumour location did not enable complete resection of contrast-enhancing tumour as decided by individual study surgeon; Karnofsky performance scale 60 or less; renal insufficiency (ie, creatinine >177 µmol/L); hepatic insufficiency (ie, gamma glutamyl transpeptidase >100 U/L, proton-beam time <60%, and bilirubin >51 µmol/L); and history of malignant tumours at any body site. All patients gave written informed consent, and the study was approved by the ethics committees of the participating centres. The study was done according to the guidelines of the International Conference on Harmonisation—Good Clinical Practice, and the German Medicines Act (AMG).

Treatment

Patients were randomly assigned to 5-aminolevulinic acid (20 mg/kg bodyweight; medac, Wedel, Germany) for fluorescence-guided resection or to conventional microsurgery with white light. Those randomly allocated to 5-aminolevulinic acid were scheduled to receive freshly prepared solutions of 5-aminolevulinic acid orally 3 h (range 2–4) before induction of anaesthesia. Solutions were prepared by dissolving the contents of a vial (1·5 g) in 50 mL of drinking water. Patients randomly allocated to white light did not receive placebo.

The study protocol stipulated resections to be as complete as thought safely feasible by the study surgeons. All resections were done by one of two designated study surgeons at every centre. The designated surgeon for a particular operation was entered into the randomisation scheme as a covariate to ensure that each surgeon did an equal number of operations in both groups. Surgery was done by use of a modified neurosurgical microscope (OPMI Neuro/NC4 system with fluorescence kit, Carl Zeiss Surgical GmbH, Oberkochen, Germany), which enabled switching from conventional white xenon illumination to violet–blue excitation light. For patients assigned white light, the tumour was resected by use of conventional illumination. For patients assigned 5-aminolevulinic acid, the microscope was switched to violet–blue illumination, as desired by the surgeon, for fluorescence visualisation. Other intraoperative diagnostic procedures (eg, sonography or neuronavigation) were permitted only for planning of the surgical approach or for initial tumour localisation.

Pretreatment with 4 mg dexamethasone three times a day (various manufacturers) was obligatory for at least 2 days before surgery (no maximum number of days was stipulated in the protocol), and was continued until early MRI had been obtained (ie, within 72 h after surgery).

For all patients, surgery was to be followed by standard[14] fractionated radiotherapy with a recommended[14] dose to the lesion of 60 Gy and saturation of the peritumoral field with about 20 Gy; radiotherapy was initiated within 14 days of surgery at 2 Gy per dose and five doses a week. The protocol recommended that chemotherapy thereafter should be given only to patients with tumours of known susceptibility to chemotherapy, such as anaplastic oligodendrogliomas. No restrictions were imposed on treatment after disease progression.

Randomisation schedule

After patients had given written informed consent and were found to fulfil all eligibility criteria according to the investigator, the randomisation centre was contacted to obtain a patient number and treatment group. Randomisation was done by a clinical research associate at the clinical research institute Clinstud GmbH, Hamburg, Germany, by use of a dynamic allocation algorithm,[15] in which patients were allocated to keep the imbalance between treatment groups to a minimum at every stage of recruitment within the covariates age (≤55 years *vs* >55 years), Karnofsky performance scale (70–80 *vs* >80), the vicinity of tumour to eloquent brain regions (ie, non-eloquent *vs* eloquent, based on the surgeon's judgment), and study surgeon. No permuted block randomisation was applied.

Treatment allocation was communicated to local investigators first by telephone and additionally by fax, notification by which was sent together with a treatment

schedule for the patient. Central neuropathological and neuroradiological reviewers, who assimilated data for assessment of endpoints, were masked as to treatment allocation. Biopsy samples for neuropathological review were obtained during surgery and placed in designated vials. They were sent directly to the pathology reviewer, and were marked only with randomisation number, the initials of the patient, date of birth, and sex. Findings were recorded on separate case-report forms by the review pathologist, which were compiled at the end of the study and sent to the data management department at medac for archiving; a copy of this form was sent to the surgeon and to the clincial-research institute. To avoid delays in additional treatment after surgery, separate samples were obtained for assessment in study centres.

MRI scans for neuroradiological review were labelled with the intials of the patient, randomisation number, and date of birth, and were sent to the radiological-review institution. MRI findings were recorded on separate case-report forms by the radiological reviewers, which were sent in batches to the data-management department at medac; a copy of this form was sent to the clinical-research institute. After central review, the digitised MRI scans were sent to the clinical-research institute for archiving of all MRI data into a study-imaging database; duplicated data were stored on an optical disc and sent for archiving at medac.

Endpoints and assessment of response

The primary endpoints were the proportion of patients with histologically confirmed malignant glioma on central neuropathological review without residual contrast-enhancing tumour on postoperative MRI, and progression-free survival at 6 months. The secondary endpoints were residual tumour volume on postoperative MRI, overall survival, type and severity of neurological deficits after surgery, and toxic effects.

Early postoperative MRI was obtained within 72 h after surgery; follow-up MRI were obtained 3, 6, 9, 12, 15, and 18 months after surgery by radiologists at the study centres. The protocol stipulated MRI to be done on a 1·5 T scanner with a head coil, and to use 0·1 mmol/kg bodyweight gadolinium-DTPA (diethylene triamine penta-acetic acid) given intravenously immediately before imaging. Unenhanced T1-weighted (ie, 256×256 matrix, rectangular field of view) and T2-weighted (ie, 512×512 matrix, rectangular field of view) axial images were obtained, followed by contrast-enhanced T1-weighted images acquired in three planes—ie, axial, coronal, and sagittal. Slice thickness was 5 mm with a 1 mm (ie, 20%) gap. MRI scans were assessed centrally at the Department of Neuroradiology, University of Frankfurt, Germany, by raters who were masked as to treatment allocation. Assessment was done at a single timepoint by two central radiologists who were required to present a unanimous assessment based on individual image assessment, with differences resolved through simultaneous assessment and professional discussion.

Residual tumour was defined as contrast-enhancement with a volume more than 0·175 cm^3. The cut-off volume was chosen on the basis of personal experience of the reference radiologist, and was the size of one voxel in the T1-weighted image—ie, the minimum resolution obtained on MRI. The cut-off was defined to prevent interpretation problems when distinguishing between tumour enhancement and that of non-specific enhancement (eg, small vessels or enhancing pia mater). The volumes of compact tumours with spherical geometry were calculated by fitting a rotational ellipsoid defined by the maximum tumour diameters in the available three dimensions. The volumes of cup-shaped, residual tumours were calculated by subtracting the volumes of the central-resection defect from the space defined by the outer boundaries of tumours. The volumes of residual tumours with complex configuration were segmented on individual scans and the individual volumes summed for the total volume.

Progression was defined as the occurrence of a new tumour lesion with a volume greater than 0·175 cm^3, or an increase in residual tumour volume of more than 25%. Progression-free survival at 6 months was defined as the proportion of patients without radiological progression at this time. Patients who died from any cause before documented progression were counted as an event for this endpoint. Overall survival was defined as the number of patients who had not died from any cause.

Time to reintervention was defined as the interval between initial surgery and radiotherapy, and the initiation of additive chemotherapy or repeat surgery after progression. The type, frequency, and timepoint of treatment initiated after surgery (ie, radiotherapy, chemotherapy, and repeat surgery) were monitored and analysed in an exploratory way.

Central neuropathological review was done at the Institute for Neuropathology, University Clinic, Bonn, Germany, and was done according to WHO classification for brain tumours.[16]

Adverse events were classified according to the US National Cancer Institute common toxicity criteria (version 1.0).[17] Laboratory tests (ie, erythrocytes, haematocrit, haemoglobin, platelets, leucocytes, creatinine, potassium, sodium, uric acid, urea, prothrombin time, partial thromboplastin time, aspartate aminotransferase, alanine transaminase, total bilirubin, gamma glutamyl transpeptidase, alkaline phosphatase, amylase, lactate dehydrogenase, creatine kinase, and glucose) for analyses of adverse drug reactions were obtained up to 14 days before surgery and at 24 h, 7 days, and 6 weeks after surgery.

General physical performance was recorded by designated study surgeons in the individual centres by

use of the Karnofsky performance scale up to 14 days before surgery until assessment of radiological progression at 6 weeks; and at 3, 6, 9, 12, 15, and 18 months after surgery. For assessment of short-term changes in neurological function as a result of surgery, the US National Institutes of Health stroke score[18–20] was adapted: this score assesses 15 neurological functions, grading the severity of impairment for every function individually. The score was measured by designated study surgeons in individual centres 2 days and 7 days after surgery until aassessment of radiological progression at 6 weeks; and at 3, 6, 9, 12, 15, and 18 months after surgery.

Statistical analysis

We defined the sample size by assuming a 20% increase in complete resections (ie, no residual contrast enhancement on early postoperative MRI)—from 30% in the white-light group to 50% in the 5-aminolevulinic-acid group. These estimates were based on historical data for microsurgery of malignant gliomas, which show complete resections of contrast-enhancing tumour in fewer than 30% of patients.[4–9] A single-centre phase II study[11] of fluorescence-guided resection by use of 5-aminolevulinic acid showed complete resection in 63% of patients who underwent surgery with 5-aminolevulinic acid; thus, we assumed 50% complete resection for 5-aminolevulinic acid.

Estimates for progression were derived from the work of Albert and co-workers,[4] who assessed the progression frequency of malignant gliomas on the basis of early postoperative MRI and CT, and of radiological follow-up. The researchers recorded that 80% of patients with residual tumours that accumulated contrast agent on early postoperative imaging progressed after 6 months, compared with 10% who did not have residual tumour. Because the frequency of residual tumour in the white-light group was expected to be 70%, the anticipated progression at 6 months was estimated to be more than 65%. For calculation of the necessary sample size for identifying a clinically relevant increase in progression-free survival, 75% progression was assumed for the white-light group and 60% for the 5-aminolevulinic-acid group—ie, a 15% increase in progression-free survival in the full-analysis population analysed by intention to treat.

The statistical-decision strategy consisted of testing of the two primary endpoints in an a-priori defined hierarchical order—ie, confirmatory testing of the second target criterion (ie, progression-free survival) was to be done only if fluoresence-guided resection had been shown to be better than conventional resection with respect to the first test criterion. This hierarchical approach was chosen to account for any long-term treatment effect that would be identified only if there were substantial treatment effects for the first (ie, short-term) primary endpoint. For the same reason, interim analysis of the coprimary endpoint was planned, with the possibility of increasing the sample size in the case of non-significance.

On the basis of these requirements, 350 patients were needed in the full-analysis set to give 80% complete power (ie, probability of rejecting all false null hypotheses) with an experiment-wise type I error of 0·05. To allow premature study termination, a confirmatory interim analysis was scheduled after 270 patients had been recruited in the full-analysis population. With 270 patients, a 20% difference in treatment-specific progression-free survival could be identified with a power of about 80%.

According to the prespecified confirmatory hierarchical-testing strategy, the first primary endpoint (ie, the proportion of patients with histologically confirmed malignant glioma without residual contrast-enhancing tumour on postoperative MRI) was to be analysed in 270 patients. If significance had not been reached, the study would have been stopped for futility. Otherwise, the coprimary endpoint (ie, 6-month progression-free survival) was to be tested in an interim analysis. To account for potential increase in the probability of overall type I error due to statistical analysis with accumulating data, nominal two-sided significance levels were calculated on the basis of power boundaries as proposed by Wang and Tsiatis[21] by use of the shape parameter of 0, generating the boundaries of O'Brien and Fleming.[21] These boundaries are

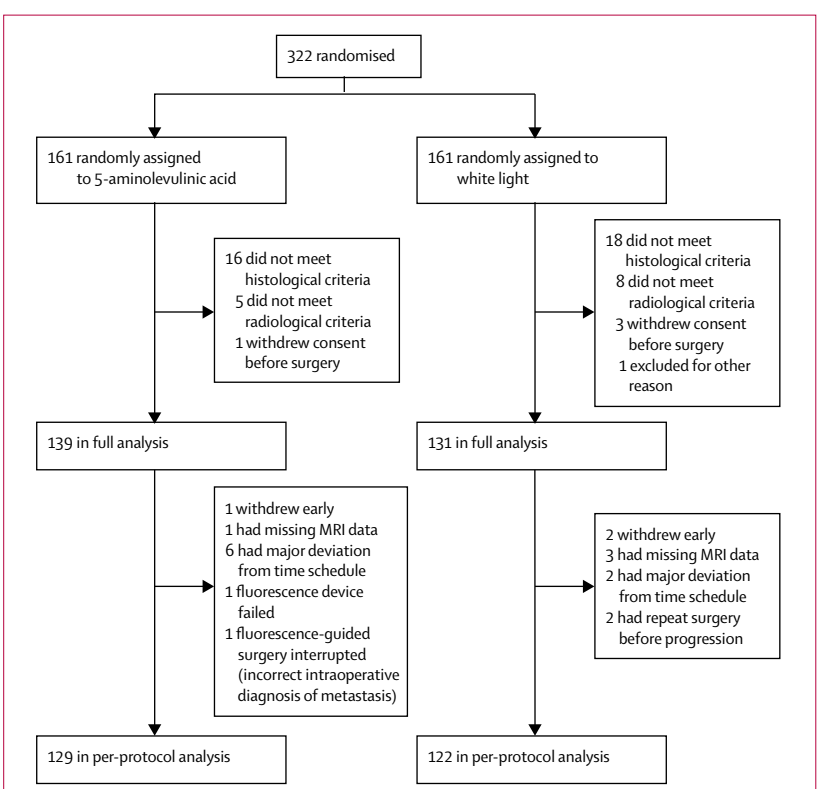

Figure 1: **Trial profile**

	5-aminolevulinic acid (n=139)	White light (n=131)
Age (years)		
Median (range)	60 (23–73)	59 (30–73)
≤55	45 (32%)	43 (33%)
>55	94 (68%)	88 (67%)
Karnofsky performance scale		
70–80	28 (20%)	31 (24%)
>80	111 (80%)	100 (76%)
Tumour location		
Non-eloquent	65 (47%)	54 (41%)
Eloquent*	74 (53%)	77 (59%)
US National Institutes of Health stroke score		
Median (range)	1 (0–10)	1 (0–8)
Pathological analysis		
Oligoastrocytoma (grade III)	0	1 (1%)
Oligodendroglioma (grade III)	0	2 (2%)
Anaplastic astrocytoma (grade III)	3 (2%)	2 (2%)
Astroblastoma (grade III)	1 (1%)	0
Gliosarcoma (grade IV)	8 (6%)	8 (6%)
Giant-cell glioblastoma (grade IV)	5 (4%)	2 (2%)
Glioblastoma multiforme (grade IV)	122 (88%)	115 (88%)
Missing data†	0	1 (1%)

*Tumour located adjacent to or in eloquent brain region (ie, region for control of sense, speech, cognition, or motor function) according to study surgeon. †Reference pathology unavailable, local pathology confirmed glioblastoma multiforme.

Table 1: **Baseline characteristics**

conservative at early analyses, and give decision rules similar to those of a fixed sample design if the final sample size is reached. This approach led to nominal significance levels of 0·05 for the first primary endpoint, and of 0·022 and 0·043 for the coprimary endpoint at the interim analysis and final analysis, respectively; all other endpoints were to be interpreted on an exploratory basis only. χ^2 test was applied for confirmatory testing of the first primary endpoint. Odds ratios (OR), risk differences, and 95% CI were used as effect measures. Cochran-Mantel-Haenszel techniques were applied, and logistic-regression models were fitted to adjust for prespecified covariates chosen within the randomisation process.

Time-to-event measures were analysed by use of Kaplan-Meier methods; comparisons were made by use of log-rank tests. Cox-regression models with all covariates used within the randomisation process were used for additional sensitivity analyses. For specific comparison of 6-month progression-free survival, we applied the *Z* test (ie, difference between treatment-specific Kaplan-Meier estimates of progression-free survival at 6 months÷√sum of associated variances).

Further exploratory testing of secondary endpoints was done by use of Mann-Whitney-Wilcoxon tests for comparison of discrete and continuous variables. χ^2 and Fisher's exact tests were used for binary data analyses. Statistical analysis was done with SAS version 8.02 and EaSt version 3.0.

The full-analysis population was defined as all randomised patients who underwent surgery and who were eligible regarding baseline histological findings and imaging; this group was analysed by intention to treat. The per-protocol set excluded patients who withdrew early from the trial, were lost to follow-up, and who had major deviations from the planned time schedule. The safety population was defined for analysis of toxicological effects of 5-aminolevulinic acid, and contained all patients in the full-analysis population and those who were initially randomised into the 5-aminolevulinic-acid group that had received 5-aminolevulinic acid, even if they were later excluded from the full-analysis set; this group was analysed per protocol.

	5-aminolevulinic acid (n=139)	White light (n=131)	OR (95% CI)	p
All patients	90 (65%)	47 (36%)	3·28 (1·99–5·40)	<0·0001*
Age (years)	..	..	3·42 (2·06–5·70)†	<0·0001†
	..	..	..	0·679‡
≤55	35/45 (78%)	20/43 (47%)	4·03 (1·60–10·14)	0·0025*
>55	55/94 (59%)	27/88 (31%)	3·19 (1·73–5·87)	0·0002*
Karnofsky performance scale	..	..	3·27 (1·98–5·40)†	0·0001†
	..	..	..	0·369‡
70–80	13/28 (46%)	9/31 (29%)	2·12 (0·72–6·20)	0·1676*
>80	77/111 (69%)	38/100 (38%)	3·70 (2·09–6·54)	<0·0001*
Tumour location	..	..	3·26 (1·97–5·40)†	<0·0001†
	..	..	..	0·351‡
Non-eloquent	45/65 (69%)	26/55 (47%)	2·51 (1·19–5·30)	0·0148*
Eloquent	45/74 (61%)	21/76 (28%)	4·06 (2·05–8·07)	<0·0001*

*Crude p value based on χ^2 test. †Common OR, adjusted for variable; p value based on Cochran-Mantel-Haenszel test. ‡Breslow-Day test for homogeneity of OR within variable.

Table 2: **Patients without residual tumour at early postoperative MRI: all patients, and stratified by age, performance status, and eloquent areas**

Role of the funding source

The sponsor of this study, medac, was responsible for study design, quality assurance, and quality control systems to ensure that the trial was done and data were generated, documented, analysed, and reported in compliance with the protocol. The CRO Clinstud GmbH (Hamburg, Germany), under contract by medac, was responsible for data monitoring and data collection. The sponsors of the study had no role in the interpretation of the data. The corresponding author had full access to all data, including those for safety, in the study, and had final responsibility to submit the paper for publication.

Results

From Oct 11, 1999, 322 patients were enrolled by 32 investigators at 17 German study centres. The last visit for the interim analysis was done on July 19, 2004. 161 patients were randomly assigned to fluorescence-guided surgery with 5-aminolevulinic acid, and 161 were randomly assigned to conventional microsurgery with white light. Figure 1 shows the trial profile.

At central review, 34 patients did not meet histological criteria and were thus excluded from the full-analysis and per-protocol sets (metastasis n=13, abscess n=3, low-grade glioma n=7, meningioma n=1, cavernoma n=2, aneurysm n=1, vasculitis n=1, lymphoma n=1, arachnoid cyst n=1, atypical meningioma n=1, primitive neuro-ectodermal tumour n=1, and non-tumour necrotic resorptive changes n=2; figure 1). 13 patients had major violations of MRI inclusion criteria and were thus excluded from the full-analysis and per-protocol sets (>1 contrast-enhancing lesion n=9 and substantial, non-contrast-enhancing tumour areas suggestive of low-grade glioma with malignant transformation n=4; figure 1). Thus, 139 patients assigned 5-aminolevulinic acid and 131 patients assigned white light were followed up for progression-free survival (figure 1); table 1 shows the baseline characteristics of this full-analysis population. 261 (97%) of 270 patients had WHO grade IV lesions.

Median follow-up was 35·4 months (95% CI 1·0–56·7). More patients assigned 5-aminolevulinic acid were without residual, contrast-enhancing tumour on early MRI compared with those assigned white-light (difference between groups 29% [95% CI 17–40], p=0·0001; table 2). Common OR for complete resection and 95% CI did not change on adjustment for age, Karnofsky performance scale, or tumour location, and were all similar to a crude OR (ie, that recorded for all patients; table 2). Logistic-regression models for estimation of the simultaneous effect of 5-aminolevulinic acid and other prognostic factors on the probability of a complete resection showed the adjusted OR to be of the same size as the crude OR. In this model, treatment had the most important effect on the probability of complete resection (3·41 [2·03–5·71], p<0·0001), followed by age (0·50 [0·28–0·87], p=0·01), and tumour location (0·58 [0·34–0·96], p=0·0358); OR for Karnofsky performance scale was 0·60 ([0·32–1·13], p=0·1).

For progression-free survival, there were 135 events in the 5-aminolevulinic-acid group and 126 events in the white-light group (ie, four and five people were censored in each group, respectively). Median progression-free survival was 5·1 months (95% CI 3·4–6·0) in the 5-aminolevulinic-acid group and was 3·6 months (3·2–4·4) in the white-light group. Progression-free survival at 6 months was higher for patients assigned 5-aminolevulinic acid than for those assigned white light (41·0% [32·8–49·2] *vs* 21·1% [14·0–28·2]; difference between groups 19·9% [9·1–30·7], p=0·0003, Z test; figure 2). 5-aminolevulinic acid led to higher 6-month progression-free survival than did white light in patients: older than 55 years (22% [9% to 35%]); with Karnofsky performance scale more than 80 (21% [9% to 33%]); without tumour in eloquent areas (24% [8% to 40%]); and with tumour in eloquent areas (16% [2% to 30%]), but not in patients aged 55 years or younger (16% [–3% to 36%]) or those with Karnofsky performance scale 70–80 (15 % [–7 to 37%]).

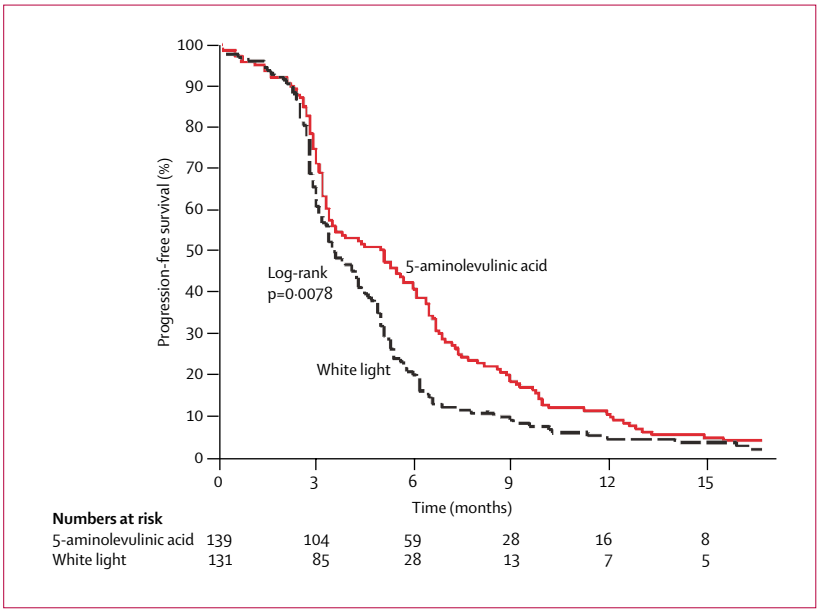

Figure 2: **Progression-free survival by surgical group**

Cox proportional-hazards modelling showed that 5-aminolevulinic acid was associated with a reduced risk of death or progression compared with white light (hazard ratio 0·73 [0·57–0·94], p=0·01). This model showed no significant effect of Karnofsky performance status (0·77 [0·57–1·04], p=0·09), age (1·09 [0·84–1·42], p=0·5), and tumour location (1·11 [0·86–1·43], p=0·4) on progression-free survival.

In the full-analysis set, residual tumour volumes were small in both groups. Median volume was significantly smaller in patients allocated 5-aminolevulinic acid than in those allocated white light (0·0 cm³ [range 0·0–25·7] *vs* 0·7 cm³ [0·0–32·6], p<0·0001).

In the full-analysis set, groups did not differ in the frequency of severe adverse events or adverse events in any organ system class reported within 7 days after surgery. The most frequent early severe adverse events were (5-aminolevulinic-acid group *vs* white-light group, Fisher's exact test): hemiparesis (4 [3%] *vs* 2 [2%], p=0·7); aphasia (3 [2%] *vs* 0, p=0·1); convulsions (3 [2%] *vs* 1 [1%], p=0·6); and epidural haematoma (1 [1%] *vs* 1 [1%], p=1·0). All dysphasia reported in patients allocated 5-aminolevulinic acid was present at baseline, but worsened after surgery and was therefore reported as a severe adverse event. By 30 days, five (4%) patients in the 5-aminolevulinic-acid group and three (2%) in the white-light group had died (difference between groups 1·3% [–3·4 to 6·2]).

Median Karnofsky performance status 6 weeks after surgery was 90 for both groups (range 20–100 for patients assigned 5-aminolevulinic acid and 10–100 for those assigned white light). At 6 months, 28% (95% CI 19–36) of patients in the 5-aminolevulinic-acid group had deterioration of Karnofsky performance scale to

	5-aminolevulinic acid (n=139)	White light (n=131)
Radiotherapy		
Number of patients (%)	126 (91%)	121 (92%)
Mean total dose, Gy (SD)*	59·8 (6·53)	60·2 (3·93)
Chemotherapy before radiological progression		
Number of patients (%)	15 (11%)	10 (8%)
Treatment after radiological progression		
Chemotherapy (overall)	73 (53%)	72 (55%)
Age ≤55 years	32/45 (71%)	28/43 (65%)
Age >55 years	41/94 (44%)	44/88 (50%)
Repeat surgery (overall)	41 (30%)	48 (37%)
Age ≤55 years	19/45 (42%)	24/43 (56%)
Age >55 years	22/94 (23%)	24/88 (27%)
Chemotherapy or repeat surgery, or both (overall)	84 (60%)	83 (63%)
Age ≤55 years	36/45 (80%)	33/43 (77%)
Age >55 years	48/94 (51%)	50/88 (57%)

*Data missing for two patients who received radiotherapy.

Table 3: **Treatment initiated after study surgery**

60 or less, compared with 31% (20–42) in the white-light group (log-rank p=0·5). 26% (17–34) of patients in the 5-aminolevulinic-acid group had a deterioration in Karnofsky performance scale of at least 30% at 6 months compared with 29% (18–39) in the white-light group (log-rank p=0·5).

The distribution of the total stroke-scale scores in the full-analysis set did not differ between groups (5-aminolevulinic acid *vs* white light) before surgery (median 1 *vs* 1, third quartile 2 *vs* 2, 90% quantile 4 *vs* 3; p=0·5); at 48 h after surgery (median 1 *vs* 0, third quartile 2·5 *vs* 2, 90% quantile 5 *vs* 4; p=0·1); 7 days after surgery (median 0 *vs* 0, third quartile 2 *vs* 1, 90% quantile 5 *vs* 3; p=0·2); and 6 weeks after surgery (median 0 *vs* 0, third quartile 1 *vs* 1, 90% quantile 3 *vs* 3; p=0·7). Thus, no significant effect of 5-aminolevulinic-acid-guided resections on the level of neurological impairment was noted.

When patients in the full-analysis set with documented stroke scores were analysed for individual changes relative to baseline on a qualitative basis, significantly more patients allocated 5-aminolevulinic acid had deteriorated than had those allocated white light (33 [24%] of 136 *vs* 19 [15%] of 130) and fewer had improved (32 [24%] of 136 *vs* 40 [31%] of 130; exact Wilcoxon-Mann-Whitney test p=0·0462) 48 h after surgery. These differences were not significant at 7 days after surgery (deterioration: 25 [18%] of 136 *vs* 13 [10%] of 128; improvement: 41 [30%] of 136 *vs* 44 [34%] of 128; p=0·2) or 6 weeks after surgery (deterioration: 21 [17%] of 122 *vs* 13 [12%] of 113; improvement: 47 [43%] of 113 *vs* 48 [43%] of 113; p=0·3). Because of missing observations, conditional analyses giving complete data for stroke score up to 48 h, 7 days, and 6 weeks after surgery were done for sensitivity purposes; results were much the same (data not shown). 6 months after surgery, the proportion of patients deteriorating by at least 2 points on the stroke scale was 31% (95% CI 22–40) in the 5-aminolevulinic-acid group and 35% (24–46) in the white-light group (log-rank p=0·7).

The safety population consisted of 289 patients (158 in the 5-aminolevulinic-acid group and 131 in the white-light group). Three patients randomly assigned to 5-aminolevulinic acid were excluded from the safety population because of withdrawal of consent before scheduled treatment (n=1), complete loss of all source data (n=1), and withdrawal of consent before scheduled treatment because of identification of multilocular tumour lesions (n=1). Analysis of the safety population showed no significant differences between groups for laboratory measurements (data not shown), except at 24 h after surgery. At this time, median gamma glutamyl transpeptidase was 0·93 times upper limit of normal value (range 0·18–9·71) in the 5-aminolevulinic-acid group and 0·72 times upper limit of normal (0·24–8·43) in the white-light group (p=0·05). Median alanine transaminase and aspartate aminotransferase were also elevated 24 h after surgery in the 5-aminolevulinic-acid group compared with the white-light group (1·05 times upper limit of normal value [0·32–8·53] *vs* 0·84 times upper limit of normal [0·18–7·36], p=0·003, and 0·72 [0·22–5·60] *vs* 0·53 [0·11–3·56], p<0·0001, respectively).

Table 3 shows treatment initiated before and after disease progression in the full-analysis set. Groups did not differ in the frequency of chemotherapy or radiotherapy. However, fewer patients assigned to 5-aminolevulinic acid had repeat surgery compared with those assigned white light.

To define better the effects of resection on additional treatment and their combined effect on survival, exploratory subgroup analyses according to subgroups defined for randomisation were done in the per-protocol set. These analyses showed differences in the frequency of reinterventions for younger patients (ie, ≤55 years, n=83) compared with the larger group (n=168) of older patients (ie, >55 years), which was 68 (82%) of 83 *vs* 93 (55%) of 168, respectively (p<0·0001). For older patients given 5-aminolevulinic acid, exploratory analysis showed a longer time to reintervention compared with that for patients given white light (10·2 months [95% CI 7·5–13·4] *vs* 7·1 months [5·7–8·7], hazard ratio 0·65 [0·43–0·99]; log-rank p=0·04); we recorded no such effect for younger patients (8·0 months [6·4–10·5] *vs* 6·7 months [5·5–10·8]; 0·86 [0·59–1·55], log-rank p=0·9).

For older patients, overall survival was 14·1 months (11·7–16·7) in the 5-aminolevulinic-acid group and was 11·5 months (8·8–13·7) in the white-light group (crude hazard ratio 0·73 [0·53–1·01], log-rank p=0·06). For younger patients, overall survival was 18 months (13·0–20·8) in the 5-aminolevulinic-acid group and was 17·5 months (14·3–21·2) in the white-light group

(1·04 [0·64–1·70], log-rank p=0·9); data for all patients 13·5 months (12·0–14·7) *vs* 15·2 months (12·9–17·5); hazard ratio 0·82 (0·62–1·07), log-rank p=0·1).

Stratification by postoperative MRI findings in the per-protocol set showed that patients without residual contrast-enhancing tumour had higher overall median survival than did those with residual-enhancing tumour (17·9 months [14·3–19·4] *vs* 12·9 months [10·6–14·0], p<0·0001). In multivariate analysis, the overall-survival advantage noted for those without contrast-enhancing tumour remained significant (hazard ratio 1·8 [1·2–2·4], p=0·0006, Wald χ^2) in addition to age (≤55 years *vs* >55 years 1·5 [1·1–2·1], p=0·01), and Karnofsky performance scale (70–80 *vs* >80 0·6 [0·4–0·9], p=0·006), but not eloquent tumour location (non-eloquent *vs* eloquent 1·2 [0·9–1·6], p=0·2).

Discussion

We have shown that use of 5-aminolevulinic acid leads to a higher frequency of complete resections of contrast-enhancing tumour on early postoperative MRI, translating into higher progression-free survival, than does conventional microsurgery guided by white light. Postoperative Karnofsky performance score, neurological status, and toxic effects were much the same for both groups, showing fluorescence-guided surgery with 5-aminolevulinic acid to be safe. Overall survival did not differ between groups as a whole or when groups were stratified by younger versus older patients. However, older patients had fewer reinterventions compared with younger patients.

The rationale for the study was based on the assumption that maximum cytoreductive treatment of malignant gliomas is of benefit to patients. However, this issue remains under debate, reports regarding this question are based on retrospective data.[3–7] These studies[3–7] have shown that removal of enhancing tumour, as shown by postoperative MRI, is an independent prognostic factor for overall survival in multivariate analysis. A large EORTC study[2] showed that patients with complete resection benefit most strongly from concomitant radiochemotherapy with temozolomide,[2] thus giving a further incentive for maximum resection.

On the basis of these findings,[2–7] most neurosurgeons attempt to remove contrast-enhancing tumour as completely as possible. However, this aim is achieved in few patients,[4–9] partly because of the difficulty in identifying marginal, contrast-enhancing tumour intraoperatively. Several methods have been introduced to help achieve this aim, such as intraoperative MRI, neuronavigation, and ultrasonography. Of these techniques, only neuronavigation has been assessed prospectively[9] as to whether it improves resection, benefits patients, or causes patients to have neurological deficits; no benefit in terms of resection was noted. Furthermore, neuronavigation has problems with brain shift (ie, intraoperative distortion of anatomy compared with that seen on preoperative imaging), intraoperative MRI is cumbersome and expensive, and intraoperative ultrasonography frequently interrupts surgery and relies on substantial experience. Therefore, none of these methods are regarded standard for use in surgery for malignant glioma.

Fluorescence-guided resection by use of 5-aminolevulinic acid is easy to do, and does not interrupt the operation. Such resection relies on specific synthesis and accumulation by 5-aminolevulinic acid of fluorescent porphyrins in malignant glioma tissue.[11,12] This technique differs from earlier attempts to contrast tumour by use of fluorescent agents (eg, fluorescein),[13] in which the fluorescing agent is in the plasma and reaches the tumour through the disrupted blood–brain barrier, thus limiting specificity.

We assessed the effectiveness of fluorescence-guided resection with 5-aminolevulinic acid by use of MRI—the standard for brain-tumour imaging;[4,22,23] assessment was done by central neuroradiological reviewers who were masked to study groups. Because the role of early MRI in the prediction of outcome after surgery is controversial, we included 6-month progression-free survival as a primary endpoint. Progression-free survival can be regarded pivotal for a patient because disease progression marks the initiation of burdening treatment (eg, repeat surgery or chemotherapy). Moreover, on radiological progression, clinical experience shows clinical deterioration to be imminent.

Use of fluorescence guidance resulted in almost a doubling of the number of patients without residual, contrast-enhancing tumour on early MRI. In patients assigned fluorescence-guided resection, we noted smaller median volumes of residual tumour compared with those assigned white light. Residual tumour volumes recorded for patients in the white-light group were much the same as those reported by a single, experienced centre[3] in a study of more than 400 patients with glioblastoma multiforme (median 0·7 cm³ [range 0–73]). Thus, the control group in our trial had a good outcome on comparison with previous data. Furthermore, in our study clinical benefit with use of 5-aminolevulinic acid was evident on analysis of progression-free survival at 6 months, which was twice that recorded with white-light microsurgery.

Because decisive diagnostic tests were not done until after randomisation, use of conventional intention-to-treat criteria to analyse our results would have included patients without malignant glioma. We did, however, assess all patients who met the inclusion criteria (full-analysis set) in accordance with their initial group of randomisation, irrespective of whether they withdrew consent, were lost-to-follow-up, or had any missing efficacy examinations. This strategy was unlikely to have biased the results because the decision for exclusion was based solely on the results of the central

neuropathology and neuroradiology, which were masked as to treatment allocation.

The study was neither designed nor powered to show differences in long-term endpoints such as overall survival. A further issue was the lack of restrictions on treatment after disease progression: we noted differences in the frequency of reinterventions (eg, chemotherapy and repeat surgery) depending on the age of patients, in that the largest subgroup of older patients had significantly fewer reinterventions than did younger patients. Assuming that reinterventions affect long-term outcome, advantages gained from better resections during initial surgery with 5-aminolevulinic acid would need to be more obvious in subgroups with less-frequent reinterventions. We recorded that time to reintervention was significantly longer in older patients who were allocated 5-aminolevulinic acid compared with those allocated white light, and we noted a crude hazard ratio of 0·73 (95% CI 0·53–1·01, log-rank p=0·06) for overall survival of older patients allocated 5-aminolevulinic acid compared with those allocated white light.

On restratification according to the results of early postoperative MRI, patients without residual tumour had a prognostic advantage compared with those who had residual tumour—an effect that was higher than that of age. Thus, this study underscores the importance of early postoperative MRI as a prognostic variable, and one that should not be ignored in future surgical trials of malignant glioma.

We analysed separately the toxicological safety of the 5-aminolevulinic acid surgical procedure and noted no concerns. Liver enzymes were mildly elevated 24 h after surgery, as reported previously.[24] For surgery-related neurological morbidity, conventional measures such as the common toxicity criteria showed no side-effects of fluorescence-guided surgery with 5-aminolevulinic acid that would cause concern.

The US National Institutes of Health stroke scale was included as an additional measure of neurological function in response to surgery. Brain surgery can result in acute impairments of individual functions, consistent with stroke. We noted a difference in neurological function from that at baseline only at 48 h after surgery; this difference was not significant 7 days or 6 weeks after surgery. Furthermore, Karnofsky performance scale showed no differences between groups. In summary, no safety concerns arose during our analyses.

In conclusion, our data show a clinical benefit for patients, in terms of completeness of tumour removal and progression-free survival, on removal of tumours by use of 5-aminolevulinic-acid-induced fluorescence guidance. Concomitant radiochemotherapy with adjuvant chemotherapy using temozolomide[1] has emerged as standard treatment for patients with malignant glioma. To define the effects of optimum resection achieved by 5-aminolevulinic acid in addition to that of concomitant radiochemotherapy, a trial is being designed in which patients are treated by both modalities.

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W Stummer was the coordinating investigator, developed the protocol, authored the report; U Pichlmeier was the study statistician; T Meinel did study organisation and protocol development; O D Wiestler did reference pathology; F E Zanella did reference neuroradiology; and H-J Reulen was the principal investigator and developed the protocol.

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Conflicts of interest

W Stummer is a paid consultant to medac and Zeiss; U Pichlmeier is a medac employee; T Meinel is under contract by medac; and H-J Reulen has received secretarial help from medac and travel reimbursement. All other authors declare no conflicts of interest.

Acknowledgments

The study was sponsored by medac GmbH, Wedel, Germany. We gratefully acknowledge Holger Thurm (medac Gesellschaft für Medizinische klinische Spezialapräparate mbH), for his work and efforts with regard to collection and analysis of safety data. We are indebted to the patients and their families for agreeing to participate in this trial, and to the study nurses, study physicians, and data managers for their collaboration.

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