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Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials

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Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials

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Details of ethical approval (or a statement that it was not required)

Ethical approval was not required.

Details of funding

The submitted work is made in frame of Author's regular activity at the Galenic Research Institute. No external funding involved.

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Galenic Research Institute as a study sponsor provided time and facilities for the work.

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No funders.

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Patient involvement statement

Patients were not involved (see also Acknowledgement).

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The trial was not registered.

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WHAT THIS PAPER ADDS

What is already known on this subject

- The prognosis for patients with recurrent GBM is still poor with MST between 3 and 6 months.
- All the modern CTX treatments like TMZ, BEV and other AAA and all their regimens are not cost-effective and mainly toxicity-limited.
- Standards of care are not yet defined for recurrent GBM. The pitiful situation with treatment of recurrent GBM requires novel approaches.

What this study adds

The application of mEHT as an enhancer of ddTMZ regimens (and, probably, of all TMZ treatments at all) can:

- improve survival since relapse up to 10 months;
- make ddTMZ regimens cost-effective;
- decrease toxicity of ddTMZ and/or restore chemosensitivity of patients.

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ABSTRACT

OBJECTIVE: To assess the efficacy and cost-effectiveness of dose-dense temozolomide (ddTMZ) 21/28d regimen with concurrent modulated electro-hyperthermia (mEHT) versus ddTMZ 21/28d alone in patients with recurrent glioblastoma (GBM). DESIGN: A cohort of fifty-four patients with recurrent or progressive GBM treated with ddTMZ+mEHT in 2000-2005 (MST of 7.7 months (95%CI: 5.7 to 9.4)) was compared retrospectively with five ddTMZ 21/28d studies completed in 2008-2013 (114 patients, pooled MST of 7.21 months (6.26 to 8.16)). RESULTS: By effect-to-treatment analysis (ETA), the median effect-treatment ratio (METR) of ddTMZ+mEHT significantly surpassed that of ddTMZ alone (1.19 LMG/ccl (0.59 to 2.40) versus 0.57 (0.39 to 0.85), p=0.011). the maximal attainable MST (MAST) was estimated of 10.10 months (9.10 to 11.10), "cycles needed to treat" suggested significant strong benefit (CNTM = 1.00 – 1.68 ccls/LMG, p<0.016) with significantly less toxicity (no grade III-IV toxicity versus 45% – 92%, p<0.0001). Cost-effectiveness analysis (CEA) suggests that ddTMZ+mEHT is cost-effective versus the applicable cost-effectiveness thresholds 25,000 – 50,000 €$/QALY, unlike ddTMZ 21/28d alone. Budget impact analysis suggests a significant economy of €8,577,947 / $11,201,761 with 29.1 – 38.5 QALY gained per 1000 patients per year. Cost-benefit analysis suggests that mEHT is profitable and will supposedly generate revenues in amount of €3,124,574 / $6,458,400, with total economic effect (economy + revenues) of €5,700,034 / $8,237,432 per a mEHT device over eight-year period. CONCLUSIONS: ETA suggests that mEHT strongly and significantly improves survival of ddTMZ 21/28d regimen. Economic evaluation suggests that ddTMZ+mEHT is cost-effective, budget-saving and profitable. After confirmation of the results, mEHT could be recommended for the treatment of recurrent GBM as a cost-effective enhancer of ddTMZ regimens, and, probably, of the regular 5/28d regimen too. MEHT is applicable as a single treatment if chemotherapy (CTX) is impossible and as a salvage treatment after the fail of CTX.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- It firstly introduces the application of the novel clinical analysis called Effect-to-Treatment Analysis (ETA).
- It firstly suggests the safe and cost-effective significant enhancement of clinical efficacy of temozolomide at recurrent glioblastoma by modulated electro-hyperthermia (mEHT).
- It includes comprehensive economic evaluation comprising consistent costs analysis, cost-efficiency analysis, budget-impact analysis and cost-benefit analysis.
- It demonstrates the possibility to extract extensive information and reliable evidences from a very limited data of retrospective cohort trial.

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BACKGROUND

Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor, accounting for 45-54% of all adult gliomas, which, in turn, hold 80-81% of all brain malignancies.[1,2] With 23,770 estimated new cases of brain and other nervous system cancers registered in the US in 2016,[3] about 10,000 new cases of GBM are diagnosed annually in the US[4] and about 2,200 cases in the UK.[5] Men have a higher incidence than women (the ratio is 1.66 times for UK), though prognosis for men is more favorable (median life expectancy is 6.5 months vs. 5.6 months in women).[5]

Median survival time (MST) of untreated or treated with radiation therapy (RT) alone patients with GBM is about 3 months.[5] Palliative surgery with/without RT extends survival to 4 – 5.5 months. Both radical surgery and non-surgical chemoradiotherapy (CRT) provide MST of 9 – 11 months. Addition of RT to radical surgery lengthens MST to 12 months. Maximal treatment (radical surgery and adjuvant CRT) provides the maximal survival of about 15 months.[5,6] MST of adult (>20 years) GBM patients in the US (2005-2007)[7] / UK (2007-2011)[5] is 9.5/6.1 months, two- and five-year survival is 17%/11.5% and 3.3%[8]/3.4%, respectively.

The standard of care first-line treatment for GBM, based on the milestone EORTC/NCICT trial,[9,10] includes a maximal possible resection consistent with the preservation of neurologic function followed by 6 weeks of adjuvant focalized fractionated RT with concurrent chemotherapy (CTX) with oral DNA-alkylating agent temozolomide (TMZ), further followed by up to 6 months of adjuvant TMZ monotherapy.[11] Nevertheless, TMZ adds only 2.5 months of MST compared to RT alone.[9,10] With more than 50% of patients which fail TMZ treatment over 6-9 months, TMZ is only a modestly effective CTX. 60-75% of patients with GBM having not methylated MGMT promoter gene derive no or limited benefit from treatment with TMZ.[12] In addition, 15-20% of patients treated with TMZ develop clinically significant toxicity.[9]

Since the introduction of TMZ in 1999, the MST in GBM patients in the US, previously stable at the level of 7.5 months, started to increase and had reached 9.5 months in overall 2005-2007 population.[7] Among patients treated with surgery and adjuvant CRT, MST increased from 9 months in 1993-1998 to 13.5 months in 2005-2007,[7] and varies from 31.9 months in patients aged 20-29 to a low of 5.5 months in patients aged 80 and older.[13] Despite uncontested significant improvement of surgery, RT and novel treatments since the introduction of TMZ, it is attempted to attribute the observed increase of survival completely to TMZ,[14] which seems somewhat ungrounded. There is

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also an attempt to connect the further rise of survival in GBM in 2009-2010 with the introduction of BEV into treatment of recurrent GBM,[15] which also seems ungrounded with respect to the recent data.

Despite the recent advances, GBM prognosis remains dismal with the median survival limited by 15 – 18 months.[11] Overall 2-year survival is 22% only and remains below 30% even in complete standard treated population (28% in 2005-2007, CI: 26 to 31%).[7] Overall 5-year survival is 6.2% according to SEER database (1998-2008 population)[16] and scarcely exceeds 10% in some subgroups, namely in patients under the age of 45 years, patients with methylated MGMT,[10] and in some countries, namely Japan (9.9 – 10.1%).[17] From the other side, there is no any progress in survival of patients aged over 80 years in the USA. Moreover, it has become even worse: hazard ratio (HR) of 2005-2007 population is 1.05 compared to 1993-1995, whereas for younger populations, HR = 0.63 – 0.70.[7]

In the EORTC/NCICT trial,[9] TMZ was given daily at 75 mg/m^2 during RT, followed by 6 cycles of adjuvant TMZ chemotherapy at 150–200 mg/m^2 for 5 days in each 28-day cycle (5/28 d) (Stupp regimen). Despite of multiple attempts to improve Stupp regimen, it remains the standard of care for the newly diagnosed GBM to the date. These attempts involved addition of anti-angiogenic agents (AAA) (mainly bevacizumab (BEV)) and increase of TMZ dosage, dose-dense TMZ (ddTMZ) regimens.[18]

The idea of ddTMZ is based on the known role of specific DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in tumor resistance to alkylating agents like TMZ, because MGMT effectively recovers TMZ-related DNA damage. Methylation of the promoter region in MGMT gene suppress MGMT expression. Methylated MGMT-promoter is observed in 30-60% of GBMs: in particular, 45% of methylation is reported in EORTS/NCIC study, and TMZ was much more effective in MGMT-methylated patients (MST 18.2 vs 12.2 months).[19] Because MGMT is a suicide enzyme and requires re-synthesis for recovery of its enzymatic activity,[20] it can be depleted by continuous alkylating pressure. Therefore, more prolonged exposure and higher cumulative doses of TMZ could sensitize tumor to the alkylating damage with toxicity as a natural limiter of such dose-escalation. Some ddTMZ regimens were clinically tested versus standard 5/28d regimen, namely 7/14d (7 days on / 7 days off), 21/28d and continuous administration (7/7d or 28/28d).[21] Multiple single-arm and retrospective studies of ddTMZ at recurrent GBM showed PFS-6m ranging from 19% to 44% and MST 7 – 10 months, similar to BEV,[18] but recent III phase RCT (RTOG

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0525)[22] on ddTMZ 21/28d vs standard 5/28d adjuvant regimen for newly diagnosed GBM after completion of concurred CRT, failed to show an advantage of ddTMZ in MST (14.9 vs 16.6 months in the standard arm, p = 0.63) though showed improvement of progression-free survival at 6 months (PFS-6m) (6.7 vs 5.5 months) with borderline significance (p = 0.06), with somewhat higher toxicity in ddTMZ arm. Efficacy did not differ by methylation status, that advocates against MGMT depletion concept. Therefore, the efficacy of ddTMZ regimens still not proven.[18]

Standards of care are not yet defined for recurrent GBM.[23] Treatment options at recurrence include surgical resection, re-irradiation and chemotherapy,[24] though all these options have significant limitations.[25] Surgery is limited by the localization of tumor in non-eloquent areas, patient performance and a potential expected benefit, and there is a controversy concerning survival benefit of salvage surgery: whereas one authors report better survival,[26,27,28] others report no benefit[29] or even increased mortality.[23] Re-irradiation is limited mainly by total equivalent radiation dose: if more than 100 Gy, the risk of radiation necrosis of normal brain tissue increases significantly.[30] Although modern conformal and radiosurgery techniques significantly reduce the risk, still 6% of radiation necrosis is reported.[31] Since an additional toxicity is suggested, patient performance is also a limiting factor for the re-irradiation. This also limits the use of adjuvant re-irradiation after re-surgery.[32]

CTX is typically administered to patients with KPS of ≥70 and an expected survival time of ≥3 months.[25] Said limitations of TMZ renewed interest to other alkylating agents such as nitrosoureas. The most commonly used regimens are carmustine (BCNU) monotherapy or lomustine (CCNU) combined with procarbazine and vincristine (PCV), though there is no proof of their advantage before TMZ.[33]

In 2009, Bevacizumab (BEV) was granted accelerated approval as a single agent for patients with progressive disease following prior therapy,[34] and it pretended to occupy the position of the standard of care for recurrent GBM.[35] Combination of BEV with irinotecan, a topoisomerase I inhibitor, was offered,[36,37] though irinotecan itself has proved to be ineffective in GBM treatment,[38] and subsequent meta-analyses didn't show a benefit of the combination over BEV alone.[35,39] BEV showed a remarkable increase of MST since relapse up to 8 – 11 months in phase II prospective and retrospective studies with PFS of 4-6 m.[40,41] At the same time, according to FDA approval report, BEV exhibits more severe toxicity with reported 3-5% of treatment-related deaths.[34]

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A recent Cochrane systematic review of seven RCTs hasn't reveal sufficient evidence to support antiangiogenic therapy in the treatment of both primary and recurrent GBM.[42] In two the most representative III phase RCTs on BEV additional to Stupp regimen (AVAGlio[43] and RTOG 0825[44]), significant increase of PFS (10.6 vs 6.2 – 7.3 months) in BEV arms without difference in MST (16.8 vs 16.7 months in AVAGlio and 15.7 vs 16.1 months in RTOG trial) was revealed against Stupp regimen alone. The discrepancy exists in the estimation of health-related quality of life (HRQoL) and performance: whereas industry-sponsored (Hoffmann-La Roche) AVAGlio trial reports the longer maintenance of baseline HRQoL and performance status in BEV arm, independently sponsored (NCI) RTOG study reports significantly worse quality of life and a significant decline in neurocognitive function in BEV arm.[44] The current common conclusion of all systematic reviews and meta-analyses is that BEV improves PFS-6 but not 1-year OS.[45]

Significantly better PFS without effect to overall survival (OS), which is reported in a majority of controlled trials with AAA, is probably caused by their ability to decrease tumor blood-vessel permeability and, therefore, to mimic a stable disease on MR imaging without a real effect on the tumor progression.[46] The reported data on 35% of nonenhancing tumor progression among the patients who stopped BEV treatment in view of progressive disease,[47] strongly support this assumption: in case of nonenhancing progression, the tumor did not demonstrate enhancement, increased blood flow on MR perfusion imaging, or hypermetabolism on FDG-PET imaging, but resection demonstrated highly aggressive and invasive sarcomatous disease.[47] Generally, BEV has been shown to induce a more invasive GBM phenotype *in vitro* and *in vivo,* reactivating angiogenesis through up-regulation of other proangiogenic factors and invading normal brain areas by upregulation of some matrix proteinases,[48] leading to an increased incidence of distal recurrence[49] and increased risk of diffuse invasive recurrence.[50]

Also, GBMs that progress during BEV therapy tend to be unresponsive to further salvage therapies with PFS-6 of 0%:[47] even with a second BEV-containing regimen, response is poor with PFS-6 of <2%.[51] With ddTMZ 7/14d regimen in recurrent GBM, in BEV-pretreated group (65%), PFS-6m was 0% and MST was 4.5 months, while in not BEV-pretreated patients PFS-6m was nearly 30% with MST 13 months.[52] Additionally, there is a concern on the possibility of rebound tumor progression (rapid re-growth) after the cessation of BEV therapy, with tumor resistance to other treatments[53] (though other authors deny such possibility.[54]) As a result, currently there are no criteria for discontinuation of BEV treatment and it tends to continue until progression or limiting toxicity.[54]

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With no standard treatment, the justified strategy of chemotherapy of recurrent GBM should imply prior use of alkylating regimens (TMZ, ddTMZ, nitrosoureas or PCV) before BEV, taking into account the similar efficacy with lower toxicity, and possibility to use BEV as a salvage treatment after failure of alkylating regimens, whereas the reverse sequence leaves no room for an effective salvage treatment. [39,51,52]

Finally, it should be noted that the modern CTX treatments like TMZ, BEV and other AAA are not cost-effective.[55,56,57,58] From the perspective of Chinese health provider (2011[55]), ICER of TMZ-based regimen was $94,968/QALY versus nitrosoureas-based one and $87,940 versus RT-based regimen. European estimations give almost twice higher cost-utility of 41,167 to 53,369 €/QALY in Switzerland (2003[56]) versus 30,000 $/QALY in China (2011[55]). The US estimations seems to be even higher.[57] The situation with cost-effectiveness of BEV is hopeless: in Canada (2014), the ICUR of BEV was $607,966/QALY (95%CI: 305,000 to 2,550,000 $/QALY), with 0% chance of being cost effective at the $100,000/QALY willingness-to-pay threshold and never going below $450,000/QALY in the one-way sensitivity analysis. The ICUR using the US costing data was $787,519/QALY.[58] Taking into account the continuously growing burden of health expenses, the development of cost-effective alternatives is of great significance in this dismal situation.

The prognosis for patients with recurrent GBM is still poor with MST between 3 and 6 months.[59] Additionally, in some significant subgroups, the treatment efficacy is even less, namely in older patients (over 50 years) and especially over 70 years, in not MGMT-methylated patients (40-70% of patients), in patients with bad performance and others unfit for CTX and/or RT, and in patients with unresectable tumors. As 20 years ago, treatment of recurrent GBM can be considered successful if stable disease is achieved.[60]

The pitiful situation with treatment of recurrent GBM requires novel approaches.[23] In fact, there still remains a significant unmet need for more effective treatments of high-grade gliomas.[12] An impressive result was shown by a novel physical treatment, tumor-treating fields (TTF). TTF, applied with NovoTTF-100A device, is an athermal technology using continuous impact of low-intensity (0.7-1 V/cm) alternating electromagnetic field with frequency of 100-200 kHz through insulated scalp cross-sectional electrodes.[61] Its effect is attributed to impairment of cell division due to dielectrophoretic suppression of the assembly of the mitotic spindle,[62] though there is a controversy on the acting mechanisms of the effect.[63] In a III phase study,[64] TTF displayed the same efficacy at recurrent GBM (median 2nd recurrence) as the best physician choice CTX (MST 6.6

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versus 6.0 months, respectively (p = 0.27), 1-year OS 20% and 20%, 6-months PFS 21.4% and 15.1% (p = 0.13), objective response 14% versus 9.6% (p = 0.19), and severe adverse events in 6% versus 16% (p = 0.022)) with better QoL, which led to FDA approval.[65] Subgroup analysis showed the significant dependence of survival from length of TTF (7.7 months in patients with ≥1 course of TTF, p<0.05) and compliance rate (7.7 months at ≥75% compliance (≥18 h/day) versus 4.5 months with <75% (p = 0.042)).[66]

There is another physical technology called modulated electro-hyperthermia (mEHT, oncothermia™), which effectiveness was shown in many I/II phase trials in recurrent brain gliomas,[67,68,69,70,71] and also in cancer of lung,[72,73,74,75] liver,[76,77,78] pancreas,[79,80] cervix,[81,82] breast,[83] esophagus,[84] colorectal cancer,[85,86,87,88] malignant ascites,[89] soft tissue sarcomas,[90,91] etc. Clinically, mEHT is typically used as an enhancer of RT[72,81] and CTX, though it possesses the own effectiveness at least of the similar magnitude. [68,85,92] Taking into account the extensive and long-term (since 1996) successful application without any negative report, a systematic review of results of mEHT is possible and necessary.

Collecting the data for the systematic review and meta-analysis on the mEHT treatment of brain gliomas, we asked for raw data whenever possible, especially when confidence intervals were not reported. The raw data of the Sahinbas et al. (2007)[68] trial including 155 patients with HGG were presented by Prof. A. Szasz, who was a co-author of the trial. In process of the data recalculation, the following shortcomings were revealed:

- Duplication of data: two patients were included twice.
- Incorrect grouping: uncontested GBM WHO IV diagnosis can be attributed to 75 adult patients only instead of 92 patients reported as GBM in the report (Table 1).
- Incorrect calculation of survival function following to incorrect processing of censoring data.

After corrections and recalculation, the results of the analysis appeared so interesting that deserved a separate paper. In this retrospective analysis, we report a results of clinical comparison and economic evaluation of ddTMZ 21/28d regimen with and without concurrent mEHT in the treatment of recurrent GBM based on the corrected results of the trial[68] for the sample of patients with GBM. No change to the original data were made except of the above mentioned corrections.

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MATERIAL AND METHODS

Objectives

The objective of the study is to assess the efficacy and cost-effectiveness of ddTMZ 21/28d regimen with concurrent mEHT versus ddTMZ 21/28d alone in patients with recurrent GBM.

Questions of the study

- Does mEHT enhance the ddTMZ 21/28d regimen significantly?
- Is the addition of mEHT to ddTMZ 21/28d regimen cost-effective?

Trial design

This retrospective clinical and economical evaluation is based on a retrospective, single-arm, two-center, phase II cohort study[68] (study of interest, SOI) performed in two German centers – Gronemeyer Institute of Microtherapy (GIM) at the University of Bochum (Bohum) and clinic "Closter Paradise" (CPC) (Soest) – in 2000-2005.

Inclusion and exclusion criteria

Patients with relapsed, or progressed after incomplete resection, or progressive inoperable, histologically confirmed GBM or gliosarcoma (WHO IV), having been underwent a complete conventional 1st-2nd-line pre-treatment, were enrolled. From those, patients treated with ddTMZ 21/28d in combination with mEHT (with or without supportive therapy but without re-irradiation and/or re-surgery and/or other CTX) were selected. No exclusion criteria were applied.

Main outcomes measures

Survival was the main outcome of the study:

- Median Survival Time (MST) is a time from initial event to the moment when value of cumulative survival function (Kaplan-Meier estimator) reaches 50%. Here and further the term MST is applied to survival since relapse/progression or the date of the first mEHT session, while survival since the date of diagnosis is defined as Median Overall Survival time (MOST).
- Overall Survival (OS) is a value of cumulative survival function (Kaplan-Meier estimator) at the set time moments from the date of the initial event.
- Overall Survival Time (OST) is a time from the initial event to death of any reason.

Interventions

The studied intervention was a combination of dose-dense temosolomide 21 days on – 7 days off regimen (100 mg/m^2/d) with concurrent mEHT as an enhancer (ddTMZ+mEHT). MEHT applied by

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virtue of EHY2000 device (Oncotherm Kft, Hungary) with 2-days interval between sessions (on each 3rd day) concurrent with TMZ and afterwards, total up to three months. Dose-escalating scheme was used with gradual increase of power from 40W to 150W, and time from 20 min to 60 min, during two weeks, adding modulation from the second week. Then, step-up heating applied, increasing power from 60W to 150W during 60-minute sessions, to ensure tumor temperature >40°C during 90% of treatment time. The mEHT course considered low-dose (LD-mEHT) if didn't exceed 8 complete 60 minute sessions. Supportive and alternative treatments (SAT) included Boswellia caterii extract 6 g/day p.o. t.i.d., Misletoe extract 15 ng/day SC 3Xw, and Selenium 300 µg/day p.o., for three months.

Intervention of interest

Modulated electro-hyperthermia (mEHT, oncothermia™) is a novel method of treatment of solid malignant tumors by local application of high-frequency electromagnetic field (13.56 MHz), modulated by 0-5 kHz fractal harmonic oscillations, by virtue of impedance-coupled functionally asymmetric electrodes.[93] MEHT is positioned as a hyperthermic technology of a new generation based on a selective heating of membranes and intracellular compartments of tumor tissue instead of heating of a bulk volume of tissue, as conventional temperature-dependent hyperthermia (HT) does.[94,95]

The difference of mEHT and HT has been well demonstrated *in vitro*:[96] mEHT caused an order of magnitude stronger activation of apoptosis of cancer cells compared to HT[97]; it significantly increased the expression of proteins of intercellular junctions (E-cadherin and ß-catenin) and heat shock proteins (HSP) on the cell membrane, while HT increased only the intracellular level of HSP;[98] it displayed another pattern of heat response[99] and generally induced other cell-damage pathways.[100]

The fundamental difference of mEHT from HT technologies of high-frequency range (HFR, 3 – 30 MHz) is a transfer of the focus from the field to the current. Alternating electromagnetic field causes orientational displacement of dipole molecules, thus effecting dielectric heating (field effect), and also induces movement of charged ions (current), thus inducing Joule (electric) heating. The balance of that components of heating critically depends on technology used: current can be either minimized, like in capacitive HT, or enhanced, like in mEHT, the difference is more than significant. There are two main reasons to emphasize currents: focusing and penetration depth. Due to a high enough wavelength at 13.56 MHz (about 2.4 m in muscles), it is hard to impossible to

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focus the energy of a field in a desired small-size volume (typically 3-10 cm in diameter). At the same time, current has a known ability to concentrate in areas with a higher conductance.[101] Increased conductance is one of the basic properties of malignant tissues: the cancerous tissue is always 2-5 times more conductive compared to its normal counterpart (i.e., the surrounding tissue).[102] This feature has long been used for electrical impedance scanning (EIS)[103] and current-density imaging (CDI).[104,105] Thus, a tumor is a natural concentrator of an electrical current (but not field). Another reason to use the current is a penetration depth. For the 13.56 MHz field, the penetration depth (i.e., the depth from the surface, at which field intensity drops for e times (1/e) compared to the surface intensity) is about 14-18 cm only,[106] which forces to use high-intensity fields to reach the effective deep heating in capacitive HT. Penetration depth of current in an impedance-matched system is 20-25 cm.[107] Therefore, the emphasis on the current allows to transfer energy selectively to the tumor for any depth and with minimal losses.

The combined set of technical solutions is used to achieve maximal electrical heating: namely, the impedance matching, based on the phase angle between voltage and current, instead of the standard capacitive matching based on the standing wave ratio (SWR); functionally asymmetric electrodes, providing the necessary stability of the field and size difference-dependent amplification of the current; physiologic skin cooling, minimizing skin losses at energy transfer; and a “skin sensor” concept, which allows to refuse thermometry without detriment to safety.[93] “Free of thermometry” use is a great advantage of mEHT, which abolishes labor-intensive thermometry planning, installation and control, thus drastically reducing time and costs, minimizing side effects, and significantly improving the perception of the treatment by a patient.[108] MEHT is the only impedance-coupled technology on the market unlike other HT technologies of HFR, which are all capacitively-coupled. That is why the technology is called “electro-hyperthermia” meaning the predominantly electric heating.[109]

The electric heating creates quasy-stable local thermal gradients on nanolevel (eg, transmembrane thermal gradient[110]), which are maintained by the balance of continuous delivery of energy by external field and energy dissipation by natural cooling mechanisms, mainly by the bloodflow. [111,112] Thus, the nanoheating, depending on the field power applied and physiological cooling power displayed, can develop even without macroscopic heating:[113] it was shown *ex vivo* that 42 □ temperature in mEHT is responsible for 25-30% of the total antitumor effect, and a significant effect remains in case of normothermia.[114]

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Thus, the effect of mEHT is thermally-induced but not temperature-dependent.[115] Nevertheless, usually mEHT causes hyperthermia-range heating[116,117,118,119,120] in accordance with a classical maxima of Schwan on impossibility to reach significant "non-thermal" effects without substantial heating.[121] Effect of mEHT is power-dependent but not signal-dependent, that is not connected with multiple tiny and questionable processes like demodulation and molecular energy uptake[122] (though doesn't exclude these possibilities and tuned to use them, if exist), and power range of mEHT (0.2 – 2 W/cm^2) is far above the "thermal noise limit" of 0.01 W/cm^2.[123]

Fractal modulation is considered the principal specific feature of mEHT. The carrying frequency is amplitude-modulated by "pink noise" (1/f)[124] which is typically emitted by all self-organized living systems and reflects their fractal organization.[125] Since a malignancy always losses organization, it more or less emits "red" or Brownian noise (1/f^2).[126] Fractal modulation allows to increase specific absorption of modulated field energy in the "red noise" sites selectively amplifying the effect of mEHT.[127] Also, the noise can amplify cancer-specific frequencies[128] by "stochastic resonance".[129] It is reported that *in vitro* modulation can amplify the effect for 20-50%.[127]

The important feature of mEHT is its selectivity, both macroscopic and cellular. Macroscopic selectivity of mEHT is expressed by selective heating of tumors based on automatic impedance-based autofocusing of electric current in tumor.[101] Cellular selectivity was displayed *in vitro* on mixed culture of cancerous and normal cells: mEHT selectively destroyed cancer cells without damage to normal cells, and the extent of the damage of the cancer cells was proportional to the degree of malignancy.[130] Exact mechanism of this cellular selectivity is unknown: this is rather a sequence of combination of membrane-acting effects of mEHT and the fractal modulation.

The exact mechanism of mEHT action is unknown. Both temperature-dependent and independent mechanisms are among possible options. Temperature-dependent mechanisms include disorder of tumor bloodflow, oxygen and glucose deprivation, depletion of intracellular ATP, influx of sodium and depolarization of cellular membrane,[131,132,133] and acidification.[134,135,136] Since these effects are present in all HT applications, and they don't lead to results characteristic for mEHT, in mEHT they are combined with other, mEHT-specific mechanisms of action.

Many so-called "non-thermal" (i.e., not associated with elevation of macroscopic temperature) effects are reported to have a peak at about 10 MHz, namely direct bactericidal effect and enhancement of antibiotics action (bioelectric effect) both in bacterial films[137] and planktonic phase,[138] dielectrophoresis,[139] damage of mitochondrial function[140] and destruction of lysosomes,[141]

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all seems to be membrane-acting. Though the frequency and field strength (2 – 5 V/cm) applied in mEHT can't cause a somewhat significant change of the membrane potential,[142] nevertheless, there are many reasons to suggest a specific membrane-acting effect of mEHT. 10 MHz is a relaxation frequency of beta-dispersion range (0.1-100 MHz) caused by Maxwell-Wagner relaxation of cell membranes,[143] which means the peak of membrane dielectric loss and selective membrane excitation (heating) at this frequency.[144] The selective heating of the cell membrane also means specific effect on its lipid bilayer, namely enhancement of its fluidity and decrease of the capacitance (though the capacitance seems to be relatively stable).[143] Also, 10 MHz is a peak of phase shift of membrane polarization under the effect of external alternative field, which nearly reaches a quadrature (-80°).[142] The upper functional limit of the β-dispersion range was revealed empirically in experiments on whole-body RF-heating of mice already in 30s: the cut-off frequency of mice killing was denoted as 50 MHz by Christie[145] and 80 MHz by Schereshewsky,[146] over this limit the irradiation of the same power was becoming not lethal.

Cell membrane relaxation is not the only process contributing to β-dispersion. Re-orientation of protein-bound water molecules, the motion of polar protein subgroups, the Maxwell-Wagner relaxation of the cell interior or the additional Maxwell-Wagner relaxations due to the non-spherical cell shape, also contribute to the β-dispersion.[143] Of interest, the relaxation frequency of re-orientational proton motion of water-bound proteins, as it was shown in a cell-free protein solution, is also peaked at about 10 MHz (with range 1 – 100 MHz).[147] This allows a selective absorption of field energy by protein macromolecules and especially their active centers, which are always polarized. Taking into account the extremely high intracellular proteins concentration (200-300 g/l[148]), this equals to cell heating. Moreover, this can be a reason of dielectric selectivity of tumor heating, because the concentration of proteins in the intercellular fluid of a normal tissue is extremely low (nearly saline), whereas in the tumor intercellular fluid, it nearly equals to blood plasma (60-80 g/l[149]).

Among other possible effects, the arrest of cell division with possible mitotic catastrophe,[138] attributable to subcellular ponderomotoric effect (dielectrophoretic forces suppress the assembly of the mitotic spindle[62]), or to membrane polarization (cell division phases are associated with changes of membrane potential, and nonlinear processes of hyperpolarization and depolarization under effect of RF-field suppress proliferation[63]), or to resonance phenomena.[150] Also, an effect to cytoskeleton[151,152,153] and selective activation of some enzymes, both conformational and voltage-dependent (in case of membrane enzymes),[154] are reported.

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The overall effect of mEHT seems to be membrane-acting because it is connected with extracellular expression of intracellular signaling molecules of "cellular stress" (HSP, p53 protein)[155] which unmasks cancer cells and initiates immune response and apoptosis.[156] It is shown *in vivo* and *in vitro* that antitumor effect of mEHT is mainly connected with significant activation of apoptosis which develops over 72 hours since single impact.[157,158,159] Some immune-dependent effects are reported, namely abscopal effect[160, 161] which is tended to be considered as a basis for "radiofrequency vaccination".[162,163] Expression of many immune-specific pathways are reported *in vitro* in mEHT.[164,165,166,167] Overexpression of cell-junction proteins with significant restoration of intercellular junctions, which can contribute to induction of apoptosis,[168,169] and reorganization of cytoskeleton[151] are reported at mEHT.

Response and survival assessment

The objective response was assessed according to MRI McDonald criteria.[170] Survivors were right-censored on the date of completion of the study, lost patients were censored on the date of the last contact, excluded patients were left-censored on the date of diagnosis/enrollment.

Statistical methods

Statistical analysis was performed using the built-in Excel 2016 analysis package using the methods of descriptive statistics, correlation and regression analysis. Normality of distribution was estimated by Kolmogorov-Smirnov test (KS-test). Confidence intervals (CI) of medians were calculated according to Conover,[171] relative risks (RR) and odds ratios (OR) according to Altman,[172] risk difference (RD) according to Newcomb and Altman,[173] product of means according to Goodman,[174] ratio of means according to Fieller[175,176] for independent means, and by Taylor approximation[177] for dependent means, ratio of two independent lognormally distributed estimates – by Newcomb's MOVER-R algorithm.[178] Inverse-variance weighting was used.[179] The significance of differences of parametric criteria was estimated by the two-sample Student t-test or Welch t-test for unequal variance;[180] of paired nonparametric criteria (proportions) – by chi-square test (χ^2) according to Campbell-Richardson.[181] Significance of rates and proportions with known 95%CI was estimated according to Altman,[182] significance of difference of two independent estimates – by two-sample z-test. All p-values are two-sided. 95% probability ($\alpha = 0.05$) was used for significance testing. Since log-transformation significantly inflates confidence intervals (up to 40 times in some cases[183]), 90% probability ($\alpha=0.1$) is considered applicable for significance of difference of estimates based on log-transformed parameters.

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Survival analysis was performed using Excel-based software package GRISA (Galenic Research Institute, 2015) by Kaplan-Meier estimate (KME) of cumulative probability of survival.[184] Standard errors and confidence intervals of KME were estimated by Greenwood's formula,[185] significance of differences – by log-rank test.[186] The hazard function was estimated by Cox proportional hazards regression analysis.[187]

Effect-to-treatment analysis

Effect-to-treatment analysis (ETA) was performed according to own algorithm[188] with the following settings: unit of treatment is a 28-days cycle, parameter of comparison is a mean survival time (mST) after relapse. Here and further we use mST for mean survival time and MST for median survival time. Medians were transformed into means with 95% confidence intervals (95%CI) using Hozo et al. (2005)[189] algorithm for medians with range and own simplified algorithm (see Supplement) for medians with 95%CI. Life months gained (LMG) parameter was calculated by subtracting the expected mST (emST). Effect-treatment ratio (ETR) was calculated by dividing LMG to mean number of cycles (mNC). Life quality adjustment was not possible due to initial differences between the cohorts. Median ETR (METR) was estimated by attenuation the ETR: $METR = ETR \times (1 - CA)^{(MNC - mNC)}$, where CA is a coefficient of attenuation. The dependence of mST from mNC was estimated by a function $mST = ETR \times (1 - CA)^{NC - mNC} \times NC + emST$ (where NC is a serial number of cycle); the extremum of the function is a maximal attainable survival time (MAST), the abscissa of the extremum is a peak number of cycle (PNC). Cost-effective number of cycles (CENC) was estimated as abscissa of cost-effective survival time value (CEST = 95%MAST). Cycles needed to treat per LMG (CNTM) was estimated as reciprocal of difference of ETRs: $CNTM = 1/\Delta ETR$. Effect enhancement ratio ($EER_{12} = ETR_1/ETR_2$) was estimated as an auxiliary parameter for calculation of CI and significance of CNTM: since EER and CNTM use the same parameters with the same null hypothesis [H_0: $ETR_1 = ETR_2$], their confidence intervals and significance are the same, and these parameters can be easily calculated for EER according to Altman.[182]

Economic evaluation

For economic evaluation, cost-effectiveness analysis (CEA) with sensitivity analysis, budget impact analysis (BIA) and cost-benefit analysis (CBA) were performed.[190,191,192,193,194,195] CEA and BIA were performed from the perspective of a health provider. CEA was based on cost-utility ratio (CUR) and incremental cost-effectiveness ratio (ICER). Ratio of CURs (CURR) and increment of CURs (ICUR) were used to compare CURs. Proportion of cost-effective cases (%CE) was estimated

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by one-tailed directional integral z-test with null hypothesis [H_0: CUR = CET] where CET is a cost-effectiveness threshold. To estimate a sensitivity of CEA, multiparametric equal cost-effectiveness test was performed exploring the value of a key parameter in which value of CURR equals 1.0 (or ICUR = 0). BIA estimated the difference of costs for treatment of 1,000 patients per year. CBA estimated the total economic effect (economy and earnings before interest and taxes (EBIT)) from the perspective of a healthcare facility.

Reporting

SOI is reported according to the STROBE statement for reporting observational studies.[196] Economic evaluation is reported according to the CHEERS standards.[197]

RESULTS

Patients characteristics

Fifty-four patients with WHO IV GBM (n = 53) and gliosarcoma (n = 1) matched the inclusion criteria (Table 2). Mean age was 48.7 ± 1.5 years, median age 49.8 years (range: 25.9 – 68.2; 95%CI: 42.2 – 52.8), including two (4%) elderly patients (≥68 years) and 26 patients (48%) over 50 years; male/female 33/21. Forty-two (78%) patients underwent complete trimodal pre-treatment including surgery and chemoradiation, four (7%) received surgery and radiation, four (7%) – surgery and chemo, three (6%) only radiation and one (2%) only chemoradiation. By modalities, 50 (93%) patients underwent previous surgery, 50 (93%) radiation and 47 (87%) chemotherapy (mainly TMZ).

Details of treatment

All patients (100%) received ddTMZ + mEHT treatment, and 43 (80%) patients received concurrent SAT. In total, 84 ddTMZ cycles were performed for 54 patients, in average 1.6 ± 0.1 cycle per patient, median 1.0 cycle (range: 1.0 – 5.0; 95%CI: 1.0 to 1.0). Average duration of treatment was 2.7 ± 0.6 months, median 1.1 months (range: 1 day – 26.4 months; 95%CI: 0.8 to 1.5 months). In eight (15%) cases treatment was terminated in view of progressive disease.

Average time elapsed since diagnosis to first mEHT session was 12.9 ± 2.1 months (Table 3), median 9.5 months (range: 0.2 – 94.2; 95%CI: 5.9 to 10.7). Total 995 mEHT sessions performed, in average 18.4 ± 0.4 per patient, median 14 (range: 3 – 65; 95%CI: 10 to 17). There were 18 (33%) patients with LD-mEHT.

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Response

Fifteen patients (28%) were assessed for response. One patient (7%) showed complete response (CR), two (13%) showed partial response (PR), so objective response rate (ORR) was 20%. Five patients (33%) showed stable disease (SD) and 7 (47%) were in progressive disease (PD) status, giving beneficial response rate (BRR) of 53%.

Survival

Average follow-up since last mEHT session (Table 4) was 5.6 ± 1.1 months, median 3.5 months (range: 1 day – 46.4 months; 95%CI: 2.2 to 5.3 months). For that period, 36 (67%) patients died, 2 (4%) were lost (censored), 16 (30%) were alive to the end of the follow-up period (right-censored). MST since first diagnosis was 20.8 months (95%CI: 15.2 to 25.1), five-year OS 13.5% (95%CI: 1.0% to 26.0%). MST since first mEHT session was 7.7 months (95%CI: 5.7 to 9.4), survival at 12 months was 29.5% (95%CI: 15.5% to 43.6%), at 24 months – 18.8% (95%CI: 6.5% to 33.1%) (Figure 1).

Safety

Unfortunately, the raw data presented doesn't contain safety data, so we should rely on the safety data of 140 patients reported in the primary paper.[68] No grade III-IV toxicity was reported. Short-term (<2h) asthenia after treatment encountered 10% of cases, rubor of the skin 8%, edema of fresh scars <1%, subcutaneous fibrosis 1%, burning blisters grade I-II – 2%, headache, fatigue and nausea (1-2 days) – 12%. In general, the toxicity profile can be assessed as extremely favorable.

ANALYSIS OF THE RESULTS

Covariates survival analysis

There was no difference in survival between patients treated with "mEHT only" (with/without SAT) and with combination treatment (Table 4, Figure 2), neither by survival (MST since 1st mEHT 6.4 months (95%CI: 3.1 to 9.9) vs 7.7 months (5.8 to 9.5), p = 0.403, hazard ratio (HR) 1.32 (95%CI: 0.92 – 1.88)) nor by response (BRR 57% vs 53%, p = 0.77), though "mEHT only" regimen was applied to significantly older patients (median 59.1 years vs. 49.8 years in combination treatment sample, p = 0.037) with KPS <60% unfit for chemotherapy and radiation.

From the other side, there was a significant difference between samples with LD-mEHT and high-dose mEHT (HD-mEHT) both in survival since 1st mEHT (p = 0.007, HR = 2.19 (95%CI: 1.21 – 3.95) and response (p = 0.003) (Table 4, Figure 3). It's hard to say, how really the difference in mEHT dose affects the response and survival because LD-mEHT sample included weakened

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patients with longer time since diagnosis to 1st mEHT (median 9.9 months (95%CI: 6.1 to 11.6)), shortest treatment time (median 0.5 months (95%CI: 0.4 to 0.6) vs. 1.9 months (95%CI: 1.2 to 2.8) in HD-mEHT sample, p = 0,0001) and highest rate of treatment termination (38% vs. 0% in HD-mEHT sample, p<0,0001) (Table 3). More correctly, LD-mEHT was rather a sequence of poor patients' state than a reason of decrease of survival. In other words, impossibility to reach the adequate mEHT dose for weakened patient makes his/her prognosis dismal.

Similar pattern was shown in the analysis of sample with SAT versus sample without SAT (Figure 4): MST since 1st mEHT was 8.7 months (95%CI: 7.2 to 11.4) with SAT versus 2.9 months (95%CI: 2.3 to 5.5) only without SAT (p = 0.004, HR = 0.40 (95%CI: 0.36 – 0.45)). Such low survival (almost twice less than expected) undisputedly indicates for selection of patients with bad prognosis and small life expectancy. Comparison of the samples showed that "No SAT" includes patients with significantly less TMZ cycles (mean 1.1 ± 0.1 cycles vs 1.7 ± 0.1, p = 0.017) and mEHT sessions (mean 11.2 ± 0.5 (median 10) vs 19.9 ± 0,4 (median 15), p = 0.013) with higher proportion of LD-mEHT (47% vs 27%, RR = 1.74 (0.90 to 3.34), p = 0.12). Therefore, this survival difference rather shows a tendency do not apply SAT in patients with bad prognosis, and that these patients were heavily undertreated, than the real survival efficacy of SAT, though the latter cannot be excluded.

Sample of younger patients (under 50 years) with HD-mEHT treatment showed the best results (Figure 5): MST since diagnosis 23.9 months (95%CI: 13.0 to Not Attained), 5-year OS 31.0% (95%CI: 5.1 to 56.8), MST since 1st mEHT session 12.8 months (95%CI: 8.2 to 48.1), and 85.7% of BRR. HR of survival since 1st mEHT versus the complete sample was 0.56 (95%CI: 0.52 to 0.87). Although overall survival didn't differ significantly from the complete sample (p = 0.32) and cumulative survival since 1st mEHT also was of borderline significance (p = 0.082), MST and BRR were significantly better (p = 0.047 and p = 0.007, respectively).

Selection of a group of comparison

Based on a systematic review[198] and a narrative review[199] of different ddTMZ regimens, five phase II, cohort, uncontrolled clinical trials studying ddTMZ 21/28d regimen were identified (Table 5).

Italian trial of Brandes et al. (2006)[200] studied highly-selected group of CTX-naïve patients with good performance status (median KPS = 90%). This was a specific design aimed to study the efficacy of TMZ at recurrent GBM in TMZ-naïve patients, and, due to this specificity, the results of Brandes are incomparable to both current trial and all other four ddTMZ trials, all made on TMZ-pretreated patients with KPS 60-80%. US Norden et al. (2013)[201] trial is one more stand-alone trial

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with median KPS 90% and extremely high share (65%) of patients with methylated MGMT promoter (excluded from the comparison, see "Bias assessment and limitations of the study"). German trial of Strik et al. (2008)[202] also stands alone: despite of the worst patients' performance status (median KPS = 60% which is usually considered unfit for CTX), the patients received the most extensive course of median 5 (mean 7.3) cycles of ddTMZ with a modest toxicity. Two remaining studies – Turkish one of Abacioglu et al. (2011)[203] and Spanish of Berrocal et al. (2010)[204] – were the "real-world"[57] studies without obvious differences from the everyday practice. Although Berrocal trial pretends to involve TMZ-resistant patients, it doesn't differ from Abacioglu trial both by extent of TMZ pre-treatment (median 6 cycles) and by time elapsed since diagnosis (14 months vs 13 months). The details of patients' characteristics and treatment schedules are presented in Table 5. Response and survival data are presented in Table 6. Strik survival data were corrected because the originally reported survival in months was derived from weeks by division to 4 (e.g., 32.8 w = 8.2 "chemo months") which overpriced survival in average for 9%.

Effect-to-treatment analysis

We used effect-to-treatment analysis (ETA) to compare the trials according to principles described in the statistics section. Mean survival time (mST) after relapse in patients receiving standard modern treatment (which can be defined as trimodal 1st-2nd-line treatment approximately equal to Stupp protocol[9]) was the parameter of comparison. Since the expected (reference) value of mST is absent in the literature, we deducted it from available data as 4.775 months (95%CI: 3.9 to 5.6) (see Supplement). Taking into account the worst MST of Berrocal study (5.1 months (95%CI: 3.7 to 8.5)), this MST expectancy seems reasonable. For the further analysis, we considered this parameter as both expected median and mean survival time (emST) since relapse (in view of supposed normal distribution according to central limit theorem). For further comparisons, meta-analysis and economic evaluations, the median parameters of all trials (MST and number of cycles) were translated into means according to the statistical methods section.

The results of ETA show the advantage of mEHT+ddTMZ regimen. The main comparator was the weighted average of three ddTMZ trials with comparable samples (WA (2-4)) (Table 7). Weighted average of all ddTMZ studies (WA (1-4)) and stand-alone Brandes and Strik studies were the additional comparators.

Mean ST in mEHT+ddTMZ sample (7.625 ± 0.57 m) was ranked third after Brandes and Srtik cohorts, and was significantly better than in Berrocal trial (5.6 ± 0.73 m, p = 0.031) and worse than

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in Brandes sample with borderline significance (9.95 ± 1.13 m, p = 0.070); other differences were not significant (Table 7). The differences by life months gained (LMG) were not significant. Mean number of treatment cycles (mNC) in mEHT+ddTMZ sample (1.56 ± 0.13) was significantly less compared to all cohorts and WAs (p ≤ 0.004). Relative survival gain has changed the ranking: ddTMZ+mEHT provided significantly better effect-treatment ratio (ETR = 1.83 LMG/ccl (95%CI: 1.04 to 4.20)) compared to all the cohorts and WAs (p < 0.022), except of Brandes cohort (ETR = 1.13 LMG/ccl (95%CI: 0.72 to 1.80), p = 0.273).

To make ETRs comparable, the common denominator was estimated as a median of mean number of cycles of all the cohorts: MNC = 4.2 ccls. To lead ETRs to the common denominator, the attenuation modelling was performed in the range of coefficients of attenuation (CA) 10-25 %×ccl^{-1} (Table 8). CA level of 15% was chosen for the following analysis as an optimal prognosis (Figure 6A). According to this scenario, median effect-treatment ratio (METR) of ddTMZ+mEHT cohort is 1,19 LMG/ccl (95%CI: 0.59 to 2.40) which is significantly more than METR of the main comparator (METR = 0.57 LMG/ccl (95%CI: 0.39 to 0.85), p = 0.011) and other cohorts (p ≤ 0.016), except Brandes (METR = 1.20 LMG/ccl (95%CI: 0.74 to 1.95), p = 0.979) and Strik (METR = 0.81 LMG/ccl (95%CI: 0.44 to 1.48), p = 0.302) cohorts. This scenario means that ddTMZ+mEHT cohort have to reach the maximal attainable survival time (MAST) of 10.10 months (95%CI: 9.10 to 11.10) at sixth cycle, which is significantly more than MAST of the main comparator (7.34 months (95%CI: 6.46 to 8.21), p < 0.001) and other cohorts (p ≤ 0.015), except Brandes cohort (10.15 months (95%CI: 9.24 to 11.06), p = 0.943).

Based on the "cycles needed to treat per LMG" criterion (CNTM) (Table 8), ddTMZ+mEHT regimen displayed strong and significant benefit versus Berrocal and Abacioglu cohorts and both WAs (CNTM = 1.00 – 1.68 ccls/LMG, p < 0.016), moderate and insignificant benefit versus Strik cohort (CNTM = 2.64 ccls/LMG, p = 0.302) and no effect versus Brandes cohort (CNTM = -90.98 ccls/LMG, p = 0.979).

Thus, ETA suggests the strong and significant enhancement of ddTMZ 21/28d regimen by concurrent mEHT.

Sensitivity analysis

Sensitivity analysis was completed to validate the robustness of the ETA results. For this purpose, the lower and upper limits of CA were estimated (Figure 6, Table 9): the lower limit at CA = 15% is defined by Abacioglu cohort, in which the ascending mST reaches cost-effective survival time level

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(CEST = 6.98 months) with other cohorts being between CEST and MAST (Figure 6A); the upper limit at CA = 19.3% is defined by Strik cohort, in which the descending mST reaches CEST = 8.35 months (Figure 6B). As if follows from Table 9, CNTM of ddTMZ+mEHT cohort versus the main comparator attenuates from strong to moderate from the lower to the upper limit (from 1.62 to 2.14 ccls/LMG) but remains significant ($p = 0.011 – 0.018$). The extremum modelling shows that CNTM of ddTMZ+mEHT cohort versus the main comparator remains significant ($p \leq 0.05$) up to CA = 24.4%. Thus, the results of the ETA are robust.

Safety comparison

Since the ddTMZ+mEHT regimen didn't displayed any grade III-IV toxicity, whereas the ddTMZ regimens generated such toxicity events at a rate of 45% – 92%, the difference was always highly significant ($p < 0.001$) (Table 10). This difference is caused by the short course of TMZ in the ddTMZ+mEHT cohort (median 1 cycle only). TMZ in known as a relatively safe alkylating drug: its toxicity appears after 2-3 cycles, and a development of the III-IV grade lymphopenia (the main adverse event) becomes virtually inevitable after 6 cycles. Grade I-II toxicity in ddTMZ+mEHT cohort was mild. Since 4% of grade I nausea can be attributed to TMZ, total 30% of the mEHT-related events encountered, the main of them are grade I-II skin reactions (12%) and grade I short-term (<2h) post-treatment asthenia (10%).

Thus, the data presented allow to conclude on high safety of mEHT *per se*, but don't allow to estimate the modifying effect of mEHT on TMZ toxicity (if exists).

Economic evaluation

Cost-effectiveness analysis

Cost-effectiveness analysis (CEA) was performed from the perspective of a health provider with lifetime horizon. The goal of the CEA was to evaluate cost-effectiveness of the ddTMZ+mEHT regimen versus ddTMZ only, so only direct costs for these two modalities were analyzed. It was considered by default that other costs are dispensed proportionally and don't affect the estimation based on the direct costs (see also "Bias assessment and limitations of the study").

Two costs models were used for the CEA, German and US (see "DISCUSSION: Economic evaluation"). The German model has lower costs level and less variance compared to the US. For both the models, enduser prices for TMZ were estimated based on open sources (as at Jan 21, 2017): mean 1.70 $/mg (95%CI: 1.44 to 1.95) in the USA[205] and 1.14 €/mg (95%CI: 1.12 to 1.17) in Germany[206] (see Supplement).

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Cost of the single mEHT session varies between countries from $100 in Russia to $500 in Israel and South Korea (as at 2016). In the European Union, it varies in the range from €145.14 per session in Germany to €300 – 400 in private clinics outside Germany. From the perspective of a health provider, this cost is limited by national regulations: eg, one deep HT session is reimbursed at a rate of €173 in Italy (National tariff nomenclature code 99.85.2) and €145.14 in Germany (GOA code 5854). In those countries where HT is not reimbursed by health insurance system (eg, Spain, Austria, etc.), the median private cost is about €300.

Thus, from the perspective of a health provider, costs of a single mEHT session in Germany was estimated as mean €145.14 with zero variance (95%CI: 145.14 to 145.14), whereas in the US the estimated mean is $300 (95%CI: 234 to 366) (Table 11).

The results of CEA are presented in Table 12 (German model) and Table 13 (US model). Along with four single cohorts of comparison, three weighted averages (WA) were assessed. WA (1-4) combines all the cohorts, WA (2-4) excludes Brandes as a selected cohort (selection bias-free average), WA (2-3) excludes also Berrocal cohort in view of its very low survival gain, which significantly affected the final results (low-result bias-free average, the main comparator).

Mean costs of ddTMZ+mEHT regimen both in the German (€9,344 (95%CI: 9,199 – 9,488)) and US ($15,378 (12,703 – 18,052)) models were significantly less versus all cohorts and WAs ($p < 0.05$ in all cases): Abacioglu cohort displayed the lowest costs (€14,379 (95%CI: 14,071 – 14,687) and $21,325 (95%CI: 18,135 – 24,515), respectively) and Strik cohort the highest (€31,539 (95%CI: 30,863 – 32,215) and $46,775 (95%CI: 39,779 – 53,772)); the main comparator WA (2-3) displayed €18,138 (95%CI: 17,750 – 18,527) and $26,901 (95%CI: 22,877 – 30,925).

For estimation of cost-utility ratio (CUR), we used weighted average index of health-related quality of life (HRQoL) of all five cohorts (0.74 QALY/LY) to counterweight the initial difference of the samples (range of median KPS 60-90%) not connected with the treatment (Table 2).

CUR of the ddTMZ+mEHT regimen both in the German (19,871 €/QALY (95%CI: 17,719 – 22,024)) and US (32,704 $/QALY (95%CI: 27,215 – 38,193)) models was also less versus all comparators. The difference was highly significant ($p \leq 0.001$), except of Brandes cohort (24,292 €/QALY (95%CI: 20,263 – 28,321), $p = 0.061$, and 36,028 $/QALY (95%CI: 28,866 – 43,189), $p = 0.472$). The main comparator WA (2-3) displayed 40,424 €/QALY (95%CI: 36,758 – 44,091) and 59,954 $/QALY (95%CI: 51,427 – 68,481), $p < 0.001$ for both.

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In the German model, versus cost-effectiveness thresholds (CET) 25,000 €/QALY (%CE_{25k}) and 30,000 € /QALY (%CE_{30k}), the proportion of cost-effective cases (%CE) for the ddTMZ+mEHT regimen was 88.8% (%CE_{25k}) and 99.2% (%CE_{30k}), i.e., it was cost-effective versus both CETs. All the other comparators showed negligible %CE (0 – 2.5%), except of Brandes cohort, which was also mainly cost-effective at both CETs (%CE_{25k} = 53.6% and %CE_{30k} = 76.5%). In the US model, versus CETs 30,000 $/QALY (%$CE_{30k}$) and 50,000 $/QALY (%$CE_{50k}$), %CE for the ddTMZ+mEHT regimen was 4.5% (%CE_{30k}) and 94.6% (%CE_{50k}), i.e., it was cost-effective versus CET = $50,000 only. Two other cohorts were also mainly cost-effective versus CET = $50,000, namely Brandes (%CE_{50k} = 84%) and Abacioglu (%CE_{50k} = 51.3%) cohorts; %CE_{50k} of all the WAs was negligible (2.0 – 2.3%).

As for comparative cost-effectiveness, only Brandes cohort showed ICER less than applied CETs (28,706 € /QALY (95%CI: -5,529 – 62,940) and 34,727 $/QALY (95%CI: -12,095 – 81,549). All the other cohorts and WAs were not cost-effective with ICER ranging from 43,717 €/QALY / 55,827 $/QALY to 367,368 €/QALY / 519,683 $/QALY.

Sensitivity analysis

For analysis of sensitivity of the CEA, we performed an equal cost-effectiveness test, that is explored the value of a key parameter in which value of the relative CUR (CURR) of the ddTMZ+mEHT regimen and the main comparator (WA (2-3)) equals to 1.0 (or ICUR = 0). For this purpose, the following variables were tested:

- price of mEHT session;
- number of TMZ application days (days on) over 28-days cycle;
- price of TMZ;
- number of cycles of ddTMX+mEHT.

The equivalent price of mEHT session is €683 in the German model and $1,013 in the US model, the coefficient of reliability of the CEA result (CR, the ratio of a key parameter of CE-equivalent model and the standard model) is 3.4/4.7 (Table 14). The equivalent price of TMZ is 0.50 $/mg in the US model and 0.24 €/mg in the German model, once again with CR = 3.4/4.7. Since these key parameters (prices) don't affect the treatment efficacy, their equivalent values don't need any size-dependent correction. The result means that the ddTMZ+mEHT regimen is cost-effective in the entire range of possible prices with double to quadruple redundancy.

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The equivalent number of TMZ "days on" is 4.46 days in the German model and 6.21 days in the US model, once again with CR = 3.4/4.7. This time, the key parameter affects the treatment efficacy, because the diminished dose (days) of ddTMZ can decrease the effectiveness and, therefore, can increase the ddTMZ+mEHT/ddTMZ CURR and can cause an offset of the equivalence point to the lower values of "days on". This means that the ddTMZ+mEHT regimen, most probably, keeps the cost-effectiveness up to the standard 5/28d regimen and below it, and the cost-effectiveness of mEHT could be generalized for the entire range of TMZ treatment of recurrent gliomas.

Maximal equivalent number of ddTMZ+mEHT cycles is 2.86 in the US model and 3.17 cycles in German model, CR = 1.8/2.1. This key parameter also affects the treatment efficacy, because with increase of number of cycles of the ddTMZ+mEHT regimen, the treatment efficacy and CUR will rise with offset of the equivalence point towards longer course. At least, the result means that the length of the ddTMZ+mEHT regimen can be doubled without loss of cost-effectiveness. The observed difference in CR values compared to those of the above parameters is also explainable. Since the other parameters are single-factor, they cause mono-factor CRm 3.4 and 4.7. Oppositely, the "number of ddTMZ+mEHT cycles" parameter includes two factors (TMZ and mEHT cycles) simultaneously, and the resulting double-factor $CR_d \approx \sqrt{CR_m}$.

Thus, the sensitivity analysis confirms that the results of the CEA are remarkably stable with from double to quadruple redundancy.

Budget impact analysis

We estimated a budget impact of the treatment of 1,000 patients per year (Table 12, Table 13) with time horizon of one year. Versus the main comparator, the economy (ΔC_{1000}) is €8,794,882 / $11,523,498 per year (German / US model) with 29.1 years of survival gain (ΔE_{1000}). Average economy ranged from €8,577,947 / $11,201,761 to €8,794,882 / $11,523,498 with 29.1 – 38.5 QALY gained. To extrapolate the economic results to a larger time horizon, depreciation rate of 20% per year must be applied.

Cost-benefit analysis

Cost-benefit analysis (CBA) was performed from the perspective of a large neurooncology center treating more than 150 patients with recurrent GBM per year (Table 15, Table 16). The main assumptions of the CBA are as follows:

- mean sessions per patient equals to that of SOI;

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- the mEHT device doesn't generate other revenues than healthcare system reimbursement for the treatment of those patients;
- the mEHT device operates in 12-hours/day mode;
- the capital costs including acquisition costs, shipment, installation and training are €300,000 in the German model and $400,000 in the US model;
- the service costs rate is 12% of the capital costs per year with 2-year free of charge guarantee service;
- the depreciation of the mEHT equipment at a rate 15% per year;
- the norm of profit of the healthcare provider is 50% (operational costs are 67% of revenues);
- the economy obtained in result of introduction of the ddTMZ+mEHT regimen depreciates at a rate of 20% per year;
- the economy is not included in earnings before interest and taxes (EBIT);
- no price discount/inflation rate used;
- time horizon is eight years.

CBA shows that use of mEHT device is profitable with above parameters and generates the total revenues in amount of €3,124,574 / $6,458,400 with EBIT €210,525 / $1,044,800 per a mEHT device over eight-year period, provided that operational costs are €2,083,049 / $4,305,600 for that period (€260,381 / $538,200 per year). With respect to the economy due to the use of the ddTMZ+mEHT regimen instead of ddTMZ only, total economic effect (economy + EBIT) over eight-year period is €5,700,034 / $8,237,432 per a mEHT device.

DISCUSSION

Clinical comparison

Trying to compare the ddTMZ+mEHT results with the other ddTMZ studies, we faced with incorrectness of the conventional comparison based on general endpoints, when compared treatment is not continued up to the maximal attainable course (MAC). E.g., in the SOI, only 15% of treatments were stopped in view of the disease progression, without any limiting toxicity. In such cases, the end of the treatment is caused either by physician's decision or economic reasons, or by patient's personal decision or economic reasons, or by applied protocol, or by combination of the reasons. In tertiary centers, like in the studied case, the treatment is typically limited by 1-2 cycles only whereas in clinics the median duration of MAC of recurrent GBM is five cycles.[56] This makes

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the obtained results incomparable. As a result, we developed an effect-to-treatment analysis (ETA) especially for such comparisons.[188]

The idea of ETA is simple and based on the effect-treatment ratio (ETR), i.e., life months gained per a typical 28-days treatment cycle, which is considered a unit of a CTX treatment. As it follows from Table 7, ETR makes ddTMZ+mEHT uncontested leader of the comparison with 1.83 LMG/ccl versus 1.13 LMG/ccl of the nearest competitor (Brandes cohort) and 0.58 LMG/ccl of the main comparator (WA 2-4), though in terms of conventional MST-based comparison, it is third in the competition (after Brandes and Strik cohorts).

The next step of ETA follows from the idea of attenuation of the treatment effect. This is a typical feature of all cancer treatments (generally, of any treatment at all, but especially expressed in cancer) in view of the ability of cancer cells to rapidly develop multiple mechanisms of acquired resistance to an applied treatment. This is especially correct for such diseases like GBM, which almost inevitably progresses, and for TMZ, for which many distinct mechanisms of acquired resistance are available,[207,208,209] so virtually all patients develop resistance to TMZ. As a result, the effectiveness of any cancer treatment decays (attenuates) soon enough.

The offered equation of the attenuation (Statistical methods) is based on ETR and coefficient of attenuation (CA). It is suggested that CA is common for all the ddTMZ cohorts. The maximum value of CA corresponds to assumption that the treatments have nearly reached the maximal attainable survival time (MAST) which equals the extremum of the function. In this case, CA = 15 %/ccl exactly matches this assumption (Figure 6A). Although Strik cohort is located after the maximum of the function, it is acceptable because this cohort is the most probably overtreated (mNC = 7.3 ccls vs. 3 – 4.5 ccls in other ddTMZ cohorts)..

The natural sequence of the attenuation idea is incomparability of ETRs obtained in different number of cycles because an early ETR with lower impact of attenuation is higher than a later one. For the correct comparison, ETRs should be led to the common denominator. The best common denominator is the median number of cycles (MNC) which equals 4.2 months. The resulting parameter median ETR (METR) allows to compare the different treatments correctly. In this comparison, the cohort of interest (COI) (METR = 1.19 LMG/ccl (95% CI: 0.59 to 2.40)) significantly surpasses the main comparator WA (2-4) (METR = 0.57 LMG/ccl (95%CI: 0.39 to 0.85), p = 0.011) and all other comparators (METR = 0.19 – 0.59, p = 0.001 – 0.016), except Brandes cohort (METR = 1.20 LMG/ccl (0.74 to 1.95), p = 0.979) and Strik cohort (METR = 0.81

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LMG/ccl (0.44 to 1.48), p = 0.302) (Table 8). In other words, the efficacy of IOI in CTX-pretreated patients with median KPS 60-70% is the same as in the selected cohort of CTX-naïve patients with median KPS 90%, and significantly better compared to TMZ-pretreated cohorts.

With CA 15%/ccl, COI shall reach the MAST of 10.10 months (95%CI: 9.10 to 11.10) at sixth cycle, which is significantly more than MAST of the main comparator (7.34 months (95%CI: 6.46 to 8.21), p < 0.001) and other cohorts, except Brandes cohort (10.15 months (95%CI: 9.24 to 11.06), p = 0.943). The next assumption is that CA of the ddTMZ+mEHT regimen is lower than that of ddTMZ only. Actually, the mechanisms of resistance to RF-field have to differ substantially from those to CTX. Little is known about such acquired resistance. One paper on TTF reports a possibility of selection or development of giant-cell GBM with syncytial-type cells,[210] which is reasonable adaptation for 100 kHz range, where the large size of a cell improves the shielding from external field, though it is a single-case observation, and it is hardly applicable to HFR, where size difference is not decisive. Taking into account the results of long-term (6 months – 3 years) mEHT treatments,[78,90,92] especially in patients with multiple liver metastases, which is virtually the same lethal condition like GBM, where mEHT displayed the ability to support PFS up to three years, and even to revert the progression happened after stop of mEHT[78] (i.e., didn't loss the efficacy over years), the assumption that CA of mEHT is lower than that of TMZ looks reasonable. If assume that the CA = 12.5 %/ccl, ddTMX+mEHT cohort can attain a MAST of 10.84 months, and of 12.13 months with CA = 10.0%.

The last parameter of ETA called "cycles needed to treat per one life month gained" (CNTM) is an analogue of the known parameter "number needed to treat" (NNT). It shows, at which number of cycles of the both compared treatments, the difference in their MST will reach one month. Positive CNTM means benefit, negative means detriment, and the value of CNTM characterizes the strength of the effect (Figure 7). In this comparison, all the cohorts displayed strong to moderate detriment versus the ddTMZ+mEHT regimen (Table 8), except the Brandes cohort (no effect).

Thus, ETA has allowed to uncover the real efficacy of ddTMZ+mEHT treatment, which was impossible to assess with the conventional comparison by general endpoints, and has suggested that mEHT strongly and significantly enhances the efficacy of ddTMZ 21/28d regimen with significantly less toxicity.

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Economic evaluation

We studied two options of mEHT application. The first one, so-called "German", is specific for a developed country with rigid governmental regulation of the medical market, which leads to relatively low prices for pharmaceuticals with low variance (mean price of TMZ is 1.14 €/mg (95%CI: 1.12 to 1.17)) and fixed and low enough prices for medical procedures (in this case, 145.14 €/sess with zero variance (95%CI: 145.14 to 145.14)). The second one, so-called "US", is specific for a developed country with lower governmental regulation, which leads to relatively high prices for pharmaceuticals with higher variance (mean price of TMZ 1.70 $/mg (95%CI: 1.44 to 1.95)) and variable and high enough prices for medical procedures (in this case, 300 $/sess (95%CI: 234 to 366)).

First, the adequacy of our costs estimation (€18,138 (95%CI: 17,750 – 18,527) (Table 12) and $26,901 (95%CI: 22,877 – 30,925) (Table 13) in the main comparator) have to be assessed. For this purpose, the result was compared with a recent study of Ray et al.[57] (2014), where expenditures for cancer drugs (without supportive drugs like antiemetics, pain killers, neutropenia related, etc.) for 6-month period were assessed as $13,555 – 17,204. Since the study was devoted to TMZ treatment and taking into account the difference in price of TMZ and other cancer drugs, these "cancer drugs" costs can be attributed to TMZ for 95-99%. Though the range $13,555 – 17,204 looks much less than the average $27,000 displayed in the current assessment, it should be noted that general practice of recurrent GBM treatment is based virtually exclusively on the standard TMZ 5/28d regimen[204] with 100-150 mg/m^2/d. The current regimen ddTMZ 21/28d 75-100 mg/m^2/d consumes 2.1 – 4.2 times more TMZ per course, therefore it is at least 2-3 times more expensive. Thus, the estimated costs range for ddTMZ 21/28d regimen is $27,000 – 50,000, and the costs estimation of the current trial is adequate. It also corresponds to other estimations.[55,56]

The result suggests the significant advantage of the ddTMZ+mEHT regimen over all the comparators ($p < 0.003$) (except Brandes cohort, against which the advantage was not significant ($p = 0.061 – 0.472$)) (Table 12, Table 13). In the German model (Table 12), the ddTMZ+mEHT regimen was cost-effective versus both 25,000 €/QALY and 30,000 €/QALY cost-effectiveness thresholds (CET) (88.8% and 99.2% of cost-effective cases, respectively), whereas the main comparator was not cost-effective (%CE of 0.0% and 0.2%). ICER versus ddTMZ+mEHT varied from 43,717 €/QALY to 367,368 €/QALY (except Brandes cohort which displayed ICER 28,706 €/QALY). In the US model (Table 13), the pattern was the same with more pronounced differences. First, the ddTMZ+mE regimen was not cost-effective versus CET = 30,000 $/QALY (%CE = 4.5%

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only), and only CET 50,000 $/QALY provides cost-effectiveness (%CE = 94.6%), whereas the main comparator showed a negligible cost-effectiveness ($\%CE_{50k}$ = 2.0%). ICER versus ddTMZ+mEHT varied from 55,827 $/QALY to 519,683 $/QALY (except Brandes cohort which displayed ICER 34,727 $/QALY).

Cost-effectiveness threshold (CET) (or willingness-to-pay, WTP) is set by National Institute for Health and Care Excellence (NICE) of £20,000-30,000 per QALY,[211] though studies show that acceptable limit can be lower up to £13-14,000.[212] In developed countries, CET of €/$/£30,000 is considered standard. CET for developing countries is suggested by WHO at the level of their triple GDP per capita for each DALY,[213] which is typically close to the above NICE WTP. For end-of-life applications, where QALY increment could be negligible, CET of £50,000 is supposed by NICE.[214] Finally, for some orphan diseases, the third CET of about £100,000 is offered.[215] Since a treatment of the recurrent GBM can be considered the end-of-life application, CET 50,000 $/QALY is applicable in the US model.

Thus, the economic evaluation suggests that inclusion of mEHT in the ddTMZ 21/28d regimen makes it cost-effective versus the applicable CET levels, whereas the ddTMZ 21/28d alone is not cost-effective. The sensitivity analysis suggests that the estimation is highly reliable with double to quadruple redundancy. It also suggests that the advantage of ddTMZ+mEHT in cost-effectiveness remains actual in the entire applicable range of prices for TMZ and mEHT procedure and TMZ intercycle variances (i.e., up to the lowest 5/28d regimen). It also suggests that the ddTMZ+mEHT course can be at least doubled without loss of cost-effectiveness. Since the cost-effective number of cycles (CENC, i.e., the number of cycles at which MST reaches 95% of MAST (Table 8)) for the ddTMZ+mEHT regimen equals 3.0, this means the all-range cost-effectiveness of the regimen.

The BIA suggests a significant economy in result of introduction of mEHT, which can be estimated of about €8,794,882 per year per 1000 patients in the German model and $11,523,498 per year per 1000 patients in the US model, with additional 29.1 – 38.5 QALY gained per 1000 patients.

Finally, CBA shows that mEHT, from the perspective of a single neurooncology center, is profitable in the both models (Table 15, Table 16).

Thus, introduction of mEHT generates both economy for budget and healthcare providers, and significant profit for the latter.

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Applicability of mEHT in GBM treatment

The result obtained in this study looks very promising, though a single retrospective trial usually doesn't provide the necessary grounds for generalization. Nevertheless, if the result is confirmed in the further meta-analysis, it will provide the excellent ground for generalization.

At least, it means that mEHT can be recommended as an enhancer of all ddTMZ regimens in the treatment of recurrent GBM, and, probably, for the regular 5/28d regimen too. Next, as it follows from the covariates survival analysis (Figure 2), mEHT can be successfully applied as a single treatment in those patients, for which CTX is impossible in view of toxicity or bad performance. Thus, mEHT has a capacity as a salvage treatment after the fail of CTX. With respect to the known low toxicity of mEHT[67,68,69,70,71] and its possibility to restore the performance and chemosensitivity, [78,90,92] this salvage treatment can, in some cases, return a possibility to continue CTX to failed patients.

Bias assessment and limitations of the study

We excluded the Norden trial[201] from ETA in view of lack of information on number of cycles and some uncertainties (namely, survival definition and some statistical uncertainties). The modest effect shown would not affect the comparison.

The main possible bias of a retrospective study is a selection bias. We consider the probability of the selection bias as minimal in the SOI because, in addition to the assurances of the authors of no exclusions from the sample, 153 patients with high-grade gliomas (HGG) is consistent with the whole amount of such patients in the enrolling centers, which are a small tertiary centers not specialized in neurooncology (and, in the case of the Institute of Microtherapy, in cancer care at all), for the five-year period. Thus, we consider the sample as consecutive patients with HGG enrolled for the stated period without exclusions and any selection. The declared inclusion criteria (recurrence/progression of HGG with KPS≥40%) rather describe the sample than limit it in any way. Absence of exclusion criteria confirms this suggestion.

At the same time, some compared ddTMZ studies showed the obvious selection bias. First, this is the Brandes study, in which the selection of CTX-naïve patients is presumed by the protocol, but the selection of patients with good performance (median KPS = 90%) also seems to be present (though it can be a natural sequence of the inclusion criteria). The same extremely favorable KPS is shown in the excluded Norden trial, which also showed extremely high share of MGMT-methylated patients (65% vs 45-46% in the other trials, which exceeds the highest historical level of about

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60%[19]) (Table 7). Also, the large share of re-operations in the Strik study (33.3%) might significantly improve the observed survival, making it hardly attributable to the applied ddTMZ treatment.

The difference in dosage between the ddTMZ regimens was not analyzed in ETA (though is considered in the economic evaluation). As many studies had displayed, there is no or negligible difference in efficacy of different doses of ddTMZ regimens, and sometimes lower doses were preferable.[216] Moreover, the possibility of dose reduction/escalation in all the protocols makes such analysis impossible. The average dose is never reported and can't be retrieved from the reported data. It is not excluded that actual doses were close.

There is an unequal MST starting point bias, because MST in the ddTMZ+mEHT cohort was calculated since 1st session of mEHT unlike since relapse/progression in the other cohorts. Since the SOI was carried out in a tertiary centers, it's normal that mEHT was applied not just after relapse but rather as second-line treatment of the relapse. Based on the median time of 9.0 months elapsed since diagnosis to 1st mEHT treatment, and estimated 7.5 months MPFS in GBM, the delay of mEHT since relapse can be 1 – 1.5 months. It can significantly change the results in favor of the ddTMZ+mEHT cohort (eg, estimated MST since relapse can reach 9 months instead 7.6 months, like in the best ddTMZ studies). At the same time, due to this delay, probably some 1st-line treatments of relapse in the SOI were not included in the assessment. Based on the delay, median one treatment cycle is supposed to be added, increasing mean CTX cycles number to 2-2.5, which can somewhat change the economic results in favor of concurrent ddTMZ studies. Thus, the bias of not equal MST starting point rather distorts the comparison in favor of ddTMZ studies, though economically it is somewhat counterbalanced.

It should be noted also that the two "real life" studies of Abacioglu and Berrocal displayed the longest time from initial diagnosis to enrollment (13 and 14 months, respectively), which is inevitably responsible for the low MST in these trials. We consider that in the weighted average assessment, this difference is counterbalanced by early enrollment in the Brandes and Strik trials and the median position of the SOI (Table 7). It is also counterbalanced (and even outbalanced) by the unequal histology bias, since Abacioglu and Berrocal trials included WHO III tumors (28% and 43%, respectively) with much longer survival, which can be, in turn, the reason of the delayed relapse.

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Nevertheless, there is a reciprocal dependence between the time to enrollment (relapse) and the MST since the enrollment (the SOI displays the medium-power correlation, Pearson 0.35), which is not considered in the ETA but seems counterbalanced or even outbalanced in favor of the ddTMZ cohorts.

Noteworthy, all the "real life" studies (Sahinbas, Berrocal and Abaciouglu) showed the same median age of 50 years, whereas the supposedly selection-biased trials included the older patients (55-57 years).

MEHT required additional visits to the hospital (2-3 times a week), which means additional transportation costs and influences cost-effectiveness from patient's perspective, though doesn't affect the assessment from the health provider perspective. At the same time, since a planned mEHT session typically doesn't require physician's involvement (a nursing procedure), we don't assume a better treatment control. Moreover, such control seems much more extensive in the compared prospective trials, where the follow-up included weekly complete blood counts,[201,200] physical and neurologic examinations every 4 weeks,[199,201] or even beweekly,[201] and brain imaging with MRI every 8 weeks[200] or earlier if indicated.[199] To compare, only 28% of patients in the SOI underwent brain imaging (the specificity of small tertiary centers). Better treatment control could significantly improve the treatment results.

Finally, all the compared ddTMZ studies recruited only patients in stable condition, whereas there was not such limitation in the SOI.

In general, although the assessment is distorted in favor of the ddTMZ studies, nevertheless it still allows to make an unambiguous conclusion on the advantage of combination of mEHT and TMZ.

Also, upon completion of the paper, we have revealed one more ddTMZ 21/28d cohort in a III phase randomized trial of Brada et al. (2010).[216] The result of this cohort (MST since relapse 6.6 months after median four ddTMZ cycles, which results in METR ≤0.5 LMG/ccl) would not in any way affect the results obtained.

Generalizability of the results

The results of the sensitivity analysis of CEA supposes the generalizability of the CEA results to the entire range of application of TMZ at recurrent GBM. There is no ground to doubt that the same or similar enhancement of TMZ efficacy and cost-efficiency by mEHT can be achieved also in the treatment of the newly diagnosed GBM, though, to the best our knowledge, mEHT still never was studied in such setting.

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Since TMZ is considered the most effective CTX treatment of GBM at the moment, the results of the covariate survival analysis (Figure 2) can be generalized to CTX at all. Thus, mEHT as a single treatment can be considered in those patients, for which CTX is impossible in view of toxicity or bad performance, and mEHT has a capacity as a salvage treatment after the fail of CTX.

Perspectives of research

This study creates a good basis for the further research on mEHT-enhancement of the GBM treatments with the possibility to develop a cost-effective alternative. First, we will estimate the other existing mEHT cohort trials, followed by systematic review with meta-analysis. Second, the new cohort and randomized trials at recurrent and newly diagnosed GBM are warranted.

Verifiability of the results

To provide the possibility to verify the results obtained, raw data of the study are available in the Supplement.

CONCLUSIONS

- In a general comparison, the ddTMZ+mEHT cohort has not revealed an improvement of the mean survival time (mST = 7.63 months (95%CI: 6.52 to 8.74)) compared to the main comparator, the pooled mST of three trials on TMZ-pretreated patients (7.16 months (95%CI: 6.25 to 8.08), p = 0.531).
- Effect-to-treatment analysis suggests that mEHT strongly and significantly enhances the efficacy of the ddTMZ 21/28d regimen: the relative efficacy (median effect-treatment ratio, METR) of the ddTMZ+mEHT regimen significantly surpassed that of the pooled ddTMZ alone (1.19 LMG/ccl (95% CI: 0.59 to 2.40) versus 0.57 LMG/ccl (95%CI: 0.39 to 0.85), p = 0.011).
- METR of ddTMZ+mEHT treatment in CTX-pretreated patients with median KPS 60-70% was the same as in the selected cohort of CTX-naïve patients with median KPS 90%, and significantly better compared to the TMZ-pretreated cohorts ($p \leq 0.015$).
- According to the attenuation modelling, in case of continuation of the treatment, the ddTMZ+mEHT cohort could supposedly reach MST of 10.10 months (95%CI: 9.10 to 11.10) in the pessimistic scenario and 11 – 12 months in optimistic scenarios.
- Sensitivity analysis shows that the result of ETA is robust.

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- The ddTMZ+mEHT regimen has displayed a significantly less toxicity compared to the ddTMZ regimens (no grade III-IV toxicity versus 45% – 92%, respectively) because of the shorter TMZ course (mean 1.56 versus 3.98 cycles).
- MEHT *per se* displays a high safety with a mild grade I-II toxicity (30% of events), mainly presented with mild skin reactions (12%) and short (<2h) post-treatment asthenia (10%).
- Cost-effectiveness analysis (CEA) suggests that the ddTMZ+mEHT regimen is cost-effective versus the applicable cost-effectiveness thresholds 25,000 – 50,000 €$/QALY, whereas ddTMZ 21/28d only is not cost-effective, with ICER versus ddTMZ+mEHT ranging from 43,717 €/QALY / 55,827 $/QALY to 367,368 €/QALY / 519,683 $/QALY.
- Sensitivity analysis suggests that the CEA result is highly reliable with double to quadruple redundancy, and the ddTMZ+mEHT regimen remains cost-effective in the entire applicable range of prices for TMZ and mEHT procedure, TMZ intercycle variances and mEHT duration.
- Budget impact analysis suggests a significant economy in result of the introduction of mEHT, which can be estimated from €8,577,947 / $11,201,761 to €8,794,882 / $11,523,498 with 29.1 – 38.5 QALY gained per 1000 patients.
- Cost-benefit analysis, from the perspective of a single neurooncology center, suggests that mEHT is profitable and will supposedly generate the total revenues in amount of €3,124,574 / $6,458,400 with EBIT €210,525 / $1,044,800 per a mEHT device over eight-year period. With respect to the economy due to the use of the ddTMZ+mEHT regimen instead of ddTMZ only, total economic effect (economy + EBIT) over eight-year period is €5,700,034 / $8,237,432 per a mEHT device.
- After confirmation of the result obtained, mEHT can be recommended as an enhancer for all ddTMZ regimens in the treatment of recurrent GBM, and, probably, for the regular 5/28d regimen too.
- MEHT can be applied as a single treatment in those patients, for which CTX is impossible in view of toxicity or bad performance, as a salvage treatment after the fail of CTX, with a possibility to restore the patient's performance and chemosensitivity and continue CTX.

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We thank all the other authors of the original study,[68] namely Dr. Hüseyin Sahinbas and Prof. Dietrich H. W. Grönemeyer from Institute of Microtherapy of University Witten-Herdecke (Bochum, Germany) and Dr. Eckhard Böcher from Clinic "Closter Paradise" (Soest, Germany) for conducting this remarkable trial, although they may not agree with all the interpretations/ conclusions of this paper.

DATA SHARING STATEMENT

Patient level data available at Supplement. Consent for data sharing was not obtained but the presented data are completely anonymised and risk of identification is absent.

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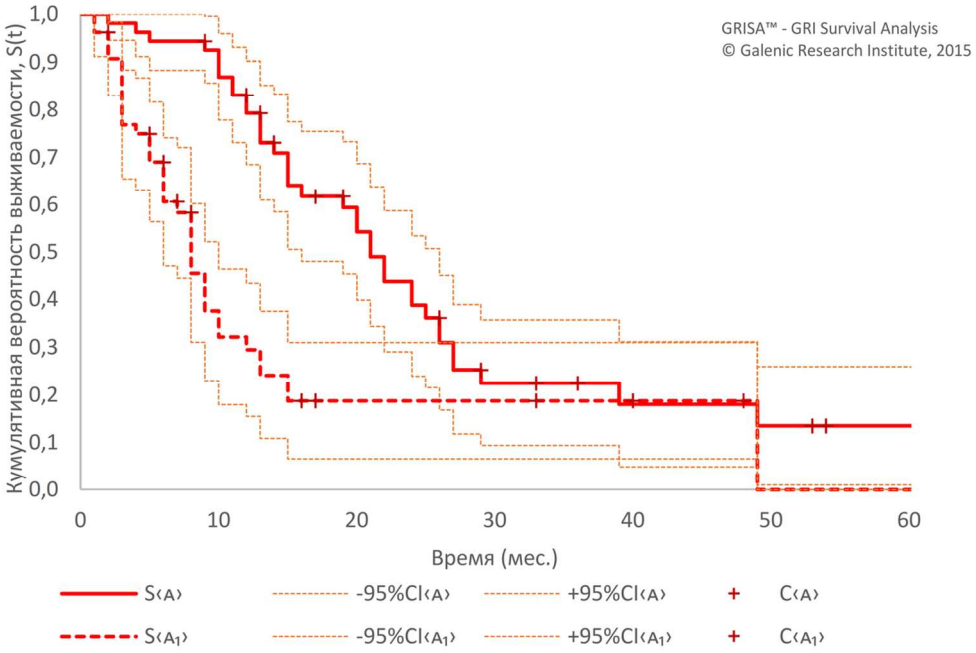

Figure 1. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

115x76mm (300 x 300 DPI)

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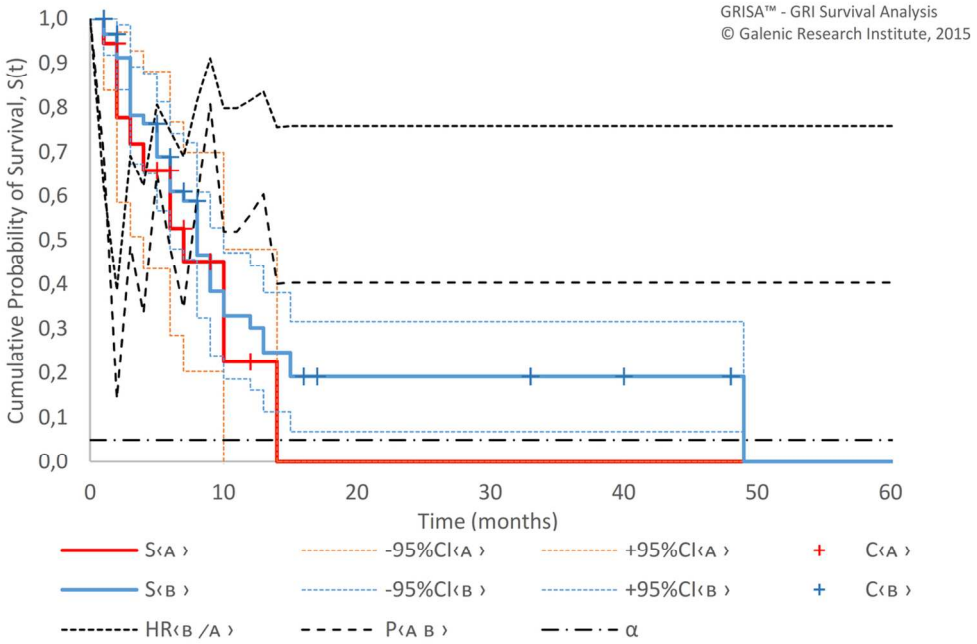

Figure 2. Survival since 1st mEHT session (Kaplan-Meier estimate) of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

114x76mm (300 x 300 DPI)

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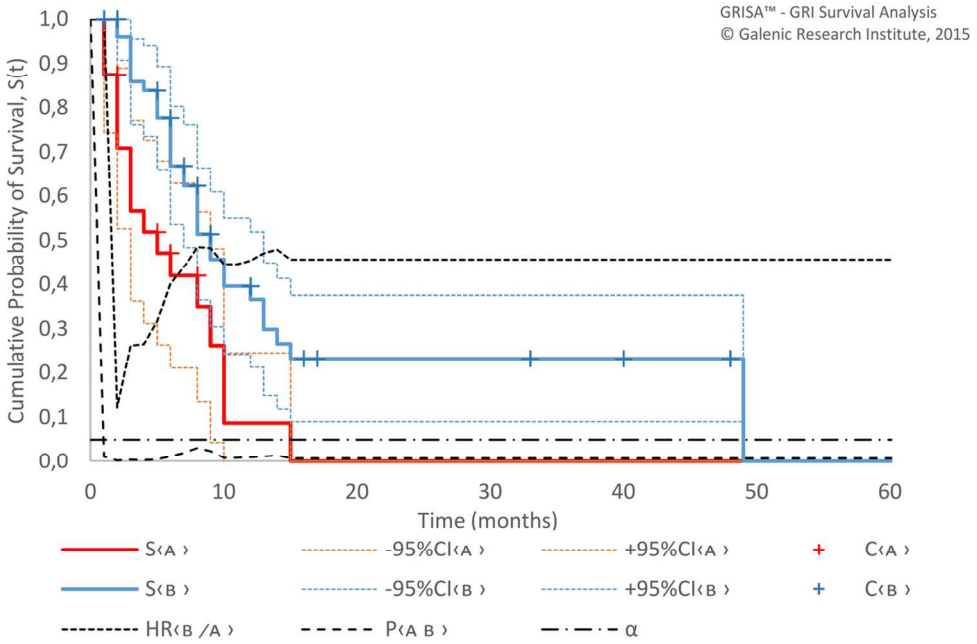

Figure 3. Survival since 1st mEHT session (Kaplan-Meier estimate) of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

115x76mm (300 x 300 DPI)

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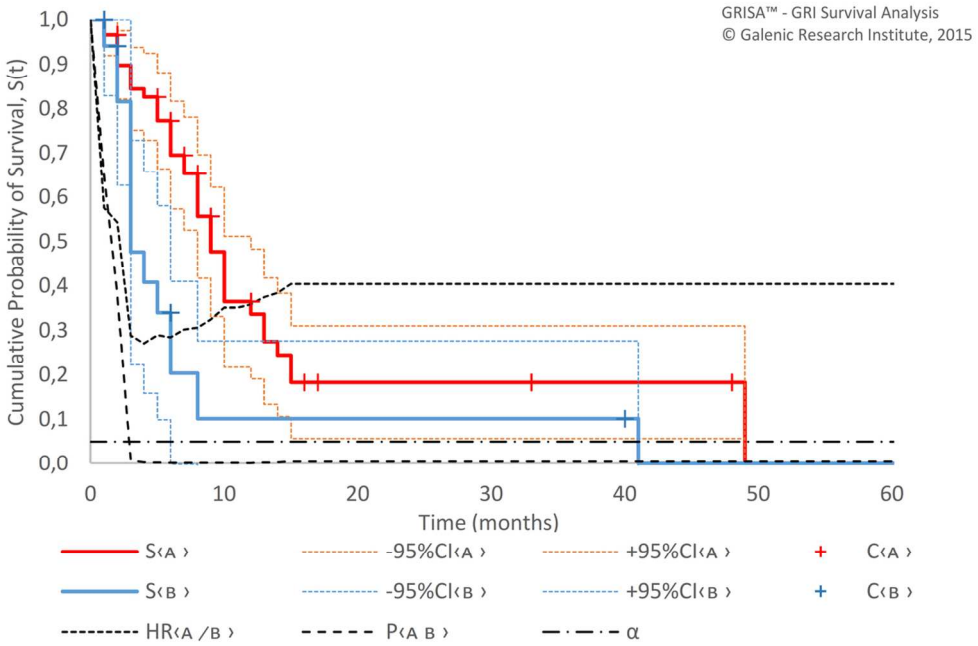

Figure 4. Survival since 1st mEHT session (Kaplan-Meier estimate) of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

115x76mm (300 x 300 DPI)

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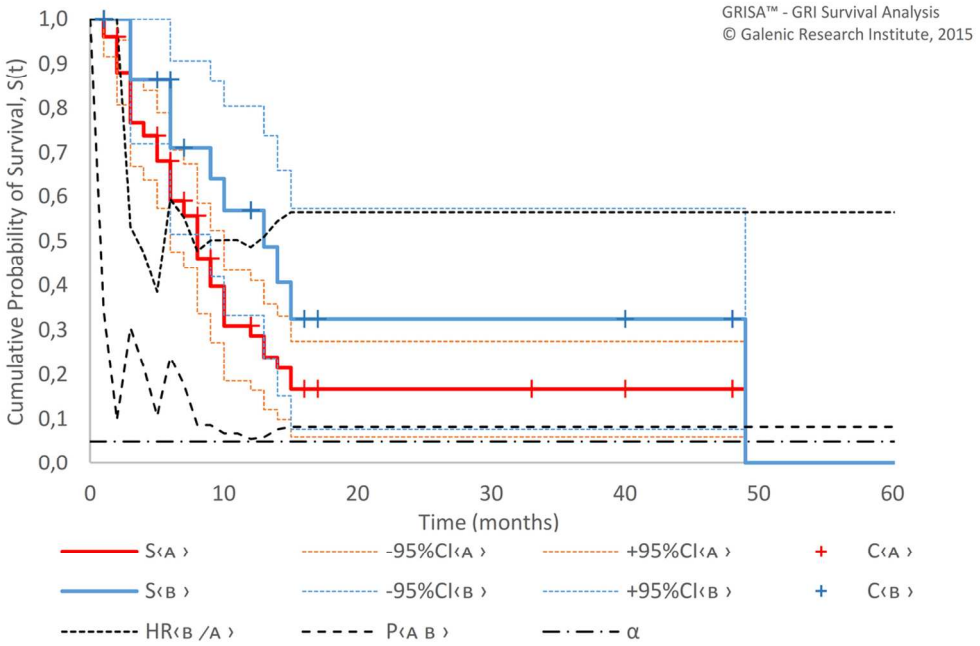

Figure 5. Survival since 1st mEHT session (Kaplan-Meier estimate) of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

115x76mm (300 x 300 DPI)

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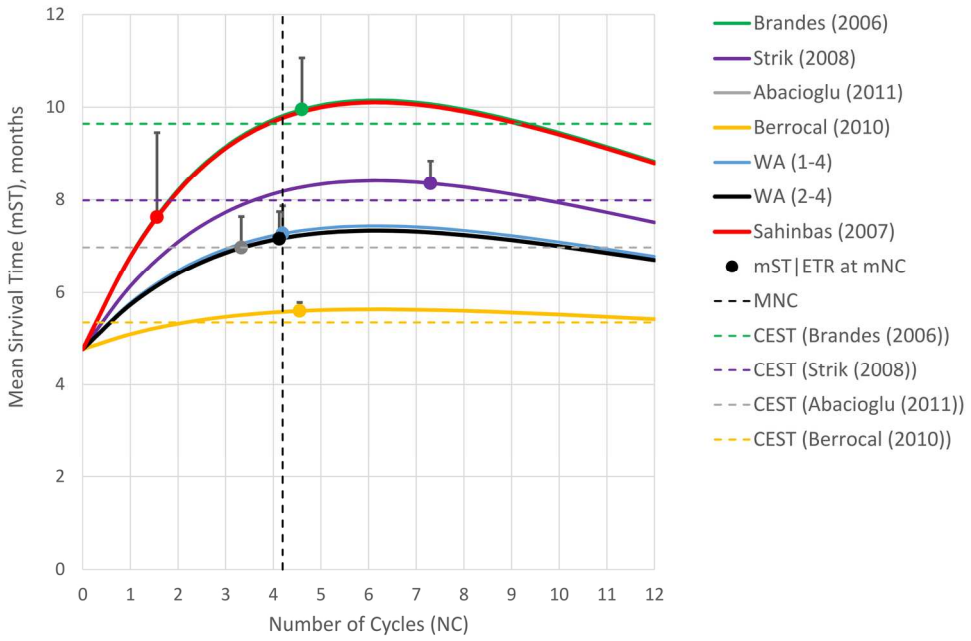

Figure 6. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

167x109mm (300 x 300 DPI)

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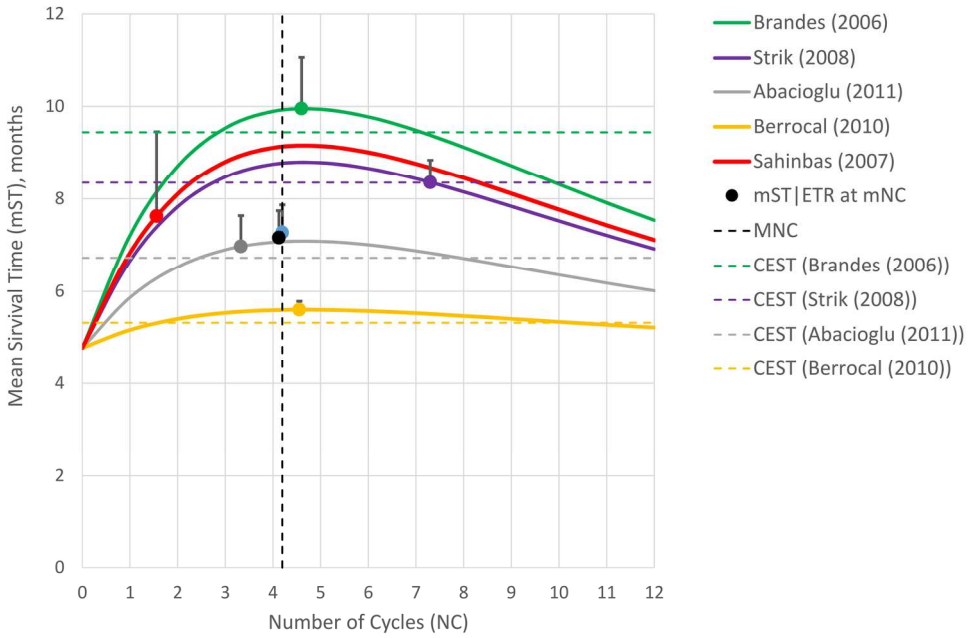

Figure 6. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

166x107mm (300 x 300 DPI)

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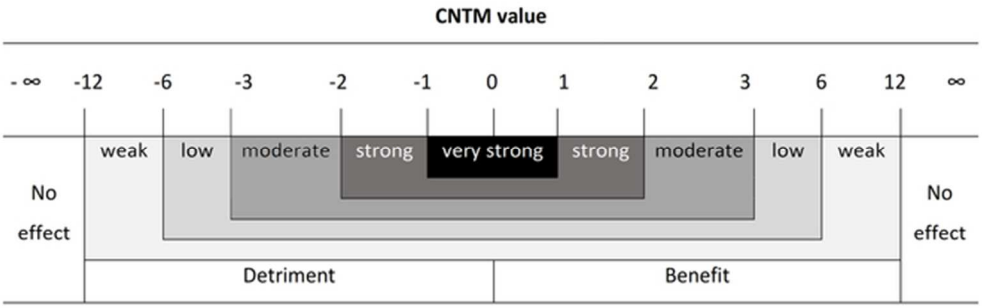

Figure 7. Cycles needed to treat per one life-month gained (CNTM) scale.

56x19mm (300 x 300 DPI)

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Table 1. Histologic types of brain tumors (Sahinbas et al., 2007[68]).

Total patients: 153

- [C71] Malignant neoplasm (MN) of brain: 137
  - WHO II: 8
    - Astrocytoma: 4
    - Mixed glioma: 4
  - WHO III: 39
    - Astrocytoma: 34
    - Mixed glioma: 3
    - Ependimoma: 1
    - Oligodendroglioma: 1
  - WHO III-IV: 4
    - Astrocytoma: 3
    - Infratentorial Glioma: 1
  - WHO IV: 86
    - **Glioblastoma: 81**
      - **Age >20: 75**
      - Age <20: 6
    - **Gliosarcoma: 1**
    - Medulloblastoma: 3
    - Primitive neuroectodermal tumor: 1
- [D43.1] Neoplasm of uncertain behavior of brain, infratentorial: 1
- [C79.3] Secondary MN of brain and cerebral meninges: 15
  - Adenocarcinoma: 12
    - MN of breast: 7
    - MN of bronchus and lung: 3
    - MN of colon: 1
    - MN of pancreas: 1
  - Ewing sarcoma: 1
  - Malignant rhabdoid tumor: 1
  - Cancer of unknown primary (CUP): 1

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Table 2. Patients characteristics (Sahinbas et al., 2007[68]).

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
No of patients (NOP)	76		18		58		54		24		52		23	
Male	46	61%	10	56%	36	62%	33	61%	16	67%	30	58%	11	48%
Female	30	39%	8	44%	22	38%	21	39%	8	33%	22	42%	12	52%
Earliest born	24.02.1932		24.02.1932		19.09.1935		19.09.1935		24.02.1932		18.06.1932		31.10.1954	
Latest born	03.04.1975		10.03.1971		03.04.1975		03.04.1975		03.04.1975		21.08.1973		21.08.1973	
Earliest diagnosed	01.08.1993		01.09.2000		01.08.1993		01.08.1993		12.07.1999		01.08.1993		01.08.1993	
Latest diagnosed	15.03.2005		03.07.2004		15.03.2005		30.08.2004		08.07.2004		15.03.2005		15.03.2005	
Age (years):														
Mean	50,2 ± 1,3		55,1 ± 2,8		48,7 ± 1,4		48,7 ± 1,5		50,9 ± 2,6		49,9 ± 1,5		39,9 ± 1,2	
Median	50,4		59,1		49,8		49,8		50,8		50,2		41,0	
Range	25,9 – 71,9		30,9 – 71,9		25,9 – 68,2		25,9 – 68,2		25,9 – 68,9		27,0 – 71,9		27,0 – 49,1	
95%CI	44,8 – 53,9		44,4 – 64,9		42,7 – 52,3		42,2 – 52,8		42,2 – 59,8		44,4 – 55,8		36,7 – 43,0	
P-value (t-test)					0,037								<0,0001*	
Elderly (over 68 years)	4	5%	2	11%	2	3%	2	4%	2	8%	2	4%	0	0%
Mature (over 50 years)	40	53%	12	67%	28	48%	26	48%	13	54%	27	52%	0	0%
Adults (over 20 years)	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
Pre-treatment:														

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
Surgery + Chemoradiation	57	75%	13	72%	44	76%	42	78%	15	63%	42	81%	20	87%
Chemoradiation	2	3%	1	6%	1	2%	1	2%	1	4%	1	2%	0	0%
Surgery + Radiation	7	9%	2	11%	5	9%	4	7%	4	17%	3	6%	2	9%
Surgery + Chemotherapy	5	7%	0	0%	5	9%	4	7%	1	4%	4	8%	1	4%
Radiaton only	5	7%	2	11%	3	5%	3	6%	3	13%	2	4%	0	0%
Chemotherapy total	64	84%	14	78%	50	86%	47	87%	17	71%	47	90%	21	91%
Radiation total	71	93%	18	100%	53	91%	50	93%	23	96%	48	92%	22	96%
Surgery total	69	91%	15	83%	54	93%	50	93%	20	83%	49	94%	23	100%

* versus all GBM sample

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Table 3. Details of treatment (Sahinbas et al., 2007[68]).

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Time to 1st mEHT since diagnosis (months):														
Mean	12,1 ± 1,6		11,2 ± 2,3		12,3 ± 1,9		12,9 ± 2,1		13,3 ± 2,4		11,5 ± 2,0		12,7 ± 4,2	
Median	8,5		8,0		9,3		9,5		9,9		8,2		5,9	
Range	0,2 – 94,2		2,3 – 44,1		0,2 – 94,2		0,2 – 94,2		1,6 – 49,1		0,2 – 94,2		1,0 – 94,2	
95%CI	6,7 – 10,6		6,1 – 15,2		5,8 – 10,7		5,9 – 10,7		6,1 – 11,6		5,1 – 10,0		4,1 – 10,0	
Earliest mEHT	01.03.2001		07.05.2001		01.03.2001		01.03.2001		07.06.2001		01.03.2001		01.03.2001	
Latest mEHT	20.05.2005		19.05.2005		20.05.2005		20.05.2005		28.04.2005		20.05.2005		20.05.2005	
Treatment combinations:														
mEHT + Chemoradiation + SAT	2	3%	0	0%	2	3%	0	0%	0	0%	2	4%	0	0%
mEHT + Chemoradiation	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy + SAT	43	57%	0	0%	43	74%	43	80%	12	50%	31	60%	13	57%
mEHT + Radiation + SAT	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy	11	14%	0	0%	11	19%	11	20%	6	25%	5	10%	3	13%
mEHT + SAT	13	17%	13	72%	0	0%	0	0%	4	17%	9	17%	5	22%
mEHT only	5	7%	5	28%	0	0%	0	0%	2	8%	3	6%	0	0%
Treatment by modality:														

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Radiation total	4	5%	0	0%	4	7%	0	0%	0	0%	4	8%	2	9%
SAT total	59	78%	13	72%	46	79%	43	80%	16	67%	43	83%	19	83%
Chemotherapy total														
NOP	57	75%	0	0%	57	98%	54	100%	18	75%	39	75%	17	74%
No of cycles	89		0		89		84		18		71		32	
Mean	1,5 ± 0,1		0		1,6 ± 0,1		1,6 ± 0,1		1,0 ± 0,0		1,8 ± 0,1		1,8 ± 0,2	
Median	1,0		1,0		1,0		1,0		1,0		1,5		2,0	
Range	1,0 – 5,0		1,0 – 3,0		1,0 – 5,0		1,0 – 5,0		1,0 – 1,0		1,0 – 5,0		1,0 – 5,0	
95%CI	1,0 – 1,0		1,0 – 2,0		1,0 – 1,0		1,0 – 1,0		1,0 – 1,0		1,0 – 2,0		1,0 – 2,0	
mEHT total:														
NOP	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
No of sessions	1367		292		1075		995		169		1198		545	
Mean	18,0 ± 0,3		16,2 ± 0,6		18,5 ± 0,4		18,4 ± 0,4		7,0 ± 0,1		23,0 ± 0,4		23,7 ± 0,6	
Median	14,0		13,5		14,0		14,0		7,0		18,0		23,0	
Range	3,0 – 65,0		4,0 – 43,0		3,0 – 65,0		3,0 – 65,0		3,0 – 9,0		10,0 – 65,0		10,0 – 65,0	
95%CI	11,0 – 16,0		7,0 – 23,0		11,0 – 17,0		10,0 – 17,0		6,0 – 9,0		15,0 – 26,0		15,0 – 27,0	
Low-dose mEHT	24	32%	6	33%	18	31%	18	33%	24	100%	0	0%	0	0%
Time of treatment (months):														
Mean	2,5 ± 0,4		1,6 ± 0,4		2,8 ± 0,5		2,7 ± 0,6		0,5 ± 0,0		3,4 ± 0,6		3,4 ± 0,7	

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Median	1,1		1,0		1,1		1,1		0,5		1,9		1,9	
Range	0,0 – 26,4		0,2 – 6,4		0,0 – 26,4		0,0 – 26,4		0,0 – 0,8		0,2 – 26,4		0,5 – 12,2	
95%CI	0,8 – 1,5		0,5 – 2,1		0,8 – 1,6		0,8 – 1,6		0,4 – 0,6		1,2 – 2,8		1,2 – 4,6	
P-value (t-test)	0,233								0,001					
Terminated (NOP)	9	12%	1	6%	8	14%	8	15%	9	38%	0	0%	0	0%
P-value (chi-square)	0,35								<0,0001				0,085*	

* versus all GBM sample

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Table 4. Survival and response rates (Sahinbas et al., 2007[68]).

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Response:														
NOP estimated	22	29%	7	39%	15	26%	15	28%	9	38%	13	25%	7	30%
CR	1	5%	0	0%	1	7%	1	7%	1	11%	0	0%	0	0%
PR	2	9%	0	0%	2	13%	2	13%	0	0%	2	15%	2	29%
OR	3	14%	0	0%	3	20%	3	20%	1	11%	2	15%	2	29%
SD	9	41%	4	57%	5	33%	5	33%	2	22%	7	54%	4	57%
BR	12	55%	4	57%	8	53%	8	53%	3	33%	9	69%	6	86%
PD	10	45%	3	43%	7	47%	7	47%	6	67%	4	31%	1	14%
P-value (χ^2)			0,77						0,003				0,007*	
Exitus	49	64%	12	67%	37	64%	36	67%	18	75%	31	60%	11	48%
Censored	27	36%	6	33%	21	36%	18	33%	6	25%	21	40%	12	52%
Lost	2	3%	0	0%	2	3%	2	4%	1	4%	1	2%	1	4%
Right-censored	25	33%	6	33%	19	33%	16	30%	5	21%	20	38%	11	48%
Overall survival (since diagnosis):**														
MST (months)	20,0		14,8		20,7		20,8		18,5		20,4		23,9	
(95%CI):**	(14,7–23,6)		(12,2–28,3)		(15,0–25,0)		(15,2–25,1)		(11,8–23,0)		(14,6–25,7)		(13,0–NR)	
Range	1,4 – 141,5		4,4 – 48,9		1,4 – 141,5		1,4 – 141,5		3,2 – 53,8		1,4 – 141,5		2,4 – 141,5	

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Parameter	All GBM (1)	mEHT ± SAT (2)	Combination treatment (3)	ddTMZ +mEHT (4)	LD-mEHT (5)	HD-mEHT (6)	HD-mEHT <50 years (7)
5-y survival (%)	13,5	0,0	13,3	13,5	0,0	16,1	31,0
(95%CI)	(2,8–24,2)	(0,0–0,0)	(1,0–25,6)	(1,0–26,0)	(0,0–0,0)	(2,0–30,1)	(5,1–56,8)
P-value (log-rank)		0,436			0,350		0,32*
Survival since 1st mEHT (months):**							
MST (months)	7,6	6,4	7,7	7,7	4,4	8,3	12,8
(95%CI):**	(5,8 – 9,3)	(3,1 – 9,9)	(5,8 – 9,5)	(5,7 – 9,4)	(2,2 – 8,8)	(6,7 – 12,3)	(8,2 – 48,1)
Range	0,3 – 47,3	0,3 – 13,6	0,7 – 47,3	0,7 – 47,3	0,3 – 14,9	1,0 – 47,3	1,0 – 47,3
1-y survival (%)	28,8	22,6	30,2	29,5	8,7	36,6	56,9
(95%CI)	(16,5–41,0)	(0,0–47,9)	(16,1–44,2)	(15,5–43,6)	(0,0–24,5)	(21,3–51,9)	(33,3–80,5)
2-y survival (%)	16,8	0,0	19,2	18,8	0,0	23,3	32,5
(95%CI)	(6,0–27,5)	(0,0–0,0)	(6,8–31,6)	(6,5–31,1)	(0,0–0,0)	(9,0–37,5)	(7,7–57,4)
P-value (log-rank)		0,403			0,007		0,047*
Survival time after the last mEHT (follow-up) (months):							
Mean	5,0 ± 0,8	3,8 ± 0,8	5,3 ± 1,0	5,6 ± 1,1	3,9 ± 0,7	5,5 ± 1,1	7,4 ± 2,4
Median	3,3	2,9	3,4	3,5	2,4	3,4	3,3
Range	0,0 – 46,4	0,0 – 12,1	0,1 – 46,4	0,1 – 46,4	0,0 – 14,3	0,1 – 46,4	0,2 – 46,4
95%CI	2,2 – 4,6	0,8 – 5,5	2,2 – 5,0	2,2 – 5,3	1,5 – 5,3	2,5 – 5,0	1,3 – 7,3

* versus all GBM sample; ** Kaplan-Meier estimation; NR – not reached.

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Table 5. Comparison of dose-dense temozolamide trials: patients characteristics.

Study (Year) (Enrollment)	NOP	Country	Study design	Inclusion	Med Age	KPS	Pre-treatment SRG	RT	TMZ	MTAD	Other	Current treatment Regimen	NOC
Brandes (2006)	33	Italy		Recurrent/ progressive GBM in chemonaïve pts with KPS≥60 in SCC; 45% of met-MGMT	57	90% (60-100)	100 %	100 %	0%	N/A	R1:100%: met 45.5%; re-op. 3%.	75 mg/m^2/d qd X21/28d	153 ccls: mean 4.6, med 3 (1-15)▪
Strik (2008) (2005-2007)	18	Germany	Phase II prospective cohort uncontrolled	Recurrent/ progressive GBM, KPS≥50 in SCC: 1st relapse 78%, 2nd – 22%	54.8	60% (50-100)	100 %	100 %	100% (≥1 adj TMZ ccls)	7.5 m[a]	R1/2: 77.8/22.2%; met.46.2%; re-op. 33.3%	100 mg/m^2 /d qd X21/28d	154 ccls, mean 7.3, med 5 (2-18)▪
Abacioglu (2011) (2006-2008)	16	Turkey		Recurrent/progressive GBM, KPS≥70 in SCC	50	80% (50-100)	100 %	100 %	100% (med 6 ccls)	13 (6-105)▪			med 2 (1-8)▪
Berrocal (2010)	47	Spain		Recurrent/progressive HGG with KPS≥60 in SCC;	50	(70-80%) ECO	81%	100 %	100% (med 6 ccls)	14 m (6-126)▪		85 mg/m^2/d qd X21/28d	med 2 (1-13)▪

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				WHO IV GBM 57%, WHO III 43%		G 1							
Norden (2013)	55	USA		Recurrent/progressive GBM with KPS≥60 in SCC, standard (Stupp) pre-treatment with ≥2 adjuvant cycles)	57	90% (60-100)	100 %	100 %	100% (≥2 adj TMZ ccls) (med 6 ccls (12-16))	N/A	R1: 100%; R/P: 48%/ 52%, met. 65%	100 mg/m^2 /d qd X21/28d X12 ccls or until PD	N/A
Sahinbas (2007) (2000-2005)	54	Germany	Retro-spective cohort uncontrolled	Recurrent/progressive GBM, KPS≥40	49.8	60% (40-100)[b]	93%	93%	87%	9.5 m (5,9-10,7)*		100 mg/m^2 /d qd X21/28d + mEHT	84 ccls, mean 1.6±0.1, med 1 (1-5)▪

SCC: stable clinical condition; HGG: high-grade glioma; GBM: glioblastoma multiforme; KPS: Karnofsky performance score; MGMT: O6-Methylguanine DNA Methyltransferase; qd: daily; MTAD: median time after diagnosis; TMZ: temozolomide; R1: first relapse/progression; R1/2: first / second relapse; R/P: relapse / progression; met.: methylated MGMT promoter gene; re-op.: re-operation; * 95% confidence interval; ▪ range; [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] estimated.

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Table 6. Comparison of dose-dense temozolamide trials: response and survival.

	NOP		Response			Overall survival	Survival since relapse		
Study	total	EFR	CR	ORR	BRR	MST mo (95%CI)	MST mo (95%CI)	1-y OS (95%CI)	MTTP (95%CI)
Brandes (2006)	33	33	3%	9%	61%	N/A	9,1 (7,1 – 14,5)	38%	3,7 (2,8 – 6,3)
Strik (2008)	18	18	17%	22%	61%	16,4[a] (17,9[b])	8,35[a] (9,1[b]) (N/A)	N/A	N/A
Abacioglu (2011)	16	14	0%	7%	57%	N/A	7 (5,7 – 8,2)	0%	3,0 (1,8 – 4,2)
Berrocal (2010)	47	27	0%	7%	38%[a]	N/A	5,1 (3,7 – 8,5)[c]	N/A	2,0 (0,9 – 3,1)
Norden (2013)	55	54	0%	13%	48%	11,7 (8,1 – 16,2)	N/A	N/A	1,8 (1,8 – 2,8)
Sahinbas (2007	54	15	7%	20%	53%	20,8 (15,2–25,1)	7,7 (5,7 – 9,4)[e]	29,5% (15,5–43,6)	N/A

EFR: Estimated for response; CR: Complete response; ORR: objective response rate (CR + partial response); BRR: beneficial response rate (ORR + stable disease); NOP: number of patients; MST: median survival time (Kaplan-Meier estimation); [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] originally reported data (without correction); [c] for the complete sample of 47 pts, including 27 GBM and 20 WHO III tumors; [d] combination treatment sample; [e] since 1st mEHT (not since relapse).

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Table 7. Effect-to-treatment analysis: basic parameters.

No	Study	NOP	mST	P-value	Rank	LMG	P-value	mNC	P-value	ETR (95%CI)	P-value	Rank
1	Brandes (2006)	33	9,95 (7,73-12,17)	0,070	1	5,18 (2,79-7,56)	0,104	4,60 (3,87-5,33)	<0.001	1,13 (0,72-1,80)	0,273	2
2	Strik (2008)	18	8,35 (7,67-9,03)	0,416	2	3,58 (1,98-5,17)	0,506	7,30 (6,05-8,55)	<0.001	0,49 (0,31-0,70)	0,001	6
3	Abacioglu (2011)	16	6,98 (6,23-7,73)	0,345	6	2,20 (1,05-3,35)	0,486	3,33 (2,43-4,22)	0,004	0,66 (0,38-1,05)	0,022	3
4	Berrocal (2010)	47	5,60 (4,16-7,04)	0,031	7	0,83 (-0,86-2,51)	0,073	4,55 (3,94-5,16)	<0.001	0,18 (-0,05-0,44)	<0,001	7
5	WA (1-4)	114	7,27 (6,30-8,24)	0,638	4	2,50 (1,20-3,80)	0,718	4,20 (3,82-4,57)	<0.001	0,59 (0,39-0,85)	0,006	4
6	WA (2-4)*	81	7,16 (6,25-8,08)	0,531	5	2,39 (1,13-3,65)	0,633	4,13 (3,68-4,57)	<0.001	0,58 (0,37-0,83)	0,005	5
7	Sahinbas (2007)	54	7,63 (6,52-8,74)	1,000	3	2,85 (1,44-4,26)	1,000	1,56 (1,31-1,81)	1,000	1,83 (1,04-4,20)	1,000	1

NOP: number of patients; WA: weighted average; mST: mean survival time since relapse; LMG: life months gained; mNC: mean number of cycles treated; * main comparator.

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Table 8. Effect-to-treatment analysis: 15% attenuation model estimation.

No	Study	MAST	p–value	PNC	CEST	CENC	METR	EER	p–value	CNTM 1	2	3	4	5	6	7
1	Brandes (2006)	10,15 (9,24-11,06)	0,943	6	9,64	4	1,20 (0,74-1,95)	1,01	0,979	∞	2,56	1,59	0,99	1,65	1,59	91
2	Strik (2008)	8,40 (7,52-9,29)	0,015	6	7,98	4	0,81 (0,44-1,48)	0,68	0,302	-2,56	∞	4,22	1,62	4,63	4,19	-2,64
3	Abacioglu (2011)	7,34 (6,46-8,22)	<0,001	6	6,98	4	0,57 (0,37-0,89)	0,48	0,016	-1,59	-4,22	∞	2,62	-47,9	592	-1,62
4	Berrocal (2010)	5,63 (4,76-6,51)	<0,001	6	5,35	3	0,19 (0,08-0,49)	0,16	<0,001	-0,99	-1,62	-2,62	∞	-2,48	-2,63	-1,00
5	WA (1–4)	7,44 (6,56-8,31)	<0,001	6	7,07	4	0,59 (0,40-0,88)	0,50	0,015	-1,65	-4,63	47,9	2,48	∞	44,3	-1,68
6	WA (2–4)*	7,34 (6,46-8,21)	<0,001	6	6,97	4	0,57 (0,39-0,85)	0,48	0,011	-1,59	-4,19	-592	2,63	-44,3	∞	-1,62
7	Sahinbas (2007)	10,10 (9,10-11,10)	1,000	6	9,5	4	1,19 (0,59-2,40)	1,00	1,000	-91	2,64	1,62	1,00	1,68	1,62	∞

WA: weighted average; * main comparator; CA: coefficient of attenuation; MAST: maximal attainable survival time; PNC: peak number of cycles; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio; EER: effect enhancement rate.

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Table 9. Effect-to-treatment analysis: sensitivity analysis.

			CA = 15%				CA = 19.3%			
No	Study	mST	CEST	METR	CNTM	p–value	CEST	METR	CNTM	p–value
1	Brandes (2006)	9,95 (7,73-12,17)	9,64	1,20 (0,74-1,95)	90,98 (48,52 – 170,60)	0,979	9,44	1,23 (0,75-2,01)	5,30 (2,97 – 9,47)	0,585
2	Strik (2008)	8,35 (7,67-9,03)	7,98	0,81 (0,44-1,48)	-2,64 (-5,43 – -1,28)	0,302	**8,35**	0,95 (0,49-1,86)	-11,73 (-24,39 – -5,64)	0,830
3	Abacioglu (2011)	6,98 (6,23-7,73)	**6,98**	0,57 (0,37-0,89)	-1,62 (-2,94 – -0,89)	0,016	6,73	0,55 (0,36-0,83)	-2,04 (-3,43 – -1,22)	0,016
4	Berrocal (2010)	5,60 (4,16-7,04)	5,35	0,19 (0,08-0,49)	-1,00 (-2,77 – -0,36)	<0,001	5,32	0,20 (0,08-0,51)	-1,19 (-3,22 – -0,44)	0,001
5	WA (1–4)	7,27 (6,30-8,24)	7,07	0,59 (0,40-0,88)	-1,68 (-2,93 – -0,96)	**0,015**	6,91	0,59 (0,40-0,88)	-2,26 (-3,70 – -1,38)	**0,027**
6	WA (2–4)*	7,16 (6,25-8,08)	6,97	0,57 (0,39-0,85)	-1,62 (-2,84 – -0,92)	**0,011**	6,82	0,57 (0,38-0,85)	-2,14 (-3,52 – -1,30)	**0,018**
7	Sahinbas (2007)	7,63 (6,52-8,74)	9,6	1,19 (0,59-2,40)	∞	1,000	8,69	1,04 (0,77-1,41)	∞	1,000

WA: weighted average; * main comparator; CA: coefficient of attenuation; mST: mean survival time; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio.

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Table 10. Comparison of dose-dense temozolamide trials: adverse events.

Adverse Event	Grade	Brandes (2006)	Strik (2008)	Abacioglu (2011)	Berrocal (2010)	Norden (2013)	Sahinbas (2007)
	NOP	33	18	16	47	55	140
Total events	I-II	122%	N/A	44%	194%	N/A	34%
	III-IV	76%	49%	92%	45%	60%	0%
	χ^2	123,721	72,196	141,308	70,654	100,593	
	p	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	
Lymphopenia	I-II	21%		12%	55%		0%
	III-IV	24%	14%	80%	28%	38%	0%
Leucopenia	I-II	21%		20%	28%		0%
	III-IV	24%	14%	4%	2%	5%	0%
Neutroopenia	I-II	9%			17%		0%
	III-IV	12%			2%	4%	0%
Trombocytopenia	I-II	3%		8%	19%		0%
	III-IV	3%	5%	8%	11%	4%	0%
Anemia	I-II	26%		4%			0%
	III-IV	3%				2%	0%
Nausea/Vomiting	I-II	6%			26%		4%
	III-IV	3%			2%	2%	0%
Fatigue	I-II						4%
	III-IV					5%	0%
Obstipation/Diarrhea	I-II	24%			15%		0%
	III-IV	3%					0%
Infection	I-II	12%					0%
	III-IV	3%	5%				0%
Headache	I-II						4%
Skin reactions	I-II						12%
Asthenia	I-II				17%		10%
Gastrointestinal	I-II				17%		0%
	III-IV		10%				0%

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Table 11. Calculated prices for economic evaluation.

	US model		German model	
	TMZ	mEHT	TMZ	mEHT
Parameter	$/mg	$/sess.	€/mg	€/sess.
Mean (95%CI)	1,70 (1,44 – 1,95)	300 (234 – 366)	1,14 (1,12 – 1,17)	145 (145 - 145)
Median (range)	1,77 (0,59 – 4,42)	300 (150 – 500)	1,14 (0,88 – 1,55)	145 (145 - 300)

TMZ: temozolomide; mEHT: modulated electro-hyperthermia.

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Table 12. Cost-effectiveness analysis (German model).

Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{25k}$	$\%CE_{30k}$	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
Brandes (2006)	14,905 (14,586 – 15,225)	<0.001	24,292 (20,263 – 28,321)	4,421 (2,090 – 6,752)	1.22 (1.10 – 1.35)	0.061	53.57%	76.5%	28,706 (-5,529 – 62,940)	5,561,695	193.8
Strik (2008)	31,539 (30,863 – 32,215)	<0.001	61,250 (53,939 – 68,561)	41,379 (37,491 – 45,267)	3.08 (2.83 – 3.34)	<0.001	0.00%	0.0%	367,368 (-710,070 – 1,444,806)	22,195,135	60.4
Abacioglu (2011)	14,379 (14,071 – 14,687)	<0.001	33,429 (30,717 – 36,141)	13,558 (11,791 – 15,325)	1.68 (1.57 – 1.80)	<0.001	0.12%	1.8%	-92,957 (-352,869 – 166,956)	5,035,150	-54.2
Berrocal (2010)	16,721 (16,362 – 17,079)	<0.001	48,419 (39,174 – 57,665)	28,548 (23,705 – 33,391)	2.44 (2.16 – 2.71)	<0.001	0.31%	0.7%	-43,717 (-91,130 – 3,697)	7,377,172	-168.8
WA (1-4)	17,922 (17,538 – 18,306)	<0.001	39,967 (35,985 – 43,949)	20,096 (17,787 – 22,405)	2.01 (1.86 – 2.16)	<0.001	0.04%	0.3%	-291,167 (-1,869,626 – 1,287,291)	8,577,947	-29.5
WA (2-4)	18,043 (17,657 – 18,430)	<0.001	40,845 (36,926 – 44,763)	20,973 (18,692 – 23,255)	2.06 (1.90 – 2.21)	<0.001	88.8%	99.2%	-226,212 (-1,153,427 – 701,004)	8,699,523	-38.5
WA (2-3)*	18,138	<0.001	40,424	20,553	2.03 (1.89	<0.001	0.02%	0.2%	-302,629	8,794,882	-29.1

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Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{25k}$	$\%CE_{30k}$	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
	(17,750 – 18,527)		(36,758 – 44,091)	(18,384 – 22,722)	– 2.18)				(-1,934,133 – 1,328,875)		
Sahinbas (2007)	9,344 (9,199 – 9,488)	1.000	19,871 (17,719 – 22,024)	0	1.00	1.000	88.8%	99.2%	0	0	0.0

TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main comparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{25k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) €25,000; $\%CE_{30k}$: %CE at CET €30,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained).

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Table 13. Cost-effectiveness analysis (US model).

Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	%CE$_{30k}$	%CE$_{50k}$	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
Brandes (2006)	22,106 (18,799 – 25,413)	0.003	36,028 (28,866 – 43,189)	3,324 (-1,280 – 7,927)	1.10 (0.96 – 1.25)	0.472	3.01%	84,02%	34,727 (-12,095 – 81,549)	6,728,332	193.8
Strik (2008)	46,775 (39,779 – 53,772)	<0.001	90,841 (76,123 – 105,558)	58,136 (50,122 – 66,151)	2.78 (2.45 – 3.11)	<0.001	0.02%	0,21%	519,683 (-1,009,423 – 2,048,790)	31,397,527	60.4
Abacioglu (2011)	21,325 (18,135 – 24,515)	0.007	49,579 (42,820 – 56,338)	16,875 (12,433 – 21,317)	1.52 (1.35 – 1.68)	<0.001	0.17%	51,27%	-109,798 (-426,187 – 206,591)	5,947,408	-54.2
Berrocal (2010)	24,799 (21,089 – 28,508)	<0.001	71,811 (56,003 – 87,619)	39,107 (30,569 – 47,644)	2.20 (1.89 – 2.51)	<0.001	0.26%	1,56%	-55,827 (-122,100 – 10,445)	9,420,880	-168.8
WA (1-4)	26,580 (22,604 – 30,555)	<0.001	59,276 (50,498 – 68,053)	26,571 (21,289 – 31,853)	1.81 (1.61 – 2.02)	<0.001	0.08%	2,34%	-380,229 (-2,447,832 – 1,687,373)	11,201,761	-29.5
WA (2-4)	26,760 (22,757 – 30,763)	<0.001	60,577 (51,756 – 69,398)	27,873 (22,572 – 33,174)	1.85 (1.64 – 2.06)	<0.001	0.06%	1,96%	-295,965 (-1,515,454 – 923,523)	11,382,070	-38.5
WA (2-3)*	26,901	<0.001	59,954	27,249	1.83	<0.001	0.06%	2,04%	-396,520	11,523,498	-29.1

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Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{30k}$	$\%CE_{50k}$	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
Sahinbas (2007)	(22,877 – 30,925) 15,378 (12,703 – 18,052)	1.000	(51,427 – 68,481) 32,704 (27,215 – 38,193)	(22,075 – 32,423) 0	(1.63 – 2.04) 1.00 (1.00 – 1.00)	1.000	4.45%	94,60%	(-2,540,572 – 1,747,533) 0	0	0.0

TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main cpmparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{30k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) $30,000; $\%CE_{50k}$: %CE at CET $50,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained).

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Table 14. Sensitivity analysis.

	US model					German model				
	TMZ					TMZ				
Parameter	Price, $/mg	Days on	mEHT $/sess	mNC	CR	Price, €/mg	Days on	mEHT €/sess	mNC	CR
Standard regimen	1.70 (1.44 – 1.95)	21	300 (234 – 366)	1.60		1.14 (1,12 – 1,17)	21	145.14 (145 – 145)	1.60	
Maximal mEHT price	NC	NC	1013.47	NC	3.38	NC	NC	683.65	NC	4.71
Minimal TMZ days on	NC	6,21	NC	NC	3,38	NC	4.46	NC	NC	4.71
Minimal TMZ price	0,50	NC	NC	NC	3.38	0.24	NC	NC	NC	4.71
Maximal TMZ+mEHT cycles	NC	NC	NC	2.86	1.79	NC	NC	NC	3.17	2.05

TMZ: temozolomide; mEHT: modulated electro-hyperthermia; mNC: mean number of cycles; CR: coefficient of reliability; NC: no change.

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Table 15. Cost-benefit analysis (US model).

		Year								
Parameter	Rate	1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		11	11	11	11	11	11	11	11	
Number of units		1								1
Capital costs[a]		400,000								400,000
Service costs	12%			48,000	48,000	48,000	48,000	48,000	48,000	288,000
Depreciation	15%		60,000	60,000	60,000	60,000	60,000	60,000	60,000	420,000
Reimbursement per session		300,00	300,00	300,00	300,00	300,00	300,00	300,00	300,00	
Reimbursement per year		807,300	807,300	807,300	807,300	807,300	807,300	807,300	807,300	6,458,400
Operational costs per year	50%	538,200	538,200	538,200	538,200	538,200	538,200	538,200	538,200	4,305,600
Economy per patient	20%	11,523	9,219	7,375	5,900	4,720	3,776	3,021	2,417	47,951
Economy per year		1,728,525	1,382,820	1,106,256	885,005	708,004	566,403	453,122	362,498	7,192,632
Earnings per year		2,535,825	2,190,120	1,913,556	1,692,305	1,515,304	1,373,703	1,260,422	1,169,798	13,651,032
Total costs per year		938,200	598,200	646,200	646,200	646,200	646,200	646,200	646,200	5,413,600
Economy & EBIT		1,597,625	1,591,920	1,267,356	1,046,105	869,104	727,503	614,222	523,598	8,237,432
EBIT		-130,900	209,100	161,100	161,100	161,100	161,100	161,100	161,100	1,044,800
Cumulative EBIT		-130,900	78,200	239,300	400,400	561,500	722,600	883,700	1,044,800	

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[a] Acquisition price + shipment + installation + training; [b] share of capital costs per year; [c] profit rate; [d] annual depreciation rate of the economy; EBIT: earnings before interest and taxes.

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Table 16. Cost-benefit analysis (German model).

Parameter	Rate	Year 1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	
Number of units		1								1
Capital costs[a]		300,000								300,000
Service costs	12,0%[b]			36,000	36,000	36,000	36,000	36,000	36,000	216,000
Depreciation	15,0%		45,000	45,000	45,000	45,000	45,000	45,000	45,000	315,000
Reimbursement per session		145.14	145.14	145.14	145.14	145.14	145.14	145.14	145.14	
Reimbursement per year		390,572	390,572	390,572	390,572	390,572	390,572	390,572	390,572	3,124,574
Operational costs per year	50%[c]	260,381	260,381	260,381	260,381	260,381	260,381	260,381	260,381	2,083,049
Economy per patient	20%[d]	8,795	7,036	5,629	4,503	3,602	2,882	2,306	1,844	36,597
Economy per year		1,319,232	1,055,386	844,309	675,447	540,358	432,286	345,829	276,663	5,489,509
Earnings per year		1,709,804	1,445,958	1,234,880	1,066,019	930,929	822,858	736,401	667,235	8,614,083
Total costs per year		560,381	305,381	341,381	341,381	341,381	341,381	341,381	341,381	2,914,049
Economy & EBIT		1,149,423	1,140,576	893,499	724,637	589,548	481,477	395,019	325,854	5,700,034
EBIT		-169,809	85,191	49,191	49,191	49,191	49,191	49,191	49,191	210,525
Cumulative EBIT		-169,809	-84,619	-35,428	13,762	62,953	112,143	161,334	210,525	

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[a] Acquisition price + shipment + installation + training; [b] share of capital costs per year; [c] profit rate; [d] annual depreciation rate of the economy; EBIT: earnings before interest and taxes.

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Table S1. Median progression-free survival after standard 1-2 line treatment of GBM (WHO IV)

Study	Tumor, state	Treatment	MPFS m
Jungk (2016)[1]	GBM, recurrent/progressive	2M (mainly no CTX)	6,10
Reithmeier (2010)[2]	GBM, recurrent/progressive	3M (mainly TMZ)	8,72
Hamza (2014)[3]	GBM, recurrent/progressive	3M	8,10
Hamza (2014)[3]	GBM, recurrent/progressive	3M	7,60
Strik (2008)[4]	GBM, recurrent/progressive	3M Stupp	7,53
Chinot (2014)[5]	GBM, newly diagnosed	3M Stupp	6,20
Gilbert (2014)[6]	GBM, newly diagnosed	3M Stupp	7,30
Gilbert (2013)[7]	GBM, newly diagnosed	3M Stupp	7,50
Gilbert (2013)[7]	GBM, newly diagnosed	3M ddTMZ	8,80
Average			7,56

Note: CTX: chemotherapy; TMZ: temozolomide; 3M – trimodal (SRG + XRT + CTX); 2M: bimodal (no CTX); Stupp: 3M SRG + (XRT 60 Gy X6w + TMZ 5/7d X 6w) + TMZ 5/28d X 6m; ddTMZ: dose-dense TMZ.

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Table S2. Inner structure of survival time.

Study	Cohort	NOP	MOST	MPFS	MST	MST%	PFS+MST	PFS+MST%
Varkoniy (2003)	HGG	24	22,0	12,2	6,5	30%	18,7	85%
	GBM (all)	76	20,0	8,5	7,6	38%	16,1	80%
Sahinbas (2007)	GBM (mEHT)	18	14,8	8,0	6,4	43%	14,4	97%
	GBM (mEHT+TMZ)	58	20,9	9,3	7,6	36%	16,9	81%
Jungk (2016)	GBM	34	15,7	6,1	8,7	56%	14,8	94%
Hamza (2014)	GBM (early BEV)	112	20,8	8,1	11,0	53%	19,1	92%
	GBM (late BEV)	133	25,9	7,6	9,9	38%	17,5	68%
Strik (2008)	GBM	18	17,9	8,2	9,1	51%	17,3	97%
	Weighted average		21,5	8,2	9,1	43%	17,3	82%
	95%CI					36,9% - 48,8%		75,3% - 88,8%

Note: NOP: number of patients; MOST: median overall survival time; MPFS: median progression-free survival; MST: median survival time since relapse; PFS: progression-free survival; HGG: high-grade gliomas; GBM: glioblastoma; mEHT: modulated electro-hyperthermia; TMZ: temozolomide; BEV: bevacizumab; CI: confidence interval.

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Table S3. Calculation of estimated mean survival time since relapse.

		95% CI		
	Mean	Lower limit	Upper limit	SE
MOST, months	13,65			
MPFS, months	7,5			
MPFS+MST (%)	82,0%	75,3%	88,8%	
MPFS+MST, months	11,2	10,3	12,1	
mST (1st estimation), months	3,7	2,8	4,6	
MST (%)	42,9%	36,9%	48,8%	
MST (2nd estimation), months	5,9	5,0	6,7	
mST (average), months	**4,775**	**3,9**	**5,6**	**0,443**

MOST: median overall survival time; MPFS: median progression-free survival; MST: median survival time since relapse.

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Table S4. Enduser price of temozolomide in USA.[205]

Provider	mg/capsule	No capsules	PPP	PPC	PPMG
Dana Farber Cancer Institute	180	5	1 347	269	1,50
Dana Farber Cancer Institute	250	5	2 471	494	1,98
Caremark	250	5	5 526	1 105	4,42
Accredo Health Group	250	5	3 158	632	2,53
Membership warehouse	180	15	1 589	106	0,59
Safeway	180	15	1 589	106	0,59
Kroger Pharmacy	180	15	1 593	106	0,59
Target (CVS)	180	15	2 034	136	0,75
Walgreens	180	15	2 468	165	0,91
CVS Pharmacy	180	15	4 781	319	1,77
Rite-Aid	180	15	5 244	350	1,94
Walmart	180	15	5 697	380	2,11
Sams Club	180	15	5 697	380	2,11
Kmart	180	15	5 763	384	2,13
Pubix	180	15	5 880	392	2,18
Membership warehouse	250	15	3 123	208	0,83
Safeway	250	15	3 123	208	0,83
Kroger Pharmacy	250	15	3 126	208	0,83
Target (CVS)	250	15	6 639	443	1,77
Walgreens	250	15	4 091	273	1,09
CVS Pharmacy	250	15	6 639	443	1,77
Rite-Aid	250	15	7 586	506	2,02
Walmart	250	15	8 243	550	2,20
Sams Club	250	15	8 243	550	2,20
Kmart	250	15	8 339	556	2,22
Pubix	250	15	8 507	567	2,27
Membership warehouse	140	15	2 604	174	1,24

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Safeway	140	15	2 604	174	1,24
Kroger Pharmacy	140	15	2 574	172	1,23
Target (CVS)	140	15	3 327	222	1,58
Walgreens	140	15	1 657	110	0,79
CVS Pharmacy	140	15	3 720	248	1,77
Rite-Aid	140	15	4 250	283	2,02
Walmart	140	15	4 431	295	2,11
Sams Club	140	15	4 615	308	2,20
Kmart	140	15	5 670	378	2,70
Min					0,59
Max					4,42
Mean					1,70 (1,44-1,95)
Median					1,77 (1,24-2,11)

Note: PPP: price per package; PPC: price per capsule; PPMG: price per milligram. Prices in US Dollars.

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Table S5. Enduser price of temozolomide in Germany.[206]

Name	Manufacturer	PPP	CIP	mg/caps	PPC	PPMG
TEMOZOLOMID HEXAL 100 mg	HEXAL AG	567,28	5	100	113,5	1,13
TEMOMEDAC 180 mg	Medac GmbH	4061,1	20	180	203,1	1,13
TEMOZOLOMID ratiophar5 mg	ratiopharm GmbH	112,13	20	5	5,61	1,12
TEMOZOLOMID ratiopharm 140 mg	ratiopharm GmbH	794,86	5	140	159	1,14
TEMOZO cell 100 mg	Cell Pharm GmbH	2258,9	20	100	112,9	1,13
TEMOZOLOMID ratiopharm 250 mg	ratiopharm GmbH	1420,7	5	250	284,1	1,14
TEMOMEDAC 100 mg	Medac GmbH	2258,9	20	100	113	1,13
TEMODAL20 mg	kohlpharma GmbH	451,61	20	20	22,58	1,13
TEMOZOLOMID Fair-Med Healthcar 5 mg	Fair-Med Healthcare GmbH	38,79	5	5	7,76	1,55
TEMODAL 100 mg	MSD Sharp & Dohme GmbH	567,61	5	100	113,5	1,14
TEMOZOLOMID Teva 100 mg	Bb Farma S.R.L.	441	5	100	88,2	0,88
TEMOMEDAC 100 mg	Medac GmbH	567,61	5	100	113,5	1,14
TEMOZOLOMIDE SUN20 mg	Sun Pharmaceuticals Germany GmbH	119,23	5	20	23,85	1,19
TEMODAL 140 mg	MSD Sharp & Dohme GmbH	794,86	5	140	159	1,14
TEMOZOLOMID Teva 140 mg	TEVA GmbH	794,86	5	140	159	1,14
TEMODAL 180 mg	kohlpharma GmbH	1022,2	5	180	204,4	1,14
TEMOZOLOMID Ribosepharm20 mg	Ribosepharm Division Hikma Pharma GmbH	452,57	20	20	22,63	1,13
TEMODA5 mg	MSD Sharp & Dohme GmbH	118,74	20	5	5,94	1,19
TEMOZOLOMID HEXAL 140 mg	HEXAL AG	794,86	5	140	159	1,14
TEMOZOLOMIDE SUN 140 mg	Sun Pharmaceuticals Germany GmbH	714,49	5	140	142,9	1,02

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Rezeptpflichtig TEMOZOLOMID ratiopharm 180 mg	ratiopharm GmbH	1022,4	5	180	204,5	1,14
TEMODAL 250 mg	Eurimpharm Arzneimittel GmbH	1410	5	250	282	1,13
TEMOZO cell 140 mg	Cell Pharm GmbH	794,85	5	140	159	1,14
TEMOZOLOMID Fair-Med Healthcare20 mg	Fair-Med Healthcare GmbH	128,57	5	20	25,71	1,29
TEMOMEDAC 140 mg	Medac GmbH	794,86	5	140	159	1,14
TEMOZOLOMID Ribosepharm 180 mg	Ribosepharm Division Hikma Pharma GmbH	1022,4	5	180	204,5	1,14
TEMOZOLOMIDE SUN 250 mg	Sun Pharmaceuticals Germany GmbH	1278,5	5	250	255,7	1,02
TEMOMEDAC 250 mg	Medac GmbH	1425	5	250	285	1,14
TEMOMEDAC 180 mg	Medac GmbH	1023,3	5	180	204,7	1,14
TEMOZOLOMID Ribosepharm 250 mg	Ribosepharm Division Hikma Pharma GmbH	1420,7	5	250	284,1	1,14
TEMOMEDAC 5 mg	Medac GmbH	37,4	5	5	7,48	1,50
TEMOZOLOMID Teva 250 mg	Bb Farma S.R.L.	1099	5	250	219,8	0,88
TEMOZO cell 250 mg	Cell Pharm GmbH	1425	5	250	285	1,14
TEMODAL 250 mg	MSD Sharp & Dohme GmbH	1425	5	250	285	1,14
TEMOZOLOMID Teva 100 mg	TEVA GmbH	567,28	5	100	113,5	1,13
TEMODAL 140 mg	Orifarm GmbH	779,82	5	140	156	1,11
TEMOZOLOMID Teva 250 mg	TEVA GmbH	1420,7	5	250	284,1	1,14
TEMODAL 180 mg	MSD Sharp & Dohme GmbH	1023,3	5	180	204,7	1,14
TEMODAL20 mg	MSD Sharp & Dohme GmbH	452,58	20	20	22,63	1,13
TEMOZO cel5 mg	Cell Pharm GmbH	118,73	20	5	5,94	1,19
TEMOZOLOMID Accord 140 mg	Accord Healthcare GmbH	2785,4	20	140	139,3	0,99
TEMODAL 100 mg	MSD Sharp & Dohme GmbH	2258,9	20	100	113	1,13

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TEMOZOLOMID Ribosepharm 100 mg	Ribosepharm Division Hikma Pharma GmbH	2251,3	20	100	112,6	1,13
TEMODAL 100 mg	MSD Sharp & Dohme GmbH	2258,9	20	100	113	1,13
TEMOZOLOMID Teva 100 mg	TEVA GmbH	2251,3	20	100	112,6	1,13
TEMOZOLOMID ratiopharm 100 mg	ratiopharm GmbH	2251,3	20	100	112,6	1,13
TEMOZOLOMIDE SUN 140 mg	Sun Pharmaceuticals Germany GmbH	2785,4	20	140	139,3	0,99
TEMOZOLOMIDE SUN 100 mg	Sun Pharmaceuticals Germany GmbH	2025,5	20	100	101,3	1,01
TEMOZOLOMID HEXAL 100 mg	HEXAL AG	2251,3	20	100	112,6	1,13
TEMOZOLOMID HEXA5 mg	HEXAL AG	112,13	20	5	5,61	1,12
TEMOZOLOMID HEXAL20 mg	HEXAL AG	452,58	20	20	22,63	1,13
TEMOZOLOMID HEXAL 140 mg	HEXAL AG	3157,7	20	140	157,9	1,13
TEMOZOLOMIDE SUN 180 mg	Sun Pharmaceuticals Germany GmbH	918,49	5	180	183,7	1,02
TEMOZOLOMID Fair-Med Healthcare 250 mg	Fair-Med Healthcare GmbH	1277,8	5	250	255,6	1,02
TEMOZOLOMID ratiopharm 100 mg	ratiopharm GmbH	567,28	5	100	113,5	1,13
TEMOZOLOMID Teva 180 mg	TEVA GmbH	1022,4	5	180	204,5	1,14
TEMOZOLOMID Ribosepharm 100 mg	Ribosepharm Division Hikma Pharma GmbH	567,28	5	100	113,5	1,13
TEMOZOLOMID Teva 100 mg	Axicorp Pharma GmbH	449	5	100	89,8	0,90
TEMODAL 100 mg	Orifarm GmbH	566,55	5	100	113,3	1,13
TEMODAL 140 mg	kohlpharma GmbH	793,84	5	140	158,8	1,13
TEMOZOLOMID HEXAL 180 mg	HEXAL AG	1022,4	5	180	204,5	1,14
TEMOZOLOMID Accord 250 mg	Accord Healthcare GmbH	1420,7	5	250	284,1	1,14
TEMOZO cell 100 mg	Cell Pharm GmbH	567,59	5	100	113,5	1,14
TEMOZOLOMID Accor5 mg	Accord Healthcare GmbH	37,4	5	5	7,48	1,50
TEMODAL 180 mg	Axicorp Pharma GmbH	1022,2	5	180	204,4	1,14

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TEMOZOLOMID Ribosepharm 140 mg	Ribosepharm Division Hikma Pharma GmbH	794,86	5	140	159	1,14
TEMODAL 250 mg	Orifarm GmbH	1424	5	250	284,8	1,14
TEMOZOLOMID Accord 180 mg	Accord Healthcare GmbH	1022,4	5	180	204,5	1,14
TEMODAL 250 mg	kohlpharma GmbH	1424	5	250	284,8	1,14
TEMODAL 100 mg	MSD Sharp & Dohme GmbH	567,61	5	100	113,5	1,14
TEMOZOLOMIDE SU5 mg	Sun Pharmaceuticals Germany GmbH	37,4	5	5	7,48	1,50
TEMOZOLOMID HEXA5 mg	HEXAL AG	37,4	5	5	7,48	1,50
TEMOZOLOMID Teva 140 mg	Axicorp Pharma GmbH	779,84	5	140	156	1,11
TEMOZOLOMID Teva 140 mg	Bb Farma S.R.L.	649,01	5	140	129,8	0,93
TEMOZOLOMID Fair-Med Healthcare 100 mg	Fair-Med Healthcare GmbH	509,12	5	100	101,8	1,02
TEMOZOLOMID HEXAL20 mg	HEXAL AG	119,23	5	20	23,85	1,19
TEMOZO cell 180 mg	Cell Pharm GmbH	1023,3	5	180	204,7	1,14
TEMOZOLOMID Accord 100 mg	Accord Healthcare GmbH	567,28	5	100	113,5	1,13
Temozolomid Teva 250 mg	Axicorp Pharma GmbH	1219	5	250	243,8	0,98
TEMODAL 100 mg	kohlpharma GmbH	566,58	5	100	113,3	1,13
TEMOZOLOMID Accord 250 mg	Accord Healthcare GmbH	1420,7	5	250	284,1	1,14
TEMOZO cell 100 mg	Cell Pharm GmbH	567,59	5	100	113,5	1,14
TEMOZOLOMID Accor5 mg	Accord Healthcare GmbH	37,4	5	5	7,48	1,50
TEMODAL 180 mg	Axicorp Pharma GmbH	1022,2	5	180	204,4	1,14
TEMOZOLOMID Ribosepharm 140 mg	Ribosepharm Division Hikma Pharma GmbH	794,86	5	140	159	1,14
TEMODAL 250 mg	Orifarm GmbH	1424	5	250	284,8	1,14
TEMOZOLOMID Accord 180 mg	Accord Healthcare GmbH	1022,4	5	180	204,5	1,14
TEMOZOLOMID HEXA5 mg	HEXAL AG	37,4	5	5	7,48	1,50

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TEMODAL 250 mg	kohlpharma GmbH	1424	5	250	284,8	1,14
TEMODAL 100 mg	MSD Sharp & Dohme GmbH	567,61	5	100	113,5	1,14
TEMOZOLOMIDE SU5 mg	Sun Pharmaceuticals Germany GmbH	37,4	5	5	7,48	1,50
TEMOZOLOMID Accord20 mg	Accord Healthcare GmbH	452,58	20	20	22,63	1,13
TEMODA5 mg	kohlpharma GmbH	117,82	20	5	5,89	1,18
TEMOZOLOMIDE SU5 mg	Sun Pharmaceuticals Germany GmbH	100,48	20	5	5,02	1,00
TEMOZOLOMID Accor5 mg	Accord Healthcare GmbH	112,13	20	5	5,61	1,12
TEMODA5 mg	MSD Sharp & Dohme GmbH	118,74	20	5	5,94	1,19
TEMOZOLOMIDE SUN20 mg	Sun Pharmaceuticals Germany GmbH	400,49	20	20	20,02	1,00
TEMOZOLOMID Fair-Med Healthcare 100 mg	Fair-Med Healthcare GmbH	2024,6	20	100	101,2	1,01
TEMOZOLOMID Teva20 mg	TEVA GmbH	452,58	20	20	22,63	1,13
TEMOZOLOMID Accord 100 mg	Accord Healthcare GmbH	2251,3	20	100	112,6	1,13
Min						0,88
Max						1,55
Mean (95%CI)						1,14 (1,12-1,17)
Median (95%CI)						1,14 (1,13-1,14)

Note: PPP: price per package; CIP: capsules in package; PPC: price per capsule; PPMG: price per milligram. Prices in EUR.

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Table S6. Raw data of ddTMZ+mEHT cohort (n = 54)

No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminated Y/N	Objective response	Last contact	EXITUS
001	W	30.4.67	1.5.03	29.9.03	2	31	Y	Y	N	NA		30.3.04
002	M	5.1.59	1.10.03	7.1.04	1	8	Y	Y	Y	PD		5.4.05
003	M	6.9.68	8.7.04	8.9.04	1	9	Y	Y	Y	NA		14.10.04
004	M	29.7.61	15.4.04	18.10.04	1	9	Y	Y	N	SD	25.5.05	
005	M	20.7.36	13.11.00	20.8.01	1	5	Y	N	Y	NA		27.10.01
006	M	28.11.53	3.5.04	12.4.05	1	9	Y	Y	N	NA	25.5.05	
007	W	12.11.62	19.6.04	15.11.04	1	11	Y	Y	N	PR	25.5.05	
008	M	9.8.50	16.5.00	3.9.01	1	14	Y	N	N	NA		15.1.02
009	W	28.1.63	13.3.03	15.7.03	2	26	Y	Y	N	NA		10.1.04
010	W	28.1.63	1.3.03	15.7.03	2	27	Y	Y	N	NA		10.1.04
011	M	21.8.73	1.6.02	14.4.04	1	16	Y	N	N	NA		19.6.04
012	W	26.12.43	12.7.99	18.6.01	1	9	Y	N	N	NA		10.7.01
013	M	21.9.38	1.5.00	30.1.02	1	13	Y	Y	N	NA		11.6.02
014	M	17.7.69	25.5.04	2.2.05	1	6	Y	Y	Y	PD		2.3.05
015	M	29.3.61	1.3.04	2.4.04	1	14	Y	Y	N	NA		15.12.04
016	M	13.8.47	8.5.04	12.10.04	1	15	Y	Y	N	NA		27.5.05
017	W	3.4.75	17.2.01	19.7.04	1	8	Y	Y	Y	PD		4.3.05
018	M	31.10.54	1.4.03	12.1.04	2	25	Y	Y	N	PD	5.5.05	
019	W	23.8.60	26.11.00	3.1.05	1	9	Y	Y	N	CR	25.5.05	

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020	M	9.8.67	1.6.04	29.11.04	2	36	Y	Y	N	NA	25.5.05	
021	M	13.5.62	13.1.03	1.12.04	1	6	Y	N	Y	NA	25.5.05	
022	M	15.1.45	1.6.03	26.1.04	1	15	Y	Y	N	NA		7.8.04
023	M	15.3.45	1.6.04	19.4.05	1	15	Y	Y	N	NA	25.5.05	
024	W	22.11.35	1.10.03	19.11.03	1	8	Y	N	Y	NA		8.2.04
025	M	29.10.41	1.12.00	5.1.04	1	12	Y	Y	N	NA		12.2.04
026	M	20.1.49	1.12.02	13.7.04	2	21	Y	Y	N	NA		15.2.05
027	M	24.4.64	1.5.00	1.3.01	1	10	Y	N	N	NA		20.5.01
028	W	3.8.66	1.8.93	13.6.01	1	12	Y	Y	N	SD	25.5.05	
029	W	15.9.51	1.11.02	22.9.03	1	3	Y	Y	N	PD		4.7.04
030	M	14.4.51	1.11.03	21.9.04	1	11	Y	Y	N	NA		19.12.04
031	M	19.9.35	1.11.03	20.9.04	1	6	Y	Y	Y	NA		8.2.05
032	M	13.12.50	1.9.03	16.8.04	1	5	Y	Y	N	NA	11.10.04	
033	M	15.10.62	8.1.04	25.10.04	2	24	Y	Y	N	PR	25.5.05	
034	M	5.12.40	1.1.02	2.12.03	1	11	Y	Y	N	NA		1.3.04
035	M	2.11.71	30.8.04	4.1.05	2	18	Y	Y	N	NA	25.5.05	
036	M	24.5.39	1.1.02	21.1.02	1	46	Y	Y	N	NA		8.9.02
037	W	17.2.55	1.8.03	1.12.03	1	9	Y	Y	N	NA		27.8.04
038	M	30.4.44	1.7.03	14.6.04	1	10	Y	N	N	PD		4.2.05
039	W	24.4.36	3.6.04	26.11.04	2	20	Y	Y	N	NA	27.5.05	
040	M	18.5.68	1.11.03	12.1.04	3	38	Y	Y	N	SD	27.5.05	
041	W	29.6.59	1.6.00	12.6.01	1	16	Y	N	N	NA	8.10.04	
042	W	9.12.64	1.4.02	27.5.02	3	44	Y	Y	N	NA		7.6.03

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043	M	20.2.45	1.4.02	24.6.02	3	29	Y	Y	N	NA		6.6.03
044	M	29.9.57	1.12.99	23.10.01	1	9	Y	N	N	NA		16.4.02
045	W	15.11.38	1.1.03	6.1.03	1	17	Y	Y	N	NA		13.2.03
046	M	30.6.50	1.8.02	13.5.03	3	34	Y	Y	N	NA		28.5.04
047	M	20.11.40	1.9.02	6.1.04	3	36	Y	Y	N	SD	30.5.05	
048	W	3.8.44	1.3.03	18.11.03	1	6	Y	Y	N	NA		24.2.04
049	W	21.9.59	1.2.02	22.11.02	5	65	Y	Y	N	NA		2.2.04
050	W	4.1.40	15.1.03	15.8.04	1	15	Y	Y	N	PD		17.4.05
051	M	11.10.57	1.11.99	7.6.01	1	6	Y	N	N	NA		13.8.01
052	W	4.2.52	1.6.02	24.9.02	2	27	Y	Y	N	SD	30.5.05	
053	M	5.1.53	1.11.03	17.2.04	3	35	Y	Y	N	NA	30.5.05	
054	W	26.9.50	1.6.00	23.4.01	5	56	Y	Y	N	NA		9.2.02

Note: CTX: chemotherapy; SAT: supportive and alternative therapy; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NA: not available.

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Figure 1. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A_1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

Figure 2. Survival since 1st mEHT session (Kaplan-Meier estimate) of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 3. Survival since 1st mEHT session (Kaplan-Meier estimate) of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 4. Survival since 1st mEHT session (Kaplan-Meier estimate) of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 5. Survival since 1st mEHT session (Kaplan-Meier estimate) of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 6. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

Figure 7. Cycles needed to treat per one life-month gained (CNTM) scale.

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Supplements

Estimation of expected mean survival time

First, we defined the expected MOST as 13.65 months.[57] This is well-established point confirmed either by official SEER data[7] and a reliable retrospective analysis.[57] Then, we defined that median PFS based on the data of 9 cohorts of 6 independent trials (Table S1) equals 7.5 months, and it well corresponds with general opinion that GBM relapses in 6-9 months after diagnosis. To define the most problematic final parameter MST since relapse, we studied the inner structure of survival time, namely time-proportions between MOST, PFS and MST, on eight cohorts for which this information was available simultaneously (Table S2). Finally, we translated these data on the established MOST and MPFS and calculated the expected MST as 4.775 months (95%CI: 3.9 – 5.6) (Table S3).

Background price information for economic evaluation

Here we report the enduser price of TMZ in USA (Table S4) and Germany (Table S5).

Raw data

Here we report the raw data of ddTMZ+mEHT cohort (Sahinbas et al., 2007[68]) (Table S6).

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21/28d: 21 days on – 7 days off

AAA: anti-angiogenic agents

BCNU: carmustine

BEV: bevacizumab

BIA: budget impact analysis

BRR: beneficial response rate

CA: coefficient of attenuation

CBA: cost-benefit analysis

ccl: cycle

CCNU: lomustine

CEA: cost-effectiveness analysis

CENC: cost-effective number of cycles

CET: cost-effectiveness threshold

CI: confidence interval

CNTM: cycles needed to treat per LMG

COI: cohort of interest

CR: coefficient of reliability

CRT: chemoradiotherapy

CTX: chemotherapy

ddTMZ: dose-dense temozolomide

EBIT: earnings before interest and taxes

EORTC: European Organisation for Research and Treatment of Cancer

ETR: effect-treatment ratio

GBM: glioblastoma multiforme

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HFR: high-frequency range

HGG: high-grade glioma

HR: hazard ratio

HRQoL: health-related quality of life

HT: hyperthermia (meaning conventional temperature-based hyperthermia)

ICER: incremental cost-effectiveness ratio

IOI: intervention of interest

LMG: life month gained

MAST: maximal attainable survival time

METR: median effect-treatment ratio

MGMT: O^6-methylguanine-DNA methyltransferase

mNC: mean number of cycles

modulated electro-hyperthermia (mEHT)

MOST: median overall survival time

MR, MRI: magnetic resonance imaging

mST: mean survival time

MST: median survival time

NCI: National Cancer institutes of USA

NCICT: The National Cancer Institute of Canada Clinical Trials

NICE: National Institute for Health and Care Excellence

OR: odds ratio

ORR: objective response rate

OS: overall survival

p.o.: orally

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PCV: procarbazine and vincristine regimen

PFS: progression-free survival

QALY: quality-adjusted life year

RCT: randomized controlled trial

RD: risk difference

RR: relative risk

RT: radiation therapy

RTOG: Radiation Therapy Oncology Group

SAT: supportive and alternative treatments

SOI: study of interest

t.i.d.: three times a day

TMZ: temozolomide

TTF: tumor-treating fields

WTP: willingness-to-pay

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STROBE Statement
Checklist of items that should be included in reports of ***cohort studies***
Title of work: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1 line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 7-12
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 13 lines 4-7
Methods			
Study design	4	Present key elements of study design early in the paper	Page 13 lines 17-18
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 13 lines 18-23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 13 lines 25-33
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 13 lines 36-50 Page 13 lines 52 – page 15 line 16 Page 18 lines 20-25
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	☒
Bias	9	Describe any efforts to address potential sources of bias	Pages 34-36
Study size	10	Explain how the study size was arrived at	Page 13 lines 17-23
Quantitative variables	11	Explain how quantitative variables were handled in the analyses.	Page 18 lines 29-55
		If applicable, describe which groupings were chosen and why	Page 21 line 39 – page 23 line 23
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 18 lines 29 – page 19 line 9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 19 lines 11-46
		(*c*) Explain how missing data were addressed	Page 18 lines 23-25
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Page 20 lines 4-7

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			Page 24 line 51 – page 25 line 12 Page 27 line 27 – page 28 line 34
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 20 line 23
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 20 lines 21-53 Pages 62-66
		(b) Indicate number of participants with missing data for each variable of interest	Pages 67-68
		(c) Summarise follow-up time (eg, average and total amount)	Page 21 lines 13-15
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 21 lines 15-23
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 21 lines 1-34
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 21-29
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 37-38
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 34-36
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 29-33
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 34 lines 1-22 Page 36 line 48 – page 37 line 7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not applicable

*Give information separately for exposed and unexposed groups.

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Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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CHEERS checklist—Items to include when reporting economic evaluations of health interventions

Title of study: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

Section/item	Item No	Recommendation	Check
Title and abstract			
Title	1	Identify the study as an economic evaluation or use more specific terms such as "cost-effectiveness analysis", and describe the interventions compared.	Page 1 line 2
Abstract	2	Provide a structured summary of objectives, perspective, setting, methods (including study design and inputs), results (including base case and uncertainty analyses), and conclusions.	Page 5
Introduction			
Background and objectives	3	Provide an explicit statement of the broader context for the study.	Page 11 lines 11-28
		Present the study question and its relevance for health policy or practice decisions.	Page 13 line 13
Methods			
Target population and subgroups	4	Describe characteristics of the base case population and subgroups analysed, including why they were chosen.	Page 20 lines 23-35 Pages 62-68 Page 13 lines 19-23
Setting and location	5	State relevant aspects of the system(s) in which the decision(s) need(s) to be made.	Page 19 lines 52-53 Page 20 lines 8-11
Study perspective	6	Describe the perspective of the study and relate this to the costs being evaluated.	Page 37 lines 10-17
Comparators	7	Describe the interventions or strategies being compared and state why they were chosen.	Page 22 line 44 – page 23 line 23
Time horizon	8	State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.	Page 25 line 42 Page 28 line 40 Page 29 line 25
Discount rate	9	Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.	Page 28 lines 45-46 Page 29 line 15, 18-19, 23
Choice of health outcomes	10	Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.	Page 13 lines 36-50
Measurement of effectiveness	11a	*Single study-based estimates:* Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	Page 13 lines 15-33 Page 34 lines 31-45
	11b	*Synthesis-based estimates*: Describe fully the methods used for identification of included studies and synthesis of clinical effectiveness data.	Page 22 line 45 – page 23 line 23 Pages 69-72
Measurement and valuation of preference based outcomes	12	If applicable, describe the population and methods used to elicit preferences for outcomes.	Pages 20-21
Estimating resources and costs	13a	*Single study-based economic evaluation:* Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any	Page 25 lines 40-48 Page 32 lines 20-41

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Section/item	Item No	Recommendation	Check
		adjustments made to approximate to opportunity costs.	
	13b	*Model-based economic evaluation:* Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	Page 25 line 50 – page 26 line 18 Page 32 lines 4-18 Pages 76-80
Currency, price date, and conversion	14	Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.	Page 25 lines 50-57 Page 26 lines 1-12 Page 32 line 23
Choice of model	15	Describe and give reasons for the specific type of decision-analytical model used. Providing a figure to show model structure is strongly recommended.	Page 25 lines 50-57 Page 32 lines 4-18
Assumptions	16	Describe all structural or other assumptions underpinning the decision-analytical model.	Page 32 lines 4-18
Analytical methods	17	Describe all analytical methods supporting the evaluation. This could include methods for dealing with skewed, missing, or censored data; extrapolation methods; methods for pooling data; approaches to validate or make adjustments (such as half cycle corrections) to a model; and methods for handling population heterogeneity and uncertainty.	Pages 18-20
Results			
Study parameters	18	Report the values, ranges, references, and, if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. Providing a table to show the input values is strongly recommended.	Pages 20-21
Incremental costs and outcomes	19	For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.	Page 26 line 29 – page 26 line 25 Pages 77-80
Characterising uncertainty	20a	*Single study-based economic evaluation:* Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness parameters, together with the impact of methodological assumptions (such as discount rate, study perspective).	NA
	20b	*Model-based economic evaluation:* Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.	Pages 27-28
Characterising heterogeneity	21	If applicable, report differences in costs, outcomes, or cost-effectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects that are not reducible by more information.	Page 25 line 50 - Page 26 lines 12
Discussion			
Study findings, limitations,	22	Summarise key study findings and describe how they support the conclusions reached. Discuss limitations	Pages 37-38 Page 35 lines 18-39

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Section/item	Item No	Recommendation	Check
generalisability, and current knowledge		and the generalisability of the findings and how the findings fit with current knowledge.	Page 36 lines 15-18 Page 36 line 40 – page 37 line 7
Other			
Source of funding	23	Describe how the study was funded and the role of the funder in the identification, design, conduct, and reporting of the analysis. Describe other non-monetary sources of support.	Information provided via the submission system
Conflicts of interest	24	Describe any potential for conflict of interest of study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors recommendations.	Information provided via the submission system

For consistency, the CHEERS statement checklist format is based on the format of the CONSORT statement checklist

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Clinical and economic evaluation of modulated electro-hyperthermia concurrent to dose-dense temozolomide 21/28d regimen in the treatment of recurrent glioblastoma: a retrospective analysis of a two-center German cohort trial with systematic comparison and effect-to-treatment analysis

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Clinical and economic evaluation of modulated electro-hyperthermia concurrent to dose-dense temozolomide 21/28d regimen in the treatment of recurrent glioblastoma: a retrospective analysis of a two-center German cohort trial with systematic comparison and effect-to-treatment analysis

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The sole author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Details of ethical approval (or a statement that it was not required)

Ethical approval was not required.

Details of funding

No external funding involved.

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Details of the role of the study sponsors

Galenic Research Institute as a study sponsor provided time and facilities for the work.

Statement of independence of researchers from funders

No funders.

Patient involvement statement

Patients were not involved (see also Acknowledgement).

Trial registration details

The trial was not registered.

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ABSTRACT

OBJECTIVE: To assess the efficacy and cost-effectiveness of modulated electro-hyperthermia (mEHT) concurrent to dose-dense temozolomide (ddTMZ) 21/28d regimen versus ddTMZ 21/28d alone in patients with recurrent glioblastoma (GBM). DESIGN: A cohort of 54 patients with recurrent GBM treated with ddTMZ+mEHT in 2000–2005 was systematically retrospectively compared with five pooled ddTMZ 21/28d cohorts (114 patients) enrolled in 2008–2013. RESULTS: The ddTMZ+mEHT cohort had a not significantly improved mean survival time (mST) versus the comparator ($p = 0.531$) after a significantly less mean number of cycles (1.56 vs. 3.98, $p < 0.001$). Effect-to-treatment analysis (ETA) suggests that mEHT significantly enhances the efficacy of the ddTMZ 21/28d regimen ($p = 0.011$), with significantly less toxicity (no grade III–IV toxicity versus 45–92%, $p<0.0001$). An estimated maximal attainable median survival time is 10.10 months (9.10 to 11.10). Cost-effectiveness analysis suggests that, unlike ddTMZ 21/28d alone, ddTMZ+mEHT is cost-effective versus the applicable cost-effectiveness thresholds 25,000–50,000 €$/QALY. Budget impact analysis suggests a significant saving of €8,577,947 / $11,201,761 with 29.1–38.5 QALY gained per 1000 patients per year. Cost-benefit analysis suggests that mEHT is profitable and will generate revenues of between €3,124,574 and $6,458,400, with a total economic effect (saving + revenues) of €5,700,034 to $8,237,432 per mEHT device over an 8 year period. CONCLUSIONS: Our ETA suggests that mEHT significantly improves survival of patients receiving the ddTMZ 21/28d regimen. Economic evaluation suggests that ddTMZ+mEHT is cost-effective, budget-saving, and profitable. After confirmation of the results, mEHT could be recommended for the treatment of recurrent GBM as a cost-effective enhancer of ddTMZ regimens, and, probably, of the regular 5/28d regimen. MEHT is applicable also as a single treatment if chemotherapy is impossible, and as a salvage treatment after the failure of chemotherapy.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study first introduces the application of a novel clinical analysis called effect-to-treatment analysis (ETA).
- The study demonstrates that using ETA, it is possible to extract extensive information and reliable evidence from a limited data source (a retrospective cohort trial).
- The study applies a systematic comparator in the form of the pooled average of a meta-analysis of a systematic review of comparable trials.
- The study includes comprehensive economic evaluation, comprising consistent costs analysis, cost-effectiveness analysis, budget-impact analysis, and cost-benefit analysis.
- Because the study is based on a single retrospective trial, future studies are needed to confirm its findings.

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ABBREVIATIONS

NICE: National Institute for Health and Care Excellence

GDP: gross domestic product

DALY: disability-adjusted life year

%CE: proportion of cost-effective cases

AAA: anti-angiogenic agents

BEV: bevazucimab, avastin

BIA: budget impact analysis

BRR: beneficial response rate (CR+PR+SD) (aka DCR)

CA: coefficient of attenuation

CBA: cost-benefit analysis

ccl, ccls: cycle, cycles

CEA: cost-effectiveness analysis

CET: cost-effectiveness threshold

CI: confidence interval

CNTM: cycles needed to treat per life month gained

COI: cohort of interest

CR: complete response

CRR: complete response rate

CRT: chemoradiation treatment

CS: censored

CT: computed tomography

CTCAE: common terminology criteria for adverse events

CTX, CTx: chemotherapy (cytotoxic drugs); common toxicity

CUR: cost-utility ratio

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CURR: ratio of cost-utility ratios

d: day

DCR: disease control rate (aka BRR)

ddTMZ: dose-dense temozolamide

DLT: dose-limiting toxicity

EBIT: economy and earnings before interest and taxes

EORTC: European Organisation for Research and Treatment of Cancer

ETA: effect-to-treatment analysis

ETR: effect-treatment ratio

FU: follow-up

GBM: glioblastoma multiforme

H_0: null hypothesis

HF: high-frequency range (3 – 30 MHz)

HGG (HGBG): high-grade (brain) glioma

HR: hazard ratio, hazard rate

HRQoL: health-related quality of life

HT: hyperthermia

ICER: incremental cost-effectiveness ratio.

ICUR: increment of cost-utility ratio

IOI: intervention of interest

KME: Kaplan-Meier estimate

KPS: Karnofsky performance score

KS-test: Kolmogorov-Smirnov test

LMG: life month gained

LYG: life year gained

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m: month

MAC: maximal attainable course

MAST: maximal attainable median survival time

mEHT: modulated electro-hyperthermia

METR: median effect-treatment ratio

MGMT: O6-methylguanine DNA methyltransferase

min: minute(s)

MN: malignant neoplasm

mNC: mean number of cycles

MNC: median number of cycles

MOST: median overall survival time

mST: mean survival time

MST: median survival time

N/A: not available

NC/SD: no change / stable disease

NNT: number needed to treat

OR: objective response (CR, PR)

OR: odds ratio

ORR: objective response rate

OS: overall survival

OST: overall survival time

p.o., p/o: per os

PD: progression of the disease / progressive disease

PFS: progression-free survival

PLT: palliative treatment

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PR: partial response; partial resection

QALY: quality-adjusted life year

qd, q.d.: every day; daily

QoL: quality of life

RD: risk difference

RF: radiofrequency

RR: relative risk

RR: response rate

RT: radiotherapy

SAT: supportive and alternative therapies

SD: stable disease (aka NC)

SOI: study of interest

t.i.d., tid: three times a day

TMZ: temozolomide

w: week

WA: weighted average

WTP: willingness to pay

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BACKGROUND

Glioblastoma multiforme (GBM) is a common and aggressive primary brain tumour, accounting for 45–54% of all adult gliomas.[1,2] About 10,000 new cases of GBM are diagnosed annually in the US[3] and about 2,200 cases in the UK.[4] Median survival time (MST) of adult (>20 years) GBM patients in the US (2005–2007)[5] and UK (2007–2011)[4] is 9.5 and 6.1 months, respectively, and the two- and five-year overall survival (OS) rates are 17% and 11.5% and 3.3% and 3.4%, respectively.[6] Maximal tri-modal treatment (radical surgery and adjuvant chemoradiation) provides the MST of about 15 months.[4,7]

The standard of first-line treatment for GBM, based on the milestone EORTC/NCICT trial,[8,9] includes a maximal possible resection consistent with the preservation of neurologic function followed by 6 weeks of adjuvant focalized fractionated RT with concurrent chemotherapy (CTX) plus oral DNA-alkylating agent temozolomide (TMZ), further followed by up to 6 months of adjuvant TMZ monotherapy.[10] Since the introduction of TMZ in 1999, the MST in GBM patients in the US, previously stable at 7.5 months, started to increase and reached 9.5 months by 2005–2007.[5] There have been attempts to attribute the observed increase in survival solely to TMZ,[11] which seems somewhat ungrounded considering uncontested, significant improvement of surgery, RT, and novel treatments since the introduction of TMZ.

Despite the recent advances, GBM prognosis remains dismal, with the MST limited to 15–18 months.[10] The 2-year OS of GBM patients is just 22% and remains below 30% even after the complete standard treatment (28% in 2005–2007, CI: 26–31%).[5] According to SEER database, the 5-year OS of GBM patients is 6.2% (1998–2008 population)[12] and scarcely exceeds 10% in some subgroups (patients under the age of 45 years, patients with methylated MGMT)[9] and in some countries, e.g., Japan (9.9–10.1%).[13] There has been no progress in the survival of patients aged over 80 years in the USA; moreover, their survival has become worse (hazard ratio [HR] of 2005–2007 population is 1.05 compared to 1993–1995).[5]

TMZ adds only about 2.5 months to the MST compared to RT alone.[8,9] Given that more than 50% of patients fail to respond to TMZ treatment over 6–9 months, TMZ should be considered a modestly effective chemotherapy. The majority (60–75%) of patients with GBM that do not have a methylated MGMT promoter derive limited benefit from TMZ treatment.[14] In addition, 15–20% of patients treated with TMZ develop clinically significant toxicity.[8]

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In the EORTC/NCICT trial,[8] TMZ was given daily at 75 mg/m^2 during RT, followed by six cycles of adjuvant TMZ chemotherapy at 150–200 mg/m^2 for 5 days in each 28-day cycle (5/28 d) (Stupp regimen).[8] Despite multiple attempts to improve the Stupp regimen, it remains the standard of care for the newly diagnosed GBM. These attempts involved the addition of anti-angiogenic agents (AAA) (mainly bevacizumab [BEV]) and increased TMZ dosage, known as dose-dense TMZ (ddTMZ) regimens.[15]

The rationale for ddTMZ is based on the known role of specific DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in tumour resistance to alkylating agents such as TMZ. MGMT effectively recovers TMZ-related DNA damage. Methylation of the promoter region of the MGMT gene suppresses MGMT expression. A methylated MGMT-promoter is observed in 30–60% of GBMs. This value was reported to be 45% in the EORTS/NCIC study, and TMZ was much more effective in MGMT-methylated patients (MST 18.2 vs. 12.2 months).[16] Because MGMT is a suicide enzyme and requires re-synthesis for recovery of its enzymatic activity,[17] it can be depleted by continuous alkylating pressure. Therefore, prolonged exposure and higher cumulative doses of TMZ could sensitize tumours to the alkylating damage, with toxicity as a natural limiter of such dose-escalation.

Some ddTMZ regimens were clinically tested versus the standard 5/28d regimen, including the 7/14d (7 days on / 7 days off), 21/28d, and continuous administration (7/7d or 28/28d) regimes.[18] Multiple single-armed and retrospective studies of ddTMZ at recurrent GBM showed progression-free survival at 6 month (PFS-6m) ranging from 19% to 44% and an MST of 7–10 months, similar to BEV.[15] However, a recent phase III RCT (RTOG 0525)[19] of ddTMZ 21/28d versus the standard 5/28d adjuvant regimen for newly diagnosed GBM patients after completion of concurred CRT, failed to show an advantage of ddTMZ in MST (14.9 vs. 16.6 months in the standard arm, $p = 0.63$), although it did show an improvement of PFS-6m (6.7 vs. 5.5 months) with borderline significance ($p = 0.06$), with somewhat higher toxicity in the ddTMZ arm. Efficacy did not differ by methylation status, which advocates against the MGMT depletion concept. Therefore, the efficacy of ddTMZ regimens remain unproven.[15]

Finally, it should be noted that the modern chemotherapies like TMZ, BEV and other AAA are not cost-effective.[20,21,22,23]

The prognosis for patients with recurrent GBM remains poor, with the MST between 3 and 6 months.[24] In some significant subgroups, the treatment efficacy is lower, e.g., in older patients (over

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50 years and especially over 70 years), in not MGMT-methylated patients (40–70% of patients), in patients with bad performance and others unfit for chemotherapy or RT, and in patients with unresectable tumours. As 20 years ago, treatment of recurrent GBM can be considered successful if the stable disease is achieved.[25]

Standards of care are not yet defined for recurrent GBM.[26] Treatment options at recurrence include surgical resection, re-irradiation, and chemotherapy, [27] though all of these options have significant limitations.[28] In fact, there remains a significant unmet need for more effective treatments of high-grade gliomas,[14] and the poor outcomes of the current treatment of recurrent GBM requires novel approaches.[26] Recently, an impressive result was shown by a novel physical treatment, tumour-treating fields (TTF), an athermal technology using continuous impact of a low-intensity (0.7–1 V/cm) alternating electromagnetic field with a frequency of 100–200 kHz through insulated scalp cross-sectional electrodes.[29,30,31,32,33,34]

There is another physical technology called modulated electro-hyperthermia (mEHT, oncothermia™), the effectiveness of which was demonstrated in many phase I/II trials in recurrent brain gliomas,[35,36,37,38,39] and also in cancer of lung, [40,41,42,43] liver,[44,45,46] pancreas,[47,48] cervix,[49,50] breast,[51] esophagus,[52] colorectal cancer,[53,54,55,56] malignant ascites,[57] and soft tissue sarcomas.[58,59] Clinically, mEHT is typically used as an enhancer of radiation[40,49] and chemotherapy, although it possesses its own effectiveness of at least a similar magnitude to these treatments.[60,36,53] Taking into account the extensive and long-term (since 1996) successful application without any negative report, a systematic review of results of mEHT is possible and necessary.

Collecting the data for the systematic review and meta-analysis on the mEHT treatment of brain gliomas, we asked for raw data whenever possible. The raw data of the Sahinbas et al. (2007)[36] trial including 155 patients with high-grade gliomas (HGG) were obtained on request. After analysis of the data, some shortcomings were revealed, namely duplications, incorrect grouping by histology, and incorrect calculation of survival function in view of incorrect processing of censoring. After corrections and recalculation, the results of this trial appeared so interesting that we believe they deserved to be re-published. In this retrospective analysis, we report the result of the systematic clinical comparison and economic evaluation of mEHT concurrent to the ddTMZ 21/28d regimen in the treatment of recurrent GBM. No change to the raw data was made.

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MATERIAL AND METHODS

Objectives

The objective of this study is to assess the efficacy and cost-effectiveness of mEHT concurrent to ddTMZ 21/28d regimen versus ddTMZ 21/28d alone in patients with recurrent GBM.

Questions of the study

- Does mEHT significantly enhance the ddTMZ 21/28d regimen?
- Is the addition of mEHT to ddTMZ 21/28d regimen cost-effective?

Trial design

This retrospective clinical and economic evaluation is based on a systematic comparison and effect-to-treatment analysis of a retrospective, single-arm study[36] (study of interest, SOI) performed in two German centres (the Gronemeyer Institute of Microtherapy at the University of Bochum and the clinic "Closter Paradise", Soest) between 2000 and 2005.

Inclusion and exclusion criteria

Patients with relapsed or progressed after incomplete resection or progressive inoperable histologically confirmed GBM or gliosarcoma (WHO IV), having undergone a complete conventional 1st–2nd-line pre-treatment were selected. From those, patients treated with ddTMZ 21/28d in combination with mEHT (with or without supportive therapy but without re-irradiation, re-surgery or other chemotherapy) were selected. No exclusion criteria were applied.

Outcomes

Survival was the main outcome of the study:

- Median survival time (MST) is the time from the initial event to the moment when the value of cumulative survival function (Kaplan-Meier estimate [KME]) reaches 50%. Here, the term MST is applied to survival since relapse/progression or the date of the first mEHT session, while survival since the date of diagnosis is defined as Median Overall Survival Time (MOST).
- Overall survival (OS) is the value of cumulative survival function (KME) at the set time moments from the date of the initial event.
- Overall survival time (OST) is the time from the initial event to the death of any reason.

No surrogate outcomes were used.

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Intervention

The studied intervention was a combination of dose-dense temosolomide 21 days on, 7 days off regimen (100 mg/m^2/d) with concurrent mEHT as an enhancer (ddTMZ+mEHT). MEHT was applied using an EHY2000 device (Oncotherm Kft, Hungary) with 2-day intervals between sessions (on each 3rd day) concurrent with TMZ and afterwards, for up to three months. A dose-escalating scheme was used with a gradual increase of power from 40 to 150W and increase of time from 20 to 60 min, during two weeks, adding modulation from the second week (Figure 1). Then, a step-up heating was applied, increasing the power from 60W to 150W during 60-min sessions, to ensure tumour temperature of >40°C during 90% of the treatment time. Dose escalation was limited by patient's individual tolerance. The mEHT course was considered low-dose (LD-mEHT) if did not exceed eight complete 60-min sessions. Supportive and alternative treatments (SAT) included *Boswellia caterii* extract 6 g/day p.o. t.i.d., mistletoe extract 15 ng/day SC 3Xw, and Selenium 300 µg/day p.o., for three months.

Intervention of interest

The intervention of interest (IOI) is modulated electro-hyperthermia (mEHT, oncothermia™), a novel method of treatment of solid malignant tumours by the local application of a high-frequency electromagnetic field (13.56 MHz), modulated by 0–5 kHz flicker noise, by virtue of impedance-coupled functionally asymmetric electrodes.[61] MEHT is positioned as a next generation hyperthermic technology based on the selective heating of intercellular compartments of tumour tissue and cell membranes, instead of the heating of a bulk volume of the tissue, as the conventional temperature-dependent hyperthermia (HT) does.[62,63]

The difference between mEHT and HT has been well demonstrated *in vitro*.[64] mEHT caused an order of magnitude stronger activation of apoptosis of cancer cells compared to HT[65]; mEHT significantly increased the expression of proteins of intercellular junctions (E-cadherin and ß-catenin) and heat shock proteins (HSP) on the cell membrane, while HT increased only the intracellular level of HSP;[66] mEHT displayed another pattern of heat response[67] and generally induced other cell-damage pathways.[65]

The fundamental difference between mEHT and HT technologies of high-frequency range (HFR, 3–30 MHz) is a transfer of the focus from the field to the current. The alternating electromagnetic field causes orientational displacement of dipole molecules, thus causing dielectric heating (field effect), and also induces movement of charged ions (current), thus inducing Joule (electric) heating. The

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balance of these components depends on the technology used: current can be either minimized, like in capacitive HT, or enhanced, like in mEHT. There are two main reasons to emphasize the current: focusing and penetration depth. Due to the high wavelength at 13.56 MHz (about 2.4 m in muscles), it is impossible to focus the energy of a field in a desired small-size volume (typically 3–10 cm in diameter). At the same time, the current has a known ability to concentrate in areas with a higher conductance.[68] Increased conductance is one of the basic properties of malignant tissue: it is always 2–5 times more conductive compared to the surrounding healthy tissue.[69] This feature has long been used for electrical impedance scanning (EIS)[70] and current-density imaging (CDI).[71,72] Thus, a tumour is a natural concentrator of electrical current (but not of a field). Another reason to use the current is the penetration depth. For the 13.56 MHz field, the penetration depth (i.e., the depth from the surface at which field intensity drops for e times [1/e] compared to the surface intensity) is only about 14–18 cm,[73] which forces to use the high-intensity field to reach the effective deep heating in the capacitive HT. The penetration depth of current in the impedance-matched system is 20–25 cm.[74] Therefore, the emphasis on the current allows transferring energy selectively to the tumour for any depth and with minimal losses. “Electro-hyperthermia” means predominantly electric heating.[75]

A combined set of technical solutions is used to achieve maximal electrical heating: namely, the impedance matching (based on the phase angle between voltage and current), instead of the standard capacitive matching (based on the standing wave ratio [SWR]); functionally asymmetric electrodes, providing the necessary stability of the field and size difference-dependent amplification of the current; physiologic skin cooling, minimizing skin losses at energy transfer; and a “skin sensor” concept, which allows for refuse thermometry without detriment to safety.[61] “Free of thermometry” use is a great advantage of mEHT, abolishing the labour-intensive thermometry planning, installation and control, thus drastically reducing time and costs, minimizing side effects, and significantly improving the perception of the treatment by a patient.[76]

The electric heating creates quasi-stable local thermal gradients at the nano level (e.g., transmembrane thermal gradient[77]), which are maintained by the balance of continuous delivery of energy by external field and energy dissipation by natural cooling mechanisms, mainly by a blood flow. [78,79] Thus, the nanoheating, depending on the field power applied and physiological cooling power displayed, can develop even without macroscopic heating.[80] It was shown *ex vivo* that a 42□ temperature in mEHT is only responsible for 25–30% of the total antitumour effect and a slightly smaller effect was shown in the case of normothermia.[81]

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Thus, the effect of mEHT is thermally-induced but not temperature-dependent.[82] Nevertheless, mEHT usually causes hyperthermia-range heating[83,84,85,86] in accordance with a classical maxima of Schwan on the impossibility to reach significant "non-thermal" effects without substantial heating.[87] The effect of mEHT is power-dependent but not signal-dependent. It is not connected with multiple tiny and questionable processes such as demodulation and molecular energy uptake[88] (although we cannot completely exclude these possibilities). The power range of mEHT (0.2–2 W/cm^2) is far above the "thermal noise limit" of 0.01 W/cm^2.[89]

Fractal modulation is a specific feature of mEHT. The carrying frequency is amplitude-modulated by "pink noise" (1/f),[90] which is typically emitted by all self-organized living systems and reflects their fractal organization.[91] Since a malignancy always losses organization, it more or less emits "red" or Brownian noise (1/f^2)[92] (correctly speaking, its noise spectrum is more "reddish"). Fractal modulation allows for increasing specific absorption of modulated field energy in the "red noise" sites, selectively amplifying the effect of mEHT.[93] Also, the noise can amplify cancer-specific frequencies[94] by "stochastic resonance".[95] It is reported *in vitro* that modulation can amplify the effect of mEHT by 20–50%.[93]

An important feature of mEHT is its selectivity, both macroscopic and cellular. Macroscopic selectivity of tumour heating is based on the automatic impedance-based autofocusing of electric current in the tumour.[68] The cellular selectivity of mEHT was demonstrated *in vitro* using a mixed culture of cancerous and normal cells. mEHT selectively destroyed malignant cells without damage to the normal cells, and the extent of the damage was proportional to the degree of malignancy.[96] The exact mechanism of this cellular selectivity is unknown but is likely a combination of the membrane-acting effects of mEHT and the fractal modulation.

The exact mechanism of mEHT action is unknown. Both temperature-dependent and independent mechanisms are among possible options. Temperature-dependent mechanisms include disorder of tumour blood flow, oxygen and glucose deprivation, depletion of intracellular ATP, the influx of sodium and depolarization of cellular membrane,[97,98,99] and acidification.[100,101,102] Since these effects are present in all HT applications, and they do not lead to results characteristic for mEHT, we propose that there must be other mEHT-specific mechanisms of action.

Many so-called "non-thermal" (i.e., not associated with elevation of macroscopic temperature) effects are reported to have a peak at about 10 MHz, namely direct bactericidal effect and enhancement of antibiotics action (bioelectric effect), both in bacterial films[103] and planktonic

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phase;[104] dielectrophoresis,[105] damage of mitochondrial function[106] and destruction of lysosomes.[107]Although the frequency and field strength (2–5 V/cm) applied in mEHT cannot cause a significant change in the membrane potential,[108] there are many reasons to suggest a specific membrane-acting effect of mEHT. The 10 MHz is a relaxation frequency of the beta-dispersion range (0.1–100 MHz) caused by Maxwell-Wagner relaxation of cell membranes,[109] which means a peak of membrane dielectric loss and selective membrane excitation (heating) at this frequency.[110] The selective heating of the cell membrane also leads to a specific effect on its lipid bilayer, namely the enhancement of its fluidity and decrease of the capacitance (though the capacitance seems to be relatively stable).[109] Also, 10 MHz is a peak of phase shift of membrane polarization under the effect of the external alternative field, which nearly reaches a quadrature (-80°).[108] Re-orientation of protein-bound water molecules, the motion of polar protein subgroups, the Maxwell-Wagner relaxation of the cell interior or the additional Maxwell-Wagner relaxations due to the non-spherical cell shape, also contribute to the β-dispersion.[109] The relaxation frequency of the re-orientational proton motion of water-bound proteins, as it was shown in a cell-free protein solution, also peaks at about 10 MHz (range, 1–100 MHz).[111] This allows a selective absorption of field energy by protein macromolecules and especially their active centres, which are always polarized. Given the extremely high intracellular protein concentration (200–300 g/l[112]), selective intracellular heating seems likely. This might also contribute to the dielectric selectivity of tumour heating, because the concentration of protein in the intercellular fluid of normal tissue is extremely low (nearly saline), whereas, in a tumour intercellular fluid, it almost equals that of blood plasma (60–80 g/l[113]).

Another possible effect of mEHT is an arrest of cell division with possible mitotic catastrophe,[104] attributable to a subcellular ponderomotoric effect (dielectrophoretic forces suppress the assembly of the mitotic spindle[30]), to membrane polarization (cell division phases are associated with changes in membrane potential, and nonlinear processes of hyperpolarization and depolarization, under the effect of RF-field, suppress proliferation[31]), or to resonance phenomena.[114] Also, effects on the cytoskeleton[115,116] and selective activation of some enzymes, both conformational and voltage-dependent (in the case of membrane enzymes),[117] are reported.

The overall effect of mEHT seems to be membrane-acting because it is connected with an extracellular expression of intracellular signalling molecules of cellular stress (e.g., HSP and p53 protein),[118] which unmask cancer cells and initiate the immune response and apoptosis.[119] It has been shown *in vivo* and *in vitro* that the antitumour effect of mEHT is mainly connected with significant activation of apoptosis, which develops over 72 h after a single impact.[119,120,121] Some

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immune-dependent effects are reported, namely the abscopal effect[122, 123] which is considered as a basis for a 'radiofrequency vaccination'.[124,125] Expression of many immune-specific pathways has been reported *in vitro* in mEHT.[118,126,127,128] Overexpression of cell-junction proteins with the significant restoration of intercellular junctions, which can contribute to the induction of apoptosis,[129,130] and reorganization of cytoskeleton[115] are reported for mEHT.

Response and survival assessment

The objective response was assessed according to the MRI McDonald criteria.[131] Survival function was assessed by the Kaplan-Meier estimate. Survivors were right-censored on the date of completion of the study (May 30, 2005), lost patients were censored on the date of the last contact, and excluded patients were left-censored on the date of diagnosis/enrolment.

Statistical methods

Statistical analysis was performed using the built-in Excel 2016 analysis package using the methods of descriptive statistics, correlation, and regression analysis. Normality of distribution was estimated by the Kolmogorov-Smirnov test (KS-test). Confidence intervals (CI) of medians were calculated according to Conover,[132] relative risks (RR) and odds ratios (OR) according to Altman,[133] risk difference (RD) according to Newcomb and Altman,[134] product of means according to Goodman,[135] ratio of means according to Fieller[136,137] for independent means, and by Taylor approximation[138] for dependent means, and the ratio of two independent lognormally distributed estimates by Newcomb's MOVER-R algorithm.[139] Inverse-variance weighting was used.[140] The significance of differences in parametric criteria was estimated by the two-sample Student t-test or Welch t-test for unequal variance;[141] and for paired nonparametric criteria (proportions) by the Pearson's chi-square test (χ^2) according to Campbell-Richardson.[142] The significance of rates and proportions with known 95% CI was estimated according to Altman,[143] and the significance of the difference of two independent estimates by the two-sample z-test. All p-values are two-sided. A 95% probability ($\alpha = 0.05$) was used for significance testing. Since log-transformation significantly inflates confidence intervals (up to 40-times in some cases[144]), 90% probability ($\alpha=0.1$) is considered applicable for the significance of the difference of estimates based on log-transformed parameters in some cases.

Survival analysis was performed using the Excel-based software package GRISA (Galenic Research Institute, 2015) by Kaplan-Meier estimate (KME) of the cumulative probability of survival.[145] Standard errors and confidence intervals of KME were estimated by Greenwood's formula,[146] and

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the significance of differences by the log-rank test.[147] The hazard function was estimated by the Cox proportional hazards regression model.[148]

Meta-analysis was performed using the Excel-based software package GRIMA (Galenic Research Institute, 2015) according to Borenstein et al.[140] and statistical algorithms of the Cochrane Collaboration.[149] The heterogeneity of studies was assessed by the I^2 criterion.[150] In view of the significant heterogeneity of the cohorts, a random effect model was applied.

Effect-to-treatment analysis

Effect-to-treatment analysis (ETA) was performed according to our own algorithm[151] with the following settings: a unit of treatment is a 28-days cycle, and the parameter of comparison is the mean survival time (mST) after relapse. Here, we use mST for mean survival time and MST for median survival time. Medians were transformed into means with 95% confidence intervals (95% CI) using the Hozo et al. (2005)[152] algorithm for medians with range and our own simplified algorithm (see Supplemental Material) for medians with 95% CI. The life months gained (LMG) parameter was calculated by subtracting the expected mST (emST). Effect-treatment ratio (ETR) was calculated by dividing the LMG by the mean number of cycles (mNC). Life quality adjustment was not possible due to significant initial differences between the cohorts. The median ETR (METR) was estimated by attenuation of the ETR according to the formula $METR = ETR \times (1 - CA)^{(MNC - mNC)}$, where CA is a coefficient of attenuation. The dependence of mST from mNC was estimated by the function $mST = ETR \times (1 - CA)^{NC - mNC} \times NC + emST$ (where NC is a serial number of cycle); the extremum of the function is a maximal attainable survival time (MAST), the abscissa of the extremum is a peak number of cycle (PNC). Cost-effective number of cycles (CENC) was estimated as abscissa of cost-effective survival time value (CEST = 95%MAST). Cycles needed to treat per LMG (CNTM) was estimated as the reciprocal of the difference of ETRs: $CNTM = 1/\Delta ETR$. The effect enhancement ratio ($EER_{12} = ETR_1/ETR_2$) was estimated as an auxiliary parameter for calculation of CI and significance of CNTM: since EER and CNTM use the same parameters with the same null hypothesis [H_0: $ETR_1 = ETR_2$], their confidence intervals and significance are the same, and these parameters can be easily calculated for EER according to Altman.[143]

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Economic evaluation

For economic evaluation, cost-effectiveness analysis (CEA) with sensitivity analysis, budget impact (BIA) and cost-benefit (CBA) analyses were performed.[153,154,155,156,157] CEA and BIA were performed from the perspective of a health provider. CEA was based on the cost-utility ratio (CUR) and incremental cost-effectiveness ratio (ICER). The ratio of CURs (CURR) and increment of CURs (ICUR) were used to compare CURs. The proportion of cost-effective cases (%CE) was estimated by one-tailed directional integral z-test with the null hypothesis [H_0: CUR = CET], where CET is a cost-effectiveness threshold. To estimate a sensitivity of CEA, a multiparametric equal cost-effectiveness test was performed exploring the value of a key parameter in which the value of CURR equals 1.0 (or ICUR = 0). The BIA estimated the difference of costs for treatment of 1,000 patients per year. CBA estimated the total economic effect (saving and earnings before interest and taxes [EBIT]) from the perspective of a healthcare facility.

Reporting

SOI is reported according to the STROBE statement for reporting observational studies.[158] Economic evaluation is reported according to the CHEERS standards.[159]

RESULTS

Patients' flow

A total of 153 patients with different brain tumours (Table 1)

Table 1. Histologic types of brain tumors (SOI).

Total patients: 153

- [C71] Malignant neoplasm (MN) of brain: 137
  - WHO II: 8
    - Astrocytoma: 4
    - Mixed glioma: 4
  - WHO III: 39
    - Astrocytoma: 34
    - Mixed glioma: 3
    - Ependimoma: 1
  - Age <20: 6
    - **Gliosarcoma: 1**
    - Medulloblastoma: 3
    - Primitive neuroectodermal tumor: 1
- [D43.1] Neoplasm of uncertain behavior of brain, infratentorial: 1
- [C79.3] Secondary MN of brain and cerebral meninges: 15
  - Adenocarcinoma: 12
    - MN of breast: 7

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- Oligodendroglioma: 1
- WHO III-IV: 4
  - Astrocytoma: 3
  - Infratentorial Glioma: 1
- WHO IV: 87
  - **Glioblastoma: 81**
    - **Age >20: 75**
  - MN of bronchus and lung: 3
  - MN of colon: 1
  - MN of pancreas: 1
- Ewing sarcoma: 1
- Malignant rhabdoid tumor: 1
- Cancer of unknown primary (CUP): 1

were enrolled in the two centres between 2000 and 2005 (Figure 2). Of those, 138 patients had primary brain tumours, and 87 were graded as WHO IV, including 81 GBM and one gliosarcoma (n = 82). Of those, 76 patients were adults (> 20 years). Fifty-eight adult GBM patients received a combination treatment (mEHT ± ddTMZ ± RT ± SAT), other 18 GBM patients were treated with mEHT only (with or without SAT). Twenty-three patients of the combination cohort were younger than 50 years and received HD mEHT. The cohort of interest (COI) included 54 patients who received mEHT + ddTMZ (with or without SAT). Four other patients of the combination cohort received RT in addition to mEHT, either alone (n = 1) or with ddTMZ (n = 3) (with or without SAT). Of the adult GMB patients (n = 76), 24 received LD mEHT and 52 received high-dose mEHT (HD mEHT); 59 received SAT vs. 17 that did not.

Patients' characteristic

Fifty-four adult patients with WHO IV GBM (n = 53) and gliosarcoma (n = 1) matched the inclusion criteria (COI). The mean age was 48.7 ± 1.5 years (median, 49.8 years; range, 25.9–68.2; 95%CI, 42.2–52.8), including two (4%) elderly patients (≥68 years) and 26 patients (48%) over 50 years. Thirty-three of the patients were male and 21 female (Table 2).

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Table 2. Patients' characteristic.

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
No of patients (NOP)	76		18		58		54		24		52		23	
Male	46	61%	10	56%	36	62%	33	61%	16	67%	30	58%	11	48%
Female	30	39%	8	44%	22	38%	21	39%	8	33%	22	42%	12	52%
Earliest born	24.02.1932		24.02.1932		19.09.1935		19.09.1935		24.02.1932		18.06.1932		31.10.1954	
Latest born	03.04.1975		10.03.1971		03.04.1975		03.04.1975		03.04.1975		21.08.1973		21.08.1973	
Earliest diagnosed	01.08.1993		01.09.2000		01.08.1993		01.08.1993		12.07.1999		01.08.1993		01.08.1993	
Latest diagnosed	15.03.2005		03.07.2004		15.03.2005		30.08.2004		08.07.2004		15.03.2005		15.03.2005	
Age (years):														
Mean	50,2 ± 1,3		55,1 ± 2,8		48,7 ± 1,4		48,7 ± 1,5		50,9 ± 2,6		49,9 ± 1,5		39,9 ± 1,2	
Median	50,4		59,1		49,8		49,8		50,8		50,2		41,0	
Range	25,9 – 71,9		30,9 – 71,9		25,9 – 68,2		25,9 – 68,2		25,9 – 68,9		27,0 – 71,9		27,0 – 49,1	
95%CI	44,8 – 53,9		44,4 – 64,9		42,7 – 52,3		42,2 – 52,8		42,2 – 59,8		44,4 – 55,8		36,7 – 43,0	
P-value (t-test)					0,037								<0,0001*	
Elderly (over 68 years)	4	5%	2	11%	2	3%	2	4%	2	8%	2	4%	0	0%
Mature (over 50 years)	40	53%	12	67%	28	48%	26	48%	13	54%	27	52%	0	0%
Adults (over 20 years)	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
Pre-treatment:														

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
Surgery + Chemoradiation	57	75%	13	72%	44	76%	42	78%	15	63%	42	81%	20	87%
Chemoradiation	2	3%	1	6%	1	2%	1	2%	1	4%	1	2%	0	0%
Surgery + Radiation	7	9%	2	11%	5	9%	4	7%	4	17%	3	6%	2	9%
Surgery + Chemotherapy	5	7%	0	0%	5	9%	4	7%	1	4%	4	8%	1	4%
Radiaton only	5	7%	2	11%	3	5%	3	6%	3	13%	2	4%	0	0%
Chemotherapy total	64	84%	14	78%	50	86%	47	87%	17	71%	47	90%	21	91%
Radiation total	71	93%	18	100%	53	91%	50	93%	23	96%	48	92%	22	96%
Surgery total	69	91%	15	83%	54	93%	50	93%	20	83%	49	94%	23	100%

Note: * versus all GBM sample.

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Forty-two (78%) patients underwent complete trimodal pre-treatment including surgery and chemoradiation, four (7%) received previous surgery and radiation, four (7%) received surgery and chemotherapy, three (6%) received only radiation and one (2%) received only chemoradiation. By modalities, 50 (93%) patients underwent previous surgery, 50 (93%) radiation, and 47 (87%) chemotherapy (mainly TMZ). The characteristics of the other cohorts are given in Table 2.

Details of treatment

All patients (100%) in the COI received ddTMZ + mEHT treatment, and 43 (80%) patients received concurrent SAT (Table 3).

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Table 3. Details of treatment.

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Time to 1st mEHT since diagnosis (months):														
Mean	12,1 ± 1,6		11,2 ± 2,3		12,3 ± 1,9		12,9 ± 2,1		13,3 ± 2,4		11,5 ± 2,0		12,7 ± 4,2	
Median	8,5		8,0		9,3		9,5		9,9		8,2		5,9	
Range	0,2 – 94,2		2,3 – 44,1		0,2 – 94,2		0,2 – 94,2		1,6 – 49,1		0,2 – 94,2		1,0 – 94,2	
95%CI	6,7 – 10,6		6,1 – 15,2		5,8 – 10,7		5,9 – 10,7		6,1 – 11,6		5,1 – 10,0		4,1 – 10,0	
Earliest mEHT	01.03.2001		07.05.2001		01.03.2001		01.03.2001		07.06.2001		01.03.2001		01.03.2001	
Latest mEHT	20.05.2005		19.05.2005		20.05.2005		20.05.2005		28.04.2005		20.05.2005		20.05.2005	
Treatment combinations:														
mEHT + CRT + SAT	2	3%	0	0%	2	3%	0	0%	0	0%	2	4%	0	0%
mEHT + Chemoradiation	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy + SAT	43	57%	0	0%	43	74%	43	80%	12	50%	31	60%	13	57%
mEHT + Radiation + SAT	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy	11	14%	0	0%	11	19%	11	20%	6	25%	5	10%	3	13%
mEHT + SAT	13	17%	13	72%	0	0%	0	0%	4	17%	9	17%	5	22%
mEHT only	5	7%	5	28%	0	0%	0	0%	2	8%	3	6%	0	0%
Treatment by modality:														
Radiation total	4	5%	0	0%	4	7%	0	0%	0	0%	4	8%	2	9%

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
SAT total	59	78%	13	72%	46	79%	43	80%	16	67%	43	83%	19	83%
Chemotherapy total														
NOP	57	75%	0	0%	57	98%	54	100%	18	75%	39	75%	17	74%
No of cycles	89		0		89		84		18		71		32	
Mean	1,5 ± 0,1		0		1,6 ± 0,1		1,6 ± 0,1		1,0 ± 0,0		1,8 ± 0,1		1,8 ± 0,2	
Median	1,0		1,0		1,0		1,0		1,0		1,5		2,0	
Range	1,0 – 5,0		1,0 – 3,0		1,0 – 5,0		1,0 – 5,0		1,0 – 1,0		1,0 – 5,0		1,0 – 5,0	
95%CI	1,0 – 1,0		1,0 – 2,0		1,0 – 1,0		1,0 – 1,0		1,0 – 1,0		1,0 – 2,0		1,0 – 2,0	
mEHT total:														
NOP	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
No of sessions	1367		292		1075		995		169		1198		545	
Mean	18,0 ± 0,3		16,2 ± 0,6		18,5 ± 0,4		18,4 ± 0,4		7,0 ± 0,1		23,0 ± 0,4		23,7 ± 0,6	
Median	14,0		13,5		14,0		14,0		7,0		18,0		23,0	
Range	3,0 – 65,0		4,0 – 43,0		3,0 – 65,0		3,0 – 65,0		3,0 – 9,0		10,0 – 65,0		10,0 – 65,0	
95%CI	11,0 – 16,0		7,0 – 23,0		11,0 – 17,0		10,0 – 17,0		6,0 – 9,0		15,0 – 26,0		15,0 – 27,0	
Low-dose mEHT	24	32%	6	33%	18	31%	18	33%	24	100%	0	0%	0	0%
Time of treatment (months):														
Mean	2,5 ± 0,4		1,6 ± 0,4		2,8 ± 0,5		2,7 ± 0,6		0,5 ± 0,0		3,4 ± 0,6		3,4 ± 0,7	
Median	1,1		1,0		1,1		1,1		0,5		1,9		1,9	

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Range	0,0 – 26,4		0,2 – 6,4		0,0 – 26,4		0,0 – 26,4		0,0 – 0,8		0,2 – 26,4		0,5 – 12,2	
95%CI	0,8 – 1,5		0,5 – 2,1		0,8 – 1,6		0,8 – 1,6		0,4 – 0,6		1,2 – 2,8		1,2 – 4,6	
P-value (t-test)	0,233								0,001					
Terminated (NOP)	9	12%	1	6%	8	14%	8	15%	9	38%	0	0%	0	0%
P-value (chi-square)	0,35								<0,0001				0,085*	

Note: * versus all GBM sample.

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In total, 84 ddTMZ cycles were performed for 54 patients, an average of 1.6 ± 0.1 cycles per patient (median, 1.0 cycles; range, 1.0–5.0; 95%CI, 1.0–1.0). The average duration of the treatment was 2.7 ± 0.6 months (median, 1.1 months; range, 1 day to 26.4 months; 95%CI: 0.8–1.5 months). In eight (15%) cases the treatment was terminated because of progressive disease. The average time elapsed since primary diagnosis to the first mEHT session was 12.9 ± 2.1 months (median, 9.5 months; range, 0.2–94.2; 95%CI, 5.9–10.7). A total of 995 mEHT sessions were performed, with a mean of 18.4 ± 0.4 per patient (median, 14; range, 3–65; 95%CI, 10–17). There were 18 (33%) patients with LD-mEHT.

Response

Fifteen patients (28%) in the COI were assessed for a response (Figure 2). One patient (7%) showed a complete response (CR) and two (13%) showed a partial response (PR) so that the objective response rate (ORR) was 20% (Table 4).

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Table 4. Survival and response rates (COI).

	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
Parameter	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Response:														
NOP estimated	22	29%	7	39%	15	26%	15	28%	9	38%	13	25%	7	30%
CR	1	5%	0	0%	1	7%	1	7%	1	11%	0	0%	0	0%
PR	2	9%	0	0%	2	13%	2	13%	0	0%	2	15%	2	29%
OR	3	14%	0	0%	3	20%	3	20%	1	11%	2	15%	2	29%
SD	9	41%	4	57%	5	33%	5	33%	2	22%	7	54%	4	57%
BR	12	55%	4	57%	8	53%	8	53%	3	33%	9	69%	6	86%
PD	10	45%	3	43%	7	47%	7	47%	6	67%	4	31%	1	14%
P-value (χ^2)				0,77						0,003			0,007*	
Exitus	49	64%	12	67%	37	64%	36	67%	18	75%	31	60%	11	48%
Censored	27	36%	6	33%	21	36%	18	33%	6	25%	21	40%	12	52%
Lost	2	3%	0	0%	2	3%	2	4%	1	4%	1	2%	1	4%
Right-censored	25	33%	6	33%	19	33%	16	30%	5	21%	20	38%	11	48%
Overall survival (since diagnosis):**														
MST (months)	20,0		14,8		20,7		20,8		18,5		20,4		23,9	
(95%CI):**	(14,7–23,6)		(12,2–28,3)		(15,0–25,0)		(15,2–25,1)		(11,8–23,0)		(14,6–25,7)		(13,0–NR)	
Range	1,4 – 141,5		4,4 – 48,9		1,4 – 141,5		1,4 – 141,5		3,2 – 53,8		1,4 – 141,5		2,4 – 141,5	

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Parameter	All GBM (1)	mEHT ± SAT (2)	Combination treatment (3)	ddTMZ +mEHT (4)	LD-mEHT (5)	HD-mEHT (6)	HD-mEHT <50 years (7)
5-y survival (%)	13,5	0,0	13,3	13,5	0,0	16,1	31,0
(95%CI)	(2,8–24,2)	(0,0–0,0)	(1,0–25,6)	(1,0–26,0)	(0,0–0,0)	(2,0–30,1)	(5,1–56,8)
P-value (log-rank)		0,436			0,350		0,32*
Survival since 1st mEHT (months):**							
MST (months)	7,6	6,4	7,7	7,7	4,4	8,3	12,8
(95%CI):**	(5,8 – 9,3)	(3,1 – 9,9)	(5,8 – 9,5)	(5,7 – 9,4)	(2,2 – 8,8)	(6,7 – 12,3)	(8,2 – 48,1)
Range	0,3 – 47,3	0,3 – 13,6	0,7 – 47,3	0,7 – 47,3	0,3 – 14,9	1,0 – 47,3	1,0 – 47,3
1-y survival (%)	28,8	22,6	30,2	29,5	8,7	36,6	56,9
(95%CI)	(16,5–41,0)	(0,0–47,9)	(16,1–44,2)	(15,5–43,6)	(0,0–24,5)	(21,3–51,9)	(33,3–80,5)
2-y survival (%)	16,8	0,0	19,2	18,8	0,0	23,3	32,5
(95%CI)	(6,0–27,5)	(0,0–0,0)	(6,8–31,6)	(6,5–31,1)	(0,0–0,0)	(9,0–37,5)	(7,7–57,4)
P-value (log-rank)		0,403			0,007		0,047*
Survival time after the last mEHT (follow-up) (months):							
Mean	5,0 ± 0,8	3,8 ± 0,8	5,3 ± 1,0	5,6 ± 1,1	3,9 ± 0,7	5,5 ± 1,1	7,4 ± 2,4
Median	3,3	2,9	3,4	3,5	2,4	3,4	3,3
Range	0,0 – 46,4	0,0 – 12,1	0,1 – 46,4	0,1 – 46,4	0,0 – 14,3	0,1 – 46,4	0,2 – 46,4
95%CI	2,2 – 4,6	0,8 – 5,5	2,2 – 5,0	2,2 – 5,3	1,5 – 5,3	2,5 – 5,0	1,3 – 7,3

Note: * versus all GBM sample; ** Kaplan-Meier estimation; NR – not reached.

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Five patients (33%) showed stable disease (SD) and seven (47%) were in progressive disease (PD) status, giving a beneficial response rate (BRR) of 53% (see Bias assessment and limitations of the study).

Survival

All of the patients of the COI were included in the survival analysis (Figure 2). Average follow-up since the 1st mEHT session was 8.4 ± 1.2 months (median, 6.0 months; range, 0.7–47.3 months; 95%CI, 4.6–7.5 months). Average follow-up since the last mEHT session (Table 4) was 5.6 ± 1.1 months (median, 3.5 months; range, 1 day to 46.4 months; 95%CI, 2.2–5.3 months). For that period, 36 (67%) patients died, two (4%) were lost (censored), and 16 (30%) were alive at the end of the follow-up period (right-censored). The MST since the first diagnosis was 20.8 months (95%CI, 15.2–25.1) and the five-year OS was 13.5% (95%CI, 1.0–26.0%). The MST since the first mEHT session was 7.7 months (95%CI, 5.7–9.4). Survival at 12 and 24 months was 29.5% (95%CI, 15.5–43.6%) and 18.8% (95%CI: 6.5–33.1%) respectively (Figure 3) (see Bias assessment and limitations of the study).

Safety

Unfortunately, the raw data presented does not contain safety data, so we rely on the safety data of the 140 patients reported in the primary paper.[36] No grade III–IV toxicity was reported. Short-term (<2 h) asthenia after treatment was encountered in 10% of the cases, rubor of the skin in 8%, edema of fresh scars in <1%, subcutaneous fibrosis in 1%, burning blisters grade I–II in 2%, and headache, fatigue and nausea (1–2 days) in 12% (see the Bias assessment and limitations of the study).

ANALYSIS OF THE RESULTS

Covariates survival analysis

There was no a difference in survival between patients treated with mEHT only (with or without SAT) and with the combination treatment (Table 4, Figure 4), neither by survival (MST since 1st mEHT 6.4 months [95% CI, 3.1 to 9.9] vs. 7.7 months [5.8 to 9.5], p = 0.403) or by response (BRR 57% vs. 53%, p = 0.77), although the mEHT only regimen was applied to significantly older patients (median 59.1 years vs. 49.8 years in the combination treatment sample, p = 0.037) with KPS <60% unfit for chemotherapy and radiation.

However, we did detect a significant difference between samples with LD-mEHT and high-dose mEHT (HD-mEHT), both in survival since 1st mEHT (p = 0.007; HR = 2.19; 95%CI, 1.21–3.95)

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and response (p = 0.003) (Table 4, Figure 5). A similar pattern was shown in the analysis of the sample treated with SAT versus the sample without SAT (Figure 6): the MST since 1st mEHT was 8.7 months (95%CI, 7.2–11.4) with SAT vs. 2.9 months (95%CI, 2.3–5.5) only without SAT (p = 0.004, HR = 0.40 [95%CI, 0.36 to 0.45]) (see DISCUSSION).

The sample of younger patients (under 50 years) with HD-mEHT treatment showed the best results (Figure 7): an MST since diagnosis of 23.9 months (95%CI, 13.0 to Not Attained); a 5-year OS of 31.0% (95%CI, 5.1 to 56.8); an MST since 1st mEHT session of 12.8 months (95%CI, 8.2 to 48.1); and a BRR of 85.7%. Although the overall survival did not differ significantly from the complete sample (p = 0.32), the survival since 1st mEHT and BRR were significantly better (p = 0.047 and p = 0.007, respectively).

Systematic comparator

Based on a systematic review[160] and a narrative review[15] of different ddTMZ regimens, five phase II, cohort, uncontrolled clinical trials addressing the ddTMZ 21/28d regime were identified (Table 5).

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Table 5. Comparison of dose-dense temozolamide trials: patients' characteristic.

Study (Year) (Enrollment)	NOP	Country	Study design	Inclusion	Med Age	KPS	Pre-treatment SRG	Pre-treatment RT	Pre-treatment TMZ	MTAD	Other	Current treatment Regimen	Current treatment NOC
Brandes (2006)	33	Italy		Recurrent/ progressive GBM in chemonaïve pts with KPS≥60 in SCC; 45% of met-MGMT	57	90% (60-100)	100 %	100 %	0%	N/A	R1:100%: met 45.5%; re-op. 3%.	75 mg/m^2/d qd X21/28d	153 ccls: mean 4.6, med 3 (1-15)▪
Strik (2008) (2005-2007)	18	Germany	Phase II prospective cohort uncontrolled	Recurrent/ progressive GBM, KPS≥50 in SCC: 1st relapse 78%, 2nd – 22%	54.8	60% (50-100)	100 %	100 %	100% (≥1 adj TMZ ccls)	7.5 m[a]	R1/2: 77.8/22.2%; met.46.2%; re-op. 33.3%	100 mg/m^2 /d qd X21/28d	154 ccls, mean 7.3, med 5 (2-18)▪
Abacioglu (2011) (2006-2008)	16	Turkey		Recurrent/progressive GBM, KPS≥70 in SCC	50	80% (50-100)	100 %	100 %	100% (med 6 ccls)	13 (6-105)▪			med 2 (1-8)▪
Berrocal (2010)	47	Spain		Recurrent/progressive HGG with KPS≥60 in SCC;	50	(70-80%) ECO	81%	100 %	100% (med 6 ccls)	14 m (6-126)▪		85 mg/m^2/d qd X21/28d	med 2 (1-13)▪

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				WHO IV GBM 57%, WHO III 43%		G 1							
Norden (2013)	55	USA		Recurrent/progressive GBM with KPS≥60 in SCC, standard (Stupp) pre-treatment with ≥2 adjuvant cycles)	57	90% (60-100)	100 %	100 %	100% (≥2 adj TMZ ccls) (med 6 ccls (12-16))	N/A	R1: 100%; R/P: 48%/ 52%, met. 65%	100 mg/m^2 /d qd X21/28d X12 ccls or until PD	N/A
Sahinbas (2007) (2000-2005)	54	Germany	Retro-spective cohort uncontrolled	Recurrent/progressive GBM, KPS≥40	49.8	60% (40-100)[b]	93%	93%	87%	9.5 m (5,9-10,7)*		100 mg/m^2 /d qd X21/28d + mEHT	84 ccls, mean 1.6±0.1, med 1 (1-5)▪

Note: SCC: stable clinical condition; HGG: high-grade glioma; GBM: glioblastoma multiforme; KPS: Karnofsky performance score; MGMT: O6-Methylguanine DNA Methyltransferase; qd: daily; MTAD: median time after diagnosis; TMZ: temozolomide; R1: first relapse/progression; R1/2: first / second relapse; R/P: relapse / progression; met.: methylated MGMT promoter gene; re-op.: re-operation; * 95% confidence interval; ▪ range; [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] estimated.

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The Italian trial of Brandes et al. (2006)[161] studied a highly-selected group of CTX-naïve patients with good performance status (median KPS = 90%). This was a specific design aimed to study the efficacy of TMZ at GBM recurrent in TMZ-naïve patients, and, due to this specificity, the results of Brandes are incomparable to both the current trial and the all other four ddTMZ trials, all made on TMZ-pretreated patients with KPS 60–80%. US trial by Norden et al. (2013)[162] is another stand-alone trial with a median KPS of 90% and an extremely high share (65%) of patients with a methylated MGMT promoter (excluded from the comparison, see Bias assessment and limitations of the study). The German trial by Strik et al. (2008)[163] also stands alone: despite the worst patients' performance status (median KPS = 60% which is usually considered unfit for CTX), the patients received the extensive course of ddTMZ (a median of five cycles; mean, 7.3) with a modest toxicity. Two other studies, a Turkish study by Abacioglu et al. (2011)[164] and a Spanish study by Berrocal et al. (2010)[165] were the real-world[22] studies without an obvious difference from everyday practice: although the Berrocal trial claims to have selected TMZ-resistant patients, its findings do not differ from those of the Abacioglu trial both by extent of TMZ pre-treatment (median of six cycles) or by the time elapsed since diagnosis (14 vs. 13 months).

The details of patients' characteristic and treatment schedules are presented in Table 5. The response and survival data are presented in Table 6.

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Table 6. Comparison of dose-dense temozolamide trials: response and survival.

	NOP		Response			Overall survival	Survival since relapse		
Study	total	EFR	CR	ORR	BRR	MST mo (95%CI)	MST mo (95%CI)	1-y OS (95%CI)	MTTP (95%CI)
Brandes (2006)	33	33	3%	9%	61%	N/A	9,1 (7,1 – 14,5)	38%	3,7 (2,8 – 6,3)
Strik (2008)	18	18	17%	22%	61%	16,4[a] (17,9[b])	8,35[a] (9,1[b]) (N/A)	N/A	N/A
Abacioglu (2011)	16	14	0%	7%	57%	N/A	7 (5,7 – 8,2)	0%	3,0 (1,8 – 4,2)
Berrocal (2010)	47	27	0%	7%	38%[a]	N/A	5,1 (3,7 – 8,5)[c]	N/A	2,0 (0,9 – 3,1)
Norden (2013)	55	54	0%	13%	48%	11,7 (8,1 – 16,2)	N/A	N/A	1,8 (1,8 – 2,8)
Sahinbas (2007	54	15	7%	20%	53%	20,8 (15,2–25,1)	7,7 (5,7 – 9,4)[e]	29,5% (15,5–43,6)	N/A

Note: EFR: Estimated for response; CR: Complete response; ORR: objective response rate (CR + partial response); BRR: beneficial response rate (ORR + stable disease); NOP: number of patients; MST: median survival time (Kaplan-Meier estimation); [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] originally reported data (without correction); [c] for the complete sample of 47 pts, including 27 GBM and 20 WHO III tumors; [d] combination treatment sample; [e] since 1st mEHT (not since relapse).

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The Strik's survival data were corrected because the originally reported survival in months was derived from weeks by the division to 4 (e.g., 32.8 w = 8.2 "chemo months"), which overrated survival by an average of 9%.

Effect-to-treatment analysis

We used effect-to-treatment analysis (ETA) to compare the trials according to the principles described in the statistics section. The mean survival time (mST) after relapse in patients receiving standard modern treatment (which can be defined as trimodal 1st–2nd-line treatment approximately equal to Stupp protocol[8]) was the parameter of comparison. Since the expected (reference) value of mST is absent in the literature, we deducted it from the available data as 4.775 months (95%CI, 3.9–5.6) (see Supplemental Material). Taking into account the worst MST of the Berrocal study (5.1 months [95%CI, 3.7–8.5]), this MST expectancy seems reasonable. For the further analysis, we considered this parameter as both the expected median and mean survival time (emST) since relapse (in view of supposed normal distribution according to central limit theorem). For further comparisons, meta-analysis and economic evaluations, the median parameters of all trials (MST and number of cycles) were translated into means according to the statistical methods section.

The results of ETA show the advantage of the mEHT+ddTMZ regimen. The main comparator was the weighted average of three ddTMZ trials with comparable samples (WA (2-4)) (Table 7).

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Table 7. Effect-to-treatment analysis: basic parameters.

No	Study	NOP	mST	P-value	Rank	LMG	P-value	mNC	P-value	ETR (95%CI)	P-value	Rank
1	Brandes (2006)	33	9,95 (7,73-12,17)	0,070	1	5,18 (2,79-7,56)	0,104	4,60 (3,87-5,33)	<0.001	1,13 (0,72-1,80)	0,273	2
2	Strik (2008)	18	8,35 (7,67-9,03)	0,416	2	3,58 (1,98-5,17)	0,506	7,30 (6,05-8,55)	<0.001	0,49 (0,31-0,70)	0,001	6
3	Abacioglu (2011)	16	6,98 (6,23-7,73)	0,345	6	2,20 (1,05-3,35)	0,486	3,33 (2,43-4,22)	0,004	0,66 (0,38-1,05)	0,022	3
4	Berrocal (2010)	47	5,60 (4,16-7,04)	0,031	7	0,83 (-0,86-2,51)	0,073	4,55 (3,94-5,16)	<0.001	0,18 (-0,05-0,44)	<0,001	7
5	WA (1-4)	114	7,27 (6,30-8,24)	0,638	4	2,50 (1,20-3,80)	0,718	4,20 (3,82-4,57)	<0.001	0,59 (0,39-0,85)	0,006	4
6	WA (2-4)*	81	7,16 (6,25-8,08)	0,531	5	2,39 (1,13-3,65)	0,633	4,13 (3,68-4,57)	<0.001	0,58 (0,37-0,83)	0,005	5
7	Sahinbas (2007)	54	7,63 (6,52-8,74)	1,000	3	2,85 (1,44-4,26)	1,000	1,56 (1,31-1,81)	1,000	1,83 (1,04-4,20)	1,000	1

Note: NOP: number of patients; WA: weighted average; mST: mean survival time since relapse; LMG: life months gained; mNC: mean number of cycles treated; * main comparator.

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The weighted average of all ddTMZ studies (WA (1-4)) and stand-alone Brandes and Strik studies were the additional comparators.

The mST in the mEHT+ddTMZ sample (7.625 ± 0.57 m) was ranked third after the Brandes and Srtik cohorts, and was significantly better than in the Berrocal trial (5.6 ± 0.73 m, $p = 0.031$) and worse than in the Brandes sample with borderline significance (9.95 ± 1.13 m, $p = 0.070$); other differences were not significant (Table 7). The differences by life months gained (LMG) were not significant. The mean number of treatment cycles (mNC) in the mEHT+ddTMZ sample (1.56 ± 0.13) was significantly less compared to all cohorts and WAs ($p \leq 0.004$). The relative survival gain changes the ranking: ddTMZ+mEHT provided significantly better effect-treatment ratio (ETR = 1.83 LMG/ccl [95%CI, 1.04–4.20]) compared to all other cohorts and WAs ($p < 0.022$), except the Brandes cohort (ETR = 1.13 LMG/ccl [95%CI, 0.72–1.80], $p = 0.273$).

To make ETRs comparable, the common denominator was estimated as a median of the mean number of cycles of all of the cohorts: MNC = 4.2 cycles. To lead ETRs to the common denominator, attenuation modelling was performed in the range of coefficients of attenuation (CA) 10–25 %×ccl^{-1} (Table 8).

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Table 8. Effect-to-treatment analysis: 15% attenuation model estimation.

No	Study	MAST	p–value	PNC	CEST	CENC	METR	EER	p–value	CNTM 1	2	3	4	5	6	7
1	Brandes (2006)	10,15 (9,24-11,06)	0,943	6	9,64	4	1,20 (0,74-1,95)	1,01	0,979	∞	2,56	1,59	0,99	1,65	1,59	91
2	Strik (2008)	8,40 (7,52-9,29)	0,015	6	7,98	4	0,81 (0,44-1,48)	0,68	0,302	-2,56	∞	4,22	1,62	4,63	4,19	-2,64
3	Abacioglu (2011)	7,34 (6,46-8,22)	<0,001	6	6,98	4	0,57 (0,37-0,89)	0,48	0,016	-1,59	-4,22	∞	2,62	-47,9	592	-1,62
4	Berrocal (2010)	5,63 (4,76-6,51)	<0,001	6	5,35	3	0,19 (0,08-0,49)	0,16	<0,001	-0,99	-1,62	-2,62	∞	-2,48	-2,63	-1,00
5	WA (1–4)	7,44 (6,56-8,31)	<0,001	6	7,07	4	0,59 (0,40-0,88)	0,50	0,015	-1,65	-4,63	47,9	2,48	∞	44,3	-1,68
6	WA (2–4)*	7,34 (6,46-8,21)	<0,001	6	6,97	4	0,57 (0,39-0,85)	0,48	0,011	-1,59	-4,19	-592	2,63	-44,3	∞	-1,62
7	Sahinbas (2007)	10,10 (9,10-11,10)	1,000	6	9,5	4	1,19 (0,59-2,40)	1,00	1,000	-91	2,64	1,62	1,00	1,68	1,62	∞

Note: WA: weighted average; * main comparator; CA: coefficient of attenuation; MAST: maximal attainable survival time; PNC: peak number of cycles; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio; EER: effect enhancement rate.

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A CA level of 15% was chosen for the following analysis as an optimal prognosis (Figure 8A). According to this scenario, the median effect-treatment ratio (METR) of the ddTMZ+mEHT cohort is 1.19 LMG/ccl (95%CI, 0.59 to 2.40), which is significantly more than the METR of the main comparator (METR = 0.57 LMG/ccl [95%CI: 0.39–0.85], p = 0.011) and other cohorts ($p \leq 0.016$), except that of Brandes (METR = 1.20 LMG/ccl [95%CI, 0.74–1.95], p = 0.979) and Strik (METR = 0.81 LMG/ccl [95%CI: 0.44 to 1.48], p = 0.302) cohorts. This scenario means that the ddTMZ+mEHT cohort would have to reach the maximal attainable survival time (MAST) of 10.10 months (95%CI, 9.10–11.10) at the sixth cycle, which is significantly more than the MAST of the main comparator (7.34 months [95%CI, 6.46–8.21] $p < 0.001$) and other cohorts ($p \leq 0.015$), except the Brandes cohort (10.15 months [95%CI, 9.24–11.06], p = 0.943).

Based on the "cycles needed to treat per LMG" criterion (CNTM) (Table 8), the ddTMZ+mEHT regimen displayed strong and significant benefit versus the Berrocal and Abacioglu cohorts and both WAs (CNTM = 1.00–1.68 ccls/LMG, $p < 0.016$), moderate and insignificant benefit versus Strik cohort (CNTM = 2.64 ccls/LMG, p = 0.302) and no effect versus the Brandes cohort (CNTM = -90.98 ccls/LMG, p = 0.979).

Thus, our ETA suggests a strong and significant enhancement of the ddTMZ 21/28d regimen by concurrent mEHT.

Sensitivity analysis

Sensitivity analysis was completed to validate the robustness of the ETA results. For this purpose, the lower and upper limits of CA were estimated (Figure 8, Table 9):

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Table 9. Effect-to-treatment analysis: sensitivity analysis.

			CA = 15%				CA = 19.3%			
No	Study	mST	CEST	METR	CNTM	p–value	CEST	METR	CNTM	p–value
1	Brandes (2006)	9,95 (7,73-12,17)	9,64	1,20 (0,74-1,95)	90,98 (48,52 — 170,60)	0,979	9,44	1,23 (0,75-2,01)	5,30 (2,97 — 9,47)	0,585
2	Strik (2008)	8,35 (7,67-9,03)	7,98	0,81 (0,44-1,48)	-2,64 (-5,43 — -1,28)	0,302	**8,35**	0,95 (0,49-1,86)	-11,73 (-24,39 — -5,64)	0,830
3	Abacioglu (2011)	6,98 (6,23-7,73)	**6,98**	0,57 (0,37-0,89)	-1,62 (-2,94 — -0,89)	0,016	6,73	0,55 (0,36-0,83)	-2,04 (-3,43 — -1,22)	0,016
4	Berrocal (2010)	5,60 (4,16-7,04)	5,35	0,19 (0,08-0,49)	-1,00 (-2,77 — -0,36)	<0,001	5,32	0,20 (0,08-0,51)	-1,19 (-3,22 — -0,44)	0,001
5	WA (1–4)	7,27 (6,30-8,24)	7,07	0,59 (0,40-0,88)	-1,68 (-2,93 — -0,96)	**0,015**	6,91	0,59 (0,40-0,88)	-2,26 (-3,70 — -1,38)	**0,027**
6	WA (2–4)*	7,16 (6,25-8,08)	6,97	0,57 (0,39-0,85)	-1,62 (-2,84 — -0,92)	**0,011**	6,82	0,57 (0,38-0,85)	-2,14 (-3,52 — -1,30)	**0,018**
7	Sahinbas (2007)	7,63 (6,52-8,74)	9,6	1,19 (0,59-2,40)	∞	1,000	8,69	1,04 (0,77-1,41)	∞	1,000

Note: WA: weighted average; * main comparator; CA: coefficient of attenuation; mST: mean survival time; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio.

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the lower limit of CA = 15% is defined by Abacioglu cohort, in which the ascending mST reaches a cost-effective survival time level (CEST = 6.98 months) with other cohorts being between CEST and MAST (Figure 8A); the upper limit at CA = 19.3% is defined by Strik cohort, in which the descending mST reaches CEST = 8.35 months (Figure 8B). The CNTM of the ddTMZ+mEHT cohort versus the main comparator attenuates from strong to moderate from the lower to the upper limit (from 1.62 to 2.14 ccls/LMG) but remains significant (p = 0.011–0.018). The extremum modelling shows that the CNTM of the ddTMZ+mEHT cohort versus the main comparator remains significant ($p \leq 0.05$) up to CA = 24.4%. Thus, the result of the ETA is robust.

Safety comparison

Since the ddTMZ+mEHT regimen did not display any grade II–-IV toxicity, whereas the ddTMZ regimens generated such toxicity events at a rate of 45–92%, the difference was always highly significant ($p < 0.001$) (Table 10).

Table 10. Comparison of dose-dense temozolamide trials: adverse events.

Adverse Event	Grade	Brandes (2006)	Strik (2008)	Abacioglu (2011)	Berrocal (2010)	Norden (2013)	Sahinbas (2007)
	NOP	33	18	16	47	55	140
Total events	I-II	122%	N/A	44%	194%	N/A	34%
	III-IV	76%	49%	92%	45%	60%	0%
	χ^2	123,721	72,196	141,308	70,654	100,593	
	p	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	
Lymphopenia	I-II	21%		12%	55%		0%
	III-IV	24%	14%	80%	28%	38%	0%
Leucopenia	I-II	21%		20%	28%		0%
	III-IV	24%	14%	4%	2%	5%	0%
Neutroopenia	I-II	9%			17%		0%
	III-IV	12%			2%	4%	0%
Trombocytopenia	I-II	3%		8%	19%		0%
	III-IV	3%	5%	8%	11%	4%	0%
Anemia	I-II	26%		4%			0%
	III-IV	3%				2%	0%
Nausea/Vomiting	I-II	6%			26%		4%

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Adverse Event	Grade	Brandes (2006)	Strik (2008)	Abacioglu (2011)	Berrocal (2010)	Norden (2013)	Sahinbas (2007)
	NOP	33	18	16	47	55	140
	III-IV	3%			2%	2%	0%
Fatigue	I-II						4%
	III-IV					5%	0%
Obstipation/Diarrhea	I-II	24%			15%		0%
	III-IV	3%					0%
Infection	I-II	12%					0%
	III-IV	3%	5%				0%
Headache	I-II						4%
Skin reactions	I-II						12%
Asthenia	I-II				17%		10%
Gastrointestinal	I-II				17%		0%
	III-IV		10%				0%

Grade I–II toxicity in the ddTMZ+mEHT cohort was mild. Since 4% of grade I nausea can be attributed to TMZ, total 30% of the mEHT-related events encountered. The main of them are grade I-II skin reactions (12%) and grade I short-term (<2h) post-treatment asthenia (10%).

Economic evaluation

Cost-effectiveness analysis

Cost-effectiveness analysis (CEA) was performed from the perspective of a health provider with a lifetime horizon. The goal of the CEA was to evaluate the cost-effectiveness of the ddTMZ+mEHT regimen versus ddTMZ only, so that only the direct costs for these two modalities were analysed. It was considered by default that other costs are dispensed proportionally and do not affect the estimation based on the direct costs (see Bias assessment and limitations of the study).

Two costs models were used for the CEA: conditionally termed ‘German’ and ‘US’ (see DISCUSSION). The German model has lower costs and less variance compared to the US model. For both the models, end user prices for TMZ were estimated based on open sources (as at Jan 21,

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2017): mean 1.70 $/mg (95%CI: 1.44 to 1.95) in the USA [166] and 1.14 €/mg (95% CI: 1.12 to 1.17) in Germany.[167]

The cost of the single mEHT session varies between countries, from $100 in Russia to $500 in Israel and South Korea (as at 2016). In the European Union, it varies in the range from €145.14 per session in Germany to €300–400 in private clinics outside Germany. From the perspective of a health provider, this cost is limited by national regulations: e.g., one deep HT session is reimbursed at a rate of €173 in Italy (National tariff nomenclature code 99.85.2) and €145.14 in Germany (GOA code 5854). In those countries where HT is not reimbursed by the health insurance system (e.g., Spain and Austria), the median private cost is about €300.

Thus, from the perspective of a health provider, the mean cost of a single mEHT session in Germany was estimated as €145.14 with zero variance (95%CI, €145.14–145.14), whereas in the US the estimated mean is $300 (95%CI, $234–366) (Table 11).

Table 11. Calculated prices for economic evaluation.

	US model		German model	
	TMZ	mEHT	TMZ	mEHT
Parameter	$/mg	$/sess.	€/mg	€/sess.
Mean (95%CI)	1,70 (1,44 – 1,95)	300 (234 – 366)	1,14 (1,12 – 1,17)	145 (145 - 145)
Median (range)	1,77 (0,59 – 4,42)	300 (150 – 500)	1,14 (0,88 – 1,55)	145 (145 - 300)

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia.

The results of the CEA are presented in Table 12 (German model)

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Table 12. Cost-effectiveness analysis (German model).

Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	%CE_{25k}	%CE_{30k}	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
Brandes (2006)	14,905 (14,586 – 15,225)	<0.001	24,292 (20,263 – 28,321)	4,421 (2,090 – 6,752)	1.22 (1.10 – 1.35)	0.061	53.57%	76.5%	28,706 (-5,529 – 62,940)	5,561,695	193.8
Strik (2008)	31,539 (30,863 – 32,215)	<0.001	61,250 (53,939 – 68,561)	41,379 (37,491 – 45,267)	3.08 (2.83 – 3.34)	<0.001	0.00%	0.0%	367,368 (-710,070 – 1,444,806)	22,195,135	60.4
Abacioglu (2011)	14,379 (14,071 – 14,687)	<0.001	33,429 (30,717 – 36,141)	13,558 (11,791 – 15,325)	1.68 (1.57 – 1.80)	<0.001	0.12%	1.8%	-92,957 (-352,869 – 166,956)	5,035,150	-54.2
Berrocal (2010)	16,721 (16,362 – 17,079)	<0.001	48,419 (39,174 – 57,665)	28,548 (23,705 – 33,391)	2.44 (2.16 – 2.71)	<0.001	0.31%	0.7%	-43,717 (-91,130 – 3,697)	7,377,172	-168.8
WA (1-4)	17,922 (17,538 – 18,306)	<0.001	39,967 (35,985 – 43,949)	20,096 (17,787 – 22,405)	2.01 (1.86 – 2.16)	<0.001	0.04%	0.3%	-291,167 (-1,869,626 – 1,287,291)	8,577,947	-29.5
WA (2-4)	18,043 (17,657 – 18,430)	<0.001	40,845 (36,926 – 44,763)	20,973 (18,692 – 23,255)	2.06 (1.90 – 2.21)	<0.001	88.8%	99.2%	-226,212 (-1,153,427 – 701,004)	8,699,523	-38.5

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Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{25k}$	$\%CE_{30k}$	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
WA (2-3)*	18,138 (17,750 – 18,527)	<0.001	40,424 (36,758 – 44,091)	20,553 (18,384 – 22,722)	2.03 (1.89 – 2.18)	<0.001	0.02%	0.2%	-302,629 (-1,934,133 – 1,328,875)	8,794,882	-29.1
Sahinbas (2007)	9,344 (9,199 – 9,488)	1.000	19,871 (17,719 – 22,024)	0	1.00	1.000	88.8%	99.2%	0	0	0.0

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main comparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{25k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) €25,000; $\%CE_{30k}$: %CE at CET €30,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained).

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and Table 13 (US model).

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Table 13. Cost-effectiveness analysis (US model).

Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	%CE_{30k}	%CE_{50k}	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
Brandes (2006)	22,106 (18,799 – 25,413)	0.003	36,028 (28,866 – 43,189)	3,324 (-1,280 – 7,927)	1.10 (0.96 – 1.25)	0.472	3.01%	84,02%	34,727 (-12,095 – 81,549)	6,728,332	193.8
Strik (2008)	46,775 (39,779 – 53,772)	<0.001	90,841 (76,123 – 105,558)	58,136 (50,122 – 66,151)	2.78 (2.45 – 3.11)	<0.001	0.02%	0,21%	519,683 (-1,009,423 – 2,048,790)	31,397,527	60.4
Abacioglu (2011)	21,325 (18,135 – 24,515)	0.007	49,579 (42,820 – 56,338)	16,875 (12,433 – 21,317)	1.52 (1.35 – 1.68)	<0.001	0.17%	51,27%	-109,798 (-426,187 – 206,591)	5,947,408	-54.2
Berrocal (2010)	24,799 (21,089 – 28,508)	<0.001	71,811 (56,003 – 87,619)	39,107 (30,569 – 47,644)	2.20 (1.89 – 2.51)	<0.001	0.26%	1,56%	-55,827 (-122,100 – 10,445)	9,420,880	-168.8
WA (1-4)	26,580 (22,604 – 30,555)	<0.001	59,276 (50,498 – 68,053)	26,571 (21,289 – 31,853)	1.81 (1.61 – 2.02)	<0.001	0.08%	2,34%	-380,229 (-2,447,832 – 1,687,373)	11,201,761	-29.5
WA (2-4)	26,760 (22,757 – 30,763)	<0.001	60,577 (51,756 – 69,398)	27,873 (22,572 – 33,174)	1.85 (1.64 – 2.06)	<0.001	0.06%	1,96%	-295,965 (-1,515,454 – 923,523)	11,382,070	-38.5

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Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{30k}$	$\%CE_{50k}$	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
WA (2-3)*	26,901 (22,877 – 30,925)	<0.001	59,954 (51,427 – 68,481)	27,249 (22,075 – 32,423)	1.83 (1.63 – 2.04)	<0.001	0.06%	2,04%	-396,520 (-2,540,572 – 1,747,533)	11,523,498	-29.1
Sahinbas (2007)	15,378 (12,703 – 18,052)	1.000	32,704 (27,215 – 38,193)	0	1.00 (1.00 – 1.00)	1.000	4.45%	94,60%	0	0	0.0

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main comparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{30k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) $30,000; $\%CE_{50k}$: %CE at CET $50,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained)

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Along with four single cohorts of comparison, three weighted averages (WA) were assessed. WA (1-4) combines all the cohorts, WA (2-4) excludes the Brandes cohort as a selected cohort (selection bias-free average), WA (2-3) also excludes the Berrocal cohort in view of its very low survival gain, which significantly affected the final results (low-result bias-free average, the main comparator).

The mean costs of ddTMZ+mEHT regimen both in the German (€9,344 [95%CI, 9,199–9,488]) and US ($15,378 [12,703–18,052]) models were significantly less versus all cohorts and WAs ($p < 0.05$ in all cases). The Abacioglu cohort displayed the lowest costs (€14,379 [95%CI, 14,071–14,687]) and $21,325 [95%CI, 18,135 – 24,515] respectively) and the Strik cohort the highest (€31,539 [95%CI, 30,863 – 32,215] and $46,775 [95%CI: 39,779–53,772]); the main comparator WA (2-3) costs were calculated to be €18,138 [95%CI: 17,750–18,527] and $26,901 [95%CI: 22,877–30,925]).

For estimation of the cost-utility ratio (CUR), we used the weighted average index of health-related quality of life (HRQoL) of all five cohorts (0.74 QALY/LY) to counterweight the initial difference of the samples (range of median KPS 60–90%) not connected with the treatment (Table 2).

The CUR of the ddTMZ+mEHT regimen, both in the German (19,871 €/QALY [95%CI, 17,719 – 22,024]) and US (32,704 $/QALY [95%CI, 27,215–38,193]) models was also less versus all comparators. The difference was highly significant ($p \leq 0.001$), except for the Brandes cohort (24,292 €/QALY [95%CI, 20,263–28,321]), $p = 0.061$; and 36,028 $/QALY [95%CI, 28,866 – 43,189], $p = 0.472$). The main comparator WA (2-3) was calculated as 40,424 €/QALY (95%CI, 36,758–44,091) and 59,954 $/QALY (95%CI, 51,427–68,481), $p < 0.001$ for both.

In the German model, versus cost-effectiveness thresholds (CET) 25,000 €/QALY ($\%CE_{25k}$) and 30,000 € /QALY ($\%CE_{30k}$), the proportion of cost-effective cases (%CE) for the ddTMZ+mEHT regimen was 88.8% ($\%CE_{25k}$) and 99.2% ($\%CE_{30k}$) (i.e., it was cost-effective versus both CETs). All the other comparators showed negligible %CE (0–2.5%), except the Brandes cohort, which was also mainly cost-effective at both CETs ($\%CE_{25k}$ = 53.6% and $\%CE_{30k}$ = 76.5%). In the US model, versus CETs 30,000 $/QALY ($\%CE_{30k}$) and 50,000 $/QALY ($\%CE_{50k}$), the %CE for the ddTMZ+mEHT regimen was 4.5% ($\%CE_{30k}$) and 94.6% ($\%CE_{50k}$) (i.e., it was cost-effective versus CET = $50,000 only). Two other cohorts were also mainly cost-effective versus CET = $50,000: namely the Brandes ($\%CE_{50k}$ = 84%) and Abacioglu ($\%CE_{50k}$ = 51.3%) cohorts; the $\%CE_{50k}$ of all of the WAs was negligible (2.0–2.3%).

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As for comparative cost-effectiveness, only the Brandes cohort showed an ICER of less than the applied CETs (28,706 € /QALY [95%CI, -5,529–62,940) and 34,727 $/QALY [95%CI, -12,095–81,549). All of the other cohorts and WAs were not cost-effective with the ICER ranging from 43,717 €/QALY / 55,827 $/QALY to 367,368 €/QALY / 519,683 $/QALY.

Sensitivity analysis

The sensitivity of the CEA was analysed by using an equal cost-effectiveness test, that is by exploring the value of a key parameter in which the value of the relative CUR (CURR) of the ddTMZ+mEHT regimen and the main comparator (WA [2-3]) equals to 1.0 (or ICUR = 0). For this purpose, the following variables were tested: the price of the mEHT session; the number of TMZ application days (days on) over a 28-days cycle; the price of TMZ; the number of cycles of ddTMX+mEHT.

The equivalent price of the mEHT session is €683 in the German model, and $1,013 in the US model and the coefficient of reliability of the CEA result (CR, the ratio of a key parameter of CE-equivalent model and the standard model) is 3.4/4.7 (Table 14).

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Table 14. Cost-effectiveness analysis: sensitivity analysis.

	US model					German model				
	TMZ		mEHT			TMZ		mEHT		
Parameter	Price, $/mg	Days on	$/sess	mNC	CR	Price, €/mg	Days on	€/sess	mNC	CR
Standard regimen	1.70 (1.44 – 1.95)	21	300 (234 – 366)	1.60		1.14 (1,12 – 1,17)	21	145.14 (145 – 145)	1.60	
Maximal mEHT price	NC	NC	1013.47	NC	3.38	NC	NC	683.65	NC	4.71
Minimal TMZ days on	NC	6,21	NC	NC	3,38	NC	4.46	NC	NC	4.71
Minimal TMZ price	0,50	NC	NC	NC	3.38	0.24	NC	NC	NC	4.71
Maximal TMZ+mEHT cycles	NC	NC	NC	2.86	1.79	NC	NC	NC	3.17	2.05

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; mNC: mean number of cycles; CR: coefficient of reliability; NC: no change.

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The equivalent price of TMZ is 0.50 $/mg in the US model and 0.24 €/mg in the German model; once again with CR = 3.4/4.7. Since these key parameters (prices) do not affect the treatment efficacy, their equivalent values do not need any size-dependent correction. The result means that the ddTMZ+mEHT regimen is cost-effective in the entire range of possible prices with double to quadruple redundancy.

The equivalent number of TMZ "days on" is 4.46 days in the German model and 6.21 days in the US model, once again with CR = 3.4/4.7. This time, the key parameter affects the treatment efficacy, because the diminished dose (days) of ddTMZ can decrease the effectiveness and, therefore, can increase the ddTMZ+mEHT/ddTMZ CURR and cause an offset of the equivalence point to the lower values of "days on". This means that the ddTMZ+mEHT regimen, most probably, keeps the cost-effectiveness up to the standard 5/28d regimen and below it, and the cost-effectiveness of mEHT could be generalized for the entire range of TMZ treatment of recurrent gliomas.

The maximal equivalent number of ddTMZ+mEHT cycles is 2.86 in the US model and 3.17 cycles in German model (CR = 1.8/2.1). This key parameter also affects the treatment efficacy, because, with an increase of cycle number of the ddTMZ+mEHT regimen, the treatment efficacy and CUR will rise with an offset of the equivalence point towards the longer course. At the least, this result means that the length of the ddTMZ+mEHT regimen can be doubled without loss of cost-effectiveness.

Thus, the sensitivity analysis confirms that the results of the CEA are remarkably stable, with double to quadruple redundancy.

Budget impact analysis

We estimated a budget impact of the treatment of 1,000 patients per year (Table 12 and 13) with a time horizon of one year. Versus the main comparator, the saving (ΔC_{1000}) is €8,794,882 / $11,523,498 per year (German / US model) with 29.1 years of survival gain (ΔE_{1000}). The average saving ranged from €8,577,947 / $11,201,761 to €8,794,882 / $11,523,498 with 29.1–38.5 QALY gained. To extrapolate the economic results to a larger time horizon, the depreciation rate of 20% per year must be applied.

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Cost-benefit analysis

Cost-benefit analysis (CBA) was performed from the perspective of a large neurooncology centre treating more than 150 patients with recurrent GBM per year (Table 15,

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Table 15. Cost-benefit analysis (US model).

Parameter	Rate	Year 1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		11	11	11	11	11	11	11	11	
Number of units		1								1
Capital costs[a]		400,000								400,000
Service costs	12%			48,000	48,000	48,000	48,000	48,000	48,000	288,000
Depreciation	15%		60,000	60,000	60,000	60,000	60,000	60,000	60,000	420,000
Reimbursement per session		300,00	300,00	300,00	300,00	300,00	300,00	300,00	300,00	
Reimbursement per year		807,300	807,300	807,300	807,300	807,300	807,300	807,300	807,300	6,458,400
Operational costs per year	50%	538,200	538,200	538,200	538,200	538,200	538,200	538,200	538,200	4,305,600
Economy per patient	20%	11,523	9,219	7,375	5,900	4,720	3,776	3,021	2,417	47,951
Economy per year		1,728,525	1,382,820	1,106,256	885,005	708,004	566,403	453,122	362,498	7,192,632
Earnings per year		2,535,825	2,190,120	1,913,556	1,692,305	1,515,304	1,373,703	1,260,422	1,169,798	13,651,032
Total costs per year		938,200	598,200	646,200	646,200	646,200	646,200	646,200	646,200	5,413,600
Economy & EBIT		1,597,625	1,591,920	1,267,356	1,046,105	869,104	727,503	614,222	523,598	8,237,432
EBIT		-130,900	209,100	161,100	161,100	161,100	161,100	161,100	161,100	1,044,800
Cumulative EBIT		-130,900	78,200	239,300	400,400	561,500	722,600	883,700	1,044,800	

Note: [a] Acquisition price + shipment + installation + training; [b] share of capital cost per year; [c] profit rate; [d] annual depreciation rate of the saving; EBIT: earnings before interest and taxes.

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Table 16).

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Table 16. Cost-benefit analysis (German model).

Parameter	Rate	Year 1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	
Number of units		1								1
Capital costs[a]		300,000								300,000
Service costs	12,0%[b]			36,000	36,000	36,000	36,000	36,000	36,000	216,000
Depreciation	15,0%		45,000	45,000	45,000	45,000	45,000	45,000	45,000	315,000
Reimbursement per session		145.14	145.14	145.14	145.14	145.14	145.14	145.14	145.14	
Reimbursement per year		390,572	390,572	390,572	390,572	390,572	390,572	390,572	390,572	3,124,574
Operational costs per year	50%[c]	260,381	260,381	260,381	260,381	260,381	260,381	260,381	260,381	2,083,049
Economy per patient	20%[d]	8,795	7,036	5,629	4,503	3,602	2,882	2,306	1,844	36,597
Economy per year		1,319,232	1,055,386	844,309	675,447	540,358	432,286	345,829	276,663	5,489,509
Earnings per year		1,709,804	1,445,958	1,234,880	1,066,019	930,929	822,858	736,401	667,235	8,614,083
Total costs per year		560,381	305,381	341,381	341,381	341,381	341,381	341,381	341,381	2,914,049
Economy & EBIT		1,149,423	1,140,576	893,499	724,637	589,548	481,477	395,019	325,854	5,700,034
EBIT		-169,809	85,191	49,191	49,191	49,191	49,191	49,191	49,191	210,525
Cumulative EBIT		-169,809	-84,619	-35,428	13,762	62,953	112,143	161,334	210,525	

Note: [a] Acquisition price + shipment + installation + training; [b] share of capital costs per year; [c] profit rate; [d] annual depreciation rate of the economy; EBIT: earnings before interest and taxes.

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The main assumptions of the CBA are as follows: mean sessions per patient is equal to that of SOI; the mEHT device does not generate revenues other than health care system reimbursement for the treatment of those patients; the mEHT device operates in 12-h/day mode; the capital costs including acquisition costs, shipment, installation and training are €300,000 in the German model and $400,000 in the US model; the service costs rate is 12% of the capital costs per year with 2-year free of charge guarantee service; the depreciation of the mEHT equipment at a rate of 15% per year; the norm of profit of the health care provider is 50% (operational costs are 67% of revenues); the saving obtained as a result of the introduction of the ddTMZ+mEHT regimen depreciates at a rate of 20% per year; the saving is not included in earnings before interest and taxes (EBIT); no price discount/inflation rate is used; the time horizon is 8 years.

Our CBA shows that use of an mEHT device is profitable with the above parameters and generates the total revenues in amount of €3,124,574 / $6,458,400 with EBIT €210,525 / $1,044,800 per mEHT device over 8 years, provided that operational costs are €2,083,049 / $4,305,600 for that period (€260,381 / $538,200 per year). With respect to the saving due to the use of the ddTMZ+mEHT regimen instead of ddTMZ only, the total economic effect (saving + EBIT) over the 8 year period is €5,700,034 / $8,237,432 per mEHT device.

DISCUSSION

Clinical evaluation

In a general comparison, the ddTMZ+mEHT cohort has revealed a non-significantly better mean survival time (mST = 7.63 months [95%CI, 6.52–8.74]) compared to the main comparator, the pooled mST of three trials on TMZ-pretreated patients (7.16 months [95%CI, 6.25 to 8.08], p = 0.531).

Covariates survival analysis has revealed the comparable efficacy of mEHT and ddTMZ, at least in weakened patients (Figure 4), suggesting the feasibility of mEHT as a single treatment in those patients, for which CTX is impossible in view of toxicity or bad performance. The advantage of mEHT over chemotherapy was shown elsewhere in GBM[35] and other cancers.[43,46,54,57]

Despite the shown significant dependence of survival from mEHT dose (p = 0.007), it is difficult to say how the difference in the mEHT dose actually affects the response and survival because the LD-mEHT sample included weakened patients with longer time since diagnosis to 1st mEHT (median

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9.9 months [95%CI, 6.1–11.6]), shortest treatment time (median 0.5 months [95%CI, 0.4–0.6) vs. 1.9 months (95%CI, 1.2–2.8) in the HD-mEHT sample, p = 0,0001) and highest rate of treatment termination (38% vs. 0% in the HD-mEHT sample, p<0,0001) (Table 3). More correctly, the LD-mEHT was rather a sequence of poor patient states, which likely accounts for the decrease in survival. In other words, the impossibility to reach an adequate mEHT dose for weakened patients made their prognosis dismal.

The dependence of survival on SAT use is questioned. The extremely low survival in the "No SAT" sample (2.9 months [95%CI, 2.3–5.5), almost 2-fold lower than the expected value) undisputedly indicates for the selection of patients with bad prognosis and small life expectancy. Comparison of the samples showed that "No SAT" includes patients with significantly less TMZ cycles (mean 1.1 ± 0.1 cycles vs. 1.7 ± 0.1, p = 0.017) and mEHT sessions (mean, 11.2 ± 0.5; median, 10 vs. 19.9 ± 0.4; median, 15, p = 0.013) with a higher proportion of LD-mEHT (47% vs. 27%, RR = 1.74 [0.90–3.34], p = 0.12). Therefore, this survival difference shows a tendency to not apply SAT to patients with a bad prognosis, and that these patients were heavily undertreated.

The shown significantly reduced toxicity of ddTMZ+mEHT is, in our opinion, caused by the short course of TMZ in the COI (median 1 cycle only). TMZ is known as a relatively safe alkylating drug. Its toxicity appears after 2–3 cycles and a development of the III–IV grade lymphopenia (the main adverse event) becomes virtually inevitable after six cycles. Thus, the data presented here allows us to conclude that mEHT *per se* is safe, but does not allow us to estimate the modifying effect of mEHT on TMZ toxicity (if such an effect exists).

Effect-to-treatment analysis

Direct comparison of the ddTMZ+mEHT results with the other ddTMZ studies is impossible because the ddTMZ+mEHT treatment in the participating tertiary centres was not continued up to the maximal attainable course (MAC). The median number of cycles was just one, and only 15% of treatments were stopped in view of the disease progression, without limiting toxicity. In tertiary centres, the end of treatment is caused either by the physician's decision, by the patient's personal decision, economic reasons, by an applied protocol, or because of a combination of these reasons. Therefore, the treatment is typically limited by 1–3 cycles only, whereas in clinics the median duration of MAC of recurrent GBM is five cycles.[21] Therefore, effect-to-treatment analysis (ETA) was used for the comparison.[151]

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The idea of ETA is simple and based on the effect-treatment ratio (ETR), i.e., life months gained per a typical 28-days treatment cycle, which is considered a unit of a CTX treatment. By ETR, we identified ddTMZ+mEHT as the uncontested leader, with 1.83 LMG/ccl versus 1.13 LMG/ccl of the nearest competitor (Brandes cohort) and 0.58 LMG/ccl of the main comparator (WA 2-4) (Table 7), although in terms of conventional MST-based comparison, ddTMZ+mEHT was ranked third (behind the Brandes and Strik cohorts).

The next step of the ETA follows from the idea of attenuation of the treatment effect. This is a typical feature of all cancer treatments because of the ability of cancer cells to rapidly develop multiple mechanisms of acquired resistance to an applied treatment. This is especially correct for diseases such as GBM, which almost inevitably progresses, and for TMZ, for which many distinct mechanisms of acquired resistance are available,[168,169,170] so that virtually all patients develop resistance to TMZ. As a result, the effectiveness of any cancer treatment decays (attenuates).

The offered equation of the attenuation is based on ETR and coefficient of attenuation (CA). It is suggested that CA is common for all the ddTMZ cohorts. The maximum value of CA corresponds to the assumption that the treatments have almost reached the maximal attainable survival time (MAST), which equals the extremum of the function. In this case, CA = 15 %/ccl exactly matches this assumption (Table 8A). Although the Strik cohort is located after the maximum of the function, it is acceptable because this cohort is likely overtreated (mNC = 7.3 ccls vs. 3–4.5 ccls in other ddTMZ cohorts).

The natural sequence of the attenuation idea is incomparability of ETRs obtained in a different number of cycles. This is because an early ETR with the lower impact of attenuation is higher than a later one. For the correct comparison, ETRs should be led to the common denominator. The best common denominator is the median number of cycles (MNC), which equals 4.2 cycles. The resulting parameter median ETR (METR) allows us to correctly compare the different treatments. In this comparison, COI (METR = 1.19 LMG/ccl [95%CI, 0.59–2.40]) significantly surpasses the main comparator WA (2-4) (METR = 0.57 LMG/ccl [95%CI, 0.39–0.85), p = 0.011) and all other comparators (METR = 0.19–0.59, p = 0.00–0.016), except the Brandes (METR = 1.20 LMG/ccl [0.74–1.95], p = 0.979) and Strik (METR = 0.81 LMG/ccl [0.44–1.48], p = 0.302) cohorts (Table 8). In other words, the efficacy of IOI in CTX-pretreated patients with a median KPS of 60–70% is the same as in the selected cohort of CTX-naïve patients with a median KPS of 90%, and significantly better compared to the TMZ-pretreated cohorts.

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With CA 15%/ccl, the COI reach a MAST of 10.10 months (95%CI, 9.10–11.10) at the sixth cycle, which is significantly more than the MAST of the main comparator (7.34 months [95%CI, 6.46–8.21], $p < 0.001$) and other cohorts, except the Brandes cohort (10.15 months [95%CI, 9.24–11.06), $p = 0.943$). The next assumption is that the CA of the ddTMZ+mEHT regimen is lower than that of the ddTMZ only regimen. Actually, the mechanisms of resistance to the RF-field have to differ substantially from those of CTX. Little is known about such acquired resistance. TTF reports a possibility of selection or development of giant-cell GBM with syncytial-type cells,[171] which is reasonable adaptation for 100 kHz range, where the large size of a cell improves the shielding from the external field, though it is a single-case observation, and it is hardly applicable to HFR, where size difference is not decisive. Taking into account the results of long-term (6 months to 3 years) mEHT treatments,[46,58,60] especially in patients with multiple liver metastases, which is a similarly lethal condition as GBM, where mEHT displayed the ability to support PFS up to three years, and even to revert the progression after stopping mEHT[46] (i.e., mEHT does not lose its efficacy over years), the assumption that the CA of mEHT is lower than that of TMZ looks reasonable. If we assume that the CA = 12.5 %/ccl, the ddTMX+mEHT cohort can attain a MAST of 10.84 months, or of 12.13 months with a CA = 10.0%.

The last parameter of ETA, called "cycles needed to treat per one life month gained" (CNTM), is an analogue of the known parameter "number needed to treat" (NNT). The CNTM shows the number of cycles of the compared treatments, at which the difference in their MST reaches one month. Positive CNTM means a benefit, negative means detriment, and the value of CNTM characterizes the strength of the effect (Figure 9). In this comparison, all of the cohorts displayed strong to moderate detriment versus the ddTMZ+mEHT regimen (Table 8), except the Brandes cohort (no effect).

Thus, the ETA has allowed us to uncover the real efficacy of the ddTMZ+mEHT treatment, which was impossible to assess with the conventional comparison by general endpoints, and has suggested that mEHT strongly and significantly enhances the efficacy of the ddTMZ 21/28d regimen with significantly less toxicity.

Economic evaluation

We studied two options for the mEHT application. The first, so-called German option, is specific for a developed country with rigid governmental regulation of the medical market, which leads to relatively low prices for pharmaceuticals with low variance (mean price of TMZ is 1.14 €/mg

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[95%CI, 1.12–1.17]) and fixed and low enough prices for medical procedures (in this case, 145.14 €/sess with zero variance [95%CI, 145.14–145.14]). The second, so-called US option, is specific for a developed country with lower governmental regulation, which leads to relatively high prices for pharmaceuticals with higher variance (mean price of TMZ 1.70 $/mg [95%CI, 1.44 to 1.95]) and variable and high enough prices for medical procedures (in this case, 300 $/sess [95%CI, 234 to 366]).

First, the adequacy of our costs estimation (€18,138 [95%CI, 17,750–18,527]) and $26,901 [95%CI, 22,877–30,925] in the main comparator) have to be assessed (Table 12 and 13). For this purpose, the result was compared with a recent study of Ray et al. (2014)[22], where expenditures for cancer drugs (without supportive drugs like antiemetics, pain killers, neutropenia related, etc.) for a 6-month period were assessed as $13,555–17,204. Since the study was devoted to TMZ treatment and taking into account the difference in price of TMZ and other cancer drugs, 95–99% of these 'cancer drugs' costs can be attributed to TMZ. Although the reported range of $13,555–17,204 appears to be much less than the average $27,000 displayed in the current assessment, it should be noted that the general practice of recurrent GBM treatment is based almost exclusively on the standard TMZ 5/28d regimen, [8] with 100–150 mg/m^2/d. The current regimen ddTMZ 21/28d 75–100 mg/m^2/d consumes 2.1–4.2 times more TMZ per course. Therefore, it is at least 2–3-times more expensive. Thus, the estimated costs range for the ddTMZ 21/28d regimen is $27,000–50,000, and the costs estimation of the current trial is adequate. It also corresponds to other estimations.[20,21]

The result suggests the significant advantage of the ddTMZ+mEHT regimen over all the comparators ($p < 0.003$) (except the Brandes cohort, against which the advantage was not significant [$p = 0.061–0.472$]). In the German model (Table 12), the ddTMZ+mEHT regimen was cost-effective versus both the 25,000 €/QALY and 30,000 €/QALY cost-effectiveness thresholds (CET) (88.8% and 99.2% of cost-effective cases, respectively), whereas the main comparator was not cost-effective (%CE of 0.0% and 0.2%). ICER versus ddTMZ+mEHT varied from 43,717 €/QALY to 367,368 €/QALY (except for the Brandes cohort, which displayed an ICER of 28,706 €/QALY).

In the US model (Table 13), the pattern was the same with more pronounced differences. The ddTMZ+mEHT regimen was not cost-effective versus CET = 30,000 $/QALY (%CE = 4.5% only), and only CET 50,000 $/QALY provides cost-effectiveness (%CE = 94.6%), whereas the main comparator showed a negligible cost-effectiveness ($\%CE_{50k}$ = 2.0%). ICER versus ddTMZ+mEHT varied from 55,827 $/QALY to 519,683 $/QALY (except for the Brandes cohort, which displayed an ICER of 34,727 $/QALY).

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The cost-effectiveness threshold (CET) (or willingness-to-pay, WTP) is set by National Institute for Health and Care Excellence (NICE) at £20,000–30,000 per QALY,[172] although studies show that the acceptable limit can be lower (up to £13–14,000).[173] In developed countries, a CET of €/$/£30,000 is considered standard. The CET for developing countries is suggested by the WHO at the level of their triple GDP per capita for each DALY,[174] which is typically close to the above NICE WTP. For end-of-life applications, where the QALY increase could be negligible, a CET of £50,000 is supposed by NICE.[175] Finally, for some orphan diseases, the third CET of about £100,000 is offered.[176] Since a treatment of the recurrent GBM can be considered an end-of-life application, a CET of 50,000 $/QALY is applicable in the US model.

Thus, the economic evaluation suggests that the inclusion of mEHT in the ddTMZ 21/28d regimen makes it cost-effective versus the applicable CET levels, whereas the ddTMZ 21/28d alone is not cost-effective. The sensitivity analysis suggests that this estimation is highly reliable, with double to quadruple redundancy. The sensitivity analysis also suggests that the advantage of ddTMZ+mEHT in cost-effectiveness remains true throughout the entire applicable range of prices for TMZ and the mEHT procedure, as well as for the TMZ intercycle variances (i.e., up to the lowest 5/28d regimen). It also suggests that the ddTMZ+mEHT course can be at least doubled without loss of cost-effectiveness. Since the cost-effective number of cycles (CENC) (i.e., the number of cycles at which MST reaches 95% of MAST) for the ddTMZ+mEHT regimen equals 3.0 (Table 8), this means the all-range cost-effectiveness of the regimen.

The BIA suggests significant savings from the introduction of mEHT, which can be estimated as about €8,794,882 per year per 1000 patients in the German model and $11,523,498 per year per 1000 patients in the US model, with an additional 29.1–38.5 QALY gained per 1000 patients.

Finally, the CBA shows that the mEHT, from the perspective of a single neurooncology centre, is profitable in both of the tested models (Table 15 and 16).

Thus, the introduction of mEHT generates savings for budget and health care providers and significant profit for the latter.

Applicability of mEHT in GBM treatment

The result obtained in this study looks promising, although a single retrospective trial does not provide the necessary grounds for generalization. Nevertheless, if the result is confirmed in a further meta-analysis, it will provide an excellent ground for generalization. At the least, it means that mEHT can be recommended as an enhancer of all ddTMZ regimens in the treatment of recurrent

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GBM, and, probably, for the regular 5/28d regimen too. Next, as shown by the covariates survival analysis (Figure 5), mEHT is feasible as a single treatment in those patients for which chemotherapy is impossible because of toxicity or bad performance. Thus, mEHT has a capacity as a salvage treatment after the failure of chemotherapy. With respect to the known low toxicity of mEHT[35,36,37,38,39] and its possibility to restore the performance and chemosensitivity,[46,58,60] this salvage treatment can, in some cases, provide an opportunity to continue chemotherapy in previously failed patients.

Bias assessment and limitations of the study

Only 15 patients (28%) in the COI were assessed for response. Although natural selection is supposed, selection bias is not excluded. Consequently, the response rate was excluded from the analysis.

Although follow-up period was short enough (median 6.0 months; range, 0.7–47.3 months; 95%CI, 4.6–7.5 months), it is close to the MST since the 1st mEHT session (7.7 months, 95%CI, 5.7–9.4), and the mean of the follow-up (8.4 ± 1.2 months) exactly fits the CI of the MST. Thus, the MST value is robust. Although 1-year and 2-year survivals since 1st mEHT are less robust in view of the short follow-up, they are also well within the range of the follow-up time (0.7–47.3 months) and, therefore, are reliable enough. Nevertheless, in view of their lower reliability, the 1-year and 2-year survivals were excluded from the comparison, which was based solely on the robust MST value.

The absence of the safety data matched to the COI is not a serious limitation because the absence of severe toxicity in the whole sample also excludes it for the sub-samples. So, the absence of grade III–IV toxicity and limited I–II toxicity (up to 30%) findings are relevant and robust, although the rate and distribution of the mild toxicity in the COI are approximate.

We excluded the Norden trial[162] from the ETA because of a lack of information on the number of cycles and some uncertainties (e.g., survival definition and some statistical uncertainties). The modest effect shown would not affect the comparison.

The main possible bias of a retrospective study is a selection bias. We consider the probability of the selection bias as minimal in the SOI because, in addition to the assurances of the authors of no exclusions from the sample, 153 patients with high-grade gliomas (HGG) is consistent with the whole amount of such patients in the enrolling centres, which are small tertiary centres not specialized in neurooncology (and, in the case of the Institute of Microtherapy, in cancer care at all), for the five-year period. Thus, we consider the sample as consecutive patients with HGG enrolled

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for the stated period without exclusions or selection. The declared inclusion criteria (recurrence/progression of HGG with KPS≥40%) rather describe the sample than limit it in any way. The absence of exclusion criteria confirms this suggestion.

At the same time, some compared ddTMZ studies showed an obvious selection bias. First, the Brandes study, in which the selection of CTX-naïve patients is presumed by the protocol, but the selection of patients with good performance (median KPS = 90%) also seems to be present (although this might be a natural sequence of the inclusion criteria). The same extremely favourable KPS is shown in the excluded Norden trial, which also showed an extremely high share of MGMT-methylated patients (65% vs. 45–46% in the other trials, which exceeds the highest historical level of about 60%[16]) (Table 7). Also, the large share of re-operations in the Strik study (33.3%) might significantly improve the observed survival, making it hardly attributable to the applied ddTMZ treatment.

The difference in dosage between the ddTMZ regimens was not analysed in the ETA (although it was considered in the economic evaluation). As many studies had displayed, there is no or negligible difference in efficacy of different doses of ddTMZ regimens, and sometimes lower doses were preferable.[177] Moreover, the possibility of dose reduction/escalation in all of the protocols makes such an analysis impossible. The average dose is never reported and cannot be retrieved from the reported data. We do not exclude the possibility that the actual doses were similar to each other.

There is an unequal MST starting point bias because the MST in the ddTMZ+mEHT cohort was calculated since the 1st session of mEHT, rather than since relapse/progression in the other cohorts. Since the SOI was carried out in tertiary centres, it is normal that mEHT was applied not just after relapse but rather as the second-line treatment of the relapse. Based on the median time of 9.0 months elapsed since diagnosis to the 1st mEHT treatment, and estimated 7.5 months MPFS in GBM, the delay of mEHT since relapse can be 1–1.5 months. This could significantly change the results in favour of the ddTMZ+mEHT cohort (e.g., estimated MST since relapse can reach 9 months instead of 7.6 months, as in the best ddTMZ studies). At the same time, due to this delay, probably some 1st-line treatments of relapse in the SOI were not included in the assessment. Based on the delay, the median one treatment cycle is supposed to be added, increasing the mean CTX cycles number to 2–2.5, which can somewhat change the economic results in favour of concurrent ddTMZ studies. Thus, the bias of not equal MST starting point rather distorts the comparison in favour of ddTMZ studies, though economically it is somewhat counterbalanced.

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It should also be noted that the two "real life" studies of Abacioglu and Berrocal displayed the longest time from initial diagnosis to enrolment (13 and 14 months, respectively), which is responsible for the low MST values in these trials. We consider that, in the weighted average assessment, this difference is counterbalanced by early enrolment in the Brandes and Strik trials and the median position of the SOI (Table 7). It is also counterbalanced (and even outbalanced) by the unequal histology bias, since the Abacioglu and Berrocal trials included WHO III tumours (28% and 43%, respectively) with much longer survival, which can be, in turn, the reason for the delayed relapse.

Nevertheless, there is a reciprocal dependence between the time to enrolment (relapse) and the MST since the enrolment (the SOI displays the medium-power correlation, Pearson 0.35), which is not considered in the ETA but seems counterbalanced or even outbalanced in favour of the ddTMZ cohorts.

It is worth noting that all of the "real life" studies (Sahinbas, Berrocal and Abaciouglu) showed the same median age of 50 years, whereas the supposedly selection-biased trials included the older patients (55–57 years).

MEHT required additional visits to the hospital (2–3 times a week), which means additional transportation costs and influences cost-effectiveness from the patient's perspective, although this does not affect the assessment from the health provider perspective. At the same time, since a planned mEHT session typically does not require the physician's involvement (a nursing procedure), we do not assume a better treatment control. Moreover, such control seems much more extensive in the compared prospective trials, where the follow-up included weekly complete blood counts,[163,162] physical and neurologic examinations every 4 weeks,[161,163] or even biweekly,[163] and brain imaging with MRI every 8 weeks[162] or earlier if indicated.[161] To compare, only 28% of patients in the SOI underwent brain imaging (the specificity of small tertiary centres). Better treatment control could significantly improve the treatment results.

Finally, all of the compared ddTMZ studies recruited only patients in a stable condition, whereas there was no such limitation in the SOI.

In general, although the assessment is distorted in favour of the ddTMZ studies, it still allows us to make an unambiguous conclusion on the advantage of the combination of mEHT and TMZ.

Also, upon completion of the paper, we have identified one additional ddTMZ 21/28d cohort in phase III randomized trial of Brada et al. (2010).[177] The result of this cohort (MST since relapse 6.6

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months after median four ddTMZ cycles, which results in METR ≤0.5 LMG/ccl) would not in any way affect the results obtained.

Generalizability of the results

The results of the sensitivity analysis of the CEA supposes the generalizability of the CEA results to the entire range of application of TMZ at recurrent GBM. There is a probability of similar enhancement of TMZ efficacy and cost-efficiency by mEHT can also be achieved in the treatment of the newly diagnosed GBM, although, to the best our knowledge, mEHT has never been studied in such a setting.

Since TMZ is considered the current most effective CTX treatment of GBM, the results of the covariate survival analysis (Figure 4) can be generalized to CTX. Thus, mEHT as a single treatment can be considered in those patients for which CTX is impossible because of toxicity or bad performance, and mEHT has a capacity as a salvage treatment after the failure of CTX.

Perspectives of research

This study creates a good basis for the further research on mEHT-enhancement of the GBM treatments with the possibility to develop a cost-effective alternative. First, we will estimate the other existing mEHT cohort trials, followed by a systematic review with meta-analysis. Second, a new cohort and randomized trials at recurrent and newly diagnosed GBM are warranted.

Verifiability of the results

To provide the possibility to verify the results obtained, raw data of the study are available in Table 17.

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Table 17. Raw data of ddTMZ+mEHT cohort (n = 54).

No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminated Y/N	Objective response	Last contact	EXITUS
001	W	30.4.67	1.5.03	29.9.03	2	31	Y	Y	N	NA		30.3.04
002	M	5.1.59	1.10.03	7.1.04	1	8	Y	Y	Y	PD		5.4.05
003	M	6.9.68	8.7.04	8.9.04	1	9	Y	Y	Y	NA		14.10.04
004	M	29.7.61	15.4.04	18.10.04	1	9	Y	Y	N	SD	25.5.05	
005	M	20.7.36	13.11.00	20.8.01	1	5	Y	N	Y	NA		27.10.01
006	M	28.11.53	3.5.04	12.4.05	1	9	Y	Y	N	NA	25.5.05	
007	W	12.11.62	19.6.04	15.11.04	1	11	Y	Y	N	PR	25.5.05	
008	M	9.8.50	16.5.00	3.9.01	1	14	Y	N	N	NA		15.1.02
009	W	28.1.63	13.3.03	15.7.03	2	26	Y	Y	N	NA		10.1.04
010	W	28.1.63	1.3.03	15.7.03	2	27	Y	Y	N	NA		10.1.04
011	M	21.8.73	1.6.02	14.4.04	1	16	Y	N	N	NA		19.6.04
012	W	26.12.43	12.7.99	18.6.01	1	9	Y	N	N	NA		10.7.01
013	M	21.9.38	1.5.00	30.1.02	1	13	Y	Y	N	NA		11.6.02
014	M	17.7.69	25.5.04	2.2.05	1	6	Y	Y	Y	PD		2.3.05
015	M	29.3.61	1.3.04	2.4.04	1	14	Y	Y	N	NA		15.12.04
016	M	13.8.47	8.5.04	12.10.04	1	15	Y	Y	N	NA		27.5.05
017	W	3.4.75	17.2.01	19.7.04	1	8	Y	Y	Y	PD		4.3.05
018	M	31.10.54	1.4.03	12.1.04	2	25	Y	Y	N	PD	5.5.05	
019	W	23.8.60	26.11.00	3.1.05	1	9	Y	Y	N	CR	25.5.05	

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No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminated Y/N	Objective response	Last contact	EXITUS
020	M	9.8.67	1.6.04	29.11.04	2	36	Y	Y	N	NA	25.5.05	
021	M	13.5.62	13.1.03	1.12.04	1	6	Y	N	Y	NA	25.5.05	
022	M	15.1.45	1.6.03	26.1.04	1	15	Y	Y	N	NA		7.8.04
023	M	15.3.45	1.6.04	19.4.05	1	15	Y	Y	N	NA	25.5.05	
024	W	22.11.35	1.10.03	19.11.03	1	8	Y	N	Y	NA		8.2.04
025	M	29.10.41	1.12.00	5.1.04	1	12	Y	Y	N	NA		12.2.04
026	M	20.1.49	1.12.02	13.7.04	2	21	Y	Y	N	NA		15.2.05
027	M	24.4.64	1.5.00	1.3.01	1	10	Y	N	N	NA		20.5.01
028	W	3.8.66	1.8.93	13.6.01	1	12	Y	Y	N	SD	25.5.05	
029	W	15.9.51	1.11.02	22.9.03	1	3	Y	Y	N	PD		4.7.04
030	M	14.4.51	1.11.03	21.9.04	1	11	Y	Y	N	NA		19.12.04
031	M	19.9.35	1.11.03	20.9.04	1	6	Y	Y	Y	NA		8.2.05
032	M	13.12.50	1.9.03	16.8.04	1	5	Y	Y	N	NA	11.10.04	
033	M	15.10.62	8.1.04	25.10.04	2	24	Y	Y	N	PR	25.5.05	
034	M	5.12.40	1.1.02	2.12.03	1	11	Y	Y	N	NA		1.3.04
035	M	2.11.71	30.8.04	4.1.05	2	18	Y	Y	N	NA	25.5.05	
036	M	24.5.39	1.1.02	21.1.02	1	46	Y	Y	N	NA		8.9.02
037	W	17.2.55	1.8.03	1.12.03	1	9	Y	Y	N	NA		27.8.04
038	M	30.4.44	1.7.03	14.6.04	1	10	Y	N	N	PD		4.2.05
039	W	24.4.36	3.6.04	26.11.04	2	20	Y	Y	N	NA	27.5.05	
040	M	18.5.68	1.11.03	12.1.04	3	38	Y	Y	N	SD	27.5.05	

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No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminated Y/N	Objective response	Last contact	EXITUS
041	W	29.6.59	1.6.00	12.6.01	1	16	Y	N	N	NA	8.10.04	
042	W	9.12.64	1.4.02	27.5.02	3	44	Y	Y	N	NA		7.6.03
043	M	20.2.45	1.4.02	24.6.02	3	29	Y	Y	N	NA		6.6.03
044	M	29.9.57	1.12.99	23.10.01	1	9	Y	N	N	NA		16.4.02
045	W	15.11.38	1.1.03	6.1.03	1	17	Y	Y	N	NA		13.2.03
046	M	30.6.50	1.8.02	13.5.03	3	34	Y	Y	N	NA		28.5.04
047	M	20.11.40	1.9.02	6.1.04	3	36	Y	Y	N	SD	30.5.05	
048	W	3.8.44	1.3.03	18.11.03	1	6	Y	Y	N	NA		24.2.04
049	W	21.9.59	1.2.02	22.11.02	5	65	Y	Y	N	NA		2.2.04
050	W	4.1.40	15.1.03	15.8.04	1	15	Y	Y	N	PD		17.4.05
051	M	11.10.57	1.11.99	7.6.01	1	6	Y	N	N	NA		13.8.01
052	W	4.2.52	1.6.02	24.9.02	2	27	Y	Y	N	SD	30.5.05	
053	M	5.1.53	1.11.03	17.2.04	3	35	Y	Y	N	NA	30.5.05	
054	W	26.9.50	1.6.00	23.4.01	5	56	Y	Y	N	NA		9.2.02

Note: CTX: chemotherapy; SAT: supportive and alternative therapy; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NA: not available.

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CONCLUSIONS

The ddTMZ+mEHT cohort revealed a non-significant improvement in the mean survival time versus the pooled mST of three trials on TMZ-pretreated patients (7.63 months [95%CI: 6.52 to 8.74] vs. 7.16 months [95%CI: 6.25 to 8.08], p = 0.531), with significantly fewer courses (1.6 vs. 3.98 cycles, p < 0.001). Our ETA suggests that mEHT strongly and significantly enhances the efficacy of the ddTMZ 21/28d regimen (p = 0.011). The effectiveness of the ddTMZ+mEHT treatment in CTX-pretreated patients with a median KPS of 60–70% was the same as in the selected cohort of CTX-naïve patients with a median of KPS 90%, and significantly better than the TMZ-pretreated cohorts (p ≤ 0.015). Attenuation modelling of continued ddTMZ+mEHT treatment suggests a maximum attainable MST of 10.10 months (95%CI: 9.10 to 11.10) in the pessimistic scenario and 11–12 months in the optimistic scenarios. Sensitivity analysis shows that the result of the ETA is robust. The ddTMZ+mEHT cohort has displayed significantly less toxicity than the ddTMZ 21/28d cohorts (no grade III–IV toxicity vs. 45–92%, respectively) because of the shorter TMZ course. MEHT *per se* displays high safety with a mild grade I–II toxicity (30% of events), mainly of mild skin reactions (12%) and short (<2 h) post-treatment asthenia (10%). Our CEA suggests that the ddTMZ+mEHT regimen is cost-effective compared to the applicable cost-effectiveness thresholds 25,000–50,000 €$/QALY, whereas ddTMZ 21/28d only is not cost-effective, with ICER versus ddTMZ+mEHT ranging from 43,717 €/QALY / 55,827 $/QALY to 367,368 €/QALY / 519,683 $/QALY. Sensitivity analysis suggests that the CEA result is highly reliable with double to quadruple redundancy, and the ddTMZ+mEHT regimen remains cost-effective in the entire applicable range of prices for TMZ and the mEHT procedure, TMZ intercycle variances, and mEHT duration. Our BIA suggests a significant saving from the introduction of mEHT, which can be estimated from €8,577,947 / $11,201,761 to €8,794,882 / $11,523,498 with 29.1–38.5 QALY gained per 1000 patients. The CBE, from the perspective of a single neurooncology center, suggests that mEHT is profitable and will generate a total revenue of €3,124,574 / $6,458,400 with EBIT €210,525 / $1,044,800 per mEHT device over an 8 year period, with total economic effect (economy + EBIT) over an 8 year period of €5,700,034 / $8,237,432 per mEHT device. After confirmation of these findings, mEHT can be recommended as an enhancer for all ddTMZ regimens in the treatment of recurrent GBM, and, probably, for the regular 5/28d regimen. MEHT can be applied as a single treatment in those patients for which chemotherapy is impossible because of its toxicity or bad performance, and as a salvage treatment after the failure of

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chemotherapy, with a possibility to restore the patient's performance and chemosensitivity and subsequently continue chemotherapy.

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DATA SHARING STATEMENT

Patient level data are available in Table 17. Consent for data sharing was not obtained but the presented data are completely anonymised, and risk of identification is absent.

FIGURE LEGEND

Figure 1. Dose-escalating scheme of mEHT.
The tenth session attains the maximum escalation, the further sessions are the same.

Figure 2. CONSORT flowchart.
Note: White: Cohort of Interest (COI); Light grey: cohorts of Covariate Survival Analysis (CSA); Dark grey: cohorts out of analysis; Black: Analyses.

Figure 3. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A_1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

Figure 4. Survival (Kaplan-Meier estimate) since 1st mEHT session of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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Figure 5. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 6. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 7. Survival (Kaplan-Meier estimate) since 1st mEHT session of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 8. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

Figure 9. Cycles needed to treat per one life-month gained (CNTM) scale.

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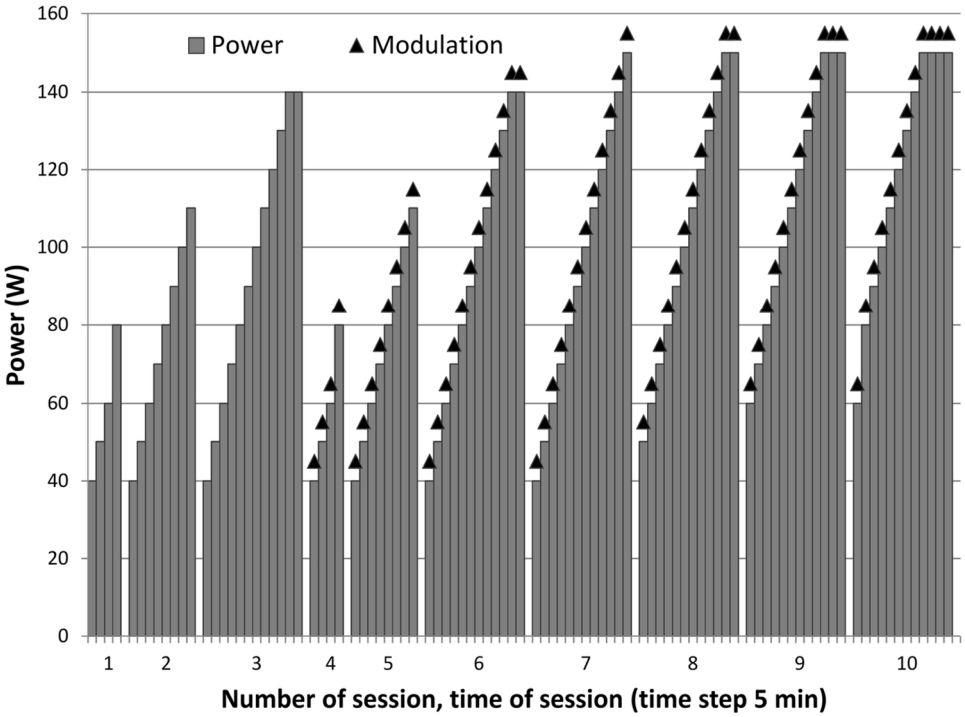

Figure 1. Dose-escalating scheme of mEHT.
The tenth session attains the maximum escalation, the further sessions are the same.

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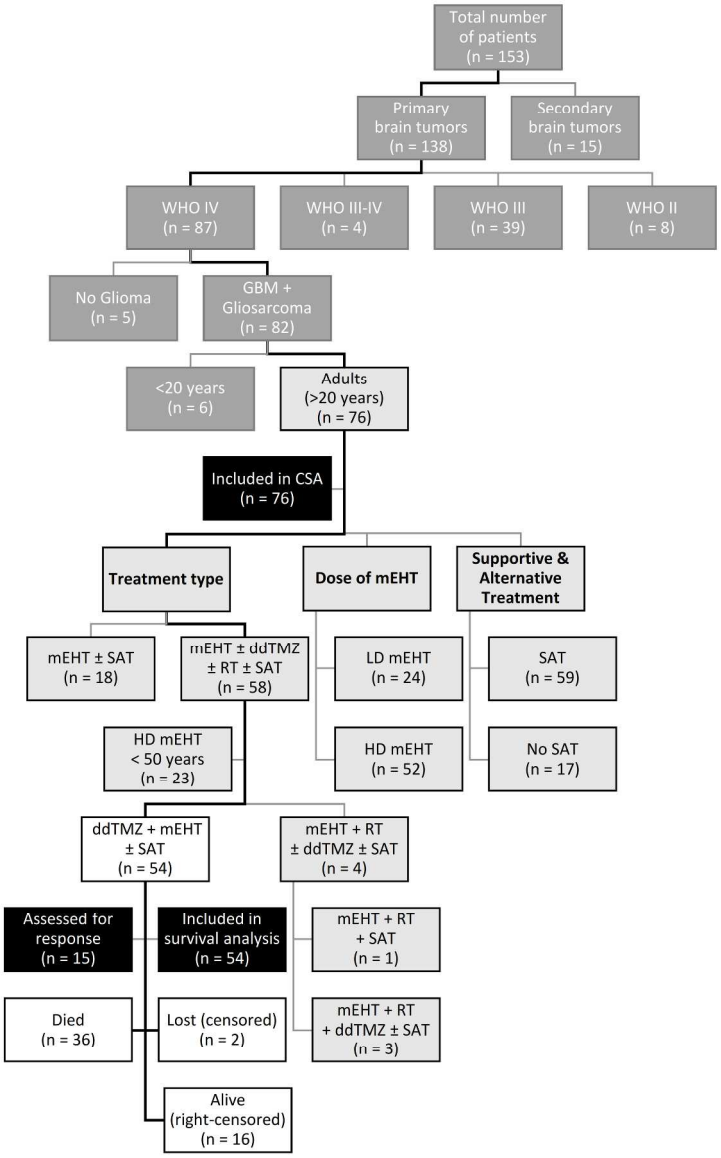

Figure 2. CONSORT flowchart.
Note: White: Cohort of Interest (COI); Light grey: cohorts of Covariate Survival Analysis (CSA); Dark grey: cohorts out of analysis; Black: Analyses.

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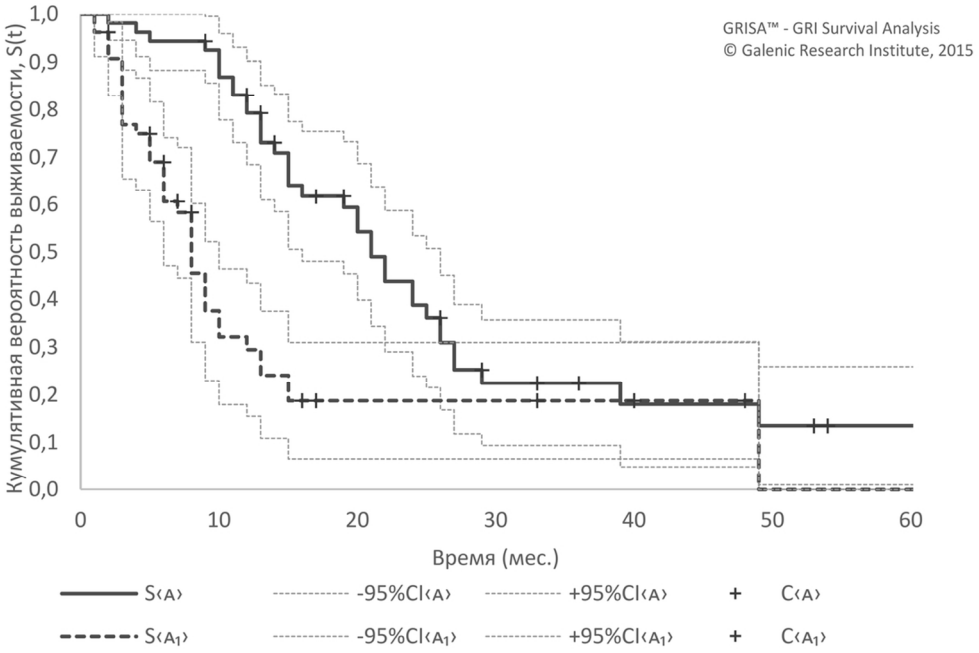

Figure 3. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

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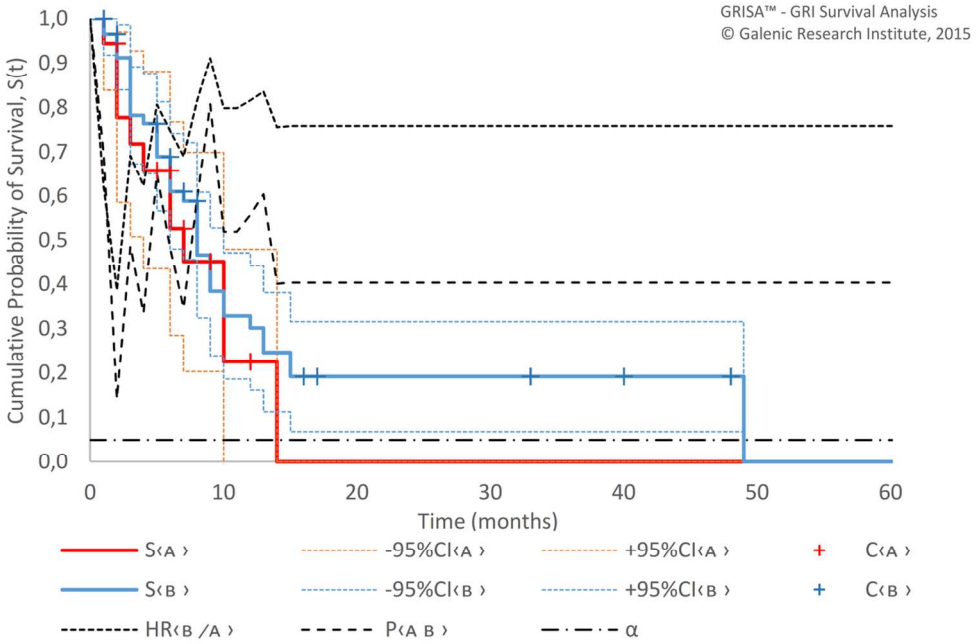

Figure 4. Survival (Kaplan-Meier estimate) since 1st mEHT session of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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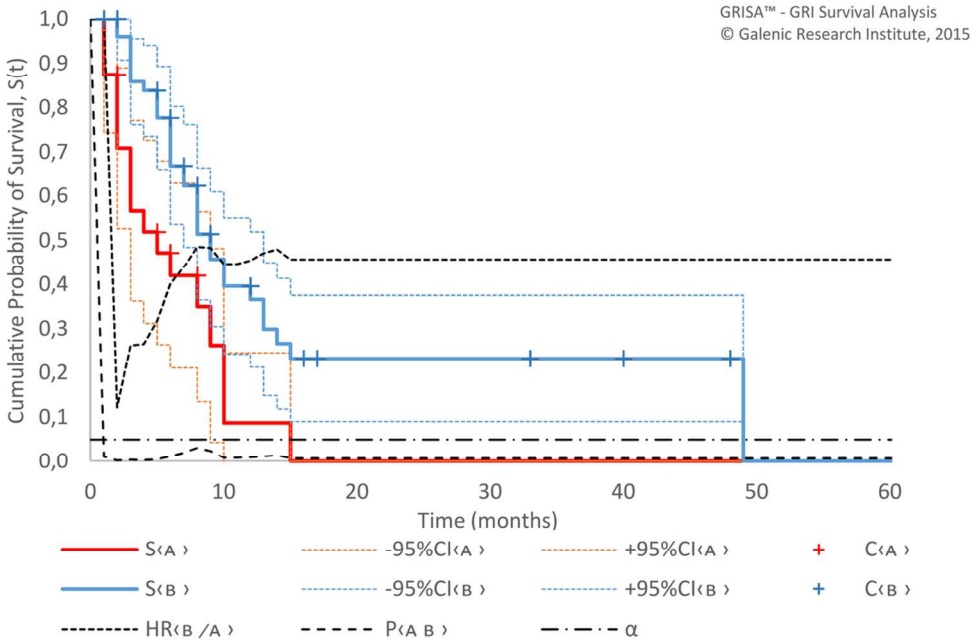

Figure 5. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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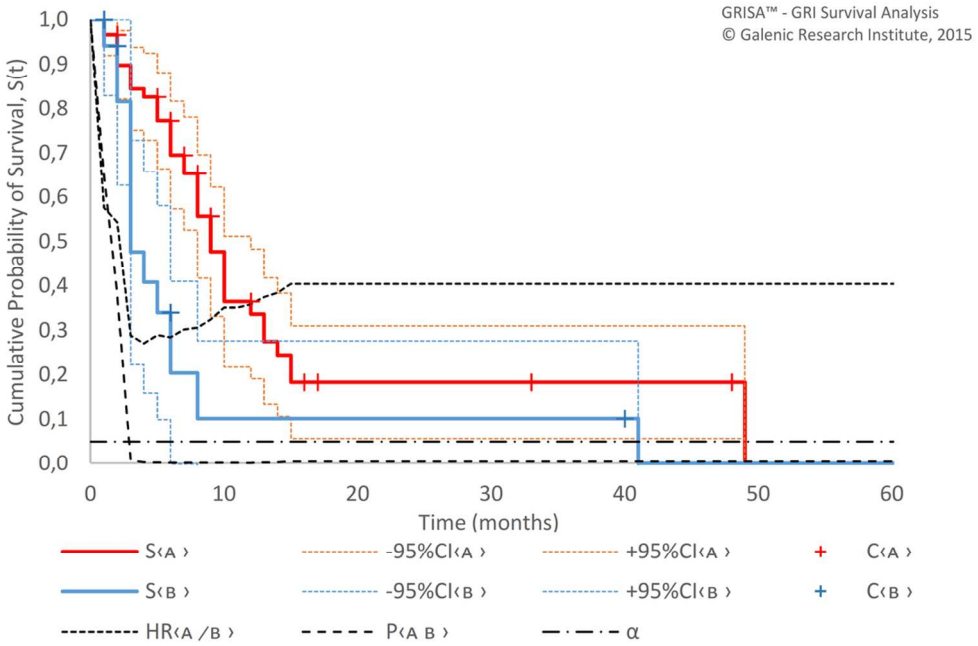

Figure 6. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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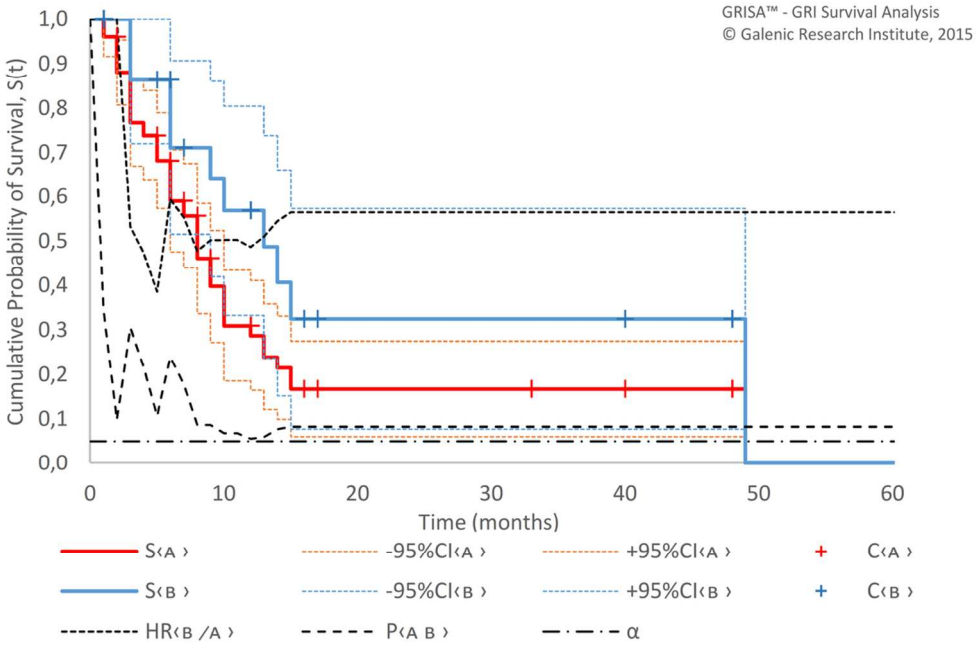

Figure 7. Survival (Kaplan-Meier estimate) since 1st mEHT session of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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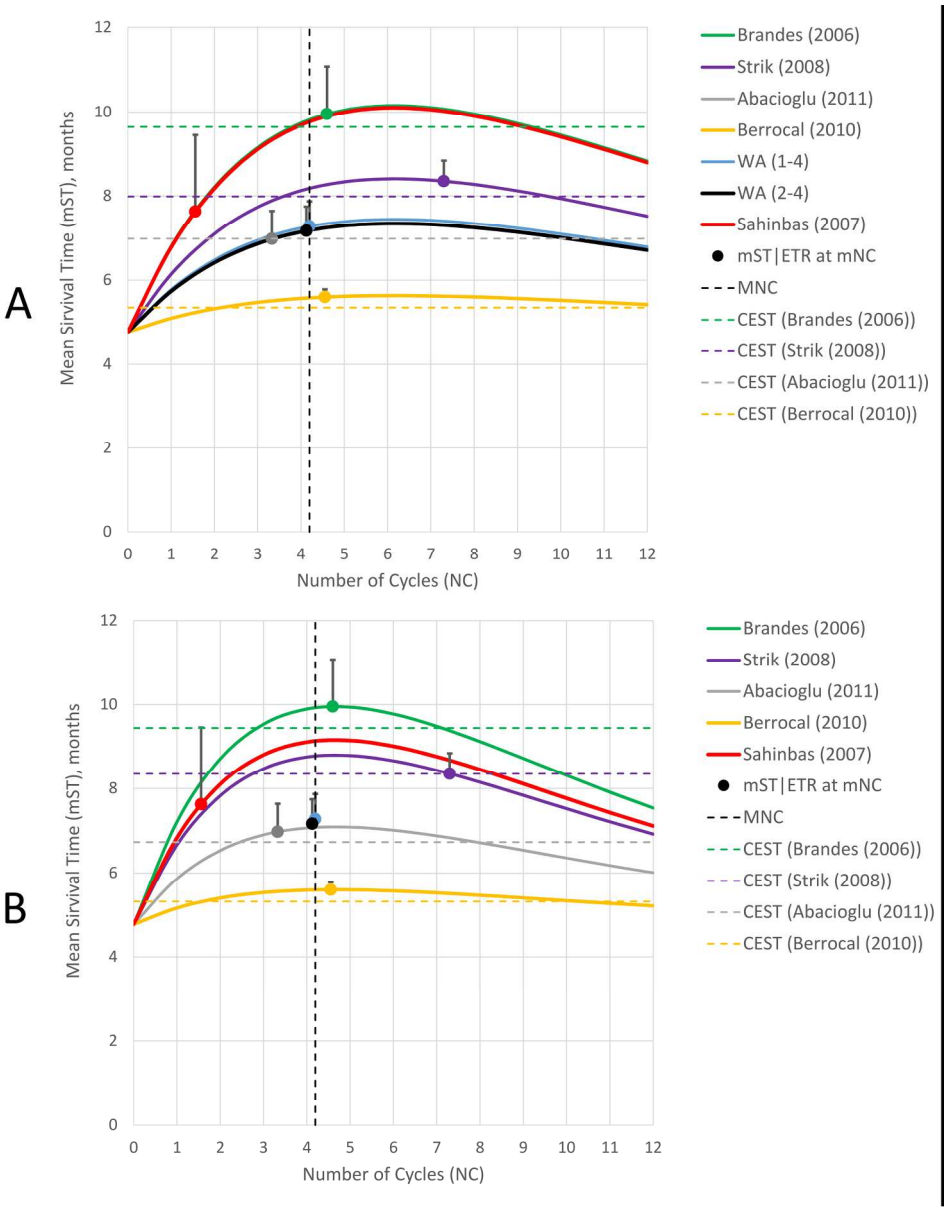

Figure 8. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

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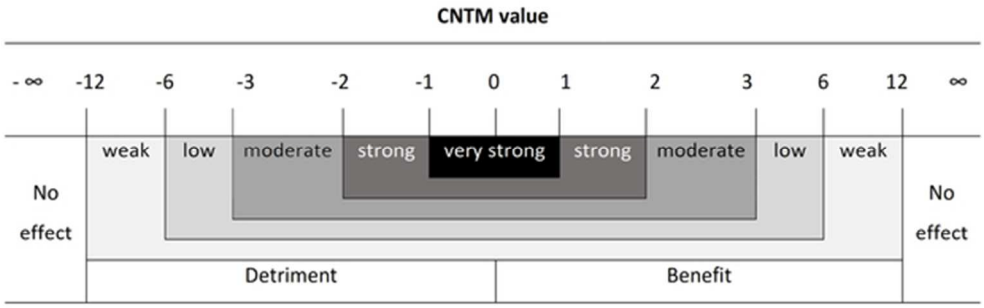

Figure 9. Cycles needed to treat per one life-month gained (CNTM) scale.

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Supplemental Material

Estimating the mean and confidence interval from the median and confidence interval

This simplified algorithm is based on the idea that the mean value of a skewed dispersion is located in the center of the confidence interval for the median with displacement towards the median value proportional to the extent of the median value displacement (Figure S1). Thus,

$$= \frac{+\frac{(\quad '' \quad)}{2}}{2}$$

where: m: mean; M: median; UL and LL: upper and lower limits of 95% CI of M.

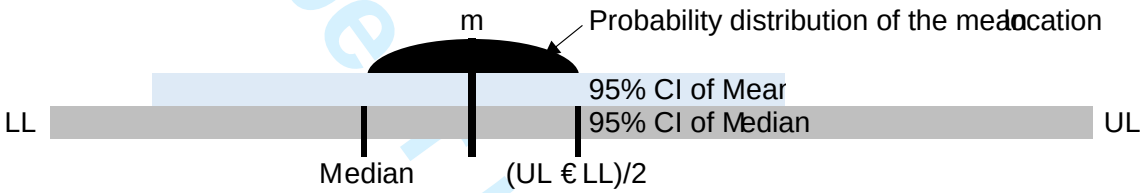

Figure S1. Graphic representation of the idea of the estimation of the mean.

Next, by the modelling on the sample of 10 – 1000 random values (Excel RANDBETWEEN(18;85) function was used to mimic the distribution of adult (18 – 85 years) patients in a clinical trial), it was revealed that 95% CI of the mean value of a sample is virtually always close to 60% of 95% CI (calculated according to Conover[132]) of the corresponding median value (mean of 100 readings, each repeated 10 times, coefficient of variation 1.5 – 3,2%), independently of the sample size (range 10 – 1000 subjects was tested) (Table S1).

Table S1. Results of modelling of 95% CI of mean to 95% CI of median ratio on different sample sizes (10-1000 subjects), mean value of n=100 readings of the ratio in each attempt

	Sample size						Weighted
Attempt	10	25	50	100	1000	Average	Average
1	57,0%	61,8%	69,8%	57,9%	60,4%		
2	57,0%	63,0%	64,6%	61,1%	58,4%		
3	58,0%	60,5%	65,3%	63,4%	61,6%		
4	55,8%	61,7%	65,6%	61,5%	57,7%		

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	Sample size						Weighted
Attempt	10	25	50	100	1000	Average	Average
5	55,8%	61,5%	68,8%	62,8%	62,2%		
6	57,3%	59,4%	66,1%	60,2%	59,1%		
7	56,8%	60,9%	66,8%	63,6%	60,1%		
8	57,3%	63,8%	63,6%	62,8%	60,9%		
9	55,2%	63,3%	67,2%	62,2%	59,9%		
10	57,0%	61,7%	69,7%	60,9%	61,6%		
Mean	56,7%	61,8%	66,8%	61,6%	60,2%	61,4%	60,6%
SD	0,9%	1,3%	2,1%	1,7%	1,5%		
CV	1,5%	2,2%	3,2%	2,8%	2,4%		

Thus,

$$95\% = \pm\frac{0{,}6\times(UL - LL)}{2}$$

where: m: mean; UL and LL: upper and lower limits of 95% CI of the median.

Checking of the algorithm on some sets of real data confirms its applicability. E.g., estimation of mean of temozolomide (TMZ) prices per mg from the median of 1.77€ (95% CI: 1.24€ - 2.11) returns mean of 1.72€ (95% CI: 1.46€ - 1.98) versus the actual mean of 1.79€ (95% CI: 1.44€ - 1.95), the error is 1.32-1.72%.

$$= \frac{1{,}77 + \frac{2{,}11 - 1{,}24}{2}}{2} = 1{,}7225$$

$$95\% = 1{,}72 \pm \frac{0{,}6\times(2{,}11 - 1{,}24)}{2} = [1{,}459 - 1{,}981]$$

Since we looked for simple and practical algorithm of translation, we consider such precision adequate both for clinical and economic evaluations.

Estimation of the expected mean survival time

First, we defined the expected MOST as 13.65 months. This is a well-established point confirmed either by official SEER data and a reliable retrospective analysis.[28] Then, we defined that mean progression-free survival after 1st-line treatment, based on the data of 9 cohorts of 6 independent trials (Table S2), equals 7.5 months, and it well corresponds with general opinion that GBM relapses

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in 6-9 months after diagnosis. To define the most problematic final parameter MST since relapse, we studied the inner structure of the survival time, namely time proportions between MOST, PFS and MST, on eight cohorts for which this information was available simultaneously (Table S3). Finally, we translated these data on the established MOST and MPFS and calculated the expected MST as 4.775 months (95%CI: 3.95-5.6) (Table S4).

Table S2. Median progression-free survival after standard 1-2 line treatment of GBM (WHO IV).

Study	Tumor state	Treatment	MPFS m
Jungk (2016)[i]	GBM, recurrent/progressive	2M (mainly no CTX)	6,10
Reithmeier (2010)[ii]	GBM, recurrent/progressive	3M (mainly TMZ)	8,72
Hamza (2014)[iii]	GBM, recurrent/progressive	3M	8,10
Hamza (2014)[iii]	GBM, recurrent/progressive	3M	7,60
Strik (2008)[iv]	GBM, recurrent/progressive	3M Stupp	7,53
Chinot (2014)[v]	GBM, newly diagnosed	3M Stupp	6,20
Gilbert (2014)[vi]	GBM, newly diagnosed	3M Stupp	7,30
Gilbert (2013)[vii]	GBM, newly diagnosed	3M Stupp	7,50
Gilbert (2013)[vii]	GBM, newly diagnosed	3M ddTMZ	8,80
Average			7,56

Note: CTX: chemotherapy; TMZ: temozolomide; 3M: trimodal (SRG + XRT + CTX); 2M: bimodal (no CTX); Stupp: 3M SRG + (XRT 60Gy X6w + TMZ 5/7d X 6w) + TMZ 5/28d X 6m; ddTMZ: dose-dense TMZ.

Table S3. Inner structure of survival time.

Study	Cohort	NOP	MOST	MPFS	MST	MST%	PFS+ MST	PFS+ MST%
Varkoniy (2003)	HGG	24	22,0	12,2	6,5	30%	18,7	85%
Sahinbas (2007)	GBM (all)	76	20,0	8,5	7,6	38%	16,1	80%
	GBM (mEHT)	18	14,8	8,0	6,4	43%	14,4	97%
	GBM mEHT+TMZ)	58	20,9	9,3	7,6	36%	16,9	81%
Jungk (2016)	GBM	34	15,7	6,1	8,7	56%	14,8	94%

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Study	Cohort	NOP	MOST	MPFS	MST	MST%	PFS+ MST	PFS+ MST%
Hamza (2014)	GBM (early BEV)	112	20,8	8,1	11,0	53%	19,1	92%
	GBM (late BEV)	133	25,9	7,6	9,9	38%	17,5	68%
Strik (2008)	GBM	18	17,9	8,2	9,1	51%	17,3	97%
	Weighted average		21,5	8,2	9,1	43%	17,3	82%
	95%CI					36,9%€ 48,8%		75,3%€ 88,8%

Note: NOP: number of patients; MOST: median overall survival time; MPFS: median progression free survival; MST: median survival time since relapse; PFS: progression free survival; HGG: high grade gliomas; GBM: glioblastoma; mEHT: modulated electrohyperthermia; TMZ: temozolomide; BEV: bevacizumab; CI: confidence interval.

Table S4. Calculation of estimated mean survival time since relapse.

	Mean	95% CI Lower limit	95% CI Upper limit	SE
MOST, months	13,65			
MPFS, months	7,5			
MPFS+MST (%)	82,0%	75,3%	88,8%	
MPFS+MST, months	11,2	10,3	12,1	
mST (1st estimation), months	3,7	2,8	4,6	
MST (%)	42,9%	36,9%	48,8%	
MST (2nd estimation), months	5,9	5,0	6,7	
mST (average), months	4,775	3,9	5,6	0,443

Note: MOST: median overall survival time; MPFS: median progression free survival; MST: median survival time since relapse.

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STROBE Statement
Checklist of items that should be included in reports of ***cohort studies***
Title of work: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1 line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 7-12
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 13 lines 4-7
Methods			
Study design	4	Present key elements of study design early in the paper	Page 13 lines 17-18
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 13 lines 18-23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 13 lines 25-33
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 13 lines 36-50 Page 13 lines 52 – page 15 line 16 Page 18 lines 20-25
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	☒
Bias	9	Describe any efforts to address potential sources of bias	Pages 34-36
Study size	10	Explain how the study size was arrived at	Page 13 lines 17-23
Quantitative variables	11	Explain how quantitative variables were handled in the analyses.	Page 18 lines 29-55
		If applicable, describe which groupings were chosen and why	Page 21 line 39 – page 23 line 23
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 18 lines 29 – page 19 line 9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 19 lines 11-46
		(*c*) Explain how missing data were addressed	Page 18 lines 23-25
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Page 20 lines 4-7

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			Page 24 line 51 – page 25 line 12 Page 27 line 27 – page 28 line 34
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 20 line 23
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 20 lines 21-53 Pages 62-66
		(b) Indicate number of participants with missing data for each variable of interest	Pages 67-68
		(c) Summarise follow-up time (eg, average and total amount)	Page 21 lines 13-15
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 21 lines 15-23
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 21 lines 1-34
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 21-29
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 37-38
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 34-36
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 29-33
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 34 lines 1-22 Page 36 line 48 – page 37 line 7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not applicable

*Give information separately for exposed and unexposed groups.

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Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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CHEERS checklist—Items to include when reporting economic evaluations of health interventions

Title of study: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

Section/item	Item No	Recommendation	Check
Title and abstract			
Title	1	Identify the study as an economic evaluation or use more specific terms such as "cost-effectiveness analysis", and describe the interventions compared.	Page 1 line 2
Abstract	2	Provide a structured summary of objectives, perspective, setting, methods (including study design and inputs), results (including base case and uncertainty analyses), and conclusions.	Page 5
Introduction			
Background and objectives	3	Provide an explicit statement of the broader context for the study.	Page 11 lines 11-28
		Present the study question and its relevance for health policy or practice decisions.	Page 13 line 13
Methods			
Target population and subgroups	4	Describe characteristics of the base case population and subgroups analysed, including why they were chosen.	Page 20 lines 23-35 Pages 62-68 Page 13 lines 19-23
Setting and location	5	State relevant aspects of the system(s) in which the decision(s) need(s) to be made.	Page 19 lines 52-53 Page 20 lines 8-11
Study perspective	6	Describe the perspective of the study and relate this to the costs being evaluated.	Page 37 lines 10-17
Comparators	7	Describe the interventions or strategies being compared and state why they were chosen.	Page 22 line 44 – page 23 line 23
Time horizon	8	State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.	Page 25 line 42 Page 28 line 40 Page 29 line 25
Discount rate	9	Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.	Page 28 lines 45-46 Page 29 line 15, 18-19, 23
Choice of health outcomes	10	Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.	Page 13 lines 36-50
Measurement of effectiveness	11a	*Single study-based estimates:* Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	Page 13 lines 15-33 Page 34 lines 31-45
	11b	*Synthesis-based estimates*: Describe fully the methods used for identification of included studies and synthesis of clinical effectiveness data.	Page 22 line 45 – page 23 line 23 Pages 69-72
Measurement and valuation of preference based outcomes	12	If applicable, describe the population and methods used to elicit preferences for outcomes.	Pages 20-21
Estimating resources and costs	13a	*Single study-based economic evaluation:* Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any	Page 25 lines 40-48 Page 32 lines 20-41

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Section/item	Item No	Recommendation	Check
		adjustments made to approximate to opportunity costs.	
	13b	*Model-based economic evaluation:* Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	Page 25 line 50 – page 26 line 18 Page 32 lines 4-18 Pages 76-80
Currency, price date, and conversion	14	Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.	Page 25 lines 50-57 Page 26 lines 1-12 Page 32 line 23
Choice of model	15	Describe and give reasons for the specific type of decision-analytical model used. Providing a figure to show model structure is strongly recommended.	Page 25 lines 50-57 Page 32 lines 4-18
Assumptions	16	Describe all structural or other assumptions underpinning the decision-analytical model.	Page 32 lines 4-18
Analytical methods	17	Describe all analytical methods supporting the evaluation. This could include methods for dealing with skewed, missing, or censored data; extrapolation methods; methods for pooling data; approaches to validate or make adjustments (such as half cycle corrections) to a model; and methods for handling population heterogeneity and uncertainty.	Pages 18-20
Results			
Study parameters	18	Report the values, ranges, references, and, if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. Providing a table to show the input values is strongly recommended.	Pages 20-21
Incremental costs and outcomes	19	For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.	Page 26 line 29 – page 26 line 25 Pages 77-80
Characterising uncertainty	20a	*Single study-based economic evaluation:* Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness parameters, together with the impact of methodological assumptions (such as discount rate, study perspective).	NA
	20b	*Model-based economic evaluation:* Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.	Pages 27-28
Characterising heterogeneity	21	If applicable, report differences in costs, outcomes, or cost-effectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects that are not reducible by more information.	Page 25 line 50 - Page 26 lines 12
Discussion			
Study findings, limitations,	22	Summarise key study findings and describe how they support the conclusions reached. Discuss limitations	Pages 37-38 Page 35 lines 18-39

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Section/item	Item No	Recommendation	Check
generalisability, and current knowledge		and the generalisability of the findings and how the findings fit with current knowledge.	Page 36 lines 15-18 Page 36 line 40 – page 37 line 7
Other			
Source of funding	23	Describe how the study was funded and the role of the funder in the identification, design, conduct, and reporting of the analysis. Describe other non-monetary sources of support.	Information provided via the submission system
Conflicts of interest	24	Describe any potential for conflict of interest of study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors recommendations.	Information provided via the submission system

For consistency, the CHEERS statement checklist format is based on the format of the CONSORT statement checklist

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Clinical and economic evaluation of modulated electro-hyperthermia concurrent to dose-dense temozolomide 21/28 days regimen in the treatment of recurrent glioblastoma: a retrospective analysis of a two-center German cohort trial with systematic comparison and effect-to-treatment analysis

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Secondary Subject Heading:	Oncology, Research methods, Neurology, Health economics, Evidence based practice
Keywords:	recurrent glioblastoma, modulated electro-hyperthermia (mEHT), oncothermia, dose-dense temozolamide (ddTMZ), effect-to-treatment analysis (ETA), cost-effectiveness analysis

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Clinical and economic evaluation of modulated electro-hyperthermia concurrent to dose-dense temozolomide 21/28 days regimen in the treatment of recurrent glioblastoma: a retrospective analysis of a two-center German cohort trial with systematic comparison and effect-to-treatment analysis

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The author has completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declares: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Details of contributors, and the name of the guarantor.

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Transparency declaration

The sole author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Details of ethical approval (or a statement that it was not required)

Ethical approval was not required.

Details of funding

No external funding involved.

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Details of the role of the study sponsors

Galenic Research Institute as a study sponsor provided time and facilities for the work.

Statement of independence of researchers from funders

No funders.

Patient involvement statement

Patients were not involved (see also Acknowledgement).

Trial registration details

The trial was not registered.

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ABSTRACT

OBJECTIVE: To assess the efficacy and cost-effectiveness of modulated electro-hyperthermia (mEHT) concurrent to dose-dense temozolomide (ddTMZ) 21/28d regimen versus ddTMZ 21/28d alone in patients with recurrent glioblastoma (GBM).

DESIGN: A cohort of 54 patients with recurrent GBM treated with ddTMZ+mEHT in 2000–2005 was systematically retrospectively compared with five pooled ddTMZ 21/28d cohorts (114 patients) enrolled in 2008–2013.

RESULTS: The ddTMZ+mEHT cohort had a not significantly improved mean survival time (mST) versus the comparator ($p = 0.531$) after a significantly less mean number of cycles (1.56 vs. 3.98, $p < 0.001$). Effect-to-treatment analysis (ETA) suggests that mEHT significantly enhances the efficacy of the ddTMZ 21/28d regimen ($p = 0.011$), with significantly less toxicity (no grade III–IV toxicity versus 45–92%, $p<0.0001$). An estimated maximal attainable median survival time is 10.10 months (9.10 to 11.10). Cost-effectiveness analysis suggests that, unlike ddTMZ 21/28d alone, ddTMZ+mEHT is cost-effective versus the applicable cost-effectiveness thresholds 25,000–50,000 €$/QALY. Budget impact analysis suggests a significant saving of €8,577,947 / $11,201,761 with 29.1–38.5 QALY gained per 1000 patients per year. Cost-benefit analysis suggests that mEHT is profitable and will generate revenues of between €3,124,574 and $6,458,400, with a total economic effect (saving + revenues) of €5,700,034 to $8,237,432 per mEHT device over an 8 year period.
CONCLUSIONS: Our ETA suggests that mEHT significantly improves survival of patients receiving the ddTMZ 21/28d regimen. Economic evaluation suggests that ddTMZ+mEHT is cost-effective, budget-saving, and profitable. After confirmation of the results, mEHT could be recommended for the treatment of recurrent GBM as a cost-effective enhancer of ddTMZ regimens, and, probably, of the regular 5/28d regimen. MEHT is applicable also as a single treatment if chemotherapy is impossible, and as a salvage treatment after the failure of chemotherapy.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- The study first introduces the application of a novel clinical analysis called effect-to-treatment analysis (ETA).
- The study applies a systematic comparator in the form of the pooled average of a meta-analysis of a systematic review of comparable trials.
- The study includes comprehensive economic evaluation, comprising consistent costs analysis, cost-effectiveness analysis, budget-impact analysis, and cost-benefit analysis.
- Because the study is based on a single retrospective trial, future studies are needed to confirm its findings.

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ABBREVIATIONS

NICE: National Institute for Health and Care Excellence

GDP: gross domestic product

DALY: disability-adjusted life year

%CE: proportion of cost-effective cases

AAA: anti-angiogenic agents

BEV: bevazucimab, avastin

BIA: budget impact analysis

BRR: beneficial response rate (CR+PR+SD) (aka DCR)

CA: coefficient of attenuation

CBA: cost-benefit analysis

ccl, ccls: cycle, cycles

CEA: cost-effectiveness analysis

CET: cost-effectiveness threshold

CI: confidence interval

CNTM: cycles needed to treat per life month gained

COI: cohort of interest

CR: complete response

CRR: complete response rate

CRT: chemoradiation treatment

CS: censored

CT: computed tomography

CTCAE: common terminology criteria for adverse events

CTX, CTx: chemotherapy (cytotoxic drugs); common toxicity

CUR: cost-utility ratio

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CURR: ratio of cost-utility ratios

d: day

DCR: disease control rate (aka BRR)

ddTMZ: dose-dense temozolamide

DLT: dose-limiting toxicity

EBIT: economy and earnings before interest and taxes

EORTC: European Organisation for Research and Treatment of Cancer

ETA: effect-to-treatment analysis

ETR: effect-treatment ratio

FU: follow-up

GBM: glioblastoma multiforme

H_0: null hypothesis

HF: high-frequency range (3 – 30 MHz)

HGG (HGBG): high-grade (brain) glioma

HR: hazard ratio, hazard rate

HRQoL: health-related quality of life

HT: hyperthermia

ICER: incremental cost-effectiveness ratio.

ICUR: increment of cost-utility ratio

IOI: intervention of interest

KME: Kaplan-Meier estimate

KPS: Karnofsky performance score

KS-test: Kolmogorov-Smirnov test

LMG: life month gained

LYG: life year gained

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m: month

MAC: maximal attainable course

MAST: maximal attainable median survival time

mEHT: modulated electro-hyperthermia

METR: median effect-treatment ratio

MGMT: O6-methylguanine DNA methyltransferase

min: minute(s)

MN: malignant neoplasm

mNC: mean number of cycles

MNC: median number of cycles

MOST: median overall survival time

mST: mean survival time

MST: median survival time

N/A: not available

NC/SD: no change / stable disease

NNT: number needed to treat

OR: objective response (CR, PR)

OR: odds ratio

ORR: objective response rate

OS: overall survival

OST: overall survival time

p.o., p/o: per os

PD: progression of the disease / progressive disease

PFS: progression-free survival

PLT: palliative treatment

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PR: partial response; partial resection

QALY: quality-adjusted life year

qd, q.d.: every day; daily

QoL: quality of life

RD: risk difference

RF: radiofrequency

RR: relative risk

RR: response rate

RT: radiotherapy

SAT: supportive and alternative therapies

SD: stable disease (aka NC)

SOI: study of interest

t.i.d., tid: three times a day

TMZ: temozolomide

w: week

WA: weighted average

WTP: willingness to pay

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BACKGROUND

Glioblastoma multiforme (GBM) is a common and aggressive primary brain tumour, accounting for 45–54% of all adult gliomas.[1,2] Despite the recent treatment advances, GBM prognosis remains dismal, with the MST limited to 15–18 months.[10] The prognosis for patients with recurrent GBM remains poor, with the MST between 3 and 6 months.[3] As 20 years ago, treatment of recurrent GBM can be considered successful if the stable disease is achieved.[4]

Standards of care are not yet defined for recurrent GBM.[5] Treatment options at recurrence include surgical resection, re-irradiation, and chemotherapy (CTX), [6] though all of these options have significant limitations.[7] The standard CTX treatment for recurrent GBM, based on the milestone EORTC/NCICT trial,[8,9] includes oral DNA-alkylating agent temozolomide (TMZ) given daily at 150–200 mg/m^2 for 5 days in each 28-day cycle (5/28 d) (Stupp regimen).[10] Unfortunately, TMZ adds only about 2.5 months to the MST compared to RT alone at first-line treatment.[8,9] Given that more than 50% of patients fail to respond to TMZ treatment over 6–9 months, and the majority (60–75%) of patients with GBM that do not have a methylated MGMT promoter derive limited benefit from TMZ treatment,[11] and 15–20% of patients treated with TMZ develop clinically significant toxicity,[8] TMZ should be considered a modestly effective chemotherapy. Attempts to improve the Stupp regimen involve, among others, the increased TMZ dosage, known as dose-dense TMZ (ddTMZ) regimens.[12]

The rationale for ddTMZ is based on the known role of specific DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in tumour resistance to alkylating agents such as TMZ. MGMT effectively recovers TMZ-related DNA damage. Methylation of the promoter region of the MGMT gene suppresses MGMT expression. A methylated MGMT-promoter is observed in 30–60% of GBMs.[13] Because MGMT is a suicide enzyme and requires re-synthesis for recovery of its enzymatic activity,[14] it can be depleted by continuous alkylating pressure. Therefore, prolonged exposure and higher cumulative doses of TMZ could sensitize tumours to the alkylating damage, with toxicity as a natural limiter of such dose-escalation. Some ddTMZ regimens were applied versus the standard 5/28d regimen, including the 7/14d (7 days on / 7 days off), 21/28d, and continuous administration (7/7d or 28/28d) regimes. [12,15] Multiple single-armed and retrospective studies of ddTMZ at recurrent GBM showed progression-free survival at 6 month (PFS-6m) ranging from 19% to 44% and an MST of 7–10 months.[12] However, a recent phase III RCT (RTOG 0525)[16] of ddTMZ 21/28d versus the standard 5/28d adjuvant regimen for newly diagnosed GBM patients

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after completion of concurred CRT, failed to show an advantage of ddTMZ in MST (14.9 vs. 16.6 months in the standard arm, p = 0.63), although it did show an improvement of PFS-6m (6.7 vs. 5.5 months) with borderline significance (p = 0.06), with somewhat higher toxicity in the ddTMZ arm. Therefore, the efficacy of ddTMZ regimens remain unproven.[12]

Finally, it should be noted that the modern chemotherapies like TMZ, bevacizumab (BEV) and other anti-angiogenic agents (AAA) are not cost-effective.[17,18,19,20] In fact, there remains a significant unmet need for more effective treatments of high-grade gliomas,[21] and the poor outcomes of the current treatment of recurrent GBM requires novel approaches.5

There is a physical technology called modulated electro-hyperthermia (mEHT, oncothermia™), the effectiveness of which was demonstrated in many phase I/II trials in recurrent brain gliomas,[22,23,24,25,26] and also in cancer of lung, [27,28,29,30] liver,[31,32,33] pancreas,[34,35] cervix,[36,37] breast,[38] esophagus,[39] colorectal cancer,[40,41,42,43] malignant ascites,[44] and soft tissue sarcomas.[45,46] Clinically, mEHT is typically used as an enhancer of radiation[27,36] and chemotherapy, although it possesses its own effectiveness of at least a similar magnitude to these treatments.[47,23,40]

MEHT is a novel method of treatment of solid malignant tumours by the local application of a high-frequency electromagnetic field (13.56 MHz), modulated by 0–5 kHz flicker noise, by virtue of impedance-coupled functionally asymmetric electrodes.[48] MEHT is positioned as a next generation hyperthermic technology based on the selective heating of intercellular compartments of tumour tissue and cell membranes, instead of the heating of a bulk volume of the tissue, as the conventional temperature-dependent hyperthermia (HT) does.[49,50,51,52,53.52]

Unlike the old HT technologies, mEHT transfers the focus from the dielectric heating (field effect) to the Joule (electric) heating in order to improve focusing and penetration depth. Since the current has a known ability to concentrate in areas with a higher conductance,[54] and the increased conductance is one of the basic properties of malignant tissue,[55] hence a tumour is a natural concentrator of electrical current. This feature has long been used for electrical impedance scanning (EIS)[56] and current-density imaging (CDI).[57,58] The penetration depth of current in the impedance-matched system is 20–25 cm[59] versus 14–18 cm only[60] in the regular capacitive HT at 13.56 MHz. Therefore, the emphasis on the current allows transferring energy selectively to the tumour for any depth and with minimal losses. “Electro-hyperthermia” means predominantly electric heating.[61]

A combined set of technical solutions is used to achieve maximal electrical heating: namely, the impedance matching based on the phase angle between voltage and current; functionally asymmetric

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electrodes, providing the necessary stability of the field and size difference-dependent amplification of the current; physiologic skin cooling, minimizing skin losses at energy transfer; and a "skin sensor" concept, which allows for refuse thermometry without detriment to safety.[48] "Free of thermometry" use is a great advantage of mEHT, abolishing the labour-intensive thermometry planning, installation and control, thus drastically reducing time and costs, minimizing side effects, and significantly improving the perception of the treatment by a patient.[62]

The electric heating creates quasi-stable local thermal gradients at the nano level (e.g., transmembrane thermal gradient[63]), which are maintained by the balance of continuous delivery of energy by external field and energy dissipation by natural cooling mechanisms, mainly by a blood flow. [64,65] Thus, the nanoheating, depending on the field power applied and physiological cooling power displayed, can develop even without macroscopic heating.[66] It was shown *ex vivo* that a 42□ temperature in mEHT is only responsible for 25–30% of the total antitumour effect and a slightly smaller effect was shown in the case of normothermia.[67] Thus, the effect of mEHT is thermally-induced but not temperature-dependent.[68]

The clinical value of the not temperature-dependent effects can no longer be questioned after the FDA approval[69] of tumour-treating fields (TTF), an athermal technology using continuous impact of a low-intensity (0.7–1 V/cm) alternating electromagnetic field with a frequency of 100–200 kHz through insulated scalp cross-sectional electrodes.[70,71,72,73,74,75] In a III phase study,[76] TTF displayed the same efficacy at recurrent GBM as the best physician choice CTX (MST 6.6 versus 6.0 months, respectively ($p = 0.27$)) with better quality of life (QoL).

Nevertheless, mEHT usually causes hyperthermia-range heating[77,78,79,80] in accordance with a classical maxima of Schwan on the impossibility to reach significant "non-thermal" effects without substantial heating.[81] The effect of mEHT is power-dependent but not signal-dependent. It is not connected with multiple tiny and questionable processes such as demodulation and molecular energy uptake[82] (although we cannot completely exclude these possibilities). The power range of mEHT (0.2–2 W/cm^2) is far above the "thermal noise limit" of 0.01 W/cm^2.[83]

Fractal modulation is a specific feature of mEHT. The carrying frequency is amplitude-modulated by "pink noise" (1/f),[84] which is typically emitted by all self-organized living systems and reflects their fractal organization.[85] Since a malignancy always losses organization, it more or less emits "red" or Brownian noise ($1/f^2$)[86] (correctly speaking, its noise spectrum is more "reddish"). Fractal modulation allows for increasing specific absorption of modulated field energy in the "red noise" sites, selectively amplifying the effect of mEHT.[87] Also, the noise can amplify cancer-specific

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frequencies[88] by "stochastic resonance".[89] It is reported *in vitro* that modulation can amplify the effect of mEHT by 20–50%.[87]

An important feature of mEHT is its selectivity, both macroscopic and cellular. Macroscopic selectivity of tumour heating is based on the automatic impedance-based autofocusing of electric current in the tumour.[54] The cellular selectivity of mEHT, based on the membrane selectivity and modulation, was demonstrated *in vitro* using a mixed culture of cancerous and normal cells. mEHT selectively destroyed malignant cells without damage to the normal cells, and the extent of the damage was proportional to the degree of malignancy. [90]

The exact mechanism of mEHT action is unknown. Both temperature-dependent and independent mechanisms are among possible options. Temperature-dependent mechanisms include disorder of tumour blood flow, oxygen and glucose deprivation, depletion of intracellular ATP, the influx of sodium and depolarization of cellular membrane,[91,92,93] and acidification.[94,95,96] Since these effects are present in all HT applications, and they do not lead to results characteristic for mEHT, we propose that there must be other mEHT-specific mechanisms of action. Many not temperature dependent (so-called "non-thermal") effects are reported to have a peak at about 10 MHz, namely direct bactericidal effect and enhancement of antibiotics action (bioelectric effect), both in bacterial films[97] and planktonic phase;[98] dielectrophoresis,[99] damage of mitochondrial function[100] and destruction of lysosomes.[101]

Although the frequency and field strength (2–5 V/cm) applied in mEHT cannot cause a significant change in the membrane potential,[102] there are many reasons to suggest a specific membrane-acting effect of mEHT. The 10 MHz is a relaxation frequency of the beta-dispersion range (0.1–100 MHz) caused by Maxwell-Wagner relaxation of cell membranes,[103] which means a peak of membrane dielectric loss and selective membrane excitation (heating) at this frequency[104] (re-orientation of protein-bound water molecules, the motion of polar protein subgroups, the Maxwell-Wagner relaxation of the cell interior or the additional Maxwell-Wagner relaxations due to the non-spherical cell shape, also contribute to the β-dispersion[103]), and also a peak of phase shift of membrane polarization under the effect of the external alternative field, which nearly reaches a quadrature (-80°).[102] The relaxation frequency of the re-orientational proton motion of water-bound proteins also peaks at about 10 MHz (range, 1–100 MHz).[105]

Another possible effect of mEHT is an arrest of cell division with possible mitotic catastrophe,[98] attributable to a subcellular ponderomotoric effect (dielectrophoretic forces suppress the assembly of

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the mitotic spindle[71]), to membrane polarization (cell division phases are associated with changes in membrane potential, and nonlinear processes of hyperpolarization and depolarization, under the effect of RF-field, suppress proliferation[72]), or to resonance phenomena.[106] Also, effects on the cytoskeleton[107,108] and selective activation of some enzymes, both conformational and voltage-dependent (in the case of membrane enzymes),[109] are reported.

The overall effect of mEHT is connected with an extracellular expression of intracellular signalling molecules of cellular stress (e.g., HSP and p53 protein),[110] which unmask cancer cells and initiate the immune response and apoptosis.[111] It has been shown *in vivo* and *in vitro* that the antitumour effect of mEHT is mainly connected with significant activation of apoptosis, which develops over 72 h after a single impact.[111,112,113] Some immune-dependent effects are reported, namely the abscopal effect[114, 115] which is considered as a basis for a 'radiofrequency vaccination'.[116,117] Expression of many immune-specific pathways has been reported *in vitro* in mEHT.[111,118,119,120] Overexpression of cell-junction proteins with the significant restoration of intercellular junctions, which can contribute to the induction of apoptosis,[121,122] and reorganization of cytoskeleton[107] are reported for mEHT.

Taking into account the extensive and long-term (since 1996) successful application without any negative report, a systematic review of results of mEHT is possible and necessary. Collecting the data for the systematic review and meta-analysis on the mEHT treatment of brain gliomas, we asked for raw data whenever possible. The raw data of the Sahinbas et al. (2007)[23] trial including 155 patients with high-grade gliomas (HGG) were obtained on request. After analysis of the data, some shortcomings were revealed, namely duplications, incorrect grouping by histology, and incorrect calculation of survival function in view of incorrect processing of censoring. After corrections and recalculation, the results of this trial appeared so interesting that we believe they deserved to be re-published. In this retrospective analysis, we report the result of the systematic clinical comparison and economic evaluation of mEHT concurrent to the ddTMZ 21/28d regimen in the treatment of recurrent GBM. No change to the raw data was made.

MATERIAL AND METHODS

Objectives

The objective of this study is to assess the efficacy and cost-effectiveness of mEHT concurrent to ddTMZ 21/28d regimen versus ddTMZ 21/28d alone in patients with recurrent GBM.

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Questions of the study

- Does mEHT significantly enhance the ddTMZ 21/28d regimen?
- Is the addition of mEHT to ddTMZ 21/28d regimen cost-effective?

Trial design

This retrospective clinical and economic evaluation is based on a systematic comparison and effect-to-treatment analysis of a retrospective, single-arm study[23] (study of interest, SOI) performed in two German centres (the Gronemeyer Institute of Microtherapy at the University of Bochum and the clinic "Closter Paradise", Soest) between 2000 and 2005.

Inclusion and exclusion criteria

Patients with relapsed or progressed after incomplete resection or progressive inoperable histologically confirmed GBM or gliosarcoma (WHO IV), having undergone a complete conventional 1st–2nd-line pre-treatment were selected. From those, patients treated with ddTMZ 21/28d in combination with mEHT (with or without supportive therapy but without re-irradiation, re-surgery or other chemotherapy) were selected. No exclusion criteria were applied.

Outcomes

Survival was the main outcome of the study:

- Median survival time (MST) is the time from the initial event to the moment when the value of cumulative survival function (Kaplan-Meier estimate [KME]) reaches 50%. Here, the term MST is applied to survival since relapse/progression or the date of the first mEHT session, while survival since the date of diagnosis is defined as Median Overall Survival Time (MOST).
- Overall survival (OS) is the value of cumulative survival function (KME) at the set time moments from the date of the initial event.
- Overall survival time (OST) is the time from the initial event to the death of any reason.

No surrogate outcomes were used.

Intervention

The studied intervention was a combination of dose-dense temosolomide 21 days on, 7 days off regimen (100 mg/m^2/d) with concurrent mEHT as an enhancer (ddTMZ+mEHT). MEHT (the intervention of interest, IOI) was applied using an EHY2000 device (Oncotherm Kft, Hungary) with

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2-day intervals between sessions (on each 3rd day) concurrent with TMZ and afterwards, for up to three months. A dose-escalating scheme was used with a gradual increase of power from 40 to 150W and increase of time from 20 to 60 min, during two weeks, adding modulation from the second week (Figure 1). Then, a step-up heating was applied, increasing the power from 60W to 150W during 60-min sessions, to ensure tumour temperature of >40°C during 90% of the treatment time. Dose escalation was limited by patient's individual tolerance. The mEHT course was considered low-dose (LD-mEHT) if did not exceed eight complete 60-min sessions. Supportive and alternative treatments (SAT) included *Boswellia caterii* extract 6 g/day p.o. t.i.d., mistletoe extract 15 ng/day SC 3Xw, and Selenium 300 µg/day p.o., for three months.

Response and survival assessment

The objective response was assessed according to the MRI McDonald criteria.[123] Survival function was assessed by the Kaplan-Meier estimate. Survivors were right-censored on the date of completion of the study (May 30, 2005), lost patients were censored on the date of the last contact, and excluded patients were left-censored on the date of diagnosis/enrolment.

Statistical methods

Statistical analysis was performed using the built-in Excel 2016 analysis package using the methods of descriptive statistics, correlation, and regression analysis. Normality of distribution was estimated by the Kolmogorov-Smirnov test (KS-test). Confidence intervals (CI) of medians were calculated according to Conover,[124] relative risks (RR) and odds ratios (OR) according to Altman,[125] risk difference (RD) according to Newcomb and Altman,[126] product of means according to Goodman,[127] ratio of means according to Fieller[128,129] for independent means, and by Taylor approximation[130] for dependent means, and the ratio of two independent lognormally distributed estimates by Newcomb's MOVER-R algorithm.[131] Inverse-variance weighting was used.[132] The significance of differences in parametric criteria was estimated by the two-sample Student t-test or Welch t-test for unequal variance;[133] and for paired nonparametric criteria (proportions) by the Pearson's chi-square test (χ^2) according to Campbell-Richardson.[134] The significance of rates and proportions with known 95% CI was estimated according to Altman,[135] and the significance of the difference of two independent estimates by the two-sample z-test. All p-values are two-sided. A 95% probability ($\alpha = 0.05$) was used for significance testing. Since log-transformation significantly inflates confidence intervals (up to 40-times in some cases[136]), 90% probability ($\alpha=0.1$) is considered applicable for the significance of the difference of estimates based on log-transformed parameters in some cases.

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Survival analysis was performed using the Excel-based software package GRISA (Galenic Research Institute, 2015) by Kaplan-Meier estimate (KME) of the cumulative probability of survival.[137] Standard errors and confidence intervals of KME were estimated by Greenwood's formula,[138] and the significance of differences by the log-rank test.[139] The hazard function was estimated by the Cox proportional hazards regression model.[140]

Meta-analysis was performed using the Excel-based software package GRIMA (Galenic Research Institute, 2015) according to Borenstein et al.[132] and statistical algorithms of the Cochrane Collaboration.[141] The heterogeneity of studies was assessed by the I^2 criterion.[142] In view of the significant heterogeneity of the cohorts, a random effect model was applied.

Effect-to-treatment analysis

Effect-to-treatment analysis (ETA) was performed according to our own algorithm[143] with the following settings: a unit of treatment is a 28-days cycle, and the parameter of comparison is the mean survival time (mST) after relapse. Here, we use mST for mean survival time and MST for median survival time. Medians were transformed into means with 95% confidence intervals (95% CI) using the Hozo et al. (2005)[144] algorithm for medians with range and our own simplified algorithm (Supplement 1) for medians with 95% CI. The life months gained (LMG) parameter was calculated by subtracting the expected mST (emST). Effect-treatment ratio (ETR) was calculated by dividing the LMG by the mean number of cycles (mNC). Life quality adjustment was not possible due to significant initial differences between the cohorts. The median ETR (METR) was estimated by attenuation of the ETR according to the formula $METR = ETR \times (1 - CA)^{(MNC-mNC)}$, where CA is a coefficient of attenuation. The dependence of mST from mNC was estimated by the function $mST = ETR \times (1 - CA)^{NC-mNC} \times NC + emST$ (where NC is a serial number of cycle); the extremum of the function is a maximal attainable survival time (MAST), the abscissa of the extremum is a peak number of cycle (PNC). Cost-effective number of cycles (CENC) was estimated as abscissa of cost-effective survival time value (CEST = 95%MAST). Cycles needed to treat per LMG (CNTM) was estimated as the reciprocal of the difference of ETRs: $CNTM = 1/\Delta ETR$. The effect enhancement ratio ($EER_{12} = ETR_1/ETR_2$) was estimated as an auxiliary parameter for calculation of CI and significance of CNTM: since EER and CNTM use the same parameters with the same null hypothesis [H_0: $ETR_1 = ETR_2$], their confidence intervals and significance are the same, and these parameters can be easily calculated for EER according to Altman.[135]

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Economic evaluation

For economic evaluation, cost-effectiveness analysis (CEA) with sensitivity analysis, budget impact (BIA) and cost-benefit (CBA) analyses were performed.[145,146,147,148,149] CEA and BIA were performed from the perspective of a health provider. CEA was based on the cost-utility ratio (CUR) and incremental cost-effectiveness ratio (ICER). The ratio of CURs (CURR) and increment of CURs (ICUR) were used to compare CURs. The proportion of cost-effective cases (%CE) was estimated by one-tailed directional integral z-test with the null hypothesis [H_0: CUR = CET], where CET is a cost-effectiveness threshold. To estimate a sensitivity of CEA, a multiparametric equal cost-effectiveness test was performed exploring the value of a key parameter in which the value of CURR equals 1.0 (or ICUR = 0). The BIA estimated the difference of costs for treatment of 1,000 patients per year. CBA estimated the total economic effect (saving and earnings before interest and taxes [EBIT]) from the perspective of a healthcare facility.

Reporting

SOI is reported according to the STROBE statement for reporting observational studies.[150] Economic evaluation is reported according to the CHEERS standards.[151]

RESULTS

Patients' flow

A total of 153 patients with different brain tumours (Table 1)

Table 1. Histologic types of brain tumors (SOI).

Total patients: 153

- [C71] Malignant neoplasm (MN) of brain: 137
  - WHO II: 8
    - Astrocytoma: 4
    - Mixed glioma: 4
  - WHO III: 39
    - Astrocytoma: 34
    - Mixed glioma: 3
    - Ependimoma: 1
  - Age <20: 6
    - **Gliosarcoma: 1**
    - Medulloblastoma: 3
    - Primitive neuroectodermal tumor: 1
- [D43.1] Neoplasm of uncertain behavior of brain, infratentorial: 1
- [C79.3] Secondary MN of brain and cerebral meninges: 15
  - Adenocarcinoma: 12
    - MN of breast: 7

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- Oligodendroglioma: 1
- WHO III-IV: 4
  - Astrocytoma: 3
  - Infratentorial Glioma: 1
- WHO IV: 87
  - **Glioblastoma: 81**
    - **Age >20: 75**
  - MN of bronchus and lung: 3
  - MN of colon: 1
  - MN of pancreas: 1
- Ewing sarcoma: 1
- Malignant rhabdoid tumor: 1
- Cancer of unknown primary (CUP): 1

were enrolled in the two centres between 2000 and 2005 (Figure 2). Of those, 138 patients had primary brain tumours, and 87 were graded as WHO IV, including 81 GBM and one gliosarcoma (n = 82). Of those, 76 patients were adults (> 20 years). Fifty-eight adult GBM patients received a combination treatment (mEHT ± ddTMZ ± RT ± SAT), other 18 GBM patients were treated with mEHT only (with or without SAT). Twenty-three patients of the combination cohort were younger than 50 years and received HD mEHT. The cohort of interest (COI) included 54 patients who received mEHT + ddTMZ (with or without SAT). Four other patients of the combination cohort received RT in addition to mEHT, either alone (n = 1) or with ddTMZ (n = 3) (with or without SAT). Of the adult GMB patients (n = 76), 24 received LD mEHT and 52 received high-dose mEHT (HD mEHT); 59 received SAT vs. 17 that did not.

Patients' characteristic

Fifty-four adult patients with WHO IV GBM (n = 53) and gliosarcoma (n = 1) matched the inclusion criteria (COI). The mean age was 48.7 ± 1.5 years (median, 49.8 years; range, 25.9–68.2; 95%CI, 42.2–52.8), including two (4%) elderly patients (≥68 years) and 26 patients (48%) over 50 years. Thirty-three of the patients were male and 21 female (Table 2).

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Table 2. Patients' characteristic.

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
No of patients (NOP)	76		18		58		54		24		52		23	
Male	46	61%	10	56%	36	62%	33	61%	16	67%	30	58%	11	48%
Female	30	39%	8	44%	22	38%	21	39%	8	33%	22	42%	12	52%
Earliest born	24.02.1932		24.02.1932		19.09.1935		19.09.1935		24.02.1932		18.06.1932		31.10.1954	
Latest born	03.04.1975		10.03.1971		03.04.1975		03.04.1975		03.04.1975		21.08.1973		21.08.1973	
Earliest diagnosed	01.08.1993		01.09.2000		01.08.1993		01.08.1993		12.07.1999		01.08.1993		01.08.1993	
Latest diagnosed	15.03.2005		03.07.2004		15.03.2005		30.08.2004		08.07.2004		15.03.2005		15.03.2005	
Age (years):														
Mean	50,2 ± 1,3		55,1 ± 2,8		48,7 ± 1,4		48,7 ± 1,5		50,9 ± 2,6		49,9 ± 1,5		39,9 ± 1,2	
Median	50,4		59,1		49,8		49,8		50,8		50,2		41,0	
Range	25,9 – 71,9		30,9 – 71,9		25,9 – 68,2		25,9 – 68,2		25,9 – 68,9		27,0 – 71,9		27,0 – 49,1	
95%CI	44,8 – 53,9		44,4 – 64,9		42,7 – 52,3		42,2 – 52,8		42,2 – 59,8		44,4 – 55,8		36,7 – 43,0	
P-value (t-test)					0,037								<0,0001*	
Elderly (over 68 years)	4	5%	2	11%	2	3%	2	4%	2	8%	2	4%	0	0%
Mature (over 50 years)	40	53%	12	67%	28	48%	26	48%	13	54%	27	52%	0	0%
Adults (over 20 years)	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
Pre-treatment:														

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
Surgery + Chemoradiation	57	75%	13	72%	44	76%	42	78%	15	63%	42	81%	20	87%
Chemoradiation	2	3%	1	6%	1	2%	1	2%	1	4%	1	2%	0	0%
Surgery + Radiation	7	9%	2	11%	5	9%	4	7%	4	17%	3	6%	2	9%
Surgery + Chemotherapy	5	7%	0	0%	5	9%	4	7%	1	4%	4	8%	1	4%
Radiaton only	5	7%	2	11%	3	5%	3	6%	3	13%	2	4%	0	0%
Chemotherapy total	64	84%	14	78%	50	86%	47	87%	17	71%	47	90%	21	91%
Radiation total	71	93%	18	100%	53	91%	50	93%	23	96%	48	92%	22	96%
Surgery total	69	91%	15	83%	54	93%	50	93%	20	83%	49	94%	23	100%

Note: * versus all GBM sample.

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Forty-two (78%) patients underwent complete trimodal pre-treatment including surgery and chemoradiation, four (7%) received previous surgery and radiation, four (7%) received surgery and chemotherapy, three (6%) received only radiation and one (2%) received only chemoradiation. By modalities, 50 (93%) patients underwent previous surgery, 50 (93%) radiation, and 47 (87%) chemotherapy (mainly TMZ). The characteristics of the other cohorts are given in Table 2.

Details of treatment

All patients (100%) in the COI received ddTMZ + mEHT treatment, and 43 (80%) patients received concurrent SAT (Table 3).

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Table 3. Details of treatment.

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Time to 1st mEHT since diagnosis (months):														
Mean	12,1 ± 1,6		11,2 ± 2,3		12,3 ± 1,9		12,9 ± 2,1		13,3 ± 2,4		11,5 ± 2,0		12,7 ± 4,2	
Median	8,5		8,0		9,3		9,5		9,9		8,2		5,9	
Range	0,2 – 94,2		2,3 – 44,1		0,2 – 94,2		0,2 – 94,2		1,6 – 49,1		0,2 – 94,2		1,0 – 94,2	
95%CI	6,7 – 10,6		6,1 – 15,2		5,8 – 10,7		5,9 – 10,7		6,1 – 11,6		5,1 – 10,0		4,1 – 10,0	
Earliest mEHT	01.03.2001		07.05.2001		01.03.2001		01.03.2001		07.06.2001		01.03.2001		01.03.2001	
Latest mEHT	20.05.2005		19.05.2005		20.05.2005		20.05.2005		28.04.2005		20.05.2005		20.05.2005	
Treatment combinations:														
mEHT + CRT + SAT	2	3%	0	0%	2	3%	0	0%	0	0%	2	4%	0	0%
mEHT + Chemoradiation	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy + SAT	43	57%	0	0%	43	74%	43	80%	12	50%	31	60%	13	57%
mEHT + Radiation + SAT	1	1%	0	0%	1	2%	0	0%	0	0%	1	2%	1	4%
mEHT + Chemotherapy	11	14%	0	0%	11	19%	11	20%	6	25%	5	10%	3	13%
mEHT + SAT	13	17%	13	72%	0	0%	0	0%	4	17%	9	17%	5	22%
mEHT only	5	7%	5	28%	0	0%	0	0%	2	8%	3	6%	0	0%
Treatment by modality:														
Radiation total	4	5%	0	0%	4	7%	0	0%	0	0%	4	8%	2	9%

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
SAT total	59	78%	13	72%	46	79%	43	80%	16	67%	43	83%	19	83%
Chemotherapy total														
NOP	57	75%	0	0%	57	98%	54	100%	18	75%	39	75%	17	74%
No of cycles	89		0		89		84		18		71		32	
Mean	1,5 ± 0,1		0		1,6 ± 0,1		1,6 ± 0,1		1,0 ± 0,0		1,8 ± 0,1		1,8 ± 0,2	
Median	1,0		1,0		1,0		1,0		1,0		1,5		2,0	
Range	1,0 – 5,0		1,0 – 3,0		1,0 – 5,0		1,0 – 5,0		1,0 – 1,0		1,0 – 5,0		1,0 – 5,0	
95%CI	1,0 – 1,0		1,0 – 2,0		1,0 – 1,0		1,0 – 1,0		1,0 – 1,0		1,0 – 2,0		1,0 – 2,0	
mEHT total:														
NOP	76	100%	18	100%	58	100%	54	100%	24	100%	52	100%	23	100%
No of sessions	1367		292		1075		995		169		1198		545	
Mean	18,0 ± 0,3		16,2 ± 0,6		18,5 ± 0,4		18,4 ± 0,4		7,0 ± 0,1		23,0 ± 0,4		23,7 ± 0,6	
Median	14,0		13,5		14,0		14,0		7,0		18,0		23,0	
Range	3,0 – 65,0		4,0 – 43,0		3,0 – 65,0		3,0 – 65,0		3,0 – 9,0		10,0 – 65,0		10,0 – 65,0	
95%CI	11,0 – 16,0		7,0 – 23,0		11,0 – 17,0		10,0 – 17,0		6,0 – 9,0		15,0 – 26,0		15,0 – 27,0	
Low-dose mEHT	24	32%	6	33%	18	31%	18	33%	24	100%	0	0%	0	0%
Time of treatment (months):														
Mean	2,5 ± 0,4		1,6 ± 0,4		2,8 ± 0,5		2,7 ± 0,6		0,5 ± 0,0		3,4 ± 0,6		3,4 ± 0,7	
Median	1,1		1,0		1,1		1,1		0,5		1,9		1,9	

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Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Range	0,0 – 26,4		0,2 – 6,4		0,0 – 26,4		0,0 – 26,4		0,0 – 0,8		0,2 – 26,4		0,5 – 12,2	
95%CI	0,8 – 1,5		0,5 – 2,1		0,8 – 1,6		0,8 – 1,6		0,4 – 0,6		1,2 – 2,8		1,2 – 4,6	
P-value (t-test)	0,233								0,001					
Terminated (NOP)	9	12%	1	6%	8	14%	8	15%	9	38%	0	0%	0	0%
P-value (chi-square)	0,35								<0,0001				0,085*	

Note: * versus all GBM sample.

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In total, 84 ddTMZ cycles were performed for 54 patients, an average of 1.6 ± 0.1 cycles per patient (median, 1.0 cycles; range, 1.0–5.0; 95%CI, 1.0–1.0). The average duration of the treatment was 2.7 ± 0.6 months (median, 1.1 months; range, 1 day to 26.4 months; 95%CI: 0.8–1.5 months). In eight (15%) cases the treatment was terminated because of progressive disease. The average time elapsed since primary diagnosis to the first mEHT session was 12.9 ± 2.1 months (median, 9.5 months; range, 0.2–94.2; 95%CI, 5.9–10.7). A total of 995 mEHT sessions were performed, with a mean of 18.4 ± 0.4 per patient (median, 14; range, 3–65; 95%CI, 10–17). There were 18 (33%) patients with LD-mEHT.

Response

Fifteen patients (28%) in the COI were assessed for a response (Figure 2). One patient (7%) showed a complete response (CR) and two (13%) showed a partial response (PR) so that the objective response rate (ORR) was 20% (Table 4).

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Table 4. Survival and response rates (COI).

Parameter	All GBM (1)		mEHT ± SAT (2)		Combination treatment (3)		ddTMZ +mEHT (4)		LD-mEHT (5)		HD-mEHT (6)		HD-mEHT <50 years (7)	
	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%	Value	%
Response:														
NOP estimated	22	29%	7	39%	15	26%	15	28%	9	38%	13	25%	7	30%
CR	1	5%	0	0%	1	7%	1	7%	1	11%	0	0%	0	0%
PR	2	9%	0	0%	2	13%	2	13%	0	0%	2	15%	2	29%
OR	3	14%	0	0%	3	20%	3	20%	1	11%	2	15%	2	29%
SD	9	41%	4	57%	5	33%	5	33%	2	22%	7	54%	4	57%
BR	12	55%	4	57%	8	53%	8	53%	3	33%	9	69%	6	86%
PD	10	45%	3	43%	7	47%	7	47%	6	67%	4	31%	1	14%
P-value (χ^2)			0,77						0,003				0,007*	
Exitus	49	64%	12	67%	37	64%	36	67%	18	75%	31	60%	11	48%
Censored	27	36%	6	33%	21	36%	18	33%	6	25%	21	40%	12	52%
Lost	2	3%	0	0%	2	3%	2	4%	1	4%	1	2%	1	4%
Right-censored	25	33%	6	33%	19	33%	16	30%	5	21%	20	38%	11	48%
Overall survival (since diagnosis):**														
MST (months)	20,0		14,8		20,7		20,8		18,5		20,4		23,9	
(95%CI):**	(14,7–23,6)		(12,2–28,3)		(15,0–25,0)		(15,2–25,1)		(11,8–23,0)		(14,6–25,7)		(13,0–NR)	
Range	1,4 – 141,5		4,4 – 48,9		1,4 – 141,5		1,4 – 141,5		3,2 – 53,8		1,4 – 141,5		2,4 – 141,5	

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Parameter	All GBM (1)	mEHT ± SAT (2)	Combination treatment (3)	ddTMZ +mEHT (4)	LD-mEHT (5)	HD-mEHT (6)	HD-mEHT <50 years (7)
5-y survival (%)	13,5	0,0	13,3	13,5	0,0	16,1	31,0
(95%CI)	(2,8–24,2)	(0,0–0,0)	(1,0–25,6)	(1,0–26,0)	(0,0–0,0)	(2,0–30,1)	(5,1–56,8)
P-value (log-rank)		0,436			0,350		0,32*
Survival since 1st mEHT (months):**							
MST (months)	7,6	6,4	7,7	7,7	4,4	8,3	12,8
(95%CI):**	(5,8 – 9,3)	(3,1 – 9,9)	(5,8 – 9,5)	(5,7 – 9,4)	(2,2 – 8,8)	(6,7 – 12,3)	(8,2 – 48,1)
Range	0,3 – 47,3	0,3 – 13,6	0,7 – 47,3	0,7 – 47,3	0,3 – 14,9	1,0 – 47,3	1,0 – 47,3
1-y survival (%)	28,8	22,6	30,2	29,5	8,7	36,6	56,9
(95%CI)	(16,5–41,0)	(0,0–47,9)	(16,1–44,2)	(15,5–43,6)	(0,0–24,5)	(21,3–51,9)	(33,3–80,5)
2-y survival (%)	16,8	0,0	19,2	18,8	0,0	23,3	32,5
(95%CI)	(6,0–27,5)	(0,0–0,0)	(6,8–31,6)	(6,5–31,1)	(0,0–0,0)	(9,0–37,5)	(7,7–57,4)
P-value (log-rank)		0,403			0,007		0,047*
Survival time after the last mEHT (follow-up) (months):							
Mean	5,0 ± 0,8	3,8 ± 0,8	5,3 ± 1,0	5,6 ± 1,1	3,9 ± 0,7	5,5 ± 1,1	7,4 ± 2,4
Median	3,3	2,9	3,4	3,5	2,4	3,4	3,3
Range	0,0 – 46,4	0,0 – 12,1	0,1 – 46,4	0,1 – 46,4	0,0 – 14,3	0,1 – 46,4	0,2 – 46,4
95%CI	2,2 – 4,6	0,8 – 5,5	2,2 – 5,0	2,2 – 5,3	1,5 – 5,3	2,5 – 5,0	1,3 – 7,3

Note: * versus all GBM sample; ** Kaplan-Meier estimation; NR – not reached.

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Five patients (33%) showed stable disease (SD) and seven (47%) were in progressive disease (PD) status, giving a beneficial response rate (BRR) of 53% (see Bias assessment and limitations of the study).

Survival

All of the patients of the COI were included in the survival analysis (Figure 2). Average follow-up since the 1st mEHT session was 8.4 ± 1.2 months (median, 6.0 months; range, 0.7–47.3 months; 95%CI, 4.6–7.5 months). Average follow-up since the last mEHT session (Table 4) was 5.6 ± 1.1 months (median, 3.5 months; range, 1 day to 46.4 months; 95%CI, 2.2–5.3 months). For that period, 36 (67%) patients died, two (4%) were lost (censored), and 16 (30%) were alive at the end of the follow-up period (right-censored). The MST since the first diagnosis was 20.8 months (95%CI, 15.2–25.1) and the five-year OS was 13.5% (95%CI, 1.0–26.0%). The MST since the first mEHT session was 7.7 months (95%CI, 5.7–9.4). Survival at 12 and 24 months was 29.5% (95%CI, 15.5–43.6%) and 18.8% (95%CI: 6.5–33.1%) respectively (Figure 3) (see Bias assessment and limitations of the study).

Safety

Unfortunately, the raw data presented does not contain safety data, so we rely on the safety data of the 140 patients reported in the primary paper.[23] No grade III–IV toxicity was reported. Short-term (<2 h) asthenia after treatment was encountered in 10% of the cases, rubor of the skin in 8%, edema of fresh scars in <1%, subcutaneous fibrosis in 1%, burning blisters grade I–II in 2%, and headache, fatigue and nausea (1–2 days) in 12% (see the Bias assessment and limitations of the study).

ANALYSIS OF THE RESULTS

Covariates survival analysis

There was no a difference in survival between patients treated with mEHT only (with or without SAT) and with the combination treatment (Table 4, Figure 4), neither by survival (MST since 1st mEHT 6.4 months [95% CI, 3.1 to 9.9] vs. 7.7 months [5.8 to 9.5], p = 0.403) or by response (BRR 57% vs. 53%, p = 0.77), although the mEHT only regimen was applied to significantly older patients (median 59.1 years vs. 49.8 years in the combination treatment sample, p = 0.037) with KPS <60% unfit for chemotherapy and radiation.

However, we did detect a significant difference between samples with LD-mEHT and high-dose mEHT (HD-mEHT), both in survival since 1st mEHT (p = 0.007; HR = 2.19; 95%CI, 1.21–3.95)

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and response (p = 0.003) (Table 4, Figure 5). A similar pattern was shown in the analysis of the sample treated with SAT versus the sample without SAT (Figure 6): the MST since 1st mEHT was 8.7 months (95%CI, 7.2–11.4) with SAT vs. 2.9 months (95%CI, 2.3–5.5) only without SAT (p = 0.004, HR = 0.40 [95%CI, 0.36 to 0.45]) (see DISCUSSION).

The sample of younger patients (under 50 years) with HD-mEHT treatment showed the best results (Figure 7): an MST since diagnosis of 23.9 months (95%CI, 13.0 to Not Attained); a 5-year OS of 31.0% (95%CI, 5.1 to 56.8); an MST since 1st mEHT session of 12.8 months (95%CI, 8.2 to 48.1); and a BRR of 85.7%. Although the overall survival did not differ significantly from the complete sample (p = 0.32), the survival since 1st mEHT and BRR were significantly better (p = 0.047 and p = 0.007, respectively).

Systematic comparator

Based on a systematic review[152] and a narrative review[12] of different ddTMZ regimens, five phase II, cohort, uncontrolled clinical trials addressing the ddTMZ 21/28d regime were identified (Table 5).

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Table 5. Comparison of dose-dense temozolamide trials: patients' characteristic.

Study (Year) (Enrollment)	NOP	Country	Study design	Inclusion	Med Age	KPS	Pre-treatment SRG	Pre-treatment RT	Pre-treatment TMZ	MTAD	Other	Current treatment Regimen	Current treatment NOC
Brandes (2006)	33	Italy		Recurrent/ progressive GBM in chemonaïve pts with KPS≥60 in SCC; 45% of met-MGMT	57	90% (60-100)	100 %	100 %	0%	N/A	R1:100%: met 45.5%; re-op. 3%.	75 mg/m^2/d qd X21/28d	153 ccls: mean 4.6, med 3 (1-15)▪
Strik (2008) (2005-2007)	18	Germany	Phase II prospective cohort uncontrolled	Recurrent/ progressive GBM, KPS≥50 in SCC: 1st relapse 78%, 2nd – 22%	54.8	60% (50-100)	100 %	100 %	100% (≥1 adj TMZ ccls)	7.5 m[a]	R1/2: 77.8/22.2%; met.46.2%; re-op. 33.3%	100 mg/m^2 /d qd X21/28d	154 ccls, mean 7.3, med 5 (2-18)▪
Abacioglu (2011) (2006-2008)	16	Turkey		Recurrent/progressive GBM, KPS≥70 in SCC	50	80% (50-100)	100 %	100 %	100% (med 6 ccls)	13 (6-105)▪			med 2 (1-8)▪
Berrocal (2010)	47	Spain		Recurrent/progressive HGG with KPS≥60 in SCC;	50	(70-80%) ECO	81%	100 %	100% (med 6 ccls)	14 m (6-126)▪		85 mg/m^2/d qd X21/28d	med 2 (1-13)▪

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				WHO IV GBM 57%, WHO III 43%		G 1							
Norden (2013)	55	USA		Recurrent/progressive GBM with KPS≥60 in SCC, standard (Stupp) pre-treatment with ≥2 adjuvant cycles)	57	90% (60-100)	100 %	100 %	100% (≥2 adj TMZ ccls) (med 6 ccls (12-16))	N/A	R1: 100%; R/P: 48%/ 52%, met. 65%	100 mg/m^2 /d qd X21/28d X12 ccls or until PD	N/A
Sahinbas (2007) (2000-2005)	54	Germany	Retro-spective cohort uncontrolled	Recurrent/progressive GBM, KPS≥40	49.8	60% (40-100)[b]	93%	93%	87%	9.5 m (5,9-10,7)*		100 mg/m^2 /d qd X21/28d + mEHT	84 ccls, mean 1.6±0.1, med 1 (1-5)▪

Note: SCC: stable clinical condition; HGG: high-grade glioma; GBM: glioblastoma multiforme; KPS: Karnofsky performance score; MGMT: O6-Methylguanine DNA Methyltransferase; qd: daily; MTAD: median time after diagnosis; TMZ: temozolomide; R1: first relapse/progression; R1/2: first / second relapse; R/P: relapse / progression; met.: methylated MGMT promoter gene; re-op.: re-operation; * 95% confidence interval; ▪ range; [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] estimated.

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The Italian trial of Brandes et al. (2006)[153] studied a highly-selected group of CTX-naïve patients with good performance status (median KPS = 90%). This was a specific design aimed to study the efficacy of TMZ at GBM recurrent in TMZ-naïve patients, and, due to this specificity, the results of Brandes are incomparable to both the current trial and the all other four ddTMZ trials, all made on TMZ-pretreated patients with KPS 60–80%. US trial by Norden et al. (2013)[154] is another stand-alone trial with a median KPS of 90% and an extremely high share (65%) of patients with a methylated MGMT promoter (excluded from the comparison, see Bias assessment and limitations of the study). The German trial by Strik et al. (2008)[155] also stands alone: despite the worst patients' performance status (median KPS = 60% which is usually considered unfit for CTX), the patients received the extensive course of ddTMZ (a median of five cycles; mean, 7.3) with a modest toxicity. Two other studies, a Turkish study by Abacioglu et al. (2011)[156] and a Spanish study by Berrocal et al. (2010)[157] were the real-world[19] studies without an obvious difference from everyday practice: although the Berrocal trial claims to have selected TMZ-resistant patients, its findings do not differ from those of the Abacioglu trial both by extent of TMZ pre-treatment (median of six cycles) or by the time elapsed since diagnosis (14 vs. 13 months).

The details of patients' characteristic and treatment schedules are presented in Table 5. The response and survival data are presented in Table 6.

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Table 6. Comparison of dose-dense temozolamide trials: response and survival.

	NOP		Response			Overall survival	Survival since relapse		
Study	total	EFR	CR	ORR	BRR	MST mo (95%CI)	MST mo (95%CI)	1-y OS (95%CI)	MTTP (95%CI)
Brandes (2006)	33	33	3%	9%	61%	N/A	9,1 (7,1 – 14,5)	38%	3,7 (2,8 – 6,3)
Strik (2008)	18	18	17%	22%	61%	16,4[a] (17,9[b])	8,35[a] (9,1[b]) (N/A)	N/A	N/A
Abacioglu (2011)	16	14	0%	7%	57%	N/A	7 (5,7 – 8,2)	0%	3,0 (1,8 – 4,2)
Berrocal (2010)	47	27	0%	7%	38%[a]	N/A	5,1 (3,7 – 8,5)[c]	N/A	2,0 (0,9 – 3,1)
Norden (2013)	55	54	0%	13%	48%	11,7 (8,1 – 16,2)	N/A	N/A	1,8 (1,8 – 2,8)
Sahinbas (2007	54	15	7%	20%	53%	20,8 (15,2–25,1)	7,7 (5,7 – 9,4)[e]	29,5% (15,5–43,6)	N/A

Note: EFR: Estimated for response; CR: Complete response; ORR: objective response rate (CR + partial response); BRR: beneficial response rate (ORR + stable disease); NOP: number of patients; MST: median survival time (Kaplan-Meier estimation); [a] corrected data (the originally reported survival in months is derived from weeks by division to 4 (e.g., 32.8 w = 8.2 m) which overprices survival for 9%); [b] originally reported data (without correction); [c] for the complete sample of 47 pts, including 27 GBM and 20 WHO III tumors; [d] combination treatment sample; [e] since 1st mEHT (not since relapse).

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The Strik's survival data were corrected because the originally reported survival in months was derived from weeks by the division to 4 (e.g., 32.8 w = 8.2 "chemo months"), which overrated survival by an average of 9%.

Effect-to-treatment analysis

We used effect-to-treatment analysis (ETA) to compare the trials according to the principles described in the statistics section. The mean survival time (mST) after relapse in patients receiving standard modern treatment (which can be defined as trimodal 1st–2nd-line treatment approximately equal to Stupp protocol8) was the parameter of comparison. Since the expected (reference) value of mST is absent in the literature, we deducted it from the available data as 4.775 months (95%CI, 3.9–5.6) (Supplement 2). Taking into account the worst MST of the Berrocal study (5.1 months [95%CI, 3.7–8.5]), this MST expectancy seems reasonable. For the further analysis, we considered this parameter as both the expected median and mean survival time (emST) since relapse (in view of supposed normal distribution according to central limit theorem). For further comparisons, meta-analysis and economic evaluations, the median parameters of all trials (MST and number of cycles) were translated into means according to the statistical methods section.

The results of ETA show the advantage of the mEHT+ddTMZ regimen. The main comparator was the weighted average of three ddTMZ trials with comparable samples (WA (2-4)) (Table 7).

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Table 7. Effect-to-treatment analysis: basic parameters.

No	Study	NOP	mST	P-value	Rank	LMG	P-value	mNC	P-value	ETR (95%CI)	P-value	Rank
1	Brandes (2006)	33	9,95 (7,73-12,17)	0,070	1	5,18 (2,79-7,56)	0,104	4,60 (3,87-5,33)	<0.001	1,13 (0,72-1,80)	0,273	2
2	Strik (2008)	18	8,35 (7,67-9,03)	0,416	2	3,58 (1,98-5,17)	0,506	7,30 (6,05-8,55)	<0.001	0,49 (0,31-0,70)	0,001	6
3	Abacioglu (2011)	16	6,98 (6,23-7,73)	0,345	6	2,20 (1,05-3,35)	0,486	3,33 (2,43-4,22)	0,004	0,66 (0,38-1,05)	0,022	3
4	Berrocal (2010)	47	5,60 (4,16-7,04)	0,031	7	0,83 (-0,86-2,51)	0,073	4,55 (3,94-5,16)	<0.001	0,18 (-0,05-0,44)	<0,001	7
5	WA (1-4)	114	7,27 (6,30-8,24)	0,638	4	2,50 (1,20-3,80)	0,718	4,20 (3,82-4,57)	<0.001	0,59 (0,39-0,85)	0,006	4
6	WA (2-4)*	81	7,16 (6,25-8,08)	0,531	5	2,39 (1,13-3,65)	0,633	4,13 (3,68-4,57)	<0.001	0,58 (0,37-0,83)	0,005	5
7	Sahinbas (2007)	54	7,63 (6,52-8,74)	1,000	3	2,85 (1,44-4,26)	1,000	1,56 (1,31-1,81)	1,000	1,83 (1,04-4,20)	1,000	1

Note: NOP: number of patients; WA: weighted average; mST: mean survival time since relapse; LMG: life months gained; mNC: mean number of cycles treated; * main comparator.

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The weighted average of all ddTMZ studies (WA (1-4)) and stand-alone Brandes and Strik studies were the additional comparators.

The mST in the mEHT+ddTMZ sample (7.625 ± 0.57 m) was ranked third after the Brandes and Srtik cohorts, and was significantly better than in the Berrocal trial (5.6 ± 0.73 m, $p = 0.031$) and worse than in the Brandes sample with borderline significance (9.95 ± 1.13 m, $p = 0.070$); other differences were not significant (Table 7). The differences by life months gained (LMG) were not significant. The mean number of treatment cycles (mNC) in the mEHT+ddTMZ sample (1.56 ± 0.13) was significantly less compared to all cohorts and WAs ($p \leq 0.004$). The relative survival gain changes the ranking: ddTMZ+mEHT provided significantly better effect-treatment ratio (ETR = 1.83 LMG/ccl [95%CI, 1.04–4.20]) compared to all other cohorts and WAs ($p < 0.022$), except the Brandes cohort (ETR = 1.13 LMG/ccl [95%CI, 0.72–1.80], $p = 0.273$).

To make ETRs comparable, the common denominator was estimated as a median of the mean number of cycles of all of the cohorts: MNC = 4.2 cycles. To lead ETRs to the common denominator, attenuation modelling was performed in the range of coefficients of attenuation (CA) 10–25 %×ccl^{-1} (Table 8).

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Table 8. Effect-to-treatment analysis: 15% attenuation model estimation.

No	Study	MAST	p–value	PNC	CEST	CENC	METR	EER	p–value	CNTM 1	2	3	4	5	6	7
1	Brandes (2006)	10,15 (9,24-11,06)	0,943	6	9,64	4	1,20 (0,74-1,95)	1,01	0,979	∞	2,56	1,59	0,99	1,65	1,59	91
2	Strik (2008)	8,40 (7,52-9,29)	0,015	6	7,98	4	0,81 (0,44-1,48)	0,68	0,302	-2,56	∞	4,22	1,62	4,63	4,19	-2,64
3	Abacioglu (2011)	7,34 (6,46-8,22)	<0,001	6	6,98	4	0,57 (0,37-0,89)	0,48	0,016	-1,59	-4,22	∞	2,62	-47,9	592	-1,62
4	Berrocal (2010)	5,63 (4,76-6,51)	<0,001	6	5,35	3	0,19 (0,08-0,49)	0,16	<0,001	-0,99	-1,62	-2,62	∞	-2,48	-2,63	-1,00
5	WA (1–4)	7,44 (6,56-8,31)	<0,001	6	7,07	4	0,59 (0,40-0,88)	0,50	0,015	-1,65	-4,63	47,9	2,48	∞	44,3	-1,68
6	WA (2–4)*	7,34 (6,46-8,21)	<0,001	6	6,97	4	0,57 (0,39-0,85)	0,48	0,011	-1,59	-4,19	-592	2,63	-44,3	∞	-1,62
7	Sahinbas (2007)	10,10 (9,10-11,10)	1,000	6	9,5	4	1,19 (0,59-2,40)	1,00	1,000	-91	2,64	1,62	1,00	1,68	1,62	∞

Note: WA: weighted average; * main comparator; CA: coefficient of attenuation; MAST: maximal attainable survival time; PNC: peak number of cycles; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio; EER: effect enhancement rate.

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A CA level of 15% was chosen for the following analysis as an optimal prognosis (Figure 8A). According to this scenario, the median effect-treatment ratio (METR) of the ddTMZ+mEHT cohort is 1.19 LMG/ccl (95%CI, 0.59 to 2.40), which is significantly more than the METR of the main comparator (METR = 0.57 LMG/ccl [95%CI: 0.39–0.85], p = 0.011) and other cohorts ($p \leq 0.016$), except that of Brandes (METR = 1.20 LMG/ccl [95%CI, 0.74–1.95], p = 0.979) and Strik (METR = 0.81 LMG/ccl [95%CI: 0.44 to 1.48], p = 0.302) cohorts. This scenario means that the ddTMZ+mEHT cohort would have to reach the maximal attainable survival time (MAST) of 10.10 months (95%CI, 9.10–11.10) at the sixth cycle, which is significantly more than the MAST of the main comparator (7.34 months [95%CI, 6.46–8.21] $p < 0.001$) and other cohorts ($p \leq 0.015$), except the Brandes cohort (10.15 months [95%CI, 9.24–11.06], p = 0.943).

Based on the "cycles needed to treat per LMG" criterion (CNTM) (Table 8), the ddTMZ+mEHT regimen displayed strong and significant benefit versus the Berrocal and Abacioglu cohorts and both WAs (CNTM = 1.00–1.68 ccls/LMG, $p < 0.016$), moderate and insignificant benefit versus Strik cohort (CNTM = 2.64 ccls/LMG, p = 0.302) and no effect versus the Brandes cohort (CNTM = -90.98 ccls/LMG, p = 0.979).

Thus, our ETA suggests a strong and significant enhancement of the ddTMZ 21/28d regimen by concurrent mEHT.

Sensitivity analysis

Sensitivity analysis was completed to validate the robustness of the ETA results. For this purpose, the lower and upper limits of CA were estimated (Figure 8, Table 9):

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Table 9. Effect-to-treatment analysis: sensitivity analysis.

			CA = 15%				CA = 19.3%			
No	Study	mST	CEST	METR	CNTM	p–value	CEST	METR	CNTM	p–value
1	Brandes (2006)	9,95 (7,73-12,17)	9,64	1,20 (0,74-1,95)	90,98 (48,52 — 170,60)	0,979	9,44	1,23 (0,75-2,01)	5,30 (2,97 — 9,47)	0,585
2	Strik (2008)	8,35 (7,67-9,03)	7,98	0,81 (0,44-1,48)	-2,64 (-5,43 — -1,28)	0,302	**8,35**	0,95 (0,49-1,86)	-11,73 (-24,39 — -5,64)	0,830
3	Abacioglu (2011)	6,98 (6,23-7,73)	**6,98**	0,57 (0,37-0,89)	-1,62 (-2,94 — -0,89)	0,016	6,73	0,55 (0,36-0,83)	-2,04 (-3,43 — -1,22)	0,016
4	Berrocal (2010)	5,60 (4,16-7,04)	5,35	0,19 (0,08-0,49)	-1,00 (-2,77 — -0,36)	<0,001	5,32	0,20 (0,08-0,51)	-1,19 (-3,22 — -0,44)	0,001
5	WA (1–4)	7,27 (6,30-8,24)	7,07	0,59 (0,40-0,88)	-1,68 (-2,93 — -0,96)	**0,015**	6,91	0,59 (0,40-0,88)	-2,26 (-3,70 — -1,38)	**0,027**
6	WA (2–4)*	7,16 (6,25-8,08)	6,97	0,57 (0,39-0,85)	-1,62 (-2,84 — -0,92)	**0,011**	6,82	0,57 (0,38-0,85)	-2,14 (-3,52 — -1,30)	**0,018**
7	Sahinbas (2007)	7,63 (6,52-8,74)	9,6	1,19 (0,59-2,40)	∞	1,000	8,69	1,04 (0,77-1,41)	∞	1,000

Note: WA: weighted average; * main comparator; CA: coefficient of attenuation; mST: mean survival time; CEST: cost-effective survival time; CENC: cost-effective number of cycles; METR: median effect-treatment ratio.

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the lower limit of CA = 15% is defined by Abacioglu cohort, in which the ascending mST reaches a cost-effective survival time level (CEST = 6.98 months) with other cohorts being between CEST and MAST (Figure 8A); the upper limit at CA = 19.3% is defined by Strik cohort, in which the descending mST reaches CEST = 8.35 months (Figure 8B). The CNTM of the ddTMZ+mEHT cohort versus the main comparator attenuates from strong to moderate from the lower to the upper limit (from 1.62 to 2.14 ccls/LMG) but remains significant (p = 0.011–0.018). The extremum modelling shows that the CNTM of the ddTMZ+mEHT cohort versus the main comparator remains significant ($p \leq 0.05$) up to CA = 24.4%. Thus, the result of the ETA is robust.

Safety comparison

Since the ddTMZ+mEHT regimen did not display any grade II–-IV toxicity, whereas the ddTMZ regimens generated such toxicity events at a rate of 45–92%, the difference was always highly significant ($p < 0.001$) (Table 10).

Table 10. Comparison of dose-dense temozolamide trials: adverse events.

	Grade	Brandes (2006)	Strik (2008)	Abacioglu (2011)	Berrocal (2010)	Norden (2013)	Sahinbas (2007)
Adverse Event	NOP	33	18	16	47	55	140
Total events	I-II	122%	N/A	44%	194%	N/A	34%
	III-IV	76%	49%	92%	45%	60%	0%
	χ^2	123,721	72,196	141,308	70,654	100,593	
	p	<0,00001	<0,00001	<0,00001	<0,00001	<0,00001	
Lymphopenia	I-II	21%		12%	55%		0%
	III-IV	24%	14%	80%	28%	38%	0%
Leucopenia	I-II	21%		20%	28%		0%
	III-IV	24%	14%	4%	2%	5%	0%
Neutroopenia	I-II	9%			17%		0%
	III-IV	12%			2%	4%	0%
Trombocytopenia	I-II	3%		8%	19%		0%
	III-IV	3%	5%	8%	11%	4%	0%
Anemia	I-II	26%		4%			0%
	III-IV	3%				2%	0%
Nausea/Vomiting	I-II	6%			26%		4%

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Adverse Event	Grade	Brandes (2006)	Strik (2008)	Abacioglu (2011)	Berrocal (2010)	Norden (2013)	Sahinbas (2007)
	NOP	33	18	16	47	55	140
	III-IV	3%			2%	2%	0%
Fatigue	I-II						4%
	III-IV					5%	0%
Obstipation/Diarrhea	I-II	24%			15%		0%
	III-IV	3%					0%
Infection	I-II	12%					0%
	III-IV	3%	5%				0%
Headache	I-II						4%
Skin reactions	I-II						12%
Asthenia	I-II				17%		10%
Gastrointestinal	I-II				17%		0%
	III-IV		10%				0%

Grade I–II toxicity in the ddTMZ+mEHT cohort was mild. Since 4% of grade I nausea can be attributed to TMZ, total 30% of the mEHT-related events encountered. The main of them are grade I-II skin reactions (12%) and grade I short-term (<2h) post-treatment asthenia (10%).

Economic evaluation

Cost-effectiveness analysis

Cost-effectiveness analysis (CEA) was performed from the perspective of a health provider with a lifetime horizon. The goal of the CEA was to evaluate the cost-effectiveness of the ddTMZ+mEHT regimen versus ddTMZ only, so that only the direct costs for these two modalities were analysed. It was considered by default that other costs are dispensed proportionally and do not affect the estimation based on the direct costs (see Bias assessment and limitations of the study).

Two costs models were used for the CEA: conditionally termed ‘German’ and ‘US’ (see DISCUSSION). The German model has lower costs and less variance compared to the US model. For both the models, end user prices for TMZ were estimated based on open sources (as at Jan 21,

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2017): mean 1.70 $/mg (95%CI: 1.44 to 1.95) in the USA [158] and 1.14 €/mg (95% CI: 1.12 to 1.17) in Germany.[159]

The cost of the single mEHT session varies between countries, from $100 in Russia to $500 in Israel and South Korea (as at 2016). In the European Union, it varies in the range from €145.14 per session in Germany to €300–400 in private clinics outside Germany. From the perspective of a health provider, this cost is limited by national regulations: e.g., one deep HT session is reimbursed at a rate of €173 in Italy (National tariff nomenclature code 99.85.2) and €145.14 in Germany (GOA code 5854). In those countries where HT is not reimbursed by the health insurance system (e.g., Spain and Austria), the median private cost is about €300.

Thus, from the perspective of a health provider, the mean cost of a single mEHT session in Germany was estimated as €145.14 with zero variance (95%CI, €145.14–145.14), whereas in the US the estimated mean is $300 (95%CI, $234–366) (Table 11).

Table 11. Calculated prices for economic evaluation.

	US model		German model	
	TMZ	mEHT	TMZ	mEHT
Parameter	$/mg	$/sess.	€/mg	€/sess.
Mean (95%CI)	1,70 (1,44 – 1,95)	300 (234 – 366)	1,14 (1,12 – 1,17)	145 (145 - 145)
Median (range)	1,77 (0,59 – 4,42)	300 (150 – 500)	1,14 (0,88 – 1,55)	145 (145 - 300)

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia.

The results of the CEA are presented in Table 12 (German model)

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Table 12. Cost-effectiveness analysis (German model).

Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	%CE_{25k}	%CE_{30k}	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
Brandes (2006)	14,905 (14,586 – 15,225)	<0.001	24,292 (20,263 – 28,321)	4,421 (2,090 – 6,752)	1.22 (1.10 – 1.35)	0.061	53.57%	76.5%	28,706 (-5,529 – 62,940)	5,561,695	193.8
Strik (2008)	31,539 (30,863 – 32,215)	<0.001	61,250 (53,939 – 68,561)	41,379 (37,491 – 45,267)	3.08 (2.83 – 3.34)	<0.001	0.00%	0.0%	367,368 (-710,070 – 1,444,806)	22,195,135	60.4
Abacioglu (2011)	14,379 (14,071 – 14,687)	<0.001	33,429 (30,717 – 36,141)	13,558 (11,791 – 15,325)	1.68 (1.57 – 1.80)	<0.001	0.12%	1.8%	-92,957 (-352,869 – 166,956)	5,035,150	-54.2
Berrocal (2010)	16,721 (16,362 – 17,079)	<0.001	48,419 (39,174 – 57,665)	28,548 (23,705 – 33,391)	2.44 (2.16 – 2.71)	<0.001	0.31%	0.7%	-43,717 (-91,130 – 3,697)	7,377,172	-168.8
WA (1-4)	17,922 (17,538 – 18,306)	<0.001	39,967 (35,985 – 43,949)	20,096 (17,787 – 22,405)	2.01 (1.86 – 2.16)	<0.001	0.04%	0.3%	-291,167 (-1,869,626 – 1,287,291)	8,577,947	-29.5
WA (2-4)	18,043 (17,657 – 18,430)	<0.001	40,845 (36,926 – 44,763)	20,973 (18,692 – 23,255)	2.06 (1.90 – 2.21)	<0.001	88.8%	99.2%	-226,212 (-1,153,427 – 701,004)	8,699,523	-38.5

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Study	Costs, € mean (95%CI)	p-value	CUR, €/QALY (95%CI)	ICUR, €/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{25k}$	$\%CE_{30k}$	ICER €/QALYG (95%CI)	ΔC_{1000} €	ΔE_{1000} QALYG
WA (2-3)*	18,138 (17,750 – 18,527)	<0.001	40,424 (36,758 – 44,091)	20,553 (18,384 – 22,722)	2.03 (1.89 – 2.18)	<0.001	0.02%	0.2%	-302,629 (-1,934,133 – 1,328,875)	8,794,882	-29.1
Sahinbas (2007)	9,344 (9,199 – 9,488)	1.000	19,871 (17,719 – 22,024)	0	1.00	1.000	88.8%	99.2%	0	0	0.0

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main comparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{25k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) €25,000; $\%CE_{30k}$: %CE at CET €30,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained).

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and Table 13 (US model).

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Table 13. Cost-effectiveness analysis (US model).

Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	%CE$_{30k}$	%CE$_{50k}$	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
Brandes (2006)	22,106 (18,799 – 25,413)	0.003	36,028 (28,866 – 43,189)	3,324 (-1,280 – 7,927)	1.10 (0.96 – 1.25)	0.472	3.01%	84,02%	34,727 (-12,095 – 81,549)	6,728,332	193.8
Strik (2008)	46,775 (39,779 – 53,772)	<0.001	90,841 (76,123 – 105,558)	58,136 (50,122 – 66,151)	2.78 (2.45 – 3.11)	<0.001	0.02%	0,21%	519,683 (-1,009,423 – 2,048,790)	31,397,527	60.4
Abacioglu (2011)	21,325 (18,135 – 24,515)	0.007	49,579 (42,820 – 56,338)	16,875 (12,433 – 21,317)	1.52 (1.35 – 1.68)	<0.001	0.17%	51,27%	-109,798 (-426,187 – 206,591)	5,947,408	-54.2
Berrocal (2010)	24,799 (21,089 – 28,508)	<0.001	71,811 (56,003 – 87,619)	39,107 (30,569 – 47,644)	2.20 (1.89 – 2.51)	<0.001	0.26%	1,56%	-55,827 (-122,100 – 10,445)	9,420,880	-168.8
WA (1-4)	26,580 (22,604 – 30,555)	<0.001	59,276 (50,498 – 68,053)	26,571 (21,289 – 31,853)	1.81 (1.61 – 2.02)	<0.001	0.08%	2,34%	-380,229 (-2,447,832 – 1,687,373)	11,201,761	-29.5
WA (2-4)	26,760 (22,757 – 30,763)	<0.001	60,577 (51,756 – 69,398)	27,873 (22,572 – 33,174)	1.85 (1.64 – 2.06)	<0.001	0.06%	1,96%	-295,965 (-1,515,454 – 923,523)	11,382,070	-38.5

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Study	Costs, $ mean (95%CI)	p-value	CUR, $/QALY (95%CI)	ICUR, $/QALY (95%CI)	CURR, (95%CI)	p-value	$\%CE_{30k}$	$\%CE_{50k}$	ICER $/QALYG (95%CI)	ΔC_{1000} $	ΔE_{1000} QALYG
WA (2-3)*	26,901 (22,877 – 30,925)	<0.001	59,954 (51,427 – 68,481)	27,249 (22,075 – 32,423)	1.83 (1.63 – 2.04)	<0.001	0.06%	2,04%	-396,520 (-2,540,572 – 1,747,533)	11,523,498	-29.1
Sahinbas (2007)	15,378 (12,703 – 18,052)	1.000	32,704 (27,215 – 38,193)	0	1.00 (1.00 – 1.00)	1.000	4.45%	94,60%	0	0	0.0

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; QALY: quality-adjusted life year; * main comparator; CUR: cost-utility ratio; RCUR: relative CUR; $\%CE_{30k}$: proportion of cost-effective cases (patients) at cost-effectiveness threshold (CET) $30,000; $\%CE_{50k}$: %CE at CET $50,000; ICER: incremental cost-effectiveness ratio; QALYG: QALY gained; ΔC_{1000}: costs difference per 1000 patients; ΔE_{1000}: effect difference per 1000 patients (QALY gained)

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Along with four single cohorts of comparison, three weighted averages (WA) were assessed. WA (1-4) combines all the cohorts, WA (2-4) excludes the Brandes cohort as a selected cohort (selection bias-free average), WA (2-3) also excludes the Berrocal cohort in view of its very low survival gain, which significantly affected the final results (low-result bias-free average, the main comparator).

The mean costs of ddTMZ+mEHT regimen both in the German (€9,344 [95%CI, 9,199–9,488]) and US ($15,378 [12,703–18,052]) models were significantly less versus all cohorts and WAs ($p < 0.05$ in all cases). The Abacioglu cohort displayed the lowest costs (€14,379 [95%CI, 14,071–14,687]) and $21,325 [95%CI, 18,135 – 24,515] respectively) and the Strik cohort the highest (€31,539 [95%CI, 30,863 – 32,215] and $46,775 [95%CI: 39,779–53,772]); the main comparator WA (2-3) costs were calculated to be €18,138 [95%CI: 17,750–18,527] and $26,901 [95%CI: 22,877–30,925]).

For estimation of the cost-utility ratio (CUR), we used the weighted average index of health-related quality of life (HRQoL) of all five cohorts (0.74 QALY/LY) to counterweight the initial difference of the samples (range of median KPS 60–90%) not connected with the treatment (Table 2).

The CUR of the ddTMZ+mEHT regimen, both in the German (19,871 €/QALY [95%CI, 17,719 – 22,024]) and US (32,704 $/QALY [95%CI, 27,215–38,193]) models was also less versus all comparators. The difference was highly significant ($p \leq 0.001$), except for the Brandes cohort (24,292 €/QALY [95%CI, 20,263–28,321]), $p = 0.061$; and 36,028 $/QALY [95%CI, 28,866 – 43,189], $p = 0.472$). The main comparator WA (2-3) was calculated as 40,424 €/QALY (95%CI, 36,758–44,091) and 59,954 $/QALY (95%CI, 51,427–68,481), $p < 0.001$ for both.

In the German model, versus cost-effectiveness thresholds (CET) 25,000 €/QALY ($\%CE_{25k}$) and 30,000 € /QALY ($\%CE_{30k}$), the proportion of cost-effective cases (%CE) for the ddTMZ+mEHT regimen was 88.8% ($\%CE_{25k}$) and 99.2% ($\%CE_{30k}$) (i.e., it was cost-effective versus both CETs). All the other comparators showed negligible %CE (0–2.5%), except the Brandes cohort, which was also mainly cost-effective at both CETs ($\%CE_{25k}$ = 53.6% and $\%CE_{30k}$ = 76.5%). In the US model, versus CETs 30,000 $/QALY ($\%CE_{30k}$) and 50,000 $/QALY ($\%CE_{50k}$), the %CE for the ddTMZ+mEHT regimen was 4.5% ($\%CE_{30k}$) and 94.6% ($\%CE_{50k}$) (i.e., it was cost-effective versus CET = $50,000 only). Two other cohorts were also mainly cost-effective versus CET = $50,000: namely the Brandes ($\%CE_{50k}$ = 84%) and Abacioglu ($\%CE_{50k}$ = 51.3%) cohorts; the $\%CE_{50k}$ of all of the WAs was negligible (2.0–2.3%).

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As for comparative cost-effectiveness, only the Brandes cohort showed an ICER of less than the applied CETs (28,706 € /QALY [95%CI, -5,529–62,940) and 34,727 $/QALY [95%CI, -12,095–81,549). All of the other cohorts and WAs were not cost-effective with the ICER ranging from 43,717 €/QALY / 55,827 $/QALY to 367,368 €/QALY / 519,683 $/QALY.

Sensitivity analysis

The sensitivity of the CEA was analysed by using an equal cost-effectiveness test, that is by exploring the value of a key parameter in which the value of the relative CUR (CURR) of the ddTMZ+mEHT regimen and the main comparator (WA [2-3]) equals to 1.0 (or ICUR = 0). For this purpose, the following variables were tested: the price of the mEHT session; the number of TMZ application days (days on) over a 28-days cycle; the price of TMZ; the number of cycles of ddTMX+mEHT.

The equivalent price of the mEHT session is €683 in the German model, and $1,013 in the US model and the coefficient of reliability of the CEA result (CR, the ratio of a key parameter of CE-equivalent model and the standard model) is 3.4/4.7 (Table 14).

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Table 14. Cost-effectiveness analysis: sensitivity analysis.

	US model					German model				
	TMZ		mEHT			TMZ		mEHT		
Parameter	Price, $/mg	Days on	$/sess	mNC	CR	Price, €/mg	Days on	€/sess	mNC	CR
Standard regimen	1.70 (1.44 – 1.95)	21	300 (234 – 366)	1.60		1.14 (1,12 – 1,17)	21	145.14 (145 – 145)	1.60	
Maximal mEHT price	NC	NC	1013.47	NC	3.38	NC	NC	683.65	NC	4.71
Minimal TMZ days on	NC	6,21	NC	NC	3,38	NC	4.46	NC	NC	4.71
Minimal TMZ price	0,50	NC	NC	NC	3.38	0.24	NC	NC	NC	4.71
Maximal TMZ+mEHT cycles	NC	NC	NC	2.86	1.79	NC	NC	NC	3.17	2.05

Note: TMZ: temozolomide; mEHT: modulated electro-hyperthermia; mNC: mean number of cycles; CR: coefficient of reliability; NC: no change.

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The equivalent price of TMZ is 0.50 $/mg in the US model and 0.24 €/mg in the German model; once again with CR = 3.4/4.7. Since these key parameters (prices) do not affect the treatment efficacy, their equivalent values do not need any size-dependent correction. The result means that the ddTMZ+mEHT regimen is cost-effective in the entire range of possible prices with double to quadruple redundancy.

The equivalent number of TMZ "days on" is 4.46 days in the German model and 6.21 days in the US model, once again with CR = 3.4/4.7. This time, the key parameter affects the treatment efficacy, because the diminished dose (days) of ddTMZ can decrease the effectiveness and, therefore, can increase the ddTMZ+mEHT/ddTMZ CURR and cause an offset of the equivalence point to the lower values of "days on". This means that the ddTMZ+mEHT regimen, most probably, keeps the cost-effectiveness up to the standard 5/28d regimen and below it, and the cost-effectiveness of mEHT could be generalized for the entire range of TMZ treatment of recurrent gliomas.

The maximal equivalent number of ddTMZ+mEHT cycles is 2.86 in the US model and 3.17 cycles in German model (CR = 1.8/2.1). This key parameter also affects the treatment efficacy, because, with an increase of cycle number of the ddTMZ+mEHT regimen, the treatment efficacy and CUR will rise with an offset of the equivalence point towards the longer course. At the least, this result means that the length of the ddTMZ+mEHT regimen can be doubled without loss of cost-effectiveness.

Thus, the sensitivity analysis confirms that the results of the CEA are remarkably stable, with double to quadruple redundancy.

Budget impact analysis

We estimated a budget impact of the treatment of 1,000 patients per year (Table 12 and 13) with a time horizon of one year. Versus the main comparator, the saving (ΔC_{1000}) is €8,794,882 / $11,523,498 per year (German / US model) with 29.1 years of survival gain (ΔE_{1000}). The average saving ranged from €8,577,947 / $11,201,761 to €8,794,882 / $11,523,498 with 29.1–38.5 QALY gained. To extrapolate the economic results to a larger time horizon, the depreciation rate of 20% per year must be applied.

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Cost-benefit analysis

Cost-benefit analysis (CBA) was performed from the perspective of a large neurooncology centre treating more than 150 patients with recurrent GBM per year (Table 15,

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Table 15. Cost-benefit analysis (US model).

Parameter	Rate	Year 1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		11	11	11	11	11	11	11	11	
Number of units		1								1
Capital costs[a]		400,000								400,000
Service costs	12%			48,000	48,000	48,000	48,000	48,000	48,000	288,000
Depreciation	15%		60,000	60,000	60,000	60,000	60,000	60,000	60,000	420,000
Reimbursement per session		300,00	300,00	300,00	300,00	300,00	300,00	300,00	300,00	
Reimbursement per year		807,300	807,300	807,300	807,300	807,300	807,300	807,300	807,300	6,458,400
Operational costs per year	50%	538,200	538,200	538,200	538,200	538,200	538,200	538,200	538,200	4,305,600
Economy per patient	20%	11,523	9,219	7,375	5,900	4,720	3,776	3,021	2,417	47,951
Economy per year		1,728,525	1,382,820	1,106,256	885,005	708,004	566,403	453,122	362,498	7,192,632
Earnings per year		2,535,825	2,190,120	1,913,556	1,692,305	1,515,304	1,373,703	1,260,422	1,169,798	13,651,032
Total costs per year		938,200	598,200	646,200	646,200	646,200	646,200	646,200	646,200	5,413,600
Economy & EBIT		1,597,625	1,591,920	1,267,356	1,046,105	869,104	727,503	614,222	523,598	8,237,432
EBIT		-130,900	209,100	161,100	161,100	161,100	161,100	161,100	161,100	1,044,800
Cumulative EBIT		-130,900	78,200	239,300	400,400	561,500	722,600	883,700	1,044,800	

Note: [a] Acquisition price + shipment + installation + training; [b] share of capital cost per year; [c] profit rate; [d] annual depreciation rate of the saving; EBIT: earnings before interest and taxes.

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Table 16).

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Table 16. Cost-benefit analysis (German model).

Parameter	Rate	Year 1	2	3	4	5	6	7	8	Total
Number of patients per year		150	150	150	150	150	150	150	150	1,200
Mean sessions per patient		17.9	17.9	17.9	17.9	17.9	17.9	17.9	17.9	
Sessions per year		2,691	2,691	2,691	2,691	2,691	2,691	2,691	2,691	
Sessions per day		10.8	10.8	10.8	10.8	10.8	10.8	10.8	10.8	
Number of units		1								1
Capital costs[a]		300,000								300,000
Service costs	12,0%[b]			36,000	36,000	36,000	36,000	36,000	36,000	216,000
Depreciation	15,0%		45,000	45,000	45,000	45,000	45,000	45,000	45,000	315,000
Reimbursement per session		145.14	145.14	145.14	145.14	145.14	145.14	145.14	145.14	
Reimbursement per year		390,572	390,572	390,572	390,572	390,572	390,572	390,572	390,572	3,124,574
Operational costs per year	50%[c]	260,381	260,381	260,381	260,381	260,381	260,381	260,381	260,381	2,083,049
Economy per patient	20%[d]	8,795	7,036	5,629	4,503	3,602	2,882	2,306	1,844	36,597
Economy per year		1,319,232	1,055,386	844,309	675,447	540,358	432,286	345,829	276,663	5,489,509
Earnings per year		1,709,804	1,445,958	1,234,880	1,066,019	930,929	822,858	736,401	667,235	8,614,083
Total costs per year		560,381	305,381	341,381	341,381	341,381	341,381	341,381	341,381	2,914,049
Economy & EBIT		1,149,423	1,140,576	893,499	724,637	589,548	481,477	395,019	325,854	5,700,034
EBIT		-169,809	85,191	49,191	49,191	49,191	49,191	49,191	49,191	210,525
Cumulative EBIT		-169,809	-84,619	-35,428	13,762	62,953	112,143	161,334	210,525	

Note: [a] Acquisition price + shipment + installation + training; [b] share of capital costs per year; [c] profit rate; [d] annual depreciation rate of the economy; EBIT: earnings before interest and taxes.

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The main assumptions of the CBA are as follows: mean sessions per patient is equal to that of SOI; the mEHT device does not generate revenues other than health care system reimbursement for the treatment of those patients; the mEHT device operates in 12-h/day mode; the capital costs including acquisition costs, shipment, installation and training are €300,000 in the German model and $400,000 in the US model; the service costs rate is 12% of the capital costs per year with 2-year free of charge guarantee service; the depreciation of the mEHT equipment at a rate of 15% per year; the norm of profit of the health care provider is 50% (operational costs are 67% of revenues); the saving obtained as a result of the introduction of the ddTMZ+mEHT regimen depreciates at a rate of 20% per year; the saving is not included in earnings before interest and taxes (EBIT); no price discount/inflation rate is used; the time horizon is 8 years.

Our CBA shows that use of an mEHT device is profitable with the above parameters and generates the total revenues in amount of €3,124,574 / $6,458,400 with EBIT €210,525 / $1,044,800 per mEHT device over 8 years, provided that operational costs are €2,083,049 / $4,305,600 for that period (€260,381 / $538,200 per year). With respect to the saving due to the use of the ddTMZ+mEHT regimen instead of ddTMZ only, the total economic effect (saving + EBIT) over the 8 year period is €5,700,034 / $8,237,432 per mEHT device.

DISCUSSION

Clinical evaluation

In a general comparison, the ddTMZ+mEHT cohort has revealed a non-significantly better mean survival time (mST = 7.63 months [95%CI, 6.52–8.74]) compared to the main comparator, the pooled mST of three trials on TMZ-pretreated patients (7.16 months [95%CI, 6.25 to 8.08], p = 0.531).

Covariates survival analysis has revealed the comparable efficacy of mEHT and ddTMZ, at least in weakened patients (Figure 4), suggesting the feasibility of mEHT as a single treatment in those patients, for which CTX is impossible in view of toxicity or bad performance. The advantage of mEHT over chemotherapy was shown elsewhere in GBM[22] and other cancers.[30,33,41,44]

Despite the shown significant dependence of survival from mEHT dose (p = 0.007), it is difficult to say how the difference in the mEHT dose actually affects the response and survival because the LD-mEHT sample included weakened patients with longer time since diagnosis to 1st mEHT (median

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9.9 months [95%CI, 6.1–11.6]), shortest treatment time (median 0.5 months [95%CI, 0.4–0.6) vs. 1.9 months (95%CI, 1.2–2.8) in the HD-mEHT sample, p = 0,0001) and highest rate of treatment termination (38% vs. 0% in the HD-mEHT sample, p<0,0001) (Table 3). More correctly, the LD-mEHT was rather a sequence of poor patient states, which likely accounts for the decrease in survival. In other words, the impossibility to reach an adequate mEHT dose for weakened patients made their prognosis dismal.

The dependence of survival on SAT use is questioned. The extremely low survival in the "No SAT" sample (2.9 months [95%CI, 2.3–5.5), almost 2-fold lower than the expected value) undisputedly indicates for the selection of patients with bad prognosis and small life expectancy. Comparison of the samples showed that "No SAT" includes patients with significantly less TMZ cycles (mean 1.1 ± 0.1 cycles vs. 1.7 ± 0.1, p = 0.017) and mEHT sessions (mean, 11.2 ± 0.5; median, 10 vs. 19.9 ± 0.4; median, 15, p = 0.013) with a higher proportion of LD-mEHT (47% vs. 27%, RR = 1.74 [0.90–3.34], p = 0.12). Therefore, this survival difference shows a tendency to not apply SAT to patients with a bad prognosis, and that these patients were heavily undertreated.

The shown significantly reduced toxicity of ddTMZ+mEHT is, in our opinion, caused by the short course of TMZ in the COI (median 1 cycle only). TMZ is known as a relatively safe alkylating drug. Its toxicity appears after 2–3 cycles and a development of the III–IV grade lymphopenia (the main adverse event) becomes virtually inevitable after six cycles. Thus, the data presented here allows us to conclude that mEHT *per se* is safe, but does not allow us to estimate the modifying effect of mEHT on TMZ toxicity (if such an effect exists).

Effect-to-treatment analysis

Direct comparison of the ddTMZ+mEHT results with the other ddTMZ studies is impossible because the ddTMZ+mEHT treatment in the participating tertiary centres was not continued up to the maximal attainable course (MAC). The median number of cycles was just one, and only 15% of treatments were stopped in view of the disease progression, without limiting toxicity. In tertiary centres, the end of treatment is caused either by the physician's decision, by the patient's personal decision, economic reasons, by an applied protocol, or because of a combination of these reasons. Therefore, the treatment is typically limited by 1–3 cycles only, whereas in clinics the median duration of MAC of recurrent GBM is five cycles.[18] Therefore, effect-to-treatment analysis (ETA) was used for the comparison.[143]

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The idea of ETA is simple and based on the effect-treatment ratio (ETR), i.e., life months gained per a typical 28-days treatment cycle, which is considered a unit of a CTX treatment. By ETR, we identified ddTMZ+mEHT as the uncontested leader, with 1.83 LMG/ccl versus 1.13 LMG/ccl of the nearest competitor (Brandes cohort) and 0.58 LMG/ccl of the main comparator (WA 2-4) (Table 7), although in terms of conventional MST-based comparison, ddTMZ+mEHT was ranked third (behind the Brandes and Strik cohorts).

The next step of the ETA follows from the idea of attenuation of the treatment effect. This is a typical feature of all cancer treatments because of the ability of cancer cells to rapidly develop multiple mechanisms of acquired resistance to an applied treatment. This is especially correct for diseases such as GBM, which almost inevitably progresses, and for TMZ, for which many distinct mechanisms of acquired resistance are available,[160,161,162] so that virtually all patients develop resistance to TMZ. As a result, the effectiveness of any cancer treatment decays (attenuates).

The offered equation of the attenuation is based on ETR and coefficient of attenuation (CA). It is suggested that CA is common for all the ddTMZ cohorts. The maximum value of CA corresponds to the assumption that the treatments have almost reached the maximal attainable survival time (MAST), which equals the extremum of the function. In this case, CA = 15 %/ccl exactly matches this assumption (Table 8A). Although the Strik cohort is located after the maximum of the function, it is acceptable because this cohort is likely overtreated (mNC = 7.3 ccls vs. 3–4.5 ccls in other ddTMZ cohorts).

The natural sequence of the attenuation idea is incomparability of ETRs obtained in a different number of cycles. This is because an early ETR with the lower impact of attenuation is higher than a later one. For the correct comparison, ETRs should be led to the common denominator. The best common denominator is the median number of cycles (MNC), which equals 4.2 cycles. The resulting parameter median ETR (METR) allows us to correctly compare the different treatments. In this comparison, COI (METR = 1.19 LMG/ccl [95%CI, 0.59–2.40]) significantly surpasses the main comparator WA (2-4) (METR = 0.57 LMG/ccl [95%CI, 0.39–0.85), p = 0.011) and all other comparators (METR = 0.19–0.59, p = 0.00–0.016), except the Brandes (METR = 1.20 LMG/ccl [0.74–1.95], p = 0.979) and Strik (METR = 0.81 LMG/ccl [0.44–1.48], p = 0.302) cohorts (Table 8). In other words, the efficacy of IOI in CTX-pretreated patients with a median KPS of 60–70% is the same as in the selected cohort of CTX-naïve patients with a median KPS of 90%, and significantly better compared to the TMZ-pretreated cohorts.

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With CA 15%/ccl, the COI reach a MAST of 10.10 months (95%CI, 9.10–11.10) at the sixth cycle, which is significantly more than the MAST of the main comparator (7.34 months [95%CI, 6.46–8.21], $p < 0.001$) and other cohorts, except the Brandes cohort (10.15 months [95%CI, 9.24–11.06), $p = 0.943$). The next assumption is that the CA of the ddTMZ+mEHT regimen is lower than that of the ddTMZ only regimen. Actually, the mechanisms of resistance to the RF-field have to differ substantially from those of CTX. Little is known about such acquired resistance. TTF reports a possibility of selection or development of giant-cell GBM with syncytial-type cells,[163] which is reasonable adaptation for 100 kHz range, where the large size of a cell improves the shielding from the external field, though it is a single-case observation, and it is hardly applicable to HFR, where size difference is not decisive. Taking into account the results of long-term (6 months to 3 years) mEHT treatments,[33,45,47] especially in patients with multiple liver metastases, which is a similarly lethal condition as GBM, where mEHT displayed the ability to support PFS up to three years, and even to revert the progression after stopping mEHT[33] (i.e., mEHT does not lose its efficacy over years), the assumption that the CA of mEHT is lower than that of TMZ looks reasonable. If we assume that the CA = 12.5 %/ccl, the ddTMX+mEHT cohort can attain a MAST of 10.84 months, or of 12.13 months with a CA = 10.0%.

The last parameter of ETA, called "cycles needed to treat per one life month gained" (CNTM), is an analogue of the known parameter "number needed to treat" (NNT). The CNTM shows the number of cycles of the compared treatments, at which the difference in their MST reaches one month. Positive CNTM means a benefit, negative means detriment, and the value of CNTM characterizes the strength of the effect (Figure 9). In this comparison, all of the cohorts displayed strong to moderate detriment versus the ddTMZ+mEHT regimen (Table 8), except the Brandes cohort (no effect).

Thus, the ETA has allowed us to uncover the real efficacy of the ddTMZ+mEHT treatment, which was impossible to assess with the conventional comparison by general endpoints, and has suggested that mEHT strongly and significantly enhances the efficacy of the ddTMZ 21/28d regimen with significantly less toxicity.

Economic evaluation

We studied two options for the mEHT application. The first, so-called German option, is specific for a developed country with rigid governmental regulation of the medical market, which leads to relatively low prices for pharmaceuticals with low variance (mean price of TMZ is 1.14 €/mg

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[95%CI, 1.12–1.17]) and fixed and low enough prices for medical procedures (in this case, 145.14 €/sess with zero variance [95%CI, 145.14–145.14]). The second, so-called US option, is specific for a developed country with lower governmental regulation, which leads to relatively high prices for pharmaceuticals with higher variance (mean price of TMZ 1.70 $/mg [95%CI, 1.44 to 1.95]) and variable and high enough prices for medical procedures (in this case, 300 $/sess [95%CI, 234 to 366]).

First, the adequacy of our costs estimation (€18,138 [95%CI, 17,750–18,527]) and $26,901 [95%CI, 22,877–30,925] in the main comparator) have to be assessed (Table 12 and 13). For this purpose, the result was compared with a recent study of Ray et al. (2014)[19], where expenditures for cancer drugs (without supportive drugs like antiemetics, pain killers, neutropenia related, etc.) for a 6-month period were assessed as $13,555–17,204. Since the study was devoted to TMZ treatment and taking into account the difference in price of TMZ and other cancer drugs, 95–99% of these 'cancer drugs' costs can be attributed to TMZ. Although the reported range of $13,555–17,204 appears to be much less than the average $27,000 displayed in the current assessment, it should be noted that the general practice of recurrent GBM treatment is based almost exclusively on the standard TMZ 5/28d regimen, 8 with 100–150 mg/m^2/d. The current regimen ddTMZ 21/28d 75–100 mg/m^2/d consumes 2.1–4.2 times more TMZ per course. Therefore, it is at least 2–3-times more expensive. Thus, the estimated costs range for the ddTMZ 21/28d regimen is $27,000–50,000, and the costs estimation of the current trial is adequate. It also corresponds to other estimations.[17,18]

The result suggests the significant advantage of the ddTMZ+mEHT regimen over all the comparators ($p < 0.003$) (except the Brandes cohort, against which the advantage was not significant [$p = 0.061–0.472$]). In the German model (Table 12), the ddTMZ+mEHT regimen was cost-effective versus both the 25,000 €/QALY and 30,000 €/QALY cost-effectiveness thresholds (CET) (88.8% and 99.2% of cost-effective cases, respectively), whereas the main comparator was not cost-effective (%CE of 0.0% and 0.2%). ICER versus ddTMZ+mEHT varied from 43,717 €/QALY to 367,368 €/QALY (except for the Brandes cohort, which displayed an ICER of 28,706 €/QALY).

In the US model (Table 13), the pattern was the same with more pronounced differences. The ddTMZ+mEHT regimen was not cost-effective versus CET = 30,000 $/QALY (%CE = 4.5% only), and only CET 50,000 $/QALY provides cost-effectiveness (%CE = 94.6%), whereas the main comparator showed a negligible cost-effectiveness ($\%CE_{50k}$ = 2.0%). ICER versus ddTMZ+mEHT varied from 55,827 $/QALY to 519,683 $/QALY (except for the Brandes cohort, which displayed an ICER of 34,727 $/QALY).

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The cost-effectiveness threshold (CET) (or willingness-to-pay, WTP) is set by National Institute for Health and Care Excellence (NICE) at £20,000–30,000 per QALY,[164] although studies show that the acceptable limit can be lower (up to £13–14,000).[165] In developed countries, a CET of €/$/£30,000 is considered standard. The CET for developing countries is suggested by the WHO at the level of their triple GDP per capita for each DALY,[166] which is typically close to the above NICE WTP. For end-of-life applications, where the QALY increase could be negligible, a CET of £50,000 is supposed by NICE.[167] Finally, for some orphan diseases, the third CET of about £100,000 is offered.[168] Since a treatment of the recurrent GBM can be considered an end-of-life application, a CET of 50,000 $/QALY is applicable in the US model.

Thus, the economic evaluation suggests that the inclusion of mEHT in the ddTMZ 21/28d regimen makes it cost-effective versus the applicable CET levels, whereas the ddTMZ 21/28d alone is not cost-effective. The sensitivity analysis suggests that this estimation is highly reliable, with double to quadruple redundancy. The sensitivity analysis also suggests that the advantage of ddTMZ+mEHT in cost-effectiveness remains true throughout the entire applicable range of prices for TMZ and the mEHT procedure, as well as for the TMZ intercycle variances (i.e., up to the lowest 5/28d regimen). It also suggests that the ddTMZ+mEHT course can be at least doubled without loss of cost-effectiveness. Since the cost-effective number of cycles (CENC) (i.e., the number of cycles at which MST reaches 95% of MAST) for the ddTMZ+mEHT regimen equals 3.0 (Table 8), this means the all-range cost-effectiveness of the regimen.

The BIA suggests significant savings from the introduction of mEHT, which can be estimated as about €8,794,882 per year per 1000 patients in the German model and $11,523,498 per year per 1000 patients in the US model, with an additional 29.1–38.5 QALY gained per 1000 patients.

Finally, the CBA shows that the mEHT, from the perspective of a single neurooncology centre, is profitable in both of the tested models (Table 15 and 16).

Thus, the introduction of mEHT generates savings for budget and health care providers and significant profit for the latter.

Applicability of mEHT in GBM treatment

The result obtained in this study looks promising, although a single retrospective trial does not provide the necessary grounds for generalization. Nevertheless, if the result is confirmed in a further meta-analysis, it will provide an excellent ground for generalization. At the least, it means that mEHT can be recommended as an enhancer of all ddTMZ regimens in the treatment of recurrent

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GBM, and, probably, for the regular 5/28d regimen too. Next, as shown by the covariates survival analysis (Figure 5), mEHT is feasible as a single treatment in those patients for which chemotherapy is impossible because of toxicity or bad performance. Thus, mEHT has a capacity as a salvage treatment after the failure of chemotherapy. With respect to the known low toxicity of mEHT[22,23,24,25,26] and its possibility to restore the performance and chemosensitivity,[33,45,47] this salvage treatment can, in some cases, provide an opportunity to continue chemotherapy in previously failed patients.

Bias assessment and limitations of the study

Only 15 patients (28%) in the COI were assessed for response. Although natural selection is supposed, selection bias is not excluded. Consequently, the response rate was excluded from the analysis.

Although follow-up period was short enough (median 6.0 months; range, 0.7–47.3 months; 95%CI, 4.6–7.5 months), it is close to the MST since the 1st mEHT session (7.7 months, 95%CI, 5.7–9.4), and the mean of the follow-up (8.4 ± 1.2 months) exactly fits the CI of the MST. Thus, the MST value is robust. Although 1-year and 2-year survivals since 1st mEHT are less robust in view of the short follow-up, they are also well within the range of the follow-up time (0.7–47.3 months) and, therefore, are reliable enough. Nevertheless, in view of their lower reliability, the 1-year and 2-year survivals were excluded from the comparison, which was based solely on the robust MST value.

The absence of the safety data matched to the COI is not a serious limitation because the absence of severe toxicity in the whole sample also excludes it for the sub-samples. So, the absence of grade III–IV toxicity and limited I–II toxicity (up to 30%) findings are relevant and robust, although the rate and distribution of the mild toxicity in the COI are approximate.

We excluded the Norden trial[154] from the ETA because of a lack of information on the number of cycles and some uncertainties (e.g., survival definition and some statistical uncertainties). The modest effect shown would not affect the comparison.

The main possible bias of a retrospective study is a selection bias. We consider the probability of the selection bias as minimal in the SOI because, in addition to the assurances of the authors of no exclusions from the sample, 153 patients with high-grade gliomas (HGG) is consistent with the whole amount of such patients in the enrolling centres, which are small tertiary centres not specialized in neurooncology (and, in the case of the Institute of Microtherapy, in cancer care at all), for the five-year period. Thus, we consider the sample as consecutive patients with HGG enrolled

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for the stated period without exclusions or selection. The declared inclusion criteria (recurrence/progression of HGG with KPS≥40%) rather describe the sample than limit it in any way. The absence of exclusion criteria confirms this suggestion.

At the same time, some compared ddTMZ studies showed an obvious selection bias. First, the Brandes study, in which the selection of CTX-naïve patients is presumed by the protocol, but the selection of patients with good performance (median KPS = 90%) also seems to be present (although this might be a natural sequence of the inclusion criteria). The same extremely favourable KPS is shown in the excluded Norden trial, which also showed an extremely high share of MGMT-methylated patients (65% vs. 45–46% in the other trials, which exceeds the highest historical level of about 60%[13]) (Table 7). Also, the large share of re-operations in the Strik study (33.3%) might significantly improve the observed survival, making it hardly attributable to the applied ddTMZ treatment.

The difference in dosage between the ddTMZ regimens was not analysed in the ETA (although it was considered in the economic evaluation). As many studies had displayed, there is no or negligible difference in efficacy of different doses of ddTMZ regimens, and sometimes lower doses were preferable.[169] Moreover, the possibility of dose reduction/escalation in all of the protocols makes such an analysis impossible. The average dose is never reported and cannot be retrieved from the reported data. We do not exclude the possibility that the actual doses were similar to each other.

There is an unequal MST starting point bias because the MST in the ddTMZ+mEHT cohort was calculated since the 1st session of mEHT, rather than since relapse/progression in the other cohorts. Since the SOI was carried out in tertiary centres, it is normal that mEHT was applied not just after relapse but rather as the second-line treatment of the relapse. Based on the median time of 9.0 months elapsed since diagnosis to the 1st mEHT treatment, and estimated 7.5 months MPFS in GBM, the delay of mEHT since relapse can be 1–1.5 months. This could significantly change the results in favour of the ddTMZ+mEHT cohort (e.g., estimated MST since relapse can reach 9 months instead of 7.6 months, as in the best ddTMZ studies). At the same time, due to this delay, probably some 1st-line treatments of relapse in the SOI were not included in the assessment. Based on the delay, the median one treatment cycle is supposed to be added, increasing the mean CTX cycles number to 2–2.5, which can somewhat change the economic results in favour of concurrent ddTMZ studies. Thus, the bias of not equal MST starting point rather distorts the comparison in favour of ddTMZ studies, though economically it is somewhat counterbalanced.

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It should also be noted that the two "real life" studies of Abacioglu and Berrocal displayed the longest time from initial diagnosis to enrolment (13 and 14 months, respectively), which is responsible for the low MST values in these trials. We consider that, in the weighted average assessment, this difference is counterbalanced by early enrolment in the Brandes and Strik trials and the median position of the SOI (Table 7). It is also counterbalanced (and even outbalanced) by the unequal histology bias, since the Abacioglu and Berrocal trials included WHO III tumours (28% and 43%, respectively) with much longer survival, which can be, in turn, the reason for the delayed relapse.

Nevertheless, there is a reciprocal dependence between the time to enrolment (relapse) and the MST since the enrolment (the SOI displays the medium-power correlation, Pearson 0.35), which is not considered in the ETA but seems counterbalanced or even outbalanced in favour of the ddTMZ cohorts.

It is worth noting that all of the "real life" studies (Sahinbas, Berrocal and Abaciouglu) showed the same median age of 50 years, whereas the supposedly selection-biased trials included the older patients (55–57 years).

MEHT required additional visits to the hospital (2–3 times a week), which means additional transportation costs and influences cost-effectiveness from the patient's perspective, although this does not affect the assessment from the health provider perspective. At the same time, since a planned mEHT session typically does not require the physician's involvement (a nursing procedure), we do not assume a better treatment control. Moreover, such control seems much more extensive in the compared prospective trials, where the follow-up included weekly complete blood counts,[155,154] physical and neurologic examinations every 4 weeks,[153,155] or even biweekly,[155] and brain imaging with MRI every 8 weeks[154] or earlier if indicated.[153] To compare, only 28% of patients in the SOI underwent brain imaging (the specificity of small tertiary centres). Better treatment control could significantly improve the treatment results.

Finally, all of the compared ddTMZ studies recruited only patients in a stable condition, whereas there was no such limitation in the SOI.

In general, although the assessment is distorted in favour of the ddTMZ studies, it still allows us to make an unambiguous conclusion on the advantage of the combination of mEHT and TMZ.

Also, upon completion of the paper, we have identified one additional ddTMZ 21/28d cohort in phase III randomized trial of Brada et al. (2010).[169] The result of this cohort (MST since relapse 6.6

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months after median four ddTMZ cycles, which results in METR ≤0.5 LMG/ccl) would not in any way affect the results obtained.

Generalizability of the results

The results of the sensitivity analysis of the CEA supposes the generalizability of the CEA results to the entire range of application of TMZ at recurrent GBM. There is a probability of similar enhancement of TMZ efficacy and cost-efficiency by mEHT can also be achieved in the treatment of the newly diagnosed GBM, although, to the best our knowledge, mEHT has never been studied in such a setting.

Since TMZ is considered the current most effective CTX treatment of GBM, the results of the covariate survival analysis (Figure 4) can be generalized to CTX. Thus, mEHT as a single treatment can be considered in those patients for which CTX is impossible because of toxicity or bad performance, and mEHT has a capacity as a salvage treatment after the failure of CTX.

Perspectives of research

This study creates a good basis for the further research on mEHT-enhancement of the GBM treatments with the possibility to develop a cost-effective alternative. First, we will estimate the other existing mEHT cohort trials, followed by a systematic review with meta-analysis. Second, a new cohort and randomized trials at recurrent and newly diagnosed GBM are warranted.

Verifiability of the results

To provide the possibility to verify the results obtained, raw data of the study are available in Supplement 3.

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CONCLUSIONS

Our ETA suggests that mEHT strongly and significantly enhances the efficacy of the ddTMZ 21/28d regimen (p = 0.011), with a maximum attainable MST of 10.10 months (95%CI: 9.10 to 11.10). The ddTMZ+mEHT cohort has displayed significantly less toxicity than the ddTMZ 21/28d cohorts (no grade III–IV toxicity vs. 45–92%, respectively) because of the shorter TMZ course. MEHT *per se* displays high safety with a mild grade I–II toxicity (30% of events), mainly of mild skin reactions (12%) and short (<2 h) post-treatment asthenia (10%). Our CEA suggests that the ddTMZ+mEHT regimen is cost-effective compared to the applicable cost-effectiveness thresholds 25,000–50,000 €$/QALY, whereas ddTMZ 21/28d only is not cost-effective, with ICER versus ddTMZ+mEHT ranging from 43,717 €/QALY to 367,368 €/QALY. This CEA result is highly reliable with double to quadruple redundancy. Our BIA suggests a significant saving from the introduction of mEHT, which can be estimated from €8,577,947 to $11,523,498 with 29.1–38.5 QALY gained per 1000 patients. The CBA, from the perspective of a single neurooncology center, suggests that mEHT is profitable and will generate a total revenue of €3,124,574 – $6,458,400 with total economic effect (economy + EBIT) of €5,700,034 – $8,237,432 per mEHT device over an 8 year period,. After confirmation of these findings, mEHT can be recommended as an enhancer for all ddTMZ regimens in the treatment of recurrent GBM, and, probably, for the regular 5/28d regimen. MEHT can be applied as a single treatment in those patients for which chemotherapy is impossible because of its toxicity or bad performance, and as a salvage treatment after the failure of chemotherapy, with a possibility to restore the patient's performance and chemosensitivity and subsequently continue chemotherapy.

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DATA SHARING STATEMENT

Patient level data are available in Supplement 3. Consent for data sharing was not obtained but the presented data are completely anonymised, and risk of identification is absent.

FIGURE LEGEND

Figure 1. Dose-escalating scheme of mEHT.
The tenth session attains the maximum escalation, the further sessions are the same.

Figure 2. CONSORT flowchart.
Note: White: Cohort of Interest (COI); Light grey: cohorts of Covariate Survival Analysis (CSA); Dark grey: cohorts out of analysis; Black: Analyses.

Figure 3. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A_1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

Figure 4. Survival (Kaplan-Meier estimate) since 1st mEHT session of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 5. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 6. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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Figure 7. Survival (Kaplan-Meier estimate) since 1st mEHT session of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

Figure 8. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

Figure 9. Cycles needed to treat per one life-month gained (CNTM) scale.

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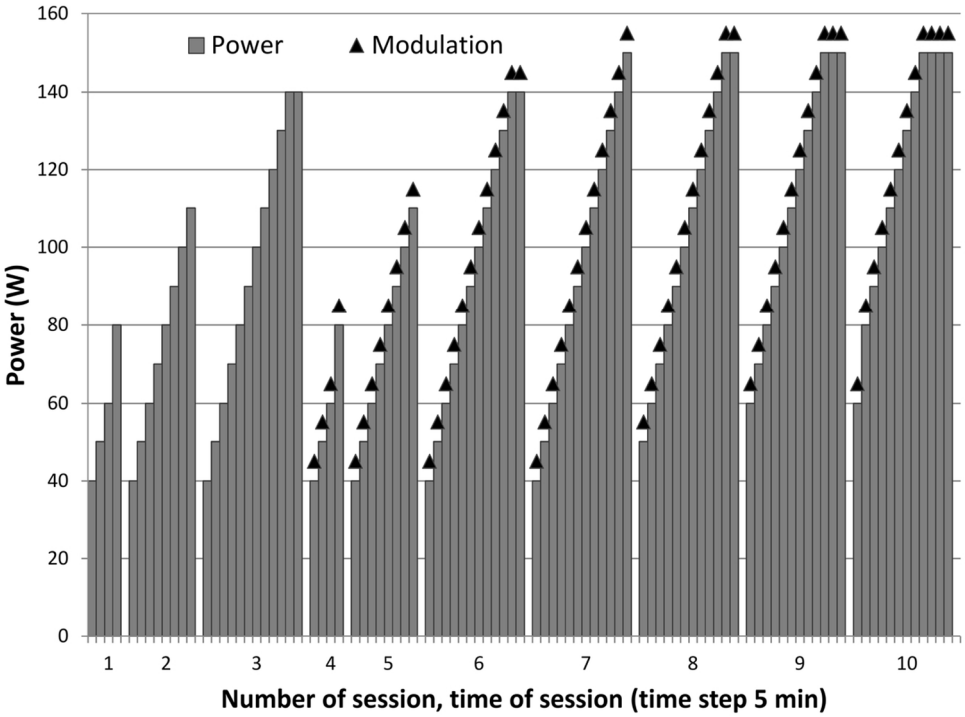

Figure 1. Dose-escalating scheme of mEHT.
The tenth session attains the maximum escalation, the further sessions are the same.

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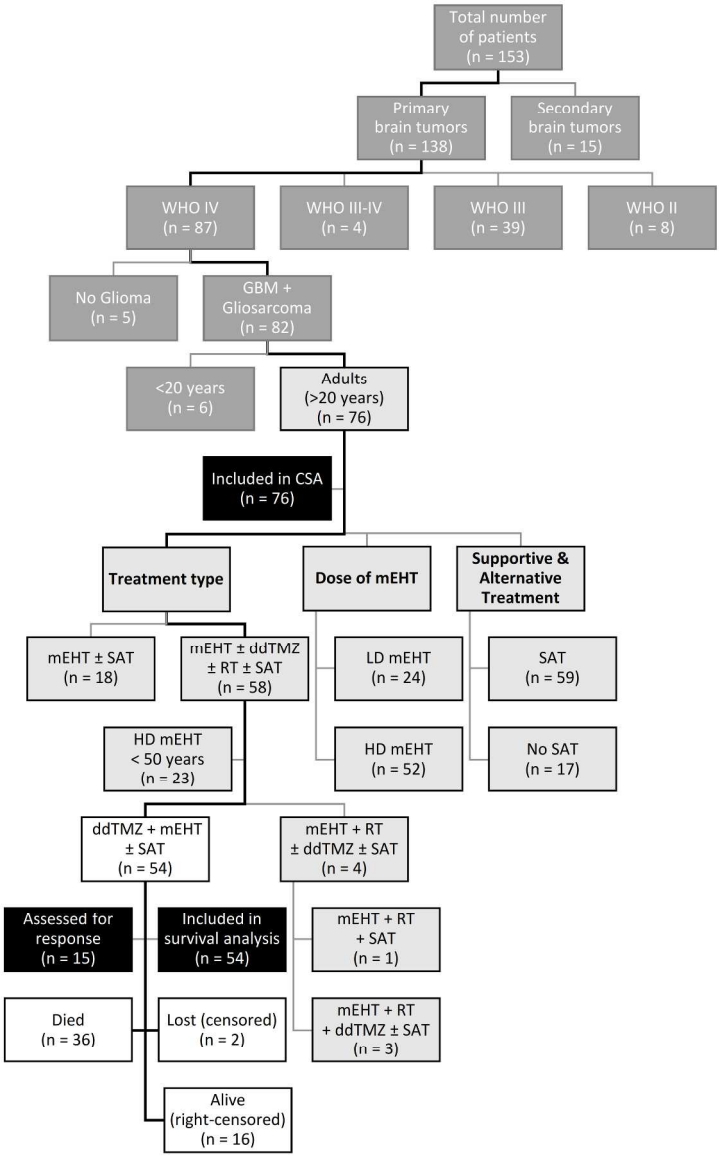

Figure 2. CONSORT flowchart.
Note: White: Cohort of Interest (COI); Light grey: cohorts of Covariate Survival Analysis (CSA); Dark grey: cohorts out of analysis; Black: Analyses.

229x359mm (300 x 300 DPI)

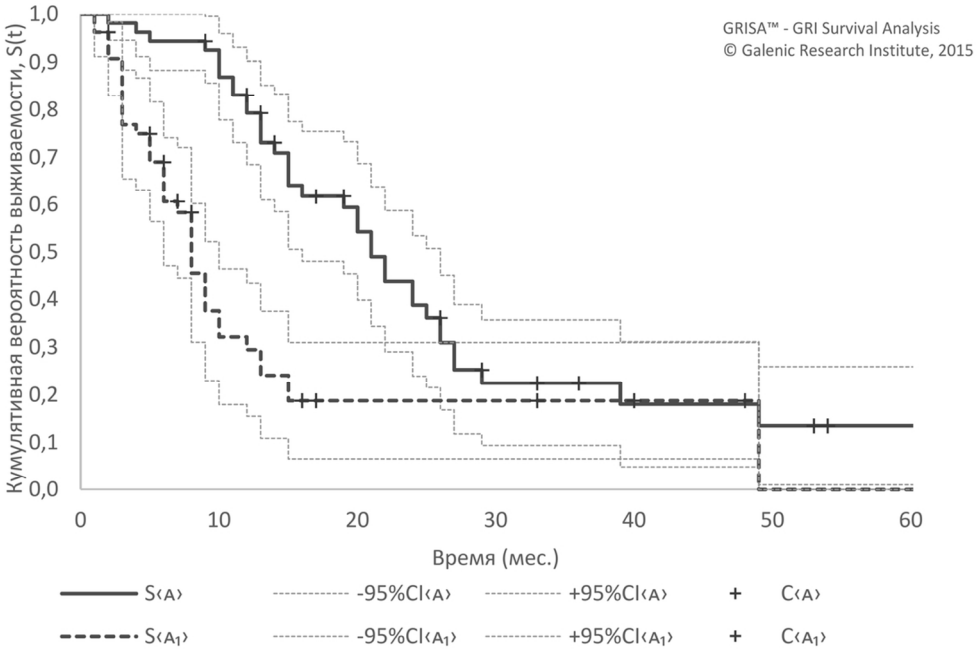

Figure 3. Kaplan-Meier survival function of the patients treated with ddTMZ + mEHT (n = 54) since diagnosis (A) and since 1st mEHT session (A1).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored.

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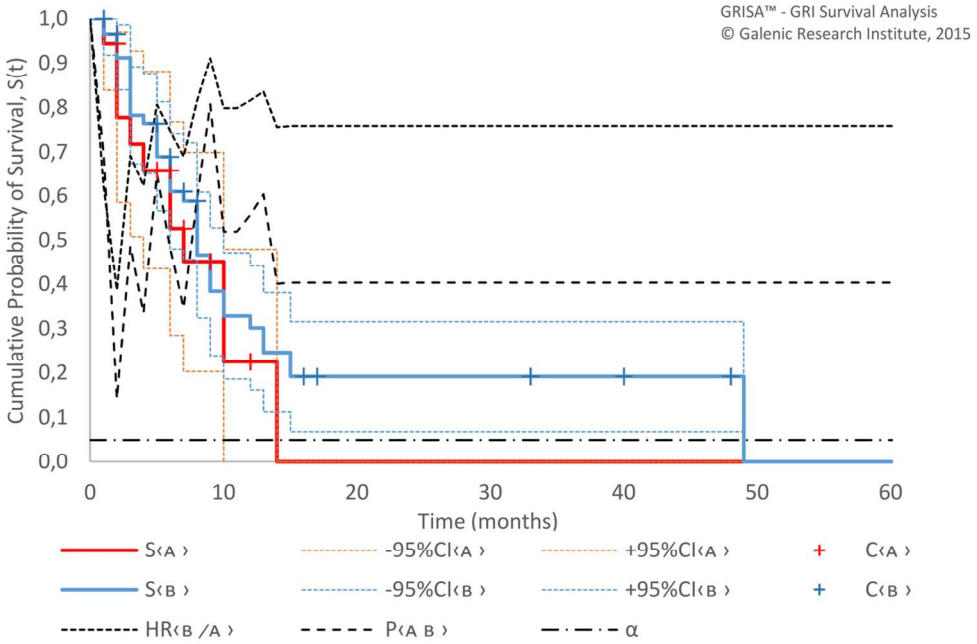

Figure 4. Survival (Kaplan-Meier estimate) since 1st mEHT session of "mEHT only" (A, n = 18) and combination treatment (B, n = 58) samples.
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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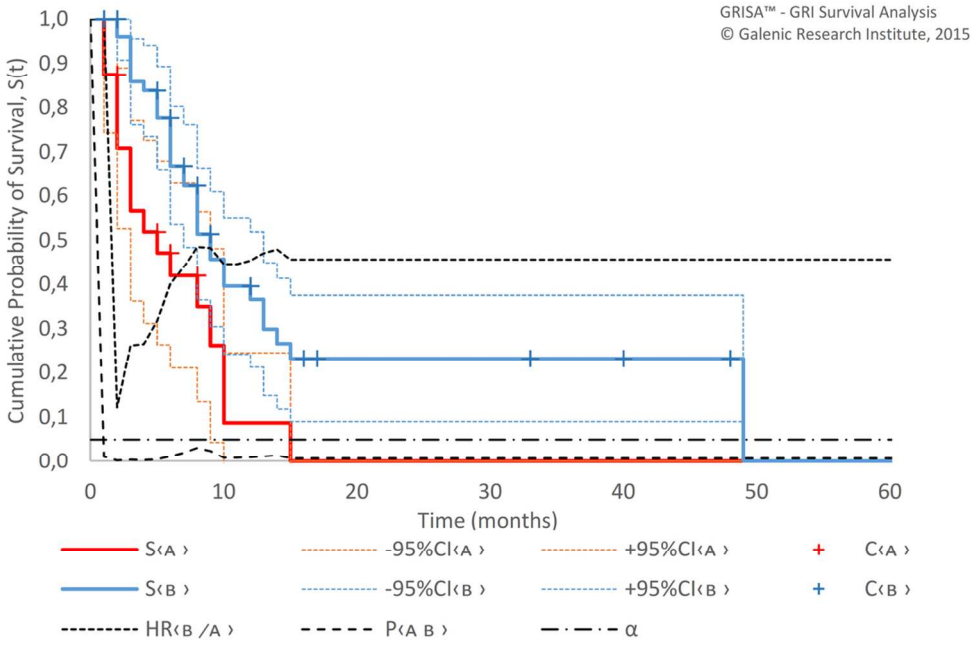

Figure 5. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients treated with low-dose mEHT (A, n = 24) and high-dose mEHT (B, n = 52).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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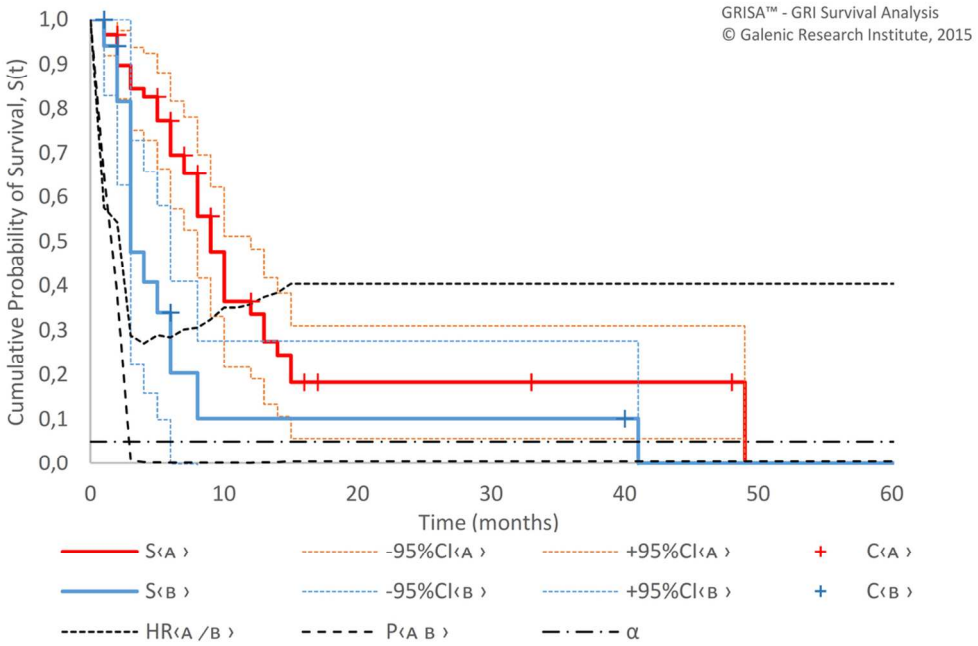

Figure 6. Survival (Kaplan-Meier estimate) since 1st mEHT session of patients with SAT (A, n = 59) and without SAT (B, n = 17).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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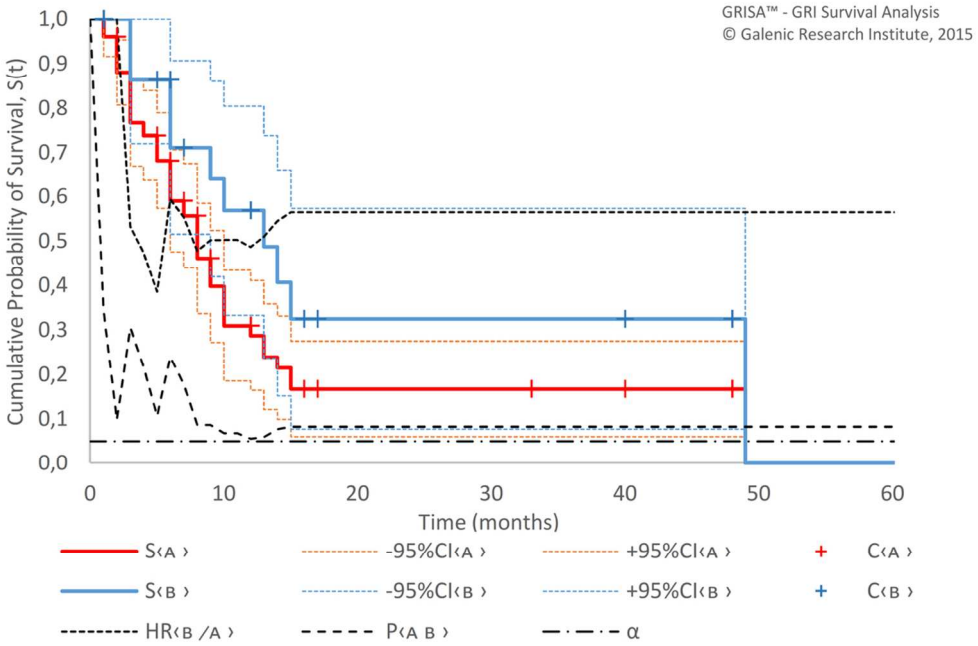

Figure 7. Survival (Kaplan-Meier estimate) since 1st mEHT session of all GBM patients (A, n = 76) and younger (<50 years) patients with high-dose mEHT (B, n = 23).
Note: S: survival function; 95%CI: 95% confidence interval; C: censored; HR: hazard ratio; P: p-value; α: probability of type I error.

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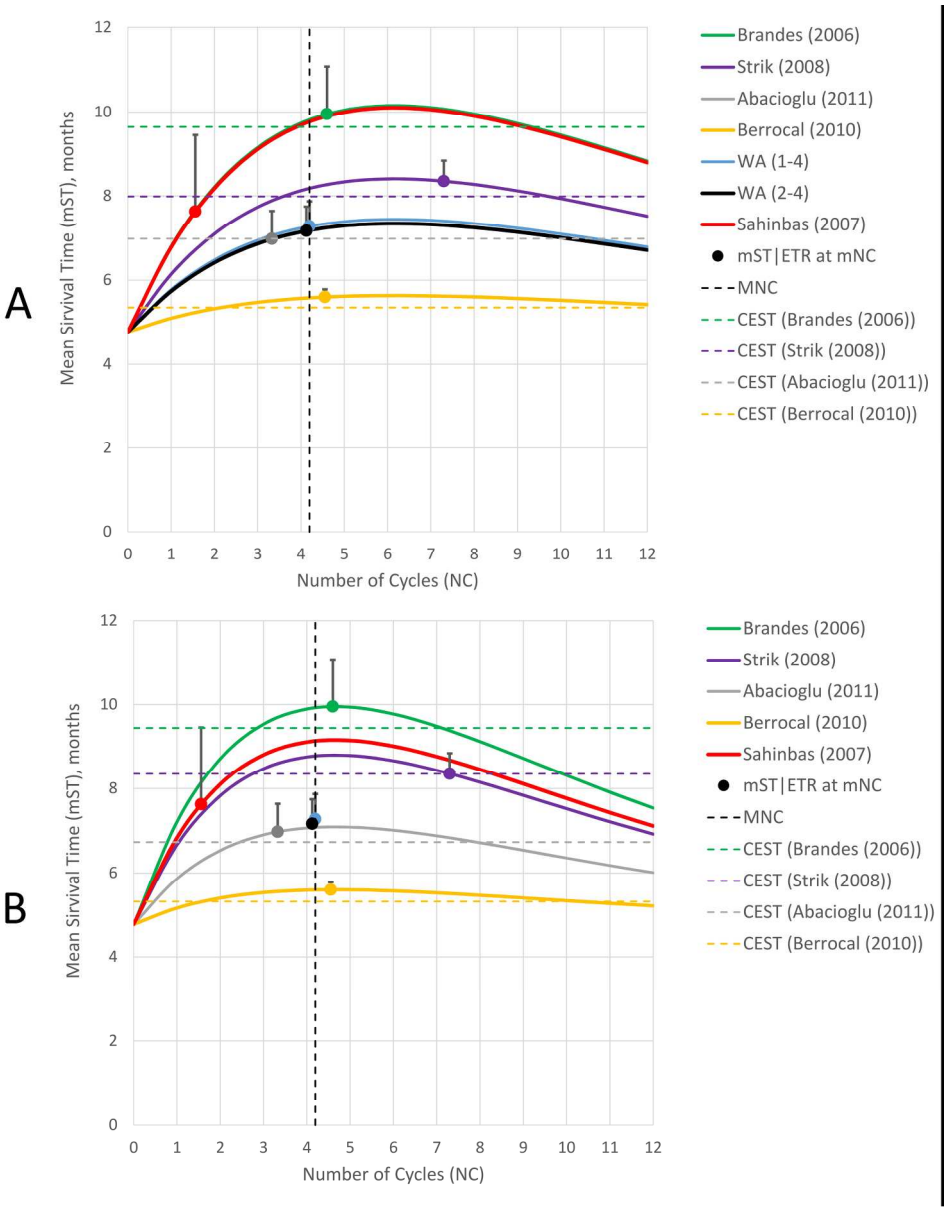

Figure 8. Effect-to-treatment analysis, attenuation modelling. (A) CA = 15.0%; (B) CA = 19.3%.
Note: MNC: median number of cycles; mNC: mean number of cycles; mST | ETR: dot – mean survival time (mST), line segment – effect-tretament ratio (ETR).

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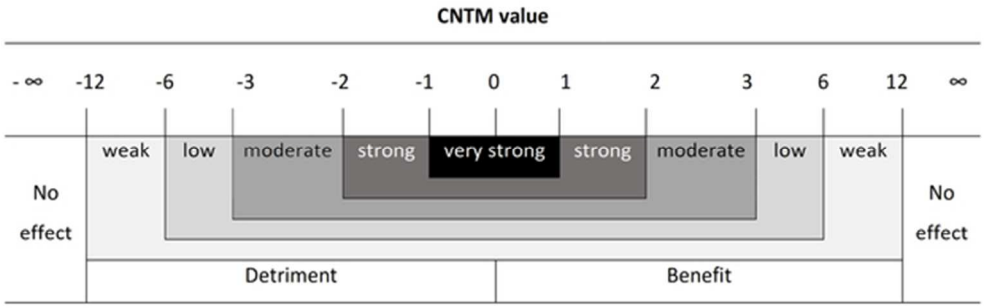

Figure 9. Cycles needed to treat per one life-month gained (CNTM) scale.

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Supplement 1

Estimating the mean and confidence interval from the median and confidence interval

This simplified algorithm is based on the idea that the mean value of a skewed dispersion is located in the center of the confidence interval of the median with displacement towards the median value proportional to the extent of the median value displacement (Figure S1). Thus,

$$= \frac{+\frac{(\quad " \quad)}{2}}{2}$$

where: m: mean; M: median; UL and LL: upper and lower limits of 95% CI of M.

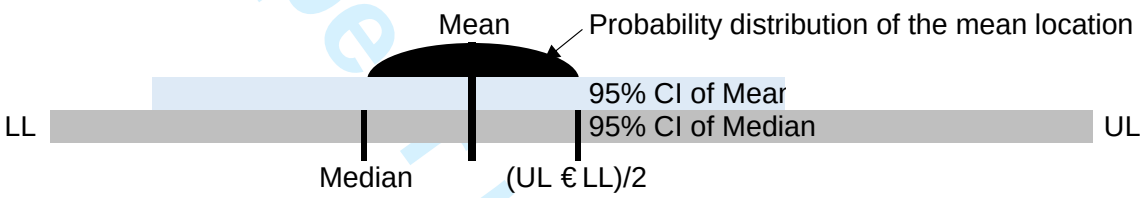

Figure S1. Graphic representation of the idea of the estimation of the mean.

Next, by the modelling on the sample of € 10-1000 random values (Excel RANDBETWEEN(18;85) function was used to mimic the distribution of adult (€ 18-85 years) patients in a clinical trial), it was revealed that 95% CI of the mean value of a sample is virtually always close to 60% of 95% CI (calculated according to Conover[1]) of the corresponding median value (mean of 100 readings, each repeated 10 times, coefficient of variation € 3,2%), independently of the sample size (range € 10-1000 subjects was tested) (Table S1).

Table S1. Results of modelling of 95% CI of mean to 95% CI of median ratio on different sample sizes (10-1000 subjects), mean value of n=100 readings of the ratio in each attempt.

	Sample size						Weighted
Attempt	10	25	50	100	1000	Average	Average
1	57,0%	61,8%	69,8%	57,9%	60,4%		
2	57,0%	63,0%	64,6%	61,1%	58,4%		
3	58,0%	60,5%	65,3%	63,4%	61,6%		
4	55,8%	61,7%	65,6%	61,5%	57,7%		

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Attempt	Sample size 10	25	50	100	1000	Average	Weighted Average
5	55,8%	61,5%	68,8%	62,8%	62,2%		
6	57,3%	59,4%	66,1%	60,2%	59,1%		
7	56,8%	60,9%	66,8%	63,6%	60,1%		
8	57,3%	63,8%	63,6%	62,8%	60,9%		
9	55,2%	63,3%	67,2%	62,2%	59,9%		
10	57,0%	61,7%	69,7%	60,9%	61,6%		
Mean	56,7%	61,8%	66,8%	61,6%	60,2%	61,4%	60,6%
SD	0,9%	1,3%	2,1%	1,7%	1,5%		
CV	1,5%	2,2%	3,2%	2,8%	2,4%		

Thus,

$$95\% = \pm\frac{0{,}6\times(\text{UL}-\text{LL})}{2}$$

where: m: mean; UL and LL: upper and lower limits of 95% CI of the median.

Checking of the algorithm on some sets of real data confirms its applicability. E.g., estimation of mean of temozolomide (TMZ) prices per mg from the median of 1.77 (95%CI: 1.14-2.21) returns mean of 1.72 (95%CI: 1.46-1.98) versus the actual mean of 1.7 (95%CI: 1.44-1.95), the error is 1.32-1.72%.

$$= \frac{1{,}77+\frac{2{,}11-1{,}24}{2}}{2} = 1{,}7225$$

$$95\% = 1{,}72\pm\frac{0{,}6\times(2{,}11-1{,}24)}{2} = [1{,}459-1{,}981]$$

Since we looked for simple and practical algorithm of translation, we consider such precision adequate both for clinical and economic evaluations.

[1] Conover WJ. Practical nonparametric statistics. 3rd ed. New York: Wiley; 1980: 592 p. ISBN 978-0-471-16068-7.

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Supplement 2

Estimation of the expected mean survival time

First, we defined the expected MOST as 13.65 months. This is a well-established point confirmed either by official SEER data and a reliable retrospective analysis[1]. Then, we defined that median progression-free survival after 1st line treatment based on the data of 9 cohorts of 6 independent trials (Table S1), equals 7.5 months, and it well corresponds with general opinion that GBM relapses in 6-9 months after diagnosis. To define the most problematic final parameter MST since relapse, we studied the inner structure of the survival time, namely time proportions between MOST, PFS and MST, on eight cohorts for which this information was available simultaneously (Table S2). Finally, we translated these data on the established MOST and MPFS and calculated the expected MST as 4.775 months (95%CI: 3.95-5.6) (Table S3).

Table S1. Median progression-free survival after standard 1-2 line treatment of GBM (WHO IV).

Study	Tumor, state	Treatment	MPFS m
Jungk (2016)[2]	GBM, recurrent/progressive	2M (mainly no CTX)	6,10
Reithmeier (2010)[3]	GBM, recurrent/progressive	3M (mainly TMZ)	8,72
Hamza (2014)[4]	GBM, recurrent/progressive	3M	8,10
Hamza (2014)[4]	GBM, recurrent/progressive	3M	7,60
Strik (2008)[5]	GBM, recurrent/progressive	3M Stupp	7,53
Chinot (2014)[6]	GBM, newly diagnosed	3M Stupp	6,20
Gilbert (2014)[7]	GBM, newly diagnosed	3M Stupp	7,30
Gilbert (2013)[8]	GBM, newly diagnosed	3M Stupp	7,50
Gilbert (2013)[8]	GBM, newly diagnosed	3M ddTMZ	8,80
Average			7,56

Note: CTX: chemotherapy; TMZ: temozolomide; 3M: trimodal (SRG + XRT + CTX); 2M: bimodal (no CTX); Stupp: 3M SRG + (XRT 60 Gy X6w + TMZ 5/7d X 6w) + TMZ 5/28d X 6m; ddTMZ: dose-dense TMZ.

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Table S2. Inner structure of survival time.

Study	Cohort	NOP	MOST	MPFS	MST	MST%	PFS+ MST	PFS+ MST%
Varkoniy (2003)	HGG	24	22,0	12,2	6,5	30%	18,7	85%
Sahinbas (2007)	GBM (all)	76	20,0	8,5	7,6	38%	16,1	80%
	GBM (mEHT)	18	14,8	8,0	6,4	43%	14,4	97%
	GBM mEHT+TMZ)	58	20,9	9,3	7,6	36%	16,9	81%
Jungk (2016)	GBM	34	15,7	6,1	8,7	56%	14,8	94%
Hamza (2014)	GBM (early BEV)	112	20,8	8,1	11,0	53%	19,1	92%
	GBM (late BEV)	133	25,9	7,6	9,9	38%	17,5	68%
Strik (2008)	GBM	18	17,9	8,2	9,1	51%	17,3	97%
	Weighted average		21,5	8,2	9,1	43%	17,3	82%
	95%CI					36,9%€ 48,8%		75,3%€ 88,8%

Note: NOP: number of patients; MOST: median overall survival time; MPFS: median progression free survival; MST: median survival time since relapse; PFS: progression free survival; HGG: high grade gliomas; GBM: glioblastoma; mEHT: modulated electro-hyperthermia; TMZ: temozolomide; BEV: bevacizumab; CI: confidence interval.

Table S3. Calculation of estimated mean survival time since relapse.

		95% CI	
	Mean	Lower limit	Upper limit	SE
MOST, months	13,65			
MPFS, months	7,5			
MPFS+MST (%)	82,0%	75,3%	88,8%	
MPFS+MST, months	11,2	10,3	12,1	
mST (1st estimation), months	3,7	2,8	4,6	
MST (%)	42,9%	36,9%	48,8%	

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		95% CI		
	Mean	Lower limit	Upper limit	SE
MST (2nd estimation), months	5,9	5,0	6,7	
mST (average), months	4,775	3,9	5,6	0,443

Note: MOST: median overall survival time; MPFS: median progression free survival; MST: median survival time since relapse.

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Supplement 3

Raw data old TMZ+mEHT cohort (n = 54)

No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminated Y/N	Objective response	Last contact	EXITUS
001	W	30.4.67	1.5.03	29.9.03	2	31	Y	Y	N	NA		30.3.04
002	M	5.1.59	1.10.03	7.1.04	1	8	Y	Y	Y	PD		5.4.05
003	M	6.9.68	8.7.04	8.9.04	1	9	Y	Y	Y	NA		14.10.04
004	M	29.7.61	15.4.04	18.10.04	1	9	Y	Y	N	SD	25.5.05	
005	M	20.7.36	13.11.00	20.8.01	1	5	Y	N	Y	NA		27.10.01
006	M	28.11.53	3.5.04	12.4.05	1	9	Y	Y	N	NA	25.5.05	
007	W	12.11.62	19.6.04	15.11.04	1	11	Y	Y	N	PR	25.5.05	
008	M	9.8.50	16.5.00	3.9.01	1	14	Y	N	N	NA		15.1.02
009	W	28.1.63	13.3.03	15.7.03	2	26	Y	Y	N	NA		10.1.04
010	W	28.1.63	1.3.03	15.7.03	2	27	Y	Y	N	NA		10.1.04
011	M	21.8.73	1.6.02	14.4.04	1	16	Y	N	N	NA		19.6.04
012	W	26.12.43	12.7.99	18.6.01	1	9	Y	N	N	NA		10.7.01
013	M	21.9.38	1.5.00	30.1.02	1	13	Y	Y	N	NA		11.6.02
014	M	17.7.69	25.5.04	2.2.05	1	6	Y	Y	Y	PD		2.3.05
015	M	29.3.61	1.3.04	2.4.04	1	14	Y	Y	N	NA		15.12.04
016	M	13.8.47	8.5.04	12.10.04	1	15	Y	Y	N	NA		27.5.05
017	W	3.4.75	17.2.01	19.7.04	1	8	Y	Y	Y	PD		4.3.05

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No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminatec Y/N	Objective response	Last contact	EXITUS
018	M	31.10.54	1.4.03	12.1.04	2	25	Y	Y	N	PD	5.5.05	
019	W	23.8.60	26.11.00	3.1.05	1	9	Y	Y	N	CR	25.5.05	
020	M	9.8.67	1.6.04	29.11.04	2	36	Y	Y	N	NA	25.5.05	
021	M	13.5.62	13.1.03	1.12.04	1	6	Y	N	Y	NA	25.5.05	
022	M	15.1.45	1.6.03	26.1.04	1	15	Y	Y	N	NA		7.8.04
023	M	15.3.45	1.6.04	19.4.05	1	15	Y	Y	N	NA	25.5.05	
024	W	22.11.35	1.10.03	19.11.03	1	8	Y	N	Y	NA		8.2.04
025	M	29.10.41	1.12.00	5.1.04	1	12	Y	Y	N	NA		12.2.04
026	M	20.1.49	1.12.02	13.7.04	2	21	Y	Y	N	NA		15.2.05
027	M	24.4.64	1.5.00	1.3.01	1	10	Y	N	N	NA		20.5.01
028	W	3.8.66	1.8.93	13.6.01	1	12	Y	Y	N	SD	25.5.05	
029	W	15.9.51	1.11.02	22.9.03	1	3	Y	Y	N	PD		4.7.04
030	M	14.4.51	1.11.03	21.9.04	1	11	Y	Y	N	NA		19.12.04
031	M	19.9.35	1.11.03	20.9.04	1	6	Y	Y	Y	NA		8.2.05
032	M	13.12.50	1.9.03	16.8.04	1	5	Y	Y	N	NA	11.10.04	
033	M	15.10.62	8.1.04	25.10.04	2	24	Y	Y	N	PR	25.5.05	
034	M	5.12.40	1.1.02	2.12.03	1	11	Y	Y	N	NA		1.3.04
035	M	2.11.71	30.8.04	4.1.05	2	18	Y	Y	N	NA	25.5.05	
036	M	24.5.39	1.1.02	21.1.02	1	46	Y	Y	N	NA		8.9.02
037	W	17.2.55	1.8.03	1.12.03	1	9	Y	Y	N	NA		27.8.04

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No	Sex	Birth Date	Date of Diagnosis	Date of 1st mEHT	Number of cycles	No of mEHT sessions	CTX Y/N	SAT Y/N	Terminatec Y/N	Objective response	Last contact	EXITUS
038	M	30.4.44	1.7.03	14.6.04	1	10	Y	N	N	PD		4.2.05
039	W	24.4.36	3.6.04	26.11.04	2	20	Y	Y	N	NA	27.5.05	
040	M	18.5.68	1.11.03	12.1.04	3	38	Y	Y	N	SD	27.5.05	
041	W	29.6.59	1.6.00	12.6.01	1	16	Y	N	N	NA	8.10.04	
042	W	9.12.64	1.4.02	27.5.02	3	44	Y	Y	N	NA		7.6.03
043	M	20.2.45	1.4.02	24.6.02	3	29	Y	Y	N	NA		6.6.03
044	M	29.9.57	1.12.99	23.10.01	1	9	Y	N	N	NA		16.4.02
045	W	15.11.38	1.1.03	6.1.03	1	17	Y	Y	N	NA		13.2.03
046	M	30.6.50	1.8.02	13.5.03	3	34	Y	Y	N	NA		28.5.04
047	M	20.11.40	1.9.02	6.1.04	3	36	Y	Y	N	SD	30.5.05	
048	W	3.8.44	1.3.03	18.11.03	1	6	Y	Y	N	NA		24.2.04
049	W	21.9.59	1.2.02	22.11.02	5	65	Y	Y	N	NA		2.2.04
050	W	4.1.40	15.1.03	15.8.04	1	15	Y	Y	N	PD		17.4.05
051	M	11.10.57	1.11.99	7.6.01	1	6	Y	N	N	NA		13.8.01
052	W	4.2.52	1.6.02	24.9.02	2	27	Y	Y	N	SD	30.5.05	
053	M	5.1.53	1.11.03	17.2.04	3	35	Y	Y	N	NA	30.5.05	
054	W	26.9.50	1.6.00	23.4.01	5	56	Y	Y	N	NA		9.2.02

Note: CTX: chemotherapy; SAT: supportive and alternative therapy; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease; NA: not available.

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STROBE Statement
Checklist of items that should be included in reports of ***cohort studies***
Title of work: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

	Item No	Recommendation	Check
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 1 line 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 7-12
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 13 lines 4-7
Methods			
Study design	4	Present key elements of study design early in the paper	Page 13 lines 17-18
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 13 lines 18-23
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 13 lines 25-33
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 13 lines 36-50 Page 13 lines 52 – page 15 line 16 Page 18 lines 20-25
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	☒
Bias	9	Describe any efforts to address potential sources of bias	Pages 34-36
Study size	10	Explain how the study size was arrived at	Page 13 lines 17-23
Quantitative variables	11	Explain how quantitative variables were handled in the analyses.	Page 18 lines 29-55
		If applicable, describe which groupings were chosen and why	Page 21 line 39 – page 23 line 23
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 18 lines 29 – page 19 line 9
		(*b*) Describe any methods used to examine subgroups and interactions	Page 19 lines 11-46
		(*c*) Explain how missing data were addressed	Page 18 lines 23-25
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable
		(*e*) Describe any sensitivity analyses	Page 20 lines 4-7

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			Page 24 line 51 – page 25 line 12 Page 27 line 27 – page 28 line 34
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 20 line 23
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 20 lines 21-53 Pages 62-66
		(b) Indicate number of participants with missing data for each variable of interest	Pages 67-68
		(c) Summarise follow-up time (eg, average and total amount)	Page 21 lines 13-15
Outcome data	15*	Report numbers of outcome events or summary measures over time	Page 21 lines 15-23
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page 21 lines 1-34
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Pages 21-29
Discussion			
Key results	18	Summarise key results with reference to study objectives	Pages 37-38
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Pages 34-36
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Pages 29-33
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 34 lines 1-22 Page 36 line 48 – page 37 line 7
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Not applicable

*Give information separately for exposed and unexposed groups.

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Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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CHEERS checklist—Items to include when reporting economic evaluations of health interventions

Title of study: **Clinical and economic evaluation of dose-dense temozolomide 21/28d regimen with and without concurrent modulated electro-hyperthermia in the treatment of recurrent glioblastoma: a retrospective comparison of cohort trials**

Section/item	Item No	Recommendation	Check
Title and abstract			
Title	1	Identify the study as an economic evaluation or use more specific terms such as "cost-effectiveness analysis", and describe the interventions compared.	Page 1 line 2
Abstract	2	Provide a structured summary of objectives, perspective, setting, methods (including study design and inputs), results (including base case and uncertainty analyses), and conclusions.	Page 5
Introduction			
Background and objectives	3	Provide an explicit statement of the broader context for the study.	Page 11 lines 11-28
		Present the study question and its relevance for health policy or practice decisions.	Page 13 line 13
Methods			
Target population and subgroups	4	Describe characteristics of the base case population and subgroups analysed, including why they were chosen.	Page 20 lines 23-35 Pages 62-68 Page 13 lines 19-23
Setting and location	5	State relevant aspects of the system(s) in which the decision(s) need(s) to be made.	Page 19 lines 52-53 Page 20 lines 8-11
Study perspective	6	Describe the perspective of the study and relate this to the costs being evaluated.	Page 37 lines 10-17
Comparators	7	Describe the interventions or strategies being compared and state why they were chosen.	Page 22 line 44 – page 23 line 23
Time horizon	8	State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.	Page 25 line 42 Page 28 line 40 Page 29 line 25
Discount rate	9	Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.	Page 28 lines 45-46 Page 29 line 15, 18-19, 23
Choice of health outcomes	10	Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.	Page 13 lines 36-50
Measurement of effectiveness	11a	*Single study-based estimates:* Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	Page 13 lines 15-33 Page 34 lines 31-45
	11b	*Synthesis-based estimates*: Describe fully the methods used for identification of included studies and synthesis of clinical effectiveness data.	Page 22 line 45 – page 23 line 23 Pages 69-72
Measurement and valuation of preference based outcomes	12	If applicable, describe the population and methods used to elicit preferences for outcomes.	Pages 20-21
Estimating resources and costs	13a	*Single study-based economic evaluation:* Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any	Page 25 lines 40-48 Page 32 lines 20-41

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Section/item	Item No	Recommendation	Check
		adjustments made to approximate to opportunity costs.	
	13b	*Model-based economic evaluation:* Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	Page 25 line 50 – page 26 line 18 Page 32 lines 4-18 Pages 76-80
Currency, price date, and conversion	14	Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.	Page 25 lines 50-57 Page 26 lines 1-12 Page 32 line 23
Choice of model	15	Describe and give reasons for the specific type of decision-analytical model used. Providing a figure to show model structure is strongly recommended.	Page 25 lines 50-57 Page 32 lines 4-18
Assumptions	16	Describe all structural or other assumptions underpinning the decision-analytical model.	Page 32 lines 4-18
Analytical methods	17	Describe all analytical methods supporting the evaluation. This could include methods for dealing with skewed, missing, or censored data; extrapolation methods; methods for pooling data; approaches to validate or make adjustments (such as half cycle corrections) to a model; and methods for handling population heterogeneity and uncertainty.	Pages 18-20
Results			
Study parameters	18	Report the values, ranges, references, and, if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. Providing a table to show the input values is strongly recommended.	Pages 20-21
Incremental costs and outcomes	19	For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.	Page 26 line 29 – page 26 line 25 Pages 77-80
Characterising uncertainty	20a	*Single study-based economic evaluation:* Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness parameters, together with the impact of methodological assumptions (such as discount rate, study perspective).	NA
	20b	*Model-based economic evaluation:* Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.	Pages 27-28
Characterising heterogeneity	21	If applicable, report differences in costs, outcomes, or cost-effectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects that are not reducible by more information.	Page 25 line 50 - Page 26 lines 12
Discussion			
Study findings, limitations,	22	Summarise key study findings and describe how they support the conclusions reached. Discuss limitations	Pages 37-38 Page 35 lines 18-39

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Section/item	Item No	Recommendation	Check
generalisability, and current knowledge		and the generalisability of the findings and how the findings fit with current knowledge.	Page 36 lines 15-18 Page 36 line 40 – page 37 line 7
Other			
Source of funding	23	Describe how the study was funded and the role of the funder in the identification, design, conduct, and reporting of the analysis. Describe other non-monetary sources of support.	Information provided via the submission system
Conflicts of interest	24	Describe any potential for conflict of interest of study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors recommendations.	Information provided via the submission system

For consistency, the CHEERS statement checklist format is based on the format of the CONSORT statement checklist

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