Clinical Neurology and Neurosurgery 196 (2020) 105890

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

The efficacy and safety of radiotherapy with adjuvant temozolomide for glioblastoma: A meta-analysis of randomized controlled studies

Yan Wang, Yimo Feng*

Department of Neurosurgery, Chongqing General Hospital, University of Chinese Academy of Science , Chongqing, PR China

ARTICLE INFO

Keywords:
Temozolomide
Radiotherapy
Glioblastoma
Randomized controlled trials

ABSTRACT

The efficacy of radiotherapy with adjuvant temozolomide for glioblastoma remains controversial. We conduct a systematic review and meta-analysis to explore the influence of radiotherapy with adjuvant temozolomide on treatment efficacy for glioblastoma.We search PubMed, EMbase, Web of science, EBSCO, and Cochrane library databases through November 24, 2019 for randomized controlled trials (RCTs) assessing the efficacy and safety of adjuvant temozolomide to radiotherapy for glioblastoma. This meta-analysis is performed using the random-effect model.Five RCTs are included in the meta-analysis. Overall, compared with radiotherapy for glioblastoma, adjuvant temozolomide is associated with substantially improved overall survival (HR = 0.63; 95% CI = 0.52–76; P < 0.00001) and 2-year survival rate (3.25 = 1.76; 95% CI = 2.13–4.94; P < 0.00001), with no increase in adverse events (RR = 0.76; 95% CI = 0.40–1.45; P = 0.41). However, adjuvant temozolomide appears to increase the incidence of haematological complications than only radiotherapy (RR = 3.58; 95% CI = 1.10–11.59; P = 0.03).Adjuvant temozolomide to radiotherapy may provide better efficacy for the treatment of glioblastoma.

1. Introduction

Glioblastoma is known as the most common malignant primary brain tumor in adults [1–3]. Median time of survival after diagnosis is estimated not to exceed 12 months, and most patients would die within 24 months [4,5]. The current standard treatment of glioblastoma is maximally radical surgical excision of the tumor followed by post-operative radiotherapy [6]. Various chemotherapy combined with surgery and radiotherapy are used for the treatment of glioblastoma [6–9].

Despite the development of surgical techniques, radiotherapy and various chemotherapies, high-grade glioblastoma still has poor prognosis and improvement of survival is modest [10,11]. Temozolomide is an oral alkylating agent, a derivative of imidazotetrazine with a broad spectrum of antineoplastic activity [12,13]. In patients with glioblastoma recurrence and treatment failure by other therapies, temozolomide has shown some efficacy [14]. The concomitant use of temozolomide and radiotherapy may decrease active clonogenic cells more efficiently than only radiotherapy. Synergic action of radiotherapy and temozolomide was observed during human glioma cell cultures. Adjunctive temozolomide to radiotherapy may delay the recurrence and prolong the progression-free period [6].

Current evidence is insufficient for routine clinical use of adjuvant temozolomide to radiotherapy for glioblastoma. Recently, several studies have investigated the efficacy and safety of adjuvant temozolomide to therapy for these patients, but the results are conflicting [6,15,16]. This systematic review and meta-analysis of RCTs aims to assess the efficacy and safety of adjuvant temozolomide to radiotherapy for glioblastoma.

2. Materials and methods

This systematic review and meta-analysis are performed based on the guidance of the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement and Cochrane Handbook for Systematic Reviews of Interventions [17,18]. No ethical approval and patient consent are required because all analyses are based on previous published studies.

2.1. Literature search and selection criteria

We systematically search several databases including PubMed, EMbase, Web of science, EBSCO, and the Cochrane library from inception to November 24, 2019 with the following keywords: temozolomide, and radiotherapy, and glioblastoma. The reference lists of retrieved studies and relevant reviews are also hand-searched and the

* Corresponding author at: No. 104 Pibashan Street, Yuzhong district, Chongqing 400500, PR China.
E-mail addresses: 731779068@qq.com (Y. Wang), kongkong20180707@163.com (Y. Feng).

https://doi.org/10.1016/j.clineuro.2020.105890
Received 25 December 2019; Received in revised form 27 February 2020; Accepted 1 May 2020
Available online 26 May 2020

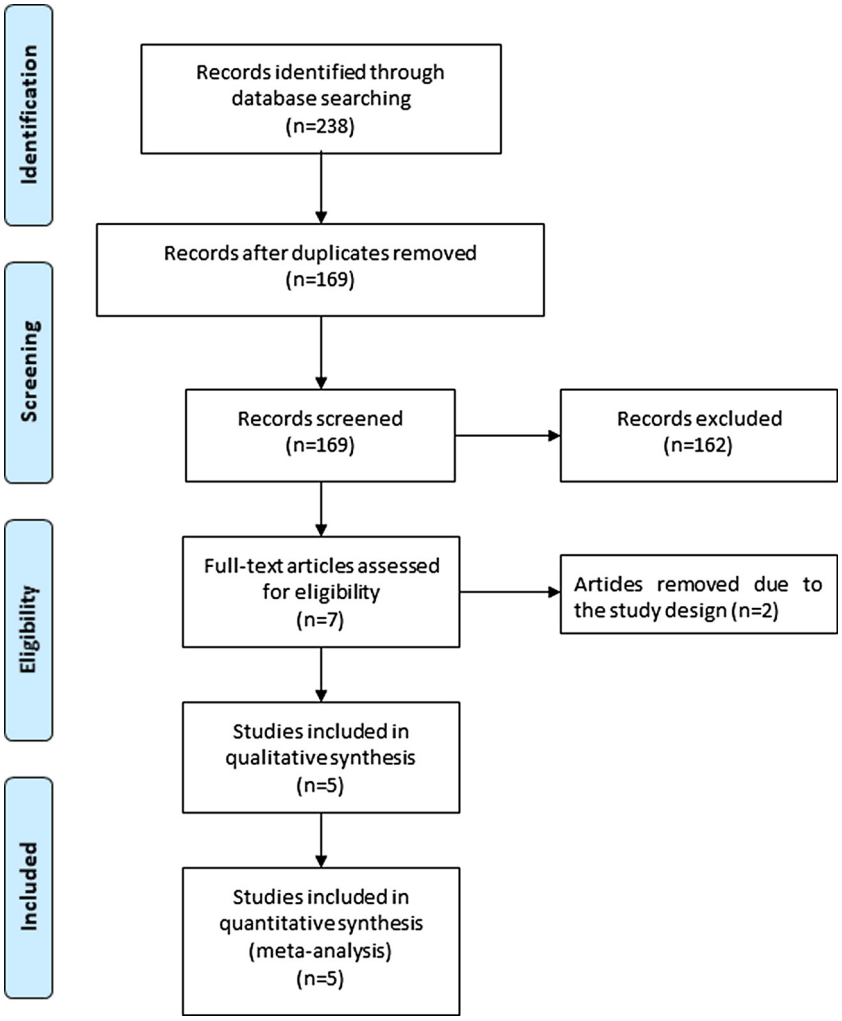

Fig. 1. Flow diagram of study searching and selection process.

process above is performed repeatedly in order to include additional eligible studies.

The inclusion criteria are presented as follows: (1) study design is RCT, (2) patients are newly diagnosed as glioblastoma, and (3) intervention treatments are adjuvant temozolomide to radiotherapy versus only radiotherapy.

2.2. Data extraction and outcome measures

Some baseline information is extracted from the original studies, and they include first author, number of patients, age, World Health Organization (WHO) grade, complete resection and detail methods in two groups. Data are extracted independently by two investigators, and discrepancies are resolved by consensus. We have contacted the corresponding author to obtain the data when necessary.

The primary outcome is overall survival. Secondary outcomes include 2-year survival rate, adverse events and haematological complications.

2.3. Quality assessment in individual studies

The methodological quality of each RCT is assessed by the Jadad Scale which consists of three evaluation elements: randomization (0–2 points), blinding (0–2 points), dropouts and withdrawals (0–1 points) [19]. One point would be allocated to each element if they have been conducted and mentioned appropriately in the original article. The score of Jadad Scale varies from 0 to 5 points. An article with Jadad score ≤ 2 is considered to be of low quality. The study is thought to be of high quality if Jadad score ≥ 3 [20].

2.4. Statistical analysis

We assess risk ratios (RR) or hazard ratio (HR) with 95% confidence interval (CI) for dichotomous outcomes (overall survival, 2-year survival rate, adverse events and haematological complications). Heterogeneity is evaluated using the I^2 statistic, and $I^2 > 50\%$ indicates significant heterogeneity [21]. The random-effects model is used for all meta-analysis. We search for potential sources of heterogeneity for significant heterogeneity. Sensitivity analysis is performed to detect the influence of a single study on the overall estimate via omitting one study in turn or performing the subgroup analysis. Owing to the limited number (< 10) of included studies, publication bias is not assessed. Results are considered as statistically significant for $P < 0.05$. All statistical analyses are performed using Review Manager Version 5.3 (The Cochrane Collaboration, Software Update, Oxford, UK).

3. Results

3.1. Literature search, study characteristics and quality assessment

Fig. 1 shows the detail flowchart of the search and selection results. 238 potentially relevant articles are identified initially. Finally, three

Table 1
Characteristics of included studies.

No.	author	Temozolomide group						Control group						Jada scores
		Number	Age	Female (n)	WHO grade (0/1/2)	Complete resection (n)	Methods	Number	Age	Female (n)	WHO grade (0/1/2)	Complete resection (n)	Methods	
1	Perry 2016	281	73 (range 65 − 90)	–	–	–	Radiotherapy plus monthly adjuvant temozolomide until progression	281	73 (range 65 − 90)	–	–	–	Only radiotherapy	3
2	Szczepanek 2013	28	55 (range 18 − 65)	10	11/15/2	18	Radiotherapy in 2 Gy fractions daily five times a week up to the total of 60 Gy over 6 weeks of treatment plus temozolomide, initially in the dose of 200 mg/m 2 during 5 postoperative days and after 23 days followed by 75 mg/m 2 of body surface area daily, 7 days a week	30	56 (range 20 − 68)	14	14/12/2	18	Only radiotherapy	4
3	Athanassiou 2005	57	11 (≤50 years)/42 (> 50 years)	21	–	10	Temozolomide 75 mg/m 2 /d orally, concomitantly with RT (60 Gy in 30 fractions), followed by six cycles of temozolomide (150 mg/m 2 on days 1 through 5 and 15–19 every 28 days)	53	9 (≤50 years)/48 (> 50 years)	19	–	8	Only radiotherapy	5
4	Cohen 2005	287	55 (range 18 − 70)	103	116/136/36	116	Radiation therapy plus six cycles of temozolomide, 150 or 200 mg/m2 daily for 5 days, every 4 weeks	286	56 (range 23 − 70)	109	112/136/34	113	Only radiotherapy	3
5	Stupp 2004	287	55 (range 18 − 70)	103	116/136/36	116	Radiation therapy plus six cycles of temozolomide, 150 or 200 mg/m2 daily for 5 days, every 4 weeks	286	56 (range 23 − 70)	109	112/136/34	113	Only radiotherapy	3

WHO, World Health Organization.

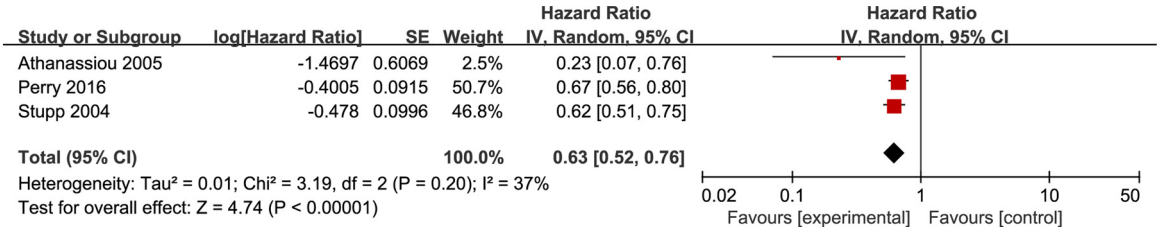

Fig. 2. Forest plot for the meta-analysis of overall survival.

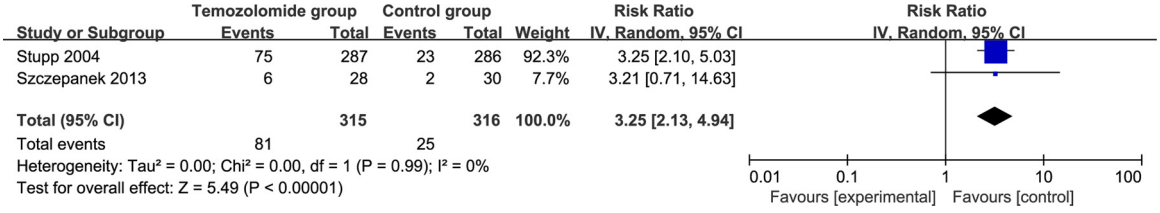

Fig. 3. Forest plot for the meta-analysis of 2-year survival rate.

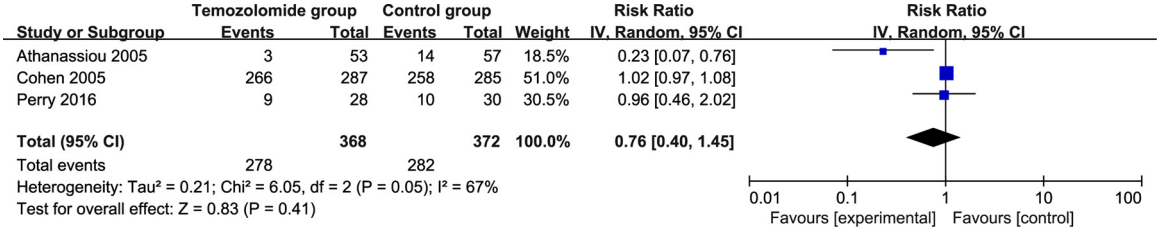

Fig. 4. Forest plot for the meta-analysis of adverse events.

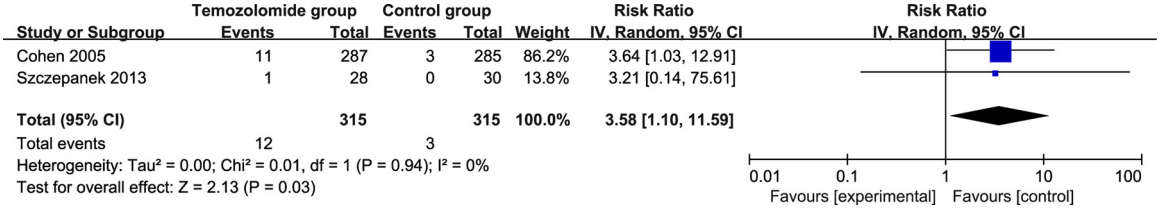

Fig. 5. Forest plot for the meta-analysis of haematological complications.

RCTs and two abstracts are included in the meta-analysis [6,15,16,22,23].

The baseline characteristics of five included RCTs are shown in Table 1. These studies are published between 2004 and 2016, and the total sample size is 1303. Among the included RCTs, two studies report different outcomes of the same patient sample [16,23]. Temozolomide at different doses serves as the adjunctive therapy to radiotherapy.

Three studies report overall survival [15,22,23], two studies report 2-year survival rate [6,23], three studies report adverse events [15,16,22] and two studies report haematological complications [6,16]. Jadad scores of the five included studies vary from 3 to 5, and all five studies have high-quality based on the quality assessment.

3.2. Primary outcome: overall survival

The random-effect model is used for the analysis of primary outcome. The results find that compared to control group for glioblastoma, adjuvant temozolomide is associated with substantially increased overall survival (HR = 0.63; 95% CI = 0.52–76; P < 0.00001), with low heterogeneity among the studies (I^2 = 37%, heterogeneity P = 0.20, Fig. 2).

3.3. Sensitivity analysis

There is low heterogeneity for overall survival, and thus we do not perform sensitivity analysis by omitting one study in each turn to detect the source of heterogeneity.

3.4. Secondary outcomes

In comparison with radiotherapy for glioblastoma, adjuvant temozolomide can significantly improve 2-year survival rate (3.25 = 1.76; 95% CI = 2.13–4.94; P < 0.00001; Fig. 3), but demonstrates no substantial impact on adverse events (RR = 0.76; 95% CI = 0.40–1.45; P = 0.41; Fig. 4). Adjuvant temozolomide appears to result in the increase in haematological complications than only radiotherapy (RR = 3.58; 95% CI = 1.10–11.59; P = 0.03; Fig. 5).

4. Discussion

Many researches have been conducted to combine new chemotherapy with radiotherapy for neoplasms, and the results reveal the improvement of efficacy [4,24–26]. Patients with additional chemotherapy have prolonged time of survival [27,28]. Temozolomide has been introduced to clinical treatment for glia-derived brain neoplasms [25,29]. It can substantially inhibit the activity of O^6-methylguanine-

DNA methyltransferase (MGMT) which plays important role in DNA repair [30]. These provide the theoretical support for the reported association between low activity of MGMT in tumor cells and the longer survival of patients with glioblastoma multiforme treated with additional regimens of chemotherapy with nitrosourea derivatives [31,32].

Our meta-analysis suggests that adjuvant temozolomide to radiotherapy can substantially improve overall survival and 2-year survival rate for glioblastoma patients as compared to only radiotherapy. In addition, in one RCT involving 562 glioblastoma patients, adjuvant temozolomide therapy (3 weeks of concomitant temozolomide plus monthly adjuvant temozolomide until progression or 12 cycles) can significantly improve progression-free survival (median 5.3 m versus 3.9 m, HR 0.50, 95% CI = 0.41–0.60, $p < 0.0001$) than radiotherapy [15]. One recent meta-analysis aimed to explore the molecularly targeted drugs as the adjunctive therapy to radiotherapy plus temozolomide for newly diagnosed glioblastoma, and the results revealed that the addition of molecularly targeted drugs could increase the progression-free survival and adverse events [33].

Methylation of the MGMT promoter can preclude transcription (i.e. production of MGMT), and induce the repair in TMZ-alkylated DNA [4]. This may improve the efficacy of temozolomide and increase the survival of patients with gliomas. In contrast, patients who lack methylation of the MGMT promoter maybe have poor prognosis [4]. In MGMT methylated patients, overall survival for temozolomide plus radiotherapy versus radiotherapy was 13.5 m and 7.7 m respectively (HR: 0.53 (95% CI = 0.38–0.73, $p = 0.0001$). In MGMT unmethylated patients, overall survival for temozolomide plus radiotherapy versus radiotherapy was 10.0 m vs 7.9 m respectively (HR 0.75 (95% CI = 0.56–1.01, $p = 0.055$). These indicated that patients with MGMT methylated tumors benefited the most from the addition of temozolomide plus radiotherapy and median overall survival was nearly doubled [15]. In both subgroups of patients with IDH mutant type (IDHmt) and IDH wild type (IDHwt) of lower grade gliomas, progression-free survival was reported to be similar between patients treated with radiotherapy and temozolomide [34]. In addition, patients with IDHmt tumors demonstrated better prognosis than those with IDHwt tumors [34,35].

There are similar adverse events between adjunctive temozolomide and radiotherapy in this meta-analysis, but the haematological complications appear to be higher than only radiotherapy for glioblastoma patients. These haematological complications are generally tolerated [6,16]. Several limitations exist here. Firstly, our analysis is based on only five RCTs, and more RCTs with large sample size should be conducted to explore this issue. Next, different doses of drugs and treatment methods may have some influence on the pooling results. Finally, among the five included RCTs, only one RCT is published after 2016, and involves patients with histologically confirmed newly diagnosed glioblastoma, Eastern Cooperative Oncology Group (ECOG) 0–2 [15]. No information about WHO classification of brain tumors is reported. Treatment recommendations before 2016 for glioblastoma are different from those after 2016 based on the WHO classification of brain tumors [9,36], which may produce some bias to influence the pooling analysis.

5. Conclusion

Adjuvant temozolomide may have additional efficacy for glioblastoma patients.

Research involving human participants and/or animals

Not applicable.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

[1] J.D. Lathia, S.C. Mack, E.E. Mulkearns-Hubert, C.L. Valentim, J.N. Rich, Cancer stem cells in glioblastoma, Genes Dev. 29 (12) (2015) 1203–1217.

[2] J. Wang, E. Cazzato, E. Ladewig, V. Frattini, D.I. Rosenbloom, S. Zairis, F. Abate, Z. Liu, O. Elliott, Y.-J. Shin, Clonal evolution of glioblastoma under therapy, Nat. Genet. 48 (7) (2016) 768.

[3] Z. Qian, L. Ren, D. Wu, X. Yang, Z. Zhou, Q. Nie, G. Jiang, S. Xue, W. Weng, Y. Qiu, Overexpression of FoxO3a is associated with glioblastoma progression and predicts poor patient prognosis, Int. J. Cancer 140 (12) (2017) 2792–2804.

[4] R. Stupp, M.E. Hegi, B. Neyns, R. Goldbrunner, U. Schlegel, P.M. Clement, G.G. Grabenbauer, A.F. Ochsenbein, M. Simon, P.-Y. Dietrich, Phase I/IIa study of cilengitide and temozolomide with concomitant radiotherapy followed by cilengitide and temozolomide maintenance therapy in patients with newly diagnosed glioblastoma, J. Clin. Oncol. 28 (16) (2010) 2712–2718.

[5] J. Frenel, M. Botti, D. Loussouarn, M. Campone, Prognostic and predictive factors for gliomas in adults, Bull. Cancer 96 (4) (2009) 357–367.

[6] D. Szczepanek, A. Marchel, M. Moskała, M. Krupa, P. Kunert, T. Trojanowski, Efficacy of concomitant and adjuvant temozolomide in glioblastoma treatment. A multicentre randomized study, Neurol. Neurochir. Pol. 47 (2) (2013) 101–108.

[7] P. Kattner, L. Nonnenmacher, K. Buljovcic, S. Bartholomä, K. Zeiler, G. Karpel-Massler, M. Halatsch, M. Westhoff, K. Debatin, Combination therapy as a potential strategy in glioblastoma treatment, Klin. Pädiatrie 230 (06) (2018) 1.

[8] T. Kazda, A. Dziacky, P. Burkon, P. Pospisil, M. Slavik, Z. Rehak, R. Jancalek, P. Slampa, O. Slaby, R. Lakomy, Radiotherapy of glioblastoma 15 years after the landmark Stupp's trial: more controversies than standards? Radiol. Oncol. 52 (2) (2018) 121–128.

[9] L. Nayak, D.A. Reardon, High-grade gliomas, Contin. Lifelong Learn. Neurol. 23 (6) (2017) 1548–1563.

[10] G.M.-a.T.G. Group, Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient data from 12 randomised trials, Lancet 359 (9311) (2002) 1011–1018.

[11] C. Nieder, M. Mehta, R. Jalali, Combined radio-and chemotherapy of brain tumours in adult patients, Clin. Oncol. 21 (7) (2009) 515–524.

[12] Y. He, W.P. Roos, Q. Wu, T.G. Hofmann, B. Kaina, The SIAH1–HIPK2–p53ser46 damage response pathway is involved in temozolomide-induced glioblastoma cell death, Mol. Cancer Res. 17 (5) (2019) 1129–1141.

[13] A. Omuro, K. Beal, K. McNeill, R.J. Young, A. Thomas, X. Lin, R. Terziev, T.J. Kaley, L.M. DeAngelis, M. Daras, Multicenter phase IB trial of carboxyamidotriazole orotate and temozolomide for recurrent and newly diagnosed glioblastoma and other anaplastic gliomas, J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 36 (17) (2018) 1702–1709.

[14] R. Stupp, M. Gander, S. Leyvraz, E. Newlands, Current and future developments in the use of temozolomide for the treatment of brain tumours, Lancet Oncol. 2 (9) (2001) 552–560.

[15] J.R. Perry, N. Laperriere, C.J. O'Callaghan, A.A. Brandes, J. Menten, C. Phillips, M.F. Fay, R. Nishikawa, J.G. Cairncross, W. Roa, D. Osoba, A. Sahgal, H.W. Hirte, W. Wick, F. Laigle-Donadey, E. Franceschi, O.L. Chinot, C. Winch, K. Ding, W.P. Mason, A phase III randomized controlled trial of short-course radiotherapy with or without concomitant and adjuvant temozolomide in elderly patients with glioblastoma (CCTG CE.6, EORTC 26062-22061, TROG 08.02, NCT00482677), J. Clin. Oncol. 34 (Suppl 18) (2016) LBA2–LBA2.

[16] M.H. Cohen, J.R. Johnson, R. Pazdur, Food and Drug Administration Drug approval summary: temozolomide plus radiation therapy for the treatment of newly diagnosed glioblastoma multiforme, Clin. Cancer Res. 11 (19) (2005) 6767–6771.

[17] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, P. Group, Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement, BMJ 339 (2009) b2535.

[18] G. Higgins JPT, Cochrane Handbook for Systematic Reviews of Interventions Version 5.1. 0 [updated March 2011], The cochrane collaboration, 2011.

[19] A.R. Jadad, R.A. Moore, D. Carroll, C. Jenkinson, D.J.M. Reynolds, D.J. Gavaghan, H.J. McQuay, Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control. Clin. Trials 17 (1) (1996) 1–12.

[20] L.L. Kjaergard, J. Villumsen, C. Gluud, Reported methodologic quality and discrepancies between large and small randomized trials in meta-analyses, Ann. Intern. Med. 135 (11) (2001) 982–989.

[21] J.P. Higgins, S.G. Thompson, Quantifying heterogeneity in a meta-analysis, Stat. Med. 21 (11) (2002) 1539–1558.

[22] H. Athanassiou, M. Synodinou, E. Maragoudakis, M. Paraskevaidis, C. Verigos, D. Misailidou, D. Antonadou, G. Saris, K. Beroukas, P. Karageorgis, Randomized phase II study of temozolomide and radiotherapy compared with radiotherapy alone in newly diagnosed glioblastoma multiforme, J. Clin. Oncol. 23 (10) (2005) 2372–2377.

[23] R. Stupp, W.P. Mason, M.J. Van Den Bent, M. Weller, B. Fisher, M. Taphoorn, A.A. Brandes, G. Cairncross, D. Lacombe, R.O. Mirimanoff, Concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) for newly diagnosed glioblastoma multiforme (GBM). Conclusive results of a randomized phase III trial by the EORTC Brain & RT Groups and NCIC Clinical Trials Group, J. Clin. Oncol. 22 (Suppl 14) (2004) 2–2.

[24] T. Weiss, H. Schneider, M. Silginer, A. Steinle, M. Pruschy, B. Polić, M. Weller, P. Roth, NKG2D-dependent antitumor effects of chemotherapy and radiotherapy against glioblastoma, Clin. Cancer Res. 24 (4) (2018) 882–895.

[25] R. Stupp, M.E. Hegi, W.P. Mason, M.J. van den Bent, M. Taphoorn, R.C. Janzer, S.K. Ludwin, A. Allgeier, B. Fisher, K. Belanger, European organisation for research and treatment of cancer brain tumour and radiation oncology groups; national

cancer institute of Canada clinical trials group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial, Lancet Oncol. 10 (5) (2009) 459–466.

[26] J. Evans, D. Gillespie, S. Erridge, L. Vale, A. Kernohan, R. Grant, T.A. Lawrie, Long-term side effects of radiotherapy, with or without chemotherapy, for glioma, Cochrane Database Syst. Rev. 2018 (6) (2018).

[27] R. Stupp, M. Hegi, Recent developments in the management of maligant glioma, J. Clin. Oncol. (2003) 779–788.

[28] M. Glas, C. Happold, J. Rieger, D. Wiewrodt, O. Bähr, J.P. Steinbach, W. Wick, R. Kortmann, G. Reifenberger, M. Weller, Long-term survival of glioblastoma patients treated with radiotherapy and lomustine plus temozolomide, J. Clin. Oncol. 27 (8) (2009) 1257–1261.

[29] R. Stupp, W.P. Mason, M.J. Van Den Bent, M. Weller, B. Fisher, M.J. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, N. Engl. J. Med. 352 (10) (2005) 987–996.

[30] A. Tolcher, S. Gerson, L. Denis, C. Geyer, L. Hammond, A. Patnaik, A. Goetz, G. Schwartz, T. Edwards, L. Reyderman, Marked inactivation of O 6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules, Br. J. Cancer 88 (7) (2003) 1004.

[31] M. Esteller, J. Garcia-Foncillas, E. Andion, S.N. Goodman, O.F. Hidalgo, V. Vanaclocha, S.B. Baylin, J.G. Herman, Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents, N. Engl. J. Med. 343 (19) (2000) 1350–1354.

[32] M.E. Hegi, A.-C. Diserens, S. Godard, P.-Y. Dietrich, L. Regli, S. Ostermann, P. Otten, G. Van Melle, N. De Tribolet, R. Stupp, Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide, Clin. Cancer Res. 10 (6) (2004) 1871–1874.

[33] W.-L. Wang, N. Aru, Z. Liu, X. Shen, Y.-M. Ding, S.-J. Wu, H.-H. Qin, W.-Y. Jin, Prognosis of patients with newly diagnosed glioblastoma treated with molecularly targeted drugs combined with radiotherapy vs temozolomide monotherapy: A meta-analysis, Medicine 98 (45) (2019).

[34] B.G. Baumert, M.E. Hegi, M.J. van den Bent, A. von Deimling, T. Gorlia, K. Hoang-Xuan, A.A. Brandes, G. Kantor, M.J. Taphoorn, M.B. Hassel, Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033-26033): a randomised, open-label, phase 3 intergroup study, Lancet Oncol. 17 (11) (2016) 1521–1532.

[35] H. Suzuki, K. Aoki, K. Chiba, Y. Sato, Y. Shiozawa, Y. Shiraishi, T. Shimamura, A. Niida, K. Motomura, F. Ohka, Mutational landscape and clonal architecture in grade II and III gliomas, Nat. Genet. 47 (5) (2015) 458.

[36] D.N. Louis, A. Perry, G. Reifenberger, A. Von Deimling, D. Figarella-Branger, W.K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World Health Organization classification of tumors of the central nervous system: a summary, Acta Neuropathol. 131 (6) (2016) 803–820.