[Frontiers in Bioscience 11, 502-505, January 1, 2006]

Phase I study of temozolomide and lomustine in the treatment of high grade malignant glioma

Salvatore Tafuto, Paolo Muto, Anna Tortoriello, Agata Pisano, Pasquale Comella, Roberta Formato, Stefano Quattrin and Rosario Vincenzo Iaffaioli

Department of Medical Oncology, "S. Maria delle Grazie" Hospital, Pozzuoli; O.C.U. of Radiotherapy, Ascalesi Hospital, Naples; Department of Medical Oncology, National Cancer Institute, Naples, Italy

TABLE OF CONTENTS

1. ABSTRACT

Systematic reviews and meta-analysis have demonstrated an improved prognosis by chemotherapy of malignant glioma patients. The effects of clinical research therefore have the aim to find more active drugs or new combination therapies. The combination of Temozolomide (TMZ) and nitrosoureas was evaluated preclinically with an evidence of therapeutic synergy. Based on these findings, we have carried out a phase I study with TMZ administered in low, prolonged doses of 75 mg/m^2 per day, once a day for 21 days, escalated in cohorts of 3 patients, in combination with a fixed dose of Lomustine (CCNU) 100 mg/m^2 orally on day 1. MTD was evident. The treatment was generally well tolerated. We did not observe bleeding or severe infections, as described for several combination chemotherapies with TMZ and other agents. In this study, for the first time in high grade malignant glioma, two orally administrated drugs were associated .TMZ 75 mg/m^2 for 28 consecutive days and CCNU 100 mg/m^2 on day 1 of every 6 weeks could be recommended as a safe treatment dosage. One of the ten patients evaluated for clinical response showed a partial response, while nine showed stability of disease, with a median duration of from 5 to 6 months.

2. INTRODUCTION

Although it is universally accepted that standard treatment of high grade gliomas is based on surgery and radiation therapy (1), many researchers now think that chemotherapy should be included in high grade, malignant glioma (HHG) treatment. Recent systematic reviews and meta-analysis data have demonstrated that chemotherapy can improve HGG patient's prognosis. These evaluations were made after observing that a combination of procarbazine, lomustine and vincristine (PCV) or a nitrosoureas treatment were associated with a significant increase in survival, offering an absolute improvement of 6% at 1 year and 5% at 2 years for glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA) respectively, with 17% reduction in the risk of progression or death. (2-3).

With this results, the efforts of clinical research to find more active drugs or new combination therapies have been continued. As previously demonstrated in recent studies, temozolomide (TMZ) used as single agent showed greater antitumoral activity then PCV, with an increase of the overall response rate and progression free survival, preserving and improving the quality of life (QoL) (4-5).

Table 1. Patient characteristics

Median age (years) (range)	52 (43-70)
Sex ratio (M:F)	3 (9:3)
Histopathological diagnosis (%)	
- Glioblastoma multiforme	9 (75%)
- Anaplastic Astrocytoma	3 (25%)
Prior treatment (%)	
- Macroscopically complet resection	7 (58%)
- Partial resection	3 (25%)
- Biopsy	2 (16%)
- Radiotherapy	12 (100%)
- Chemotherapy	0
Associated treatment	
- Anti-edema	12 (100%)
- anticonvulsants	10 (83%)

Table 2. Dose levels escalation

Dose level 1 : CCNU 100mg/m^2 d 1 + TMZ 75 mg/m^2 d 1 -> 21, q 4ws
Dose level 2 : CCNU 100mg/m^2 d 1 + TMZ 75 mg/m^2 d 1 -> 28, q 6ws
Dose level 3 : CCNU 100mg/m^2 d 1 + TMZ 100 mg/m^2 d 1 -> 28, q 6ws

However, the results obtained with TMZ could be improved; the therapeutic activity of TMZ is, in fact, reduced by the development of drug resistance. High levels of a repair enzyme, O^6-alkylguanine DNAalkyl transferase (AGAT), in glioblastoma cell lines are correlated to lower TMZ cytotoxicity *in vitro*. It does appear as if the additive or synergistic activity of combination therapy and/or the extended/prolonged administration of the drug could improve the effectiveness of single agent TMZ treatment (6-7).

TMZ is a second-generation alkylating agent that has shown activity in recurrent human malignant gliomas. It is completely absorbed after oral administration with almost 100% bioavailability and easily penetrates in the central nervous system (CNS) . TMZ is well tolerated with a favourable toxicity profile (8-9).

Nitrosoureas are highly lipophilic drugs that readily cross the blood-brain barrier and achieve effective concentrations in the CNS that spontaneously convert to the methyl-(1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) (methylCCNU) and than degrade at physiologic pH to a reactive and cytotoxic agents, with alkilating properties. Lomustine (CCNU) is a structural analogue of Carmustine (BCNU). It is an integral part of the commonly used procarbazine, CCNU, and vincristine combination chemotherapy regimen (PCV) against malignant glioma (10).

The combination of TMZ and nitrosoureas was evaluated preclinically. These experimental results indicated a therapeutic synergy when TMZ is combined with nitrosoureas (11-13). The administration of prolonged low doses of TMZ may deplete AGAT levels, thus reducing the probability of development of primary resistance to TMZ (14-15).

Based on these findings, the primary endpoint of the phase I study was to determine the dose limiting toxicity (DLT) and maximum tolerated dose (MTD), the safety and the feasibility of a new treatment schedule of TMZ administered in low prolonged doses with a fixed dose of CCNU. The secondary objective was to evaluate the preliminary data on the efficacy of this combination therapy.

3. MATERIAL AND METHODS

Eligibility criteria included a histopathology diagnosis of GBM, AA, age $\geq$ 18 years, patients in recurrent or progressive disease. Table 1 shows the characteristics of the patients.

Disease recurrence or progression was defined with McDonald criteria, with an increase in tumour size on MRI scan or axial tomography. Patients with a life expectancy of less than three months and ECOG performance status > 1 were excluded. At least one month from last treatment (surgery and radiation therapy) was required before starting the TMZ treatment. Patients were required to have absolute neutrophil count $\geq$ 1000 neutrophil/mm^3, platelet count $\geq$ 100.000 platelet/ mm^3 , haemoglobin levels $\geq$ 10 gr/dL, serum creatinine and bilirubin levels $\leq$ 1,5 times the upper limit of normal (ULN), serum levels of aspartate and alanine aminotransferase $\leq$ 3 times the ULN, and serum alkaline phosphatase $\leq$ 3 times the ULN. Concurrent malignancies (except basal or squamous cell carcinoma of the skin) and severe co-morbidities were reasons of ineligibility. Informed consent was obtained from all participating subjects. The study was approved by the institutional ethic committee. All patients received a fixed dose of Lomustine 100 mg/m^2 orally on day 1. Patients also received TMZ starting at 75 mg/m^2 per day, once a day for 21 days. The dose of TMZ at 75 mg/m^2 was escalated in cohorts of 3 patients by extending the number of days and increasing the doses (table 2).

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria version 2.0 . The efficacy evaluation was not a primary aim of the study. However we evaluated the responses by McDonald criteria (16,17), performing an MRI scan every two months from start.

4. RESULTS

Twelve patients (8 GBM and 4 AA) were recruited for the present study. It was possible to evaluate drug-related toxicity in all patients. As reported above, none of them had been previously treated with chemotherapy.

Seven patients were in relapse after a three – six months interval from previous surgery and standard radiation therapy. Four patients were in progression disease after about two-four months interval from previous cytoreduction and radiation treatment. One patient was considered ineligible for surgery and was in progression

Table 3. Toxicity (NCI-CTC Version 2.0)

	Grade 1	**2**	**3**	**4**
Neutropenia	3	3	4	2
Thrombocytopenia	6	2	3	1
Anemia	0	0	1	1
Nausea	4	2	0	0
Vomiting	1	1	0	0
ALT	2	0	0	0
Infection	0	0	0	0
Costipation	0	4	0	0
Fatigue	5	3	0	0

disease after radiation palliative radiotherapy. All patients were treated with low doses of dexamethasone, ten patients received also anticonvulsivant therapy.

No DLT was observed in the cohorts at dose level 1 and dose level 2. Notably, grade 1-2 vomiting for few days after day one and transient neutropenia were observed and at level 3, thrombocytopenia grade 2..

At level 3, two of six patients showed DLT because of grade 4 neutropenia lasting ten days and one of these patients had thrombocytopenia which lasted for more than 14 days. In other study where TMZ was tested with other agents, we have often found Pneumocystis carinii pneumonia. Never we observed bleeding and/or infections. Table 3 shows the patient toxicities.

With regard to non-hemathological DLT, only two patients showed a transient grade 1-2 increase in ALT serum levels. Therapeutic response was evaluated in 10 patients; one patient showed partial response and nine stability of disease.

5. DISCUSSION

The rationale of our study was based on several biological and clinical studies that suggested the antitumoral activity of nitrosoureas and TMZ as a single agent and in combination regimen. Although these molecules are both alkylating agents, they showed synergistic activity on *in vitro* glioma cell culture systems when administered together. The primary endpoint of our phase I study was to determine the DLT, the MTD, safety and feasibility of a new schedule of TMZ administered in low prolonged doses with a fixed dose of CCNU: the secondary endpoint was to evaluate the possible activity of this multiagent regimen. We also choose to escalate the TMZ doses by increasing the number of days of treatment, according to Raja et al. who recently showed that the administration of TMZ, in prolonged, low doses may deplete the AGAT level, reducing the probability of primary TMZ resistance development. We enrolled twelve recurrent patients, affected by high-grade glioma (8 GBM, 4AA). They received a total of 40 courses of TMZ + CCNU.

At dose level 1 and dose level 2, we did not observe DLT. The treatment was well tolerated and grade 1-2 vomiting and/or hematologic toxicity were recorded. The main toxicity in 15 courses in patients at lower dose levels consisted of grade 1-2 vomiting, constipation, neutropenia and thrombocytopenia. On the contrary, we discontinued the treatment in three out six dose level 3 patients because of grade 4 hematologic toxicities (neutropenia and thrombocytopenia). In these patients, granulocyte-colony stimulating factor administration for five days or more was needed and the treatment was continued at lower doses. We did not observe bleeding or severe infections, the latter, in particular, being a common adverse effect of several combination chemotherapies with TMZ and other agents (18-19).

In conclusion, as per our experience, TMZ 75 mg/m^2 for 28 consecutive days and CCNU 100 mg/m^2 day1 every 6 weeks could be recommended as a safe treatment dosage. At these doses, the addition of CCNU does not substantially modify the tolerability of TMZ with respect to the standard single agent TMZ schedule (TMZ 200 mg/m^2/day x 5 days). Moreover, the dose-intensity of schedule we recommend is higher in comparison to standard TMZ doses.

One of the ten patients evaluated for clinical response showed a partial response, while nine showed stability of disease, with a median duration of from 5 to 6 months. These data, although not conclusive about efficacy, seem to confirm the safety and the activity, with a satisfactory control of disease, of a TMZ + CCNU combination regimen. We also stress the fact that TMZ and CCNU is first combination therapy in the HGG where both drugs are orally administered drugs. This might be very important in the treatment strategy and in the QoL improvement of HGG. Now a Phase II trial of this combination at safe dose to prove efficacy in HGG patients is ongoing.

6. ACNOWLEDGMENTS

We acknowledge substantial contribution of our collegue and friend Gianvincenzo Barba, M.D. for statistical and linguistic support.

7. REFERENCES

Simpson JR, J. Horton, C. Scott, W.J. Curran, P. Rubin, J. Fischbach, S. Isaacson, M. Rotman, S.O. Asbell, J.S. Nelson Influence of location and extent resection on survival of patients with glioblastoma multiforme: results of three consecutive RTOG clinical trials. *INT J Radiat Oncol Biol Phys*, 26:239-244 (1993)

Prados MD, C. Scott, W.J. Jr Curran, D.F.Nelson, S. Leibel , S. Kramer: Procarbazine, lomustine, and vincristine (PCV) for anaplastic astrocytoma: a retrospective review of radiation therapy Oncology group protocols comparing survival with carmustine or PCV adjuvant chemotherapy. *J Clin Oncol*, 17: 3389-3395 (1999)

Stewart L.A.: Chemotherapy in adult high-grade glioma: a systematic review and meta-analysis of individual patient

data from 12 randomized trials. *Lancet*, 359:1011-1018 (2002)

Brandes AA, M. Ermani, U. Basso, M.K. Paris, F.Lumachi, F. Berti, P. Amista, M. Gardiman, P. Iuzzolino, S.Turazzi & S. Monfardini S.: Temozolomide in patients with glioblastoma at second relapse after first line nitrosourea-procarbazine failure: a phase II study. *Oncology*, 63: 38-41 (2002)

Brandes AA, F.Vastola, U. Basso, F. Berti, G. Pinna, A. Rotilio, M. Gardiman., R. Scienza, S. Monfardini & M. Ermani: A prospective study on glioblastoma in the elderly. *Cancer*, 97: 657-662 (2003)

Friedman HS, R.E. McLendon, T. Kerby, M. Dugan , S.H. Bigner,A.J. Henry, D.M. Ashley, J. Krischer, S. Lovell, K. Rasheed, F. Marchev, A.J. Seman , I. Cokgor , J. Rich, E. Stewart , O.M. Colvin , J.M. Provenzale, D.D. Bigner, M.M. Haglund, A.H. Friedman & P.L. Modrich ::DNA mismatch repair and O^6alkylguanine DNA alkyltransferase analysis and response to temodal in newly diagnosed malignant glioma. *J Clin Oncol* 16: 3851 – 3857 (1998)

Brock CS, E.S. Newlands, S.R. Weige, M. Bower, H. Evans, I. Colquhoun, M, Roddie, M. Glaser, M.H. Brampton, G.J. & Rustin GJ: Phase I Trial of Temozolomide using an extended continuous oral schedule. *Cancer Res.* 58, 4363-4367 (1998)

Friedman HS, M.E. Dolan, A.E. Pegg, S. Marcelli, S. Keir, J.J. Catino, D.D. Bigner & S.C. Jr Schold: Activity of temozolomide in the treatment of central nervous system tumour xenografts. *Cancer Res* 55:2853-2857 (1995)

Yung WK, R.E. Albright, J. Olson, R. Fredericks, K. Fink, M.D. Prados, M. Brada, A. Spence , R.J. Hohl, W. Shapiro, M. Glantz, H. Greenberg, R.G. Selker, N.A. Vick, R. Rampling, H. Friedman, P. PhillipS, J. Bruner, N. Yue, D. Osoba, S. Zaknoen & V.A. Levin: A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *British J Canc* 83, 588-593 (2000)

Levin VA, P. Silver, J. Hannigan, W.M. Wara, P.H. Gutin, R.L. Davis, C.B. Wilson: Superiority of post-radiotherapy adjiuvant chemotherapy with CCNU, procarbazine and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. *Int J Radiat Oncol Biol Phys* 18:321-324 (1990)

Plowman J, W.R. Waud, A.D. Koutsoukos, L.V. Rubinstein, T.D. Moore & M.R. Grever: Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chlorethyl)-1-nitrosourea. *Cancer Res.* 54, 3793-3799 (1994)

Rohn TA, B. Wagenknecht,W. Roth, U.Naumann , E. Gulbins, P.H. Krammer, M. Walkz Weller:CCNU-dependent potentiation of TRAIL/Apo2L-induced apoptosis in human glioma cells is p53-independent but may involve enhanced cytochrome c release. *Oncogene* 20(31):4128-37 (2001)

Friedman HS, S. Keir, A.E. Pegg,P.J. Houghton, O.M. Colvin, R.C. Moschel, D.D. Bigner, M.E. Dolan**:** O^6-Benzylguanine-mediated enhancement of chemotherapy. *Molecular Cancer Therapy* 1, 943-948 (2002)

Khan RB, J.J. Raizer, M.G. Malkin, K.A. Bazylewicz, L.E. Abrey: A phase II study of extended low-dose temozolomide in recurrent malignant gliomas. *Neurooncology* 4(1):39-43 (2002)

Schold SC Jr, J.G. Kuhn, S.M. Chang, M.E. Bosik, H.I. Robins, M.P. Mehta, A.M. Spence, D. Fulton, K.L. Fink & Prados MD : A phase I trial of 1,3-bis-(-2-chloroethyl)-1-nitrosourea plus temozolomide: A North American Brain Tumor Consortium Study. *Neuro-Oncology* 2: 34-39 (2000)

National Cancer Institute. Common toxicity criteria, version 2.0. In: Common toxicity criteria manual. Bethesda: National Cancer Center Institute Cancer Therapy Evaluation Program, (1999)

Macdonald DR, T.L. Cascino, S.C. Jr Schold, J.G. Cairncross: Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 8: 1277-1280 (1990)

Schiff D.: Pneumocystis pneumonia in brain tumor patients: risk factors and clinical features. *J Neurooncol* 27:235-240, (1996)

Korones DN, M. Benita-Weiss, T.E. Coyle, L. Mechtler, p. Bushunow, B. Evans, D.A. Reardon, J.A. Quinn, H. Friedman.: Phase I study of Temozolomide and escalating doses of oral Etoposide for adults with recurrent malignant glioma. *Cancer* 97(8),1963-1968 (2003)

Chang SM, M.D. Prados, W.K. Yung, H. Fine, L. Junck, H. Greenberg , H.I. Robins, M. Mehta, K.L.Fink, K.A. Jaeckle, J. Kuhn, K. Hess & C. Schold : Phase II of neoadjuvant 1,3-bis (2-chloroethyl)-nitrosourea and temozolomide for new diagnosed anaplastic glioma: a North American Brain Tumor Consortium Trial. *Cancer* 100(8):1712-6 (2004)

Key Words: High Grade Glioma, Temozolomide, Lomustine, Nitrosoureas, Phase I Study, Combination Chemotherapy

Send correspondence to: Salvatore Tafuto, MD, Division of Haematology Oncology, Department of Medical Oncology,"S. Maria delle Grazie" Hospital - ASL Na2 Via Domitiana - Pozzuoli, 80072 – Naples, Italy. Tel: 39 081 8552363; Fax: 39 081 8552286, E-mail: salvatore.tafuto@libero.it

http://www.bioscience.org/current/vol11.htm