Phase II Trial of the Association of a Methionine-free Diet with Cystemustine Therapy in Melanoma and Glioma

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Abstract. *Background: In a previous phase I clinical trial of dietary methionine (MET) restriction with cystemustine treatment for melanoma or glioma, we determined the optimal MET-free diet duration to be 1 day. On this basis, a phase II clinical trial was initiated to evaluate safety and efficacy of this combination. Patients and methods: Twenty-two patients (20 with metastatic melanoma and 2 with recurrent gloma) received a median of 4 cycles of the association of a 1-day MET-free diet with cystemustine (60 mg/m^2) every two weeks. Results: This association was well tolerated (toxicity and nutritional status). Toxicity remained mainly hematological and consisted of WHO grade 3-4 thrombocytopenia, leucopenia and neutropenia in 36, 27 and 27% of patients respectively. The median disease-free survival was 1.8 months and the median survival was 4.6 months, with 2 long-duration stabilizations. The plasmatic MET depletion obtained was of 40±31%.*

Among the few effective chemotherapy agents used in the treatment of glioma and melanoma, chloroethylnitrosoureas (CENUs), such as carmustine (BCNU), fotemustine, nimustine (ACNU) and cystemustine, have documented activity in metastatic malignant melanoma and in recurrent glioma, with an objective response rate of 10-25% (1-4). However, their clinical usefulness has been limited both by their toxicity and chemoresistance mainly due to the 'suicide' DNA repair protein O^6-methylguanine-methyltransferase (MGMT) (5).

In contrast with normal cells, studies have revealed that numerous human tumor cells are characterized by their methionine (MET) dependency (6-9). This metabolic difference has been extensively studied and offers interesting perspectives for targeting tumor cells (10). MET depletion induces many modifications in tumor cells, including cell arrest in the S and G_2 phases of the cell cycle, apoptosis, decrease of glutathione (GSH) content and reduced activity of MGMT (11, 12).

Furthermore, since the alterations induced by MET restriction may sensitize tumors to alkylating agents, numerous preclinical studies have tested the association of MET restriction and CENUs. These studies have demonstrated that MET depletion substantially improves the therapeutic index of CENUs (11-14). The combination of MET stress and CENU treatment increases sensitivity to BCNU and cystemustine in human brain tumors xenografted into athymic mice and in B16 melanoma tumors grafted into syngenic mice, respectively (12, 14).

To evaluate the potential synergistic effect of the association of MET restriction and nitrosourea treatment in cancer patients, first we carried out a phase I clinical trial of dietary MET restriction with cystemustine in 10 patients (metastatic melanoma or recurrent glioma) to determine the optimal duration of the MET-free diet. With a good tolerance of the association (acceptability of the diet, nutritional status), the optimal plasmatic MET depletion (41%) was obtained on the first day of the diet (15).

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Based on these results, we then initiated a phase II clinical trial. The aim of the current study was to estimate the safety of the association of a 1-day dietary MET restriction with cystemustine therapy (toxicity and nutritional status) and its activity, defined by the determination of the response rate and the median survival. The plasma MET depletion was also explored.

Patients and Methods

Patients. Patients presenting with histological proof of metastatic melanoma or recurrent high-grade glioma were enrolled in this study. Eligibility criteria included at least one measurable metastatic lesion as evaluated by gadolinium-enhanced magnetic resonance imaging (MRI) or computed tomography (CT) scan, an estimated life expectancy of ≥2 months, a World Health Organization (WHO) performance status of 0-2, and age >18 years. Patients with comorbidity factors that required dietary restriction (*i.e.* diabetes, celiac disease) or who were unable to meet nutritional requirements were excluded. All patients provided written informed consent for the trial. The study protocol was reviewed and approved by local ethical committees.

Treatment schedule. Patients received 4 cycles of cystemustine every two weeks as conventionally used (4, 16, 17) associated with a MET-free diet. Each patient had to receive at least 4 cycles of treatment to be assessed for response evaluation and at least 1 cycle for the assessment of other studied parameters (toxicity, plasma MET concentration, and nutritional status). At each cycle, during the hospitalization period, patients received a standard diet on the first day (D0) and the MET-free diet on the following day (D1). Cystemustine treatment was administered at 12 a.m. on the day of MET-free diet.

Cystemustine was administered *i.v.* at 60 mg/m^2, being infused for 15 min in 100 ml of 5% dextrose. The treatment plan consisted of one administration every 2 weeks. In the event of a granulocyte count below 1,500/μl or a platelet count below 100,000/μl on the day before the forecast date of cystemustine administration, the treatment was delayed for a minimum of 1 week until blood parameters recovered acceptability. However, a delay greater than 5 weeks between two cycles led to premature treatment discontinuation. Treatment was prolonged until progression or unacceptable toxicity occurred.

Concerning the MET-free diet, the main source of dietary nitrogen was provided by XMET Maxamum (SHS, Liverpool, UK), an amino acid-modified medical food. This was supplemented with soluble DUOCAL (SHS), which is a medical food providing fat and carbohydrate, in order to reach the estimated energy requirements. The mixture of XMET Maxamum and soluble DUOCAL contains no methionine and was formulated to meet the energy and nitrogen requirements of a 70-kg man. It supplied a total energy intake of 2,510 kcal/day and 1 g of protein/kg body weight per day. Patients were advised to spread the MET-free diet throughout the day, *i.e.* from 8 a.m. to 12 p.m.

Patient monitoring. The clinical and biological characteristics of the patients were assessed before each cycle. The following measurements were made: i) Nutritional parameters: Patients were monitored by a dietician the day of MET-free diet to collect the food record. Compliance with the MET-free diet was evaluated by calculating the percentage of MET-free diet ingested *versus* MET-free diet administered (theoretical intake). Nutritional status was evaluated daily during the hospitalization period *via* physical (weight) and biological (albumin and prealbumin) assessments. During hospitalization, the patients were weighed every morning to the nearest 0.1 kg, and the body mass index (BMI) [weight (kg)/height (m^2)] was calculated. ii) Toxicity: This was assessed by clinical examination, peripheral blood count and standard biochemistry. The toxicity assessment was performed weekly before each chemotherapy administration and during the rest period. Clinical toxicity was graded retrospectively according to WHO scoring system (18). iii) Disease response: This was assessed by CT scans or MRI after 4 cycles. To qualify as a confirmed response, 2 objective assessments at least 4 weeks apart were required. Responses were recorded using the WHO criteria (18). Complete response (CR) was defined as disappearance of all clinical and radiological evidence of target lesions. Partial response (PR) was defined as a 50% or greater decrease in the overall sum of the products of diameters of all target lesions in reference to the baseline sum. Stable disease (SD) was defined as neither a sufficient increase to qualify for PD nor sufficient shrinkage to qualify for PR. Progressive disease (PD) was defined as 25% or greater increases in the overall sum of the products of diameters of all target lesions taking as reference the smallest sum recorded at or following baseline. The overall response rate (ORR) was defined as the percentage of patients with confirmed CR or PR. iv) Plasma MET concentrations: During the hospitalization period, blood samples collected in heparinized tubes were taken every day at 8 a.m (just before the first meal) and at 12 a.m. (4 h after the start of the MET-free diet). Plasma samples were treated as described previously (19). Briefly, all samples were immediately deproteinized using sulfosalicylic acid (50 mg/ml plasma). After centrifugation (4˚C, 3,500 rpm, 10 min), the supernatants were stored at –80˚C until analysis. Plasma MET concentrations were quantified in the supernatant by ion exchange chromatography using high-performance liquid chromatography (P680 HPLC pump, ASI-100 automated sample injector; Dionex, Voisin-Le-Bretonneux, France) with post-column ninhydrin derivatization, model PCX5200 (Pickering Laboratories, Mountain View, CA, USA), with a coefficient of variation of the internal standard within and between runs of <5%.

Statistical analysis. Data are presented as means±standard deviation. To evaluate the impact of the association of the MET-free diet with cystemustine on nutritional status (weight, albumin and prealbumin levels) or the impact of the MET-free diet on plasmatic MET concentration, values after treatment were compared to those prior to treatment using a paired *t*-test. The evaluation of the cycle effect during the 2 months of treatment on MET concentration was made using analysis of variance (ANOVA or a Kruskal-Wallis H test). The level of significance was set at $p<0.05$. Time to progression was measured from the initiation of cystemustine chemotherapy to the first observation of PD. Survival was calculated from the date chemotherapy was started until death or the last observation. Median overall survival (OS) and median time to progression were estimated by Kaplan-Meier methods. Data were analyzed using SEM software (20).

Results

Patients characteristics. Patient characteristics are given in Table I. Twenty-two patients, 20 presenting with metastatic melanoma (among whom 6 with choroid melanoma) and 2 with recurrent glioma, were recruited between December 2001 and November 2006 at the Jean Perrin Center in Clermont-Ferrand. Sixteen had previously received chemotherapy either

Table I. *Patient characteristics*.

Characteristic	n
Included patients	22
Gender	
Male	13
Female	9
Median age, years (range)	62 (35-76)
WHO performance status	
0	8
1	9
2	5
Primitive tumor sites	
Melanoma (choroid)	20 (6)
Glioma	2
Metastatic sites	
Liver	11
Lung, pleura	10
Central nervous system	9
Lymph node, skin	4
Bone	2
Other	
Previous treatments	
Prior surgery	21
Prior radiotherapy	14
Prior chemotherapy	16
Adjuvant treatment	6
Metastatic treatment line	
1	7
2	4
3	2
4	1

Table II. *Maximal toxicity observed per patient*.

	WHO grade			
	1	2	3	4
Hematological toxicity, n				
Anemia	7	6	0	0
Leukopenia	2	6	5	1
Neutropenia	1	5	6	0
Thrombocytopenia	7	1	6	2
Non-hematological toxicity, n				
Nausea/vomiting	5	3	0	0
Diarrhea	3	0	0	0
Hepatic	2	3	0	0
Renal	2	1	0	0

Table III. *Responses, time to progression and survival data from the start of cystemustine treatment*.

Response	n	Median time to progression (months) (range)	Median survival (months) [range]
Included patients	22	1.8 (0.4-29.4)	4.6 (0.4-55)
CR	0	-	-
PR	0	-	-
SD	3	7 (3.4-29-4)	12.1 (9.6-55)
PD	19	1.7 (0.4-2.4)	4.1 (0.4-8.8)

CR: Complete response; PR: partial response; SD: stable disease; PD: progression disease.

in an adjuvant setting (n=6) or at a metastatic state (n=14). The latter patients received a median of 4 cycles (range from 1 to 7 cycles) of the association of cystemustine with a 1-day dietary MET restriction. Because of hematological toxicity, 11 of them completed the last cycle with a MET-free diet only (*i.e*. without chemotherapy treatment).

Nutritional status. Patients consumed 78±27% of the MET-free diet administered, *i.e*. an energy intake of 2,265±802 kcal. The initial BMI of patients was normal (24.6±3.1 kg/m^2). During the 2-month treatment period, body weight (68.8±11.5 kg before treatment *vs*. 67.8±11.4 kg after treatment, p=0.11) and BMI (24.6±3.1 kg/m^2 before treatment *vs*. 24.5±3.0 kg/m^2 after treatment, p=0.63) remained normal and stable. Plasmatic albumin (from 37.8±5.6 g/l to 36.6±6.8 g/l, p=0.09) and prealbumin (from 0.25±0.1 g/l to 0.23± 0.1 g/l, p=0.32) levels were normal and unchanged comparing before and after treatment.

Toxicity. Toxicity data are given in Table II. Hematological toxicity was the main toxicity and consisted of thrombocytopenia (WHO grade 3-4 in 8/22 of patients), leukopenia (WHO grade 3-4 in 6/22 of patients) and neutropenia (WHO grade 3-4 in 6/22 of patients). Non-hematological toxicity remained limited, with no WHO grade 3-4 reported.

Response and survival. Among the 22 patients included, 17 were evaluable for response after the fourth cycle by CT scans or MRI assessement. Three patients showed a disease stabilization of 3.4, 7 and 29.4 months duration, respectively. Four patients died because of disease progression. One patient, not evaluated by medical imaging, was considered as having disease progression after clinical evaluation. The median time to progression of the 22 patients was 1.8 months (range: 0.4 to 29.4 months) (Table III), and median overall survival was 4.6 months (range: 0.4 to 55 months) (Figure 1).

MET variations. The fasting MET concentrations were unchanged on the MET-free diet. During the MET-free diet day, the plasma MET fall was of 53.1±21.8% after 4 h from the start of the MET-free diet (from 25.1±8.7 μmol/l at 8 a.m. D1 to 12.5±7.1 μmol/l at 12 a.m. D1; p<0.05).

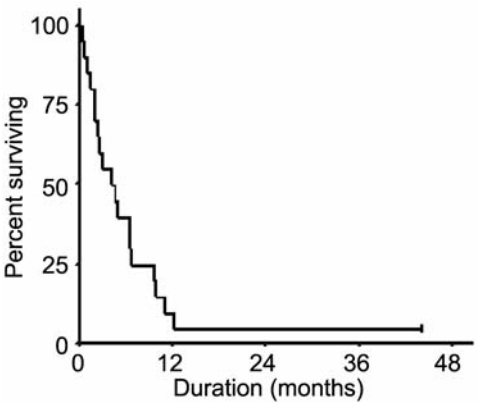

Figure 1. *Survival of 22 patients treated by the association of cystemustine and MET-free diet.*

Comparing methioninemia in the fed stade (12 a.m.) from D0 to D1, a MET depletion of 40.0±31.3% was obtained (from 20.3±7.4 μmol/l at 12 a.m. D0 to 12.5±7.1 μmol/l at 12 a.m. D1; $p<0.05$). During the 2-month period of treatment, no cycle effect was observed on plasma MET concentrations, neither of fasting ($p=0.13$) nor fed state concentrations ($p=0.51$), nor on values determined on the day of the standard diet ($p=0.09$) nor during the MET-free diet ($p=0.75$).

Discussion

Numerous experimental studies have demonstrated a synergistic effect of MET restriction and alkylating agents on tumor regression (12, 14). In cancer patients, only few studies have tested the effects of MET depletion (21, 22). In a previous phase I clinical trial testing the association of a short dietary MET-free period (1 to 4 days) with nitrosourea treatment for metastatic melanoma or recurrent glioma, we demonstrated that an optimal plasmatic MET depletion was obtained on the first day of the diet (of 41% comparing 12 a.m. D1 *vs.* 12 a.m. D0, or of 55% comparing 12 a.m. D1 *vs.* 8 a.m. D1), without effect by extending the MET-free diet period to 2, 3 or 4 days (15). The lack of cumulative effect of the MET-free diet on plasma MET concentrations could be linked to the daily increase in MET level during the night fasting. Moreover, we also showed that the association of a MET-free diet with cystemustine reduced MGMT activity, a main mechanism of nitrosourea resistance, in peripheral blood mononuclear cells (23). Based on these different results, a phase II clinical trial combining a 1-day MET-free diet with cystemustine treatment was initiated. The primary objective of this phase II trial was to assess safety and efficacy of the association in metastatic melanoma and recurrent glioma.

In the current phase II clinical trial, we confirmed the feasibility of the association with a good compliance of the MET-free diet by patients. Moreover, as previously shown (15), the successive short period of MET-free diet associated with chemotherapy did not alter the nutritional status of patients as seen by the stability of their body weight and by the relatively constant level of plasma albumin and prealbumin.

The association also showed acceptable toxicity, mainly hematological, especially on platelets as already shown in the previous trials with cystemustine treatment (4, 16, 24). WHO grade 3-4 toxicity for leukocytes, observed in 27% of the patients, appeared slightly more frequent compared with the prior trial using cystemustine at 60 mg/m^2 in second-line treatment of metastatic melanoma (13%) (4) but inferior to results reported in the trial testing cystemustine at 90 mg/m^2 for the 3 first cycles and then 60 mg/m^2 (44%) (16). However, no life-threatening side-effect was registered with the association of the MET-free diet and cystemustine treatment. Non-hematological toxicity remained limited, with no WHO grade 3-4 reported, and consisted mainly of WHO grade 1-2 nausea and vomiting (37%). Comparing with WHO grade 1-2 nausea and vomiting observed in previous trials with cystemustine treatment, the MET-free diet seemed not to increase the gastrointestinal toxicity of cystemustine (4, 16, 24). These results need to be confirmed in a randomised trial *versus* cystemustine treatment.

Among the 22 patients of this study, we reported 3 cases of SD and 19 PD. No CR or PR was observed. The median OS and the median time to progression, of 4.6 months and 1.8 months, respectively, were comparable to those observed with cystemustine alone as second-line treatment of metastatic melanoma (4 months and 1 month respectively) (4). Interestingly, we observed two long-duration stabilizations of 7 and 29 months. Survival of patients with SD (12 months) treated with this combination seems to be comparable with the median survival of responders with cystemustine alone (11 months) (4). The patient characteristics, *i.e.* mixed population with melanoma or glioma previously treated with different numbers of chemotherapy lines in a metastatic state, make it difficult to compare these results with those from previous trials with cystemustine and also with literature focused on one localization (melanoma or glioma) and mainly on first- or second-line of metastatic treatment. In the current trial, 16/22 of patients had already been treated by chemotherapy and 7/22 of patients had already received more than 2 lines of metastatic treatment and had a limited life expectancy. Moreover, a large proportion of patients of this phase II trial already had a poor prognosis. Our population comprised a high proportion of patients with choroid melanoma (30%) with a low median OS of 2 to 6 months (25-27). Numerous patients presented hepatic metastasis (50%), which is currently associated with a median survival of around 3 to 4 months (28).

As observed in the phase I clinical trial, a plasmatic MET depletion of 40% from the first day of MET-free diet *vs*. D0 was obtained (53% *vs*. D1 8 a.m.). The reduction threshold for plasma MET was consistent with previous reports for dietary MET restriction in healthy men (49%) (29), in patients with metastatic solid cancer (42%) (22) and also in animal models (50%) (30). In mice bearing human glial tumor xenograpfts, a dietary MET restriction induced regression of some tumor lines, whereas some others did not respond to a MET-free diet alone (plasmatic MET restriction of 50%) but did to a drastic depletion of plasma MET of 93% achieved by an enzymatic method with methioninase (30). From these experimental results, we wondered if an optimal plasmatic methionine depletion of 40-50% with a MET-free diet was sufficient to obtain a tumor effect. Recombinant methioninase infusion reduced serum MET levels to 0.1 μM within 2 hours in cancer patients (31). Methioninase may be able to offer the possibility to potentiate greater efficacy of nitrosourea treatment but at the moment, further investigations are needed before it can be used clinically especially because of its antigenicity and the risk of anaphylatic reaction with repeated administrations, as reported in primates (32-33).

In conclusion, the association of a 1-day MET-free diet with cystemustine treatment in patients with metastatic melanoma or recurrent glioma was well tolerated (toxicity, and nutritional status) and while producing no objective response gave a median survival comparable to the previous trial using cystemustine treatment, with an interesting long-duration stabilization for 2 patients. These results might be confirmed in a larger randomized trial.

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References

1 Jacquillat C, Khayat D, Banzet P, Weil M, Fumoleau P, Avril MF, Namer M, Bonneterre J, Kerbrat P, Bonerandi JJ *et al*: Final report of the French multicenter phase II study of the nitrosourea fotemustine in 153 evaluable patients with disseminated malignant melanoma including patients with cerebral metastases. Cancer *66*: 1873-1878, 1990.

2 Bajetta E, Del Vecchio M, Bernard-Marty C, Vitali M, Buzzoni R, Rixe O, Nova P, Aglione S, Taillibert S and Khayat D: Metastatic melanoma: chemotherapy. Semin Oncol *29*: 427-445, 2002.

3 Galanis E and Buckner JC: Chemotherapy of brain tumors. Curr Opin Neurol *13*: 619-625, 2000.

4 Thivat E, Durando X, D'Incan M, Cure H, Mouret-Reynier MA, Madelmont JC, Souteyrand P and Chollet P: Second-line chemotherapy of disseminated malignant melanoma with cystemustine at 60 mg/m^2: a phase II trial. Anticancer Drugs *16*: 1003-1007, 2005.

5 Belanich M, Pastor M, Randall T, Guerra D, Kibitel J, Alas L, Li B, Citron M, Wasserman P, White A, Eyre H, Jaeckle K, Schulman S, Rector D, Prados M, Coons S, Shapiro W and Yarosh D: Retrospective study of the correlation between the DNA repair protein alkyltransferase and survival of brain tumor patients treated with carmustine. Cancer Res *56*: 783-788, 1996.

6 Halpern BC, Clark BR, Hardy DN, Halpern RM and Smith RA: The effect of replacement of methionine by homocystine on survival of malignant and normal adult mammalian cells in culture. Proc Natl Acad Sci USA *71*: 1133-1136, 1974.

7 Stern PH and Hoffman RM: Enhanced *in vitro* selective toxicity of chemotherapeutic agents for human cancer cells based on a metabolic defect. J Natl Cancer Inst *76*: 629-639, 1986.

8 Breillout F, Antoine E and Poupon MF: Methionine dependency of malignant tumors: a possible approach for therapy. J Natl Cancer Inst *82*: 1628-1632, 1990.

9 Guo HY, Herrera H, Groce A and Hoffman RM: Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. Cancer Res *53*: 2479-2483, 1993.

10 Cellarier E, Durando X, Vasson MP, Farges MC, Demiden A, Maurizis JC, Madelmont JC and Chollet P: Methionine dependency and cancer treatment. Cancer Treat Rev *29*: 489-499, 2003.

11 Poirson-Bichat F, Goncalves RA, Miccoli L, Dutrillaux B and Poupon MF: Methionine depletion enhances the antitumoral efficacy of cytotoxic agents in drug-resistant human tumor xenografts. Clin Cancer Res *6*: 643-653, 2000.

12 Kokkinakis DM, Hoffman RM, Frenkel EP, Wick JB, Han Q, Xu M, Tan Yand Schold SC: Synergy between methionine stress and chemotherapy in the treatment of brain tumor xenografts in athymic mice. Cancer Res *61*: 4017-4023, 2001.

13 Goseki N and Endo M: Thiol depletion and chemosensitization on nimustine hydrochloride by methionine-depleting total parenteral nutrition. Tohoku J Exp Med *161*: 227-239, 1990.

14 Morvan D, Papon J, Madelmont JC and Demidem A: Methionine deprivation potentiates the effect of cystemustine treatment on B16 melanoma tumor in syngenic recipients. AACR Proceedings 2002, 3822, 2002.

15 Durando X, Thivat E, Farges MC, Cellarier E, D'Incan M, Demidem A, Vasson MP, Barthomeuf C and Chollet P: Optimal methionine-free diet duration for nitrosourea treatment: a phase I clinical trial. Nutr Cancer *60*: 23-30, 2008.

16 Cure H, Souteyrand P, Ouabdesselam R, Roche H, Ravaud A, D'Incan M, Viens P, Fargeot P, Lentz MA, Fumoleau P, Hanauske A and Chollet P: Results of a phase II trial with cystemustine at 90 mg/m^2 as a first- or second-line treatment in advanced malignant melanoma: a trial of the EORTC Clinical Studies Group. Melanoma Res *9*: 607-610, 1999.

17 Durando X, Thivat E, Roche H, Bay JO, Lemaire JJ, Verrelle P, Chazal J, Cure H and Chollet P: Cystemustine in recurrent high grade glioma. J Neurooncol *79*: 33-37, 2006.

18 WHO Handbook for Reprinting Results of Cancer Treatment. WHO Offset Publication No. 48. Neoplasma *20*: 37-46, 1990.

19 Minet-Quinard R, Van Praagh I, Kwiatkowski F, Beaujon G, Feillel V, Beaufrere B, Bargnoux PJ, Cynober L and Vasson MP: Pre- and postoperative aminoacidemia in breast cancer: a study *vs*. matched healthy subjects. Cancer Invest *22*: 203-210, 2004.

20 Kwiatkowski F, Girard M, Hacene K and Berlie J: SEM: A suitable statistical software adaptated for research in oncology. Bull Cancer *10*: 715-721, 2000.

21 Goseki N, Yamazaki S, Shimojyu K, Kando F, Maruyama M, Endo M, Koike M and Takahashi H: Synergistic effect of methionine-depleting total parenteral nutrition with 5-fluorouracil on human gastric cancer: a randomized, prospective clinical trial. Jpn J Cancer Res *86*: 484-489, 1995.

22 Epner DE, Morrow S, Wilcox M and Houghton JL: Nutrient intake and nutritional indexes in adults with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Nutr Cancer *42*: 158-166, 2002.

23 Thivat E, Durando X, Demidem A, Farges MC, Rapp M, Cellarier E, Guenin S, D'Incan M, Vasson MP and Chollet P: A Methionine-free diet associated with nitrosourea treatment down-regulates methylguanine-DNA methyl transferase activity in patients with metastatic cancer. Anticancer Res *27*: 2779-2783, 2007.

24 Urosevic V, Chollet P, Adenis A, Chauvergne J, Fargeot P, Roche H, Kerbrat P, Lentz MA, Fumoleau P and Chevallier B: Results of a phase II trial with cystemustine in advanced malignant melanoma. A trial of the EORTC Clinical Screening Group. Eur J Cancer *32*: 181-182, 1996.

25 Bedikian AY, Legha SS, Mavligit G, Carrasco CH, Khorana S, Plager C, Papadopoulos N and Benjamin RS: Treatment of uveal melanoma metastatic to the liver: a review of the M.D. Anderson Cancer Center experience and prognostic factors. Cancer *76*: 1665-1670, 1995.

26 Gragoudas ES, Egan KM, Seddon JM, Glynn RJ, Walsh SM, Finn SM, Munzenrider JE and Spar MD: Survival of patients with metastases from uveal melanoma. Ophthalmology *98*: 383-389, 1991.

27 Kath R, Hayungs J, Bornfeld N, Sauerwein W, Höffken K and Seeber S: Prognosis and treatment of disseminated uveal melanoma. Cancer *72*: 2219-2223, 1993.

28 Lens MB and Eisen TG: Systemic chemotherapy in the treatment of malignant melanoma. Expert Opin Pharmacother *4*: 2205-2211, 2003.

29 Lakshmanan FL, Perera WD, Scrimshaw NS and Young VR: Plasma and urinary amino acids and selected sulfur metabolites in young men fed a diet devoid of methionine and cystine. Am J Clin Nutr *29*: 1367-1371, 1976.

30 Kokkinakis DM, Wick JB and Zhou QX: Metabolic response of normal and malignant tissue to acute and chronic methionine stress in athymic mice bearing human glial tumor xenografts. Chem Res Toxicol *15*: 1472-1479, 2002.

31 Tan Y, Zavala J Sr, Han Q, Xu M, Sun X, Tan X, Tan X, Magana R, Geller J and Hoffman RM: Recombinant methioninase infusion reduces the biochemical endpoint of serum methionine with minimal toxicity in high-stage cancer patients. Anticancer Res *17*: 3857-3860, 1997.

32 Yang Z, Wang J, Yoshioka T, Li B, Lu Q, Li S, Sun X, Tan Y, Yagi S, Frenkel EP and Hoffman RM: Pharmacokinetics, methionine depletion, and antigenicity of recombinant methioninase in primates. Clin Cancer Res *10*: 2131-2138, 2004.

33 Yang Z, Wang J, Lu Q, Xu J, Kobayashi Y, Takakura T, Takimoto A, Yoshioka T, Lian C, Chen C, Zhang D, Zhang Y, Li S, Sun X, Tan Y, Yagi S, Frenkel EP and Hoffman RM: PEGylation confers greatly extended half-life and attenuated immunogenicity to recombinant methioninase in primates. Cancer Res *64*: 6673-6678, 2004.

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