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Optimal Methionine-Free Diet Duration for Nitrourea Treatment: A Phase I Clinical Trial

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In animal models, methionine (MET) restriction in association with chloroethylnitrosoureas led to a substantial improvement. On this basis, we initiated a Phase I clinical trial of dietary MET restriction in association with chloroethylnitrosourea (cystemustine) treatment for patients with recurrent glioma or metastatic melanoma. Our purpose was 1) to determine the optimal MET-free diet duration for a maximum depletion of plasma MET and 2) to evaluate the feasibility of this association. A total of 10 patients received 4 cycles of 2 wk of an association of a MET-free diet of 1, 2, 3, or 4 consecutive days and cystemustine (60 mg/m^2). For each cycle, plasma MET concentrations, nutritional status (weight, albumin, prealbumin) and toxicity were measured. Conversely, fed-state concentrations of plasma MET (12 AM) were reduced by dietary MET restriction, with an optimal depletion of 41% at the 1st day of MET-free diet without effect of the extending MET-free diet period. Indeed, we demonstrated the feasibility, that is, good diet acceptability and good tolerance (nutritional status and toxicity), of the association of a MET-free diet and cystemustine treatment. Based on these results, a Phase II clinical trial has been initiated to test the activity of the association of a 1-day MET-free diet with cystemustine treatment.

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INTRODUCTION

Several malignant tumors are characterized by a high rate of growth and specific amino acids requirements. Methionine (MET) is an essential amino acid with at least 4 major functions. First, MET contributes to protein synthesis. Second, MET is a precursor of glutathione (GSH), a tripeptide that reduces reactive oxygen species and thereby protects cells from oxidative stress (1). Third, MET is required for the formation of the polyamines, spermine and spermidine, which have far-ranging effects on nuclear and cell division (2). Fourth, MET is the major source of methyl groups for methylation of DNA and other molecules.

Studies have revealed that unlike normal cells, numerous human tumor cells are characterized by their "MET dependency" (3–6). This metabolic difference has been extensively studied and opens interesting perspectives for targeting tumor cells (7). In addition, MET depletion induces many modifications in tumor cells including cell arrest in the S and G2 phases of the cell cycle, apoptosis, decrease of GSH content, and reduced activity of the DNA repair protein O6-methylguanine-methyltransferase (MGMT) (8–9).

Among the few effective chemotherapy agents in the treatment of glioma and melanoma, chloroethylnitrosoureas such as carmustine (BCNU), fotemustine, nimustine, and cystemustine are currently used with an objective response rate of around 20% (10–13). However, their clinical usefulness has been limited by their toxicity and chemoresistance mainly due to MGMT activity.

Furthermore, because MET restriction induces numerous alterations including downregulation of MGMT, MET depletion could potentially act synergistically with chloroethylnitrosourea treatment. Preclinical studies have demonstrated that MET restriction substantially improves the therapeutic index of chloroethylnitrosoureas (8,9,14,15). Indeed, the combination of MET stress and chloroethylnitrosourea treatment increases sensitivity to BCNU or cystemustine in human brain tumors xenografted into athymic mice and in B16 melanoma tumors grafted into syngeneic mice, respectively (9,15).

We initiated a Phase I clinical trial associating dietary MET restriction with chloroethylnitrosourea (cystemustine) treatment in patients presenting with metastatic melanoma and recurrent glioma. The aim of this study was to determine the MET-free diet duration required for an optimal plasma MET depletion. We have also assessed the feasibility of the association of a dietary MET restriction with a chloroethylnitrosourea agent to treat advanced cancer with recurrent glioma and metastatic melanoma by determining the impact of this association on nutritional status and toxicity.

SUBJECTS 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 1 measurable metastatic lesion as evaluated by gadolinium-enhanced magnetic resonance imaging (MRI) or computed tomography (CT) scan, an estimated life expectancy of $\geq$ 2 mo, 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 to be included in the study. The study protocol was reviewed and approved by local ethical committees (Consultative Committee of Persons' Protection in Biomedical Research of Auvergne).

Treatment Schedule

Patients received 4 cycles of 2 wk of chemotherapy as conventionally used (13,16,17) associated with a MET-free diet. At each cycle during the hospitalization period, patients received a standard diet on the 1st day (DO) and the MET-free diet on the following days (D1, D2, D3, D4). Four periods of 1, 2, 3, or 4 days of MET-free diet were randomly tested during the 4 cycles. As shown by Lakshmanan et al. (18) on healthy subjects, a significant plasma MET depletion was observed 3 h after the beginning of a similar oral MET-free diet. We chose to administer cystemustine treatment at 12 AM (i.e., 4 h after diet beginning) on the last day of the MET-free diet period.

Cystemustine Treatment

Cystemustine was administered iv at 60 mg/m^2, being infused for 15 min in 100 ml of 5% dextrose. The treatment plan consisted of 1 administration every 2 wk. In case of granulocytopenia ($<$1,500/μl) or in case of thrombocytopenia ($<$100,000/μl) on the day before the forecasted date of cystemustine administration, the treatment was delayed for a minimum of 1 wk until blood parameters recovered higher values. However, a delay greater than 5 wk between 2 cycles led to premature treatment discontinuation. Treatment was continued until progression or unacceptable toxicity occurred. Each patient had to receive at least 4 cycles of treatment to be assessed.

MET-Free Diet

The main source of dietary protein was provided by XMET Maxamum (SHS, Liverpool, England), a MET-free powder. This was supplemented with soluble DUOCAL (SHS), which is a medical food providing fat and carbohydrate (CHO), to reach the estimated energy requirements. The mixture of XMET Maxamum and soluble DUOCAL was formulated on the basis of the energy requirements for a 70-kg man with a total energy intake of 2,510 kcal/day, providing 1 g protein kg^{-1} body weight per day. Patients were advised to spread the MET-free diet throughout the day, that is, from 8 AM to 12 PM.

Patient Monitoring

The clinical and biological characteristics of the patients were assessed before each cycle. The following measurements were made:

Plasma and Urinary MET Concentrations. Plasma MET was measured just before the first meal (8 AM) and at 12 AM on each day of the hospitalization period. Blood samples were collected in heparinized tubes. Urinary MET was determined by daily collection of 24-h urine outputs throughout the hospitalization period. Plasma and urine samples were treated as described previously (19). Briefly, all samples were immediately deproteinized using sulfosalicylic acid (50 mg/ml plasma or urine). After centrifugation (4°C, 3,500 rpm/min, 10 min), the supernatants were stored at −80°C until analysis. Plasma and urinary MET concentrations were quantified in the supernatant by ion exchange chromatography with ninhydrin detection using an amino acid autoanalyzer (System 6300, Beckman instruments, Palo Alto, CA).

Nutritional Parameters. Patients were monitored by a dietitian who evaluated dietary intake for each day of the MET-free diet period.

Nutritional status was evaluated daily during the hospitalization period via physical (weight) and biological (albumin and prealbumin) assessments. During hospitalization, the subjects were weighed every morning to the nearest 0.1 kg, and the body mass index (BMI) [weight (kg)/height (m^2)] was calculated. The nutrition risk index (NRI) described by Buzby et al. (20) was used to determine degree of malnutrition as either borderline mild (>97.5), moderate (83.5–97.5), or severe (<83.5).

Protein Metabolism. Nitrogen balance was calculated as the difference between total nitrogen intake and total urinary nitrogen excretion. Nitrogen excretion was determined by the Lee and Hartley method (21) using urea determination. Nitrogen intake was calculated as the product of the volume and nitrogen contents of the MET-free diet administered.

Urinary creatinine excretion was determined to evaluate muscular mass after checking that the patient did not suffer from renal insufficiency.

Urinary 3-methylhistidine (3MH) was measured to evaluate myofibrillar protein degradation, and 1-methylhistidine (1MH) was determined as an indicator of exogenous 3MH and meat intake. Urinary 3MH and 1MH concentrations were quantified using an amino acid autoanalyzer (System 6300, Beckman instruments, Palo Alto, CA). 3MH:creatinine ratio was calculated and used as an index of muscular proteolysis.

Toxicity. T oxicity 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 the WHO scoring system (22).

Response Assessments. Disease response was assessed by CT scans or MRI after Cycles 2 and 4. To qualify as a confirmed response, 2 objective assessments at least 4 wk apart were required.

Responses were recorded using WHO criteria and defined as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The overall response rate was defined as percentage of patients with confirmed CR or PR.

Statistical Analysis

Data are presented as means ± SD. Group comparisons were made using analysis of variance (ANOVA or multivariate ANOVA or a Kruskal–Wallis H test) and a paired t-test, and the level of significance was set at $P < 0.05$. Correlation analyses were evaluated using Spearman's correlation coefficient. Time to progression was measured from initiation of cystemustine chemotherapy to the first observation of PD. Survival was calculated from the date of chemotherapy was started until death or last observation. Median survival and median time to progression were estimated by Kaplan–Meier methods. Data were analyzed using SEM software version 3.5 (23).

RESULTS

Patients Characteristics

A total of 10 patients, 9 presenting with metastatic melanoma (among whom 5 with choroid melanoma) and 1 with recurrent glioma (oligodendroglioma), were recruited between December 2001 and May 2003 at the Jean Perrin Center in Clermont-Ferrand. Patient characteristics are given in Table 1. These patients received a median number of 4 ± 1 cycles of treatment; 2 of them (patient 6 and patient 8) received 1 cycle with MET-free diet only (i.e., without chemotherapy treatment) because of hematological toxicity.

Dietary Intake and Patient Compliance With MET-Free Diet

Figure 1 represents the individual energy intake of patients and the theoretical daily dietary energy intake of 2,510 kcal (11.3% proteins, 32% fat, 56.7% CHO). Patient compliance with the MET-free diet was evaluated by calculating the percentage of MET-free diet ingested vs. MET-free diet administered (theoretical intake). Thus, patients have consumed 72.4% ± 31.5% of the MET-free diet administered (theoretical intake), that is, an energy intake of 2,139 ± 933 kcal with a energy percentage of 9.9 ± 1.8 for proteins, 28.4 ± 5.4 for fat, and 61.8 ± 8.9 for CHO. The real dietary intake was higher than the theoretical one for CHO because patients were offered the option of adding syrup to improve the acceptability of the MET-free diet. Patient 10 presented a high energy intake due to added sugar. Only 3 patients (Patient 4, Patient 7, and Patient 9) ingested less than 50% of the theoretical intake.

MET Variations

During the 2-mo period of treatment, no additive cycle effect was observed on plasma MET concentrations, neither on fasting, nor fed state concentrations, nor on values determined at the day of the standard diet and during the MET-free diet. Plasma

TABLE 1
Patient characteristics[a]

Patient	Sex	Age (Years)	WHO Performance Status	Diagnosis	Previous Chemotherapy (Number of Courses)
1	m	45	0	CM	Dacarbazine/Vindesine/Platine Interferon α (4)
2	m	46	0	CM	—
3	f	63	2	M	—
4	f	76	0	M	—
5	m	74	1	M	Dacarbazine/Vindesine (6)
6	m	75	0	CM	—
7	f	69	2	CM	—
8	m	68		M	Dacarbazine (3) Fotemustine (9) Cysplatin
9	f	35	1	G	Temozolomide (4)
10	f	68	1	CM	—
	f: 50%	Median: 68	0: 40% 1: 30%	M: 40 % CM: 50%	0: 60% 1: 30%
	m: 50%	Range: 35 to 76	2: 20%	O: 10%	3: 10%

[a]Abbreviations are as follows: WHO, World Health Organization, m, male; f, female; M, melanoma; CM, choroid melanoma; G, glioma.

MET concentrations during the cycles at fasting and fed state are shown in Fig. 2, top. Fasting MET concentrations were not affected by the MET-free diet. However, plasma MET concentrations in fed state showed a sharp decline after administration of the MET-free diet ($P < 0.05$). The optimal MET depletion of 40.7 ± 36.9% was obtained on the 1st day of MET-free diet. No further changes were observed during the following days of the MET-free diet period.

During cycles, MET excretion (Fig. 2, bottom) was affected by MET-free diet ($P < 0.05$), with an optimal decline achieved on first day of MET-free diet.

Nutritional Status

The initial BMI of patients was normal (25.3 ± 3.2 kg/m^2) except for Patient 6 who was obese. During the 2-mo treatment period, BMI, plasmatic albumin levels, and NRI remained stable and normal. There was no significant difference in plasmatic prealbumin levels comparing before and after diet administration plus chemotherapy (from 0.25 ± 0.1 g/l to 0.26± 0.1 g/l; $P = 0.45$). C-reactive protein, which is an inflammatory marker, remained stable over the cycles. Patient 7 presented low plasmatic albumin levels and NRI, but the results were not conclusive because there were concomitant high levels of C-reactive protein indicating inflammatory syndrome.

Protein metabolism assessments are shown in Fig. 3. Regardless of MET-free diet duration, nitrogen balance was stable and negative during the MET-free diet period (–2.24 ± 3.16 g/24 h). Daily 3MH:creatinin ratio remained normal but decreased progressively during the hospitalization period from 29.9 ± 14.9 $\times 10^{-3}$ at D0 to 15.9 ± 4.9 $\times 10^{-3}$ at D4 ($P < 0.05$), and 1MH:creatinine ratio followed a similar pattern ($P < 0.05$). There was a positive correlation between urinary 3MH and 1MH ($r = .675$, $P < 0.05$).

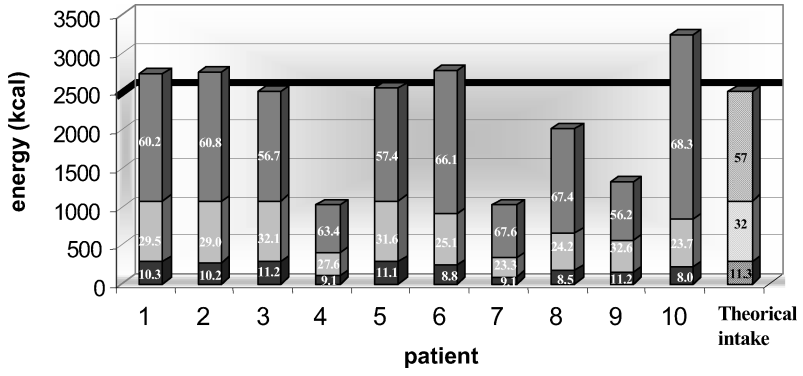

FIG. 1. Dietary energy intake. carbohydrate, ■ fat, ■ protein. The numbers in columns show percentage energy intake, striped boxes show the theoretical energy intake given daily.

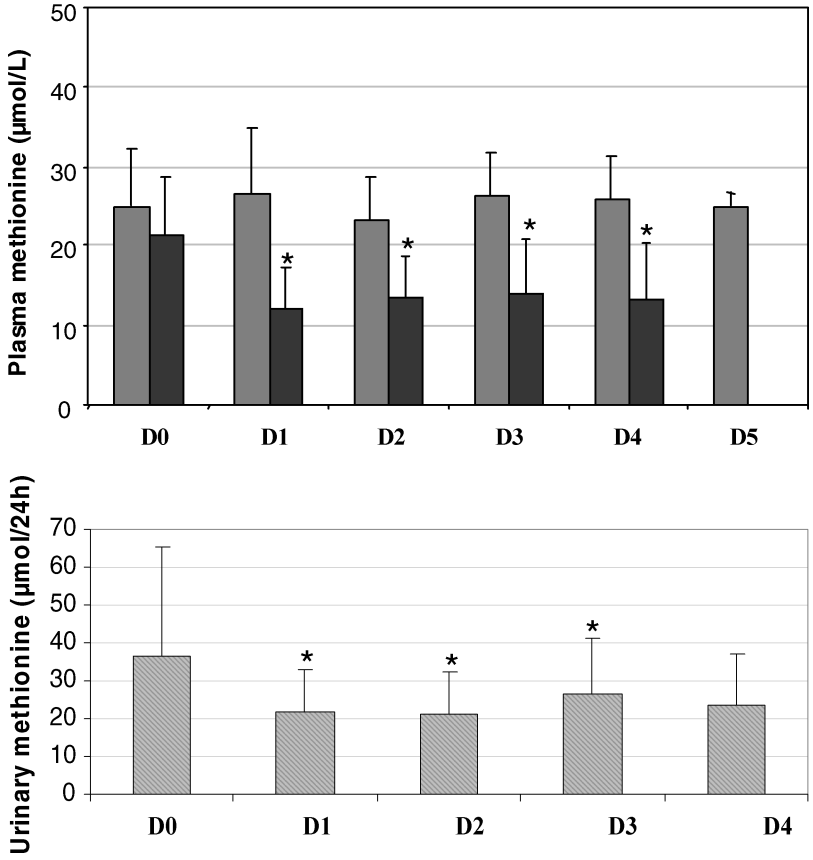

FIG. 2. Plasma and urinary methionine (MET) variation during cycles. Top: Plasma MET concentrations in fasting and fed state. Bottom: Urinary MET concentrations. D0, standard diet; D1, D2, D3, D4, MET-free diet. ■ plasma MET concentrations in fasting state (8 AM); ■ plasma MET concentrations in fed state (12 AM); urinary MET concentrations; *****, $P < 0.05$ vs. D0 (paired t-test).

Toxicity

The main toxicity was hematological and was responsible for an early treatment interruption (Patient 10). WHO Grade 3 thrombocytopenia occurred in 3 patients (Patient 8, Patient 7, and Patient 10). Three patients presented WHO Grade 3 neutropenia (Patient 8, Patient 9, and Patient 10). WHO Grade 3 and Grade 4 leucopenia was observed in Patient 9 and Patient 8, respectively.

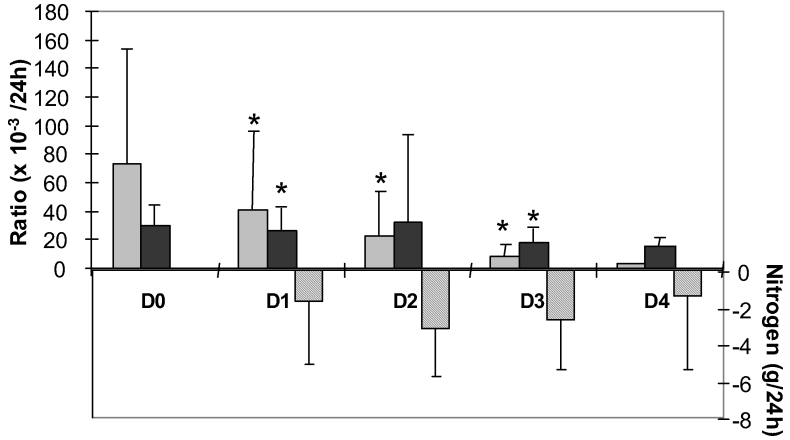

FIG. 3. Evaluation of protein metabolism: nitrogen balance, urinary 3-methylhistidine (3MH):creatinine and 1MH:creatinine ratio variations during cycles. D0, standard diet; D1, D2, D3, D4, MET-free diet. ■ 1MH:creatinine ratio; ■ 3MH:creatinine ratio; ■ nitrogen balance; *, $P < 0.05$ vs. D0 (paired t-test).

Nonhematological toxicity remained limited, with no WHO Grade 3–4 reported and only Grade 1 nausea and vomiting.

Response and Survival

Among the 10 patients included, all were evaluable for response after Cycle 2. After Cycle 2, disease stabilization was shown in 8 patients and disease progression in 2 patients (Patient 3 and Patient 7). After Cycle 4, 7 patients were evaluable for response. Patient 10 was excluded from the study for unacceptable toxicity. Two patients (Patient 2 and Patient 9) showed a disease stabilization of 7- and 29-mo duration, respectively. The median time to progression was 2 mo (range = 0.8–28.9 mo), and median overall survival was 6.5 mo (range = 1.2–32.7 mo).

DISCUSSION

Numerous experimental studies have demonstrated a synergistic effect of MET restriction and alkylating agents on tumor regression (9,15). In cancer patients, only few studies have tested MET depletion (24–26). This study is the first to report MET depletion with a synthetic MET-free solution administered via oral route in association with chloroethylnitrosoureas treatment.

We have demonstrated that a MET-free diet was able to reduce plasma MET levels (after feeding) of 41% from the 1st day of MET-free diet, that is, 4 h after the beginning of diet administration, without a significant effect of extending the MET-free diet period to 2, 3 or 4 days. The observed reduction threshold for plasma MET was consistent with previous reports (18–26). In a clinical trial performed in healthy men, Lakshmanan et al. (18) reported a 49% decrease of plasma MET levels after 3 h of an oral MET-free diet and a 60% decrease after 2 days (fed state). In a Phase I clinical trial in 8 patients with a variety of metastatic solid cancer, Epner et al. (26) reported a 42% decline of plasma MET at fed state after 4 days of enteral MET restriction and a reduction threshold of 58% within 2 wk.

Moreover, the absence of a cumulative effect of the MET-free diet on plasma MET concentrations could be linked to the daily MET level increase during the night fasting. The decrease in urinary MET excretion (23%) observed on the 1st day of MET-free diet is consistent with a successful accommodation to dietary amino acid deprivation to maintain the stability of the amino acids pools. An adaptive decrease of MET excretion and MET catabolism and (or) an increase of MET release from peripheral tissues could explain the night increase of plasma MET.

This absence of a cumulative effect of the MET-free diet on plasma MET concentration presented the advantage of limiting the potential toxicity of a depletion of an essential amino acid. In addition, in spite of the circadian fluctuation of plasma MET (increase of MET level during the night fasting), a significant MET depletion was observed during 16 h (from 12 AM to 4 AM; data not shown). This long MET depletion might be sufficient to cover the cytotoxic activity period of cystemustine (12 h) (27) to have a synergistic effect and confirmed our choice to administer cystemustine at 12 AM.

Because it is difficult to obtain metastatic samples (often hepatic or cerebral), tumoral explorations (MET level and MGMT activity) have not been realized in this study. Concerning MGMT, we have already shown that association of a MET-free diet with cystemustine induced a downregulation of MGMT activity in white blood cells of patients with metastatic cancer (28), which could reflect MGMT expression in tumors (29,30).

In this trial, the therapeutic strategy used was based on metabolism defect of tumor cells, MET dependency of which the biochemical mechanism remains unclear (7). Besides, in this Phase I trial, we have also studied the eventual undesirable effects on normal cells of a depletion of an essential amino acids.

Experimental studies have shown that mice receiving limited exogenous MET through low-protein diets experienced rapid body weight loss and profound alterations in their health status (31). Moreover, several authors have used synthetic diets that supply a high-calorie ration (5,6,32,33). With long depletion periods, rats deprived of MET show weight loss starting 14 days after initiation of the MET-free diet (32). As observed in animal models, Epner et al. (26) reported weight loss in cancer patients given a MET-free enteral diet for several weeks, not only in patients with an energy intake of 25 kcal/kg/day but also in patients with a higher energy intake of 35 kcal/kg/day. However, the difference between the baseline BMI and BMI before diet administration was significant from 4 wk of MET-free diet. In our cohort of patients, the energy intake of 32 $\pm$ 11 kcal/kg/day seems to be adequate to maintain baseline body weight throughout the 2-mo treatment. Thus, this precedent result demonstrated the weight safety of successive short periods of MET-free diet.

Plasma albumin levels were not affected by the MET-free diet and remained normal. Catabolism of muscle proteins, actin and myosin, were monitored by calculating urinary 3MH:creatinin ratio, which remained normal but decreased progressively during the hospitalization period. This progressive decrease could be due to a change in nitrogen form provided by the food. Indeed, proteolysis is not the only source of 3MH because significant amounts of 3MH can be taken up with the food. Endogenous 3MH can be distinguished from the exogenous 3MH by screening for 1MH, a methylated derivative of histidine that is not formed in humans, although common in other animals (34). Because meat was replaced by a mixture of amino acids, the urinary 1MH:creatinine ratio decreased from the 1st day of MET-free diet, and this decrease was correlated with urinary 3MH:creatinine ratio.

Nitrogen balance remained stable but moderately negative, thus showing that MET depletion did not alter protein mass.

Furthermore, the association of a MET-free diet and cystemustine treatment showed acceptable toxicity. Drug toxicity was mainly hematological, affecting platelets and neutrophils

as previously described with cystemustine treatment (13,16,17). No life-threatening side effects were registered. Nevertheless, to conclude on the absence of toxicity improvement with the association of MET-free diet with cystemustine, toxicity should be verified in a larger trial.

In conclusion, in this Phase I clinical trial, we determined 1 day of MET-free diet as the optimal duration for a maximal plasma MET depletion. Moreover, we demonstrated the feasibility (good acceptability of the diet) and nutritional tolerance of the association of a MET-free diet administered for a short period with chloroethylnitrosourea treatment in patients with melanoma and glioma. A Phase II clinical trial has been initiated to assess the therapeutic index (efficacy and toxicity) of the association of a 1-day MET-free diet with cystemustine treatment.

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