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# Phase I Study of Alpha-difluoromethylornithine and Methyl-GAG*

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**Abstract**—*α-Difluoromethylornithine (DFMO) is a potent inhibitor of the synthesis of putrescine (pu) and spermidine (sd) in some benign and malignant tissues. Intracellular deprivation of pu and sd has been shown to induce an enhanced uptake of polyamine-analogs such as methyl-GAG (MGBG). The purpose of this study was to investigate the tolerance and the toxicity of the combination of DFMO and MGBG. Thirty-six patients received* 4 × 2 g *of DFMO*/day *orally and every* 2 weeks 250–500 mg/m$^2$ *of MGBG as a* 2*-hr infusion, starting on day* 14. *Besides the well known acute and late side-effects of methyl-GAG, dose-limiting toxicity consisted also of thrombocytopenia, leucopenia, dyspnea, hemolysis and jaundice. The maximal tolerated dose of MGBG for one course was* 350 mg/m$^2$ *and for repeated courses* 250 mg/m$^2$, *due to cumulative toxicity.*

*Furthermore, after* 8 weeks *of continuous administration of DFMO* 70% *of the patients had a severe hearing loss, which was reversible after a treatment delay of* 4–6 weeks. *Since the hearing loss prohibited the continuous use of DFMO, two different schedules of intermittent DFMO-administration together with two different infusion periods of MGBG have been investigated in* 15 *patients. In none of these patients did hearing loss occur. The schedule of continuous administration of* 4 × 2 g *of DFMO*/day *orally for* 21 days *and* 250 mg/m$^2$ *of MGBG as a* 24*-hr infusion on days* 7, 14 *and* 21, *repeated on day* 42, *was tolerated best. In* 28 *evaluable patients two partial remissions were seen. Pretreatment with DFMO significantly enhanced the toxicity of MGBG and the combination of both drugs produced side-effects not seen with either drug alone.*

## INTRODUCTION

THE POLYAMINES (putrescine, spermidine and spermine) are small polycations which have been implicated in the control processes for cell growth and/or differentiation [1].

α–Difluoromethylornithine (DFMO) is a specific irreversible inhibitor of ornithine decarboxylase (ODC), the rate limiting enzyme in the polyamine biosynthetic pathway [2]. Inhibition of ODC leads to a depletion of the polyamines, putrescine and spermidine, *in vitro* and *in vivo* [3, 4]. Polyamine depleted cells show an enhanced uptake of exogeneous polyamines as well as of methyl-GAG (MGBG), which may be regarded as a structural analog of spermidine [5]. In mice, treatment with DFMO led to a varied pattern of decrease of putrescine and/or spermidine in different organs [4]. The accumulation of MGBG after DFMO-treatment was selectively enhanced in the small intestine, bone marrow and inoculated Ehrlich ascites tumor cells [3, 4]. Five children with heavily pretreated acute leukemia received the combination of DFMO, followed by MGBG. The concentration of MGBG in the circulating blast cells was increased 3- to 4-fold by pretreatment with DFMO. Side-effects were absent or mild and all patients achieved a remission [6]. The report of Knight [7], showing that a weekly schedule of 500–700 mg/m$^2$ of MGBG was tolerated reasonably well, has revived interest in the use of MGBG in malignant lymphomas and solid tumors. As a single agent it has substantial antitumor activity in patients with malignant lymphoma and moderate activity in cancer of the esophagus, head and neck, and lung (non-small cell) (reviewed in [8]). The main toxicity consists of fatigue, fever, nausea, vomiting, anorexia, mucositis, diarrhea, myalgia, neuropathy and moderate myelosuppression. Higher doses of MGBG are tolerated when the duration of infusion is increased to 24 or 120 hr [9]. Remarkably, the ratio between the maximal

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tolerable dose and minimal toxic dose is small, approx. 1.7 [7].

DFMO has recently been tested in a phase I study [10]. Twenty patients received doses of 0.75–3.0 g/m$^2$ every 6 hr for 28 days. Thrombocytopenia was the dose-limiting toxicity. Other side-effects included diarrhea, anorexia, anemia, nausea and fatigue. Clinical hearing loss was noted in another study (Merrell Dow Research Institute investigational brochure).

Since DFMO selectively enhanced the uptake of MGBG in tumors implanted in mice, and possibly also in leukemia cells in children, it seemed very interesting to investigate such a combination in cancer patients. Therefore a phase I study was started to test the toxicity of continuous oral administration of DFMO together with increasing intravenous doses of MGBG. During the study Warrell *et al.* reported that the combination of DFMO and MGBG caused substantially more hematologic and gastro-intestinal toxicity than MGBG alone [11].

## MATERIALS AND METHODS

*Patient population*

The population for this study consisted of patients between 15 and 75 yr of age with histologically proven cancer, which had been treated conventionally and was shown to be progressive. Other eligibility criteria were a performance status (Karnofsky) $\geq$ 50%, life expectancy of at least 3 months, platelet count $> 100 \times 10^9$/l, WBC count $> 3 \times 10^9$/l, serum creatinine $< 150$ μmol/l, no active infection, absence of clinical symptoms of brain metastases and verbal informed consent, according to the rules of the faculty ethical committee.

*Pretreatment and follow-up studies*

Before the start of treatment all patients had a complete history and physical examination, hemogram, kidney- and liver function tests, serum electrolytes, calcium, phosphate, total protein and albumin, chest X-ray and audiometry.

Measurable lesions were documented by the appropriate techniques, such as X-rays, CT-scanning and ultra-sonography. Every 2 weeks physical examination, hemogram and serum creatinine were repeated. Every 6 weeks the other investigations were repeated.

*Treatment regimen*

The treatment regimens are shown in Fig. 1.

*Regimen A.* DFMO sachets were supplied by Merrell Dow Research Institute in sachets, each containing 2 g. Starting on day 0, every 6 hr one sachet (2 g), dissolved in a glass of water, was taken by mouth. Every 2 weeks, starting on day 14,

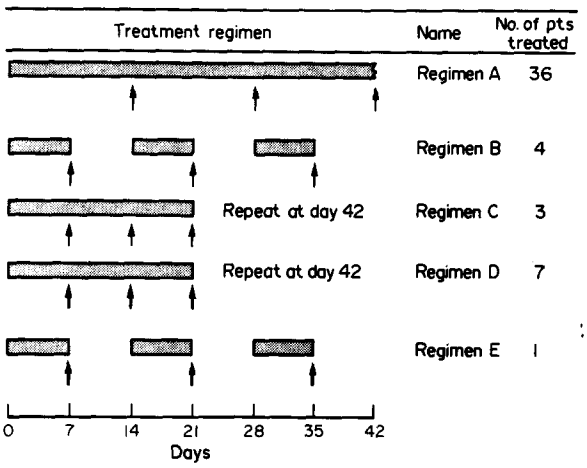


*Fig. 1. Treatment regimen of DFMO and MGBG;* ▬: *DFMO 4 × 2 g/day orally;* ↑: *2-hr infusion of MGBG;* ↑: *24-hr infusion of MGBG.*

patients received MGBG dissolved in 500 ml of a 5% glucose solution as an intravenous infusion during 2 hr.

The starting dose of MGBG in the first five patients was 500 mg/m$^2$, in the next patients 200–300 mg/m$^2$. At each course the dose of MGBG was increased by 50 mg/m$^2$ if toxicity of the previous course was tolerable.

*Regimen B–E*: DFMO (4 × 2 g/day) was administered intermittently, as depicted in Fig. 1. MGBG was dissolved in 500 or 1000 ml of 5% glucose solution for a 2- or 24-hr i.v. infusion respectively, at a dose of 250 mg/m$^2$.

*Evaluation of toxicity and response*

The maximum tolerable dose of MGBG was defined as the dose, which induced grade 3 (WHO) toxicity [12]. The standard WHO-criteria of response were used [12].

## RESULTS

The primary tumor sites of all patients are listed in Table 1. Thirty-six patients were treated according to regimen A (Fig. 1). The median age of these patients was 55 yr (range 16–73), median performance status 60% (Karnofsky) (range 50–100), 28 had measurable disease, two received prior radiotherapy and 31 prior chemotherapy. Five patients were not evaluable: two due to early non-toxic death, one developed an ileus attributable to the disease, one had severe radiation esophagitis and one was a protocol violation. The remaining 31 patients received 99 courses of MGBG.

During the first 14 days of treatment with DFMO alone no toxicity was observed. The number of courses and the number of patients treated with different doses of MGBG are shown in Table 2, together with the grade (WHO) of toxicity, which was evaluated 14 days after the first course

*Table 1. Sites of the primary tumor*

| | |
|---|---|
| Non-small cell lung cancer | 14 |
| Small cell lung cancer | 6 |
| Prostatic cancer | 4 |
| Hypernephroma | 5 |
| Colon cancer | 3 |
| Ovarian cancer | 3 |
| Bladder cancer | 2 |
| Cervix cancer | 1 |
| Breast cancer | 1 |
| Pancreatic cancer | 1 |
| Osteosarcoma | 1 |
| Leiomyosarcoma | 1 |
| Gastric cancer | 2 |
| Primary undetermined | 1 |
| Follicular thyroid cancer | 1 |
| Esophagus cancer | 1 |
| Carcinoid | 1 |

of MGBG. The first five patients were treated with 500 mg/m$^2$ of MGBG as a starting dose. All five patients experienced a severe toxicity consisting of nausea, vomiting, diarrhea, mucositis, dehydration, thrombocytopenia and leucopenia. One of them developed a severe hemolysis, subsiding spontaneously after approximately 5 days, but requiring a transfusion of 13 units of blood. Two patients died. Both patients developed an icterus (highest serum bilirubin levels of 534 and 618 μmol/l respectively), not due to hemolysis or cholestasis, in the presence of normal liver function tests, i.e. transaminases, alkaline phosphatase, fibrinogen, clotting factor V and ammonia. Approximately 14 days after the infusion they became somnolent, followed by coma and death from circulatory failure. At post-mortem examination of one of them, no abnormalities could be detected by light microscopy, except for an accumulation of bile pigment in the liver cells. Electron microscopy showed a severe destruction of mitochondria in the liver, but not in the heart. Of the remaining three patients, one refused further treatment, while the other two accepted further treatment with lower doses of MGBG after recovery. Up to a dose of 400 mg/m$^2$ of MGBG no leucopenia, thrombocytopenia or hemolysis were observed.

Thereafter the initial dose of MGBG varied between 200 and 350 mg/m$^2$. Anemia, anorexia and (peri)anal inflammation were encountered at doses of $\geq$ 300 mg/m$^2$. One patient with renal insufficiency developed a severe mucositis and thrombocytopenia ($< 5 \times 10^9$/l) and a slight leucopenia ($2.0 \times 10^9$/l) at a dose of 300 mg/m$^2$. After recovery repeated infusions of 200 mg/m$^2$ did not induce these side-effects.

In Table 3 the toxicity and frequency of toxicity after one or more infusions of MGBG during continuous DFMO administration, i.e. regimen A, are listed. The dose-dependent or cumulative character was deducted from the appearance of the side-effects after the first or subsequent courses of MGBG and from the lessening of the specific-side-effect(s) after reduction of the MGBG dose. The long list of toxic side-effects has been divided into three groups in order to distinguish between side-effects probably induced mainly by DFMO alone (I), by DFMO and MGBG together (II) and by MGBG alone (III).

Hearing loss was the dose-limiting toxicity for DFMO alone. Seventy per cent of the patients who had continuously used DFMO for 8 weeks or more experienced a rapidly progressive hearing loss, first of the high tones and then complete. After stopping treatment hearing often got worse subjectively during the first 2 weeks and then recovered. Four to six weeks later hearing was normal again in all patients except one.

In the second group the most important side-effects were anemia, thrombocytopenia and leucopenia. The median decrease of the hemoglobin level was 1.5 mmol/l (range 0–3.6). The anemia was not dependent on the dose of MGBG, but increased with the number of courses. After the observation of hemolysis in one patient, serum haptoglobin, plasma hemoglobin and iron excretion in the urine were measured before and after

*Table 2. Number of courses per dose of MGBG and maximal grade of toxicity after the first course in treatment regimen A*

| Dose (mg/m$^2$) | No. of patients | No. of courses | Toxicity after the *first* course of MGBG | |
|---|---|---|---|---|
| 200 | 3 | 3 | grade 0 | 3/3 |
| 250 | 9 | 19 | grade 0 | 4/4 |
| 300 | 17 | 37 | grade 2 | 9/13, grade 4 1/13 |
| 350 | 11 | 17 | grade 2 | 7/8 |
| 400 | 9 | 16 | — | |
| 450 | 2 | 2 | — | |
| 500 | 5 | 5 | grade 2 | 1/5, grade 4 2/5 |
| | | | toxic death | 2/5 |

*Table 3. Evaluation of the toxicity of DFMO and MGBG in treatment regimen A*

| Toxicity | No. of patients* | Dose dependent | Cumulative |
|---|---|---|---|
| I | | | |
| Hearing loss | 17 of 24 with 6 weeks treatment | | × |
| II | | | |
| Anaemia | 29 | | × |
| Thrombocytopenia | 10 | × | |
| Leucopenia | 5 | × | |
| Local tumor pain | 7 | | |
| Effect on brain metastases | 4 | | |
| Hemolysis | 1 | × | |
| Icterus | 2 | × | |
| III | | | |
| Anorexia | 18 | × | × |
| Nausea | 7 | × | |
| Diarrhea | 10 | × | × |
| Stomatitis | 14 | × | × |
| (Peri)anal inflammation | 15 | × | × |
| Decrease of perform. status | 21 | × | × |
| Peripheral neuropathy | 2 | | × |
| Myalgia | 12 | × | × |
| Arthralgia | 4 | × | × |
| Skin rash | 3 | | ×? |
| Dyspnea | 5 | × | |

*Total No. of evaluable patients was 31.

MGBG infusion in five patients. All these parameters of hemolysis were found to be normal, indicating that hemolysis is probably not a significant factor in the causation of anemia.

Thrombocytopenia occurred in ten patients. The nadir was observed between 3 and 7 days after MGBG infusion and recovery took place in 7–14 days. In three patients with a severe thrombocytopenia a bone marrow aspiration was performed. Normal numbers of megakaryocytes were counted. In the same patients no indications of intravascular clotting could be detected by examination of fibrinogen, fibrin split products, clotting factor V and the ethanol gelation test. Anti-platelet antibodies could also not be demonstrated. In seven of these patients DFMO treatment was continued and the platelet number returned to normal. Leucopenia occurred 14–21 days after MGBG infusion and was always preceded by a severe thrombocytopenia.

Seven patients suffered a very severe exacerbation of pain, localized at the site of the primary tumor or metastases, which started immediately after the infusion of MGBG and lasted for 1–2 hr.

Four patients, who had no symptoms of brain metastases, developed an acute transient neurological syndrome 6–12 hr after the MGBG infusion, indicating focal brain injury. A CT-scan of the brain showed metastases, the localization of which was compatible with the neurological symptoms. Seventy-two hours later the symptoms had disappeared spontaneously.

The patient with hemolysis and the two patients with icterus have been described above.

The third group consists of side-effects, which are known from the use of MGBG alone. They all seem to be cumulative. Pulmonary toxicity is an uncommon reaction to MGBG. However, three patients with primary lung cancer or lung metastases experienced transient episodes of dyspnea, starting 24–48 hr after MGBG infusion. No clinical explanation for this side-effect could be found. Two patients died from acute respiratory failure 2 and 3 days after the second and sixth course of MGBG, respectively. After the previous courses no pulmonary toxicity was observed. Post-mortem examination in one of the patients did not provide an explanation for the acute respiratory failure.

The median duration of treatment of the 31 patients was 9 weeks (range 2–14). The main reasons for stopping treatment were symptomatic

brain metastases (3), toxicity (13) and progression of the disease (11), while in two cases death from toxicity and in two others from acute respiratory failure occurred.

Since the large percentage of patients with a hearing loss prohibited the continuous administration of DFMO, the intermittent use of DFMO was investigated in two different schedules, together with two different durations of MGBG infusion at a dose of 250 mg/m$^2$. Four patients were treated according to regimen B. Three of them had been treated in regimen A and two had experienced a severe hearing loss. All three patients experienced the same toxicity after MGBG infusion as in regimen A.

Three patients were treated according to regimen C. All three patients experienced the same toxicity as described above for regimen A. The same was true for one patient treated according to regimen E.

Seven patients received regimen D. The median number of MGBG infusions was eight (range 2–12), the median duration of treatment 15 weeks (range 3–24). The main reasons for stopping treatment were toxicity (one), progressive disease (four) and death not related to treatment (1). One patient is still on treatment. At a dose of 250 mg/m$^2$ of MGBG anorexia, anemia, slight pretibial pain, diarrhea and peri-anal inflammation were observed. In two patients 300 mg/m$^2$ of MGBG induced thrombocytopenia.

None of the 15 patients treated with intermittent DFMO showed any hearing loss.

*Therapeutic effects*

Of 48 patients 28 were evaluable for response, as shown in Table 4. One patient with hypernephroma had a minor remission of his lung metastases and progression of liver metastases, which was called a mixed response. One patient with a previously treated non-small cell lung cancer and one patient with advanced prostate cancer with bone metastases and measurable lymph node involvement had a partial remission after two cycles of chemotherapy according to regimen A. The duration of response could not be assessed due to intolerable cumulative toxicity but was at least 12 weeks.

*Table 4. Evaluation of tumor response*

| Type of response | No. of patients | Pathological diagnosis |
|---|---|---|
| Progressive disease | 15 | |
| Stable disease | 10 | 1 colon, 1 bladder, 4 nsclc,* 2 sclc,† 1 hypernephroma, 1 papillary ca. of the thyroid |
| Mixed response | 1 | hypernephroma |
| Partial remission | 2 | 1 nsclc, 1 prostate |

*nsclc, non-small cell lung cancer.
†sclc, small cell lung cancer.

## DISCUSSION

This phase I trial of DFMO and MGBG was based upon the hypothesis that inhibition of polyamine synthesis by DFMO will lead to a depletion of intra-cellular polyamines and a subsequent enhanced uptake of polyamine-analogs, such as MGBG [5]. Furthermore, from investigations with mice inoculated with Ehrlich ascites tumor cells [4], and a trial with a small number of children with leukemia [6], it was suggested that DFMO selectively enhanced the uptake of MGBG in certain tissues.

It is important to realize that in the animal experiments mice were treated with 3% DFMO in the drinking water (5 g/kg/day) [13] and in *in vitro* studies concentrations of 0.5–5mM were used [14]. In order to achieve the latter serum concentration in humans prolonged intravenous administration of 20–30 g/m$^2$/day seems to be necessary [15, 16]. In this study 4 × 2 g/day of DFMO (approx. 5 g/m$^2$) was orally administered since little or no side-effects had been observed at this dose [10].

After 6–8 weeks of continuous DFMO administration 70% of patients showed a significant hearing loss clinically and/or by audiometry starting at the higher tones and rapidly involving the whole hearing range. Interestingly, most patients complained of an increasing hearing loss in the first 1 or 2 weeks after withdrawal of DFMO, followd by a complete subjective and objective recovery in the next 2–4 weeks. This side effect has also been observed by others [17, Merrell Dow Pharmaceuticals, Inc., personal communications] and prohibits long-term administration of DFMO.

The first five patients were treated with a starting dose of 500 mg/m$^2$ of MGBG after pretreatment with DFMO because of a report by Siimes [6] stating that after 3 days of DFMO treatment MGBG could be infused in children at a dose of 500 mg/m$^2$ without serious side-effects. In these five patients the whole range of toxic side-effects (Table 3, II + III) of the combination of DFMO and MGBG were seen. Two patients died from circulatory collapse after 18 and 20 days of severe thrombocytopenia, leucopenia, mucositis, diarrhea and icterus. Hyperbilirubinemia without hemolysis or abnormal liver function tests is a rare side-effect. Severe destruction of mitochondria in the liver cells of one of these patients was the only abnormality found by electron microscopy. This effect has been described earlier as the probable mechanism of

cytotoxic action of MGBG [18] and may be correlated with the observed icterus. One patient recovered but refused further therapy. The remaining two patients recovered completely and were treated again at lower doses of MGBG without major side-effects. Another worrying side-effect was the observed pulmonary toxicity. Three patients experienced transient episodes of dyspnea and two patients died from acute respiratory failure. Bronchospasm has been described as a side-effect of MGBG [19] but it may only partly explain the clinical picture seen in these patients. This experience was in large contrast to the report by Siimes [6]. A possible explanation may be that children can tolerate higher doses of MGBG, since the same group from Helsinki described severe myalgia and mucositis in a 49-yr-old man and acute hemolysis in a child without any other side-effects, using their original therapy regimen [20, 21].

At lower doses of MGBG two major problems were encountered. Firstly, a very sharp limit was found in almost all patients between a slightly or non-toxic first dose of 250 mg/m$^2$ and a clearly toxic first dose of 300–350 mg/m$^2$.

Secondly, cumulative toxicity, mainly consisting of mucositis, diarrhea, general malaise and a decrease of performance status, even at a dose of 250 mg/m$^2$, limited the maximal tolerated number of courses. These two problems have been observed by others with MGBG alone [7, 9]. Furthermore, acute toxicity (within 48 hr after infusion of MGBG), consisting of myalgia, arthralgia, nausea, severe pain in the primary tumor or metastases and acute neurological symptoms in patients with previously unsuspected brain metastases, was often difficult to tolerate, although all side-effects were transient within 10 days. Some of the above-mentioned side-effects were also reported by Warrell *et al.* [11]. In the second phase of the study the toxicity of intermittent DFMO and MGBG was investigated, after each of two different ways of discontinuous administration of DFMO (see Fig. 1) plus infusion of MGBG (250 mg/m$^2$) during 2 or 24 hr. The latter choice was made because Hart *et al.* [9] showed that higher doses of MGBG were tolerated if infused during 24 or 120 hr.

Fifteen patients were entered into this part of the study. No hearing loss was observed objectively and/or subjectively after $\geq$6 weeks of treatment. MGBG infusion during 24 hr considerably lessened acute toxicity but did not seem to decrease cumulative toxicity. Dose-escalation to 300 mg/m$^2$ in two patients induced a thrombocytopenia, which suggests that in the presence of DFMO no higher doses of MGBG were tolerated despite infusion over 24 hr. Regimen D was tolerated best because of little acute toxicity and a no-treatment period of 3 weeks to recover.

In 28 evaluable patients two objective responses were seen, which is certainly not better than the results of phase I–II trials with MGBG alone [7, 9, 22].

In conclusion, this study has shown that DFMO at a dose of 4 × 2 g/day enhances the toxicity of MGBG but not preferentially in tumors. Recently Romijn *et al.* [23] reported that DFMO could induce an 8-fold increase of MGBG uptake in a hypernephroma cell line without affecting the tissue polyamine levels. This datum may indicate that the enhancement of the toxicity of MGBG by DFMO *in vivo* may not be due to polyamine depletion.

Therefore it seems important to investigate first the mechanism(s) of the potentiation of drug effects by DFMO more extensively *in vitro*. Besides, if DFMO is to become a useful drug in cancer treatment, it will be necessary to find out whether and how some degree of selective polyamine depletion can be induced *in vivo*. Preliminary data from Maddox *et al.* [15] suggest that prolonged i.v. infusion of 20–30 g/m$^2$ of DFMO is needed to induce polyamine depletion in leukemia cells *in vivo*.

Although treatment regimen D was well tolerated, a phase II study of the combination of DFMO and MGBG cannot yet be recommended.

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## REFERENCES

1. Williams-Ashman HG, Canellakis ZN. Polyamines in mammalian biology and medicine. *Perspect Biol Med* 1979, **22**, 421–453.
2. Metcalf R, Bey P, Danzin C *et al.* Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analoges. *J Am Chem Soc* 1978, **100**, 2551–2553.
3. Seppänen P, Alhonen-Hongisto L, Jänne J. Polyamine deprivation-induced enhanced uptake of methylglyoxal bis (guanyl hydrazone) by tumor cells. *Biochim Biophys Acta* 1981, **674**, 169–177.

4. Kallio A, Seppännen P, Alhonen-Hongisto L *et al.* Modulation of the tissue disposition of methylglyoxal bis (guanyl hydrazone) in mice by polyamine depletion and by polyamine administration. *Cancer Res* 1983, **43**, 324–327.
5. Alhonen-Hongisto L, Seppänen P, Jänne J. Intracellular putrescine and spermidine deprivation induces increased uptake of the natural polyamines and methylglyoxal bis (guanyl hydrazone). *Biochem J* 1980, **192**, 941–945.
6. Siimes M. Synergistic action of two polyamine antimetabolites leads to a rapid therapeutic response in childhood leukemia. *Int J Cancer* 1981, **28**, 567–570.
7. Knight WA. Phase I–II trial of methyl-GAG: a South-west Oncology Group pilot study. *Cancer Treat Rep* 1979, **63**, 1933–1937.
8. Warrell RP, Burchenal JH. Methylglyoxal bis (guanyl hydrazone) (methyl-GAG): current status and future prospects. *J Clin Oncol* 1983, **1**, 52–65.
9. Hart RD, Ohnuma T, Holland JF *et al.* Methyl-GAG in patients with malignant neoplasms: a phase I re-evaluation. *Cancer Treat Rep* 1982, **66**, 65–71.
10. Abeloff MD, Slavik M, Luk GD *et al.* Phase I trial and pharmacokinetic studies of α-difluoromethylornithine, an inhibitor of polyamine biosynthesis. *J Clin Oncol* 1984, **2**, 124–130.
11. Warrell RP, Coonly CJ. Burchenal JH. Sequential inhibition of polyamines synthesis. A phase I trial of DFMO (α-difluoromethylornithine) and methyl-GAG [methylglyoxal-bis(guanyl hydrazone)]. *Cancer Chemother Pharmacol* 1984, **11**, 134–136.
12. *WHO Handbook for Reporting Results of Cancer Treatment.* Geneva, WHO offset publication No 48. 1979.
13. Luk GD, Abeloff MD, Griffin CA *et al.* Successful treatment with DL- α-difluoromethylornithine in established human small cell variant lung carcinoma implants in athymic mice. *Cancer Res* 1983, **43**, 4239–4243.
14. Luk GD, Goodwin G, Marton LJ *et al.* Polyamines are necessary for the survival of human small cell lung carcinoma culture. *Proc Natl Acad Sci* 1981, **78**, 2355–2358.
15. Maddox AM, Freireich EJ, Keating MJ *et al.* Pharmacology of the polyamine inhibitor α-difluoromethylornithine in patients with hematologic malignancies. *Proc ASCO* 1984, **3**, 42.
16. Griffin C, Abeloff MD, Slavik M *et al.* Phase I trial & pharmacokinetic study of i.v. & high dose oral α-difluoromethylornithine (DFMO). *Proc ASCO* 1984, **3**, 34.
17. Talpaz M, Quesada J, Gutterman JU. Phase I study of α-difluoromethylornithine (DFMO) and leucocyte interferon (IFN) in cancer patients. *Proc ASCO* 1984, **3**, 63.
18. Kramer DL, Zychlinski L, Wisemann, A *et al.* Biochemical and ultrastructural characterization of human cell variants resistant to the antiproliferative effects of methylglyoxal bis (guanyl hydrazone). *Cancer Res* 1983, **43**, 5943–5950.
19. Warrell RP, Lee BJ, Kempin SJ *et al.* Effectiveness of methyl-GAG (methylglyoxal-bis[guanyl hydrazone]) in patients with advanced malignant lymphoma. *Blood* 1981, **57**, 1011–1014
20. Seppänen P, Alhonen-Hongisto L, Siimes M *et al.* Quantitation of methylglyoxal (bis guanyl) hydrazone in blood plasma and leukemia cells of patients receiving the drug. *Int J Cancer* 1980, **26**, 571–576.
21. Jänne J, Alhonen-Hongisto L, Seppänen P *et al.* Use of polyamine antimetabolites in experimental tumours and in human leukemia. *Med Biol* 1981, **59**, 448–457.
22. Killen JY, Mitchell EP, Hoth DF *et al.* Phase II studies of methylglyoxal bis guanyl hydrazone (NSC 32946) in carcinoma of the colon and lung. *Cancer* 1982, **50**, 1258–1261.
23. Romijn JC, Splinter TAW, Verkoelen CF *et al.* Stimulation of the uptake of MGBG in tumor cells by DFMO, unrelated to polyamine depletion. *In: Book of Abstracts of the International Conference on Polyamines,* Budapest, 1984, 133.