Review

Mafosfamide as a New Anticancer Agent: Preclinical Investigations and Clinical Trials

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Abstract. *Mafosfamide (4-thioethane sulfonic acid salt of 4-hydroxy-cyclophosphamide, MAF) belongs to a new generation of the oxazaphosphorine agents. MAF is a cyclophosphamide analog which spontaneously degrades to 4-hydroxy-cyclophosphamide. The effects of MAF on various types of cancer cells were determined during preclinical investigations and clinical trials. The positive results from in vitro and in vivo anticancer studies promoted MAF to a good candidate for phase I trials. Clinical experience with intrathecal MAF, used for patients with neoplastic meningitis due to leukemia, lymphoma, and solid tumors, indicated good tolerability and efficacy. The recommended phase II doses of intrathecally administered MAF were determined. Clinical trials using intrathecal MAF are now underway. To obtain a better therapeutic index, a strategy to alternate dosing between the intraventricular and intralumbar routes is also being tested. MAF is an attractive agent for regional cancer therapy. The current available knowledge on MAF as a new anticancer agent is based on a collection of the original published studies, conference abstracts and relevant articles.*

New Oxazaphosphorine Anticancer Agents

Oxazaphosphorines represent a class of alkylating agents widely used in the treatment of hematological malignancies and solid tumors. Nonetheless, the clinical use of the oxazaphosphorine drugs cyclophosphamide (CP), ifosfamide and trofosfamide, is limited due to their toxicity to normal cells and cancer cell resistance against their action. Recently,

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a new generation of oxazaphosphorines have been developed in order to improve the therapeutic index and increase the effectiveness of chemotherapy (1-6).

Mafosfamide as a New Oxazaphosphorine Agent

Mafosfamide (CAS number 88859-04-5, MAF) is one of the novel oxazaphosphorine agents (2, 4, 5). MAF is a preactivated cyclophosphamide analog (Figure 1). It is a chemically stable 4-thioethane sulfonic acid salt of 4-hydroxy-cyclophosphamide (4-HO-CP). Mafosfamide cyclohexylamine salt and mafosfamide lysine salt (Figure 2) were synthesized and tested in preclinical and clinical studies (6-14).

Metabolism and Transport of MAF

In comparison with CP, MAF does not require hepatic activation. MAF breaks down, spontaneously, to 4-HO-CP and a thiol, mesna (2-mercaptoethanesulfonate) cyclohexylamine or lysine salt. The released 4-OH-CP is in equilibrium with its tautomer aldophosphamide that can be decomposed by β-elimination to generate cytotoxic phosphoramide mustard and acrolein (Figure 3). Therefore, MAF is directly active in intracellular fluids and cells. 4-OH-CP, phosphoramide mustard and acrolein can enter cells *via* passive diffusion and transporter-mediated translocation (2-5, 9, 15, 16).

Mechanisms of MAF Action on Cells

The mechanisms of MAF action on cells have not yet been fully explained (3, 15). However, the effects of MAF are accepted as being mainly dependent on its reactive alkylating agents – phosphoramide mustard and acrolein. The metabolites of cyclophosphamide also include phosphoramide mustard and acrolein. Thus, the mechanisms of MAF action are similar to those of CP (1, 2, 4, 17). In the case of MAF, it has been shown that mesna, increased the therapeutic effects without interfering in MAF anticancer activity (7).
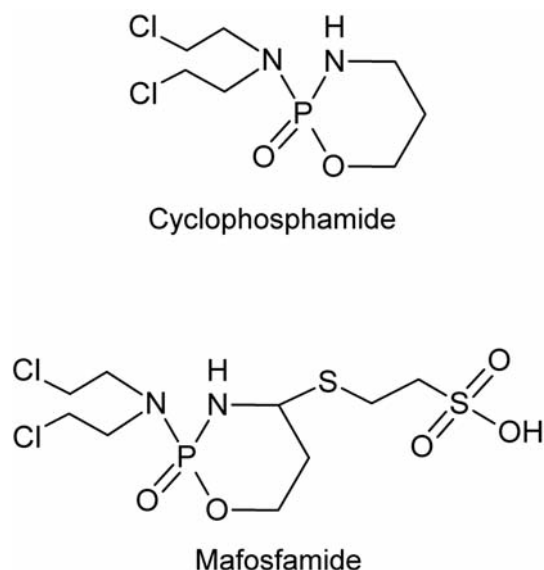
Figure 1. *Chemical structures of cyclophosphamide and mafosfamide.*

Figure 2. *Chemical structures of mafosfamide cyclohexylamine salt and mafosfamide (L-lysine) salt.*

The reactive alkylating agents, phosphoramide mustard and acrolein can bind covalently to a variety of molecules. MAF metabolites can react with available groups of amino acids, proteins and peptides, such as NH_2, COOH, SH, and with the primary phosphate, hydroxyl and amino groups of the bases of nucleic acids. Nevertheless, the DNA binding site is the most important. The biological effects of MAF are generally considered to originate from damage to cellular DNA (16, 18, 19). After MAF application, DNA breaks were observed in lymphocytes and leukemia cells (20, 21). The DNA molecular damage caused by reactive alkylating agents involving bonds between atoms, formation of cross-bridges and strand breaks, is believed to lead to the disruption of DNA function, inhibition of cell division and cell death (1, 18, 22, 23). Mitotic catastrophe, a process preceding cell death (11) and programmed cell death were triggered by MAF (Figure 4). After exposure of hematopoietic cells to MAF, these cells underwent apoptosis, necrosis (10, 23-25) and autophagy (25). MAF also affected the viability and the size of leukemia cells (12-14, 25).

MAF in Preclinical Studies

The effects of MAF on various types of cancer cells were determined during preclinical investigations. MAF has been found to have significant activity *in vitro* against leukemia and solid tumor cell lines such as U-937, ML-1, MOLT-4, rhabdomyosarcoma and MCF-7, and also *in vivo* against several transplantable murine tumors, including P388 and
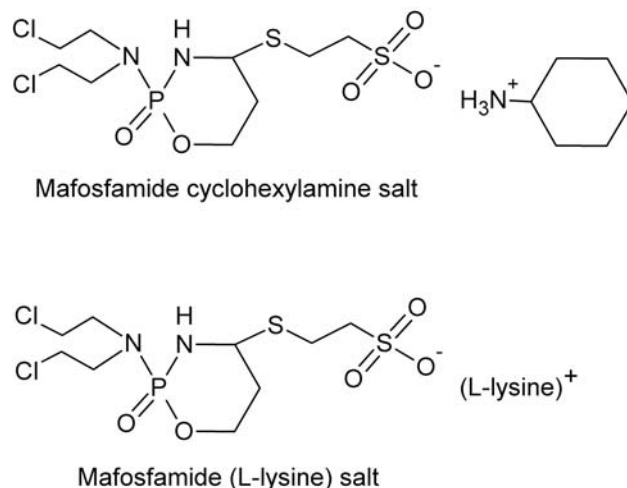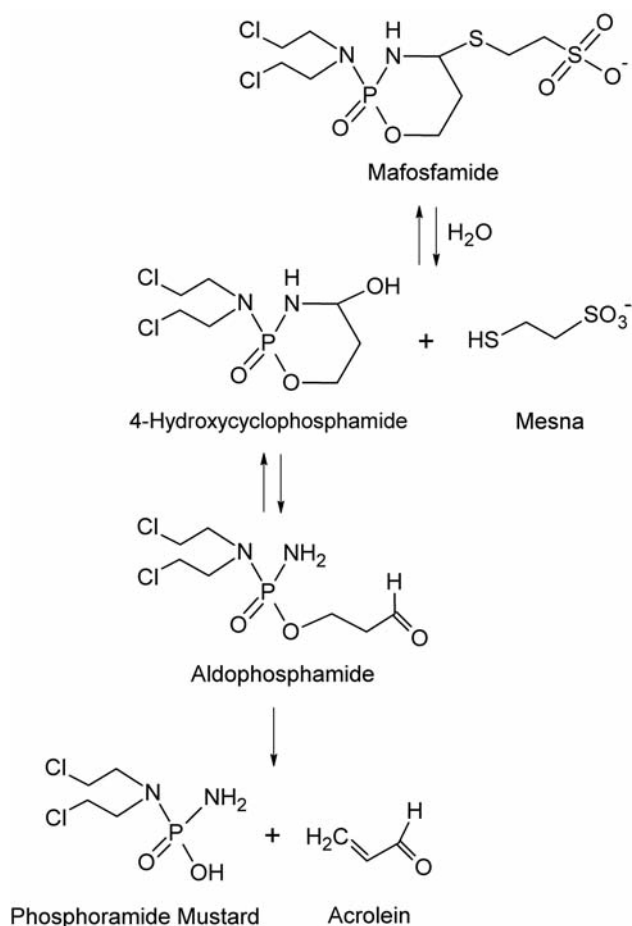

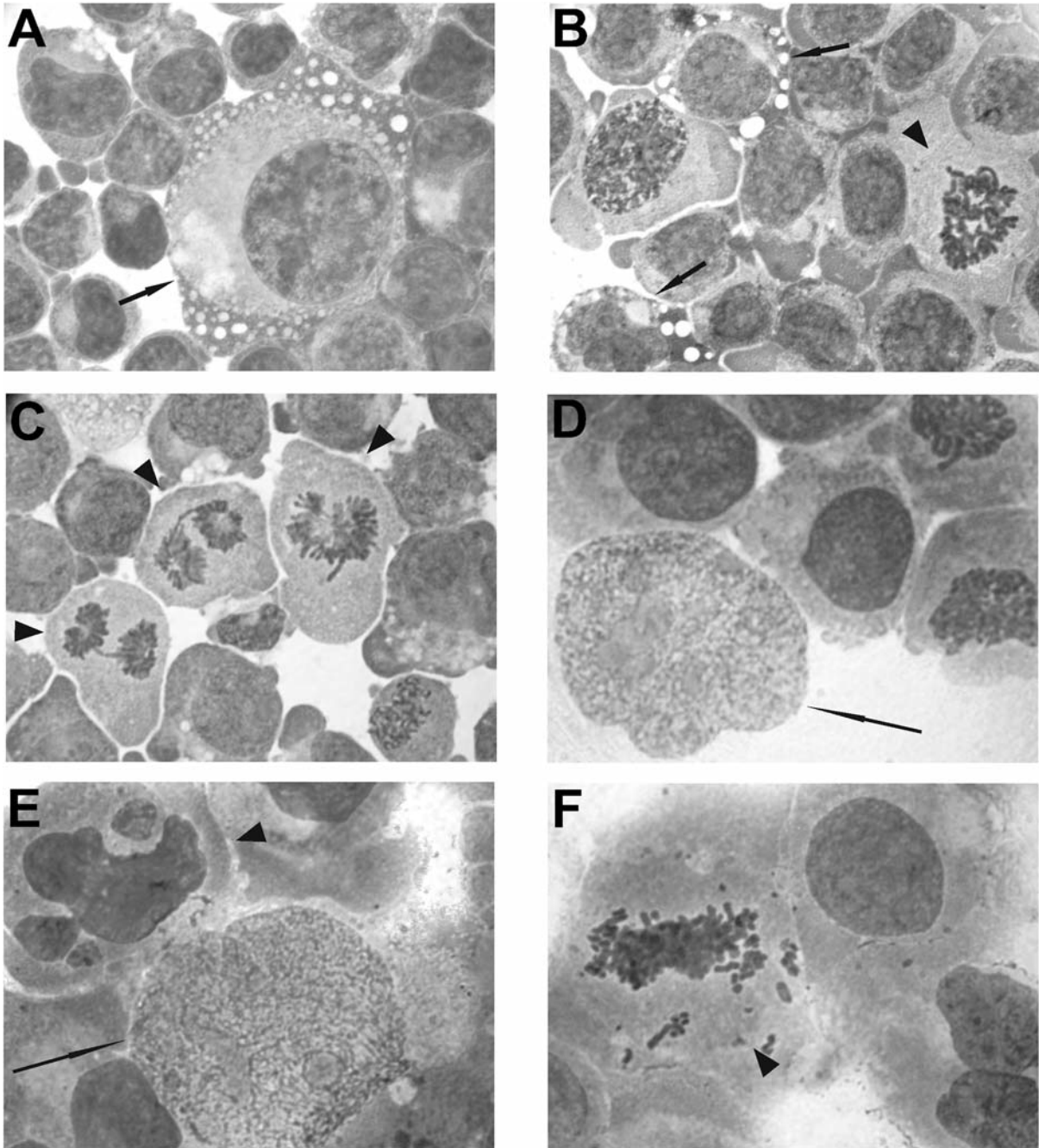Figure 3. *Mafosfamide and its metabolites.*

Figure 4. *Morphology of human histiocytic lymphoma U-937 cells undergoing autophagy (A, B, short arrow), mitotic catastrophe (B, C, E, F, arrow head) and necrosis (D, E, long arrow) after their exposure to the action of mafosfamide cyclohexylamine salt. U-937 cells visible under a light microscope (A, B, C – magnification ×400; D, E, F – magnification ×1000).*

L1210 leukemia, B16 melanoma, Lewis lung carcinoma, colon 38 tumor, and cyclophosphamide-resistant P388 leukemia. MAF has demonstrated anticancer activity similar to or better than CP in mouse models and in a cyclophosphamide-resistant P388 subline (2-5, 11, 13-15, 21, 26-28).

The *in vitro* combined action of MAF with various chemotherapeutic agents, was studied. The *in vitro* drug interactions were analyzed in acute lymphoblastic leukemia samples of children, obtained during the initial diagnosis. The combination of prednisolone and MAF generally showed additive and even synergistic interaction (29). The cytotoxic

combination of loxoribine with fludarabine and MAF on freshly isolated B-chronic lymphocytic leukemia cells was also tested. The combination of fludarabine and MAF was synergistic towards CLL cells, and the cytotoxic activity was increased by the addition of loxoribine (30). The cytotoxic effects of the *in vitro* combined action of karenitecin and MAF against leukemia, medulloblastoma and neuroblastoma cell lines, were also determined (28). These investigations provide important support for the development of the karenitecin and mafosfamide combination for use in intrathecal therapy.

Toxicological investigations carried out on rodents revealed that MAF given intravenously was more toxic in rats than in mice. The toxic LD_{50} lethal dose for mafosfamide was determined to be between 500 and 625 mg/kg in mice and between 250 and 310 mg/kg in rats, after intravenous administration. These values were much higher than the corresponding ones for 4-OH-CP. Moreover, in a single intravenous injection of CP (Endoxan), the LD_{50} was about 160 mg/kg in the rat, and 400 mg/kg in the mouse (31). MAF was clearly less toxic with respect to the bone marrow, the immune system and the urinary tract. However, the predominant toxicity in rodents was myelosuppression. When comparing equimolar doses, it was found that myelo- and urotoxicity caused by MAF were less severe than with CP (5, 8, 9, 32). Because of these marked differences in cytotoxicity, a phase I trial with MAF was warranted.

The development of animal models of neoplastic meningitis has been reported (33-35). The usefulness of the athymic rat model of human neoplastic meningitis was shown by Fuchs *et al.* (34). The activity and efficacy of intrathecal 4-hydroperoxy-CP against the human rhabdomyosarcoma cell line TE-671 and the human glioma cell line D-54 MG, grown in the subarachnoid space of athymic rats and the lack of toxicity at therapeutic levels of 4-hydro-peroxy-CP in normal athymic rats were demonstrated. Preclinical studies using a non-human primate model were also carried out (15, 33, 36, 37). The Rhesus monkey (*Macaca mulatta*) model of neoplastic meningitis, using MAF, was described. Application of a silicone catheter, surgically placed into the fourth ventricle, and attached to a subcutaneously implanted Ommaya reservoir, allowed for the determination of the acute and cumulative toxicity of mafosfamide (15, 33, 36). It has been demonstrated that MAF was well tolerated, when applied intrathecally in *Macaca mulatta*. The preclinical pharmacological studies based on the Rhesus monkey model of neoplastic meningitis showed that intrathecal mafosfamide administration was safe, and no systemic, nor neurologic toxic effects were observed (2, 15, 33, 36, 37).

MAF in Clinical Trials

The effects of mafosfamide were determined during several phase I clinical trials. The action of MAF was analyzed in patients with leukemia, lymphoma and central nervous system tumors, as well as in children younger than 3 years old who had newly diagnosed embryonal tumors. Systemic administration of MAF by an intravenous route and regional administration by intrathecal, intralumbar, and intraventricular routes were tested (2-5, 8, 9, 15, 38, 39).

Systemic intravenous administration of MAF. Mafosfamide cyclohexylamine salt (ASTA-Z-7557) was chosen for phase I clinical testing because of an expected higher therapeutic index and lack of complete cross resistance in animal tumors, compared to those observed after CP application. ASTA-Z-7557 was given to patients with histologically proven malignancies which either had become resistant to standard chemotherapy or were known to be highly resistant to cytotoxic drugs. The patients were also not eligible for surgery or radiotherapy. The schedule consisted of a single intravenous dose, repeated every three weeks. The maximum tolerated dose was approximately 1000 mg/m², given as a slow infusion over 2-3 h. Severe pain along the injected vein and acute irritation of mucous membranes were found to cause ASTA-Z-7557 dose-limiting toxicity. The severe venous pain and the mucosal irritation were probably caused by the high local concentration of 4-OH-CP or by its metabolites. Hematological toxicity was mild. A limited phase I study with the lysine salt of MAF (ASTA-Z-7654) demonstrated an identical type of toxicity. MAF given in a high-dose intermittent schedule was of little interest for further clinical trials (8).

A phase I trial with cyclohexylamine and lysine salts of MAF was also carried out on patients with advanced malignancies which were not amenable to standard treatments. An intravenous MAF infusion was administered weekly for at least 5 min at doses of 200 mg/m², 400 mg/m² and 700 mg/m². A dose of 700 mg/m² per week represents the maximum tolerated dose on a weekly schedule. Dose-limiting toxicities were observed in the form of severe pain along the vein during administration. After application of cyclohexylamine salt of MAF, moderate anemia was noted at all dose levels tested. Hematological toxicity was not encountered with the lysine salt applied at these dose levels. A particular mucosal syndrome with sneezing and conjunctivitis was seen only after the administration of the lysine salt. These results confirmed that systemic administration of MAF by the intravenous route was unacceptable in patients of phase I trials due to severe local pain at the injection site (9).

Regional intrathecal administration of MAF. The simple and rapid hydrolysis of MAF in biological fluids made this alkylating agent a suitable candidate for regional use. During the phase I clinical trials, MAF was selected and tested as a potential candidate agent for intrathecal dosing (4, 5, 15, 39).

A phase I clinical study assessed the effects of the intrathecal MAF in patients with refractory neoplastic meningitis who were treated with five doses ranging from 1.0 mg to 6.5 mg. Most of the patients had leukemia and lymphoma neoplastic meningitis. MAF demonstrated good activity. Chemotherapy was generally well-tolerated and there were no reported drug-related adverse events at lower doses. In this phase I clinical trial, headache was the dose-limiting toxicity. Most patients experienced headaches, probably related to the fast rate of drug infusion, associated with the 5 mg dose. Headache was ameliorated at 5 mg by prolonging the infusion rate to 20 min, but the dose-limiting headache occurred at a 6.5 mg dose with prolonged infusion. Thus, the final recommended phase II dose for intrathecal MAF, administered without concomitant analgesia, was 5 mg with a constant infusion rate of over 20 min (15, 37, 40).

Another phase I trial was carried out using intrathecal MAF in combination with multiagent systemic chemotherapy for children younger than 3 years old, who had newly diagnosed embryonal tumors of the central nervous system. Patients received intrathecal MAF at one of the six dose levels ranging from 5 to 17 mg. Patients were also premedicated with dexamethasone at 0.15 mg/kg and morphine at 0.1 mg/kg before receiving intrathecal MAF dosing. Irritability, presumably secondary to pain or headache, observed during the administration of MAF was the dose-limiting toxicity in patients at the 17 mg dose level. In these patients, the maximum-tolerated and recommended phase II dose of intrathecal MAF, following premedication with dexamethasone and morphine was establish to be 14 mg (3, 37, 39).

Regional intraventricular and intralumbar administration of MAF. During the phase I clinical trials, a strategy to alternate dosing between the intraventricular and intralumbar routes was tested. The cytotoxic target exposure for mafosfamide was determined to be 10 μmol/l. The results of pharmacokinetic investigations performed in patients with Ommaya reservoirs indicated that ventricular cerebrospinal fluid (CSF) MAF concentrations at 5 mg dosing, exceeded the target cytotoxic concentrations after an intraventricular dose, but lumbar CSF concentrations 2 h after the dose were less than 10 μmol/l (3, 15). The results of these clinical studies indicated that MAF dosing should be alternated between the ventricular and lumbar space.

Further Development of MAF

Mafosfamide was developed by Baxter-Oncology (www.baxter-oncology.com). Clinical studies using MAF are among the most recent clinical trials performed and described in the current literature (41-43). During phase I clinical trials, MAF appeared to be an excellent candidate for regional administration. The recommended phase II doses of MAF were determined (15, 39). However, the mode of MAF action is still under experimental and clinical investigation.

Opportunity to improve mafosfamide efficacy. Elucidating the action mechanisms of MAF on pathological and normal cells is important for its optional use in cancer therapy. For this reason, the response of various types of cells to the action of this oxazaphosphorine agent should be analyzed in further preclinical and clinical investigations.

Current and future clinical research should focus on methods allowing for the most effective delivery of MAF into the CSF, in which an appropriate concentration of this agent is maintained for extended periods. The combined intraventricular and intralumbar routes of MAF administration should be taken into consideration. During further clinical trials, the ventriculolumbar cerebrospinal application technique, using MAF, should be described in detail. A better understanding of the pharmacokinetics and the CSF pharmacology of MAF and of other chemotherapeutic agents used regionally, in combination with MAF, is an essential prerequisite for safe, effective administration of these drugs. Investigational efforts are underway to verify the feasibility and efficacy of MAF application at different dosages, schedules and combinations with both other intra-CSF agents and drugs used in systemic therapy (44-46).

Potential of MAF for therapy of neoplastic meningitis. Neoplastic meningitis is a problem in neuro-oncology. Nowadays, because of new diagnostic methods introduced into everyday clinical practice, the occurrence of leptomeningal carcinomatosis has been noted in approximately 5% of patients with cancer. Neoplastic meningitis results from the leptomeningeal dissemination of a variety of cancer types. Pathological cells spread in the leptomeninges and the CSF. Leukemia and lymphoma, lung and breast cancer, melanoma, and brain tumor, are the primary diseases commonly associated with leptomeningeal carcinomatosis. In general, the survival period of most patients with neoplastic meningitis is short and averages only 3 to 4 months (5, 36, 43, 47-51).

New and more effective agents, without severe local neurotoxicity, are needed for the management of leptomeningeal metastases. The development of new regional agents for the treatment of neoplastic meningitis is extremely challenging because of the limited number of drugs currently available and the increasing frequency of patients with neoplastic meningitis (37, 40, 43, 52, 53).

MAF may be potentially utilized for regional chemotherapy in the treatment of neoplastic meningitis (www.baxter-oncology.com). MAF is believed to be a potent candidate agent for this therapy because its mechanisms of

action differ from those of most available intrathecal drugs that are primarily antimetabolites. Clinical experience with MAF in neoplastic meningitis from leukemia, lymphoma and solid tumors indicated good tolerability and efficacy. Combined MAF application along with the currently used drugs could improve the therapeutic index of the treatment of neoplastic meningitis (2, 15, 45, 50, 51).

Conclusion

Mafosfamide represents an attractive new agent for cancer therapy. An assessment of the anticancer potential of MAF against various types of pathological cells is of key importance in chemotherapy. Further development of MAF is very important for its optional use in regional cancer therapy, and especially in the treatment of neoplastic meningitis.

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