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A Risk-Benefit Assessment of Amifostine in Cytoprotection

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Abstract

Recent advances in chemotherapy have focused on the benefit of high dose regimens, increasing the dose intensity of conventional chemotherapy and using intensified chemotherapy with or without autologous bone marrow rescue. Dose intensity usually increases objective response rates of antineoplastic drugs and might, in some circumstances, improves survival. However, unacceptable acute and/or cumulative toxicity often impairs the proper management of patients, leading to dose reduction or treatment delay, thus reducing the efficacy and potentially the quality of life of patients. Therefore, considerable efforts have been made to manage, to prevent, and to delay many acute and cumulative treatment-related toxicities.

Amifostine (WR-2721) is a multiorgan cytoprotector which has demonstrated cytoprotective effects, *in vitro* and *in vivo*, against the most common cytotoxic

drug-related toxicities and against radiation-induced adverse effects in healthy tissues. *In vitro* and *in vivo*, cytoprotection was observed in several organs including kidney, haematopoietic stem cells, myocardial cells, neural cells, and mucosa, without detectable protection of malignant cells. In addition, in preclinical studies, amifostine appeared to be able to reduce the risk of radiation-induced secondary neoplasms. Phase I studies showed that nausea/vomiting and hypotension are the dose-limiting toxicities of amifostine and these may be controlled by reducing the duration of injection of amifostine. Phase II and randomised studies have confirmed the efficacy of amifostine in protecting against radiotherapy-induced mucositis, cisplatin-induced nephrotoxicity, cyclophosphamide-induced neutropenia and carboplatin-induced thrombocytopenia. Importantly, the cytoprotection of healthy tissues occurred without any significant deleterious effect on response rate, time to progression, and survival of patients receiving amifostine.

However, in addition to the potential quality of life benefit, the most important question of whether the use of a cytoprotective agent might translate into the possibility of maintaining the dose intensity of anticancer therapies has still to be answered. The real benefit of amifostine in the overall management of patients with cancer requires additional studies to determine whether this chemoprotective approach can be of benefit to patients by increasing response rate, time to progression, and long term survival in patients receiving the more recent combination therapies involving new drugs such as the taxanes and oxaliplatin.

With the use of conventional chemotherapy, activity against malignant cells is counterbalanced by toxicity towards healthy cells. A tremendous amount of attention has been paid towards antineoplastic drug–related toxicity over the last 10 years allowing the reduction of adverse effects such as nausea and vomiting, neutropenia, infection and renal toxicity. This has allowed chemotherapy to be better tolerated and while preserving the quality of life and maintaining efficient doses and schedules.

However, several toxicities remain major problems in the management of patients receiving chemotherapy. Neurotoxicity often limits the proper use of cisplatin, paclitaxel, and more recently introduced agents such as oxaliplatin. Moreover, despite good hydration, some patients develop severe nephrotoxicity after receiving an injection of cisplatin thus precluding future use of this cytotoxic agent and sometimes leading to an increase in neurotoxicity, anaemia or ototoxicity. This is particularly important for patients with curable diseases such as germ cell cancer who require cisplatin-based treatment.

There are many theoretical possibilities that may help to balance the risk-benefit of chemotherapy. One of them involves increasing the specificity of antineoplastic drugs for cancer cells. The development of new antineoplastic agents with a selective mechanism of action, such as prodrugs, or drugs specifically targeted to tumour cell receptors is ongoing, but is currently of little clinical relevance. Secondly, improvement in the management of adverse effects with supportive care agents, such as antiemetics, bone marrow support, haematopoietic growth factors or antibacterials has demonstrated a real impact on the overall treatment of malignant diseases. At last, cytoprotectants may offer broad protection for tissues against the damage induced by cytotoxic drugs. One of the most relevant examples is mesna, a sulfhydryl compound, which demonstrated specific urinary bladder cytoprotection against bladder damages induced by the acrolein metabolites of ifosfamide and cyclophosphamide, without reducing the anticancer efficacy of these agents.[1] Subsequently, dexrazoxane (ICRF-187) showed selective protection of myocardial cells from anthracycline-related

toxicities. In patients with breast cancer treated with doxorubicin-based chemotherapy, dexrazoxane demonstrated significant protection against cumulative cardiac toxicity, without affecting the antitumour effect of doxorubicin, allowing an increase in the cumulative dose of doxorubicin administered.[2,3]

Interestingly, use of amifostine produces multiorgan and multidrug cytoprotection. Amifostine, formerly known as WR-2721, was initially developed by the Walter Reed Army Institute of Research as a US army project aiming to protect military soldiers and the population against atomic radiation in the event of a nuclear warfare. Among more than 4000 compounds tested in this project, amifostine was selected for its superior protective activity and for its safety profile. Since the main toxic effect of radiation relies on the production of free radicals in cells, this gave a rational for reversing toxicity by using other molecules that scavenge free radicals.[1] The first evidence of a selective protection of healthy cells without protection of malignant cells by amifostine was presented in 1969, by the works of Yuhas and Storer[4] Further preclinical studies have shown that amifostine offers a broad spectrum of protection against the toxicity of radiation, alkylating agents and cisplatin.[1] Amifostine efficacy and tolerance were then evaluated in several studies involving the use of amifostine as a cytoprotective agent in patients with advanced ovarian cancer treated with cisplatin + cyclophosphamide chemotherapy.[1] Several questions remain to be answered:

- does amifostine protect against major acute and cumulative toxicities induced by current chemotherapeutic agents?
- are the effects of amifostine selective enough to protect healthy cells without impairing the antitumour effects of cytotoxic drugs?
- are the toxicity and the cost of amifostine acceptable for routine practice?

In this review, we assess the risk-benefit profile of amifostine according to current preclinical and clinical data. The effectiveness of amifostine in the protection of several tissues, the toxicity, and the risk of tumour protection of amifostine are discussed.

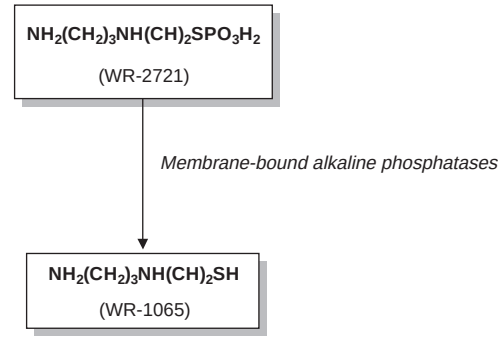

Fig. 1. Structures of amifostine (WR-2721) and its active metabolite WR-1065.

1. Mechanism of Action

Amifostine is a phosphorylated aminothiol inactive prodrug which is converted to a main active metabolite WR-1065 after dephosphorylation by a membrane-bound alkaline phosphatase (fig. 1). WR-1065 is believed to protect cells from damaging agents and radiation by scavenging free radicals, donating hydrogen ions to free radicals, depleting oxygen, and binding to active derivatives of cytotoxic drugs, either avoiding or repairing DNA damages.[1]

Yuhas and Storer[4] first noticed the differential chemoprotection of healthy from malignant tissues by amifostine and that, as a consequence, the effectiveness of radiation therapy could be improved if damage to healthy tissues could be preferentially prevented.

Surprisingly, preclinical studies showed a greater uptake of amifostine in healthy tissues than in tumours.[5,6] Therefore, the kinetics of absorption of amifostine were determined both in healthy and tumour tissues of mice, rats, and rabbits. Interestingly, healthy tissues actively concentrate amifostine against the gradient of concentration (i.e. when the serum concentration is declining, the healthy tissue concentration continues to increase). In contrast, solid tumours passively absorb the compound at low concentrations.[7] This observa-

tion was crucial to explain the ability of amifostine to selectively protect healthy tissues against radiation and alkylating lesions. The transport of amifostine into healthy cells seemed to be related to an active membrane transport system, probably after dephosphorylation of amifostine into WR-1065 (fig. 2). Thus, preferential uptake of amifostine by healthy tissues may be explained by a high ratio of membrane-bound alkaline phosphatase between healthy tissues and tumours.[8-10]

Yet, despite a considerable work, the basis for the selective cytoprotection of healthy tissue by amifostine is only partially explained. The selectivity of amifostine is also believed to be associated with several mechanisms:[11]

- high pH of healthy cells facilitating both thiol formation and intracellular uptake
- accumulation of healthy cells in G2/M phase contributing to sensitivity toward thiol compounds
- promotion of haematopoietic progenitors protecting bone marrow against cytostatic drugs
- oxygen dependence of the protective effect of amifostine; oxygenation is reduced in tumour cells.

2. Pharmacokinetics and Pharmacokinetic Interactions of Amifostine

In early preclinical studies, pharmacokinetic and tissue distribution of amifostine was investigated in mice. Amifostine and its active metabolite WR-1065 were observed to be present in significant amounts in the kidney, lung, liver, skin, bone marrow, bowel, and spleen for 60 to 90 minutes, whereas they were nearly nondetectable in brain, muscle and tumour tissues.[5] Elimination was essentially renal.[5,6]

The pharmacokinetics of amifostine were also studied by Shaw et al.[12] in 13 patients with cancer.

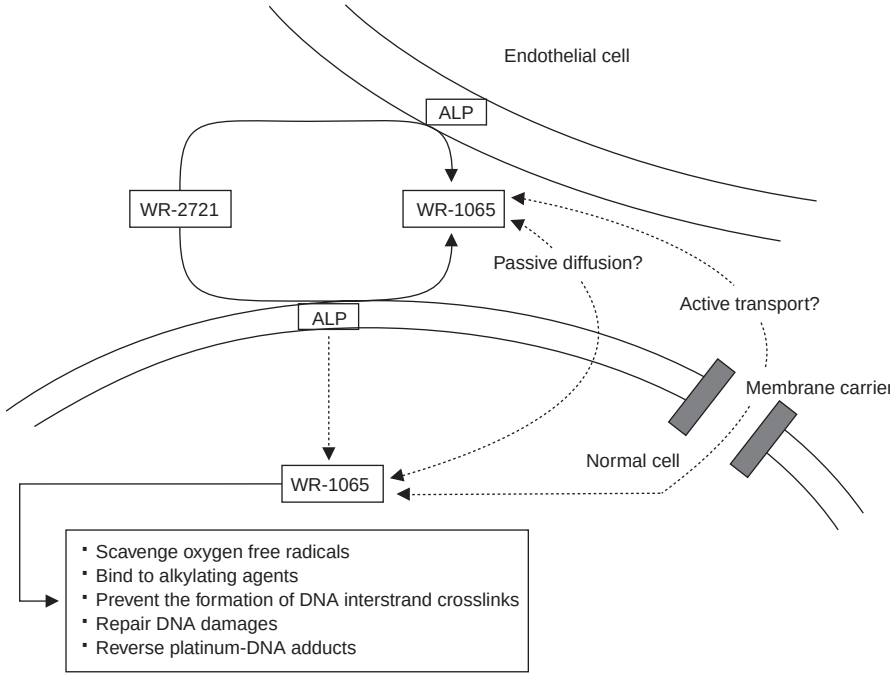

Fig. 2. Uptake and mechanism of action of amisfostine (WR-2721) and of its active metabolite WR-1065. **ALP** = membrane-bound alkaline phosphatase.

After a 150 mg/m^2 intravenous bolus injection, maximal concentrations of amifostine and its active metabolite WR-1065 were observed at the end of the infusion; they then decreased rapidly. Elimination half-life was very short (2 to 9 minutes) and less than 10% of the drug remained in the plasma compartment 6 minutes after the administration of the dose. An average plasma clearance value of 2.17 L/min was obtained. Together with the small average volume of distribution (6.4L), these results suggested that amifostine is immediately taken up by tissues and converted to metabolites.

Pharmacokinetic interactions of amifostine with chemotherapeutic agents were investigated in several preclinical studies, pointing out the original proprieties of this compound. The protective effects of amifostine have been investigated primarily against the damaging effects of alkylating agents. *In vitro*, the influence of amifostine on the formation and stability of cisplatin adducts is well documented.[13] On isolated DNA incubated with cisplatin, amifostine and its active metabolite WR-1065 were remarkably active in the inhibition of DNA platination. Formation of adducts decreased in the order of 74% with WR-1065 and 51% with amifostine, in a concentration-dependent manner. Furthermore, WR-1065 and amifostine were respectively able to remove up to 28% and 13 to 14% of adducts in pre-platinated DNA. These results suggested the high potential of amifostine to protect from cisplatin-induced DNA damages, but stressed at the same time the importance of a selective formation and uptake of the active metabolite WR-1065 by nontumour tissues when combined with chemotherapeutic agents.

The concept of selective protection was further confirmed by testing the effect of amifostine toward platinum compounds using *in vivo* models.[14,15] When administered 30 minutes prior cisplatin in mice, amifostine selectively protected healthy tissues from cisplatin-induced toxicity, allowing an increase in platinum doses before the occurrence of nephrotoxicity.[14] Regarding carboplatin pharmacokinetics, a single dose of amifostine increased the area under the curve of carboplatin in mice. The concentration of platinum adducts was decreased in healthy tissues (liver, kidney and bone marrow) whereas there was a significant increase in tumour tissue that may explain the increase of antitumour activity of carboplatin.[15]

Preclinical results are consistent with clinical findings. Korst et al.[16,17] investigated the pharmacokinetics of platinum compounds with and without amifostine in patients with cancer. For cisplatin, the final half-life of unchanged cisplatin did not increase, and even slightly decrease after treatment with amifostine. Because area under the curve values and platinum-DNA adduct concentration in leucocytes did not change significantly, the authors concluded that the change in the pharmacokinetics of cisplatin has no significant impact on the efficacy of cisplatin.[16] For carboplatin, the pharmacokinetic interaction consisted of an increase of the final half-life and the area under the curve value of ultra-filterable platinum in plasma after treatment with carboplatin in combination with amifostine.[17]

Only sparse data are available regarding the protective effects of amifostine toward other cytotoxic agents and further studies are warranted with novel antitumour agents presenting cumulative toxicities including anthracyclines and taxanes.[18-21]

3. Risks Associated with Amifostine

3.1 Risk of Tumour Protection

The major question with any chemoprotective agent is whether the protection of healthy tissues extends to protecting tumour cells from the cytotoxic effects of chemotherapy and radiotherapy. Cytoprotection is efficient if a very high degree of specificity is achieved to protect healthy cells and not cancer cells.

Yuhas and Storer[4] first described the differential chemoprevention by amifostine of healthy and malignant tissues in animal studies. In this model, injection of amifostine in mice with 67% of the maximum tolerated dose increased the radiation resistance of the skin by a factor of 2.4 and of the

Table I. Summary of preclinical studies investigating the selectivity of amifostine for healthy cells

Reference	Tumour	Recipient	Anticancer agent	Cytoprotective agent tested	Results
Yuhas et al.[4]	Murine mammary tumour	C57BL/6J mice	Radiation	Amifostine	Amifostine increased the $LD_{50/30}$ by 160-170%, the x-ray dose required to induce skin ulceration by 140%, and to inhibit tumour transplantability by 15%
Yuhas et al.[24]	Murine lung carcinoma cell line	Balb/c mice	HN2	Amifostine	Amifostine failed to alter HN2-induced growth delays, except when given within 5 to 15 mins prior the injection of HN2. HN2 inhibition appeared to result from direct inactivation of amifostine in the blood
Yuhas et al.[22]	Mammary carcinoma	Balb/c mice and fischer rats	Cisplatin + cyclophosphamide	Amifostine	No evidence for tumour protection
Valeriote et al.[25]	AKR leukaemia cell line	AKR mice	HN2	Amifostine	Amifostine potentiated the cytotoxicity of HN2 on leukaemia cells, while protecting haematopoietic cells
Treskes et al.[14]	OVCAR-3	Nude mice	Cisplatin	Amifostine	No evidence of tumour protection
Treskes et al.[26]	OVCAR-3	Nude mice	Carboplatin	Amifostine	Amifostine had a potentiating effect on the tumour growth inhibition of a standard dose of carboplatin, while allowing a 1.5 fold increase in the MTD
Wittenkeller et al.[28]	NSCLC	Nude mice	Cisplatin = vinblastine	Amifostine	Amifostine enhanced antitumour effect of cytotoxic drugs as assessed by inhibition of tumour growth
Alberts et al.[30]	2780 cells, MCF7	*In vitro*	16 drugs tested[a]	WR-1065	WR-1065 had no effect on the IC_{50} values of any of the drugs tested
Dunn et al.[29]	H12.1	Nude mice	Cisplatin	Amifostine	No evidence of tumour protection as assessed by the tumour volumes at days 14 and 30
Fulda et al.[31]	2 neuroblastoma cell lines: IMR-5 and CHP-100	*In vitro*	Perfosfamide,[b] 4HI, cisplatin, etoposide, vincristine, doxorubicin	Amifostine or WR-1065	Addition of amifostine or WR-1065 did not reduce cytotoxicity of all 6 agents tested, as assessed by the dose-response curves and the ED_{50} values
Fichtner et al.[11]	Human neuroblastoma cells: IMR5-75 and Kelly	Nude mice	Cyclophosphamide, ifosfamide, cisplatin, etoposide, vincristine, doxorubicin	Amifostine	Amifostine did not interact with the antitumour effect of any cytostatic used in combination, while it mitigated vincristine-induced bodyweight loss and leucopenia related to cisplatin, ifosfamide or cyclophosphamide
Taylor et al.[27]	NSCLC	*In vitro*	Paclitaxel	Amifostine	Amifostine enhanced cytotoxicity in NSCLC, while protecting healthy human lung fibroblasts
Taylor et al.[27]	A2780 cells	Scid mice	Paclitaxel	Amifostine	Amifostine did not interfere with paclitaxel antitumour activity

a Bleomycin, carboplatin, cisplatin, cytarabine, daunorubicin, doxorubicin, etoposide, fluorouracil, idarubicin, melphalan, mitomycin, mitoxantrone, paclitaxel, taxotere, vinblastine, vincristine.

b 4-hydroperoxycyclophosphamide.

A2780 cells = human ovarian cancer cells; **ED_{50}** = *in vitro* concentration for 50% growth inhibition; **H12.1** = human non-seminomatous germ cell tumour; **4HI** = 4-hydroperoxyifosfamide **HN2** = nitrogen mustard; **IC** = inhibitory concentration; **LD** = lethal dose; **MCF7** = human breast cancer cells; **MTD** = maximum tolerated dose; **NSCLC** = human non–small cell lung cancer cells; **OVCAR-3** = subcutaneous human ovarian carcinoma xenografts; **WR-1065** = active metabolite of amifostine.

bone marrow cells by a factor of 2.7, but it did not increase the resistance of a transplantable mammary carcinoma.

Thereafter, the absence of cytoprotection of malignant cells has been assessed in many *in vitro* models. Early studies have investigated the activity of amifostine on malignant cells obtained from animal models. The absence of protection by amifostine was demonstrated in several tumours cell lines, in *in vitro* culture and/or in *in vivo* models including numerous mammary,[4,22,23] lung,[23,24] and leukaemia murine cell lines.[25] Early data were summarised by Yuhas et al.[23] Further preclinical studies are listed in table I.

Recent studies listed in table I investigated the effect of amifostine on human malignant cell lines. When amifostine was administered to nude mice bearing subcutaneous human ovarian cancer OVCAR-3 xenografts, no evidence of tumour protection against cisplatin[14] or carboplatin[26] was observed, as assessed by the delay of tumour growth. Furthermore, amifostine demonstrated a potentiating effect on the tumour growth inhibition of standard doses of carboplatin, while protection of healthy tissues by amifostine allowed a 1.5-fold increase in the maximum tolerated dose of carboplatin.[26] Recently, similar results were obtained in nude mice bearing A2780 human ovarian cancer cells treated with amifostine and paclitaxel. Amifostine did not reduce the antitumour effect of paclitaxel in this model.[27] The administration of amifostine to nude mice bearing human non–small cell lung cancer before the injection of cisplatin and vinblastine demonstrated an enhancement of the antitumour effect of cytotoxic drugs as assessed by tumour growth inhibition.[28] Surprisingly, the enhancement of the antitumour effect was not observed in the *in vitro* model[28] while it was demonstrated in *in vitro* culture of non–small cell lung cancers cell lines incubated with paclitaxel and amifostine.[27]

Since testicular germ cell tumours showed high sensitivity to cisplatin, and since cisplatin-based chemotherapy demonstrated high response rates, allowing long term survival, it would be of interest to investigate the activity of amifostine on the cytotoxic effects of cisplatin in this tumour. Testicular germ cell tumours are known to express alkaline phosphatase, suggesting a potential protection of tumour cells by amifostine. This question was addressed in a preclinical study. In this study, nude mice bearing human alkaline phosphatase-positive embryonal carcinoma xenografted tumours received cisplatin with or without amifostine. Tumour volumes were measured on days 14 and 30. Cisplatin showed good antitumour activity. Mean tumour volumes were not significantly different between mice treated with amifostine and cisplatin versus cisplatin alone, suggesting the absence of tumour protection by amifostine. Despite high rate of alkaline phosphatases, germ cell tumours were not protected by amifostine against cisplatin-toxicity, neither *in vitro* nor in nude mice models.[29] In the light of these results, it would appear that most malignant tumours are not protected by amifostine. Furthermore, in several animal models the tumour control seemed to be improved by amifostine. Despite the positive results of these studies, data are still warranted to confirm the absence of tumour protection when amifostine is given to patients.

The absence of tumour protection of amifostine has been assessed in randomised clinical trials.[32-42] In these trials, which are summarised in table II, tumour response and survival were found to be similar in patients treated with and without amifostine and amifostine allowed protection of several types of healthy tissues without any effect on tumour protection. Furthermore, in most of these studies, response rates were slightly higher in patients receiving amifostine. However, with the exception of the trial conducted by Kemp et al.,[32] the number of patients in the trials was too small to detect a significant difference in survival between patients treated with or without amifostine. Long term results of further phase III randomised studies are warranted to assess definitively the absence of tumour protection when amifostine is given to patients with a variety of tumours.

Table II. Summary of phase II and III randomised studies evaluating amifostine activity

Reference	No. patients in amifostine group/control group	Type of tumours	Cytotoxic regimens	Amifostine dose (mg/m^2)	Tissues protected	Survival (months) or RR (amifostine group/control group)	Comments
Kemp et al.[32]	122/120	Ovarian cancer	Cisplatin 100 mg/m^2 CPM 1 g/m^2 q21d	910	Kidney Bone marrow Peripheral nerves	MS: 31/31	Tissues protected without impairment of antitumour activity
Glover et al.[36]	21	Several solid tumours	CPM 1500 mg/m^2 q28d	0 at C1 740 at C2	Neutrophilic cells	ND	Reduction of neutropenia, data on survival not available
Aviles et al.[37]	40	NHL	EEIC	910	Neutrophilic cells	ND	Reduction of neutropenia, data on survival not available
Poplin et al. [41]	48/49	colorectal cancer	Mitomycin	910	Platelet	MS: 7.2/6.3	No evidence of tumour protection; however, objective response was not observed in any patient
Planting et al. [42]	74/74	Non-operable head and neck	Cisplatin 70 mg/m^2/week	740	Platelets: Auditory cells	RR: 57/70%	Nonsignificant reduction of response rate
Budd et al.[33]	30/23	Several solid tumours	Carboplatin 500 mg/m^2	910 H0+H2	Platelets	ND	No significant protection of neutrophilic cells; data on survival not available
Anderson et al.[34]	24/21	NSCLC	Carboplatin AUC 9	740 H0+H2 (or G-CSF)	Platelets	RR: 55/22%	No significant protection of neutrophilic cells; data on survival not available. Amifostine reduced severity of thrombocytopenia without a reduction in response rate
Betticher et al.[35]	21	NSCLC	Carboplatin 600 mg/m^2	910 or 683 H0+H2+H4	Platelets	MS: 14/9	Amifostine reduced the duration of thrombocytopenia without a reduction in survival
Liu et al. [40]	49/51	Rectum	45 + 15Gy RT	340	Mucosa (late toxicity)	MS: 15/12.6	Nonsignificant reduction in survival
Brüntzel et al.[39]	25/14	Head and neck cancers	60Gy RT + carboplatin	500[a]	Mucosa Salivary glands	DFS: 80/78% at 6 months	No evidence of tumour protection
Brizel et al.[38]	300	Head and neck cancers	60-70Gy RT	200	Mucosa Salivary glands	DFS: 86/79% at 12 months	No evidence of tumour protection

a Prior the injection of carboplatin.

AUC = area under the curve; **C1** = first course; **C2** = second course; **CPM** = cyclophosphamide; **DFS** = disease free survival; **EEIC** = etoposide 1 g/m^2, epirubicin 180 mg/m^2, ifosfamide 5 g/m^2, cyclophosphamide 1.5 g/m^2; **G-CSF** = granulocyte colony-stimulating factor; **H0**+**H2** = amifostine administration before and 2 hours after carboplatin injection; **H0**+**H2**+**H4** = H0+H2 + amifostine administration 4 hours after carboplatin injection; **MS** = median survival; **ND** = data not available; **NHL** = non–Hodgkin's lymphoma; **NSCLC** = non–small cell lung cancer; **qxd** = every x days; **RT** = radiotherapy; **RR** = response rate.

3.2 Toxicity of Amifostine

The toxicity profile of amifostine has been elucidated from phase I clinical trials[43] and has been further confirmed in recent phase II and III studies. In all studies, the principal toxicities were emesis and hypotension.

In phase I trials, hypotension was a dose-limiting toxicity of amifostine, occurring in 60% of patients at doses up to 910 mg/m^2. Hypotension con-

sisted of a decrease in systolic blood pressure of 20 to 50mm Hg and occurred in about 40% of patients at doses ranging between 740 and 910 mg/m^2. Rate and severity of hypotension were correlated to the dose and to the duration of exposure to amifostine. Hypotension occurred more often when the infusions of amifostine lasted more than 15 minutes. Hypotension was usually asymptomatic when patients were hydrated and kept in supine position during drug administration. Therefore, it is that blood pressure is monitored every 5 minutes during amifostine administration and the infusion should be stopped if there is a 20% decrease in systolic blood pressure. Symptomatic hypotension or a major decrease in blood pressure should be treated by rapid saline infusion.[32,43] Hypotension seemed to be more frequent in patients receiving doses above 740 mg/m^2.

Emesis was noticed in 80 to 90% of patients at doses above 740 mg/m^2 prior to the systematic use of prophylactic antiemetics.[43] In recent studies, patients received a cocktail of a serotonin-receptor antagonist and dexamethasone to prevent amifostine-induced nausea and vomiting. Despite this precaution, grade 3 or 4 nausea and vomiting were observed in 23% of patients, in a phase III study.[32] In this study, the incidence of nausea and vomiting was significantly higher in patients receiving amifostine and chemotherapy than in patients receiving chemotherapy alone.[32] Emesis was mainly observed in patients treated with high doses, i.e. amifostine >740 mg/m^2, and in young patients (mainly women with breast or ovarian cancer). Emesis is a major concern in the treatment with amifostine since concomitant medications aiming to prevent toxicity should not increase the toxicity of chemotherapy. This is particularly important in combination with cisplatin, which is already known to induce high grade nausea and vomiting. Surprisingly, as described above, the usual cocktail used to prevent chemotherapy-induced nausea and vomiting were not efficient in preventing amifostine nausea and vomiting. To date, the physiopathology and the treatment of this particular adverse effect is poorly understood. Considering the relatively low efficacy of current premedication, it could be speculated that the mechanism of amifostine-induced emesis differs from that of chemotherapy. Since this could clearly alter the quality of treatment, more work needs to be done to understand and efficiently prevent this adverse effect.

Minor adverse effects were also noticed in 10 to 30% of patients and consisted of sneezing, flushing, dizziness, somnolence, hiccups, and minor allergic reactions such as skin rashes or rigors.[32,43]

Hypocalcaemia occurred in less than 1% of patients after they received a single dose of amifostine.[43] Symptomatic hypocalcaemia was described in studies where patients were given multiple infusions of amifostine.[44] Hypocalcaemia was always well corrected with calcium administration and should be prevented with by use of oral calcium supplementation and serum calcium level monitoring, when multiple doses of amifostine are given.

In summary, the adverse effects of amifostine are usually minor and always transient. However, they are quite frequent and potentially affect the quality of life of patients. Hypotension and emesis should be always adequately prevented.

4. Benefits of Amifostine in Cytoprotection

4.1 Nephroprotection

Dose-limiting nephrotoxicity is a major stumbling block in the use of cisplatin. Both acute and chronic forms of renal injury have been described. The rate of moderate to severe acute nephrotoxicity has been reported to be less than 5% after administration of medium dose of cisplatin 50 to 60 mg/m^2 and 1 to 10% after treatment with cisplatin 100 mg/m^2.[45-47] Acute toxicity can be reduced by adequate hydration,[48] while chronic toxicity does not seem to be related to the degree of hydration but to the cumulative dose of cisplatin. In almost all patients, it consists of a decrease in glomerular filtration rate ranging from 12 to 23% of the baseline level.[49,50]

Protection by amifostine against renal damage induced by cisplatin was first established in several

preclinical studies.[14,22] Lethal doses of cisplatin were administered to amifostine pretreated rats and did not induce renal toxicity.[22] Similar effects were observed in mice treated with cisplatin allowing an increase of the cisplatin doses by a factor of 1.6 (5 to 8 mg/kg).[14] Nephroprotection was only obtained when amifostine was administered either 5 or 30 minutes before cisplatin injection, but not when amifostine was given 30 minutes after cisplatin.[14] These data strongly support the hypothesis that amifostine prevents damage rather than repairs damage to renal cells.

Pharmacological data predicted that amifostine could protect the renal tubules against damage caused by cisplatin. Tubular renal cells have been shown to express a large amount of alkaline phosphatases. As expected, this translates into a high level of conversion of amifostine into the WR-1065 form in the kidneys of mice receiving radiolabelled amifostine.[5,6,12] In these studies, a maximum accumulation of amifostine in proximal tubules was observed 30 minutes after amifostine injection then decreased rapidly after 90 minutes.[12]

Based on preclinical data, the cytoprotection produced by amifostine was then evaluated in several phase I-II trials with cisplatin.[44,51-57,61] Results of these trials are summarised in table III. Overall in the phase II studies more than 150 patients with metastatic melanoma,[51,52] head and neck tumours,[52] and non–small cell lung cancers,[52-54] received cisplatin at doses ranging from 120 to 150 mg/m^2 in combination with amifostine. No significant renal damage was observed when amifostine was given at doses of 740 or 910 mg/m^2 15 minutes prior to the administration of high dose cisplatin. In these studies, despite the administration of high doses of cisplatin, 10 to 15% of patients experienced an increase in serum creatinine level and in those who did, the increases were mild and transient.[51,55]

Conversely, in 1 study, 5 of 19 patients treated for advanced uterine cervical cancer with a combination of cisplatin + radiation therapy, presented with serious nephrotoxicity, despite amifostine administration before cisplatin injection.[44] The serum creatinine level increased to 55 to 132 mg/L and recovered to less than 20 mg/L in all patients. In this study, the planned cumulative cisplatin dose was 400 mg/m^2 over a 12-week period. Pelvic radiotherapy was incriminated as a potential factor to explain the high rate of nephrotoxicity observed in this study. However, the study authors have compared the results to a historical group of 43 patients with advanced cervical cancer treated with the same cisplatin-radiation schedule but without amifostine. Only 3 out of these 43 patients experienced serious nephrotoxicity (defined as a more than 25 mg/L increase in serum creatinine level). Thus, in the small subset of patients treated by Wadler et al.,[44] amifostine did not appear effective in protecting against renal toxicity induced by cisplatin. Similarly, in another study, 20 patients with metastatic melanoma were treated with amifostine 910 mg/m^2 prior to receiving high doses cisplatin ranging from 135 to 150 mg/m^2. In this study, 14 of 20 patients experienced grade 3 or 4 severe nephrotoxicity.[56] Thus, nonrandomised phase II studies should be interpreted with caution, given the small number of patients involved and the absence of a control group.

In addition to the results of the first investigational phase I/II studies, a phase III trial comparing cisplatin-based chemotherapy, with or without amifostine was performed. This randomised study was conducted by Kemp et al.,[32] in 242 patients with advanced ovarian cancers. All patients were treated with a combination of cisplatin 100 mg/m^2 and cyclophosphamide 1000 mg/m^2 every 3 weeks for 6 courses. After randomisation, 122 patients received amifostine 910 mg/m^2 as a 15-minute infusion before receiving the cisplatin injection. In this study, both acute and cumulative toxicities were studied. In the control group, administration of cisplatin had to be delayed in 15% of patients, because of a serum creatinine level of >1.5 mg/dl, compared with the 5% of patients in the amifostine group ($p = 0.014$). By the last cycle of chemotherapy, 30% of patients in the control group compared with 13% in the amifostine group experienced a >40% reduction from baseline in creatinine clearance ($p = 0.001$).

This study was the first to confirm the ability of amifostine to reduce cisplatin-induced nephrotoxicity. However, several biases are present in this study. In particular, the aim of the study was to investigate the protecting effect of amifostine against chemotherapy-induced haematotoxicity not nephrotoxicity. Therefore, the observation of nephroprotection is a retrospective observation that needs to be confirmed in prospective clinical trials specifically addressing this question. Furthermore, data about severe nephrotoxicity were not reported in the study.[32] Nevertheless, based on this study, it was concluded that amifostine has the potential to reduce the nephrotoxicity induced by cisplatin in patients with advanced ovarian cancers, without reducing efficacy.

4.2 Haematoprotection

Early *in vitro* studies demonstrated the ability of amifostine to protect haematopoietic progenitors from the adverse effects of several alkylating drugs. Usually, most alkylating agents are capable of inducing cellular damage at concentrations ranging from 0.01 to 10 μmol/L. Wasserman et al.[58] demonstrated that the concentrations of cytotoxic drugs required to reduce stem cell viability

Table III. Studies assessing the efficacy of amifostine in cisplatin-induced nephrotoxicity

Reference	Study phase	No. of pts	Type of tumours	Cisplatin regimen (mg/m^2)	Amifostine dose (mg/m^2)	Nephrotoxicity according to CTC grading	Comments
Glover et al.[51]	I	36	Metastatic melanoma	60 to 150 q21d or q28d	740	Grade 2 NT in 5 of 126 courses	Suggested nephroprotection
Demchak et al.[56]	I/II	27	Metastatic melanoma	135 or 150 q21d + IL-2	910	Grade 3 or 4 NT in 14 of 20 pts receiving amifostine	No evidence of protection
Wadler et al.[44]	II	19	Uterine cervical cancer	100 q21d + RT	340 to 910	Grade 3 or 4 NT in 5 pts	No evidence of protection
Capizzi et al.[52]	II	74	Metastatic melanoma, head and neck tumours, NSCLC	120 q28d	740 or 910	3 of 49 pts experienced >40% reduction from baseline in creatinine clearance following 4 cycles or more	Suggested nephroprotection
Schiller et al.[53]	II	25	Stage IV NSCLC	120 q28d + vinblastine 5 mg/m^2/week	740 or 910	12% grade 3 NT, reversible 28% grade 2 NT	Suggested nephroprotection
Tannehill et al.[54]	II	26	Stage III NSCLC	120 q28d + vinblastine followed by RT 60Gy	740 or 910	12% grade 2 NT	Few NT, but only 2 courses of cisplatin
De la Garza et al.[57]	II-R	20	Uterine cervical cancer	100 q21d + RT	825 (10 pts) or 0 (10 pts)	Grade 2 NT in 2 of 10 pts	No evidence of protection
Breier et al.[55]	II	15	Stage IIIb or IV NSCLC	120 q28d + vinorelbine 60	740	No toxicity	Suggested nephroprotection
Bokemeyer et al.[61]	II-R	25	Different solid tumours	50 + ifosfamide + etoposide or teniposide	910 or G-CSF	No toxicity	Cisplatin doses too low to make conclusions
Kemp et al.[32]	III	242	Ovarian cancer	100 q21d + cyclophosphamide	910	5% grade 1 NT (versus 15% in the control arm, p = 0.014) 13% of >40% reduction from baseline creatinine clearance (versus 30%, p = 0.001)	Demonstrated nephroprotection

CTC = common toxicity criteria; **G-CSF** = granulocyte colony-stimulating factor; **IL-2** = interleukin-2; **NSCLC** = non–small cell lung cancer; **NT** = nephrotoxicity; **pts** = patients; **qxd** = every x days; **II-R** = randomised phase II study; **RT** = radiotherapy.

were increased by 1.5- to 4.6-fold when alkylating agents were give in combination with amifostine. Further studies showed that the cytoprotection was selective for healthy stem cells. For example, healthy AKR-mice stem cells cultured *in vitro* were selectively protected against nitrogen mustard toxicity while AKR-leukaemia cells were not.[25]

This selective protective effect on healthy stem cells did not seem to impair the antitumour activity of alkylating agents. For example, Treskes et al.[26] treated nude mice bearing an ovarian cancer with carboplatin alone or in combination with amifostine. In this study, haematotoxicity was reduced but the antitumour effect was not significantly altered.[26] More recently, a similar *in vitro* protection of granulocyte-erythroid-macrophage-megakaryocyte colony-forming units and erythroid burst-forming units was observed in mice treated with paclitaxel and amifostine while anticancer efficacy was not significantly altered.[27]

Amifostine appears to act as a bone marrow cytoprotectant against the toxicity of several alkylating drugs.[59,60] Interestingly, amifostine might be considered a multilineage cytoprotectant, giving it potential superiority over granulocyte colony-stimulating factors (G-CSF) for both alkylating agent–induced neutropenia and carboplatin-induced thrombocytopenia. Because of its preventive mechanism of action, amifostine is supposed to 'preserve' bone marrow, by protecting stem cells progenitors, while conversely haematopoietic growth stimulating factors might not fully preserve bone marrow from cumulative toxicity. Furthermore, amifostine has been shown to increase and stimulate the stem cell growth in *in vitro* cultures of healthy haematopoietic cells and in cultures of stem cells from patients with myelodysplastic syndromes.[59,60]

4.2.1 Protection of Erythropoiesis

The ability of amifostine to protect against the development of anaemia has not been fully investigated and no specific phase I or II study has been designed to investigate this particular effect. Indirect information can be found in the study by Kemp et al (see section 4.1).[32] In this phase III trial, a 29% reduction in the need for red blood cell transfusions was observed in the amifostine pretreated group. However, the reduction in the need for red blood cell transfusions was not significant when compared with the need for such transfusions in the control group.

4.2.2 Protection of Thrombopoiesis

Hundreds of patients have been treated in several randomised trials to evaluate the protective effect of amifostine against carboplatin-induced thrombocytopenia.[33-35] In a study by Budd et al.,[33] carboplatin 500 mg/m^2 was administered with or without an infusion of amifostine 910 mg/m^2 given 15 minutes before and 2 hours after the carboplatin injection in patients with a variety of solid tumours. Median platelet nadir and incidence of grade 3 thrombocytopenia were not significantly modified by amifostine administration after the first course of treatment. However, the median platelet nadir for all courses was higher in the amifostine-treated patients (127×10^9/L) than in the control group (88×10^9/L, $p = 0.023$).[33]

In another trial, 45 patients with non-resectable non–small cell lung cancer were randomised to receive high dose carboplatin (area under the curve = 9) in combination with either amifostine or G-CSF. Amifostine 740 mg/m^2 was given as a 15-minute infusion 15 minutes before and 2 hours after carboplatin injection. The rate of grade 4 thrombocytopenia was significantly lower in patients receiving amifostine (0 *vs* 45%, $p < 0.001$) as was the number of patients requiring a platelet transfusion (1 *vs* 7 patients, $p = 0.017$).[34]

In a small randomised trial,[61] 25 patients received a multidrug regimen combining cisplatin 50 mg/m^2, ifosfamide and etoposide or teniposide. Amifostine was administered at a dose of 910 mg/m^2 to 14 patients immediately before cisplatin while other patients received G-CSF after the completion of chemotherapy. In this study, thrombocytopenia was significantly reduced by amifostine as assessed by the median platelet nadir between the 2 groups.[61] However, this biological parameter could not be considered clinically relevant and larger trials are needed to confirm the impact of

amifostine in the prevention of bleeding and in the reduction of platelet transfusion requirements in patients treated with chemotherapy.

4.2.3 Protection of Granulopoiesis

The protective effect of amifostine against neutropenia induced by chemotherapy was first investigated in patients treated with alkylating agents such as cyclophosphamide. In an early controlled phase II study, 21 patients with a variety of solid tumours were treated with cyclophosphamide 1500 mg/m^2 for 2 courses at 4-week intervals. Amifostine 740 mg/m^2 was administered to all patients before the second injection of cyclophosphamide only. Amifostine improved white blood cell and granulocyte counts in 90% of patients. The mean granulocyte count was 541/ml and 1247/ml in cyclophosphamide and amifostine plus cyclophosphamide treated patients, respectively ($p < 0.0005$). These data suggest that amifostine provides protection against cyclophosphamide-induced haematological toxicity.[36]

Conversely, protection of granulopoiesis was not observed in an earlier similar study in which 13 patients received cyclophosphamide 1000 mg/m^2 and, following recovery of their blood cell counts, received a second course of cyclophosphamide preceded by amifostine at doses ranging from 250 and 1000 mg/m^2. In this study, amifostine did not reduce the bone marrow toxicity of cyclophosphamide.[62] More recently, Aviles et al.,[37] conducted a trial in 40 patients with high risk malignant lymphoma. Ten patients received amifostine 910 mg/m^2 before receiving cyclophosphamide 1500 mg/m^2 for 2 cycles at 14-day intervals, 20 patients received amifostine prior to cyclophosphamide either at first or at second cycle of cyclophosphamide, and 10 patients received cyclophosphamide alone with no amifostine pretreatment. Patients who received amifostine had fewer days of severe granulocytopenia (3.6 *vs* 8.5 days), fewer infectious episodes (0 *vs* 4 episodes), and the delay of treatment due to haematological toxicity was reduced (2 *vs* 11 patients).[37]

The main evidence of amifostine protection against chemotherapy-related neutropenia was obtained in a randomised phase III study comparing cisplatin-cyclophosphamide chemotherapy with or without amifostine 910 mg/m^2 in patients with ovarian cancers.[32] The authors demonstrated that amifostine was able to reduce significantly the rate of grade 4 neutropenia, the time to recovery from toxicity, the rate of delay or reduction of chemotherapy due to neutropenia, the number of days requiring antibacterials, and the number of days of hospitalisation. As a result of this study, amifostine became registered in several countries as a protective agent against the myelotoxicity of cisplatin-based chemotherapy in patients with advanced ovarian cancer. Nevertheless, confirmatory studies are warranted to definitely address the preventive effect of amifostine.

4.2.4 Other Effects on Bone Marrow

In addition to its cytoprotective effects, amifostine appears to be also able to stimulate healthy stem cells *in vitro*.[59,60]

The haematopoietic stem cell stimulating effects of amifostine have been evaluated in patients with myelodysplastic syndromes.[63] Increasing doses of amifostine from 100 mg/m^2 to 740 mg/m^2 were administered as a 15-minute intravenous infusion 3 times a week for 3 consecutive weeks to 18 patients. A 50% increase in neutrophil count was observed in 13 patients and a 50% increase in platelet count was seen in 6 patients. The need for red blood cell transfusion was reduced in 5 patients. At doses of 100 and 200 mg/m^2, the toxicity of amifostine was mild. Nausea, vomiting, and fatigue were noticed in most patients receiving 400 to 740 mg/m^2 of amifostine. In this small population of patients, an amifostine dose of 200 mg/m^2 seemed efficient enough to observe an increase in platelet and neutrophil cell counts. As cell blood count may vary spontaneously in patients with myelodysplastic syndromes, further randomised studies have been planned to assess the real benefit of amifostine in myelodysplastic diseases.[63]

High dose chemotherapy with autologous stem cell support has been demonstrated to be an effective treatment for patients with haematological malignancies such as leukaemia or lymphoma and

could possibly be of interest in patients with tumours associated with a high risk of recurrence such as breast cancer.[64] In order to decrease the contamination of the graft recipient by residual malignant cells, which increase the risk of recurrence, several techniques of bone marrow purging have been developed. One of them consisted of the treatment of the graft with high concentration of alkylating agents, such as perfosfamide (4-hydroperoxycyclophosphamide) or mafosfamide. This approach was limited by the toxic effect of the drug on healthy stem cells. Thus, the effect of amifostine on healthy stem cells, before negative selection (i.e. removal of tumour cells) of the graft recipient with mafosfamide was evaluated *in vitro*. In this study, incubation of bone marrow with amifostine, before purging with mafosfamide, demonstrated that amifostine protected the healthy progenitor cells, without protecting the leukaemic clones. This selective protection allowed an improvement in the therapeutic index of mafosfamide.[65,66]

This approach was further evaluated in a randomised phase II study of patients with poor-prognosis breast cancer. The bone marrow of the 15 patients was treated with perfosfamide alone or in combination with amifostine. The time to leucocyte engraftment, the average number of platelet transfusions, and the number of days of antibacterial treatment were significantly reduced in patients whose marrow was exposed to amifostine. In this small group of patients, there was no difference in response rate, neither in the disease-free survival between the 2 groups.[67]

4.3 Neuroprotection

Neurotoxicity is of frequent occurrence in patients treated with chemotherapy. The clinical use of the following drugs in limited by neurotoxicity: cisplatin, plant alkaloids such as vincristine, paclitaxel and oxaliplatin. Cisplatin remains a major drug in the treatment of ovarian and germ cell tumours and is frequently used to treat several other tumour types including lung, bladder, gastric, breast, lymphoma, head and neck, and cervical cancer. When nephrotoxicity is controlled, the predominant cumulative dose-limiting toxicity of cisplatin is neurotoxicity. It usually consists of a peripheral sensitive neuropathy, which is dose-related and cumulative at dose of up to 300 to 600 mg/m^2. Symptoms can begin or progress even after cisplatin discontinuation. The incidence of neuropathy was evaluated in 292 patients with ovarian cancer treated with cisplatin-based chemotherapy.[68] In this study, neurotoxicity developed in 47% of patients. Neurotoxicity-free survival decreased below to 50% at a cumulative dose of cisplatin between 500 and 600 mg/m^2.[68]

Therefore, several studies have focused on the neuroprotective effects of amifostine given before cisplatin administration. The potential protective effect of amifostine against cisplatin-induced neuropathy was first suggested by a comparative study by Mollmann et al.[69] In a small group of 69 patients treated with several cisplatin-regimens, the incidence of neuropathy was significantly reduced in the group of patients who also received amifostine (25 *vs* 49%). Neurotoxicity occurred at cumulative doses of cisplatin that were significantly higher within the group of patients treated with amifostine compared with patients who did not receive amifostine (635 *vs* 383 mg/m^2).

Thereafter, neuroprotection has been reported in the phase III randomised study conducted by Kemp et al.[32] Study design is described in section 4.1. The incidence of cisplatin-induced grade 3 neurotoxicity was significantly reduced by the use of amifostine (7.4 *vs* 12.5%, $p < 0.03$). However, the incidence of grade 2 neurotoxicity was not significantly reduced.

When the dose of cisplatin is increased, the neuroprotective effect of amifostine might became only partial. In a phase II study of non–small cell lung cancer, Schiller et al.[53] reported a 28% rate of grade 3 neuropathy in 25 patients treated with amifostine 740 or 910 mg/m^2 before they received cisplatin 120 mg/m^2. In another study,[51] 36 patients with metastatic melanoma were treated with amifostine 740 mg/m^2 before receiving different doses of cisplatin (60 to 150 mg/m^2). Neurotoxicity

was mild, including only 25% grade 1 to 2 neuropathy.

However, clinical data about neurotoxicity might be criticised. Neurotoxicity is mainly subjective and the grading of neurotoxicity is very investigator-dependent. Because of this, neurotoxicity can be easily underestimated if not specifically investigated by the same investigator and confirmed by electromyogram. Furthermore, the development of neurotoxicity might be delayed from 6 to 12 months after the cisplatin discontinuation. To date, few studies have reported the effects of amifostine on cisplatin-induced delayed toxicity. Therefore, it does not seem reasonable to use amifostine to protect against neurotoxicity until the results of studies specifically addressing the question of neuroprotection are available.

The neuroprotective effect of amifostine has evaluated with other chemotherapeutic agents, such as paclitaxel. Mitchell[70] treated patients with non–small cell lung cancer with amifostine 740 mg/m^2 before they received high dose paclitaxel (200 mg/m^2) and carboplatin (area under the curve = 6). After a median of 3 treatment cycles, the author reported that 5 out of 11 patients had grade 3 neuropathy. While it seems too early to come to a definitive conclusion, amifostine did not appear to protect against paclitaxel neurotoxicity in this study.[70] A randomised trial of high dose paclitaxel (250 mg/m^2) alone or in combination with amifostine 910 mg/m^2, in 41 patients with recurrent breast cancer is currently ongoing to address the question.[71]

4.4 Protection of Healthy Tissues Against Radiation Toxicity

Acute radiation effects occur in renewing tissues, such as skin oropharyngeal mucosa, small intestine, rectum, bladder mucosa, and vaginal mucosa. However, late effects are dose-limiting factors in radiation therapy. These late effects include necrosis, fibrosis, fistula formation, nonhealing ulceration and damage to specific organs, such as spinal cord transection and blindness. Although the mechanisms behind these phenomena are not clear, they do not appear to depend primarily on cell proliferation.

Yuhas and Storer,[4] first described the differential protection of healthy and malignant tissues achieved with amifostine against radiation injuries which provided the rational for clinical studies. Balb/c mice bearing murine mammary tumours were exposed to several doses of radiation after receiving an intraperitoneal injection of amifostine. In this model, amifostine increased the 50% lethal dose (LD_{50}) by 160 to 170%, the x-ray dose required to induce skin ulceration by 140% and the x-ray dose required to inhibit tumour transplantability by only 115%.

4.4.1 Protection of Salivary Glands and Mucosa Involved in Irradiation of Head and Neck

Salivary gland injury is one of the most frequent toxicities experienced with radiotherapy in patients with head and neck cancer. A mean dose of 50Gy induces symptomatic xerostomia by a direct toxic effect of radiation on salivary glands. Xerostomia is only partially regressive 6 to 12 months after radiotherapy discontinuation.

When radiolabelled amifostine is administered to C3H mice, high concentrations of amifostine are detected in submandibular glands.[4,5]

Several studies have been conducted in patients with head and neck cancer to assess the role of amifostine in protection of salivary glands and mucosa against radiation injuries.[38,39,72,73] In a multicentre randomised trial, 315 patients with squamous cell carcinoma of the head and neck were treated with 1.8 to 2Gy fractions of radiation therapy up to a total dose of 60 to 70Gy, with or without daily administration of amifostine 200 mg/m^2, given 30 minutes before radiation. To date, preliminary data are available on 234 patients. The incidence of grade 2 xerostomia was reduced in patients treated with amifostine compared with those not treated with amifostine (50 *vs* 76%, p = 0.0001). The median cumulative dose of radiation received at the onset of xerostomia was 60Gy for the group of patients treated with amifostine compared with 42Gy for those not treated with amifostine (p = 0.0001). Amifostine also improved de-

layed symptoms related to xerostomia. At 6 months follow up there was no difference in loco-regional tumour control between the 2 treatment groups. Therefore, amifostine reduced the incidence of acute xerostomia without reducing the antitumour efficacy.[38] Further studies are ongoing to address the effect of amifostine against acute toxicities on the mucosa of patients treated with accelerated schedules.

With the aim of improving local tumour control and radiation efficacy, the concomitant use of chemotherapy such as carboplatin and or fluorouracil has been investigated. However, when carboplatin was administered during radiation therapy in patients with head and neck cancer, grade 3 or 4 mucositis was frequent (about 80% of patients) and dose limiting.

Amifostine has been shown to protect tissues from the effect of both carboplatin and radiation, therefore a randomised phase II study was conducted to assess the effect of amifostine in protecting salivary glands and mucosa against damage related to concomitant chemoradiotherapy. In this study,[39] 41 patients with head and neck cancer were treated with a standard-fractionation 60Gy radiation, along with carboplatin 70 mg/m^2/day given from day 1 to 5 and from day 21 to 25. Amifostine was administered to 25 randomly assigned patients as a 500mg infusion prior to carboplatin injection, only. Amifostine significantly reduced the incidence of mucositis (86 *vs* 0%, $p < 0.0001$), and on a lower extent, acute xerostomia (100 *vs* 12%, $p < 0.0001$), late xerostomia, dermatitis, dysphagia, and loss of taste. Tumour control was 72% in the amifostine arm compared with 43% in patients who did not receive amifostine.[39]

In a further study, 43 patients were randomly assigned to 3 different regimens of chemotherapy (cumulative dose of 700 mg/m^2 carboplatin, or cumulative dose of 1400 mg/m^2 carboplatin, or 700 mg/m^2 carboplatin with fluorouracil).[73] All patients received radiotherapy at a total dose of 70Gy and amifostine 500mg by injection prior to carboplatin administration, only. The incidence of grade 2 xerostomia and grade 3 or 4 mucositis was dramatically low in this study (37 and 9%, respectively). The overall response rate to treatment was 91%, confirming the fact that amifostine does not protect malignant tissues.[73]

In summary, amifostine appears to be effective in protecting against the damage caused by radiation and chemoradiotherapy in patients with head and neck cancer without impairing tumour control. As evaluations of xerostomia and mucositis are mainly qualitative and could be biased by investigators, such trials should be double-blinded. Thus, we expect final results of the ongoing phase III trial[38] and new placebo-controlled, multicentre phase III trials to confirm the results discussed.

4.4.2 Protection of Tissues Involved in Pelvic Radiation

After pelvic radiation, patients may experience local injuries such as cystitis, proctitis, rectitis, damage to the small bowel, and skin reactions. In a randomised study, 100 patients with adenocarcinoma of the rectum, were treated with radiation therapy to a total dose of 45Gy, with or without prior administration of amifostine.[40] Amifostine was given at a dose of 340 mg/m^2, 15 minutes before each 2.25Gy fraction, 4 days a week for 5 weeks. In addition, patients received a cone-down of 7.2Gy followed by surgery or 14.4Gy, without amifostine administration. No difference was seen between the 2 groups of patients regarding acute toxicities to the urinary bladder, lower gastrointestinal tract, mucous membrane, and skin. Data on delayed toxicity were only available for 34 patients in the amifostine group and 37 patients in the control group. Despite the small number of evaluable patients, amifostine demonstrated tissue protection. Moderate-to-severe delayed toxicities occurred in 5 of 34 patients treated with radiotherapy alone, such as skin reactions (2 patients), genitourinary damages (2) and small bowel (1), while no moderate to severe toxicity was seen in patients treated with amifostine. There was no evidence of tumour protection by amifostine.[40]

Unfortunately, to date no other randomised studies have been conducted to assess the effect of amifostine against damage related to radiation in

the treatment of patients with carcinoma of the uterus. Radiation doses are usually higher, delivering up to 70Gy by external beam and brachytherapy. Acute and delayed toxicities are frequent and seriously impair the patient's quality of life. In the small group of 20 patients with carcinoma of the uterine cervix treated by Wadler et al.,[44] with radiotherapy in combination with cisplatin and amifostine 823 mg/m^2 given prior to cisplatin administration, the rate of grade 3 or 4 genitourinary toxicities was 25%. These data do not allow any definitive conclusion, as amifostine was given only prior to cisplatin administration.[44]

4.4.3 Protection of Tissues Involved in Chest Radiation

No randomised trial has been conducted to assess the effect of amifostine on the damage induced by chest radiation. However, in a phase II study,[54] 26 patients with unresectable stage III non–small cell lung cancer were treated with 2 courses of cisplatin plus vinblastine chemotherapy followed by thoracic radiation up to a total dose of 60Gy for 6 weeks. Amifostine was given as a 7-minute infusion at doses of 200 mg/m^2 or 340 mg/m^2 15 minutes before each daily 2Gy fraction. No grade 3 or 4 acute oesophagitis was seen nor were there any delays to treatment or treatment discontinuation because of toxicity. Only 20% of patients experienced grade 2 oesophagitis and this resolved in all patients. Data on changes in pulmonary function were available in too few patients to allow any conclusion.[54]

4.4.4 Prevention of Tumourigenic Effects

Apart from acute adverse effects which are usually managed with supportive treatment, one of the most serious events in the management of patients with cancer is the occurrence of a second malignancy related to treatment with radiation or chemotherapy after a patient has been cured of their primary cancer. Second malignancies are believed to be induced by the high rate of mutations in cells exposed to radiation and/or alkylating drugs. Amifostine has been shown in several preclinical studies to protect cells against radiation- and chemotherapy-induced mutagenesis. The antimutagenic effects have been studied against fission-spectrum-neutron- and 60Co-gamma-ray-induced mutagenesis in mice.[74] Mutagenesis at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus was measured in splenic T lymphocytes from B6CF1 mice 56 days following whole body irradiation of mice. The mutation frequency increased 100-fold with doses of 150cGy neutrons or 750cGy 60Co photons. When animals were injected with 400 mg/kg of amifostine, 30 minutes before irradiation, mutation frequencies were significantly reduced (protection factors of 1.4 and 2.4).

Amifostine has also been evaluated for its ability to protect against cyclophosphamide-induced mutagenesis at the HGPRT locus in mouse splenocytes.[75] In C3H mice injected with viable fibrosarcoma cells, amifostine 100 mg/kg was effective in reducing cyclophosphamide-induced HGPRT mutation frequency from 160×10^{-5} to 35×10^{-5} per cell without affecting the therapeutic efficacy of cyclophoshamide. In *in vitro* studies, the mechanism of protection from mutagenesis seemed different from the cytoprotective mechanism of amifostine. It did not appear to be linked to a modulation of glutathion or cysteine levels,[76] but appeared be related to a repression of c-myc gene expression.[77] Further studies are needed to understand the mechanism of reduction of mutagenesis and assess its potential benefit in patients with cancer.

Proof of the effectiveness of amifostine in the prevention of second malignancies would be very difficult to obtain because of the need for long term follow-up and the difficulty in proving the role of chemotherapy or radiotherapy in the occurrence of a second malignancy. However, prevention of anticancer treatment–related malignancies could be of particular interest in patients with curable diseases, such as haematological or paediatric malignancies.

5. Cost-Benefit Assessment

In addition to considerations related to effectiveness, the decision as whether to prescribe new cytoprotective agents requires a consideration of

the costs and cost-effectiveness. The cost-utility of amifostine has been assessed in 1 study.[78] This study was based on a cost-utility model of an economic evaluation of amifostine from the perspective of the payer. This model described the additional incremental costs that society must spend to buy an additional amount of life, with adjustments made for anticipated changes in health status. The relative efficacy of amifostine in reducing chemotherapy related toxicities was estimated from the results of the phase III licensing trial for the drug.[32] Based on an amifostine dose of 910 mg/m^2 and an average wholesale price, costs of amifostine were estimated to be $US832 (1998 values) per cycle. In this model, the authors evaluated the incremental cost of amifostine to $US3146 per complete course of chemotherapy. However, after adjustment for potential health status changes from reductions in toxicities, the use of amifostine was estimated to cost $US36 161 in direct medical costs per quality-adjusted life year saved. Thus, the authors concluded that, based on the phase III licensing trial, the use of amifostine was associated with a favourable cost-utility profile. However, the study was performed in the US and the cost for treatment in that country may not reflect the cost-utility profile in other countries depending on the healthcare insurance system.

6. Conclusion

Amifostine has been shown to be capable of protecting the kidney against acute and cumulative toxicities induced by cisplatin, and to reduce slightly the incidence of cisplatin-related neuropathy. Amifostine has demonstrated an ability to reduce cyclophosphamide-induced neutropenia and carboplatin-induced thrombocytopenia. Amifostine has also been shown to protect salivary glands and mucosa against damage related to radiation in patients treated for head and neck cancers and to slightly protect against the late adverse effects of pelvic radiation.

However, the major question with any chemoprotective agent remains whether the protection of healthy tissues extends to protecting tumour cells from the cytotoxic effects of chemotherapy and radiotherapy. Obviously, there would be no benefit in using a drug that protects against toxicity in both healthy tissue and tumours. The risk of protection of malignant cells seems to be low with amifostine according to the results of preclinical studies. Furthermore, the results of several randomised clinical trials demonstrated similar overall response rates and overall survival in patients receiving or not amifostine. These studies strongly suggested the absence of tumour protection at least in patients with ovarian cancers treated with cisplatin and cyclophosphamide, in patients with head and tumours treated with radiotherapy, and in patients with non–small cell lung cancer treated with carboplatin. However, these results cannot be extended to other malignant tumours and/or other treatment schedules without further studies.

To date, with the exception of clinical trials, and according to the US Food and Drug Administration recommendations, the use of amifostine is limited in the US to the prevention of renal cumulative toxicity related to repeated doses of cisplatin in patients with advanced ovarian cancer and non–small cell lung cancer. In France, amifostine is approved to reduce neutropenia associated with cisplatin plus cyclophosphamide in patients with advanced ovarian cancers. However, in several European countries, in this indication G-CSFs are often preferred by physicians because of their known safety and tolerability.

Despite its broad spectrum multiorgan, multidrug cytoprotectant profile, and despite encouraging preclinical and clinical studies, amifostine is rarely given to patients outside of clinical trials. This is the consequence of several independent events including the cost of amifostine, its toxicity profile, and because several new anticancer drugs have not yet been properly investigated with amifostine. For example in France, the recommended use of amifostine is currently limited to patients with advanced ovarian cancers treated with cisplatin and cyclophosphamide while the current management of patients with ovarian cancers has considerably evolved during the last 5

years, with the development of new drugs such as paclitaxel. Preclinical studies suggest the absence of interaction of amifostine on the cytotoxic effects of paclitaxel. However, additional phase III studies are warranted to investigate the protective effect and the safety of amifostine when given with recent gold standard regimen such as cisplatin plus paclitaxel. Many phase II studies have investigated the effect of amifostine in patients with non–small cell lung cancer treated with high dose cisplatin. However, whether a regimen with high dose cisplatin can lead to an increase in survival is unknown.[45-47] Furthermore, new drugs such as gemcitabine have demonstrated efficacy in the treatment of non–small cell lung cancer. Regimens including standard dose cisplatin and gemcitabine are being currently investigated. Amifostine has not yet been evaluated with gemcitabine.

Testicular germ cell tumours have shown high sensitivity to cisplatin and cisplatin-based therapy has demonstrated a high response rate allowing increased long term survival. However, the repeated use of cisplatin might be limited by cumulative neurotoxicity, neuropathy and anaemia. Thus, it would be of great interest to investigate the effect of amifostine when given to patients with germ cell tumours. Clinical trials are warranted to allow the use of amifostine in such patients.

The treatment of patients with colorectal cancer has considerably evolved with the development of new anticancer drugs such as oxaliplatin and irinotecan. However, the use of oxaliplatin is limited in some patients because of the occurrence of severe neuropathy. To date, no study has been conducted to investigate the effect of amifostine in patients treated with oxaliplatin.

In conclusion, the usefulness of amifostine remains to be confirmed with a variety of new drugs and malignancies.

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