Anti-Cancer Drugs 2002, **13,** pp. 181–209

Review paper

Amifostine in clinical oncology: current use and future applications

MI Koukourakis[1]
[1]Department of Radiation Oncology, 'Democritus' University of Thrace, Alexandroupolis 68100, Greece.

Amifostine (Ethyol®), an inorganic thiophosphate, is a selective broad-spectrum cytoprotector of normal tissues that provides cytoprotection against ionizing radiation and chemotherapeutic agents, thus preserving the efficacy of radiotherapy and chemotherapy. This review summarizes the preclinical data and clinical experience with amifostine, and provides insight into future clinical directions. Amifostine, an inactive pro-drug, is transformed to an active thiol after dephosphorylation by alkaline phosphatase found in the normal endothelium. The absence of alkaline phosphatase in the tumoral endothelium and stromal components, and the hypovascularity and acidity of the tumor environment, may explain its cytoprotective selectivity. The cytoprotective mechanism of amifostine is complicated, involving free radical scavenging, DNA protection and repair acceleration, and induction of cellular hypoxia. Intravenous administration of amifostine 740–900 mg/m^2 before chemotherapy and 250–350 mg/m^2 before each radiotherapy fraction are widely used regimens. The US Food and Drug Administration has approved the use of amifostine as a cytoprotector for cisplatin chemotherapy and for radiation-induced xerostomia. Ongoing trials are being conducted to determine the efficacy of amifostine in reducing radiation-induced mucositis and other toxicities. Novel schedules and routes of administration are under investigation, and may further simplify the use of amifostine and considerably broaden its applications. [© 2002 Lippincott Williams & Wilkins.]

Key words: Amifostine, chemotherapy, cytoprotection, radiation therapy.

Introduction

Cytoprotection refers to the reduction of cytotoxic damage induced by ionizing radiation or by chemotherapeutic drugs to normal tissues by chemical or physical agents. Selectivity is a prerequisite for cytoprotective agents to be of value in clinical oncology, in that such agents should not interfere with the antineoplastic activity of oncologic therapies or, even better, should augment tumor responsiveness. Furthermore, cytoprotective agents should be well tolerated and should not be associated with severe systemic toxicity. Despite progress in the discovery of new antineoplastic agents and in the development of new radiotherapy planning techniques, the side effects of anticancer therapies remain a considerable burden to patients. Life-threatening complications or irreparable tissue damage that severely affect patient quality of life is a daily challenge in oncology practice. Moreover, dose-limiting toxicities prevent the application of appropriate therapeutic schedules and mask the curative potential of the available weapons in the war against cancer. The introduction of cytoprotective agents into clinical practice is therefore of considerable importance, as the therapeutic index of current and experimental regimens could be improved. Table 1 summarizes the benefits expected from the use of cytoprotective agents in the oncology setting.

5-Hydroxytryptamine (serotonin; 5-HT$_3$) was one of the first agents that protected tissues against radiation, probably through reduction of the intracellular levels of oxygen.[1] Vitamins E and C, and β-carotene also have shown scavenger activity against free radicals.[2] Sodium thiosulfate has been used to prevent platinum toxicity. However, studies showing platinum inactivation and reduction of its antitumor activity made sodium thiosulfate inappropriate for clinical use.[3] Another compound, diethyldithiocarbamate (Immuthiol®), was also active against platinum-induced nephrotoxicity, but was associated with considerable neurologic toxicity.[4] Mesna is a characteristic example of an excellent cytoprotector that has a very narrow spectrum of cytoprotection confined to the protection of the urothelial tract mucosa against acrolein, a toxic metabolite of cyclophosphamide.[5] Folinic acid rescue is yet

Correspondence to MI Koukourakis, Tumor and Angiogenesis Research Group, PO Box 12, Alexandroupolis 68100, Greece.
Tel: (+30) 933-480808; Fax: (+30) 5510-31522;
E-mail: targ@her.forthnet.gr

Table 1. Expected benefits from cytoprotection

1. Reduction of the early effects of radiotherapy and chemotherapy
Prevention of myelotoxicity (neutropenia, thrombocytopenia, anemia)
Prevention of immunologic toxicity (T and B lymphocyte number and function)
Prevention of radiation or chemical mucositis (stomatitis, esophagitis, colitis, cystitis)
Prevention of skin toxicity (radiation toxicity, erythrodysesthesia)
Prevention of neurotoxicity (platinum, taxanes, radiation)
Prevention of cardiac and pulmonary toxicity (anthracyclines, bleomycin, radiation)
2. Reduction of late sequelae of radiotherapy and chemoradiotherapy
Prevention of skin, muscle, breast or lung fibrosis
Prevention of esophageal or colon/intestinal stenosis
Prevention of xerostomia
Prevention of bone and soft tissue necrosis
Prevention of neuronal dysfunction and nerve demyelination
3. Reduction of the stochastic effects of radiation and chemotherapy
Prevention of leukemogenesis and carcinogenesis
4. Enhancement of the antineoplastic activity of radiotherapy and chemotherapy
Increased dose intensity of chemotherapy
Avoidance of unnecessary delays of radiotherapy
Feasibility of highly toxic accelerated radiotherapy schemes
Feasibility of highly toxic combinations of radiotherapy with chemotherapy

another established cytoprotective strategy that prevents mucosal toxicity associated with methotrexate administration.[6] The hematopoietic growth factors recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF), recombinant human granulocyte colony stimulating factor (rhG-CSF) and recombinant human erythropoietin (rhEPO) are also considered cytoprotective agents with established activity against neutropenia and anemia, respectively.[7,8] The activity of rhGM-CSF against mucositis is also under investigation.[9,10]

The aminothiols may be the most interesting group of broad-spectrum cytoprotective agents—their activity being mainly attributed to the thiol group, which acts as a free radical scavenger. Cysteine was the first aminothiol with proven activity against ionizing radiation[11] and was the basic molecule for the subsequent development of new, more potent analogs of aminothiols. Amifostine (WR-2721, Ethyol®), the most active aminothiol, was isolated from 4400 tested compounds during a long research program conducted at the Walter Reed Army Institute of Research.[12]

Amifostine

Amifostine (WR-2721, Ethyofos; Ethyol®, 2-[(3-aminopropyl)amino]ethylphosphorothioic acid is an inorganic thiophosphate with a molecular weight of 214 kDa. The drug was initially developed as a classified agent for the US army, designed to provide a military advantage in the case of a non-conventional war. Its subsequent declassification made the drug available for medical experimentation. WR-2721 is a non-active pro-drug which, following dephosphorylation by the enzyme alkaline phosphatase, is transformed to WR-1065 (2-[(aminoprolylamino]ethanthiol; WR-1065), its active metabolite (Figure 1).

The thiol group is primarily a scavenger of free radicals produced by ionizing radiation or chemotherapeutic drugs such as anthracyclines, bleomycins and bioreductive compounds. WR-1065 is further metabolized to the disulfide molecule (WR-33278; *N*,*N*′-(dithiodi-2,1-ethanediyl)bis-1,3-propanediamine), which is involved in additional cytoprotective pathways (Figure 1).

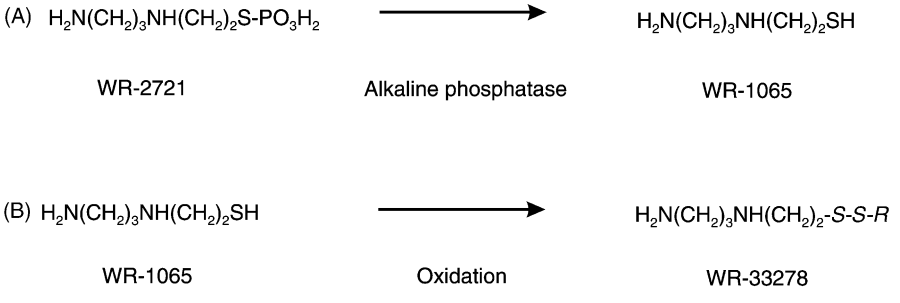

Figure 1. (A) Conversion of WR-2721, a non-active pro-drug, to the active metabolite WR-1065. (B) Oxidation of WR-1065 to WR-33278.

Amifostine is a broad-spectrum cytoprotective agent, with activity directed to all normal tissues; the only exception is the central nervous system because the blood–brain barrier prevents the accumulation of clinically relevant concentrations of amifostine metabolites in neural and glial cells. Early military experiments demonstrated an important protective activity of amifostine in animals (including dogs and monkeys) exposed to ionizing radiation.[13] A significant protection of skin, mucosa, hair follicles, intestinal wall, salivary glands and growing cartilage against radiation in experimental animals was subsequently reported.[14–20] Although most experiments have been conducted with X- or γ-rays, cytoprotection against densely ionizing radiation has been also reported.[21] WR151327, another aminothiol, has possibly even more potent cytoprotective activity against neutron radiotherapy.[22]

Overall, the protection factor against radiation toxicity at an equitoxic endpoint conferred by amifostine ranges from 1.2 to 3. The highest values have been documented for the endothelium (3.0), salivary glands (2.9), connective tissue (2.6) and spermatogonial cells (2.4), while lower values between 1.5 and 2 are reported for lungs, kidneys, esophagus, small intestine and colon.[23]

Important experimental evidence has been also provided on the protective role of amifostine against leukemogenesis and carcinogenesis. Amifostine prevents the induction of mutations of the hypoxanthine–guanine phosphoribosyl transferase (HGPRT) gene[24,25] induced by radiation and chemicals (platinum, nitrosureas and bleomycin).[26,27] Results of *in vitro* experiments show that incubation of cell lines with WR-1065 (before or for a prolonged time after irradiation) prevents their transformation.[28,29] Indeed, results of *in vivo* experiments in mice showed a significant reduction in the rate of development of leg fibrosarcomas when amifostine 400 mg/kg was administered before a single dose of 35–57 Gy γ-radiation.[30] As the development of secondary malignancies after chemotherapy or combined chemoradiotherapy is quite high,[31] amifostine may also prove of value in the prevention of stochastic effects of anticancer therapies, especially for patients with long survival expectancies.

Cysteine has been well known to protect normal tissues against nitrogen mustard since 1950.[32] In 1979, Yuhas *et al.* showed that amifostine reduces the toxicity of alkylating agents, without affecting their antineoplastic efficacy.[33] Experiments in mice performed during 1980–1982 showed that amifostine reduces the renal toxicity of cisplatin.[34,35] In 1988, Allalunis-Turner *et al.* reported a reduction of the pulmonary toxicity of cyclophosphamide when combined with amifostine.[36] An important protective effect of amifostine against the toxicity of doxorubicin on cardiac myocytes has been recently noted in cell cultures.[37]

Stimulation of hemopoiesis by amifostine has been also recognized and the therapeutic role of prolonged amifostine regimens in the management of myelodysplastic syndromes is under investigation.[38–41]

Pharmacokinetics of amifostine

The standard route of administration for amifostine is i.v.; however, s.c. administration has recently received attention. Immediately following administration of amifostine, alkaline phosphatase rapidly hydrolyzes the pro-drug WR-2721 to the active thiol metabolite WR-1065.[42–43] WR-1065 is also oxidized to the symmetric disulfide WR-33278, which binds to non-histonic areas of DNA.[44] The dephosphorylation of WR-2721 by alkaline phosphatase is optimal under alkaline conditions (optimal pH 9.0).[42] Acidic phosphatase does not dephosphorylate WR-2721.[45] The plasma concentrations of alkaline phosphatase are not sufficient for this transformation;[43] dephosphorylation primarily occurs in the normal tissues and especially in normal endothelium,[46,47] which is in fact the first tissue that amifostine contacts immediately following i.v. injection. Indeed, the addition of alkaline phosphatase in culture media rapidly increases the intracellular accumulation of WR-1065 and enhances the cytoprotective efficacy of amifostine.[48] There are four different genes encoding four different alkaline phosphatases.[49] Three of these genes are located on chromosome 2 and encode the intestinal, placental and placental-like alkaline phosphatase. The fourth gene is found on chromosome 1 and encodes the liver/bone/kidney alkaline phosphatase. Both the liver and the intestinal type of alkaline phosphatase hydrolyze WR-2721, although the hydrolysis seems to be more rapid in the presence of the liver type enzyme.[45]

Immediately following the administration of WR-2721 150 mg/m^2, the maximum concentration (100–800 μmol/l) is achieved within 1 min. WR-2721 disappears rapidly from the plasma, with a half-life of 0.88 min. Less than 5% of the drug is found in the plasma 6 min following administration.[50] The concentration of WR-1065 reaches 22 μmol/l within 1 min and decreases to 8 μmol/l within 4 min after the administration of WR-2721. The plasma levels of

WR-33278 reach 11 μmol/l within 1 min and 7 μmol/l within 3 min following i.v. injection of amifostine. No more than 2.2% of the administered drug and its metabolites is excreted in urine, indicating that more than 90% of the drug and its metabolites enter rapidly intracellularly. In pharmacokinetic studies in experimental animals, the concentration of WR-2721 and its metabolites increased sharply after amifostine administration.[51] By increasing the dose of amifostine, an increasing accumulation of the unmetabolized form is noted in the plasma, which is compatible with saturable kinetics of the hydrolysis of the drug. This means that the amount of alkaline phosphatase available in the body is not enough to rapidly hydrolyze amifostine when its dose exceeds certain levels, such that the excess amount of the inactive pro-drug is accumulated in the plasma and tissues for a certain period before it becomes dephosphorylated.[52]

The pharmacokinetics of [^{35}S]WR-2721 in experimental animals show a rapid distribution in the tissues except the brain where, because of the blood–brain barrier, the amifostine levels achieved are very low.[53] Amifostine is also very well distributed in embryos, because it easily passes through the placental barrier.[23]

The s.c. administration of amifostine is of clinical interest because of the simplicity of administration and the convenience when protracted schedules of daily use are required (i.e. radiotherapy or treatment of myelodysplastic syndromes). A pharmacokinetic study in patients with myelodysplastic disease showed that the area under the concentration–time curve (AUC) of WR-1065 obtained after s.c. injection of amifostine 500 mg is in the range of 50–80% of the AUC achieved after the i.v. administration of amifostine 200 mg/m^2.[54] This equivalence, however, should not be considered an isoeffect formula between the two routes of administration. Such a formula should be developed only after the comparative quantitation of the intracellular concentration of WR-1065 and of disulfides; however, results of such studies are not yet available. In a recent study presented at the 2001 annual meeting of the American Society of Clinical Oncology, the concentration of WR-1065 in the salivary glands of rats was compared following the i.v. or s.c. administration of amifostine 200 mg/kg.[55] Similar concentrations of drug were achieved by these two routes of administration. It is noteworthy that the s.c. administration of amifostine protected against radiation-induced mucositis for up to 8 h after administration; whereas the i.v. administration of the same dose did not seem to protect mucosa for more than 4 h.

Cytoprotective mechanism of amifostine

The cytoprotective mechanism of amifostine is quite complex. WR-2721 is the inactive pro-drug, while WR-1065, the active metabolite, is a thiol with potent scavenger activity against free radicals produced by ionizing radiation or chemicals. For example, superoxide anions produced by anthracyclines, which are the main cause of anthracycline-mediated cardiotoxicity, are deactivated by the thiol groups of WR-1065.[56] Radiation-induced DNA single and double-strand breaks are also prevented by WR-1065 by a factor of 2.[57,58] Moreover, WR-1065 reduces the amount of DNA cross-links induced by the NH_2 group of alkylating agents[59] and prevents the formation of platinum–DNA adducts.[60]

The oxidation of amifostine to its symmetric disulfide WR-33278 is also a relevant step in the metabolism of amifostine. WR-33278 is structurally similar to the polyamine spermine and binds to DNA. Once bound, it accelerates the repair of DNA strand breaks produced by cytotoxic agents. Incubation of cell lines with WR-1065 following irradiation (after the period of free radical formation and action) significantly reduces their apoptotic rate.[61] Although this pathway remains quite obscure in nature, some studies suggest that WR-33278 cooperates with topoisomerase I in the opening and uncoiling of DNA strands to allow the repair process to occur.[62] An important role for WR-33278 in preventing cellular transformation has been also reported.[63]

Microcalorimetric studies show that incubation of cells with WR-2721 results in heat production that lasts for at least 90 min.[64] This heat, which is probably a result of WR-1065 oxidation to disulfides, leads to a rapid consumption of the intracellular oxygen and to hypoxia. This may be also relevant to amifostine-mediated cytoprotection.

Nevertheless, the induction of tissue hypoxia by amifostine seems to follow more complicated pathways. The consumption of intracellular oxygen by WR-1065 oxidation could be easily overcome by an increase in oxygen extraction from the blood. Studies in humans show that, in contrast, the oxygen tension and the hemoglobin oxygen saturation of the peripheral blood increases following amifostine administration.[65] As amifostine does not interfere with oxygen dissociation from hemoglobin and neither does it change the pH in red cells, the increased oxygen saturation of the peripheral blood should be attributed to a reduced consumption of oxygen. White cells exhibit significantly reduced consumption of oxygen following administration of amifostine,[65] which suggests that amifostine interferes

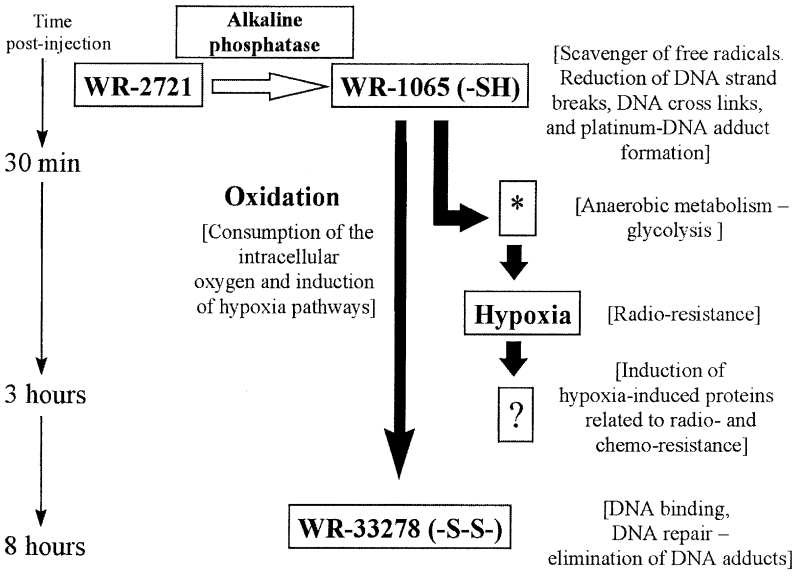

Figure 2. Schematic representation of the cytoprotective pathways and events following administration of amifostine: '*' = clinical and experimental evidence, but unknown mechanism; '?' = under investigation.

with cellular metabolism, probably by switching on anaerobic pathways.

In an unpublished study we conducted in breast cancer patients receiving amifostine 1000 mg (flat dose) daily during hypofractionated radiotherapy, oxygen tension and hemoglobin saturation rapidly increased 30 min following drug administration, and were restored to lower than pretreatment values within 1 h. Glucose levels decreased as the oxygen tension increased, suggesting that amifostine triggers glycolytic pathways in normal tissues. It may, therefore, be suggested that reduced oxygen consumption and activation of glycolytic pathways prevail during the first 30–40 min after amifostine injection, while a rapid consumption of oxygen follows thereafter, which is probably the result of WR-1065 oxidation or even stimulation of aerobic metabolism. In both cases, tissue hypoxia occurs and this may have great significance in cytoprotection. The activation of glycolytic pathways may be a result of the release of the hypoxia cascade regulated by the hypoxia inducible factors (HIF).[66] Upregulation of hypoxia-related proteins may further contribute to the mechanism of amifostine-related cytoprotection.

Figure 2 summarizes the amifostine-related cytoprotective pathways. The cytoprotective events that are triggered last for varying amounts of time after the administration of WR-2721. The free radical scavenger activity appears 5 min after administration and lasts 20–40 min thereafter. Hypoxia appears rapidly 10–15 min after administration and lasts 30–40 min, although WR-1065 oxidation may maintain the hypoxic stress for up to 90 min. Eventual induction of hypoxia-regulated proteins occurs 1–2 h after the hypoxic stress and may last several hours thereafter. The levels of WR-33278 in the nuclei seem to prolong the cytoprotection for several hours. Nevertheless, experimental studies are required to further elucidate these steps.

Selectivity of the cytoprotection of amifostine

The therapeutic index of a regimen in clinical oncology refers to the ratio 'percentage of tumor control/percentage of complications from normal tissues'. The objective in using cytoprotective agents is to increase the therapeutic index by reducing the denominator. Selectivity is a critical feature of cytoprotection, as tumor protection could reduce the numerator and therefore reduce the therapeutic index of an agent. Non-selective cytoprotective agents cannot be incorporated into clinical practice because they reduce the efficacy of anticancer treatment.

Although a long list of experimental and clinical studies show that amifostine does not reduce the efficacy of radiotherapy and chemotherapy, and that, in some circumstances, it augments their antineoplastic activity, concerns regarding tumor protection have not been completely eliminated. In a recent review, Lindergard *et al.* suggested that amifostine should still remain an experimental drug.[67] This

opinion, however, is not well justified and is not accepted by the majority of oncologists. Moreover, the US Food and Drug Administration (FDA) approved the routine use of amifostine during platinum chemotherapy and during radiotherapy for head and neck cancer; and the drug is licensed and widely used all over the world. The cost-effectiveness of the drug has been also well established; therefore financial reasons should not prevent the broad use of amifostine. Table 2 summarizes the existing experimental and clinical experience relevant to the 'tumor protection' issue.

Animal studies with amifostine

The historical experiments using amifostine in mice conducted by Yuhas *et al.* in 1969 demonstrated significant skin and bone marrow protection against radiation, with no tumor protection noted.[68] Utey *et al.* in 1976 reported a selective accumulation of radiolabeled amifostine in normal mice tissues but not in tumors.[69] In 1980 further experimentation by Yuhas *et al.*[70] in Fischer 344 rats bearing squamous 3M2N cancer cells showed that i.p. administration of [^{35}S]amifostine (200 mg/kg) resulted in 50- to 100-times higher accumulation in normal versus tumor tissues. One hour following administration of amifostine, active drug concentrations remained at least 10 times higher in normal versus tumor tissues.

Results of similar experiments conducted by Rasey *et al.* in 1985 in rats with RIF-1 sarcomas showed a dramatically increased accumulation of radiolabeled [^{35}S]amifostine and metabolites in normal tissues after i.p. injection of amifostine 1200 mg/m^2.[71] [^{35}S]Amifostine concentration was 12 times higher in the kidneys, 8 times higher in the salivary glands and 5 times higher in the lungs compared to concentrations in tumor tissue. The blood–brain barrier prevented the accumulation of [^{35}S]amifostine in the brain, where the levels achieved were similar to those in the tumors. The 'tumor barrier' was therefore equally potent as the blood–brain barrier.

In 1983, Mediondo *et al.* demonstrated that amifostine 150–500 mg/kg had a minimal protective effect (protection factor 1.04–1.15) when BALB/c mice bearing 6 mm EMT6 were treated with radiotherapy. Moreover, amifostine did not compromise the sensitization efficacy of misonidazole.[72] However, in a randomized study conducted by McChensey *et al.* in 1986, amifostine did not protect normal tissues and compromised the therapeutic efficacy of radiotherapy in dogs with sarcomas.[73] In 1993, Besa *et al.* confirmed the early results reported by Mediondo *et al.* In their study, amifostine significantly protected normal skin and the musculoskeletal system of mice with sarcomas against γ-radiation, while it did not change the antitumor efficacy of the radiation.[74]

Results of similar experiments confirmed the selective cytoprotective efficacy of amifostine against various cytotoxic agents. In 1980, Yuhas *et al.* showed that amifostine protects against the nephrotoxicity of cisplatin and allows the administration of a 3-fold higher dose, without protection of tumors.[34,75] In the same studies, a selective cytoprotection of normal tissues against cyclophosphamide was also reported.

In 1988 DeNeve *et al.* studied the development of DNA cross-links induced by nitrogen mustard[59] in AKR rats with leukemia. A 50% reduction in DNA cross-links was noted in normal cells of the bone marrow after cytoprotection with amifostine, while no such change was noted in leukemia cells. The DNA repair rate was increased in normal cells in the presence of amifostine, while the repair rate was reduced in leukemia cells. Indeed, in AKR experimental models, amifostine enhanced the antileukemic activity of alkylating agents.[76] Results of recent studies have demonstrated that amifostine dramatically sensitizes leukemia progenitor cells against mafosfamide.[77]

Using nude mice with OVCAR-2 human ovarian xenotransplanted cancer, Treskes *et al.* reported a 1.5-fold increase in the maximum tolerated dose of carboplatin when given after the administration of amifostine 200 mg/kg, which leads to increased antitumor activity of carboplatin.[78] Similar experiments conducted by van Laar *et al.* in BALB/c and C57B1/6 mice bearing colon tumors showed that amifostine increases the dose intensity and the antitumor activity of a carboplatin/5-fluorouracil combination.[79]

In 1996, Paine *et al.* administered amifostine 100–200 mg/kg i.p. to immunocompromised mice with ovarian carcinoma type 2780, which did not affect the weight of the mice nor their spleen. Mice treated with paclitaxel 27 mg/kg with or without amifostine had a similar survival rate, with no signs of tumor protection.[80] In 1997, Fichtner *et al.* reported the results of an extensive study on the antitumor efficacy of a variety of chemotherapeutic agents (cyclophosphamide, doxorubicin, cisplatin, ifosfamide, vincristine and etoposide) in IMR-75 and Kelly neuroblastoma-bearing nude mice.[81] Amifostine 200 mg/kg i.p. did not affect the antineoplastic

Table 2. Studies focusing on the cytoprotective selectivity of amifostine

Author	Year	Reference	Tumor model	Agent/ endpoint	Tumor protection
(A) Experimental tumors					
Yuhas *et al.*	1969	68	spontaneous mammary tumor	RT	no
Lowy *et al.*	1973	86	KHT sarcoma	RT	yes
Yuhas *et al.*	1976	69	3M2N tumors	(accumulation)	no
Moulder	1977	88	rhabdomyosarcoma	RT	yes
Yuhas *et al.*	1979	201	Line1 lung cancer	meclorethamine	no
Yuhas *et al.*	1980,1983	34,75	(various)	cisplatin	no
Twentyman *et al.*	1981,1983	202,203	RIF-1 and KHT sarcoma	cyclophosphamide	yes
Valeriote	1982	76	AKR leukemia	nitrogen mustard	Pn
Clement *et al.*	1982	87	Lewis lung cancer	melphalan	no
			P388 leukemia	melphalan	yes
Milas *et al.*	1982	204	mouse fibrosarcoma	RT	no
Mediondo *et al.*	1983	72	EMT6 tumors	RT	no
Williams *et al.*	1984	85	mouse fibrosarcoma	RT	yes
Penhaligon *et al.*	1984	90	RIF-1 tumors	RT	no, variable
Milas *et al.*	1984	205	fibrosarcoma, mammary	RT	variable
Milas *et al.*	1984	206	immunogenic fibrosarcoma	RT, cyclophosphamide	Pn
Wist	1985	89	Lewis lung cancer	cyclophosphamide	yes
			Lewis lung cancer	melphalan	no
Rasey	1985	71	RIF-1 sarcomas	(accumulation)	no
Rojas	1986	85	anaplastic murine tumor	RT	yes
McChensey	1986	73	canine sarcoma	RT	yes
DeNeve	1988	59	rat leukemia	nitrogen mustard	no
Besa	1993	74	murine sarcoma	RT	no
Treskes	1994	78	OVCAR-2 tumors	carboplatin	no
Paine	1996	80	ovarian 2780 tumors	paclitaxel	no
Dunn	1996	82	embryonic carcinoma	cisplatin	no
Fichtner	1997	81	Kelly neuroblastoma	(various)	no
Bergstrom	1999	83	BT4C intracranial glioma	cisplatin	no
Grdina	1999	207	fibrosarcoma	cyclophosphamide	no
(B) Cell lines					
Treskes	1992	208	OVCAR-3, V79	cisplatin	no (WR-2721) yes (WR-1065)
Douey	1995	77	leukemia	mafosfamide	Pn (NS)
Alberts	1996	94	ovarian and breast	(various)	no
Taylor	1997	168	ovarian	paclitaxel	no
Ng	1999	95	ovarian	various agents	no
Kataoka	2000	96	glioma	RT	yes (WR-1065)
(C) Clinical studies					
[1]Glover *et al.*	1987	109	melanoma	cisplatin	no
[3]Kligerman	1992	97,98	rectal	RT	Pn (NS)
[1]Coia	1992	100	hematologic	RT (TBI)	no
[1]Arvil	1992	110	melanoma	cisplatin	no
[1]Mitsuhashi	1993	99	cervical	RT	Pn (S)
[2]Betticher	1995	119	NSCLC	carboplatin	Pn (NS)
[3]Kemp	1996	113	ovarian	cisplatin/ cyclophosphamide	Pn (NS)
[1]Schiller	1996	115	NSCLC	cisplatin/vinblastine	no
[1]Tannehill	1997	106	NSCLC	chemotherapy+RT	no
[2]Budd	1997	118	NSCLC	carboplatin	Pn (NS)
[1]Aviles	1997	122	lymphomas	cyclophosphamide	no
[2]Buntzel .	1998	101,102	HNC	RT/carboplatin	Pn (NS)
[2]Koukourakis	1998	137,138	lung, pelvic	RT/carboplatin	Pn (NS)
[2]Peters	1999	103	HNC	RT/carboplatin	Pn (NS)
[2]Gelmon	1999	116	breast	paclitaxel	Pn (NS)
[2]Plating	1999	117	HNC	cisplatin	Pn (NS)
[2]Fahlke	1999	121	colorectal	5-fluorouracil	no

Table 2. continued

Author	Year	Reference	Tumor model	Agent/ endpoint	Tumor protection
[3]Antonadou	1999	107	NSCLC	RT	no
[2]Koukourakis	2000	104	HNC	RT	no
			NSCLC	RT	Pn (NS)
[1]Koukourakis	2000	138	(various)	docetaxel/carboplatin	no
[3]Britzel	2000	105	HNC	RT	Pn (NS)
[1]Gridelli	2000	114	NSCLC	cisplatin/vinorlebine	no
[2]Antonadou	2000	164	NSCLC	RT/carboplatin/paclitaxel	no
[3]Johnson	2001	120	NSCLC	carboplatin/etoposide/ ifosfamide	no
[1]Koukourakis	2001	194	breast	RT	no
[2]Komaki	2001	108	NSCLC	RT/carboplatin/etoposide	Pn (MS)
[1]Koukourakis	2001	129	breast	post-operative RT	Pn (NS)

Pn, potentiation; NS, not significant; MS, marginal significance; S, significant.Subscripts indicate study type: 1, phase II: comparison with historical controls; 2, randomized phase II; 3, randomized phase III.

activity of any of the tested drugs. In contrast, amifostine prevented the weight loss induced by vincristine and leukopenia induced by cisplatin, cyclophosphamide and ifosfamide. Results of another study conducted in nude mice bearing xenografts from embryonic carcinoma expressing alkaline phosphatase also confirmed that amifostine does not compromise the antitumor activity of cisplatin.[82]

In 1999, results of novel experiments in mice with BT4C intracranial glioma were reported.[83] Mice received cisplatin 5 mg/kg with or without amifostine 200 mg/kg i.p. Ten days later the mice were sacrificed. Cisplatin–DNA adducts in normal brain and kidneys were significantly more abundant in mice that did not receive amifostine, while the formation of DNA adducts was quite similar in the gliomas in both groups. The striking finding that amifostine reduced the DNA adducts in the normal brain shows that, although the blood–brain barrier prevents the accumulation of amifostine metabolites in the intact brain, blood–brain barrier disruption in the tumor area allows the substantial passage of amifostine into the normal brain. If this also applies in humans, then amifostine could be of value in preventing the radiation-induced damage of the normal brain in patients treated with radiotherapy for primary or metastatic brain tumors.

Despite the plethora of experimental data showing that amifostine does not protect tumors against radiotherapy and chemotherapy, results of a small number of studies (mainly performed in the 1970s) did not preclude a protective effect of amifostine in some tumor models.[84–89] The tumor protection factor in all these experimental studies is between 1.1 and 1.4. Penhaligon *et al.* showed that the manner in which experimental animals are handled (i.e. immobilization procedure) may well bias the assessment of the cytoprotective effect of amifostine.[90] Hypothermia and hypotension, common events in animals treated with amifostine, may enhance tumor hypoxia. Thus, reduced tumor sensitivity due to hypoxia may erroneously be attributed to amifostine.[91] Clearly, it is quite difficult to extrapolate results of animal experiments to humans, and the radio- and chemosensitivity of experimental tumors cannot be practically extrapolated to human carcinomas. A common cause of frustration is when cytotoxic agents fail to confirm in humans the excellent tumor control rates obtained in the laboratory. The isoeffective doses of chemotherapy (and of amifostine) between animals and humans are rather unknown, and the non-conventional radiotherapy schemes used in animal experiments further compromise the value of the findings. Clinical trials are the only tools that can provide convincing answers.

Apart from the benefit expected from cytoprotection, recent experiments by Grdina support an additional role of amifostine as an antimetastatic agent.[92] Administration of amifostine 50 mg/kg significantly reduces the metastatic property of tumors. Assessment of plasma levels of angiostatin (a potent endogenous suppressor of angiogenesis) showed a rapid increase following the administration of amifostine. Amifostine-mediated inhibition of metalloproteinase activity or even downregulation of the manganese superoxide dismutase gene (involved in the metastatic process) has been also implied.

Amifostine in cell culture

Although thiols protect normal ovarian cells in cultures, WR-1065 does not protect the A2780 ovarian cancer cells or the MCF7 breast cancer cells against the activity of 16 chemotherapeutic agents (bleomycin, carboplatin, cisplatin, cytarabine, daunorubicin, doxorubicin, etoposide, 5-fluorouracil, idarubicin, melphalan, mitomycin C, mitoxantrone, paclitaxel, docetaxel, vinblastine and vincristine).[93,94] In a study conducted with three different ovarian cancer cell lines (SKOV3, line 420 and line 429), WR-2721 did not affect the antineoplastic activity of various chemotherapeutic agents (cisplatin, paclitaxel, doxorubicin, bleomycin, etoposide and vincristine).[95] Nevertheless, Kataoka *et al.* found that the active metabolite WR-1065 protects glioma cell lines, which is an event independent of p53 activity.[96]

Clinical data: amifostine and radiotherapy studies

During the past decade, several clinical studies have investigated the cytoprotective efficacy of amifostine. One of the first studies, published in 1992, was a randomized trial involving 100 patients with locally advanced inoperable rectal cancer treated with radical radiotherapy with or without WR-2721. The response rate was 16% in the amifostine group versus 10% in the control arm; however the sample size was too small to test for statistical significance.[97,98] Similar results were found in a retrospective study in patients with cervical cancer, where 37 patients received radiotherapy with low dose amifostine (75 mg/m^2/day). The 5-year survival rate for patients with stage II disease was 88% in the amifostine group versus 72% in the historical control group (p=0.02). The 4-year survival rates for patients with stages III and IV a disease were 50 and 40%, respectively, in both treatment groups (p=NS).[99] The 11-month median survival time for patients with advanced hematologic malignancies treated with total body irradiation (15–20 cGy/fraction) and amifostine 740–910 mg/m^2, reported by Coia *et al.*,[100] does not suggest any tumor protection.

All studies performed in patients with head and neck cancer (HNC) treated with radiotherapy show a slightly increased control rate in patients treated with amifostine. Buntzel *et al.* conducted a randomized trial in 39 patients with locally advanced HNC treated with standard radiotherapy and carboplatin radiosensitization. The 1-year control rate was 79% in amifostine-treated patients versus 64% in the control arm.[101] Similar results were reported in another study from the same group (complete response rate 85 versus 78%; p values were not significantly different due to the small number of patients).[102] In a trial conducted by Peters *et al.*, 28 HNC patients were assigned randomly to receive radiotherapy and carboplatin with or without amifostine. Although the toxicity was not different between the two groups, the median survival was 19 months in the amifostine arm versus 10 months in the control arm (p=NS due to small sample size).[103] We conducted a randomized phase II study of 40 HNC patients treated with standard radiotherapy with or without s.c. administration of amifostine 500 mg before each radiotherapy fraction. The complete response rates were 58 and 54% (p=NS) in the amifostine and control groups, respectively, suggesting no tumor protection.[104] In a recent phase III trial performed by Brizel *et al.* in 315 HNC patients, the administration of amifostine 200 mg/m^2 i.v. before each radiotherapy fraction did not compromise the results of radiotherapy. On the contrary, an 8% higher 2-year survival rate was noted in the amifostine arm compared with that of the control arm, although this difference was not statistically significant (81 versus 73%; p=NS).[105]

Similar values have been reported for non-small cell lung cancer (NSCLC). Tannehill *et al.* reported a 60% response rate in 26 patients with stage III NSCLC treated with induction cisplatin/vinorelbine chemotherapy supported by amifostine, followed by definitive radiotherapy supported by daily amifostine. These results do not justify any concern regarding tumor protection.[106] We conducted a randomized phase II study in which amifostine was administered s.c. during standard radiotherapy in 35 patients with inoperable, locally advanced NSCLC. Complete and partial responses were achieved in 10 of 18 (55%) patients in the amifostine arm versus six of 17 (35%) patients in the control arm (p=0.33). The reduced rate and severity of esophagitis, which resulted in the prevention of delays in radiotherapy, may account for the higher control rate in the amifostine group.[104] In a randomized study of 145 patients with NSCLC treated with radiotherapy with or without amifostine 340 mg/m^2, the response rate was similar in both treatment groups.[107] In a recent study from the MD Anderson Cancer Center, 60 patients with inoperable NSCLC received concurrent chemotherapy (cisplatin and etoposide) and radiotherapy with or without amifostine 500 mg i.v. twice weekly. The complete response rate was almost significantly greater in the amifostine group versus the control group (27 versus 7%; p=0.07).[108]

Clinical data: amifostine and chemotherapy studies

A large amount of clinical data suggests that amifostine does not interfere with the antineoplastic efficacy of various chemotherapeutic agents. In a study conducted by Glover *et al.* in 1987, amifostine 740–1100 mg/m^2 allowed the administration of high doses of cisplatin (120–150 mg/m^2) in melanoma patients. The 53% response rate achieved in this study is high enough to preclude a tumor protection effect by amifostine.[109] Similar results have been reported in another study of high-dose cisplatin supported by amifostine in melanoma patients.[110] Amifostine does not interfere with the pharmacokinetics of platinum. In contrast, some studies suggest that amifostine prolongs the half-life of platinum and increases the exposure of tissues and tumors to the drug.[111,112]

In a large randomized study performed by Kemp *et al.* in 1996, 242 patients with advanced ovarian carcinoma were treated with cisplatin/cyclophosphamide with or without amifostine. In patients who underwent second-look surgery, histopathologic confirmation of complete response was noted in 43% (26 of 60) of patients in the amifostine group versus 36% (19 of 52) in the control group (p=NS). The median survival was 31 months in both treatment groups.[113]

The high response rate of NSCLC to cisplatin/vinorelbine and cisplatin/vinblastine regimens supported by amifostine does not support tumor protection by amifostine against these chemotherapeutic agents.[114,115] In a randomized study of 40 breast cancer patients treated with paclitaxel with or without amifostine, the complete response rate was higher in the group pretreated with amifostine (38 versus 22%; p=NS).[116] In another randomized study of 74 patients with advanced HNC, the efficacy of the cisplatin/amifostine regimen was also higher than that of the control arm (63 versus 50%), although the difference was not significant.[117] In a small randomized study, patients with NSCLC treated with carboplatin and amifostine had a median survival of 52 versus 39 weeks for those receiving carboplatin alone (p=0.11).[118] Betticher *et al.* randomly assigned 21 NSCLC patients to carboplatin with or without amifostine.[119] The median survival was 14 months in the amifostine arm versus 9 months in the control arm (p=NS). In a very recent randomized trial, 84 patients with advanced NSCLC received a regimen of carboplatin/etoposide/ifosfamide with or without amifostine. The median survival was 14 months in the amifostine arm and 11 months in the control arm, while the 12-month overall survival was 60 and 42% in the amifostine and control arms, respectively (p=NS).[120]

In a trial involving patients with colorectal cancer, the efficacy of 5-fluorouracil/leucovorin plus amifostine remained unchanged.[121] The 72% complete response rate obtained in a study in high-risk malignant lymphoma patients treated with cyclophosphamide and amifostine precludes any tumor protection effect of amifostine.[122]

Table 3 summarizes the results of randomized phase II and III studies on the efficacy (local control and survival) of radiotherapy or chemotherapy with or without amifostine. In all studies, a consistent 2–20% benefit was reported; however, in most studies, this difference did not reach statistical significance.

Mechanisms involved in the selective cytoprotection of amifostine

Vascularity of normal versus tumor tissue

It is well known that tumors bear poorly vascularized and necrotic areas. The reduced blood flow and therefore the reduced accessibility of amifostine in the tumor environment has been proposed as a possible mechanism to explain the cytoprotective selectivity of amifostine. In a recent study, we investigated the vascular density in, 1459 human carcinomas and relevant normal tissues.[123] The vascular density was found to vary up to 22-fold even among tumors of the same histology. Although the vascular density was very low in 50% of tumors examined, 10–20% of tumors had a very high vascularization, up to 3 times higher than the normal tissue vascularization. This high vascular density was primarily localized to the invading tumor front suggesting that, indeed, the poor vascular density in inner tumor areas may contribute to the lack of tumor protection. However, the very high vascular density of the invading front noted in 20% of tumors shows that this tumor area, which is the most active part of the tumor,[124,125] is well exposed to high WR-2721 concentrations. Indeed, color Doppler ultrasonography often shows a high blood flow in the tumor periphery of breast carcinomas, which is used as a sign of malignancy.[126] Thus, low blood flow can only partly explain the selective activity of amifostine.

Alkaline phosphatase and tumor pH

Since WR-2721 is an inactive compound, the selective cytoprotection of normal tissues would be

Table 3. Randomized phase II/III clinical studies comparing the efficacy of radiotherapy or chemotherapy with or without amifostine

Author	Reference	Tumor	Therapy	Endpoint	Amifostine/control	*p*
[3]Kligerman *et al.*	97, 98	rectal	RT	CR rate	16 versus 10%	NS
[2]Mitsuhashi *et al.*	99	cervix stage II	RT	5-year survival	88 versus 72%	0.04
[2]Betticher *et al.*	119	NSCLC	carboplatin	median survival	14 versus 9 months	NS
[3]Kemp *et al.*	113	ovarian	cisplatin/ cyclophosphamide	CR rate	43 versus 36%	NS
[2]Budd *et al.*	118	NSCLC	carboplatin	median survival	52 versus 39 months	0.11
[2]Buntzel *et al.*	102	HNC	RT/carboplatin	CR rate	85 versus 75%	NS
[2]Koukourakis *et al.*	148	lung, pelvic	RT/carboplatin	CR rate	better[a]	NS
[2]Peters *et al.*	103	HNC	RT/carboplatin	median survival	19 versus 10 months	NS
[2]Gelmon *et al.*	116	breast	paclitaxel	CR/PR rate	36 versus 22%	NS
[2]Planting *et al.*	117	HNC	cisplatin	CR/PR rate	63 versus 50%	NS
[3]Antonadou *et al.*	107	NSCLC	RT	CR/PR rate	83 versus 83%	NS
[2]Koukourakis *et al.*	104	HNC	RT	CR rate	58 versus 54%	NS
		NSCLC	RT	CR/PR rate	55 versus 35%	NS
[3]Britzel *et al.*	105	HNC	RT	2-year survival	81 versus 73%	NS
[2]Antonadou *et al.*	164	NSCLC	RT/carboplatin or paclitaxel	CR/PR rate	89 versus 81%	NS
[3]Johnson *et al.*	120	NSCLC	carboplatin/etoposide/ ifosfamide	1-year survival	60 versus 42%	NS
[2]Komaki *et al.*	108	NSCLC	RT/carboplatin/ etoposide	CR rate	26 versus 7%	0.07

[a]Better but not significantly in all tumor subtypes assessed (locally advanced NSCLC, cervix, prostate and bladder).
Subscripts indicate study type: 2, randomized phase II; 3, randomized phase III.

explained if, for some reason, the hydrolysis of WR-2721 by alkaline phosphatase cannot occur in the tumor environment. Alkaline phosphatase is indeed abundant in normal endothelium but not the cancerous endothelium.[127,128] We investigated the expression of alkaline phosphatase in a large number of tumor and normal tissues using immunohistochemistry with antibodies recognizing the intestinal type of the enzyme.[129] A striking loss of alkaline phosphatase expression in the tumor vessels, the fibroblasts of the tumor stroma and the cancer cells themselves was noted. Even in the invading tumor front, no more than 20% of the vessels expressed alkaline phosphatase. The depletion of alkaline phosphatase from all tumor components strongly supports the concept that amifostine is unlikely to be hydrolyzed in the tumor environment.

The reason that alkaline phosphatase expression is lost in tumors is unknown. The acidic tumor conditions induced by the activation of glycolytic pathways by cancer cells may contribute to this phenomenon.[127,128,130,131] WR-2721 is rapidly hydrolyzed under alkaline conditions, while low pH levels prevent this reaction.[42] The acidity of the tumor environment may therefore contribute to the selectivity of amifostine cytoprotection.

Active uptake of amifostine by normal cells

In a study conducted by Treskes *et al.* WR-1065 (but not WR-2721) equally protected normal and cancer cells against cisplatin.[132] The investigators suggested that the selective cytoprotection by amifostine is mainly attributed to its selective dephosphorylation in normal tissues and not to a selective uptake of its active form WR-1065. Nevertheless, studies with cancer cell cultures showed no protection conferred by WR-1065.[94,95] This suggests that, at least in the majority of the cancer cell lines examined, the uptake of WR-2721 by cancer cells is low. Yuhas *et al.* reported that there is in fact a difference in the kinetics of intrusion of WR-2721 between normal and cancer cells.[70] Normal cells actively absorb WR-2721, while a passive process occurs in cancer cells. Such a mechanism may be of particular importance in the case of leukemia, where a solid 'tumor environment' is absent.

Administration of amifostine

Intravenous administration

The maximum tolerated dose of amifostine has not been established. The recommended dose of

amifostine for chemotherapy is 740–910 mg/m^2 and 200–340 mg/m^2 before each radiotherapy fraction.[133–136] The dose of amifostine can be reduced to 500–1000 mg flat dose in case of fractionation of the dose of chemotherapy (weekly or biweeky schedules).[137,138] For hypofractionated radiotherapy regimens, it would be reasonable to increase the dose to 750–1000 mg.The most appropriate schedule of amifostine as a supportive regimen for hyperfractionated regimens is unknown. Splitting the dose of amifostine (i.e. 250–300 mg before the first and second fraction in the case of twice daily fractionation) has been suggested, although the tolerability of this regimen is questionable. Administration of amifostine 750 mg immediately before the first fraction is an alternative schedule.

The guidelines for i.v. and s.c. amifostine administration, herein described, have been established in our Department after 5 years of experience with different schedules of amifostine administration. Doses of amifostine >1500 mg are diluted in 50 ml of 0.9% normal saline and are given as a 5-min infusion, 15–20 min before chemotherapy. Doses between 500–1000 mg are diluted in 50 ml of 0.9% normal saline and are given as a 3-min infusion, 15–20 min before radiotherapy or chemotherapy (that precedes radiotherapy).

Amifostine is highly emetogenic; grade 2–3 nausea and vomiting are experienced by 10–40% of patients depending upon the dose of amifostine. The antiemetic policy of our Department follows the guidelines used for highly emetogenic chemotherapy regimens, when amifostine is used before such regimens. For an amifostine dose above 1000 mg, high doses of a 5-HT_3 antagonist (e.g. granisteron 4 mg i.v.) and dexamethasone (8–16 mg i.v.) should be given 15 min before the administration of amifostine. Amifostine doses between 750–1000 mg should be also preceded by administration of high-dose 5-HT_3 antagonists. Dexamethasone is not necessary and should be omitted in the case of protracted daily schedules (e.g. radiotherapy). Approximately 10% of patients receiving daily doses of amifostine 500–1000 mg will develop grade 3 emesis that may lead to refusal of further cytoprotection. In these patients, dexamethasone 8 mg i.v. 15 min before amifostine administration may reduce emesis. The dose of dexamethasone can be decreased gradually during the subsequent days of amifostine administration, to 4 and 2 mg. Reduction of the dose of amifostine by 35% may also be necessary. For low-dose amifostine regimens (300–500 mg), oral administration of 5-HT_3 antagonists (e.g. granisteron 2 mg p.o.) 1 h before amifostine is recommended. Omission of any antiemetic policy is justified in some patients with good tolerance.

Hypotension is another clinically relevant side effect of amifostine. Up to 40% of patients receiving an amifostine dose of 1000–1500 mg will develop hypotension, which is reversed rapidly within 2–15 min after infusion interruption. The mechanism of hypotension induced by amifostine is unclear. WR-1065 induces relaxation of the smooth muscles of the small arteries, which is independent of nitric oxide or prostaglandin production, α-adrenergic receptors, cyclic nucleotides or calcium pump activity.[139] Ephedrine reduces the magnitude of hypotension in experimental animals.

Amifostine should be administered to patients while in a supine position; otherwise, the incidence of clinical hypotension may exceed 20%. Measurement of blood pressure immediately before i.v. amifostine administration is required and a low systolic value may necessitate dose reduction or even omission of amifostine. In patients with systolic pressure below 90 mmHg, the dose of amifostine should not exceed 1000 mg. In patients with systolic blood pressure between 80 and 90 mmHg, the dose of amifostine should not exceed 500 mg. A systolic pressure below 80 mmHg requires interruption of amifostine administration. Blood pressure is monitored every 1 min during drug administration. A reduction in systolic pressure by 20% or below 80 mmHg should be immediately followed by infusion interruption. The infusion may be continued, using the same infusion rate, immediately following restoration of the systolic pressure to values above 90 mmHg. After the end of amifostine infusion, blood pressure is measured every 2 min for 6 min with the patient in a supine position and for 8 min with the patient in a seated position. If blood pressure is stabilized to values above 90 mmHg, then the patient can stand up. Clinically evident orthostatic hypotension may occur more frequently after amifostine administration and clinicians responsible for radiation treatment should be aware of this potential event. Patients receiving standard antihypertensive drugs should not interrupt their therapy when the dose of amifostine does not exceed 1000 mg.If higher doses of amifostine are prescribed (i.e. chemotherapy every 2–4 weeks), the patient should omit the morning dose of antihypertensive medication for the day of amifostine administration only.

Asthenia, which usually accompanies persistent nausea, may occur and last for 2–3 days following amifostine administration. During protracted administration of low doses (500–1000 mg) of amifostine, asthenia occurs in 30% of patients. In such cases, asthenia is cumulative, with a progressive worsening

of the symptomatology. Interruption of amifostine for 2 days may be useful. A 35% dose reduction should be tried if the symptoms are severe or if the patient refuses to continue therapy. Overall, 5–10% of patients on protracted regimens will interrupt amifostine therapy because of nausea or asthenia.

Generalized rash accompanied by high fever (38.5–40°C) may be experienced in 2% of patients receiving daily i.v. amifostine at doses up to 500 mg. Occasionally, rash may not be accompanied by fever. The incidence of this symptomatology increases to 7% if the daily dose of amifostine is increased to 1000 mg.[129] The symptoms may appear even after the 20th injection of amifostine (usually after the fourth to seventh injection and rarely after the first injection). Fever and rash are apparent within 2–4 h after amifostine administration. Fever regresses rapidly with paracetamol or within 2 h if the patient does not receive antipyretic medication. The rash is pink, with a diffuse character; it may appear over the whole body and is not itchy. The rash regresses within 2 days, even without medication. Oral antihistamines or cortisone may accelerate the regression of the rash. These symptoms are not life threatening. Patients should be informed of the eventual appearance of such symptoms. Amifostine administration should be interrupted, or the symptomatology will become more severe during the subsequent administrations.[140] No eosinophilia or rise of IgE levels is noted in these patients, while a 2- to 3-fold rise of CRP concentration is consistently noted.[104] The development of a real allergic reaction to amifostine, with signs of collapse, is very rare (less than 1 in 250).

Clinical hypoglycemia may also occur within 15–30 min following amifostine administration in 1–2% of patients treated with a dose above 1000 mg. Glucose levels below 70 mg/dl before amifostine administration should be used as a sign for an eventual hypoglycemic condition and hypertonic glucose solutions should be available for immediate use. Such patients may also be advised to consume sweets 1 h before therapy.

The reduction of calcium serum levels is quite common in patients receiving amifostine but is rarely of clinical importance. Hot flushes, sneezing, rigors, and headaches may also be reported. Metallic taste, sometimes paradoxically accompanied by xerostomia, may be experienced by 1–5% of patients.[104]

Subcutaneous administration

The s.c. administration of amifostine, although its pharmacokinetics are still unclear, presents several distinct advantages over the i.v. route,[104] especially for protracted daily schedules used in radiotherapy or in the treatment of myelodysplastic syndromes. These advantages are summarized below.

- Subcutaneous administration is simple. The total administration procedure (dilution and injection) does not take more than 2 min (versus 15–30 min for the i.v. procedure).
- No vein catheterization is required, which is important in patients heavily pretreated with chemotherapy, whose superficial vein system of the hands is not easily accessible.
- There is no need for a day clinic attached to the radiotherapy unit. As clinical hypotension never occurs when amifostine is administered s.c., the patient is injected while in a seated position.
- There is no need for specialized nurses who are familiar with the quite troublesome i.v. administration procedure.
- Subcutaneous administration facilitates the synchronization of the patient flux to the radiotherapy units and does not jeopardize the scheduled order of patients to be treated in the radiotherapy units.
- The cost of the administration procedure is decreased (pharmacoeconomic study in progress).

For conventionally fractionated radiotherapy, the dose of amifostine recommended for s.c. schedules is 500 mg flat dose. For hyperfractionated twice-a-day regimens, an amifostine dose of 200–250 mg can be used before each fraction. Amifostine 500 mg is diluted in 2.5 ml normal saline. For hypofractionated regimens, the dose may be increased to 1000 mg, diluted in 4.5 ml normal saline. The dose of 500 mg (2–2.5 ml volume) is injected with a fine needle to one site on the shoulder; 1000 mg should be injected split in two sites (two shoulders). The abdominal area may also be used as an injection site. Amifostine is administered while the patient is in a seated position. There is no need for blood pressure measurement. Rather, this should be performed once a week before amifostine administration and after radiotherapy. Oral 5-HT_3 antagonists (e.g. granisteron 2 mg) 1 h before amifostine is the recommended antiemetic policy. Radiotherapy follows 20–30 min after the injection of amifostine.

Clinical hypotension never occurs, but the risk of orthostatic hypotension may be increased with s.c. administration. Between 30 and 40% of patients develop mild nausea and malaise 0.5–4 h following amifostine injection. Patients should be informed of these effects and should be encouraged to tolerate them in view of the major benefits expected from

reduced toxicity. Approximately 5–10% of patients develop grade 2–3 emesis. Reduction of the amifostine dose to 300 mg should be considered in such cases. Slow released cortisone injection (once a week) may also be of clinical benefit. Approximately 7% of patients will develop rash/fever symptomatology. Local rash at the site of injection is a common event. Extensive local rash, which does not itch and is accompanied by dry desquamation around the area of injection, appears in 5% of cases. When this occurs, multiple areas of injection should be used and local antihistamine or cortisone creams can restore the normal skin condition within 2 days.

Alternative schedules and routes of administration

The bolus i.v. administration of amifostine 200 mg/m^2 has been applied in a study by Wagner *et al.*[141] This certainly simplifies the administration procedure. Another yet unanswered question is whether the number of days of amifostine administration during radiotherapy may be reduced. For example, if amifostine could be given on alternating days, the cost of therapy would be reduced by 40% and the regimen may be better tolerated. Peters *et al.* administered amifostine to 14 patients with HNC receiving carboplatin chemo-radiotherapy only during the days of carboplatin administration (days 1–5 and 19–33). With this schedule, no signs of mucosal protection were reported.[103] We conducted a study in which amifostine was given i.v. (500 mg flat dose) before carboplatin and radiotherapy during the first 10 and the last 10 fractions of radiotherapy. A significant protection of the esophageal and rectal mucosa (p=0.05), as well as maintenance of platelet counts ($p<0.001$) was noted.[137] Thus, a total dose of amifostine reduced by 30% seemed to maintain a good cytoprotective effect. In a recent report by Komaki *et al.*,[108] the administration of amifostine 500 mg i.v. twice weekly effectively protected normal esophageal mucosal (p=0.03) and normal lung (p=0.03) during an aggressive regimen of concurrent platinum/etoposide chemoradiotherapy. Results of this study suggest that a 60% reduction in the total amifostine dose is compatible with good mucosal and lung cytoprotection.

The local application of amifostine (mouth washes, enemas, local application on the skin) could be of clinical value, if a substantial intracellular concentration of WR-1065 could be achieved. Because WR-2721 is an inactive compound requiring dephosophorylation for its activation, it is unlikely that such a biotransformation could efficiently take place on the mucosa and skin surface. Ben-Josef *et al.* demonstrated that WR-1065 levels achieved in the rectal wall after WR-2721 enema in rats is high 20–40 min after administration.[142] In a clinical trial, however, an enema of amifostine (100–400 mg) failed to protect the rectal mucosa in 31 patients treated with standard radiotherapy.[143] Nevertheless, if WR-1065, the active metabolite of amifostine, was available for local application, it would be of great clinical interest.

Clinical experience with amifostine

Phase I/II studies of radiotherapy and chemo-radiotherapy

The role of low doses of amifostine as a cytoprotective regimen for radiotherapy has been examined in two phase II studies. McDonald *et al.* treated patients with head and neck cancer undergoing radiotherapy with amifostine 100 mg/m^2 administered before each radiotherapy fraction. The parotid gland was scheduled to receive more than 45 Gy. No protective effect against xerostomia was noted in this study, suggesting that a higher amifostine dose should be investigated in subsequent trials.[144] In another phase II study, 37 patients with stage III/IV a cervical cancer treated with radiotherapy received amifostine 75 mg/m^2 before each radiotherapy fraction.[99] After a 5-year follow-up period, the investigators concluded that this low-dose regimen does not prevent early or late radiation sequelae.

In a phase II study in 25 HNC patients receiving standard radiotherapy and carboplatin (70 mg/m^2 days 1–5 and 21–26), Buntzel *et al.* evaluated the efficacy of amifostine (500 mg flat dose i.v.) in preventing early toxicity. Incidences of mucosal toxicity, xerostomia, thrombocytopenia, and leukopenia were reduced compared with that of patients that did not receive amifostine (p values ranging from 0.05 to <0.001).[101,102]

Similar results have been reported by Schonekas *et al.* Amifostine 500 mg flat dose was administered to 20 patients with HNC undergoing standard radiotherapy. Although the expected incidence of grade 3–4 mucositis was 35%, none of the patients recruited in this phase II study experienced >above grade 2 mucositis (p=0.02). Xerostomia was also reduced.[145] Similar results were reported in a subsequent study from the same group.[146] In a separate pilot study, patients receiving standard radiotherapy concurrently with 5-fluorouracil given with a portable pump as a daily continuous infusion (250 mg/m^2/day) received amifostine at a flat dose of 300 mg

before each radiotherapy fraction. Significantly reduced mucosal toxicity was noted compared to that of a historical control group of patients (p=0.05).[147]

In NSCLC, two phase II studies reported prevention of mucositis with amifostine. Tannehill *et al.* treated 26 patients with stage III NSCLC with standard 60 Gy radiotherapy supported by amifostine 340 mg/m^2 before each radiotherapy fraction. Concurrent chemotherapy with cisplatin/vinblastine supported with amifostine (740–910 mg/m^2) was also given to these patients. The investigators observed a significant prevention of esophagitis and nephrotoxicity.[106] We also conducted a study in which amifostine provided significant protection from the development of grade 3 esophagitis and diarrhea in patients treated with standard radiotherapy and a high total dose of carboplatin (590 mg/m^2 every 4 weeks, fractionated in 10 doses before 10 radiotherapy fractions; p=0.01).[138,148]

Phase I/II studies of chemotherapy

An early phase II study evaluating cisplatin administration in conjunction with amifostine, reported by Glover *et al.* found a reduction of nephrotoxicity and neurotoxicity, which encouraged further trials.[109] In a study by Schiller *et al.* 25 patients with NSCLC received amifostine 740–910 mg/m^2 before cisplatin 120 mg/m^2 on day 1. Vinblastine 5 mg/m^2 was also given weekly without amifostine. The high toxicity of this regimen was reversible in all patients.[115] In a separate study, 13 women with advanced breast cancer received cisplatin 120 mg/m^2 every 3 weeks supported by amifostine 910 mg/m^2 i.v. before chemotherapy. No apparent benefit in terms of renal toxicity and ototoxicity was noted.[149] In contrast, in another study of 11 patients treated with high-dose cisplatin supported with amifostine, audiometric tests revealed a substantial reduction of ototoxicity.[150]

In a prospective study, 12 patients with metastatic colon cancer received 5-fluorouracil 2.6 g/m^2 and leucovorin 500 mg/m^2 on a weekly basis for 6 consecutive weeks. Amifostine 740 mg/m^2 was given before each 5-fluorouracil dose. Severe mucositis that necessitated delays in chemotherapy administration was noted in six of 12 patients versus 13 of 15 patients in a control group.[121]

Fotemustine (Muphoran) is a drug widely used in the treatment of melanoma outside the US. The dose-limiting toxicity is myelotoxicity. In a phase II study, 10 patients were treated with amifostine 740 mg/m^2 i.v. before fotemustine 100 mg/m^2. Although the expected incidence of severe neutropenia was 40–45%, according to the previous experience of the investigators with fotemustine, none of the patients treated with amifostine presented with grade 3–4 neutropenia.[151]

In contrast, Kusenda *et al.* reported no protection against myelotoxicity in patients treated with a regimen of mitomycin C (10 mg/m^2 i.v. day 1) and vinblastine (5 mg/m^2 i.v. days 1 and 15) repeated every 4 weeks, in which amifostine 910 mg/m^2 was given before mitomycin C. However, this study was performed only in six heavily pretreated patients.[152]

Glover *et al.* treated 21 patients with a high dose of cyclophosphamide (1500 mg/m^2) without amifostine. Four weeks later, the same patients received the same dose of cyclophosphamide supported by amifostine 740 mg/m^2 i.v. A significant improvement of the hematologic toxicity was noted. The median neutrophil nadir was increased from 541 to 1247/ml in the cycle supported with amifostine (p=0.0005) and the incidence of septic fever was also reduced (0 of 21 versus three of 21).[153] Similar results have been reported in a study of 40 patients with high-risk lymphomas treated with cyclophosphamide 1500 mg/m^2 with or without amifostine 910 mg/m^2. The incidences of both neutropenia and infections were significantly reduced in patients receiving amifostine compared with that of the control group.[122]

The effects of amifostine on the efficacy and tolerability of intensified chemotherapy schedules supported by bone marrow transplantation has also been investigated in several studies. In a study by De Souza *et al.* 29 patients with non-Hodgkin's lymphoma were treated with high-dose cyclophosphamide (7 g/m^2) supported by amifostine and autologous peripheral stem cell transplantation. The incidence of cardiac, pulmonary and liver toxicity, and of mucositis was significantly lower compared with the toxicity noted in a historical group of patients treated with the same regimen without amifostine. Amifostine did not prevent bone marrow aplasia. The incidence of treatment-related mortality in the amifostine group was 0% compared with 7% in the control group.[154] In a retrospective study, patients receiving high-dose melphalan and transplantation of peripheral bone marrow progenitor cells with or without amifostine 740 mg/m^2 were analyzed. Grade 2–4 stomatitis was noted in 21% of patients in the amifostine arm versus 53% of patients in the control arm. The median duration of stomatitis was significantly reduced in the amifostine arm compared with that of the control arm (0 versus 7

days; $p=0.0001$). Severe diarrhea was experienced by 3% of patients in the amifostine arm versus 34% of patients in the control arm ($p=0.01$). The bone marrow recovery was similar in both treatment arms.[155] Chauncey *et al.* treated 21 patients with a high dose of busulfan, melphalan, and thiotepa supported with transplantation of peripheral bone marrow progenitor cells. Amifostine 910 mg/m^2 i.v. was given before melphalan/thiotepa. The investigators concluded that amifostine did not prevent the mucositis caused by this regimen.[156]

An alternative approach in intensified chemotherapy regimens is the *ex vivo* incubation of the progenitor cells with amifostine. Activity of amifostine in increasing the ability of bone marrow progenitor cells to restore bone marrow function has been reported by Shpall *et al.*[157] *Ex vivo* pretreatment of progenitor cells with amifostine resulted in a significant reduction of the period required for the recovery of neutrophil counts to values above 1000/ml (26 versus 36 days). The requirements for platelet transfusion and administration of antibiotics were also significantly reduced.

Randomized phase II and III radiotherapy and chemo-radiotherapy studies

Xerostomia is an important side effect in HNC patients treated with radiotherapy and in patients with thyroid cancer treated with radioiodine therapy. Approximately 40–60% of HNC patients are cured with radiotherapy; however, because the rate of severe xerostomia is as high as 60%, quality of life for these patients is severely affected. There is no effective therapy available for this complication. Therefore, the choice of protection against xerostomia as the primary endpoint in trials with amifostine is well justified. Experimental studies showed that, indeed, amifostine is highly accumulated in the salivary glands.[70,71] In 1998, investigators conducting a randomized, double-blind study of 50 patients receiving radioiodine therapy (3–6 GBq) concluded that amifostine provided significant protection against xerostomia (0 of 25 versus nine of 25 patients experienced xerostomia; $p=0.001$).[158]

The largest randomized multicenter trial ever published with amifostine is a recent one by Brizel *et al.* who studied 315 HNC patients treated with standard radiotherapy. Prevention of xerostomia was the primary endpoint of this trial.[105] All recruited patients received a radiation dose above 40 Gy to more than 75% of the parotid gland area. Amifostine 200 mg/m^2 i.v. was administered before each radiotherapy fraction. Total saliva was quantified before, during, and after therapy. A significant reduction in grade 2–4 acute xerostomia was observed in the amifostine arm compared with that of the control arm (51 versus 78%; $p<0.0001$). In addition, the incidence of late xerostomia (grade 2–4) was significantly reduced by amifostine administration (57 versus 34%; $p=0.002$). Although the incidence of mucositis was similar in both treatment groups, a significant reduction in mucositis was reported in patients treated in the European Institutes that participated in the study.[159] The lack of clear evidence of prevention of mucositis should be attributed to the relatively low dose of amifostine administered (350–380 mg) and to the wide variation in the location of the primary tumors of the head and neck area, which demands the application of different localization and fields of radiation. If mucositis is the primary endpoint of trials for HNC, strictly homogeneous tumor entities and homogeneous radiotherapy techniques should be applied in order to extract reliable conclusions.

Based on the study by Brizel, a pharmacoeconomic study conducted by Mackoviak *et al.* showed that the cost of amifostine for the prevention of xerostomia is significantly lower than the cost of treatment for established xerostomia.[160] These studies led to the FDA approval of amifostine for the prevention of radiotherapy-related xerostomia. In a recent study, prevention of xerostomia by amifostine led to reduced dental complications in HNC patients treated with radiotherapy.[161]

Several randomized phase II studies in HNC patients have focused on the role of amifostine in the prevention of radiation mucositis. Bourhis *et al.* treated 26 patients with locally advanced inoperable HNC with an intensively accelerated radiotherapy scheme (1.7 Gy $\times$ 2 per day; 64 Gy total dose within 3.5 weeks). Half of these patients received amifostine 150 mg/m^2 before each radiotherapy fraction (twice daily). Although five of 13 patients in the amifostine arm interrupted cytoprotection due to generalized rash and emesis, grade 3–4 mucositis was noted in only one of eight patients treated with amifostine versus eight of 12 patients in the control arm. The median duration of severe mucositis was 25 days in the amifostine arm versus 49 days in the control arm ($p=0.03$). Feeding tubes were maintained for a median of 1 and 2.5 months in the amifostine and control arms ($p=0.01$), respectively.[162]

A significant protection against radiation-induced mucositis has been also confirmed in a randomized phase II study we conducted in 40 HNC patients treated with standard radiotherapy with or without

amifostine 500 mg flat dose, delivered s.c. before each radiotherapy fraction. None of the patients in the amifostine arm showed grade 3–4 mucositis versus 30% of patients in the control arm (p=0.02). As feeding tubes are not used in our Institute, a split in radiotherapy is interposed until grade 3–4 mucositis regresses to grade 2. A total of 20% of patients treated without amifostine had to interrupt their treatment for 8–14 days, while none of the patients treated with amifostine had radiotherapy protracted for more than 7 days (p=0.07).[104] A trend toward reduction in incidence of cervical/pharyngeal strictures in 54 patients with locally advanced HNC treated with concurrent chemo-radiotherapy (cisplatin, 5-fluorouracil and paclitaxel) with or without amifostine was recently reported (17% in the amifostine arm versus 37% in the control arm; p=0.06).[163]

In another study, we investigated the cytoprotective effect of amifostine in 60 patients with locally advanced thoracic tumors treated with standard radiotherapy (64 Gy).[104] Grade 3–4 esophagitis was documented in 20% of patients in the control arm versus 4% of patients in the amifostine arm (p=0.02). The 3- to 7-day and 8- to 14-day split inserted due to severe esophagitis was 37 and 17% in the control group versus 20 and 0% in the amifostine group (p=0.04). In another randomized trial conducted by Antonadou *et al.*, 145 patients with NSCLC were treated with standard radiotherapy (60 Gy) with or without amifostine 340 mg/m^2 i.v. The incidence of grade 3–4 esophagitis documented during the fourth week of radiotherapy was 4 versus 44% in the control arm (p=0.001). Radiation-induced pneumonitis was also significantly reduced with amifostine administration (p=0.001).[107] A significantly reduced incidence of esophagitis was reported by the same group in a randomized trial of NSCLC patients treated with radio-chemotherapy (with paclitaxel or carboplatin) when amifostine 340 mg/m^2 was administered before radiotherapy ($p<0.01$).[164]

Liu *et al.* published the first study on the role of amifostine in the prevention of toxicity associated with radiotherapy in patients with pelvic tumors in 1992. One hundred patients with inoperable rectal cancer treated with radiotherapy (45 Gy pelvic irradiation, 2.25 Gy/fraction, 4 fractions/week followed by a booster dose of 4 fractions of 2.8 Gy to the tumor area) were assigned randomly to receive amifostine 340 mg/m^2 or nothing before each radiotherapy fraction. A significant reduction in the moderate-to-severe late effects of radiotherapy was reported in patients receiving amifostine (none of 34 versus five of 37; p=0.03).[97] We conducted a phase II randomized study to evaluate the s.c. administration of amifostine in 40 patients with locally advanced miscellaneous pelvic tumors. The dose of pelvic radiation was 50 Gy and the tumor dose was 64–70 Gy, using standard radiotherapy. The incidence of grade 2–4 diarrhea was 50% in the control group versus 13% in patients receiving amifostine. An 8- to 14-day split insertion was deemed necessary in 20% of patients in the control arm to allow recovery from the colitis versus 0% of patients in the amifostine arm. Of importance, grade 2–3 perineal and skin toxicity, a very common and unpleasant effect of pelvic radiotherapy, was prevented in all patients receiving amifostine.[104]

Randomized phase II and III chemotherapy studies

In 1996, a large randomized study of 242 patients with advanced ovarian carcinomas treated with cisplatin 100 mg/m^2 and cyclophosphamide 1000 mg/m^2 every 3 weeks, with or without amifostine 910 mg/m^2 i.v., was conducted by Kemp *et al.*[113] The incidence of septic neutropenia was significantly reduced in the amifostine arm compared with that of the control arm (10 versus 21%; p=0.01), as was the use of antibiotics and the number of days of hospitalization required ($p<0.03$). The number of platelet and red cell transfusions was also reduced (p=NS). Amifostine provided significant protection against cisplatin nephrotoxicity. Reductions in creatinine clearance (above 40%) were noted in 12% of patients receiving amifostine versus 60% of patients in the control group (p=0.03). Hypomagnesemia and high plasma levels of creatinine that required delays in the chemotherapy schedule were also less frequent in the amifostine arm compared with that of the control arm. Amifostine also prevented the occurrence of ototoxicity (p=NS) and peripheral neuropathy (p=0.01).

Planting *et al.* investigated the cytoprotective efficacy of amifostine in patients with advanced HNC receiving high weekly doses of cisplatin (70 mg/m^2/week). Patients were assigned randomly to receive amifostine 740 mg/m^2 or nothing before cisplatin. Thrombocytopenia and peripheral neuropathy were significantly reduced in the amifostine arm compared with that of the control arm ($p<0.04$); whereas the incidence of renal toxicity and ototoxicity was similar in both arms.[117]

Hartmann *et al.* investigated the efficacy of a relatively low dose of amifostine (1000 mg flat) as a cytoprotective regimen against a VIP regimen (cisplatin 50 mg/m^2 plus ifosfamide 4 g/m^2 plus etoposide

500 mg/m^2) every 3 weeks. Renal toxicity was significantly reduced, since the excretion of *N*-acetyl-glucosaminidase and albumin was low and the glomerular filtration rate (GFR) was stable in all patients receiving amifostine. A reduced GFR was reported in 30% of patients in the control arm.[165]

In another phase II randomized trial conducted by Betticher *et al.* in 1995, 21 patients with NSCLC were treated with carboplatin 600 mg/m^2 every 4 weeks for 3 cycles with or without amifostine 910 mg/m^2 before carboplatin. Although the myelotoxicity was similar in both treatment groups, the median time for platelet recovery was 13 days in the amifostine arm versus 21 days in the control arm (p=0.04). Hospitalization for septic neutropenia was more frequent in the control arm (none of 25 versus six of 25 cycles).[119] Budd *et al.* reported that amifostine significantly protects against carboplatin-induced thrombocytopenia in a randomized study in 55 patients receiving carboplatin 500 mg/m^2 with or without amifostine 910 mg/m^2 (p=0.02).[118] In a recent study, 84 patients with NSCLC were assigned randomly to receive ifosfamide 3 g/m^2 plus carboplatin AUC 6 plus etoposide 50 mg p.o. × 2/day × 7 days (every 3 weeks), with or without amifostine 740 mg/m^2 before ifosfamide/carboplatin. In this study, amifostine did not protect against leukopenia and thrombocytopenia.[120] The investigators concluded that oral etoposide may have been associated with the failure of the study to show a benefit from amifostine. In addition, no clear benefit from amifostine against paclitaxel cytotoxicity was found in a randomized study of 40 women with advanced breast carcinoma treated with paclitaxel 250 mg/m^2 every 3 weeks with or without amifostine 910 mg/m^2.[116] Another study, however, conducted by DiPaola *et al.* in 22 patients with advanced malignancies treated with escalating doses of paclitaxel, demonstrated that amifostine 910 mg/m^2 permits a dose escalation to 270 mg/m^2 with no grade 3–4 toxicity.[166]

Overall, studies that failed to show a clear cytoprotective benefit for amifostine were those that included taxanes or epipodophylotoxins (etoposide, vinblastine) in the chemotherapy regimen.[114,116,120,153] The main activity of these drugs is confined to the polymerization or depolymerization of microtubules formed during the G_2/mitotic phase of the cell cycle.[167] Although taxanes induce DNA strand breaks and experimental studies confirm that amifostine protects normal (and not tumor) cells against this type of paclitaxel-induced damage,[168] taxanes are also involved in the phosphorylation of the anti-apoptotic Bcl-2 protein,[169] which is found on the walls of mitochondria, on microtubules and on the nuclear envelope. Bcl-2 phosphorylation leads to apoptosis through pathways independent of DNA damage. The role of amifostine in protecting cells against this specific cellular damage is still unknown.

New clinical directions

Increasing the dose intensity and the efficacy of therapy

The dose intensity of chemotherapy and radiotherapy, or the total time within which the total dose of these agents is delivered, is an important parameter that defines the antineoplastic efficacy of a regimen. Protracted overall treatment time results in a substantial compromise of radiotherapy efficacy, because rapid tumor repopulation starts within the third week of radiotherapy.[170] The combination of chemotherapy with radiotherapy, especially in recent years using novel drugs with radiosensitizing properties, is a promising field of clinical research. The dose intensity of radiotherapy and chemotherapy given concurrently may be an important factor relevant to the efficacy of such regimens in controlling local and disseminated micrometastatic disease in locally advanced tumors.

As a selective cytoprotector of normal tissues, amifostine could significantly facilitate the application of aggressive chemo-radiotherapy schemes, the feasibility of which is impossible without effective cytoprotection. Experimental studies have shown that amifostine allows the intensification of doses of chemotherapeutic agents. van Laar *et al.* demonstrated that amifostine allows an increase in the dose of carboplatin from 45 to 60 mg/kg in BALB/c mice treated with carboplatin/5-fluorouracil chemotherapy. This dose intensification resulted in an increased antitumor efficacy.[79] In another study conducted by van der Wilt *et al.* similar results were reported.[171] Treskes *et al.* demonstrated that amifostine allowed a 2.2-fold increase in the dose of cisplatin given to BALB/c mice, before renal toxicity became dose limiting.[172] In another study conducted by the same group, amifostine pretreatment allowed the delivery of a 1.5-fold increase in the dose of carboplatin in nude mice.[78]

The possibility of dose intensification of chemotherapy or radiotherapy using amifostine as a cytoprotector has not been extensively studied. Gridelli *et al.* failed to achieve intensification of the dose of vinorelbine given concurrently with radiotherapy in NSCLC patients.[173] Nevertheless,

neutropenia was the dose limiting toxicity and no amifostine was given before vinorelbine. Still, the efficacy of amifostine against non-DNA-damaging agents remains unknown. In another study in children, amifostine did not allow intensification of the dose of melphalan beyond 35 mg/m^2 and neutropenia was the dose-limiting toxicity.[174]

Neutrophils are very sensitive to most cytotoxic drugs. The relatively reduced efficacy of amifostine in protecting white cells, at least as well as GM-CSF, should not stop studies aiming at the dose intensification of chemotherapy schedules. The administration of GM-CSF together with amifostine can help to overcome the problem of neutropenia and unmask the real protective efficacy of amifostine on other tissues. In a phase I dose escalation study conducted by our group, amifostine allowed the intensification of the dose of carboplatin during fractionated radiotherapy. Eighty-four patients with NSCLC or pelvic tumors were recruited. Twenty-four patients received standard radiotherapy without chemotherapy. Thereafter, a dose escalation of carboplatin began with a starting dose of 38 mg/m^2/day for 10 consecutive fractions of radiotherapy at the beginning (weeks 1 and 2) and the end (weeks 5 and 6) of the radiotherapy schedule. The carboplatin dose was escalated by 7 mg/m^2/day dose increments. The maximum tolerated dose was 45 mg/m^2/day and the dose-limiting toxicity was neutropenia. When G-CSF (380 μg s.c. every Saturday and Sunday) was added, the maximum tolerated dose of carboplatin increased to 52 mg/m^2, where thrombocytopenia was the dose-limiting toxicity. Addition of amifostine (500 mg flat dose before each dose of carboplatin) allowed the escalation of the dose to 59 mg/m^2/day. The maximum tolerated dose and dose-limiting toxicity were not reached because the total dose of carboplatin achieved without any myelotoxicity was very high (590 mg/m^2 every 4 weeks). At this high dose level, 24 patients were recruited (12 with NSCLC and 12 with locally advanced pelvic cancer).[149] Esophagitis and diarrhea (grade 3) were noted in 20–25% of patients treated with radiotherapy alone or with radiotherapy/carboplatin without amifostine versus 0–5% of patients in the amifostine cohorts (p=0.01). Forty percent of patients treated in the non-amifostine groups had to interrupt their treatment for 1 week due to mucosal toxicity versus 13% of patients in the amifostine group (p=0.05). We calculated that the median dose of radiation wasted to compensate for rapid tumor repopulation due to delays of radiotherapy was 5 Gy in the non-amifostine arm versus 1.5 Gy in the amifostine arm. This study clearly shows that if amifostine is combined with G-CSF, the dose intensity of carboplatin and of radiotherapy may be substantially increased. Similar results have been reported in a study by Budd *et al.* in which 35 patients were treated with escalating doses of carboplatin. Prevention of thrombocytopenia by amifostine allowed a substantial increase in the maximum tolerated dose from 400 to 500 mg/m^2.[175]

In a subsequent phase I dose escalation study, we investigated the maximum tolerated dose of a docetaxel/carboplatin combination given once every 2 weeks. Amifostine 1000 mg flat dose was given before chemotherapy and GM-CSF 400 μg was given for 2 days every week. The maximum tolerated doses were docetaxel 50 mg/m^2 and carboplatin AUC 4 every 2 weeks. There were no cases of hematologic, neurologic or other toxicity in this group. Further escalation of the dose of docetaxel to 60 mg/m^2 resulted in severe asthenia, which is considered the dose-limiting toxicity of the schedule.[139] In the same study, evidence of an eventual immunologic protection by amifostine against docetaxel/radiotherapy was provided. Prevention of the severe immunologic toxicity associated with taxanes may be of importance in the natural history of various carcinomas such as breast or lung cancer, where taxanes already have an established therapeutic role.[176,177]

Radiotherapy combined with novel drugs

The combination of novel chemotherapy drugs with radiotherapy is a promising field of clinical research. Nevertheless, the feasibility of such combinations is often questioned, as unacceptable toxicity masks the increased efficacy of the regimens.[178,179] Feasibility is achieved at the cost of reduced chemotherapy dose and severe acute toxicity that substantially affects patient quality of life during therapy, and results in prolonged delays in the radiotherapy schedule, which minimize the eventual therapeutic benefit.[180–182]

The combination of taxanes with radiotherapy has resulted in impressive complete response rates of 25–30% in patients with locally advanced NSCLC and other tumors, at the cost of severe mucositis, pneumonitis and immunological suppression.[177,183,184] Prolonged delays in the docetaxel chemo-radiotherapy schedule due to mucositis resulted in reduced efficacy in patients with NSCLC.[182] Topoisomerase I inhibitors are also important radiosensitizers.[185] Their concurrent administration, however, with radiotherapy is problematic because of severe mucositis, and the dose of drugs should be reduced to achieve acceptable tolerability.[181] We conducted a phase I study in 12 patients

with locally advanced HNC. Concurrent chemo-radiotherapy with docetaxel and irinotecan resulted in severe oropharyngeal mucositis, often complicated with resistant fungal infection, which required more than 2-week treatment delays.[180] Nevertheless, complete responses were achieved in nine of 12 patients, demonstrating the high efficacy of such combination regimens. Mucositis is also the dose-limiting toxicity in concurrent chemo-radiotherapy regimens with liposomal doxorubicin.[186] The combination of gemcitabine, another important radiosensitizer,[187] with radiotherapy is clinically feasible only at a cost of a more than 80% reduction in the recommended weekly chemotherapy dose (60–100 mg/m^2 in chemoradiotherapy schedules versus 800 mg/m^2/week in chemotherapy).[188]

The risk of rejecting such aggressive, still highly effective, chemo-radiotherapy regimens can be averted by incorporating amifostine for cytoprotection. In the study by Abitbol *et al.*[163] amifostine substantially reduced the rate of acute cervical esophageal stricture in patients treated with concurrent cisplatin/5-fluorouracil/paclitaxel chemo-radiotherapy (p=0.06). We also found that the s.c. administration of amifostine during radiotherapy allows the safe administration of a high dose of docetaxel/liposomal doxorubicin or topotecan/liposomal doxorubicin concurrently with radiation in thoracic, head and neck, and pelvic tumors, producing grade 3 mucositis in less than 20% of patients.[148] As a new era of chemo-radiotherapy has already begun, amifostine may become pivotal for major advances in the control of locally advanced tumors.

Protracted infusion chemotherapy, liposomal drugs and amifostine

The cytoprotective activity of amifostine appears to be exhausted within 2–4 h following its administration, although some cytoprotective components may be active for several additional h. Administration of a single dose of amifostine before chemotherapy is therefore justified when drugs with a relatively short plasma half-life of some minutes or up to 2 h are administered (e.g. 5-fluorouracil, cisplatin and camptothecins). In contrast, drugs with a prolonged plasma half-life would necessitate a longer period of cytoprotection. For example, carboplatin has a half-life of 6–8 h; therefore, even if the amifostine cytoprotective activity lasts 6 h, half of the carboplatin dose will be in the circulation at this time point and tissues will be exposed to this high remnant dose in the absence of cytoprotection. Other examples include the liposomal drugs (e.g. Caelyx®), which have a plasma half-life of 71–96 h, and the continuous infusion regimens (e.g. 72-h cisplatin infusion), which are commonly used as radiosensitization regimens. A single amifostine administration is unlikely to significantly prevent toxicity from such drugs or schedules.

The s.c. route of administration allows the investigation of new cytoprotective schedules for these drugs. For example, administration of amifostine 740 mg/m^2 i.v. before carboplatin followed by s.c. administration of amifostine 500 mg at 6 and 12 h after chemotherapy may be a more reasonable cytoprotective approach. Administration of amifostine 500 mg s.c. twice a day for 4–5 days could prove a useful regimen in the case of liposomal drugs or in the case of protracted infusions. Indeed, in an experimental study in mice, i.v. injection of amifostine 100 mg/kg for 4 consecutive days following the administration of liposomal doxorubicin significantly reduced the rate of erythrodysesthesia without reducing the antineoplastic activity of the drug.[189] In a recent study conducted by Lyass *et al.*,[190] the administration of amifostine on days 3, 4, and 8 after the administration of escalated doses of liposomal doxorubicin (45–60 mg/m^2 every 3 weeks) provided convincing evidence that such an amifostine schedule can protect against palmar–plantar erythrodysesthesia. As a large number of liposomal drugs are under development for clinical use, the evaluation of such 'prolonged' cytoprotective regimens is warranted.

Re-evaluation of hypofractionation

Hypofractionated radiotherapy, despite its established efficacy, has been abandoned by clinicians because of the high rate of severe late sequelae.[191] Nevertheless, large radiation fractions (4–5 Gy) may be more active in certain conditions, where tumors bear a low inherent radiosensitivity (e.g. melanomas or sarcomas). Tumor hypoxia is also a factor that increases the β value of the α/β ratio and broadens the shoulder of the survival curves. Poorly vascularized tumors are not sensitive to standard radiotherapy, probably because of the profound hypoxia and the poor ability of re-oxygenation during radiotherapy.[192] If amifostine could maintain a low late radiation toxicity, then hypofractionation could become the treatment of choice for certain tumors. The results from the CHART study in HNC patients were frustrating despite the intense acceleration of radiotherapy.[193] The lack of a clear benefit from such regimens was attributed to the short schedule of 12

days that did not allow re-oxygenation of tumors. The use of accelerated hypofractionated schemes could be effective as an alternative regimen, as hypoxic cells are more vulnerable to large radiation doses. In a pilot study, we investigated the feasibility of such hypofractionated accelerated schemes with cytoprotection (HypoARC regimen) in locally advanced chemoresistant breast carcinomas. Amifostine was given at a flat dose of 1000 mg i.v. before each of 12 consecutive radiotherapy fractions (3.5–4 Gy).[194] An 80% complete response rate was achieved. An impressive prevention of the early skin toxicity was also noted. Half of the patients survived for more than 16 months and none developed any severe late toxicity in lung, musculoskeletal or neural tissues. Application of such regimens in locally advanced tumors, such as HNC or NSCLC, is justified as hypoxia is a well-known feature of these tumor types.

Seventy-two high-risk breast cancer patients treated with surgery and FEC adjuvant chemotherapy were treated with the above-mentioned HypoARC regimen directed to the breast/chest wall, axilla and supraclavicular area.[129] A dramatic reduction in acute skin toxicity was noted (p=0.0001). A minimum of 18 months and a median of 28 months of follow-up are now available. Acute pneumonitis and late toxicity from breast, chest wall, axillary and lung tissues in the HypoARC regimen were lower than the toxicity observed in two matched control cohorts treated with standard fractionation (p=NS). From this study, we concluded that the HypoARC regimen is convenient for both patients and radiotherapy departments. The regimen is well tolerated and shows a significantly better profile in terms of early toxicity, while a reduced rate of late sequelae may be expected. A marginal improvement (not statistically significant) in the local relapse rate compared with conventional radiotherapy was also noted and current molecular analysis shows that HypoARC significantly improves local control in subgroups of patients (e.g. high MIB1 cancer cell proliferation index; unpublished data).

Amifostine, brachytherapy and intraoperative radiotherapy

Intracavitary and interstitial radiotherapy are important components of the standard treatment of cervical and uterine carcinomas,[195] and their use is currently expanding to the treatment of localized prostate cancer, esophageal cancer, head and neck cancer, and other tumors. Bladder and rectum late radiation toxicity is a major problem in patients with gynecologic tumors that are highly curable with radiotherapy. Approximately 40% of the total radiotherapy dose is given with intracavitary techniques that overload rectum and bladder areas proximal to the cervix and uterus. A total of 10–20% of these patients will experience severe rectal/bowel stenosis and necrosis, or bladder complications.[196] High-dose rate brachytherapy is also often followed by late sequelae.[197,198] Intraoperative radiotherapy also delivers high doses (10–15 Gy) within one fraction during surgery, and its use is expanding for the treatment of pancreatic, gastric/biliary and locally advanced rectal cancer.[199] Development of amifostine schedules to protect normal tissues against these highly effective invasive radiotherapy techniques may substantially improve the therapeutic index of this treatment strategy.

Conclusions

Amifostine is a unique drug with broad-spectrum, selective cytoprotective activity. None of the randomized phase II or III trials conducted to date provide any evidence that amifostine counteracts the efficacy of radiotherapy or chemotherapy. This concept is also supported by a large body of experimental studies conducted during the last 30 years. On the contrary, most of the clinical studies show a constant, still not significant, 2–20% benefit in terms of response rate or survival, which could be attributed to the higher dose intensity achieved in the patients receiving amifostine. To date, the drug is licensed for the prevention of cisplatin-related toxicity[200] and radiation-induced xerostomia. Large randomized trials focusing on mucositis are ongoing and experience to date shows that soon amifostine will be a routine component of standard radiotherapy practice. Combination of amifostine with GM-CSF is expected to allow further intensification of chemotherapy schedules, which may be of importance in the adjuvant setting or for the treatment of chemosensitive tumors such as small cell lung cancer or hematologic malignancies. A major benefit is also expected from novel amifostine studies focusing on the feasibility of aggressive chemo-radiotherapy schemes and radiotherapy schedules aiming to increase the local control rate of incurable tumors.

References

1. Steffer C. Studies on the mechanism of 5-hydroxytryptamine in radioprotection of mammals. In: Locker A, Flemming K, eds. *Radioprotection*. Basel: Stuttgard Verlag 1977: 71.

2. Packer JE, Slatter TF, Wilson RL. Direct observation of a free radical interaction between vitamine E and vitamine C. *Nature* 1979; **278**: 737–8.
3. Howell SB, Pfeifle CE, Wung WE, Olshen RA. Intraperitoneal *cis*-diaminochloroplatinum with systemic thiosulfate protection. *Cancer Res* 1983; **43**: 1426–31.
4. Berry JM, Jacobs C, Sikic B, Halsey J, Borch RF. Modification of cisplatin toxicity with diethyldithio carbamate. *J Clin Oncol* 1990; **8**: 1585–90.
5. Cox P. Cyclophosphamide cystitis: identification of acrolein as the causative agent. *Biochem Pharmacol* 1979; **28**: 2045–9.
6. Flombaum CD, Meyers PA. High dose leucovorin as sole therapy for methotrexate toxicity. *J Clin Oncol* 1999; **17**: 1589–94.
7. Rubenstein EB. Colony stimulating factors in patients with fever and neutropenia. *Int J Antimicrob Ag* 2000; **16**: 117–21.
8. Henke M, Guttenberger R, Barke A, Pajonk F, Potter R, Frommhold H. Erythropoietin for patients undergoing radiotherapy: a pilot study. *Radiother Oncol* 1999; **50**: 185–90.
9. Nikolatou O, Sotiropoulou-Londou A, Skarlatos J, Kyprianou K, Kolitsi G, Dardoufas K. A pilot study of the effect of granulocyte-macrophage colony stimulating factor on oral mucositis in head and neck cancer patients during X-radiation therapy: a preliminary report. *Int J Radiat Oncol Biol Phys* 1998; **42**: 551–6.
10. Koukourakis MI, Flordelis CS, Giatromanolaki A, *et al*. Oral administration of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) in the management of radiotherapy induced esophagitis. *Clin Cancer Res* 1999; **5**: 3970–6.
11. Patt HN, Tyree R, Straube RL, Smith DE. Cysteine protection against irradiation. *Science* 1949; **110**: 213.
12. Grdina DJ, Sigdestad CP. Radiation protectors: the unexpected benefits. *Drug Metab Rev* 1989; **20**: 13–42.
13. Davidson DE, Grenan MM, Sweeney TR. Biological characteristics of some improved radioprotectors. In: Brady L, ed. *Radiation sensitizers*. New York: Masson Press 1980: 309–20.
14. Yuhas JM, Storer JB. Chemoprotection against three models of radiation death in the mouse. *Int J Radiat Biol* 1969; **15**: 233–7.
15. Sigdestad CP, Connor AM, Scott RM. The effect of *S*-2-(3-aminopropylamino) ethylphosphorothioic acid (WR-2721) on intestinal crypt survival. I. 4 MeV X-rays. *Radiat Res* 1975; **62**: 267–75.
16. Ito H, Meistrich ML, Barkley Jr HT, Thames Jr HD, Milas L. Protection of acute and late radiation damage of the gastrointestinal tract by WR-2721. *Int J Radiat Oncol Biol Phys* 1986; **12**: 211–9.
17. Milas L, Hunter N, Reid BO, Thames Jr HD. Protective effects of *S*-2-(3-aminopropylamino)ethyl and phosphorothioic acid against radiation damage of normal tissues and a fibrosarcoma in mice. *Cancer Res* 1982; **42**: 1888–97.
18. Geng L, Hanson WR, Malkinson FD. Topical or systemic 16,16 DM prostaglandin E_2 or WR-2721 (WR-1065) protects mice from alopecia after fractionated irradiation. *Int J Radiat Oncol Biol* 1992; **61**: 533–7.
19. Sodicoff M, Conger AD, Pratt NE, Trepper P. Radioprotection by WR-2721 against long term chronic damage to the rat parotid gland. *Radiat Res* 1978; **76**: 172–9.
20. Damron TA, Spadaro JA, Tamurian RM, Damron LA. Sparing of radiation-induced damage to the physis: fractionation alone compared to amifostine pretreatment. *Int J Radiat Oncol Biol Phys* 2000; **47**: 1067–71.
21. Steel LK, Jacobs AJ, Giambarresi LI, Jackson III WE. Protection of mice against fission neutron irradiation by WR-2721 or WR-151327. *Radiat Res* 1987; **109**: 469–78.
22. Sigdestad CP, Grdina DJ, Connor AM, Hanson WR. A comparison of radioprotection from three neutron sources and Co-60, of WR-2721 and WR-151327. *Radiat Res* 1986; **106**: 224–33.
23. Spencer CM, Goa KL. Amifostine: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential as a radioprotector and cytotoxic chemoprotector. *Drugs* 1995; **50**: 1001–31.
24. Grdina DJ, Nagy B, Hill CK, Wells RL, Peraino C. The radioprotector WR-1065 reduces radiation-induced mutations at the hypoxanthine–guanine phosphoribosyl transferase locus in V79 cells. *Carcinogenesis* 1985; **6**: 929–31.
25. Grdina DJ, Nagy B, Sigdestad CP. Radioprotectors in treatment therapy to reduce risk of secondary tumor induction. *Pharmacol Ther* 1988; **37**: 21–5.
26. Nagy B, Dale PJ, Grdina DJ. Protective effects of 2-[(aminopropyl)amino]ethanethiol against bleomycine and nitrogen mustard-induced mutagenicity in V79 cells. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1475–8.
27. Nagy B, Dale PJ, Grdina DJ. Protection against *cis*-diaminodichloroplatinum cytotoxicity and mutagenicity in V79 cells by 2-[(aminopropyl)amino] ethanethiol. *Cancer Res* 1986; **45**: 1132–5.
28. Hill CK, Nagy B, Peraino C, Grdina DJ. WR-1065 is antineoplastic and antimutagenic when given during ^{60}Co-gamma ray irradiation. *Carcinogenesis* 1986; **7**: 665–8.
29. Borek C, Troll W. Modifiers of free radicals inhibit *in vitro* the oncogenic actions of X-rays, bleomycin and the tumor promotor 12-*O*-tetradecanoylphorbol-13-acetate. *Proc Natl Acad Sci USA* 1983; **80**: 1304–7.
30. Milas L, Hunter NR, Stephens LC, Peters LJ. Inhibition of radiation carcinogenesis in mice by *S*-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res* 1984; **44**: 5567–9.
31. Upton A, ed. *Radiation carcinogenesis*. New York: Elsevier 1986.
32. Weisberger AS, Heinle RW. The protective effect of cysteine on leukopenia induced by nitrogen mustard. *J Lab Clin Med* 1950; **36**: 872–6.

33. Yuhas JM. Differential protection of normal and malignant tissue against the cytotoxic effects of mechlorethamine. *Cancer Treat Rep* 1979; **63**: 971–6.
34. Yuhas JM, Culo F. Selective inhibition of nephrotoxicity of *cis*-platinum without altering its antitumor effectiveness. *Cancer Treat Rep* 1980; **64**: 57–64.
35. Jordan SW, Yuhas JM, Glick J. Modulation of *cis*-platinum renal toxicity by the radioprotective agent WR-2721. *Exp Mol Pathol* 1982; **36**: 297–305.
36. Allalunis-Turner, Siemann DW. Modification of cyclophosphamide-induced pulmonary toxicity in normal mice. *NCI Monog* 1988; **6**: 51–3.
37. Dorr RT, Lagel K, McLean S. Cardioprotection of rat myocytes with amifostine (Ethyol®) and its free thiol, WR-1065, *in vitro*. *Eur J Cancer* 1996; **32**(suppl 4): 21–5.
38. List AF, Brasfield F, Heaton R, *et al*. Stimulation of haematopoiesis by amifostine in patients with myelodysplastic syndrome. *Blood* 1997; **90**: 3364–9.
39. List AF. Haematopoietic stimulation by amifostine and sodium phenylbutyrate: what is the potential in MDS? *Leuk Res* 1998; **22**: S7–11.
40. Klimecki W, Heaton R, Glinsmann-Gibson B, List AF. Amifostine suppresses apoptosis in myelodysplastic $CD34^+$ cells and promotes progenitor growth via polyamine-like effects. *Blood* 1997; **90**(suppl I) abstr 2317.
41. Bowen DT, Denzlinger C, Brugger W, *et al*. Poor response rate to a continuous schedule of amifostine therapy for low/intermediate risk myelodysplastic patients. *Br J Haematol* 1998; **103**: 785–7.
42. Nakamura J, Shaw LM, Brown DQ. Hydrolysis of WR-2721 by mouse liver cell fractions. *Radiat Res* 1987; **109**: 143–52.
43. Mori T, Nikaido O, Sughara T. Dephosphorylation of WR-2721 with mouse tissue homogenates. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1529–31.
44. Shaw LM, Bonner HS, Brown DQ. Metabolic pathways of WR-2721 (Ethyol, amifostine) in BALB/c mouse. *Drug Metab Disp* 1994; **22**: 895–902.
45. Shaw LM, Bonner HS, Turssi A, Norfleet AL, Glover DJ. A liquid chromatographic electrochemical assay for *S*-2-(3-aminopropylamino)ethylphosphorythiate (WR-2721) in human plasma. *J Liq Chromatog* 1984; **7**: 2447–65.
46. Romanul FCA, Bannister RG. Localized areas of high alkaline phosphatase activity in endothelium of arteries. *Nature* 1962; **195**: 611–2.
47. McComb RB, Bowers GN, Posen S. *Alkaline phosphatase*. New York: Plenum Press 1979.
48. Calabro-Jones PM, Fahey R, Smoluk GD, Ward JF. Alkaline phosphatase promotes radioprotection and accumulation of WR-1065 in V79-171 cells incubated in medium containing WR-2721. *Int J Radiat Biol Relat Stud Phys Chem Med* 1985; **47**: 23–7.
49. Henthorn PS, Raducha M, Kadesch T, Weiss MJ, Harris H. Sequence and characterization of human intestinal alkaline phosphatase gene. *J Biol Chem* 1988; **263**: 12011–9.
50. Shaw LM, Turrisi AT, Glover DJ, *et al*. Human pharmacokinetics of WR-2721. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1501–4.
51. Shaw MS, Glover D, Turrisi A, *et al*. Pharmacokinetics of WR-2721. *Pharmacol Ther* 1988; **39**: 195–201.
52. Shaw LM, Bonner HS, Schuchter L, Schiller J, Lieberman R. Pharmacokinetics of amifostine: effects of dose and method of administration. *Semin Oncol* 1999; **26**(2 suppl 7): 34–6.
53. Rasay JS, Spence AM, Badger CC, Krohn KA, Vera DM, Livesey JC. Specific protection of different normal tissues. *Pharmacol Ther* 1988; **39**: 33–43.
54. Shaw L, Brown W, Schein P, *et al*. A phase I study comparing bioavailability of intravenous and subcutaneous administration of amifostine (Ethyol). *Proc Am Soc Clin Oncol* 1997; **16**: 250a (abstr 883).
55. Cassatt DR. Extended window of mucosal radioprotection with intravenous or subcutaneous amifostine in rats. *Proc Am Soc Clin Oncol* 2001; **20**: 279a (abstr 1114).
56. Dorr RT. Cytoprotective agents for anthracyclines. *Semin Oncol* 1996; **23**(suppl 8): 23–4.
57. Sigdestad CP, Treacy SH, Knapp LA, Grdina DJ. The effect of 2-[(aminoprolylamino] ethanthiol (WR-1065) in radiation induced double strand damage and repair in V79 cells. *Br J Cancer* 1987; **55**: 477–82.
58. Meyn RE, Jenkins WT. Variation in normal and tumor tissue sensitivity of mice to ionizing radiation-induced DNA strand breaks *in vivo*. *Cancer Res* 1983; **43**: 5668–73.
59. DeNeve WJ, Everett CK, Suminski JE, Valeriote FA. Influence of WR-2721 on DNA-cross linking by nitrogen mustard in normal mouse bone marrow and leukemia cells *in vivo*. *Cancer Res* 1988; **48**: 6002–5.
60. Treskes M, Nijtmans LG, Fichtinger-Schepman AM, van der Vijgh WJ. Effects of modulating agent WR-2721 and its main metabolites on the formation and stability of cisplatin–DNA adducts *in vitro* in comparison to the effects of thiosulphate and diethyldithiocarbamate. *Biochem Pharmacol* 1992; **43**: 1013–9.
61. Romakrishnan N, Catravas GN. *N*-(2-mercaptoethyl)-1,3-propanediamine (WR-1065) protects thymocytes from programmed cell death. *J Immunol* 1992; **148**: 1817–21.
62. Holwitt EA, Koda E, Swenberg CE. Enhancement of toposiomerase I-mediated unwinding of super-coiled DNA by the radioprotector WR-33278. *Radiat Res* 1990; **124**: 107–9.
63. Shigematsu N, Schwartz JL, Grdina DJ. Protection against radiation induced mutagenesis at the HPRT locus by spermine and *N*,*N*′(dithiodi-2,1-ethanediyl) bis1,3-propanediamine (WR-33278). *Mutagenesis* 1994; **9**: 355–60.
64. Purdie JW, Inhaber ER. Interaction of cultured mammalian cells with WR-2721 and its thiol, WR-1065: implications for mechanisms of radio; protection. *Int J Radiat Biol* 1983; **43**: 517–27.

65. Glover D, Negendank W, Delivoria-Papadopoulos M, Glick JH. Alterations in oxygen transport following WR-2721. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1565–8.
66. Blancher C, Harris AL. The molecular basis of hypoxia response pathway: tumour hypoxia as a therapy target. *Cancer Metast Rev* 1998; **17**: 187–94.
67. Lindegaard JC, Grau C. Has the outlook improved for amifostine as a clinical radioprotector? *Radiother Oncol* 2000; **57**: 113–8.
68. Yuhas JM, Storer JB. Differential cytoprotection of normal and malignant tissues. *J Natl Cancer Inst* 1969; **42**: 331–5.
69. Utey JF, Marlowe C, Waddell WJ. Distribution of ^{35}S-labeled WR-2721 in normal and malignant tissues of the mouse. *Radiat Res* 1976; **68**: 284–91.
70. Yuhas JM. Active versus passive absorption kinetics as the basis for selective protection of normal tissue by *S*-2-(3-aminopropylamino)-ethylphosphorothioic acid. *Cancer Res* 1980; **40**: 1519–24.
71. Rasey JS, Grunbaum Z, Krohn KA, Menard TW, Spence AM. Biodistribution of the radioprotective drug ^{35}S-labeled 3-amino-2-hydroxypropyl phosphorothioate (WR-77913). *Radiat Res* 1985; **102**: 130–7.
72. Mendiondo OA, Grigsby PW, Beach JL. Radioprotection combined with hypoxic sensitization during radiotherapy of a solid murine tumor. *Radiology* 1983; **148**: 291–3.
73. McChesney SL, Gillette EL, Dewhirst MW, Withrow SJ. Influence of WR-2721 on radiation response of canine soft tissue sarcomas. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1957–63.
74. Besa PC, Hunter NR, Milas L. Improvement in radiotherapy for a murine sarcoma by indomethacin plus WR-2721. *Radiat Res* 1993; **135**: 93–7.
75. Yuhas JM, Spellman JM, Jordan SW, Pardini MC, Afzal SM, Culo F. Treatment of tumours with the combination of WR-2721 and *cis*-dichlorodiammine-platinum (II) or cyclophosphamide. *Br J Cancer* 1980; **42**: 574–85.
76. Valeriote F, Grates HE. Dose and interval relationship for the interaction of WR-2721 and mustard with normal and malignant cells. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1561–4.
77. Douay L, Hu C, Giarratana MC, *et al*. Amifostine improves the antileukemic therapeutic index of mafosfamide: implications for bone marrow purging. *Blood* 1995; **86**: 2849–55.
78. Treskes M, Boven E, van de Loosdrecht AA, *et al*. Effects of the modulating agent WR-2721 on myelotoxicity and antitumour activity in carboplatin-treated mice. *Eur J Cancer* 1994; **30A**: 183–7.
79. van Laar JA, van der Wilt CL, Treskes M, van der Vijgh WJ, Peters GJ. Effect of WR-2721 on the toxicity and antitumor activity of the combination of carboplatin and 5-fluorouracil. *Cancer Chemother Pharmacol* 1992; **31**: 97–102.
80. Paine GD, Taylor CW, Lopez MH, Johnson CS, Capizzi RL. Effects of amifostine and paclitaxel on growth of human ovarian carcinoma xenografts in the severe combined immune-deficient mouse: preliminary results. *Semin Oncol* 1996; **23**(4 suppl 8): 35–9.
81. Fichtner I, Lemm M, Becker M, Berthold F. Effects of amifostine (WR-2721, ethyol) on tumor growth and pharmacology of cytotoxic drugs in human xenotransplanted neuroblastomas. *Anti-Cancer Drugs* 1997; **8**: 174–81.
82. Dunn TA, Schmoll HJ, Grunwald V, Bokemeyer C, Casper J. Amifostine does not alter the antitumor activity of cisplatin in a pre-clinical model of testicular cancer. *Anti-Cancer Drugs* 1996; **7**: 795–9.
83. Bergstrom P, Johnsson A, Bergenheim T, Henriksson R. Effects of amifostine on cisplatin induced DNA adduct formation and toxicity in malignant glioma and normal tissues in rat. *J Neurooncol* 1999; **42**: 13–21.
84. Williams MV, Rojas A, Denekamp J. Tumor sensitization and protection: influence of stromal injury on estimates of dose modification. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1545–9.
85. Rojas A, Stewart FA, Soranson JA, Smith KA, Denekamp J. Fractionation studies with WR-2721: normal tissues and tumour. *Radiother Oncol* 1986; **6**: 51–60.
86. Lowy RO, Baker DG. Effect of radioprotective drugs on the therapeutic ratio for a mouse tumor system. *Acta Radiol Ther Phys Biol* 1973; **12**: 425–33.
87. Clement JJ, Johnson RK. Influence of WR 2721 on the efficacy of radiotherapy and chemotherapy of murine tumors. *Int J Radiat Oncol Biol Phys* 1982; **8**: 539–42.
88. Moulder JE, Lo PS, Fischer JJ. Effect of the radioprotective drugs MEA, DMSO, and WR-2721 on tumor control and skin tolerance in the rat. *Cancer Treat Rep* 1977; **61**: 825–33.
89. Wist EA. Effect of the radioprotector WR 2721 on the response of metastatic Lewis lung carcinoma colonies to alkylating agents. *Acta Radiol Oncol* 1985; **24**: 259–62.
90. Penhaligon M. Radioprotection of mouse skin vasculature and the RIF-1 fibrosarcoma by WR-2721. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1541–4.
91. Cappizzi RL. Amifostine: the preclinical basis for broad spectrum selective cytoprotection of normal tissues from cytotoxic therapies. *Semin Oncol* 1996; **23**: 2–17.
92. Grdina DJ. Amifostine: mechanisms of action. Presented at *XVI Meeting of the European Association of Cancer Research*, Chalkidiki, Greece 2000.
93. Murray D, Alyschuler EM, Kerr MS, Vanankeren SC, Meyn RE, Milas L. Radioprotection of cultured Chinese hamster ovary cells by WR-255591. *Int J Radiat Biol* 1988; **54**: 269–83.
94. Alberts DS, Speicher LA, Krutzsch M, *et al*. WR-1065, the active metabolite of amifostine (Ethyol), does not inhibit the cytotoxic effects of a broad range of standard anticancer drugs against human ovarian and breast cancer cells. *Eur J Cancer* 1996; **32A**(suppl 4): 17–20.
95. Ng TY, Ngan HY, Cheng DK, Wong LC. The effects of amifostine on the *in vitro* cytotoxicity of chemotherapeutic agents in three epithelial ovarian carcinoma cell lines. *Gynecol Oncol* 1999; **75**: 194–7.

96. Kataoka Y, Murley JS, Patel R, Grdina DJ. Cytoprotection by WR-1065, the active form of amifostine, is independent of p53 status in human malignant glioma cell lines. *Int J Radiat Biol* 2000; **76**: 633–9.
97. Liu T, Liu Y, He S, Zhang Z, Kligerman MM. Use of radiation with or without WR-2721 in advanced rectal cancer. *Cancer* 1992; **69**: 2820–5.
98. Kligerman MM, Liu T, Liu Y, Scheffler B, He S, Zhang Z. Interim analysis of a randomized trial of radiation therapy of rectal cancer with/without WR-2721. *Int J Radiat Oncol Biol Phys* 1992; **22**: 799–802.
99. Mitsuhashi N, Takahashi I, Takahashi M, Hayakawa K, Niibe H. Clinical study of radioprotective effects of amifostine (YM-08310, WR-2721) on long-term outcome for patients with cervical cancer. *Int J Radiat Oncol Biol Phys* 1993; **26**: 407–11.
100. Coia L, Krigel R Hanks G. A phase I study of WR-2721 in combination with total body irradiation (TBI) in patients with refractory lymphoid malignancies. *Int J Radiat Oncol Biol Phys* 1992; **22**: 791–4.
101. Buntzel J, Kuttnet K, Frohlich D, Glatzel M. Selective cytoprotection with amifostine in concurrent radiochemotherapy for head and neck cancer. *Ann Oncol* 1998; **9**: 505–9.
102. Buntzel J, Schuth J, Kuttner K, Glatzel M. Radiochemotherapy with amifostine cytoprotection for head and neck cancer. *Support Care Cancer* 1998; **6**: 155–60.
103. Peters K, Mucke R, Hamann D, Ziegler PG, Fietkau R. Supportive use of amifostine in patients with head and neck tumors undergoing radio-chemotherapy. Is it possible to limit the duration of the application of amifostine? *Strahlenther Onkol* 1999; **175**(suppl 4): 23–6.
104. Koukourakis MI, Kyrias G, Kakolyris S, *et al*. Subcutaneous administration of amifostine during fractionated radiotherapy: a randomized phase II study. *J Clin Oncol* 2000; **18**: 2226–33.
105. Brizel DM, Wasserman TH, Henke M, *et al*. Phase III randomized trial of amifostine as a radioprotector in head and neck cancer. *J Clin Oncol* 2000; **18**: 3339–45.
106. Tannehill SP, Mehta MP, Larson M, *et al*. Effect of amifostine on toxicities associated with sequential chemotherapy and radiation therapy for unresectable non-small-cell lung cancer: results of a phase II trial. *J Clin Oncol* 1997; **15**: 2850–7.
107. Antonadou D. Expanded application for Ethyol (amifostine): the role of Ethyol (amifostine) as a radioprotector in lung cancer, in Radioprotection: a new option for the treatment of head and neck cancers. Presented at *Ethyol Satellite Symposium (ECCO10)*, Vienna, Austria 1999.
108. Komaki R, Lee JS, Kaplan B, *et al*. Randomized phase III study of chemoradiation ± amifostine in patients with inoperable stage II–III non-small cell lung cancer (NSCLC). *Proc Am Soc Clin Oncol* 2001; **20**: 325a (abstr 1298).
109. Glover D, Glick JH, Weiler C, Fox K, Guerry D. WR-2721 and high dose cisplatin: an active combination in the treatment of metastatic melanoma. *J Clin Oncol* 1987; **5**: 574–8.
110. Arvil MF, Ortoli JC, Fortier-Beauliew M, *et al*. High dose cisplatin and WR-2721 in metastatic melanoma. *Proc Am Soc Clin Oncol* 1992; **11**: 344.
111. Korst AE, van der Sterre ML, Gall HE, Fichtinger-Schepman AM, Vermorken JB, van der Vijgh WJ. Influence of amifostine on the pharmacokinetics of cisplatin in cancer patients. *Clin Cancer Res* 1998; **4**: 331.
112. Korst AE, van der Sterre ML, Eeltink CM, Fichtinger-Schepman AM, Vermorken JB, van der Vijgh WJ. Pharmacokinetics of carboplatin with and without amifostine in patients with solid tumors. *Clin Cancer Res* 1997; **3**: 697–703.
113. Kemp G, Rose P, Lurain J, *et al*. Amifostine pretreatment for protection against cyclophosphamide-induced and cisplatin-induced toxicities: results of a randomized controlled trial in patients with advanced ovarian cancer. *J Clin Oncol* 1996; **14**: 2101–12.
114. Gridelli C, Cigolari S, Maiorino A, *et al*. Amifostine plus cisplatin plus vinorelbine in the treatment of advanced non-small cell lung cancer: a multicenter phase II study. *Lung Cancer* 2000; **28**: 237–44.
115. Schiller JH, Storer B, Berlin J, *et al*. Amifostine, cisplatin, and vinblastine in metastatic non-small-cell lung cancer: a report of high response rates and prolonged survival. *J Clin Oncol* 1996; **14**: 1913–21.
116. Gelmon K, Eisenhauer E, Bryce C, *et al*. Randomized phase II study of high-dose paclitaxel with or without amifostine in patients with metastatic breast cancer. *J Clin Oncol* 1999; **17**: 3038–47.
117. Planting AS, Catimel G, de Mulder PH, *et al*. Randomized study of a short course of weekly cisplatin with or without amifostine in advanced head and neck cancer. EORTC Head and Neck Cooperative Group. *Ann Oncol* 1999; **10**: 693–700.
118. Budd GT, Ganapathi R, Adelstein DJ, *et al*. Randomized trial of carboplatin plus amifostine versus carboplatin alone in patients with advanced solid tumors. *Cancer* 1997; **80**: 1134–40.
119. Betticher DC, Anderson H, Ranson M, Meely K, Oster W, Thatcher N. Carboplatin combined with amifostine, a bone marrow protectant, in the treatment of non-small-cell lung cancer: a randomised phase II study. *Br J Cancer* 1995; **72**: 1551–5.
120. Johnson PWM, Muers MF, Peake MD, *et al*. A randomized trial of amifostine as a cytoprotective agent in patients receiving chemotherapy for small cell lung cancer. *Br J Cancer* 2001; **84**: 19–24.
121. Fahlke J, Ridwelski K, Lippert H. High-dose therapy with combined 5-fluorouracil and folinic acid with and without amifostine in the treatment of patients with metastatic colorectal carcinoma. *Int J Colorectal Dis* 1999; **14**: 128–30.
122. Aviles A, Diaz-Maqueo JC, Talavera A, Garcia EL, Guzman R, Nambo MJ. Bone marrow protection with amifostine in the treatment of high-risk malignant lymphoma. *Eur J Cancer* 1997; **33**: 1323–5.
123. Koukourakis MI, Giatromanolaki A, Sivridis E, Fezoulidis I. Cancer vascularization: implications in radiotherapy? *Int J Radiat Oncol Biol Phys* 2000; **48**: 545–53.

124. Giatromanolaki A, Koukourakis MI, Sivridis E, O'Byrne K, Gatter KC, Harris AL. 'Invading edge vs. inner' (edvin) patterns of vascularisation: an interplay between angiogenic and vascular survival factors defines clinical behaviour of non small cell lung cancer. *J Pathol* 2000; **192**: 140–9.
125. Koukourakis MI, Giatromanolaki A, Thorpe PE, *et al*. Prognostic and pathologic comparison of VEGF/KDR activated microvessel density (aMVD) vs. CD31 standard MVD (sMVD) in non-small cell lung cancer. *Cancer Res* 2000; **60**: 3088–95.
126. Cosgrove DO, Kedar RP, Vagios E, *et al*. Color doppler in differential diagnosis of breast masses. *Radiology* 1993; **189**: 99–104.
127. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res* 18989; **49**: 6465–9.
128. Yamagata M, Hasuda K, Stamato T, Tannock IF. The contribution of lactic acid to acidification of tumours: studies of variant cells lacking lactate dehydrogenase. *Br J Cancer* 1998; **77**: 1726–31.
129. Koukourakis MI, Giatromanolaki A, Kouroussis C, *et al*. Hypofractionated and accelerated radiotherapy and cytoprotection (HypoARC). A short, safe and effective post-operative regimen for high-risk breast cancer patients. *Int J Radiat Oncol Biol Phys* 2002; **52**: 144–55.
130. Parkins CS, Strattford MR, Dennis MF, Stubbs M, Chaplin DJ. The relationship between extracellular lactate and tumour pH in a murine tumour model of ischaemia-reperfusion. *Br J Cancer* 1997; **75**: 319–23.
131. Wykoff CC, Beasley NJP, Watson PH, *et al*. Hypoxia inducible regulation of tumor associated carbonic anhydrases. *Cancer Res* 2000; **60**: 7075–83.
132. Treskes M, Nijtmans L, Fichtinger-Schepman AM, van der Vijgh WJ. Cytostatic activity of cisplatin in the presence of WR-2721 and its thiol metabolite WR-1065 in OVCAR-3 human ovarian cancer cells as compared to V79 fibroblasts. *Anticancer Res* 1992; **12**: 2261–5.
133. Kligerman MM, Glover DJ, Turrisi A, *et al*. Toxicity of WR-2721 administered in single and multiple doses. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1773–6.
134. Kligerman MM, Turrisi AT, Urtasun RC, *et al*. Final report on phase I trial of WR-2721 before protracted fractionated radiation therapy. *Int J Radiat Oncol Biol Phys* 1988; **14**: 1119–22.
135. Glick JH, Glover D, Weiler C, Norfleet L, Yuhas J, Kligerman MM. Phase I controlled trials of WR-2721 and cyclophosphamide. *Int J Radiat Oncol Biol Phys* 1984; **10**: 1777–80.
136. Constine LS, Zagars G, Rubin P, Kligerman M. Protection by WR-2721 of human bone marrow function following irradiation. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1505–8.
137. Koukourakis M, Stefanaki I, Giatromanolaki A, *et al*. Fractionated carboplatin radiosensitization. A phase I dose escalation study. *Am J Clin Oncol* 1998; **21**: 595–601.
138. Koukourakis MI, Giatromanolaki A, Kakolyris S, *et al*. A phase I/II dose escalation study of docetaxel and carboplatin combination supported with amifostine and GM-CSF, in patients with incomplete response following docetaxel chemo-radiotherapy. Additional chemotherapy enhances regression of residual cancer. *Med Oncol* 2000; **17**: 135–43.
139. Ryan SV, Carrithers SL, Parkinson SJ, *et al*. Hypotensive mechanism of amifostine. *J Clin Pharmacol* 1996; **36**: 365–73.
140. Shaw PJ, Bleakley M. Systemic inflammatory response syndrome associated with amifostine. *Med Pediatr Oncol* 2000; **34**: 309–10.
141. Wagner W, Radmard A, Schonekaes KG. A new administration schedule for amifostine as a radioprotector in cancer therapy. *Anticancer Res* 1999; **19**: 2281–3.
142. Ben-Josef E, Mesina J, Shaw LM, Bonner HS, Shamsa F, Porter AT. Topical application of WR-2721 achieves high concentrations in the rectal wall. *Radiat Res* 1995; **143**: 107–10.
143. Montana GS, Anscher MS, Mansbach CM, *et al*. Topical application of WR-2721 to prevent radiation-induced proctosigmoiditis. A phase I/II trial. *Cancer* 1992; **69**: 2826–30.
144. McDonald S, Meyerowitz C, Smudzin T, Rubin P. Preliminary results of a pilot study using WR-2721 before fractionated irradiation of the head and neck to reduce salivary gland dysfunction. *Int J Radiat Oncol Biol Phys* 1994; **29**: 747–54.
145. Schonekas KG, Wagner W, Prott FJ. Amifostine–a radioprotector in locally advanced head and neck tumors. *Strahlenther Onkol* 1999; **175**(suppl 4): 27–9.
146. Wagner W, Prott FJ, Schonekas KG. Amifostine: a radioprotector in locally advanced head and neck tumors. *Oncol Rep* 1998; **5**: 1255–7.
147. Trog D, Bank P, Wendt TG, Koscielny S, Beleites E. Daily amifostine given concomitantly to chemo-radiation in head and neck cancer. A pilot study. *Strahlenther Onkol* 1999; **175**: 444–9.
148. Koukourakis MI. Subcutaneous injection of Ethyol: the emerging role of Ethyol. *Advances in Radiation Oncology: Proceedings of the Ethyol Symposium, ESTRO 18th Congress*, Istanbul, Turkey 2000.
149. Ramnath N, LoRusso P, Simon M, Martino S. Phase II evaluation of cisplatin and WR-2721 for refractory metastatic breast cancer. *Am J Clin Oncol* 1997; **20**: 368–72.
150. Rubin JS, Wadler S, Beitler JJ, *et al*. Audiological findings in a phase I protocol investigating the effect of WR 2721, high-dose cisplatin and radiation therapy in patients with locally advanced cervical carcinoma. *J Laryngol Otol* 1995; **109**: 744–77.
151. Mohr P, Makki A, Breitbart E, Schadendorf D. Combined treatment of stage IV melanoma patients with amifostine and fotemustine—a pilot study. *Melanoma Res* 1998; **8**: 166–9.

152. Kusenda Z, Kerger J, Awada A, *et al*. Mitomycin C and vinblastine in combination with amifostine in metastatic breast cancer. A feasibility study of the EORTC–Investigational Drug Branch for Breast Cancer (IDBBC). *Support Care Cancer* 1997; **5**: 414–6.
153. Glover D, Glick JH, Weiler C, Hurowitz S, Kligerman MM. WR-2721 protects against the hematologic toxicity of cyclophosphamide: a controlled phase II trial. *J Clin Oncol* 1986; **4**: 584–8.
154. De Souza CA, Santini G, Marino G, *et al*. Amifostine (WR-2721), a cytoprotective agent during high-dose cyclophosphamide treatment of non-Hodgkin's lymphomas: a phase II study. *Braz J Med Biol Res* 2000; **33**: 791–8.
155. Capelli D, Santini G, De Souza C, *et al*. Amifostine can reduce mucosal damage after high-dose melphalan conditioning for peripheral blood progenitor cell autotransplant: a retrospective study. *Br J Haematol* 2000; **110**: 300–7.
156. Chauncey TR, Gooley TA, Lloid ME, *et al*. Pilot trial of cytoprotection with amifostine given with high-dose chemotherapy and autologous peripheral blood stem cell transplantation. *Am J Clin Oncol* 2000; **23**: 406–11.
157. Shpall EJ, Stemmer SM, Hami L, *et al*. Amifostine (WR-2721) shortens the engraftment period of 4-hydroperoxycyclophosphamide-purged bone marrow in breast cancer patients receiving high-dose chemotherapy with autologous bone marrow support. *Blood* 1994; **83**: 3132–7.
158. Bohuslavizki KH, Klutmann S, Brenner W, Mester J, Henze E, Clausen M. Salivary gland protection by amifostine in high-dose radioiodine treatment: results of a double-blind placebo-controlled study. *J Clin Oncol* 1998; **16**: 3542–9.
159. Eshwege F. The selective cytoprotection of Ethyol (amifostine) in head and neck cancers: 18 months follow-up data, in Radioprotection: a new option for the treatment of head and neck cancers. Presented at *Ethyol Satellite Symposium* (*ECCO10)*, Vienna, Austria 1999.
160. Mackoviak JI. Ethyol (amifostine) a new standard for head and neck cancer patients: patients' perspectives, in Radioprotection: a new option for the treatment of head and neck cancers. Presented at *Ethyol Satellite Symposium* (*ECCO10)*, Vienna, Austria 1999.
161. Rudat V, Meyer J, Momm F, *et al*. A. Protective effect of amifostine on dental health after radiotherapy of the head and neck. *Int J Radiat Oncol Biol Phys* 2000; **48**: 1339–43.
162. Bourhis J, De Crevoisier R, Abdulkarim B, *et al*. A randomized study of very accelerated radiotherapy with and without amifostine in head and neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 2000; **46**: 1105–8.
163. Abitbol A, Lewin A, Rodrigues MA, *et al*. Sequential trial of concurrent hyperfractionated radiation therapy and 5-fluorouracil, cisplatin and paclitaxel with and without amifostine in patients with locally advanced head and neck squamous cell carcinoma. *Proc Am Soc Clin Oncol* 2001; **20**: 228a (abstr 909).
164. Antonadou D, Synodinou M, Boufi M, *et al*. Amifostine reduces acute toxicity during radiochemotherapy in patients with localized advanced stage NSCLC. *Proc Am Soc Oncol* 2000; **19**: 501a (abstr 1960).
165. Hartmann JT, Knop S, Fels LM, *et al*. The use of reduced doses of amifostine to ameliorate nephrotoxicity of cisplatin/ifosfamide-based chemotherapy in patients with solid tumors. *Anti-Cancer Drugs* 2000; **11**: 1–6.
166. DiPaola RS, Rodriguez R, Goodin S, *et al*. Amifostine and dose-intense paclitaxel in patients with advanced malignancies. *Cancer Ther* 1998; **1**: 11–7.
167. Ringel I, Horwitz SB. Studies with RP-56976 (taxotere): a semi-synthetic analogue of Taxol. *J Natl Cancer Inst* 1991; **83**: 288–91.
168. Taylor CW, Wang LM, List AF, *et al*. Amifostine protects normal tissues from paclitaxel toxicity while cytotoxicity against tumour cells is maintained. *Eur J Cancer* 1997; **33**: 1693–8.
169. Haldar S, Chintapalli J, Croce CM. Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. *Cancer Res* 1996; **56**: 1253–5.
170. Withers HR, Taylor JMG, Maciejewski B. The hazards of accelerated tumor clonogene repopulation during radiotherapy. *Acta Oncol* 1988; **27**: 131–46.
171. van der Wilt CL, van Laar JA, Gyergyay F, Smid K, Peters GJ. Biochemical modification of the toxicity and the anti-tumour effect of 5-fluorouracil and *cis*-platinum by WR-2721 in mice. *Eur J Cancer* 1992; **28A**: 2017–24.
172. Treskes M, Boven E, Holwerda U, Pinedo HM, van der Vijgh WJ. Time dependence of the selective modulation of cisplatin-induced nephrotoxicity by WR-2721 in the mouse. *Cancer Res* 1992; **52**: 2257–60.
173. Gridelli C, Guida C, Barletta E, *et al*. Thoracic radiotherapy and daily vinorelbine as a radiosensitizer in locally advanced non small cell lung cancer: a phase I study. *Lung Cancer* 2000; **29**: 131–7.
174. Adamson PC, Balis FM, Belasco JE, *et al*. A phase I trial of amifostine (WR-2721) and melphalan in children with refractory cancer. *Cancer Res* 1995; **55**: 4069–72.
175. Budd GT, Ganapathi R, Bauer L, *et al*. Phase I study of WR-2721 and carboplatin. *Eur J Cancer* 1993; **29A**: 1122–7.
176. Kotsakis A, Sarra E, Peraki M, *et al*. Docetaxel-induced lymphopenia in patients with solid tumors. A prospective phenotypic analysis. *Cancer* 2000; **89**: 1380–6.
177. Koukourakis MI, Giatromanolaki A, Schiza S, Kakolyris S, Georgoulias V. Concurrent twice-a-week docetaxel and radiotherapy. A dose escalation trial with immunological toxicity evaluation. *Int J Radiat Oncol Biol Phys* 1999; **43**: 107–14.
178. Talamonti MS, Catalano PJ, Vaughn DJ, *et al*. Eastern Cooperative Oncology Group phase I trial of protracted venous infusion of fluorouracil plus weekly gemcitabine with concurrent radiation therapy in patients with locally advanced pancreas cancer: a regimen with unexpected early toxicity. *J Clin Oncol* 2000; **18**: 3384–9.

179. Ohe Y, Yamamoto S, Suzuki K, *et al*. Risk factors of treatment-related death in chemotherapy and thoracic radiotherapy for lung cancer. *Eur J Cancer* 2001; **37**: 54–63.
180. Koukourakis MI, Bizakis JG, Skoulakis CE, *et al*. Combined irinotecan, docetaxel and conventionally fractionated radiotherapy in locally advanced head and neck cancer. A phase I dose escalation study. *Anticancer Res* 1999; **19**: 2305–9.
181. Graham MV, Jahanzeb M, Dresler CM, Cooper JD, Emami B, Mortimer JE. Results of a trial with topotecan dose escalation and concurrent thoracic radiation therapy for locally advanced, inoperable nonsmall cell lung cancer. *Int J Radiat Oncol Biol Phys* 1996; **36**: 1215–20.
182. Koukourakis MI, Bahlitzanakis N, Froudarakis M, *et al*. Concurrent conventionally factionated radiotherapy and weekly docetaxel in the treatment of stage IIIb non-small cell lung carcinoma. *Br J Cancer* 1999; **80**: 1792–6.
183. Reckzeh B, Merte H, Pfluger K, Pfab R, Wolf M, Havemann K. Severe lymphocytopenia and interstitial pneumonia in patients treated with paclitaxel and simultaneous radiotherapy for non-small cell lung cancer. *J Clin Oncol* 1996; **14**: 1071–6.
184. Koukourakis M, Koukourakis C, Kamilaki M, *et al*. Weekly docetaxel and concomitant boost radiotherapy for non-small-cell lung cancer. A phase I/II dose escalation trial. *Eur J Cancer* 1998; **34**: 838–44.
185. Tamura K, Takada M, Kawase I, *et al*. Enhancement of tumor radio-response by irinotecan in human lung tumor xenografts. *Jpn J Cancer Res* 1997; **88**: 218–23.
186. Koukourakis MI, Koukourakis S, Giatromanolaki A, *et al*. Liposomal doxorubicin and conventionally fractionated radiotherapy in the treatment of locally advanced non-small cell lung cancer and head and neck cancer. *J Clin Oncol* 1999; **17**: 3512–21.
187. Milas L, Fuji T, Hunter N, *et al*. Enhancement of tumor radioresponse *in vivo* by gemcitabine. *Can Res* 1999; **59**: 107–14.
188. Eisbruch A, Schewach DS, Bradford CR, *et al*. Radiation concurrent with gemcitabine for locally advanced head and neck cancer: a phase I trial and intracellular drug incorporation study. *J Clin Oncol* 2001; **19**: 792–9.
189. Colbern G, Steinmetz K, Musterer R, *et al*. Amifostine reduces severity and incidence of Doxil-induced palmar/plantar erythrodysesthesia in rats but does not reduce Doxil antitumor efficacy or alter pharmacokinetics in mice. *Proc Am Soc Clin Oncol* 2000; **19**: abstract 815.
190. Lyass O, Lotem M, Edelmann E, *et al*. Protective effect of amifostine on Doxil/Caelyx-induced palmar–plantar erythrodysesthesia in patients with advanced cancer. *Proc Am Soc Clin Oncol* 2001; **20**: 996 (abstr 2148).
191. Fletcher GH. Hypofractionation: lessons from complications. *Radiother Oncol* 1991; **20**: 10–5.
192. Giatromanolaki A, Koukourakis MI, Georgoulias V, Gatter KC, Harris AL, Fountzilas G. Angiogenesis vs. response after combined chemo-radiotherapy of squamous cell head and neck cancer. *Int J Cancer* 1999; **80**: 810–7.
193. Saunders MI, Dische S, Barrett A, Parmar MK, Harvey A, Gibson D. Randomized multicenter trials of CHART vs. conventional radiotherapy in head-neck and non-small cell lung cancer. An interim report. *Br J Cancer* 1996; **73**: 1455–62.
194. Koukourakis MI, Yannakakis D. Hypofractionated and accelerated radiotherapy with Ethyol® cytoprotection (HypoARC) for locally advanced breast cancer. *Proc Am Soc Clin Oncol* 2001; **21**: 2973–8.
195. Coia L, Won M, Lanciano R, Marcial VA, Martz K, Hanks G. The patterns of care outcome study for cancer of the uterine cervix. Results of the Second National Practice Survey. *Cancer* 1990; **66**: 2451–6.
196. Perez CA, Breaux S, Madoc-Jones H, *et al*. Radiation therapy alone in the treatment of carcinoma of the uterine cervix. II. Analysis of complications. *Cancer* 1984; **54**: 235–46.
197. Scalliet P, Gerbaulet A, Dubray B. HDR versus LDR gynecological brachytherapy revisited. *Radiother Oncol* 1993; **28**: 118–26.
198. Gaspar LE, Winter K, Kocha WI, Coia LR, Herskovic A, Graham M. A phase I/II study of external beam radiation, brachytherapy, and concurrent chemotherapy for patients with localized carcinoma of the esophagus (Radiation Therapy Oncology Group Study 9207): final report. *Cancer* 2000; **88**: 988–95.
199. Azinovic I, Calvo FA, Puebla F, Aristu J, Martinez-Monge R. Long-term normal tissue effects of intraoperative electron radiation therapy (IOERT): late sequelae, tumor recurrence, and second malignancies. *Int J Radiat Oncol Biol Phys* 2001; **49**: 597–604.
200. Hensley ML, Schuchter LM, Lindley C, *et al*. American Society of Clinical Oncology clinical practice guidelines for the use of chemotherapy and radiotherapy protectants. *J Clin Oncol* 1999; **17**: 3333–55.
201. Yuhas JM. Differential protection of normal and malignat tissues against the cytotoxic effects of mechlorethamine. *Cancer Treat Rep* 1979; **63**: 971–6.
202. Twentyman PR. Modification of tumour and host response to cyclophosphamide by misonidazole and by WR2721. *Br J Cancer* 1981; **43**: 745–55.
203. Twentyman PR. Modification by WR2721 of the response to chemotherapy of tumours and normal tissues in the mouse. *Br J Cancer* 1983; **47**: 57–63.
204. Milas L, Hunter N, Reid BO, Thames Jr HD. Protective effects of *S*-2-(3-aminopropylamino) ethylphosphorothioic acid against radiation damage of normal tissues and fibrosarcoma in mice. *Cancer Res* 1982; **42**: 1888–97.
205. Milas L, Hunter N, Ito H, Peters LJ. Effect of tumor type, size and endpoint on tumor radioprotection by WR-2721. *Int J Radiat Oncol Biol Phys* 1984; **10**: 41–8.

206. Milas L, McBride WH, Hunter N, Ito H. Protection by *S*-2-(3-aminopropylamino) ethylphosphorothioic acid against radiation and cyclophosphamide-induced attenuation in antitumor resistance. *Cancer Res* 1984; **44**: 2382–6.
207. Grdina DJ, Hunter N, Kataoka Y, Murley JS, Milas L. Chemopreventive doses of amifostine confer no cytoprotection in tumor nodules growing in the lungs of mice treated with cyclophosphamide. *Semin Oncol* 1999; **26**(2 suppl 7): 22–7.
208. Treskes M, Nijtmans L, Fichtinger-Schepman AM, van der Vijgh WJ. Cytostatic activity of cisplatin in the presence of WR2721 aminothiol metabolite WR1065 in OVCAR-3 human ovarian cancer as compared to V79 fibroblasts. *Anticancer Res* 1992; **12**: 2261–5.

(Received 12 December 2001; accepted 18 December 2001)