Pergamon

European Journal of Cancer Vol. 31A, Suppl. 1, pp. S8–S13, 1995

0959–8049/95 $9.50+0.00

0959-8049(95)00144-1

Protection of Normal Tissue from the Cytotoxic Effects of Chemotherapy and Radiation by Amifostine: Clinical Experiences

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The clinical trials described in this review indicate that amifostine protects normal tissues from the toxicities of various antitumour regimens. In a controlled trial, pretreatment with amifostine reduced the frequency of cyclophosphamide-induced neutropenia. Comparisons of the effects of cisplatin with and without pretreatment with amifostine indicated that patients pretreated with amifostine had fewer nephrotoxic and neurotoxic effects and tolerated higher doses of cisplatin before the onset of neurotoxic effects. In a randomised trial, patients who received amifostine prior to treatment with cyclophosphamide and cisplatin discontinued chemotherapy because of haemato, nephro- or ototoxicity less frequently than patients treated with cyclophosphamide and cisplatin alone. Tumour response rates and survival were comparable in both groups indicating that amifostine selectively protects only normal tissues. A regimen of amifostine plus cisplatin and vinblastine followed by amifostine plus radiation in patients with non-small cell lung cancer revealed a 73% response to treatment. Other studies showed that amifostine protected against late radiation toxicity to pelvic organs without interfering with the antitumour effect of radiotherapy, and decreased the haematological and mucosal toxicity of combined treatment with cisplatin and radiation therapy.

Key words: amifostine, chemotherapy, radiation, toxicity
Eur J Cancer, Vol. 31A, Suppl. 1, S8–S13, 1995

INTRODUCTION

THE RESULTS of extensive, worldwide preclinical studies of amifostine have laid a strong foundation for clinical trials of amifostine as a cytoprotector. Initial clinical trials of amifostine have focused on the haematotoxicity of cyclophosphamide, carboplatin, and cisplatin; the haematotoxicity and mucosal toxicity of radiation therapy; and the nephro-, neuro-, and ototoxicity of cisplatin.

THE EFFECT OF AMIFOSTINE ON CYCLOPHOSPHAMIDE-INDUCED HAEMATOTOXICITY

The initial clinical development of amifostine took place at the University of Pennsylvania in Philadelphia. One of the earliest clinical trials assessed the capacity of amifostine to ameliorate the haematotoxic effects of cyclophosphamide [1]. In this study, 21 patients with diverse neoplasms were treated initially with 1500 mg/m^2 cyclophosphamide alone as a 1 h infusion. 4 weeks later, after full haematological recovery, patients received an infusion of 740 mg/m^2 amifostine over 15 min, followed 15 min later by administration of 1500 mg/m^2 cyclophosphamide over 1 h. Each patient served as his or her own control. Haematological profiles including granulocyte counts were monitored three times weekly until full haematological recovery was observed.

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When patients were pretreated with amifostine, there were reductions in both the depth of the nadir and in the duration of neutropenia following cyclophosphamide therapy (Figure 1). When cyclophosphamide was given alone, the median granulocyte count nadir was 400/mm^3, whereas when amifostine pretreatment was given, the nadir increased to 1167/mm^3; the difference between the groups was significant ($P < 0.001$). After treatment with cyclophosphamide alone, 67% of the patients had life-threatening neutropenia with a granulocyte

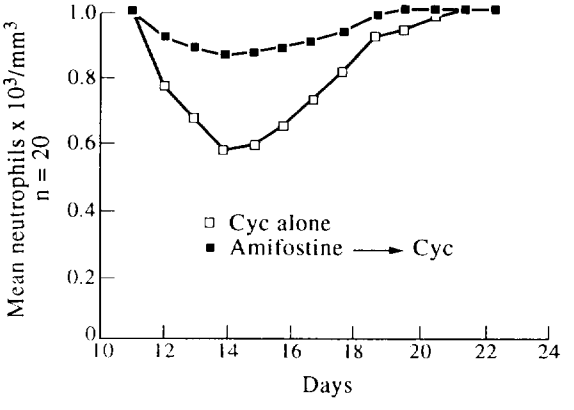

Figure 1. Cyclophosphamide-induced neutropenia in 20 patients following treatment with 1500 mg/m^2 cyclophosphamide alone or 1500 mg/m^2 cyclophosphamide preceded by 740 mg/m^2 amifostine (data on file at U.S. Bioscience).

count less than 500/mm^3, but when amifostine was given before cyclophosphamide, only 24% of the patients were so affected ($P = 0.004$). Similarly, 86% of the patients had a granulocyte count less than 1000/mm^3 following treatment with cyclophosphamide alone, compared with only 38% of the patients following treatment with cyclophosphamide preceded by amifostine ($P = 0.002$). The median number of days when the granulocyte count was less than 1000/mm^3 was reduced from 5 following treatment with cyclophosphamide alone to less than 1 following cyclophosphamide with amifostine pretreatment ($P < 0.001$). Furthermore, when cyclophosphamide alone was given, 14.3% of the patients required hospitalisation for neutropenia-related fever, but when amifostine was administered prior to cyclophosphamide, none of the patients required hospitalisation for this reason. Although cyclophosphamide does not have a profound effect on the platelets, thrombocytopenia was noted following treatment with cyclophosphamide alone in 9.5% of patients. In contrast, thrombocytopenia was not evident in any of the patients when amifostine was administered before cyclophosphamide [1]. These differences were not statistically significant.

THE EFFECT OF AMIFOSTINE ON CISPLATIN-INDUCED NEPHROTOXICITY AND NEUROTOXICITY

The nephrotoxicity associated with cisplatin therapy is well recognised and has been a dose-limiting side effect of cisplatin therapy. Glover and colleagues [2] conducted trials to explore the effects of amifostine pretreatment on cisplatin toxicity in patients with metastatic melanoma. In these studies, patients initially underwent a regimen of appropriate hydration and osmotic diuresis. They then received a 15 min infusion of amifostine, followed 15 min later by 12.5 g mannitol immediately before administration of 120–150 mg/m^2 cisplatin over 30 min. This regimen was repeated every 3–4 weeks. A similar regimen was utilised by Avril and associates at the Institute Gustave Roussy, France (data on file at U.S. Bioscience).

Table 1 compares the frequency and degree of nephrotoxicity observed following 148 courses of amifostine plus 120 mg/m^2 cisplatin with those observed following 147 courses of 100 mg/m^2 cisplatin in another previously published study of patients with metastatic melanoma with similar patient characteristics and that used similar hydration regimens with mannitol diuresis. When 100 mg/m^2 cisplatin was given without amifostine in this historical series, severe or life-threatening nephrotoxicity was observed in approximately 3% of the courses, and fatalities were noted in 2%. In contrast, when amifostine was administered prior to the higher dose of cisplatin, 120 mg/m^2, the occurrence of life-threatening and severe nephrotoxicity was substantially lower and no deaths occurred.

Neurotoxicity is another well recognised side effect of cisplatin therapy and may replace nephrotoxicity as the dose-limiting toxic effect of cisplatin treatment. Mollman and colleagues studied the neurotoxic effects of cisplatin in patients treated with various cisplatin regimens with and without pretreatment with amifostine [3]. Neuropathy was observed in 27 of the 41 (66%) patients treated with cisplatin without amifostine, compared with only 7 of the 28 (25%) patients treated with cisplatin plus amifostine, and the difference between the groups was statistically significant ($P = 0.001$). The median cumulative cisplatin dose at the onset of neurotoxicity in patients given cisplatin without amifostine was 350 mg/m^2. This dose was significantly lower than the median cumulative cisplatin dose of 600 mg/m^2 received by patients given cisplatin plus amifostine ($P = 0.0001$). These findings indicate that patients who were pretreated with amifostine were able to tolerate larger doses of cisplatin before the onset of neurotoxic effects.

THE EFFECT OF AMIFOSTINE ON THE TOXICITY TO NORMAL ORGANS AND TUMOUR RESPONSE TO CYCLOPHOSPHAMIDE/CISPLATIN COMBINATION THERAPY IN PATIENTS WITH ADVANCED OVARIAN CARCINOMA

The clinical data reviewed above indicate that amifostine pretreatment can protect against the toxicities associated with cyclophosphamide (C) and cisplatin (P) and led to a randomised clinical trial employing both of these drugs in women with Stage III/IV ovarian cancer [4]. The objectives of the trial were to determine the efficacy of amifostine in protecting patients at risk of serious CP-related toxicities, including haematological toxicity, nephrotoxicity, neurotoxicity, and ototoxicity. Additional objectives were to determine the effect of amifostine on the preservation of tumour response.

Patients were stratified according to residual tumour size (<2 cm or ≥2 cm, as measured following debulking laparotomy) and cancer centre. They were then randomly

Table 1. Nephrotoxic effect of cisplatin administered with amifostine to patients with metastatic melanoma: comparison of two published reports using 100 mg/m^2 or 120 mg/m^2 with similar hydration and osmotic diuresis

	Percentage of courses of therapy	
Nephrotoxicity	Amifostine plus cisplatin (120 mg/m^2)* $n = 148$	Cisplatin (100 mg/m^2)† $n = 147$
None	87.2	78.9
Mild	6.8	11.6
Moderate	5.4	4.8
Severe	0.7	2.0
Life-threatening	0	0.7
Fatal	0	2.0‡

*Data from [2] and Avril (data on file at U.S. Bioscience); †Data from [11]; ‡One death with hydration plus mannitol, two deaths with hydration alone.

assigned to receive six cycles of chemotherapy with or without amifostine every 3 weeks. The chemotherapy regimen consisted of 1 g/m^2 cyclophosphamide plus 100 mg/m^2 cisplatin. Patients in the group to receive amifostine were also treated with 910 mg/m^2 amifostine prior to administration of chemotherapy. The two groups were well matched with respect to age, FIGO stage, performance status, histological characteristics, extent of residual disease, and measurable disease. After completion of six cycles of therapy, those patients who had no clinically detectable disease underwent second-look laparotomy to allow determination of pathologically confirmed complete response rates.

The results of the interim analysis performed after accrual and assessment of 121 patients are presented in Table 2. Discontinuation of chemotherapy due to haematological toxicity, nephrotoxicity, or ototoxicity was necessary in 15 of the 58 (26%) patients treated with cisplatin and cyclophosphamide alone, compared with only 4 of 63 (6%) patients treated with these agents plus amifostine ($P = 0.003$). All 4 of the patients in the group treated with amifostine discontinued because of ototoxicity.

The most serious early toxicity associated with combined treatment with cisplatin and cyclophosphamide was neutropenia, defined as a neutrophil count less than 500/mm^3, and fever and/or signs and symptoms of infection requiring antibiotic therapy. 16 of the 58 (28%) patients treated with cyclophosphamide and cisplatin alone were hospitalised for neutropenia and fever compared with only 5 of 63 (8%) patients who also received amifostine; the difference between the two groups was statistically significant ($P = 0.004$). The total and mean numbers of days of hospitalisation were also significantly greater for patients treated with cyclophosphamide and cisplatin alone than for patients who also received amifostine ($P < 0.05$). The total number of days of hospitalisation was 137 for patients treated with cyclophosphamide and cisplatin alone compared with only 26 for patients who also received amifostine. The mean duration of hospitalisation (per hospitalisation) was 8.6 days for patients who received cyclophosphamide plus cisplatin alone compared with only 5.2 days for patients who also received amifostine. Finally, patients given cyclophosphamide and cisplatin alone received antibiotic treatment for 150 days, whereas patients who also received amifostine were on antibiotics for only 38 days.

The amifostine-induced haematoprotective effects noted with the CP regimen and that discussed earlier with cyclophosphamide ± amifostine differ from the haematological effects associated with haematopoietic growth factors. Characteristics of the haematopoietic growth factors are as follows: these agents stimulate residual progenitor cells that remain following cytotoxic chemotherapy. Their most prominent effect follows the initial course of chemotherapy and their efficacy diminishes following multiple cycles of chemotherapy. Indeed, the stimulatory effects on progenitor cells following an initial cycle of chemotherapy may actually enhance the cytotoxic effects of a subsequent cycle of chemotherapy. Additionally, the monolineage effect of the growth factors primarily focused on the myeloid series and provides no protection of the erythroid and megakaryocytic series. These effects result in a further decrease in bone marrow reserve following multiple cycles of chemotherapy. In contrast, amifostine administered before chemotherapy protects the progenitor cells from the cytotoxic effects. This cytoprotection is evident in the amifostine/cyclophosphamide study noted previously and the amifostine/CP trial in patients with advanced ovarian cancer discussed above, both in the early cycles as well as following multiple cycles of chemotherapy, indicating that amifostine protects the bone marrow from both the acute and cumulative haematotoxic effects of these agents.

Although amifostine greatly diminished the toxic effects of chemotherapy, it did not affect its therapeutic benefit. The pathologically confirmed complete response rates were similar in the two treatment groups both for all 121 patients evaluated and for the 64 patients who underwent second-look laparotomy (Table 3). That response rates were comparable for patients in the two treatment groups indicates that amifostine provided no

Table 2. Number (%) of patients treated with amifostine plus cyclophosphamide and cisplatin or cyclophosphamide and cisplatin alone who discontinued chemotherapy before completion of six cycles of treatment and the reasons for discontinuation [4]

Toxicity	Amifostine plus cyclophosphamide + cisplatin $n = 63$ (%)	Cyclophosphamide + cisplatin $n = 58$ (%)	P*
Study endpoints			
Haematological toxicity			
Septic death	0 (0)	1 (2)	—
Granulocytopenia and thrombocytopenia	0 (0)	1 (2)	—
Granulocytopenia	0 (0)	3 (5)	—
Nephrotoxicity	0 (0)	3 (5)	—
Ototoxicity (clinical hearing loss)	4 (6)	7 (12)	—
Total	4 (6)	15 (26)	0.003
Other toxicities			
Nausea/vomiting	4 (6)	3 (5)	—
Decrease in systolic blood pressure	2 (3)	0 (0)	—
Any toxicity	10 (16)	18 (31)	0.048

*Two-sided Pearson Chi-squared statistic.

Table 3. Histologically confirmed response to treatment of patients treated with amifostine plus cyclophosphamide and cisplatin or cyclophosphamide and cisplatin alone [4]

	Percentage of patients	
	Amifostine plus cyclophosphamide + cisplatin	Cyclophosphamide + cisplatin
All patients (n = 121)		
Complete response	22	19
Complete and partial response	43	36
Patients undergoing second-look surgery (n = 64)		
Complete response	42	36
Complete and partial response	82	68

protection from the antitumour effects of the cyclophosphamide and cisplatin combination therapy.

The lack of tumour protection by amifostine pretreatment was also reflected in the survival of these patients. As shown in Figure 2, the survival curves for the two groups of patients were identical over a median follow-up period of 40 months. The median survival period was approximately 35 months in both groups, a further indication that amifostine exerted no protective effect on the tumour.

THE EFFICACY OF A REGIMEN OF AMIFOSTINE AND CISPLATIN/VINBLASTINE FOLLOWED BY AMIFOSTINE AND RADIOTHERAPY IN NON-SMALL CELL LUNG CANCER

In an ongoing trial at the University of Wisconsin, the efficacy of a regimen of amifostine and high-dose cisplatin plus vinblastine followed by amifostine and radiotherapy is being studied in patients with Stage III non-small cell lung cancer [5]. Patients first undergo induction chemotherapy consisting of treatment on days 1 and 29 with 740 mg/m^2 amifostine, followed by 120 mg/m^2 cisplatin, and weekly treatment with 5 mg/m^2/week vinblastine for 5 weeks starting on day 1. This regimen is followed on day 50 by a regimen of consolidation radiotherapy consisting of pretreatment with 340 mg/m^2 amifostine followed by thoracic radiation at a dose of 6000 rads delivered over a 6 week period. A response to the chemotherapy portion of this

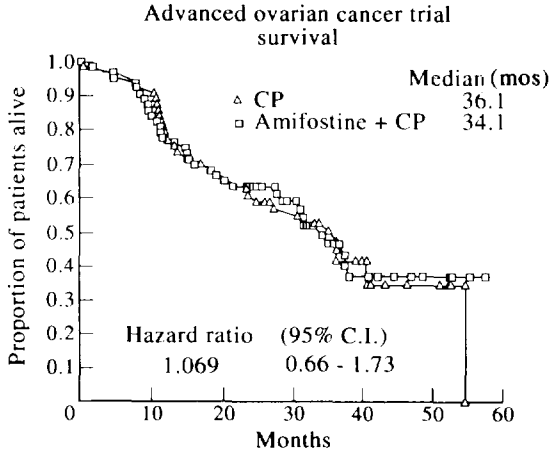

Figure 2. Overall survival in patients with advanced ovarian cancer who were treated with cyclophosphamide and cisplatin alone or cyclophosphamide and cisplatin plus amifostine.

treatment prior to radiation therapy has been obtained in 12 of 16 patients (75%) [5]. This response to high dose cisplatin/vinblastine testifies to the lack of tumour protection by amifostine.

RADIOPROTECTIVE EFFECTS OF AMIFOSTINE IN PATIENTS WITH ADVANCED RECTAL CANCER UNDERGOING PALLIATIVE RADIATION THERAPY

Liu and associates conducted a trial in patients undergoing palliative radiation therapy to determine whether pretreatment with amifostine would protect normal tissue from radiation damage without loss of antitumour effect [6]. 49 patients with inoperable, unresectable, or recurrent rectal adenocarcinoma were randomly assigned to amifostine plus radiation treatment, and 51 similar patients were assigned to radiation treatment alone. All patients underwent radiation to the pelvis at a dose of 225 cGy for 5 days per week for 5 weeks. For patients in the amifostine plus radiation treatment group, 340 mg/m^2 amifostine was injected intravenously over 7 min, 15 min before each radiation treatment. Because of the unique effect of amifostine on blood pressure, this randomised trial was not double blinded nor was a placebo used.

The groups were compared with respect to late radiation toxicity to skin and mucous membranes of the genitourinary tract and lower gastrointestinal tract. Five of the evaluable 37 patients who underwent radiation treatment alone and had assessable data had moderate to severe damage to one or more of the normal tissues examined. In contrast, none of the evaluable 34 patients who received amifostine plus radiation treatment had moderate or severe late radiation toxicity to any of these tissues. The difference between the two groups was significant (P = 0.026).

4 of the 51 (7.8%) patients treated with radiation alone had a complete tumour response compared with 8 of 49 (16.3%) patients treated with amifostine plus radiation (95% confidence intervals: −4.2–21.2%). These findings indicate that amifostine had no protective effect against the cytotoxic effects of radiation on the advanced rectal cancer while protecting normal pelvic organs from late radiation-induced toxicities. Similarly, survival of the patients in the two groups was comparable as shown in Figure 3. Median survival was 12.3 months in the radiation treatment alone group and 15.0 months in the amifostine plus radiation treatment group. These survival data provide further evidence that amifostine did not alter the effect of radiation on advanced rectal cancer.

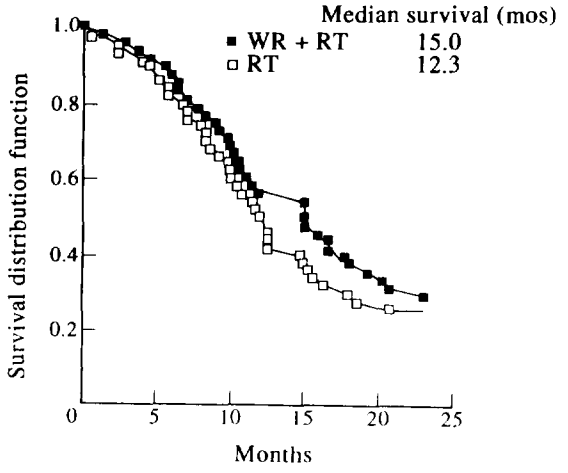

Figure 3. Survival of patients with inoperable, unresectable, or recurrent rectal adenocarcinoma treated with amifostine plus radiation (RT) or radiation alone. Adapted with permission from Liu T *et al. Cancer* 1992, 69, 2820–2825 [6].

AMELIORATION BY AMIFOSTINE OF THE TOXICITY INDUCED BY CISPLATIN AND RADIATION IN PATIENTS WITH CARCINOMA OF THE UTERINE CERVIX

Wadler and associates conducted a Phase I trial of amifostine as a means of ameliorating the toxicity of concurrent cisplatin and radiation therapy in women with locally advanced carcinoma of the uterine cervix [7]. 20 patients were treated with external beam followed by brachytherapy radiation to the pelvis. Cisplatin 20 mg/m^2 per day was administered for 5 days beginning on days 1 and 22. An additional dose of 100 mg/m^2 cisplatin was administered intravenously over 30 min following each brachytherapy treatment. Finally, five doses of 20 mg/m^2 cisplatin were administered concurrent with external-beam therapy in those patients who received boost therapy. Amifostine was administered over 15 min before each cisplatin infusion at increasing doses from 340 to 910 mg/m^2 preceding cisplatin.

Table 4 contrasts the toxic effects of this aggressive regimen in the 19 assessable patients in this study and a historical comparison with those in 43 other patients treated with the same regimen without amifostine in another earlier study [8]. The median number of days that treatment was delayed because of bone marrow suppression was substantially less when amifostine was included in the treatment regimen. More importantly, the longest treatment delay because of haematological suppression was more than 101 days among patients treated with radiation and cisplatin alone compared with only 32 days among patients who additionally received amifostine. Evidence of toxicity associated with pelvic mucosal tissue, including rectovaginal fistulas, proctitis, enteritis, vaginitis, and cystitis, occurred more frequently among patients treated with radiation and cisplatin alone than when amifostine was included in the regimen. This retrospective historical comparison suggests that amifostine may offer protection against the toxicity of radiation and cisplatin even when these cytotoxic treatments are administered concurrently in an extremely aggressive regimen. These preliminary findings must be validated in a prospective, randomised trial before definitive conclusions can be drawn.

SAFETY PROFILE OF AMIFOSTINE

The safety profile of amifostine in patients undergoing chemotherapy is shown in Table 5. The two most important toxic effects associated with amifostine treatment are vomiting and hypotension. These effects, however, have led to treatment discontinuation in only a very few patients. As shown in Table 5, treatment has been discontinued because of hypotension in 1.2% patients. Many of these discontinuations occurred in the earlier clinical trials when investigators had not yet learned how to manage this effect. Systolic hypotension typically occurs during infusion or immediately thereafter, and recovery usually occurs within 10 min. There have been no reports of delayed hypotension.

Hypothermia has been noted in mice [9]. This effect appears to be species and strain-specific since hypothermia has not been reported in the clinic, and affects certain strains of mice more than others. Indeed, a sensation of warmth or flushing, which might coincide with hypotension, has been noted in the clinical setting, rather than hypothermia. Other effects of amifostine that have been noted include sleepiness, hiccups, sneezing, and hypocalcaemia. Hypocalcaemia is not observed when amifostine is used intermittently, but it may be noted with long-term daily use of high doses of amifostine such as would be used during

Table 4. Comparison of the toxic effects of a regimen of cisplatin and radiation therapy with and without amifostine in patients with advanced cervical carcinoma

Toxic effects	Amifostine plus cisplatin and radiation $n = 19$*	Cisplatin and radiation alone $n = 43$†
Grade 3/4 thrombocytopenia	0	2
Treatment delay secondary to marrow suppression		
Median days	13	23
Range (days)	4–32	>101
Rectovaginal fistulas	0	3
Radiation proctitis	0	2
Hospitalised for radiation enteritis	1	1
Radiation vaginitis	0	1
Radiation cystitis	0	1

*Data from [7].
†Data from [8].

Table 5. Frequency of adverse experiences associated with amifostine infusion when given with chemotherapy

Adverse event	Per patient (n = 408) %	Per cycle (n = 1240) %	Patients taken off study because of adverse experience
Vomiting*	74.3	57.6	1.5
Flushing/feeling of warmth	43.1	24.9	0
Sneezing	30.4	18.0	0
Hypotension†	17.6	6.9	1.2
Sleepiness/somnolence	10.0	4.4	0
Dizziness/lightheadedness	10.3	3.8	0
Hiccups	2.9	1.3	0
Chills	1.5	0.5	0
Hypocalcaemia	1.7	0.6	0
Allergy/rash	0	0	0

*All patients did not receive optimal anti-emetic therapy; includes effects of cyclophosphamide and cisplatin on day of therapy; †Episodes which resulted in early termination of amifostine infusion.

radiation therapy. Although a reduction in serum calcium level occurs, no clinical symptomatology has been associated with this change. Indeed, this depression in calcium has been shown to be related to a amifostine-mediated inhibition of parathyroid hormone release [10].

SUMMARY

In summary, clinical experience indicates that the spectrum of biological effects of amifostine include the protection of normal tissues from early and late radiation damage, as well as the protection of normal tissues from the toxicities of various chemotherapeutic agents.

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