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Value of Granulocyte Colony Stimulating Factor in Radiotherapy Induced Neutropenia: Clinical and Laboratory Studies

M.P. Mac Manus, D. McCormick, A. Trimble and W.P. Abram

We report the effect of granulocyte colony stimulating factor (G-CSF) on neutropenia occurring during extended field radiotherapy in two groups of patients. The first group comprised 8 patients receiving craniospinal irradiation for a variety of central nervous system (CNS) neoplasms. None of these patients received cytotoxic chemotherapy. G-CSF was administered when the absolute neutrophil count (ANC) approached 1.5 × 10^9/l. Neutropenia was promptly corrected in all cases, thereby avoiding unscheduled interruptions in radiotherapy. Following each G-CSF administration, ANC reached a peak on the following day and then declined steadily. Mean ANC rose from 1.33 × 10^9/l on the day of G-CSF treatment to 7.07 × 10^9/l the next day. Patients received 2–6 G-CSF injections during radiotherapy. Experiments were carried out *in vitro* to assess the risk of G-CSF causing increased CNS tumour cell proliferation. 11 human CNS tumour cultures (2 medulloblastomas, 2 primitive neuroectodermal tumours and 7 astrocytic tumours) were cultured in the presence of G-CSF at a range of concentrations up to 100 ng/ml. Their proliferation was compared with that of a G-CSF dependent murine leukemia cell line (NFS-60). None of the human tumour cultures demonstrated a significant increase in proliferation in response to G-CSF. 4 patients undergoing "mantle" type radiotherapy for Hodgkin's Disease or Non-Hodgkin's Lymphoma also received G-CSF treatment for neutropenia. All 4 had previously received cytotoxic chemotherapy. The number of G-CSF injections given per patient during radiotherapy ranged from 3–6. Mean ANC rose from 1.76 × 10^9/l to 10.8 × 10^9/l the next day. These results suggest that G-CSF is a reliable treatment for radiotherapy induced neutropenia and that an intermittent dosage schedule is effective.

Key words: G-CSF, radiotherapy, CNS tumours, lymphoma, Hodgkin's Disease, tumour cell cultures
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INTRODUCTION

NEUTROPENIA IS a common complication of extended field radiotherapy. Neutropenic patients are at risk of infection and may have unscheduled interruptions in radiotherapy treatment protocols to allow bone marrow recovery. In an earlier study [1], we reported that granulocyte colony stimulating factor (G-CSF) caused rapid recovery of peripheral blood neutrophil counts when given to 4 patients developing neutropenia during craniospinal irradiation (CSI) for central nervous system (CNS) tumours. Patients receiving extended field radiotherapy for Hodgkin's Disease (HD) and Non-Hodgkin's Lymphomas (NHL) are also at risk of neutropenia, particularly those who have previously received cytotoxic chemotherapy. Cytotoxic drugs can cause a sustained reduction in bone marrow tolerance to radiation in these patients [2].

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In this paper, we report the effect of G-CSF in 3 cases of HD and 1 case of NHL, previously treated with cytotoxic chemotherapy, in which neutropenia occurred during extended field radiotherapy. We also report results for 8 consecutive patients who received treatment with G-CSF for neutropenia occurring during CSI for CNS tumours, updating our initial results. Unlike Marks and Halperin [3] and Schmidberger and associates [4] who gave daily G-CSF injections, we administered G-CSF only on days when the white cell count fell below a threshold level. G-CSF was given in the hope that interruptions in radiotherapy treatment protocols due to neutropenia could be entirely eliminated.

Several investigators have reported that haematopoietic growth factors can stimulate proliferation of non-haematopoietic tumour cells *in vitro*. Most of these studies used immortalised cell lines rather than primary cultures, and emphasised granulocyte macrophage stimulating factor (GM-CSF) rather than G-CSF. In a study which used primary cultures, Foulke and associates [5] showed that only 2 of 48 human tumours showed significant growth stimulation by GM-CSF *in vitro*. Stimulation of clonal growth of colonic adenocarcinoma by G-CSF [6] and functional G-CSF receptors on bladder carcinoma cell lines have also been reported [7]. Thacker and associates [8] have described an osteosarcoma cell line in which G-CSF and GM-CSF both caused

an increase in proliferation that could be blocked by specific antibodies. Retroviral insertion of human GM-CSF and G-CSF genes into tumour cells caused autocrine stimulation of cellular proliferation.

We were concerned by the remote possibility that G-CSF could promote the growth of tumour cells lying outside radiation treatment portals in CSI patients who received no systemic chemotherapy. To date, there have been no reports of the effects of G-CSF on the proliferation of brain tumours and for this reason we have conducted studies in which a number of primary CNS tumours were exposed *in vitro* to a range of concentrations of recombinant human G-CSF. Our results are reported in this paper.

PATIENTS AND METHODS

Craniospinal radiotherapy patients

8 consecutive patients, with a wide range of primary CNS tumours who developed neutropenia during CSI, were treated with recombinant human G-CSF (Neupogen, Amgen, Thousand Oaks, California, U.S.A.) by subcutaneous injection. Details of these patients are shown in Table 1. Full peripheral blood counts, including differential white cell counts, were measured frequently during radiotherapy. All patients had normal blood counts before radiotherapy commenced. On occasions where blood smears were unsuitable, an estimate of the ANC was made, using the total white cell count and based on previous differential counts.

After our first case, G-CSF was administered on days when the absolute neutrophil count in peripheral blood (ANC) fell below or approached 1.5×10^9/l. Radiotherapy was not interrupted unless there were other compelling reasons (e.g. thrombocytopenia). The criteria for G-CSF administration were relaxed when the spinal phase of radiotherapy was completed, because it was considered that the probability of a further significant fall in ANC was low. A G-CSF dose of 4–5 μg/kg was used. Adults treated most recently in this study have simply received the contents of a 300 μg vial for reasons of cost-effectiveness. No CSI patient received cytotoxic drugs before or during radiotherapy.

A standard radiotherapy technique was used. The spinal cord and its meningeal coverings were treated using direct posterior fields. The skull and upper cervical cord were treated by opposed lateral fields, matched to the spinal field. The radiation source was either a linear accelerator or a telecobalt machine. A variety of total tumour doses were employed, depending on the diagnosis. All fields were treated once daily and the dose per fraction for the spinal fields for each patient is shown in Table 1. It has been usual practice at our centre to interrupt radiotherapy if the ANC falls below 1.5×10^9/l or if the platelet count is $< 80\,000 \times 10^9$/l.

Hodgkin's Disease/lymphoma patients

4 consecutive patients with lymphorecticular malignancies, who developed neutropenia during extended field radiotherapy, were treated with G-CSF according to the protocol outlined above. All of these patients had previously received cytotoxic drugs; HD patients were treated with chlorambucil, procarbazine, prednisone and either vinblastine or vincristine, using either the ClVPP [9] or LOPP [10] regimens; the single lymphoma patient received cyclophosphamide, vincristine and prednisolone [11]. Chemotherapy was not given concurrently with radiotherapy. Details of these patients are shown in Table 2.

The three HD patients were treated using standard "mantle" radiotherapy fields to a dose of 35 Gy in 20 fractions in 4 weeks. Of these patients, 1 had a boost of 4 Gy to the mediastinum. The patient with NHL received 41.25 Gy in 21 daily fractions to a similar radiation target volume, although the axillae were not treated in this case.

Cell cultures

Cells from a range of primary human CNS tumours were grown *in vitro* and exposed to G-CSF at different concentrations. NFS-60, a murine leukaemia cell line which exhibits dose-dependent stimulation of proliferation in the presence of G-CSF [12] was used as a positive control. This was a gift from Dr Sandra Irvine, Department of Haematology, Queen's University, Belfast. The IPPN-8 medulloblastoma culture [13] was a gift from Dr G.J. Pilkington, Institute of Psychiatry, London, U.K. All other cultures were derived from tumour biopsies received from the neurosurgical unit, Royal Victoria Hospital, Belfast. These comprised two primitive neuroectodermal tumours, a medulloblastoma and seven astrocytic tumours (Table 3). Tumours were analysed in the Northern Ireland Regional Neuropathology Service, Royal Victoria Hospital, Belfast, using pathological, immunohistochemical and ultrastructural techniques. Cell lines were established *in vitro* using previously described procedures [14] and characterised morphologically and by immunocytochemistry, including demonstration of glial fibrillary acid protein [15]. All cell cultures were

Table 1. Craniospinal radiotherapy patients

Patient number	Age (years)	Diagnosis	Radiation dose per fraction to spinal field (cGy)
1	4	Medulloblastoma	123
2	6	Primitive neuroectodermal tumour	135
3	2	Medulloblastoma	115
4	21	Pineocytoma	135
5	1	Ependymoblastoma	115
6	20	Spinal cord ependymoma, CSF seeding	135
7	22	Germinoma	115
8	6	Medulloblastoma	135

Table 2. Hodgkin's Disease/lymphoma patients

Patient numbers	Age (years)	Diagnosis	Chemotherapy (CT) regime	Interval between last CT dose and radiotherapy
1	22	Hodgkin's Disease Stage IIA	LOPP 6 cycles	6 weeks
2	52	Non-Hodgkin's Lymphoma Stage IV	CVP 6 cycles	4 years
3	22	Hodgkin's Disease Stage IIA	ClVPP 8 cycles	4 weeks
4	33	Hodgkin's Disease Stage IIA	ClVPP 2 cycles	4 weeks

Table 3. Cell lines cultured with G-CSF in proliferation assay

Tumour culture	Histological diagnosis	Passage number
NFS-60	Murine myeloid leukaemia	Unknown
IPPN-8	Medulloblastoma	8
NP113/84	Medulloblastoma	3
NP325/84	Primitive neuroectodermal tumour	6
NP283/92	Primitive neuroectodermal tumour	6
NP493/92	Oligoastrocytoma	4
NP351/92	Pilocytic astrocytoma	6
NP365/92	Astrocytoma	3
NP287/92	Astrocytoma	6
NP473/92	Anaplastic astrocytoma	5
NP602/91	Glioblastoma multiforme	13
NP306/92	Glioblastoma multiforme	5

used at low passage numbers so that cells growing *in vitro* would reflect as closely as possible the characteristics of the original tumour. Human cultures were maintained as monolayers in DMEM 1640 medium supplemented with 4 mM glutamine and 10% fetal calf serum. The NSF-60 cell line was routinely maintained as a suspension culture under conditions which were identical, except for the addition of G-CSF to the medium.

Cell proliferation assay

Cells were subcultured into 96-well plates at a density of 2×10^4 cells/well. After 24 h, G-CSF was added to the wells at final concentrations ranging from 0.1 to 100 ng/ml. The maximum *in vitro* G-CSF concentration greatly exceeded maximum serum G-CSF concentrations which would be encountered clinically [16]. Control wells received no G-CSF. After 24 h exposure to G-CSF, the number of viable cells in each well was estimated using a modification of the tetrazolium salt, (3-(4,5-dimethylthiazol-2-yl)--2,5-diphenyltetrazolium bromide) (MTT) reduction method [17]. Following the addition of 50 μl of MTT (2 mg/ml) to each well, the plates were incubated for 5 h at 37°C to allow uptake of MTT and its enzymatic reduction to blue-coloured formazan. After withdrawal of MTT from the wells, the formazan product was dissolved in 100 μl dimethylsulphoxide. Optical density (OD), which is proportional to the number of living cells, was determined using a Thermomax microplate reader set at 570 nm (reference wavelength—650 nm). The OD was converted to cell number using calibration lines prepared for each culture. Because the NSF-60 cells grow in suspension, centrifugation was necessary before the MTT assay. All experiments were performed in triplicate and response to G-CSF was calculated as follows:

$$\frac{\text{Number of cells in G-CSF treated well}}{\text{Number of cells in control well}} \times 100 = \% \text{ Control proliferation}$$

RESULTS

Treatment with G-CSF caused a marked increase in neutrophil counts in all patients in this study. No adverse effects were encountered. No effect on platelet counts was observed.

Craniospinal radiotherapy patients

The effect of G-CSF on neutrophil counts in patients receiving CSI is illustrated in Figure 1, which documents each individual episode of neutropenia before and after G-CSF. On average 4.1 G-CSF doses were required per patient, with a range of 2–6. Following the introduction of this treatment policy, no patient experienced an unscheduled break in radiotherapy due to neutropenia. Nevertheless, 3 of the 8 patients had interruptions in radiotherapy for other causes. Of these patients, 2 developed thrombocytopenia (platelets $< 80 \times 10^9$/l) and the third, who had a pineal germinoma, had treatment delays due to thrombocytopenia, obstructive hydrocephalus, diabetes insipidus and urinary catheter infection. Another patient with an indwelling catheter developed a urinary infection, but this did not delay radiotherapy. Both urinary infections began early in the course of radiotherapy, before neutropenia occurred.

The mean ANC for blood samples measured immediately before each G-CSF administration was 1.33×10^9/l (S.E.M. = 0.059). The mean ANC rose to 7.07×10^9/l (S.E.M. = 0.705) on the subsequent day. The mean rise in ANC was 5.77×10^9/l (S.E.M. = 0.701). These results are based on a total of 29 G-CSF injections. After reaching a peak on the day after each G-CSF injection, the ANC declined steadily. Further G-CSF injections were administered if the ANC again reached the threshold level.

Hodgkin's Disease/lymphoma patients

Results were similar to those observed in the CSI patients. In all 4 cases, G-CSF rapidly corrected neutropenia occurring during radiotherapy. Pooled results for each G-CSF administration for all 4 patients are shown in Figure 2. Each G-CSF injection was followed by a rise in ANC. The mean ANC prior to G-CSF administration was 1.76×10^9/l (S.E.M.= 0.149). This rose to 10.8×10^9/l (S.E.M. = 1.158) on the subsequent day. The mean elevation in ANC was 9.05×10^9/l (S.E.M. = 1.073). These results are based on a total of 14 injections. On average, 3.5 G-CSF injections were given per patient, with a range of 3–6. In Figure 3, sequential neutrophil and platelet counts of a representative HD patient are illustrated. Radiotherapy was administered 5 days per week, commencing on day 1.

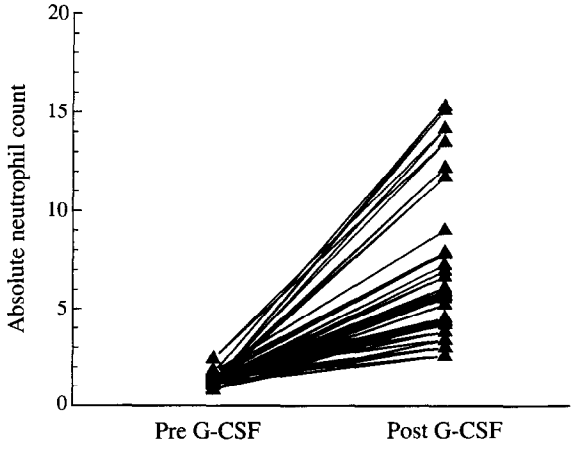

Figure 1. Craniospinal patients—absolute neutrophil counts immediately before and one day after each G-CSF dose given during craniospinal irradiation.

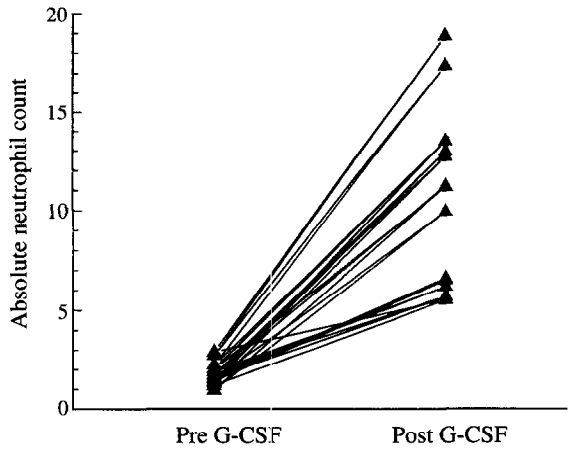

Figure 2. Hodgkin's Disease/lymphoma patients—absolute neutrophil counts immediately before and one day after each G-CSF dose (pooled results of 4 patients) given during mantle radiotherapy.

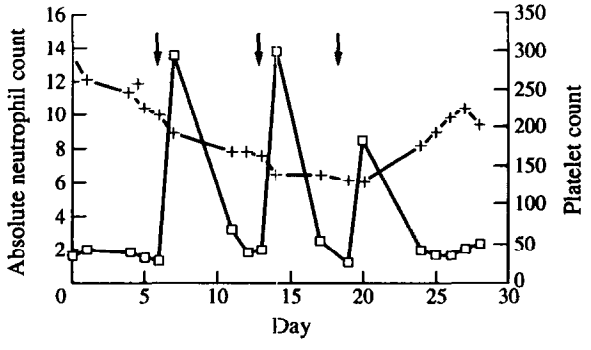

Figure 3. Variation in absolute neutrophil count (□-□) and platelet count (+-+) (× 10^9/l) during mantle radiotherapy in a representative patient with Hodgkin's Disease. Arrows indicate G-CSF doses.

Cell culture studies

The results of G-CSF treatment of the CNS tumour cell cultures are shown in Figure 4 as a ratio of the control values (proliferation achieved in the absence of G-CSF). Each value represents a mean of triplicate assays and the coefficient of variation in each case was < 10%. As expected, the G-CSF dependent control cell line responded to the recombinant human G-CSF with a marked increase in proliferation. The stimulation was dose-dependent, with a 1.25-fold increase at a G-CSF concentration of 0.1 ng/ml, a 1.45-fold increase at 1 ng/ml, reaching a plateau with an increase of 2.7-fold at 10 and 100 ng/ml. Neither of the two medulloblastoma cultures (IPPN-8 and NP113/84) showed any increase in proliferation at G-CSF concentrations below 10 ng/ml. A small increase of 1.28-fold was noted at 100 ng/ml for IPPN-8 only. Neither of the PNET cultures (NP325/84, NP283/84) nor any of the seven astrocytic tumours showed any evidence of enhanced proliferation in response to G-CSF.

DISCUSSION

Although there is a burgeoning literature on the use of G-CSF in chemotherapy induced neutropenia, data on G-CSF in radiation induced neutropenia remain scarce. A recombinant leucocyte growth factor (GM-CSF) was used to treat radiation induced leucopenia in humans following the radiation accident in Brazil [18]. Published experience with the use of G-CSF in radiotherapy initially took the form of case reports [19–21]. More recently, small groups of patients have been described [1, 4]. Schmidberger and associates described 11 assorted cases in which G-CSF was given during radiotherapy, although in 6 of these it is likely that chemotherapy administered concurrently with radiotherapy was the principal cause of neutropenia [4]. Recently Knox and colleagues from Stanford have reported that daily treatment, with a low dose of G-CSF, prevented neutropenia in 7 patients with HD who received subdiaphragmatic irradiation immediately after mantle irradiation [22]. Studies in which chemotherapy and radiation have been administered concurrently [23, 24] give little information on the value of G-CSF in radiotherapy, because the irradiated volumes often include a relatively small proportion of the active marrow, and the chemotherapy component would probably have produced neutropenia by itself.

There can be little doubt that G-CSF is effective in radiotherapy induced neutropenia, but uncertainty remains as to the optimum dosage regime and treatment schedule. In our G-CSF protocol, we have not sought to maintain the ANC at normal levels for the duration of radiotherapy. This approach is cost-effective and avoids the extreme neutrophil leucocytosis which can occur with daily G-CSF treatment schedules. It is known that "the risk of infection is in fact not appreciably increased at neutrophil counts in the range 1–2 × 10^9/l in the absence of additional complicating factors" [25]. The risk of infection is also related to the duration of neutropenia. Because of the rapid effect of G-CSF, no patient has had neutropenia lasting more than 24 h since we commenced the regime described in this paper. We have encountered no case of neutropenic sepsis in this study, although 2 of our CSI patients did have problems with urinary infections due to indwelling catheters which occurred before there was a significant reduction in neutrophil count. Such infections are common in patients with normal immune systems.

Our cell culture studies of 11 primary CNS neoplasms, comprising 2 medulloblastomas, 2 PNETs and 7 astrocytic tumours, have shown no significant evidence of enhanced tumour cell proliferation in the presence of G-CSF. The maximum concentration of G-CSF used in these studies exceeded the maximum serum concentration of G-CSF which is encountered clinically. We are unaware of any investigation of the capacity of G-CSF to cross the blood/brain or blood/tumour barrier in humans. Clearly a larger study would be required to definitively exclude an effect on CNS tumour cells, but our results are nevertheless reassuring.

There is compelling evidence that unscheduled interruption of radiotherapy treatment schedules can lead to reduction in the probability of local control [26, 27] although, as pointed out by Marks and Halperin [3], much of this evidence is derived from studies of epithelial cancers of the head and neck. One study of medulloblastoma has shown an association between interruptions in radiotherapy and reduced survival [28]. The use of G-CSF may allow treatment delays due to neutropenia to be eliminated entirely.

The purpose of this investigation was to determine whether or not G-CSF treatment would allow patients to proceed safely with radiotherapy under conditions where, in the past, treatment would have been interrupted. In the two groups of patients that we studied, G-CSF was extremely effective in this regard. We cannot tell what difference G-CSF treatment will make to overall treatment time, local tumour control and infection rates until a randomised trial, comparing G-CSF with "no treatment" is

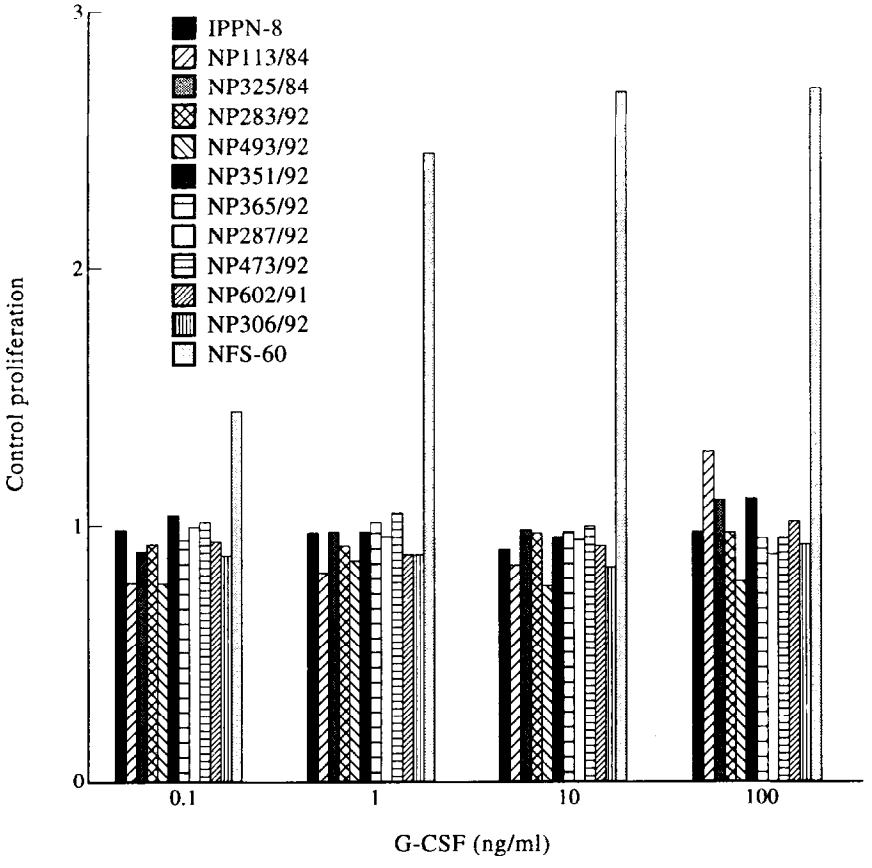

Figure 4. Effect of G-CSF on CNS tumour cell growth *in vitro*. Only the control cell line NFS-60 showed a dose-dependent enhancement in proliferation.

carried out in patients receiving extended field radiotherapy. Nevertheless, with widespread use of G-CSF, radiation induced neutropenia could cease to be a common cause of treatment interruption in extended field radiotherapy. Thrombocytopenia would then become the major acute haematological toxicity of radiotherapy.

1. Mac Manus MP, Clarke J, McCormick D, *et al.* Use of recombinant granulocyte-colony stimulating factor to treat neutropenia occurring during craniospinal irradiation. *Int J Radiat Oncol Biol Phys* 1993, **26**, 845–850.
2. Trainor KJ, Morley AA. Screening of cytotoxic drugs for residual bone marrow damage. *J Natl Cancer Inst* 1976, **57**, 1237–1239.
3. Marks LB, Halperin EC. The use of G-CSF during craniospinal irradiation (editorial). *Int J Radiat Oncol Biol Phys* 1993, **26**, 905–906.
4. Schmidberger H, Hess CF, Hoffman W, *et al.* Granulocyte colony-stimulating factor treatment of leucopenia during radiotherapy. *Eur J Cancer* 1993, **29A**, 1927–1931.
5. Foulke RS, Marshall MH, Trotta PP, *et al. In vitro* assessment of the effects of granulocyte macrophage-colony stimulating factor on primary human tumours and derived lines. *Cancer Res* 1990, **50**, 6264–6267.
6. Berdel WE, Danhauser-Reidl S, Stenhauser G, *et al.* Various human hematopoietic growth factors (interleukin 3, GM-CSF, G-CSF) stimulate clonal growth of nonhematopoietic tumor cells. *Blood* 1989, **73**, 80–83.
7. Ohigashi TP, Tachibana M, Tazaki H, *et al.* Bladder cancer cells express functional receptors for granulocyte-colony stimulating factor. *J Urol* 1992, **147**, 283–286.
8. Thacker JD, Dedhar S, Hogge DE. The effect of GM-CSF and G-CSF on the growth of human osteosarcoma cell lines *in vitro* and *in vivo*. *Int J Cancer* 1994, **56**, 236–243.
9. McElwain TJ, Toy J, Smith IE, *et al.* A combination of chlorambucil, vinblastine, procarbazine and prednisone for treatment of Hodgkin's Disease. *Br J Cancer* 1977, **36**, 276–280.
10. Hancock BW, Vaughan Hudson G, Vaughan Hudson B, *et al.* British National Lymphoma Investigation randomised study of MOPP (mustine, oncovin, procarbazine, prednisone) against LOPP (Leukeran substituted for mustine) in advanced Hodgkin's Disease—long term results. *Br J Cancer* 1991, **63**, 579–582.
11. Bagley CM, De Vita VT, Berard CW, *et al.* Advanced lymphosarcoma: intensive cyclical combination chemotherapy with cyclophosphamide, vincristine and prednisone. *Ann Intern Med* 1972, **76**, 227–234.
12. Hara K, Suda T, Suda J, *et al.* Bipotential murine hemopoietic cell line (NFS-60) that is responsive to IL-3, GM-CSF, G-CSF and erythropoietin. *Exp Hematol* 1988, **16**, 256–261.
13. Pilkington GJ, Akinwunmi J, Ognjenovic N, *et al.* Differential binding of anti-CD44 on human gliomas *in vitro*. *Neuro Rep* 1993, **4**, 259–262.
14. McCormick D, Wallace I, Kirk J, *et al.* The establishment and characterisation of a cell line and mouse xenografts from a human malignant melanoma. *Br J Exp Path* 1983, **64**, 103–115.
15. Eng LF, Rubenstein LJ. Contribution of immunohistochemistry to diagnostic problems of human cerebral tumours. *J Histochem Cytochem* 1978, **26**, 513–522.
16. Morstyn G, Campbell L, Souza LM, *et al.* Effect of granulocyte colony stimulating factor on neutropenia induced by cytotoxic chemotherapy. *Lancet* 1988, 667–672.
17. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.

18. Gale RP, Butturini A. Use of hematopoietic growth factors in radiation accidents. *Int J Radiat Oncol Biol Phys* 1990, **19**, 1291–1295.
19. MacManus MP, Clarke J, Abram WP. Use of G-CSF to treat neutropenia occurring during craniospinal irradiation in children with brain tumours (abstract). *Br J Cancer* 1992, **66** (Suppl. XVII), 27.
20. Zachariah B. Case Report: role of granulocyte-colony stimulating factor in radiotherapy. *Am J Med Sci* 1992, **304**, 252–253.
21. Marks LB, Friedman HS, Kurtzburg J, *et al.* Reversal of radiation-induced neutropenia by granulocyte-colony stimulating factor. *Med Ped Oncol* 1992, **20**, 240–242.
22. Knox SJ, Fowler S, Marquez C, *et al.* Effect of filgrastim (G-CSF) in Hodgkin's Disease patients treated with radiation therapy. *Int J Radiat Oncol Biol Phys* 1994, **28**, 445–450.
23. Crown FP, Sclafani L, McCormick B, *et al.* Induction doxorubicin followed by surgery, high-dose intensity cyclophosphamide, granulocyte-colony stimulating factor and radiation for locally advanced breast cancer (abstract). *Proc Ann Mtg Am Assoc Cancer Res* 1992, **33A**, 1532.
24. Momin F, Kraut M, Lattin P, *et al.* Thrombocytopenia in patients receiving chemoradiotherapy and G-CSF for locally advanced non-small cell lung cancer (abstract). *Proc Ann Mtg Am Assoc Cancer Res* 1992, **11A**, 983.
25. Firkin F, Chesterman C, Penington D, Rush B, eds. *de Gruchy's Clinical Haematology in Medical Practice*, 5th edn. Oxford, Blackwell Scientific Publications, 1989, 22.
26. Barton MB, Keane TJ, Gadalla T, *et al.* The effect of treatment time and treatment interruption on tumour control following radical radiotherapy of laryngeal cancer. *Radiother Oncol* 1992, **23**, 137–143.
27. Fowler JF, Lindstrom MJ. Loss of local control with prolongation in radiotherapy. *Int J Radiat Oncol Biol Phys* 1992, **23**, 457–467.
28. Berry MP, Jenkin RDT, Keen CW, *et al.* Radiation treatment for medulloblastoma: a 21 year review. *J Neurosurg* 1981, **55**, 43–51.

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Is Neuro-ectodermal Differentiation of Ewing's Sarcoma of Bone Associated with an Unfavourable Prognosis?

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Among Ewing's sarcoma (ES) of bone and related entities are tumours with neuro-ectodermal features that could represent a biologically distinct type. In order to assess the prognostic significance of the various forms of ES, a retrospective joint study involving three cancer centres in Europe and the U.S.A. was initiated. The material from 315 primary ES was reviewed by a panel of five pathologists and classified as typical ES (220 cases), atypical ES (48 cases) or ES with neuro-ectodermal features (47 cases). Prognostic factor analysis on treatment failure-free survival was performed using the Cox model. It included histopathological classification, initial patient characteristics, clinical presentation and treatment type. After multivariate analysis, in addition to treatment type ($P < 0.001$), metastases ($P = 0.003$) and proximal tumour location ($P = 0.006$), two histopathological parameters correlated with poor treatment failure-free survival, the presence of filigree pattern ($P = 0.044$) and dark cells ($P = 0.043$). We conclude that ES with neuro-ectodermal features does not appear to have a different outcome to the other subtypes.

Key words: Ewing's sarcoma, bone, neuro-ectodermal differentiation, prognosis
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INTRODUCTION

THE SO-CALLED small round cell tumours of bone are composed of a heterogeneous group of neoplasms. Among them, the use of specialised diagnostic methods [1–12] has helped to distinguish separate entities, such as Ewing's sarcoma (ES), small cell osteosarcoma, primitive sarcoma of bone, primary non-Hodgkin's lymphoma of bone, metastatic rhabdomyosarcoma, and synoviosarcoma, involving bone by direct extension.

The histological diversity of ES has been discussed at length in several reports, and documented by electron microscopic and immunocytochemical techniques [1]. Currently, several atypical forms have been described, including large cell ES, ES with neuro-ectodermal features (or PNET of bone), and ES with endothelial features [2–8]. Despite this morphological diversity, there are no generally accepted histopathological prognostic criteria applicable to all cases of ES [13–17].