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Papers

Human Granulocyte–Macrophage Colony-stimulating Factor Modulates *in vitro* Growth in only a Minority of Continuous Human Tumour Cell Lines

Members of the EORTC Clonogenic Assay Screening Study Group

Granulocyte-macrophage colony stimulating factor (GM–CSF) has potential usefulness in a range of clinical conditions, including the treatment of patients with myelosuppression induced by chemotherapy and/or radiotherapy. Prior to any extensive use of this material, however, assessment of its effects on non-haematopoietic tumour cell growth appeared warranted. Accordingly, five laboratories, all members of the EORTC Clonogenic Assay Screening Study Group, have monitored *in vitro* responses to GM–CSF, using their own individual assay procedures, in a series of 18 human tumour cell lines, predominantly of non-haematopoietic origin, 25 tumour biopsy specimens and samples from five normal bone marrow aspirates. Significant growth stimulation by GM–CSF addition was rare, being absent in all 25 "fresh" ovarian tumour samples tested, but was consistently observed in four of the 18 continuous tumour cell lines tested (1 breast and 3 ovary) and all five normal bone marrow aspirates.
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INTRODUCTION

A NUMBER of recently identified cytokines have been implicated in regulating growth and differentiation of haematopoietic progenitor cells [1, 2]. The ability of one of them, granulocyte–macrophage colony-stimulating factor (GM–CSF) to markedly stimulate production and function of granulocytes and macrophages *in vivo* [3, 4] led to its clinical evaluation in phase I and II trials in patients with myelodysplastic syndromes, acquired immunodeficiency syndrome, as well as in cancer patients following administration of myelosuppressive chemotherapy or bone marrow transplantation [5, 6]. Initially responsiveness to GM–CSF was considered a potential marker of cells derived from haematopoietic precursors; however more recent findings have suggested a broader role for GM–CSF as also a normal regulator of certain tumour cells of non-haematopoietic origin [7, 8]. Furthermore, identification of functional GM–CSF receptors on epithelial tumour cells [9] indicated involvement in stimulation of non-haematopoietic malignant cells with possible tumour progression and suggested an unanticipated role for GM–CSF gene activation in the evolution and metastasis of solid tumours. Clearly further studies with tumour cells both responsive and unresponsive to haematopoietic growth factors could yield more insight into the biology and growth-controlling mechanisms of neoplasia as well as providing information as to the optimal clinical utility of these cytokines.

With this background the EORTC Clonogenic Assay Screening Study Group (CASSG) decided to monitor *in vitro* responsiveness to GM–CSF using a range of human tumour cell lines and freshly biopsied material. Members of CASSG have formed a network of Units with proven ability to evaluate the efficacy of antitumour agents against well characterised continuous tumour cell lines maintained in their individual laboratories and/or fresh biopsy material, utilising their own specific *in vitro* assay procedures [10]. "Normalisation" studies have been used to verify interlaboratory comparability [11]. In this manuscript we summarise data derived from five laboratories relating to the *in vitro* growth modulating effects of GM–CSF on a series of 18 human tumour cell lines, predominantly of non-haematopoietic origin, together with preliminary results from 30 biopsy specimens and five normal bone marrow aspirates from patients without malignant disease.

MATERIALS AND METHODS

GM–CSF

E. coli synthesised, non-glycosylated, recombinant human GM–CSF with a specific activity of 3×10^6 units per mg protein was kindly provided for these studies by Dr J.J. Mermod (Glaxo Institute for Molecular Biology, Geneva). Aliquots were stored

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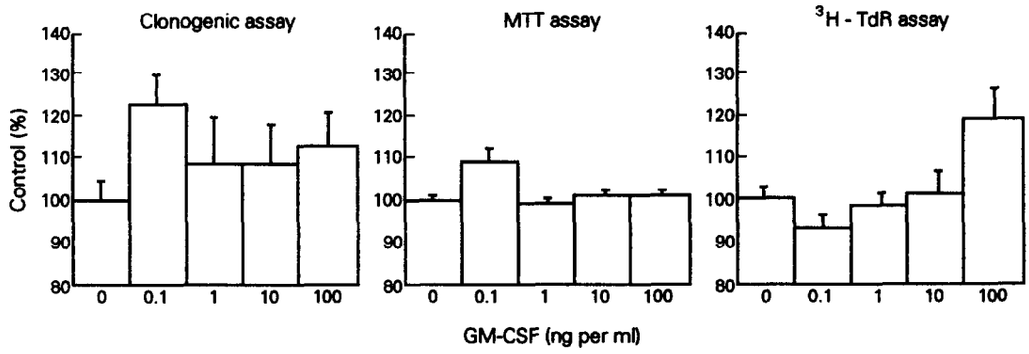

Fig. 1. Influence of GM–CSF addition, under conditions of continuous exposure, on the growth of MCF-7 cells *in vitro*, as measured by clonogenic assay, MTT assay or a ^{3}H-TdR assay. The values represent the mean (S.D.) of four experiments in triplicate.

in liquid nitrogen in phosphate buffered saline (PBS) containing 1 mg/ml bovine serum albumin. A range of concentration of GM–CSF from 0.1 to 1000 ng/ml i.e. 0.3–3000 units/ml were generally tested using conditions of continuous exposure.

Cell lines and culture conditions

The range of human tumour cell lines tested were derived from different tumour types and obtained from different sources. Some of the details are listed below and the references cited provide further information as to their origins and the culture conditions used. All tissue culture media used contained 10% foetal calf serum (FCS). Cell lines were breast carcinoma lines MCF-7 (Imperial Cancer Research Fund Laboratories, London and Department of Animal Biology, University of Torino), EVSA-T (Istituto Tumori di Genova), Hs 578T and T47D (Department of Animal Biology, University of Torino), all cultured in Dulbecco's MEM medium; colon carcinoma line WiDR (American Type Culture Collection), cultured in MEM medium with 1% non-essential aminoacids, COLO 205 cells [12] maintained in RPMI 1640 medium, lines EPC86–197, EPC86–216 and EPC85–257 (Institut fur Pathologie, Christina Albrecht Universitat zu Kiel) maintained in Leibovitz L15 medium with additions, as detailed earlier [13]; rectal carcinoma line HRT18 (Istituto Zooprofilattico de Brescia) cultured in RPMI 1640 medium; pancreatic carcinoma EPP85-181 cells (Institut fur Pathologie, Christina Albrect Universitat zu Keil) maintained in Leibovitz L15 medium with additions, as detailed earlier [13]; promyelocytic leukemia line HL60 (Department of Haematology, Hospital of Careggi, Florence) cultured in RPMI 1640 medium; ovarian carcinoma lines SKOV3 (American Type Culture Collection) cultured in RPMI 1640 medium and JAT and TR170 [14] maintained in Ham's F12 medium; testicular teratoma SuSa cells [15] cultured in RPMI 1640 medium; neuroblastoma CHP212 cells [16] cultured in RPMI 1640 medium.

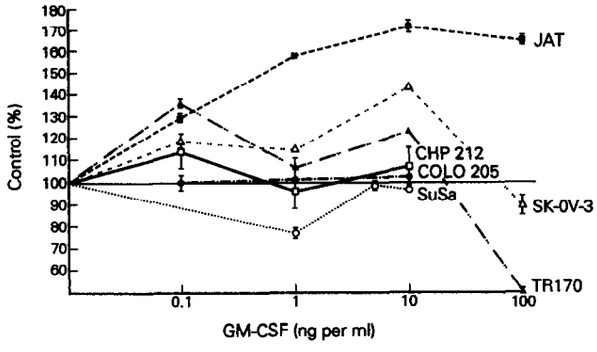

Fig. 2. Effects on the colony-forming efficiency of a range of human tumour cell lines incubated in the continuous presence of various GM–CSF concentrations. The values represent the mean (S.D.) of 2–4 experiments involving quadruple samples.

Growth modulation assays

Colony forming assays. The following procedures were adopted for the specified cell lines. The double layer soft agar assay according to Hamburger and Salmon [17] was used for MCF-7 cells, that of Courtenay *et al.* [18] for the TR170, JAT and SuSa cell lines, a soft agarose assay was employed with CHP212 [16] and COLO 205 (12) cells, whilst a methylcellulose-based clonogenic assay was employed working directly with biopsy specimens [19].

Colorimetric assays. For MCF-7 cells the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) assay, as described by Tada *et al.* [20] was followed utilising 10^5 cells/well/24-well plate in 1 ml phenol-red-free RPMI 1640 medium plus serum and a 4-day incubation with GM–CSF. For the following cell lines: WiDR, EVSA-T, HRT 18, Raji, Hs 578T, T47D, HL-60, as well as MCF-7 cells and cells obtained from bone marrow aspirates a (2,4-iodophenyl)-3-4-nitrophenyl)-5-phenyl-tetrazolium chloride (INT) (Sigma) assay, as described by Bernabei *et al.* [21] was used, involving the plating of 5×10^3 cells/well/96-well plate in phenol red-free RPMI 1640 medium plus serum and involving a 72-h incubation with GM–CSF.

Monolayer proliferation assay. The procedure adopted with the cells is detailed in an earlier publication [13, 22]. Serum-containing medium was used with continuous incubation with GM–CSF and both haemocytometer cell counts and DNA content/dish were monitored over a 9-day period.

Tritiated thymidine incorporation assay. MCF-7 cells were incubated with GM–CSF for 4 days and then pulsed with tritiated-thymidine (^{3}H-TdR) for 4 h before quantitating acid-precipitable radioactivity [23].

Statistics

Student's *t* test was used for statistical evaluations.

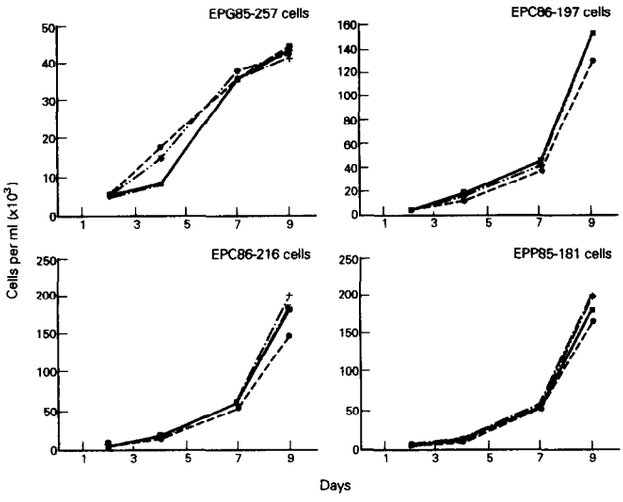

Fig. 3. Data indicating a general lack of effect of continuous exposure to a range of GM–CSF concentrations on the growth rates of four human tumour cell lines. Each point represents the mean (S.D.) cell count of triplicate cultures. • Control; * 0.5 ng/ml GM–CSF; + 1.0 ng/ml GM–CSF; ■ 10 ng/ml GM–/CSF.

RESULTS

Data shown in Fig. 1 indicate that addition of GM–CSF to cultures of MCF-7 human breast carcinoma cells failed to influence growth/survival as judged by clonogenic assay, MTT assay or incorporation of ^{3}H-TdR. Similar results were obtained using each of these differing end points.

Further evaluation by clonogenic assay of a series of cell lines, however, showed some enhancement of colony forming efficiency (CFE) following GM–CSF addition in 3/3 ovarian lines, but not in three other lines derived from colon (COLO 205), neuroblastoma (CHP212) or a teratoma (SuSa), as shown in Fig. 2. In the ovarian tumour cell lines the effect of GM–CSF addition showed some dose dependency with a definite inhibition occurring at the highest concentration tested of 100 ng/ml in two of the three lines. These effects of GM–CSF were not related to the inherent CFEs of the cell lines tested since in the three ovarian lines CFEs ranged from 0.5–15%, whilst a lack of stimulation was noted in SuSa cells with a CFE of 4% as well as in the COLO 205 cells with a 30% CFE.

Since many of these cell lines tested had been originally established in the 1970s, with the exception of lines TR170 and JAT, and had subsequently been maintained in culture for long periods, the growth modulating effects of GM–CSF were next evaluated using some recently derived lines, three from colon carcinomas and one from a pancreatic carcinoma. The results in Fig. 3 indicate that the addition of this factor did not influence the growth rates of any of these cell lines with this lack of effect being recorded, not only when the results were expressed in terms of cell number, but also cellular DNA content rather (data not shown).

Table 1 summarises data obtained using the INT assay and evaluating eight cell lines from a range of different tumour types. The lack of effect of GM–CSF addition on MCF-7 cells is again noted in this study and was apparent in six of the other seven lines tested. However, significant growth stimulation was recorded with the Hs 578T breast carcinoma cell line, at all GM–CSF concentrations tested. In this study evidence of GM–CSF growth inhibitory effects were recorded only in one of the eight lines tested, namely the RAJI cells at concentrations of 100 and 1000 ng/ml.

Overall GM–CSF addition appeared therefore rarely to stimulate cell/colony growth of human tumour cell lines, with significant positive activity being noted only in four of the eighteen lines tested. In contrast, data in Fig. 4 indicates significant effects of GM–CSF on each of the five samples of normal bone marrow aspirates evaluated. The effects of GM–CSF have also been tested on normal bone marrows using the methylcellulose assay, adapted for haematopoietic cultures. Dose-response curves indicated that the *in vitro* concentration allowing maximal stimulation of haematopoietic progenitors was 3 ng per ml (data not shown). Preliminary data, however, failed to identify any significant growth modulation of tumour cell growth when testing biopsy specimens from ovarian cancers directly in a methylcellulose clonogenic assay system (Table 2).

DISCUSSION

A survey of the literature relating to the growth modulating effects of GM–CSF in human tumour cell lines *in vitro* suggests that positive results have been obtained only in a minority of tests. Data from five recent publications and this present study, summarised in Table 3, show positive results in 28% of the lines evaluated. The number of positives recorded in this present

Table 1. In vitro *tumour cell growth modulation by continuous exposure (72 h) to a range of concentrations of GM–CSF (INT assay)*

Cell line	GM–CSF concentrations (ng per ml)					
	0	0.1	1.0	10	100	1000
HL-60	100 (5.2)	97.1 (4.9)	101.7 (4.2)	92.1 (4.7)	102.0 (5.0)	95.7 (4.1)
T47D	100 (6.8)	93.7 (6.1)	91.5 (8.3)	97.6 (4.9)	98.4 (5.7)	99.2 (6.1)
MCF-7	100 (5.2)	104.8 (4.3)	104.8 (4.5)	103.4 (3.9)	104.8 (5.1)	106.4 (5.3)
RAJI	100 (8.9)	96.6 (7.4)	90.0 (8.8)	90.3 (8.9)	*84.0 (9.1)	*86.0 (8.7)
HRT 18	100 (8.5)	105.7 (8.1)	111.4 (9.3)	94.3 (7.4)	92.9 (8.3)	99.2 (7.3)
EVSA-T	100 (7.1)	109.3 (9.9)	90.7 (9.8)	94.4 (8.6)	96.1 (7.8)	98.1 (7.0)
WiDR	100 (4.6)	104.3 (4.8)	98.5 (4.3)	103.3 (3.8)	99.2 (4.1)	101.1 (3.7)
HS578T	100 (7.0)	*116.0 (6.8)	*113.2 (7.5)	†120.6 (7.1)	†122.7 (6.8)	†121.7 (6.9)

Data are the mean (coefficient of variation) of one experiment, involving six estimations per assay. Experiments were repeated twice, with similar results obtained.
* $P < 0.05$ and † $P < 0.01$.

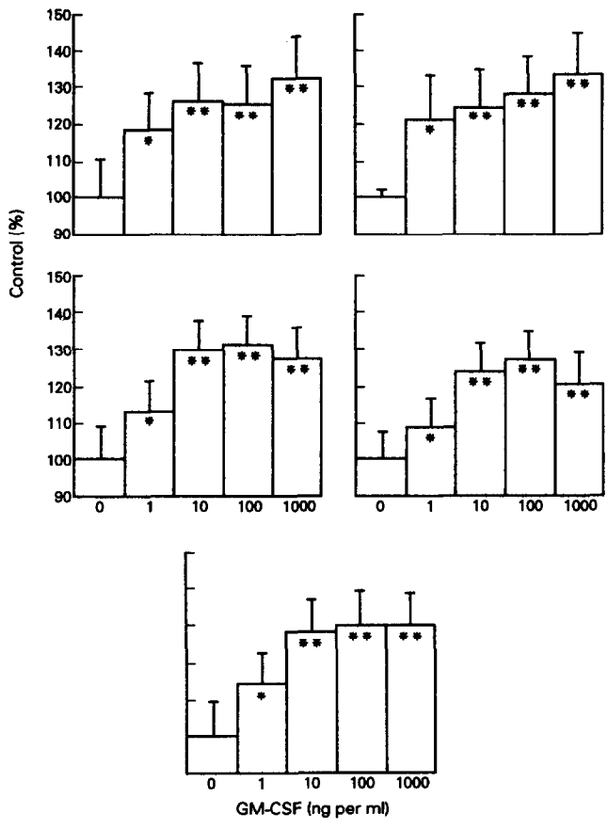

Fig. 4. Positive influence of GM–CSF addition on the proliferation of cells derived from bone marrow aspirates, as judged by the INT assay using conditions of continuous exposure. Each point represents the mean (coefficient of variation) of six estimations. Experiments were repeated twice and similar results were obtained.

study is slightly lower (22%) than those reported by certain other groups [7, 8, 24]. Our study has been more extensive investigating 18 cell lines overall. In addition, our study has involved the use of a variety of assays with different end points, which again reflect the range of procedures adopted by others. No evidence has been presented from any of these laboratories that growth factor activities can be preferentially identified by any of these specific assay methodologies, since growth modulation has been quantitated by clonogenic assay [8, 9, 25, 26] by metabolic/colorimetric assay [this study] and by assessing ^{3}H-TdR incorporation [7, 9]. Unfortunately, however, several

Table 2. In vitro tumour cell growth modulation by GM–CSF in human ovarian carcinoma tumour biopsy specimens (methylcellulose clonogenic assay)

Samples tested	25
Growth* as control	12
Growth stimulation (> 25%)	0
Growth decreased (> 25%)	4
No growth	8
Not evaluable	1†

10^5 cells were plated in serum-containing medium together with 3 ng per ml GM–CSF.
*Defined as 20 or more colonies.
†Macrophage colonies only formed.

Table 3. Overview of certain published data relating to the growth modulatory effects of GM–CSF on continuous human tumour cell lines in vitro

Tumour type	No. of lines tested	No. of lines stimulated by GM–CSF	Ref.
Breast	1	1 (MCF-7)	8
	1	1 (BT20)	23
	4	1 (Hs578T)	This study
Colon	4	0	This study
	3	2 (HTB38, CC6187)	9
Ovary	3	3 (SKOV3, TR170, JAT)	This study
Neuroblastoma	2	0	8
	1	0	This study
Osteosarcoma	2	2 (MG-63, HOS)	8
Hypernephroma	1	1	23
Lung			
Small cell	2	1 (NCT-H69)	10
	5	0	24
	6	0	25
Non-small cell	1	1 (CCL185)	23
	9	0	25
Rectum	1	0	This study
Prostate	1	0	This study
Lymphoma	1	0	This study
Leukaemia	1	0	This study
Promyelocytic	1	1 (HL60)	10

of the "positive" observations recorded have not been confirmed by other groups working with "comparable" cell lines. For example, whilst Dedhar *et al.* [7] reported growth stimulation in MCF-7 cells by GM–CSF addition, this contrasts with the lack of effect recorded by this present study by two members of our group working independently. This discrepancy however should be viewed with the knowledge that MCF-7 cell populations distributed in laboratories throughout the world are known to possess diverse characteristics [27]. Similar controversy appears to surround the data obtained with small cell lung cancer cell lines with stimulation [9], inhibition [26] or no effect [25] variously reported by groups all evaluating GM–CSF addition to cultures of NCI-H69 cells. In this study stimulation of HL60 cells was not noted, in contrast to that observed by Baldwin *et al.* [9], although this apparent difference may relate to the FCS concentrations present, since Begley *et al.* [28] reported that stimulation of proliferation of HL60 cells was most evident using 2% FCS. For colon cancer lines however, both our study and that of Berdel *et al.* [8] have reported a consistent lack of effect in WiDR cells, although these latter authors describe stimulation in two other colon lines contrasting with a general absence of effect observed in all four lines tested in this study. The possibility that response to the growth stimulatory properties of GM–CSF may be lost or diminished in cell lines passaged many times in culture also needs further evaluation by testing early passage cells from newly established lines.

In spite of certain inconsistencies however, these results from various laboratories throughout the world provide evidence that GM–CSF can stimulate the growth of certain non-haemato-

poietic malignant cell lines *in vitro*. In view of the relatively small number of tumour lines tested to date it is not possible clearly to identify any particular tumour cell type which is most likely to exhibit a proliferative response to GM–CSF addition, although further studies would appear to be warranted in tumour cell lines from breast and ovary and possibly colon cancer and perhaps also osteosarcoma. Such studies might best be devised to include an analysis of normal bone marrow aspirates as a positive control, since both we and others [29, 30] have reported consistent stimulation using this material. Further evaluation of fresh tumour biopsy specimens is also definitely indicated. Although our preliminary data failed to identify growth modulation, some growth stimulation/inhibition was observed in breast, colon and lung tumours in an earlier study by Salmon and Liu [30]. In view of its potential importance groups with access to fresh tumour material should be encouraged to pursue these investigations.

The clinical significance of any *in vitro* observations must be viewed with caution. Whilst in our study growth stimulation of some cell lines was observed at clinically obtainable sustained GM–CSF concentrations (peak plasma concentrations of 3.6–16.2 ng per ml being reported after subcutaneous administration, with levels above 2 ng per ml being observed for 6–20 hours [31]), many authors have used much higher *in vitro* concentrations. Tumour cell lines may not be representative of most tumours since they are derived from those rare samples that adapt to *in vitro* culture. Studies with fresh tissue are complicated by the presence of many accessory cells potentially capable of response to many growth factors and of producing growth factors themselves. Our data and those of other groups, point out the need for careful observation of patient outcome in clinical studies, but the very limited effects observed *in vitro* to date appear to make it unlikely that these data will be translated into *in vitro* stimulation on non-haematopoietic tumours.

1. Gabrilove J. Introduction and overview of hematopoietic growth factors. *Semin Hematol* 1989, **26**, 1–4.
2. Metcalf D. Haemopoietic growth factors 1. *Lancet* 1989, **ii**, 825–827.
3. Donahue RE, Wang EA, Stone DK, *et al.* Stimulation of haematopoiesis in primates by continuous infusion of recombinant human GM–CSF. *Nature* 1986, **321**, 872–875.
4. Mayer T, Lam C, Oberious H, Lehl E, Besemer J. Recombinant human GM–CSF induces leukocytosis and activates peripheral blood polymorphonuclear neutrophils in nonhuman primates. *Blood* 1987, **70**, 206–213.
5. Foon KA. Biological response modifiers: the new immunotherapy. *Cancer Res* 1989, **49**, 1621–1639.
6. Steward WP, Thatcher N, Kaye SB. Clinical applications of myeloid colony stimulating factors. *Cancer Treat Rev* 1990, **17**, 77–87.
7. Dedhar S, Gaboury L, Galloway P, Eaves C. Human granulocyte–macrophage colony-stimulating factor is a growth factor active on a variety of cell types of nonhemopoietic origin. *Proc Natl Acad Sci USA* 1988, **85**, 9253–9257.
8. Berdel WE, Danhauser-Reidl S, Steinhauser G, Winton EF. Various human hematopoietic growth factors (interleukin-3, GM–CSF, G-CSF) stimulate clonal growth of nonhematopoietic tumour cells. *Blood* 1989, **73**, 80–83.
9. Baldwin GC, Gasson JC, Kaufman SE, *et al.* Nonhematopoietic tumour cells express functional GM–CSF receptors. *Blood* 1989, **73**, 1033–1037.
10. Aapro MS, Eliason JF, Herman CF, Rozenweig M. The EORTC clonogenic assay screening study group program. In: Hall TC, ed. *Prediction of Response to Cancer Therapy*. New York, Alan Liss, 1988, 255–264.
11. Eliason JF, Suardet L. Normalization studies I and II. In: Dittrich C, Aapro M, eds. *Drugs, Cells and Cancer*. Vienna, Welley, 1990, 52–53, 56–57 and 60–61.
12. Hill BT, Whatley SA, Bellamy AS, Jenkins LY, Whelan RDH. Cytotoxic effects and biological activity of 2-aza-8-germanspiro-[4,5]-decane-2-propanamine-8, 8-diethyl-N,N-dimethyl dichloride (NSC 192965); Spirogermanium) *in vitro*. *Cancer Res* 1982, **42**, 2852–2856.
13. Dietel M, Arps H, Gerding D, Trapp M, Sieck M, Niedorf A. Effectiveness of mitoxantrone on the proliferation of cell cultures derived from malignant mesenchymal tumours of human origin. *J Cancer Res Clin Oncol* 1988, **114**, 197–203.
14. Hill BT, Whelan RDG, Gibby EM, *et al.* Establishment and characterisation of three new human ovarian carcinoma cell lines and initial evaluation of their potential in experimental chemotherapy studies. *Int J Cancer* 1987, **39**, 219–225.
15. Hill BT, Shellard SA, Hosking LK, Fichtinger-Schepman AM, Bedford P. Enhanced DNA repair and tolerance of DNA damage associated with resistance to *cis*-diammine-dichloroplatinum (II) after *in vitro* exposure of a human teratoma cell line to fractionated X-irradiation. *Int J Radiat Oncol Biol Phys* 1990, **19**, 75–83.
16. Hill BT, Whelan RDH, Hosking LK. Use of human neuroblastoma continuous cell lines for *in vitro* drug sensitivity screening. *Invest New Drugs* 1988, **6**, 11–18.
17. Hamburger AW, Salmon SE. Primary bioassay of human tumour stem cells. *Science* 1977, **197**, 461–463.
18. Courtenay VD, Selby PJ, Smith IE. Growth of human tumour cell colonies from biopsies using two soft-agar techniques. *Br J Cancer* 1978, **38**, 77–81.
19. Aapro MS, Eliason JF, Knauer F, Alberto P. Colony formation *in vitro*: a prognostic indicator for primary breast cancer. *J Clin Oncol* 1987, **5**, 890–896.
20. Tada H, Shiho O, Kuroshima K, Koyama M, Tsukamoto K. An improved colorimetric assay for interleukin 2. *J Immunol Meth* 1986, **93**, 157–165.
21. Bernabei PA, Santini V, Silvestro L, *et al.* *In vitro* chemosensitivity testing of leukemic cells: development of a semiautomated colorimetric assay. *Haematol Oncol* 1989, **7**, 243–253.
22. Dietel M, Arps H, Lage H, Niendorf A. Membrane vesicle formation due to acquired Mitoxantrone resistance in human gastric carcinoma cell line EPG85–257. *Cancer Res* 1990, **50**, 6100–6105.
23. Jakesz R, Smith CA, Aitken S, *et al.* Influence of cell proliferation and cell cycle phase on expression of estrogen receptor in MCF-7 breast cancer cells. *Cancer Res* 1984, **44**, 619–625.
24. Nachbaur D, Denz H, Zwierzina H, Schmalzl F, Huber H. Stimulation of colony formation of various human carcinoma cell lines by rhGM–CSF and rhIL-3. *Cancer Lett* 1990, **50**, 197–201.
25. Ruff MR, Farrar WL, Pert CB. Interferon γ and granulocyte/macrophage colony-stimulating factor inhibit growth and induce antigens characteristic of myeloid differentiation in small-cell lung cancer cell lines. *Proc Natl Acad Sci* 1986, **83**, 6613–6617.
26. Munker M, Munker R, Saxton RE, Koeffler HP. Effect of recombinant monokines, lymphokines, and other agents on clonal proliferation of human lung cancer cell lines. *Cancer Res* 1987, **47**, 4081–4085.
27. Osborne CK, Hobbs K, Trent JM. Biological differences among MCF-7 human breast cancer cell lines from different laboratories. *Breast Cancer Res Treat* 1987, **9**, 111–121.
28. Begley CG, Metcalf D, Nicola NA. Purified colony stimulating factors (G-CSF and GM–CSF) induce differentiation in human HL60 leukemic cells with supression of clonogenicity. *Int J Cancer* 1987, **39**, 99–105.
29. Tomonaga M, Golde DW, Gasson JC. Biosynthetic (recombinant) human granulocyte-macrophage colony-stimulating factor; effects on normal bone marrow and leukemia cell lines. *Blood* 1986, **67**, 31–36.
30. Salmon SE, Liu R. Effects of granulocyte-macrophage colony-stimulating factor on *in vitro* growth of human solid tumours. *J Clin Oncol* 1989, **7**, 1346–1350.
31. Thompson JA, Lee DJ, Kidd P, *et al.* Subcutaneous granulocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome: toxicity, pharmacokinetics, and hematological effects. *J Clin Oncol* 1989, **7**, 629–637.

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