Table 2. Neurological diagnoses in solid tumour (by frequency of occurrence)

	Breast	Lung	GI tract	Ovary	Head and neck	Prostate
Pain	65	12	10	4	15	9
Radiculopathy	52	11	8	2	2	10
Brachial plexopathy	44	7	1	0	5	0
Lumbosacral plexopathy	2	0	17	4	0	2
Polyneuropathy	8	1	2	23	1	1
Mononeuropathy	6	3	1	1	5	2
Brain metastasis	31	37	6	1	2	1
Spinal cord compression	24	8	5	0	4	13
Encephalopathy	6	10	5	3	3	0
Neoplastic meningitis	9	4	0	0	0	0
Other	80	18	9	25	19	11
Total	327	111	64	63	56	49

In non-Hodgkin's lymphoma, involvement of the leptomeninges (meningitis lymphomatosis) was the most frequent neurological diagnosis, followed by radiculopathy.

Paraneoplastic syndromes were rare: only 6 of our patients were presumed to have a paraneoplastic complication (mainly polyneuropathy and cerebellar ataxia). 161 patients, not included in the 1105 patients in Table 1, had neurological problems not related to their tumour (e.g. migraine, prolapsed lumbar disc or ischaemic brain infarction).

Since our survey was cross-sectional and not longitudinal, the figures refer only to those patients seen by the neurologist in 2 years and cannot indicate the risk of each patient developing neurological problems.

Our results show the spectrum of neurological disease to be expected in cancer patients. About 30% of patients seen by the neurologists had breast cancer. This high frequency was probably related to the long survival of patients with metastatic breast disease. Lung cancer is a frequent and well-known cause of neurological disease [3] and was the second most frequently encountered primary tumour in our series (10%). Nevertheless, the referral index (0.67) was low. Cisplatin-induced neuropathy is the cause of the high frequency and referral index in ovarian cancer. In addition almost all patients with ovarian cancer were examined as part of an evaluation of a new treatment to prevent such neuropathy [4].

A high referral index (1.72) was seen in acute lymphoblastic leukaemia. Since treatment of this disease includes prophylaxis of the central nervous system and is potentially neurotoxic, neurological consultation is often requested. In addition, leukaemic meningitis, opportunistic infection and vincristin-induced polyneuropathy may develop.

Pain was a common cause for neurological consultation. In about half the patients referred for pain, a specific neurological cause could be found (e.g. radiculopathy or plexopathy). Another common cause was osseous metastasis, usually in the spine or pelvis.

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Small Cell Lung Cancer Cell Line from Histologically and Immunocytochemically Negative Bone Marrow

Michele J. Everard, Valentine M. Macaulay, Toon Min, John L. Millar and Ian E. Smith

ROUTINE HISTOLOGICAL examination detects bone marrow involvement in 10–30% of untreated patients with small cell lung cancer (SCLC) [1], whereas immunocytochemistry with anti-SCLC monoclonal antibodies [1,2] detects SCLC cells in 40–75% of bone marrows. Carney *et al.* [3] were unable to culture tumour cells *in vitro* from any histologically negative samples, but SCLC cells have been grown from histologically negative bone marrow samples where immunocytochemistry has revealed tumour cell infiltration [2]. We report a SCLC cell line established from a bone marrow in which tumour cells were not detectable by histological and immunocytochemical criteria.

In February 1986, a 52-year-old woman presented with SCLC and liver metastases. Tumour cell infiltration was not found by routine histological examination of a pre-treatment bone marrow aspirate. Culture of a duplicate sample showed no tumour cell growth over 10 weeks. The patient underwent six courses of chemotherapy with carboplatin, etoposide and ifosfamide, resulting in a partial response. 1 month later she relapsed with meningeal involvement and a persistently low blood count. At this time (October 1986) a second bone marrow aspirate was taken. 2 weeks later the patient died. There was no necropsy.

Tumour cells were not detected by histological examination of smears and clot sections of the second aspirate. Immunocytochemistry with antibodies to epithelial membrane antigen (EMA) [4] and low molecular weight keratins (CAM5.2) [5] also failed to reveal malignant cells. A duplicate marrow sample was collected in RPMI 1640 containing 4 mmol/l glutamine and 50 U/ml preservative-free heparin, layered on Ficoll-Hypaque

Correspondence to: M.J. Everard, Department of Medicine, F Block, Institute of Cancer Research, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, U.K.
M.J. Everard, V.M. Macaulay, J.L. Millar and I.E. Smith are at the Department of Medicine, Institute of Cancer Research and Lung Unit, Royal Marsden Hospital, Sutton, Surrey, U.K. and T. Min is at the Department of Cytogenetics, Institute of Cancer Research and Royal Marsden Hospital, Sutton, Surrey, U.K.

and centrifuged at 90 *g* for 20 min. Nucleated cells at the interface were collected, washed in RPMI and centrifuged at 550 *g* for 10 min. The cells were inoculated into a 25 cm^2 flask containing RPMI 1640 supplemented with hydrocortisone, insulin, transferrin, oestradiol and selenium (HITES) [6] and 2% foetal calf serum (FCS) and incubated at 37°C in 85% N_2, 5% CO_2 and 10% O_2.

After 6 weeks, loose branch-like floating aggregates of cells lacking central necrosis were seen. This corresponds to the type III morphology for SCLC [3]. The cells were subcultured every 2–3 weeks by splitting 1:2 into fresh growth medium. Within 2 months growth was sustainable at an inoculum of 5×10^4–10^5/ml. Stocks were maintained in 80 cm^2 tissue culture flasks in RPMI 1640 supplemented with 5% FCS. The cell line was designated ICR-SC65. It has undergone about 60 passages and is free of mycoplasma (Flow). A cell pellet examined by electron microscopy showed no neurosecretory granules.

Growth kinetics were studied by passaging a single cell suspension at 10^5 cells per ml in RPMI plus 5% FCS. Every 2–3 days viable cells were counted by trypan blue exclusion. ICR-SC65 has a doubling time of 34 h, with 75% viability in exponential growth phase. Colony-forming efficiency (CFE) was assessed by layering 5×10^4 viable cells in 0.5 ml 0.3% agar onto an underlay of 1 ml 0.5% agar. The agar was diluted to its final concentrations with double-strength RPMI 1640 supplemented with 20% FCS (giving a final concentration of 10% FCS in the dish). Colonies containing more than 50 cells were counted after 21 days incubation at 37°C in a humidified atmosphere of 5% CO_2 in air. CFE was 2.5%.

The cell line was examined for the expression of four markers that characterise SCLC [3]. Levels of neurone specific enolase (NSE) [7] and creatine kinase-BB (CKBB) [8] were high, with undetectable levels of dopa decarboxylase (DDC) [9] and bombesin-like immunoreactivity (BLI) [10] (Table 1). Cytogenetic analysis showed a partially triploid karyotype of human origin. There was a consistent interstitial deletion in chromosome 3, which is strongly associated with SCLC [11].

On the evidence of type III morphology, lack of neurosecretory granules, growth kinetics, marker expression and karyotype, ICR-SC65 is a morphological variant SCLC cell line [3].

Table 1. *Characteristics of ICR-SC65*

	NCI reference data* [3]		
	Classic	Variant	ICR-SC65
Morphology	I II	III IV	III
Doubling-time (h)	71 (31)	32 (2)	34
CFE (%)	2.3	13.4	2.5
Biomarkers†			
DDC (nmol/h/mg)	149 (33)	<0.1	<0.1
BLI (pmol/mg)	3.7 (0.9)	<0.01	<0.01
NSE (ng/mg)	1472 (239)	422 (88)	833
CKBB (ng/mg)	6190 (903)	5878 (113)	8594
NSG	+	–	–

Mean (S.E.M.) where appropriate.
*I = tight spheroids in suspension, II = irregular dense floating aggregates, III = loose floating aggregates and IV = monolayer.
†Per mg soluble protein (Bradford reaction).
NSG = neurosecretory granules.

Bone marrow that appears histologically and immunologically normal may contain micrometastases, capable of giving rise to a cell line which possesses all the characteristics typical of SCLC.

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Changes in T Lymphocyte Subsets after Single Dose Epirubicin

P. Lissoni, G. Tancini, C. Archili, R. Rescaldani, G. Cattaneo, S. Crispino and S. Barni

THE influence of cytotoxic drugs on lymphocyte subsets, which could affect host–tumour interaction, has still to be defined [1]. Low-dose cyclophosphamide selectively reduced T suppressor cells [2, 3], while doxorubicin stimulated interleukin-2 (IL-2) production [4] and lymphokine-activated killer cell generation [5]. We have investigated early changes in lymphocyte subsets in relation to clinical response in breast cancer treated with weekly low-dose epirubicin.