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

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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.

15 consecutive patients with metastatic breast cancer (median age 62 years, range 48–72), unable to tolerate conventional chemotherapies, were treated intravenously with at least four cycles of epirubicin 25 mg/m^2 per week (median number of cycles was nine). In pretreated patients epirubicin was started at least 1 month after the last chemotherapeutic cycle. No patient received steroids during the study. Venous blood samples were collected during the morning before and 7 days after the first cycle only.

Total T (CD3), T helper (CD4), and T suppressor/cytotoxic (CD8) lymphocytes were measured by flow cytometry and monoclonal antibodies (Becton-Dickinson). Data were analysed by χ^2 and t tests.

Characteristics of patients are reported in Table 1. According to WHO criteria, a partial response was seen in 8 (53%) patients (median duration 7 months). Response rate was significantly higher in patients whose CD4/CD8 ratio increased than in those in whom it diminished after the first cycle (Table 1). Increased CD4/CD8 ratio was not different between chemotherapeutically pretreated and untreated patients (2/6 vs. 5/9). Within the responders, mean CD4/CD8 ratio increased while mean CD8 number fell (both significantly) after treatment; CD4 cell number rose non-significantly. Patients with progressive disease had lower mean CD4/CD8 ratios after than before epirubicin (not significant) whereas CD4 cell number was significantly lower after treatment. No change was seen in CD8 cells.

A single low dose of epirubicin was enough to modify T lymphocyte subsets, as previously described with doxorubicin [6]. Because of the stimulatory action of anthracyclines on IL-2 production [4, 6], the changes in CD4/CD8 ratio might be related to changes in IL-2 secretion, whose role in the regulation of T cell differentiation is well known.

The normalization of a previously reduced CD4/CD8 ratio after the first epirubicin cycle may be a favourable prognostic factor for predicting the clinical response. Also the efficacy of anthracyclines may not depend only on their cytotoxic action, but also on a possible improvement in immune status [7]. However, in our study monitoring ended after the first cycle. Since CD8 cells consist of lymphocytes with different functions, more detailed investigations with other monoclonal antibodies will be required to establish whether changes in CD8 percentage depend on variations in suppressor or cytotoxic cells.

If immunomodulating properties of epirubicin are confirmed, this drug could be used with IL-2 in the immunotherapy of cancer.

Table 1. Mean (S.E.) cell counts before and after first cycle of epirubicin

	Non-responders (n = 7)	Responders (n = 8)
White cells (×10^3/μl)		
Pre	6.9 (0.6)	7.0 (0.5)
Post	5.3 (0.5)	5.8 (0.5)
Lymphocytes (×10^3/μl)		
Pre	1.9 (0.3)	1.7 (0.3)
Post	1.2 (0.2)	1.3 (0.2)
CD3 (μl)		
Pre	1191 (204)	1088 (74)
Post	759 (165)	921 (95)
CD4 (μl)		
Pre	481 (54)	583 (62)
Post	263 (32)*	518 (43)
CD8 (μl)		
Pre	678 (139)	543 (59)
Post	490 (115)	334 (37)*
CD4/CD8		
Pre	0.9 (0.1)	1.1 (0.1)
Post	0.7 (0.2)	1.6 (0.1)*

*P <0.05 vs. pretreatment value in same group.

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Treatment of Advanced Hodgkin's Disease with Lomustine, Etoposide, and Prednimustine

Reginhard von Hirschhausen, Berthold Steinke and Johannes G. Saal

THERE IS no generally accepted chemotherapy for patients with advanced Hodgkin's disease who have not responded primarily or secondarily to cyclophosphamide/vincristine/prednisone or doxorubicin/bleomycin/vinblastine/dacarbazine (COPP/ABVD) or who had an early relapse. Santoro *et al.* [1] suggested a