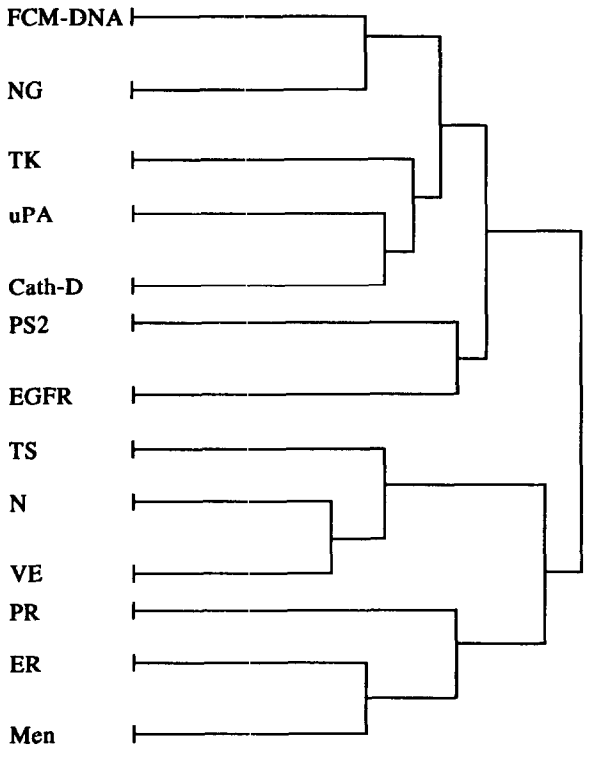


**Figure 2. Hierarchical variable clustering. Representation of the 13 variables by a hierarchical tree or dendogram which provides homogeneous partitions of the variables. Abbreviations: see Figure 1.**

hormone sensitivity, whereas EGFR and pS2 appeared relatively isolated. Figure 2 confirms the variable clustering obtained by the PCA.

This study shows the strong correlation between tumour size, node invasion and vascular emboli, and a clear separation between this group of factors and all other biological factors, including nuclear grade. These results are in keeping with Mittra and Mac Rae's meta-analysis, in which vascular emboli were not studied. They also emphasize the fact that so-called clinical factors differ in kind from other factors, although our data do not support the chronological/biological interpretation.

1. Mittra I, Mac Rae KD. A meta-analysis of reported correlations between prognostic factors in breast cancer: does axillary lymph node metasasis represent biology or chronology? *Eur J Cancer* 1991, **27**, 1574–1583.
2. Editorial. *Lancet* 1992, **340**.
3. Anderson TW. *An Introduction to Multivariate Statistical Analysis*, 2nd edn. New York, John Wiley, 1984.
4. JC Gower, GJS Ross. Minimum spanning trees and single linkage cluster analysis. *J R Stat Soc* 1969, **18**, 54–64.
5. Spyratos F, Martin PM, Hacène K, *et al.* Multiparametric prognostic evaluation of biological factors in primary breast cancer. *J Nat Cancer Inst* 1992, **84**, 1266–1272.
6. Le Doussal V, Tubiana-Hulin M, Friedman S, Hacène K, Spyratos F, Brunet M. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR). *Cancer* 1989, **64**, 1914–1921.

*European Journal of Cancer* Vol. 31A, No. 2, pp. 283–284, 1995.

0959–8049/95 $9.50 + 0.00

0959-8049(94)00482-X

# Circumvention of Doxorubicin-resistance in Tumours by Albumin-conjugated Doxorubicin

**E.W. Pommerenke, H. Sinn and M. Volm**

DOXORUBICIN (DOX) is a clinically important antineoplastic agent, but chemoresistance often prevents successful treatment of cancer patients. Although a panel of compounds (chemosensitisers) have been found to reverse drug-resistance *in vitro* [1], the results of most of the clinical studies have been inconclusive and rather disappointing [2]. Therefore, as a new approach, we analysed the reversing effect of bovine serum albumin-conjugated DOX on DOX-resistant tumours *in vivo*. Protein–drug conjugates have been shown to have a prolonged biological half-life and an increased tumour uptake [3].

The DOX-resistant tumours, murine L1210, revealed a 30-fold resistance to DOX [4]. Solid L1210 tumours were generated by subcutaneous inoculation of ascites tumour cells into mice ($1.5 \times 10^7$ tumour cells/mouse). Coupling of DOX to bovine serum albumin was carried out using the method described previously [3].

Female NMRI mice (Breeding Centre, Hannover, Germany), 6–8 weeks old, bearing DOX-resistant tumours were treated with a single intraperitoneal (i.p.) injection of DOX (8 mg/kg), bovine serum albumin-conjugated DOX (conjugate at equivalent doses of DOX) or 0.9% NaCl solution (each group 10 mice; $n = 30$ mice). Tumour growth was measured daily using calipers and the tumour volume was calculated by the formula $(a^2 \times b)/2$ where $b$ was the largest diameter and $a$ was the diameter perpendicular to $b$.

The *in vitro* effects (increase/decrease on 4th day after treatment) of DOX and bovine serum albumin-conjugated DOX (BSA-DOX) on growth of DOX-resistant solid L1210-tumours of mice compared to untreated tumours (control) is shown in Figure 1. Mice treated with the protein–DOX conjugate had a significant reduction in tumour volume compared to mice treated with DOX alone ($p = 0.023$, Kruskal Wallis test; $p = 0.029$, Wilcoxon rank sum test). Mice treated with doxorubicin alone had no significant changes in tumour volume compared to tumours of control mice. In contrast to unconjugated DOX, the conjugate was well tolerated and no loss of body weight could be found in the conjugate-treated mice (data not shown).

There is increasing evidence that several mechanisms are operational in DOX resistance [5, 6]. One of the most important resistance mechanisms is due to the expression of a 170 kDa membrane glycoprotein which functions as an energy-dependent efflux-pump [7], but glutathione S-transferase-π and topoisomerase II may be additionally involved [8]. However, in the

Correspondence to M. Volm. The authors are at the German Cancer Research Center, P.O. Box 10 19 49, W-69009 Heidelberg, Germany.
Received 12 Sep. 1994; accepted 30 Sep. 1994.

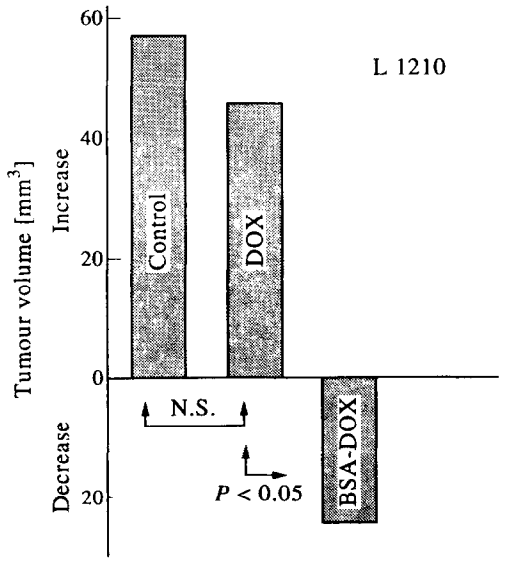


**Figure 1. Increase or decrease in tumour volumes (from day 0 to day 4 after treatment) of solid (DOX)-resistant tumours (L1210). After the solid tumours had reached an average diameter of 5 mm, the animals were treated with DOX alone (8 mg/kg) or bovine serum albumin-conjugated DOX (BSA-DOX; equivalent dose of DOX). The control animals were treated with 0.9% NaCl solution. Mean values (changes of tumour volumes during 4 days) of 10 tumours ($n$ = 30 mice).**

present investigation, we cannot decide whether circumvention of DOX resistance by albumin-conjugated DOX is due to its effects on resistance mechanisms (e.g. blockage of P-glycoprotein-efflux) or belongs to a more general effect, for example, on membrane permeability.

1. Stewart DJ, Evans WK. Non-chemotherapeutic agents that potentiate chemotherapy efficacy. *Cancer Treat Rev* 1989, **16**, 1–40.
2. Raderer M, Scheithauer W. Clinical trials of agents that reverse multidrug resistance. A literature review. *Cancer* 1993, **72**, 3553–3563.
3. Sinn H, Schrenk HH, Friedrich EA, Schilling U, Maier-Borst W. Design of compounds having an enhanced tumour uptake, using serum albumin as a carrier. Part I. *Nucl Med Biol (Int J Rad Appl Instrum Part B)* 1990, **17**, 819–827.
4. Volm M, Bak M, Efferth T, Mattern J. Induced multidrug resistance in murine leukemia L1210 and associated changes in surface membrane glycoprotein. *J Cancer Res Clin Oncol* 1989, **115**, 17–24.
5. Mattern J, Volm M. Multiple pathway drug resistance (Review). *Int J Oncol* 1993, **2**, 557–561.
6. Bradley G, Juranka PF, Ling V. Mechanisms of multidrug resistance. *Biochim Biophys Acta* 1988, **948**, 87–128.
7. Roninson IB. The role of the MDR1 (P-glycoprotein) gene in multidrug resistance *in vitro* and *in vivo*. *Biochem Pharmacol* 1992, **43**, 95–102.
8. Volm M, Mattern J, Efferth T, Pommerenke EW. Expression of several resistance mechanisms in untreated human kidney and lung carcinomas. *Anticancer Res* 1992, **12**, 1063–1068.

*European Journal of Cancer* Vol. 31A, No. 2, pp. 284–287, 1995.

0959–8049/95 $9.50 + 0.00

0959-8049(94)00471-4

# Hereditary Breast Cancer in 19 Females and 2 Males: Kindred, P.G. 1940

**P. Ghadirian, S. Narod, C. Perret, D.M. Lambert, J. Latreille, F. Letendre, M. Monté and D. Belanger**

BREAST CANCER is the most common cancer in women in Québec, with a standardised incidence rate of 70.8/100 000 women [1], and a case-control study showed that 32.6% of breast cancer cases had confirmed positive family history of the same cancer [2]. Hereditary breast cancer is characterised by early onset, bilaterality, multiple primaries, and associations with other cancers [3]. Forms of hereditary breast cancer recognised include site specific breast cancer, breast and ovarian cancer, and breast cancer with associated sarcomas, brain, lung, leukaemia and adrenocortical cancer (Li-Fraumeni syndrome).

Retrospective case-control studies have shown that a family history of breast cancer or any cancer is more common among male cases with breast cancer than controls [4–6]. A prospective population-based case-control study demonstrated that while there was an elevated risk of cancer among first degree relatives of men with breast cancer, there was no such risk among their wives, indicating a genetic, and not a shared environmental risk [7].

The standardised incidence rate of male breast cancer ranges from 0.16/100 000 (Japan) to 1.06/100 000 (Israel), at rates of approximately 1% of those of female breast cancer [8]. Factors which have been associated with male breast cancer include ethnic origin (black), remaining a bachelor, religion (Jewish), exposure to radiation, excessive weight, as well as occupational exposure to phenoxyacids, heat, dust, gasoline, grease, or electromagnetic fields [9]. Endocrine factors seem to play a large role in development of male breast cancer, with serum oestrogens being higher in affected subjects than controls. The effect of (female) hormones is evidenced by several risk factors for the development of male breast cancer, that is, Klinefelter syndrome, use of female hormones and history of orchiectomy [9].

## CASE REPORT

A large, rural dwelling family was identified through a study of nutrition and breast cancer in the Montreal region. The proband indicated an extensive family history of breast cancer,

Correspondence to P. Ghadirian. P. Ghadirian, C. Perret, D.M. Lambert, J. Latreille, F. Letendre, M. Monté and D. Belanger are at the Epidemiology Research Unit, Research Centre, Hôtel-Dieu de Montréal, 3850 st-Urbain st., Montreal (Quebec) H2W 1T8; S. Narod is at the Division of Medical Genetics, Montreal General Hospital, Montreal, Canada.
Revised 28 Oct. 1994; accepted 31 Oct. 1994.