Table 1. Patients' characteristics

	No. of patients
Total no. of patients	18
Age (years)	
Median (range)	59.5 (49–69)
Histology	
Serous	9
Mucinous	2
Clear cell	2
Endometrioid	2
Undifferentiated	3
WHO performance status (range)	1 (0–2)
No. of prior chemotherapy regimens (range)	1 (1–4)
Response to previous chemotherapy*	
Primary platinum-resistant	10
Secondary platinum-resistant	3
Potentially platinum-sensitive	5

*According to Markman classification [3].

Patient's characteristics are shown in Table 1. Median number of courses received per patient was two (range 0.5–11) at a dose intensity (mg/m^2/week delivered versus planned) of 94.20% for mitomycin C and 93.34% for 5-FU. Evaluation of response was made by computed tomography scan in 8, physical examination in 8, and by tumour marker (CA-125) in 1. There were no objective remissions with 2 patients with stable and 15 with progressive disease. Median time to progression was 10 weeks (range 3–37) and median survival time was 29 weeks (3–118+). Toxicity grade 3–4 (WHO) during the whole treatment period (% of patients) was leucopenia 12%, stomatitis 12%, diarrhoea 12%, nausea and vomiting 6% and alopecia 6%. No toxic deaths occurred.

Two additional studies using these drugs have been published since Alberts report and remission rates of 24 and 29%, respectively, were noted [4, 5]. Compared to the other series, our patients were less heavily pretreated with similar performance status and better dose intensity. In conclusion, we could not confirm the activity of the combination of mitomycin C and 5-FU as second-line therapy in patients with refractory ovarian cancer.

1. Ozols RF, Young RC. Chemotherapy of ovarian cancer. *Semin Oncol* 1991, **18**, 222–232.
2. Alberts DS, García-Kendall D, Surwit EA. Phase II trial of mitomycin C plus 5-FU in the treatment of drug-refractory ovarian cancer. *Semin Oncol* 1988, **15**(suppl. 4), 22–26.
3. Markman M, Hoskins W. Responses to salvage chemotherapy in ovarian cancer: a critical need for precise definitions of the treated population. *J Clin Oncol* 1992, **10**, 513–514.
4. Preti P, Imparato E, Pavesi L, Robustelli della Cuna G. Fluorouracil plus mitomycin C as a second-line therapy for drug-refractory ovarian cancer. In Taguchi T, Aigner KR, eds. *Mitomycin C in Cancer Chemotherapy Today*. Tokyo, Excerpta Medica, 1991, 187–192.
5. Covens A, O'Connell G, Rusthoven J, Mazurka. Phase II study of mitomycin C and 5-fluorouracil in platinum resistant ovarian cancer. *Eur J Gynaec Oncol* 1992, XIII, 125–130.

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Serological and Molecular Study on the HLA Phenotype of Female Breast Cancer Patients

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THE STUDY of susceptibility to breast cancer is clinically relevant because women may be helped by screening or by prevention advice; it is also biologically interesting because patients provide means of identifing genes of the histocompatibility complex (MHC) that may have a significant influence on immune response against this tumour, which, when faulty, can predispose to malignancy.

Over the past 10 years, tissue typing has demonstrated an alteration of MHC antigen expression of breast carcinomas, which is usually related to prognosis [1, 2]. Instead, blood cell typing has so far provided little and contradictory information regarding the association of HLA phenotypes and breast carcinoma [3, 4]. We report here a study aimed at re-evaluating, on the basis of serological and molecular techniques, data on HLA phenotype expression in peripheral blood lymphocytes (PBL) of female breast cancer patients.

From 1987 to 1992, we studied 62 patients with breast cancer, stage I or II, who were referred to our institutes. Tumours were classified histologically as infiltrating ductal carcinomas (IDC) and staged according to AJC/UICC criteria. Among the cases evaluated, HLA phenotype for 32 patients was performed concomitantly with the diagnosis; for the other 30 women, it was performed some years after the diagnosis of breast cancer and consequently after surgery and therapy.

The PBLs of these patients were typed and the HLA antigens frequencies were compared with a local control group of bone marrow donors. Using the standard cytotoxicity test to analyse HLA antigens, we detected a slightly significant decrease in the frequency of HLA-B7 and HLA-DR4 in the patients: 2/62 versus 34/269 in the control group ($P < 0.03$, according to Fisher's test), and 2/62 versus 29/242 ($P < 0.02$), respectively. Comparison for DR phenotype between patient group and control population, which was in Hardy–Weinberg equilibrium for DR antigens, showed serological homozygosity in the tumour cases (55 versus 12.3% of the controls). There was no relationship between the findings and the period of the analysis for these patients.

Molecular typing was carried out in 8 serological homozygote patients by means of restriction fragment length polymorphism (RFLP) method for DR locus [5]. We analysed *Taq* polymor-

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Table 1. Serological homozygosity and molecular heterozygosity for DR locus in patients with infiltrating ductal carcinoma (IDC)

Patient	Serologic DR	Molecular DR (RFLP)
1	7 , —	1 , 7
2	7 , —	2 , 7
3	5 , —	5 , 6
4	10 , —	1 , 10
5	5 , —	2 , 5
6	6 , —	1 , 6
7	5 , —	4 , 5
8	5 , —	3 , 5

RFLP, restriction fragment length polymorphism.

phism with a specific β probe, with standard Southern blotting hybridisation and detection methods. Surprisingly, using RFLP typing, we obtained an heterozygous DNA profile in all serological homozygote patients tested (Table 1).

In conclusion, our results clearly indicate disequilibrium of HLA information between serology and molecular typing of PBL from IDC patients. Thus, it may be hazardous to conclude that a preferential decrease in the frequencies of some HLA antigens (B7 and DR4), as defined serologically, carry an increased risk of breast cancer. According to tissue studies, however, the possibility remains that certain HLA antigen expressions influence the development of cancer [6]; the reports on immune responses controlled by MHC genes provide support for this interpretation [7–10]. Finally, in our patients, we found an elevated serological homozygosity of the DR locus that is contradicted by RFLP analysis. Since serological HLA phenotype analyses on patient and control groups were performed under the same working conditions and were consequently a reliable comparison, we would suggest that these findings are a result of perturbation of HLA gene expression with loss or aberrant translation of some HLA antigen. Further molecular study may clarify these mechanisms.

1. Concha A, Esteban F, Cabrera T, Ruiz-Cabello F, Garrido F. Tumor aggressiveness and MHC class I and II antigens in laryngeal and breast cancer. *Semin Cancer Biol* 1991, **2**, 47–54.
2. Concha A, Cabrera T, Ruiz-Cabello F, Garrido F. Can the HLA phenotype be used as a prognostic factor in breast carcinomas? *Int J Cancer* 1991, **6**, (Suppl.) 146–154.
3. Song ST. Association of HLA with breast cancer and its ER status. *Chung Hua Chung Liu Tsa Chih* 1989, **11**, 19–21.
4. Yokoe T, Ishida T, Ogawa T, Iino Y, Izuo M. Relationship of breast cancer and HLA in Japanese females. *Gan No Rinsho* 1990, **36**, 29–33.
5. Bidwell J. DNA-RFLP analyses and genotyping of HLA-DR and -DQ antigens. *Immunol Today* 1988, **9**, 18–23.
6. Glew SS, Stern P, Davidson JA, Dyer PA. HLA antigens and cervical carcinoma. *Nature* 1992, **356**, 22.
7. Doyle C, Strominger JL. Interation between CD4 and class II MHC molecules mediates cell adhesion. *Nature* 1987, **330**, 256–259.
8. Dasgupta JD, Cemach K, Dubey DP, Yunis EJ, Amos DB. The role of class I histocompatibility antigens in the regulation of T cell activation. *Proc Natl Acad Sci USA* 1987, **84**, 1094–1098.
9. Norment AM, Salter RD, Parham P, Englehard VH, Littman DR. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature* 1988, **336**, 79–81.
10. Robbins PA, McMichael AJ. Immune recognition of HLA molecules downmodulates CD8 expression on cytotoxic T lymphocytes. *J Exp Med* 1991, **173**, 221–230.

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False Negative Testicular Biopsy in an Extragonadal Germ Cell Tumour

P. Albers, R. Moll and G.E. Voges

IN 60% OF patients with extragonadal germ cell tumours, testicular biopsy reveals a testicular intraepithelial neoplasia (TIN) [1]. We report a case of a patient with the primary diagnosis of extragonadal seminoma in whom an initial testicular biopsy was unremarkable. Fourteen weeks later a solid tumour in the left testicle was detected which proved to be a "burnt-out" seminoma.

A 37-year-old male patient was admitted to an outside hospital with persistent back pain. Physical examination demonstrated a palpable mass in his left upper quadrant. Ultrasound and computerised tomography (CT) of the abdomen showed a retroperitoneal tumour (6 × 3.2 × 2 inches). Fine needle aspiration was performed and suggested a Hodgkin's lymphoma. For definitive diagnosis, a staging laparotomy and partial resection of the tumour were carried out. Histology revealed a pure seminoma. The patient was referred to the Department of Urology, Mainz University Medical School for further treatment.

Sonographic examination of both testicles was unremarkable. Lactate dehydrogenase (LDH) was increased to 1344 U/l (normal value up to 215 U/l), α-fetoprotein (AFP) and β-human chorion gonadotropin (βHCG) values were normal. CT of the abdomen showed a residual tumour mass (3.2 × 3.2 × 1.6 inches). The patient was treated with three courses of BEP (bleomycin, etoposide, cisplatin) chemotherapy. Staging after chemotherapy demonstrated tumour markers in the normal range and a volume reduction of the retroperitoneal mass of 75%. Radical retroperitoneal lymph node dissection (RPLND) at that time showed complete necrosis without histological evidence of viable tumour cells. In addition, a single biopsy of each testicle demonstrated completely atrophic seminiferous tubules without any malignant cells or testicular intraepithelial neoplasia.

Fourteen weeks after RPLND, a palpable testicular tumour on the left side was detected. Ultrasound showed a hypoechoic inhomogeneous intraparenchymal mass occupying 70% of the testis. Tumour markers were normal. Magnetic resonance tomography (MRT) confirmed the ultrasound findings. In addition, the scar of the previous biopsy in both testicles was clearly visible. The left-sided biopsy at the medial edge of the testicle was opposite the tumour (Figure 1). A left-sided radical orchiectomy was performed. Histology showed a 1.0-inch parenchymal lesion within a 1.6-inch atrophic testicle which was diagnosed as a "burnt-out" seminoma. A vascular lesion as the cause of the atrophy and scar could clearly be ruled out by histology.

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