is 1/10 000 newborn and the association of OI and malignancy has rarely been reported [4]. We were surprised to find another similar association of severe 5-FU toxicity related to DPD deficiency in a breast cancer patient with mild OI as reported by Lyss and associates [5]. The probability of a fortuitous association appears low. Specific major chromosomic alteration seems unlikely since no cytogenetic abnormality was found in this patient following high resolution karyotyping. OI type I is inherited in an autosomal dominant manner, although new mutations account for almost half of the affected individuals. With rare exceptions, OI is always the result of mutation in the genes *COL1A1* and *COL1A2* for the α1- and α2-chains of the major fibrillar collagen type I, located, respectively, on chromosomes 17q21-22 and 7q22. DPD deficiency has an autosomal recessive pattern of inheritance. The human DPD cDNA has recently been cloned and sequenced [6]. The gene was localised to the centromeric region of human chromosome 1 between 1p22 and 1q21. It is tempting to speculate that DPD activity may be abnormally regulated in OI patients.

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Vinorelbine/5-FU Combination in Metastatic Breast Cancer Chemotherapy. A Retrospective Study of 63 Cases

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VINORELBINE IS a semisynthetic vinca-alkaloid analogue, which has proved to be of interest in first-line palliative chemotherapy in patients with metastatic breast cancer, with a response rate of 41% [1]. During a phase II trial in first-line palliative breast cancer chemotherapy, it achieved a 62–72% objective response rate when combined to a 5-day continuous perfusion of 5-FU [2, 3].

Between July 1991 and November 1993, this treatment was given to 63 patients with metastatic breast cancer in our Institute. Mean age of patients was 48 years (range 29–69 years). All the patients had received at least one anthracycline-based chemotherapy regimen either during the treatment of the primary tumour or as a palliative treatment (Table 1). Among the 50 patients having undergone chemotherapy for the treatment of the primary tumour, when vinorelbine/5-FU was administered as a first-line palliative treatment, 21 patients had an early relapse (≤12 months). The median number of drugs previously received was six (range 3–11), and all the patients had received one vinka-alkaloid and/or bolus 5-FU. Most of these patients had a poor prognosis: 39% had liver metastases and 66% had at least two metastatic sites.

This chemotherapy combined vinorelbine (30 mg/m^2/day, i.v., days 1 and 6) and 5-FU (750 mg/m^2/day × 5) in continuous perfusion, every 22 days. WHO criteria were used to assess response to treatment and toxic effects [4]. The chemotherapy was continued until there was evidence of disease progression or unacceptable signs of toxicity. Patients underwent 1–25 chemotherapy cycles (median: 3). Theoretical doses of vinorelbine/5-FU were reduced immediately by 25% in 31 patients (49%) owing to their precarious performance status and/or hepatic disturbances.

One complete and five partial responses were observed giving an objective response rate of 9.5% (95% CI: 2–18%), with an average response duration of 5 months (range 3–15 months).

Disease was stabilised in 8 patients (12.7%), and time to disease progression ranged from 4 to 17 months.

The main side-effects were haematological and digestive. 13 patients experienced grade 3 or 4 leucopenia, 4 had grade 3 or 4 thrombopenia, and 6 had grade 3 anaemia. Stomatitis

Table 1. Prior treatments

Neo-adjuvant and/or adjuvant chemotherapy for the treatment of primary tumour	Palliative chemotherapy regimens delivered before vinorelbine/5-FU	Totals
Neo-adjuvant chemotherapy only* (n = 27)	none: 18 one: 4 two: 5	
Neo-adjuvant and adjuvant chemotherapy* (n = 9)	none: 5 two: 2 three: 2	none = 25 one = 7
Adjuvant chemotherapy only† (n = 14)	none: 2 one: 1 two: 8 three: 3	two = 23 three = 7 four = 1
No neo-adjuvant and/or adjuvant chemotherapy (n = 13)	one: 2 two: 8 three: 2 four: 1	

*Neo-adjuvant or adjuvant treatment: EVM (epirubicin, vincristine, methotrexate) × 3 cycles + MTV (mitomycin C, thiotepa, vindesine) × 3 cycles. †CMF IV: 9 cycles, or EVM × 3 cycles + MTV × 3 cycles.

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or mucositis grade 3 or 4 were observed for 13 patients. In 9 patients, the side-effects during the treatment made it necessary to adjust cytostatic doses.

Our response rate is low. The observed difference to previous results [2, 3] could be explained by inclusion of heavily pretreated patients, with the existence of crossresistance mechanisms. The second explanation is that the dose schedule was modified for 63% of the patients, resulting in a less intensive protocol. Using a less intensive combination in pretreated patients, Platini and associates [5] also reported a low, short-lasting response rate (10.5%).

This retrospective study does not allow us to understand the cause of failure of this combination. Such a trial should have been prospective in order to compare vinorelbine/5-FU to vinorelbine and to 5-FU alone.

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Studies of HLA-A and DR Locus Deletions in Human Liver Cancer Cell Lines by PCR

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THE HUMAN leucocyte antigen (HLA) class I and class II genes are the most polymorphic genes in the human genome. The expression of HLA molecules on tumour cells is vital for T lymphocyte recognition of tumour antigens. Altered expression of HLA antigens has been proposed as a mechanism which protects tumour cells from immunosurveillance [1, 2]. Suppression and selective loss of expression of HLA antigens on tumour cells has been described in a variety of tumour types [3–7]. The loss of HLA antigens by neoplastic cells is considered important for tumour growth and metastasis [2]. Hepatocellular carcinoma (HCC) is one of the most common cancers in males and, although expression of HLA antigens in other types of tumours has been investigated, the status in liver cancer has not been studied. In this paper, we report the results of our study of HLA-A and DR locus genotype of a panel of 14 HCC cell lines using the polymerase chain reaction (PCR) technique.

Genomic DNA was prepared from HCC cell lines by the proteinase K–phenol–chloroform extraction method. Fifteen pairs of HLA-specific primer sequences were derived from HLA class I nucleotide sequences [8]. Nineteen pairs of primers were designed for DR typing identifying polymorphism corresponding to the serologically defined series DR1-DR18, and DR52 and DR53 superspecificities [9]. PCR typing for HLA-A and DR locus was conducted according to Browning and colleagues [8] and Olerup and colleagues [9] with slight modifications. Amplified DNA products were separated on 1.5% agarose gel electrophoresis and were identified by detecting the correct size bands. A HLA homozygous individual would show only one specific band on the after PCR, representing a single specific allele, while a heterozygous individual would show two bands. Representative examples of the PCR typing for HLA-DR is shown in Figure 1. Four of the 14 (29%) HCC cell lines gave a single HLA-A locus specificity on PCR typing. HLA-DR typing showed six of the 14 cell lines (43%) were homozygous. There was no total loss of HLA-A and DR locus among the HCC cell lines studied. Results of the HLA-A and DR typings are summarised in Table 1.

Homozygosity in both HLA-A and DR loci was found in four cell lines: HA22T/VGH, HCC-M, HCC-T and HuH-7. Homozygosity in a single HLA locus in the normal population

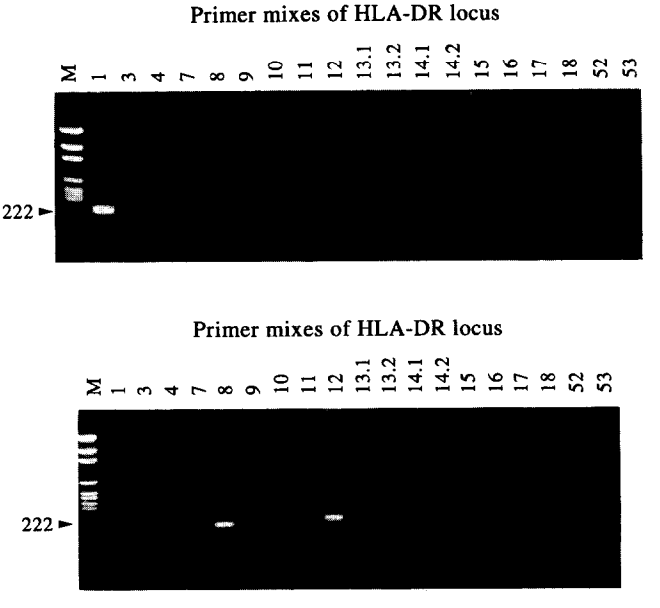

Figure 1. HLA-DR locus PCR typing of HCC cell line HCC-M (top) and PLC/PRF/5 (bottom). The target DNAs were amplified by 30 cycles. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 65°C for 50 s and extention at 72°C for 30 s. M, pGEM marker. Numbers at left indicate base pairs.

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