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

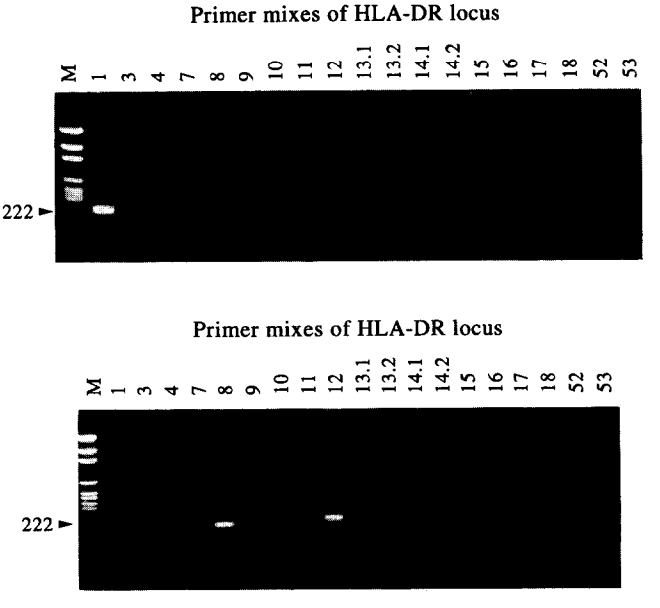

Figure 1. HLA-DR locus PCR typing of HCC cell line HCC-M (top) and PLC/PRF/5 (bottom). The target DNAs were amplified by 30 cycles. Each cycle consisted of denaturation at 94°C for 30 s, annealing at 65°C for 50 s and extention at 72°C for 30 s. M, pGEM marker. Numbers at left indicate base pairs.

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Table 1. Summary of the typing results for HLA-A and DR loci identified on HCC cell lines

Cell line	Ethnic group*	Amplified alleles A	Amplified alleles DR
HA22T/VGH	O	2,—	12,—
HCC-M	O	11,—	1,—
HCC-T	O	28,—	1,—
HuH-7	O	2,—	8,—
huH-1	O	9,29	8,—
huH-4	O	9,26	9,—
HuH-6	O	2,9	9,12
KMCH-1	O	11,31	8,17
Sk-Hep1	C	2,9	10,11
Tong/HCC	C	9,29	7,16
HepG2	C	2,9	16,18
Mahlavu	C	2,11	9,8
Hep3B	B	28,29	7,18
PLC/PRF/5	B	10,33	8,12

*O, oriental; C, caucasian; B, black. The numbers under A and DR represent the equivalent of serologically defined HLA specificities.

is approximately 10%. Due to the highly polymorphic characteristics in the HLA region, it is rare to observe homozygosity in two HLA loci at the same time. Yet in this study, a 29% (4/14) of HCC cell lines were homozygous at both HLA-A and DR. Our results therefore suggest that the high frequency of homozygosity of HLA alleles seen in HCC is a result of deletion of the genomic HLA locus. Since there is no selective advantage for the HCC cells *in vitro* to lose HLA antigens, it is most likely that the deletions occurred in the primary tumour, which is under selection pressure by the host immune system. As the human HLA gene complex is located on chromosome 6p21, deletions may occur at the short arm of chromosome 6 or possibly the entire chromosome. Whichever the mechanism, the effect of HLA loss means that the HCC cells have effectively reduced their antigenic exposure to the host T cells immune surveillance.

In conclusion, in this preliminary study, we have observed loss of heterozygosity/deletion of HLA-A and DR loci in 29% of HCC cell lines studied. Although some caution is required in evaluating these results, our findings are in agreement with those reported for other types of cancers [3–7]. Further work needs to be done to study liver cancer cells from primary tumours to determine the precise genetic alterations in HLA genes in greater detail. These findings may serve as a guide to evaluate possible mechanisms used by HCC cells to escape immune recognition *in vivo*.

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Adenocarcinoma of the Eccrine Sweat Gland: Response to Both Combination Chemotherapy and Local Field Irradiation

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ADENOCARCINOMAS OF the eccrine sweat gland are rare malignancies with a tendency to invade locally and recur; occasional

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