Pergamon

European Journal of Cancer Vol. 33, No. 9, pp. 1516–1518, 1997

Printed in Great Britain
0959-8049/97 $17.00 + 0.00

PII: S0959-8049(97)00139-1

## Original Paper

# *MDR*1 RNA Transcripts do not Indicate Long-term Prognosis in Colorectal Carcinomas

S. Zöchbauer,[1] J. Wallner,[1] K. Haider,[2] D. Depisch,[2] H. Huber[1] and R. Pirker[1]

[1]Department of Oncology, Clinic for Internal Medicine I, University of Vienna Medical School, A-1090 Vienna; and [2]Department of Surgery, General Hospital Wr. Neustadt, 2700 Wr. Neustadt, Austria

**Because P-glycoprotein expression might be associated with a more aggressive behaviour of colorectal carcinomas (Weinstein *et al.*, *Cancer Res*, 1991, 51, 2720–2726), we determined the relationship between *MDR*1 RNA expression of the carcinomas and the survival of the patients. At a median duration of follow-up of 86 months, event-free survival of patients with *MDR*1 RNA-negative tumours ($n = 35$) was not significantly different to that of patients with *MDR*1 RNA positive tumours ($n = 67$). Among the different tumour stages, event-free survival of the patients was also independent of *MDR*1 gene expression of the tumours. Thus, these findings do not support the hypothesis that local aggressiveness of P-glycoprotein positive tumour cells translates into worse clinical outcome.** © **1997 Elsevier Science Ltd.**

**Key words: colorectal carcinoma, multidrug resistance, MDR1 gene, P-glycoprotein, survival, prognosis**

*Eur J Cancer*, Vol. 33, No. 9, pp. 1516–1518, 1997

## INTRODUCTION

COLORECTAL CARCINOMAS show intrinsic resistance to most anticancer drugs, notably anthracyclines. Expression of the *MDR*1 gene, a multidrug resistance gene coding for P-glycoprotein, has been detected in approximately two-thirds of primary colorectal carcinomas [1–3]. P-glycoprotein functions as an energy-dependent drug efflux pump for natural hydrophobic substances including several anticancer drugs (e.g. anthracyclines, Vinca alkaloids, epipodophyllotoxins) [4, 5].

*MDR*1 RNA expression of carcinomas was independent of both lymph node and distant metastases in our previous study [1], but Weinstein and associates [6] found a more frequent lymph node involvement for P-glycoprotein positive tumours as compared to P-glycoprotein negative tumours, and detected P-glycoprotein predominantly in invasively growing tumour cells, suggesting an association of P-glycoprotein expression with a more aggressive phenotype [6]. Thus, we hypothesised that patients with *MDR*1 RNA/P-glycoprotein positive tumours might have a worse clinical outcome. In order to prove or exclude this, we studied the relationship between *MDR*1 RNA expression of the primary tumours and the survival of the patients. Here we report the long-term results of this study.

Correspondence to R. Pirker.
Received 4 Nov. 1996; revised 26 Feb. 1997; accepted 12 Mar. 1997.

## PATIENTS AND METHODS

### *Patients*

Between 1988 and 1991, 113 patients with colorectal carcinomas entered into a study on the clinical significance of the *MDR*1 gene [1]. The clinical data of the patients have previously been described [1]. Because 11 patients had been lost for follow-up, 102 patients were included in the present analysis.

All patients had a resection of the primary tumour. Tumour stages I, II, III and IV were present in 33, 33, 25 and 11 patients, respectively. 30 patients with tumour stages II or III received adjuvant chemotherapy and 10 patients in relapse received palliative chemotherapy. Chemotherapy mainly consisted of 5-fluorouracil with/without leucovorin and did not include MDR drugs except mitomycin C in few patients.

### MDR1 *gene expression*

Total RNA was isolated from surgically obtained colorectal carcinoma specimens by standard techniques. *MDR*1 RNA expression of tumour specimens was assessed by slot-

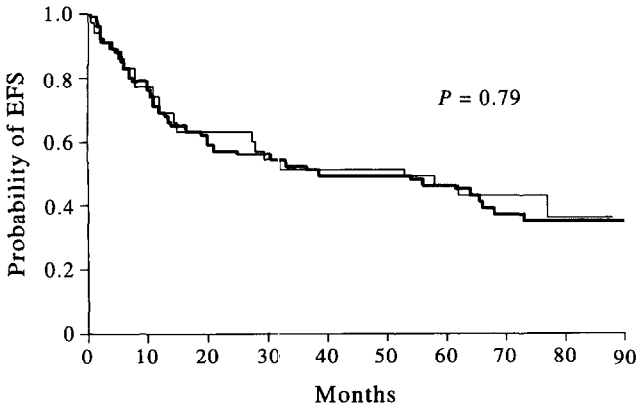


**Figure 1. Event-free survival according to *MDR*1 RNA expression of the tumours. Event-free survival of 102 patients was calculated according to Kaplan–Meier [7]. All patients had undergone a complete resection of their primary tumours. Survival curves are shown for patients with negative (—) and those with positive (-) tumours.**

blot analysis by means of a radiolabelled *MDR*1 cDNA (probe 5A, provided by I. Pastan and M. Gottesman, NCI, Bethesda, Maryland, U.S.A.) as previously described [1].

*Survival analysis*

Durations of event-free, overall and disease-free survival were calculated according to Kaplan–Meier [7]. Events were defined as either relapse or death due to any cause and, in the case of stage IV patients, only deaths were chosen as events. Event-free survival was measured from the time of primary surgery until the time of the first event. Disease-free survival and overall survival were calculated from the time of surgery until relapse and death, respectively. Survival curves were compared by the log-rank test.

## RESULTS

Results on *MDR*1 gene expression of colorectal carcinomas ($n = 113$) have previously been reported [1]. 35 (34%) patients with *MDR*1 RNA negative tumours and 67 (66%) patients with *MDR*1 RNA positive tumours were included in the present survival analysis. Treatment did not differ between patients with *MDR*1 RNA negative and positive tumours (data not shown).

For patients with tumour stages I, II, III and IV, the median durations of overall survival were >90, 73, 37 and 7 months, respectively, and the corresponding 5-year survival rates were 74%, 60%, 44% and 0%, respectively (data not shown). These survival data are consistent with data reported in the literature.

A total of 62 events (23 relapses, 39 deaths) occurred. Relapses and deaths were observed in 7 (20%) and 14 (40%) patients with *MDR*1 RNA negative tumours and in 16 (24%) and 25 (37%) patients with *MDR*1 RNA positive tumours.

At a median duration of follow-up of 86 months, the median duration of event-free survival was 53 months for the total study population (data not shown). Event-free survival of patients with *MDR*1 RNA negative tumours was not significantly different from that of patients with *MDR*1 RNA positive tumours (Figure 1). Survival was also similar between these two groups when patients with tumour stage

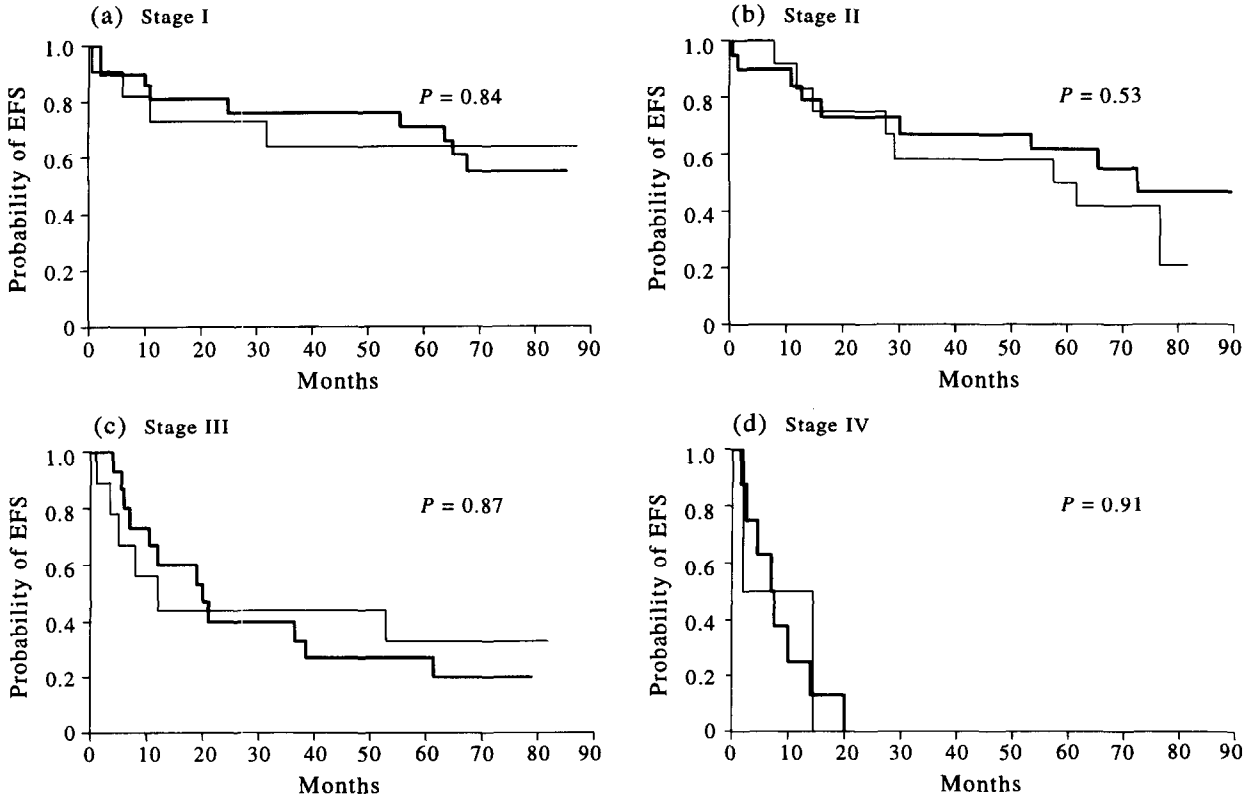


**Figure 2. Stage-dependent event-free survival according to *MDR*1 RNA expression of the tumours. Event-free survival of patients was also calculated according to Kaplan–Meier [7] for the different tumour stages. Event-free survival was similar for patients with negative (—) and those with positive (-) tumours.**

IV were excluded from analysis (data not shown). Among the different tumour stages, event-free survival was also independent of *MDR*1 gene expression (Figure 2). In addition, overall and disease-free survival were not affected by *MDR*1 RNA expression of the tumours (data not shown).

## DISCUSSION

In our study, *MDR*1 RNA expression of primary colorectal carcinomas did not predict survival of the patients. These long-term results confirm our preliminary findings after short follow-up [1] and are in agreement with a recent immunohistochemistry study which reported no association of P-glycoprotein expression with survival [2]. No survival data were presented both in the study by Weinstein and associates [6], who reported an association of P-glycoprotein expression with local tumour aggressiveness [6] and in another study in Dukes' B2 patients, which found a higher relapse rate for patients with P-glycoprotein positive tumours [3].

Several explanations exist for the observed lack of an association of *MDR*1 gene expression of the carcinomas with survival of the patients. Firstly, the clinical behaviour of *MDR*1 RNA negative tumours is similar to that of their positive counterparts, despite both the earlier report on the more aggressive nature of P-glycoprotein positive colorectal carcinoma cells [6] and the findings in osteosarcomas where P-glycoprotein positive tumours were associated with poor outcome due to a more aggressive behaviour of these tumours [8]. Secondly, *MDR*1 gene expression had no clinical impact because most patients were not treated with MDR drugs. Preferential treatment with MDR drugs most likely explains the differences in survival observed between patients with *MDR*1 negative and those with *MDR*1 positive diseases in childhood soft tissue sarcomas [9] and acute myeloid leukaemia [10–12]. In neuroblastomas, P-glycoprotein expression was associated with worse outcome in one study [13] but not in a recent study [14]. Thirdly, additional resistance mechanisms are active in colorectal carcinomas [15]. Finally, RNA slot-blot analysis has its limitations and thus our results will have to be confirmed by studies using other techniques for the detection of *MDR*1 gene expression.

In conclusion, while the high percentage of *MDR*1 RNA positive colorectal carcinomas stresses the importance of this gene with regard to the clinical resistance to anticancer drugs transported by P-glycoprotein, *MDR*1 RNA expression of the primary tumours does not indicate prognosis. Thus, these findings do not support the hypothesis that the reported local aggressiveness of P-glycoprotein positive tumour cells results in a worse clinical outcome.

1. Pirker R, Wallner J, Gsur A, *et al.* MDR1 gene expression in primary colorectal carcinomas. *Br J Cancer* 1993, **68**, 691–694.
2. Mayer A, Takimoto M, Fritz E, Schellander G, Kofler K, Ludwig H. The prognostic significance of proliferating cell nuclear antigen, epidermal growth factor receptor, and MDR gene expression in colorectal cancer. *Cancer* 1993, 71, 2454–2460.
3. Sinicrope FA, Hart J, Brasitus TA, Michelassi F, Lee J, Safa AR. Relationship of P-glycoprotein and carcinoembryonic antigen expression in human colon carcinoma to local invasion, DNA ploidy, and disease relapse. *Cancer* 1994, **74**, 2908–2917.
4. Pastan I, Gottesman MM. Multiple-drug resistance in human cancer. *N Engl J Med* 1987, **316**, 1388–1393.
5. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989, **81**, 116–124.
6. Weinstein RS, Jakate SM, Dominguez JM, *et al.* Relationship of the expression of the multidrug resistance gene product (P-glycoprotein) in human colon carcinoma to local tumor aggressiveness and lymph node metastasis. *Cancer Res* 1991, **51**, 2720–2726.
7. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958, **53**, 457–481.
8. Baldini N, Scotlandi K, Barbanti-Brodano G, *et al.* Expression of P-glycoprotein in high-grade osteosarcomas in relation to clinical outcome. *N Engl J Med* 1995, **333**, 1380–1385.
9. Chan HSL, Thorner PS, Haddad G, Ling V. Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. *J Clin Oncol* 1990, **8**, 689–704.
10. Pirker R, Wallner J, Geissler K, *et al.* MDR1 gene expression and treatment outcome in acute myeloid leukemia. *J Natl Cancer Inst* 1991, **83**, 708–712.
11. Marie JP, Zittoun R, Sikic BI. Multidrug resistance (mdr1) gene expression in adult acute leukemias: correlations with treatment outcome and *in vitro* drug sensitivity. *Blood* 1991, **78**, 586–592.
12. Zöchbauer S, Gsur A, Brunner R, Kyrle PA, Lechner K, Pirker R. P-glycoprotein expession as unfavorable prognostic factor in acute myeloid leukemia. *Leukemia* 1994, **8**, 974–977.
13. Chan HSL, Haddad G, Thorner PS, *et al.* P-glycoprotein expression as a predictor of the outcome of therapy for neuroblastoma. *N Engl J Med* 1991, **325**, 1608–1614.
14. Murray DN, Bordow SB, Marshall GM, Haber PS, Cohn SL, Haber M. Expression of the gene for multidrug-resistance-associated protein and outcome in patients with neuroblastoma. *N Engl J Med* 1996, **334**, 231–238.
15. Filipits M, Suchomel RW, Dekan G, *et al.* Expression of the multidrug-resistance-associated protein (MRP) gene in colorectal carcinomas. *Br J Cancer* 1997, **75**, 208–212.

**Acknowledgements**—R Pirker was supported by the 'Fonds zur Förderung der wissenschaftlichen Forschung' (Project P8752).