European Journal of Cancer Vol. 30A, No. 1, p. 129, 1994.
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*NF*1 Gene Mutation and Acute Myelogenous Leukaemia

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WE READ with interest the report by Tenan and colleagues in the recent issue of the *European Journal of Cancer* [1] on the low frequency of *NF*1 gene mutation in malignant gliomas. We have performed a similar study concerning the mutation of *NF*1 gene in acute myelogenous leukaemia (AML).

*NF*1 gene is responsible for von Recklinghausen's neurofibromatosis (neurofibromatosis type 1: NF1), which is an autosomal dominant disease associated with an increased risk of benign and malignant neoplasms [2]. The product of *NF*1 gene, neurofibromin, contains a domain, structurally and functionally homologous to GTPase activating protein (GAP), which negatively regulates the *ras* oncogene product ($p21^{ras}$) [2]. Since activated $p21^{ras}$ has been found in human tumours, and implicated in the pathogenesis of many cancers [3], the *NF*1 gene is considered to be a tumour suppressor gene.

Alterations of the first nucleotide position of the Lys-1423 codon, which results in the amino acid change and the loss of GAP activity of the mutant neurofibromin, have been reported in three tumour types: colon adenocarcinoma, anaplastic astrocytoma and myelodysplastic syndrome (1/28 patients) [4]. Considering that mutational activation of *ras* is found in approximately one third of myelodysplastic syndrome (preleukaemia) and AML patients [3], there is also a possibility that *ras* activation, through impaired negative regulation by mutated neurofibromin, is involved in the development of these two myeloid disorders.

In addition to the first nucleotide position of Lys-1423 codon (AAG) in exon 24, as described above, we and others [5–8] identified another hotspot in NF1 patients. The first nucleotide position of Arg-1947 codon (CGA) in exon 31 is converted to T, which results in the generation of a stop codon (TGA), thereby approximately one third of neurofibromin is not translated.

In order to clarify the role of *ras* activation in AML, we studied 23 AML patients (3 patients of M1, 15 patients of M2, 2 patients of M3 and 3 patients of M4) for the mutations at these two hotspots in the *NF*1 gene.

DNA samples were prepared from peripheral blood mononuclear cells (containing 50–90% of blasts) from patients, and analysed by polymerase chain reaction using single strand conformation polymorphism analysis (PCR-SSCP) method [9], using intron-based primer pairs [4, 5] for amplifying exons 24 and 31, which contain the hotspots. The PCR-SSCP analysis did not reveal any band of altered mobility, suggesting that there were no mutations in either of the hotspots in the *NF*1 gene in our 23 AML patients.

Although we investigated only a limited region of the large *NF*1 gene, and still have to expand the number of patients, both of the hotspots so far identified in *NF*1 gene were not mutated in our AML patients. Therefore, we suggest that *NF*1 gene mutation does not play an important role in the pathogenesis of AML.

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Revised 19 Aug. 1993; accepted 23 Sep. 1993.

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European Journal of Cancer Vol. 30A, No. 1, pp. 129–130, 1994.
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High Cell Proliferation Activity Determined by DNA Flow Cytometry Predicts Poor Prognosis After Relapse in Prostate Cancer

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SEVERAL STUDIES have shown that DNA aneuploidy and/or high cell proliferation activity determined by DNA flow cytometry is

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Revised 29 July 1993; accepted 6 Sep. 1993.

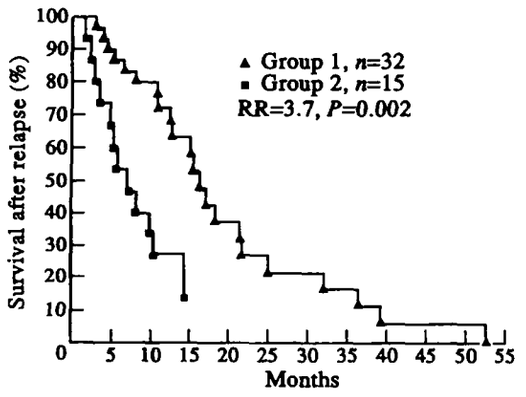

Fig. 1. Prostate cancer-specific survival after relapse. Group 1 = S+G2/M fraction less than 17% for diploid tumours or less than 20% for aneuploid tumours. Group 2 = S+G2/M more than 17% for diploid tumours or more than 20% for aneuploid tumours. RR, univariate relative risk.

Table 1. Independent predictors of survival after relapse in prostate cancer according to a multivariate Cox regression analysis

Prognostic parameters	RR	CI	P
S+G2/M fraction*	4.2	1.7–10.2	0.001
Histological grade (III vs. I+II)	2.8	0.97–8.3	0.038

RR, relative risk; CI, 95% confidence interval. *Cut-off points 17% for DNA diploid tumours and 20% for DNA aneuploid tumours. Factors also tested included M-stage, T-stage, DNA ploidy, age and response to primary treatment.

associated with poor prognosis in prostate cancer [1, 2]. However, there are no data comparing DNA flow cytometry parameters with survival after relapse in prostate cancer. The patient material in this study represented a group of patients who all suffered bone progression. We investigated retrospectively the impact of DNA ploidy and cell proliferation activity of the primary tumour on survival after relapse in this patient group.

53 prostate cancer patients, who suffered bone progression at some time during the disease, were all recruited to a randomised clinical trial of dinatriumclodronate. This drug has no effect on prostate cancer cells or survival rates of patients [3]. Patients were staged according to UICC recommendations [4] and tumours were graded according to WHO recommendations [5]. 28 (53%) patients already had metastatic disease (M1 stage) at the time of diagnosis, whereas 25 (47%) patients had local disease (M0 stage). The primary treatment after diagnosis was as follows: endocrine therapy, 49 cases; combination of radical prostatectomy and oestrogen, 1 case; radical radiation therapy, 2 cases; and combination of radical radiation therapy and oestrogen, 1 case. At the time of bone progression, all patients started to receive estramustine phosphate orally (280 mg twice daily). Simultaneously, they were randomised into two groups so that one group was treated with dinatriumclodronate and the other one with placebo.

Paraffin-embedded primary prostate tumour blocks were processed using a modification of the method described by Heiden *et al.* [6]. The stained nuclear suspensions were run in a flow cytometer and DNA histograms were analysed as described earlier [7]. Cell proliferation activity was defined as percentage of cells in S and G2/M phases (S+G2/M). Statistical analyses were conducted using the BMDP Statistical Software Package [8].

Forty nine per cent of the tumours were DNA aneuploid. The mean S+G2/M (±S.D.) was 16.3 ± 7.0% for all tumours, and 16.0 ± 5.9 and 16.5 ± 7.8% for DNA aneuploid and diploid tumours, respectively. DNA aneuploidy was associated with high T stage ($P<0.05$), but not with patients' age, histological grade or presence of distant metastases. Cell proliferation activity was not associated with any of the clinicopathological parameters mentioned above.

High cell proliferation activity defined a group of patients who had poor cancer-specific survival after relapse (Fig. 1). The strongest impact on survival for S+G2/M was reached when the approximate highest tertile was used as cut-off point (S+G2/M over 17% for diploid tumours and over 20% for aneuploid tumours). Also, patients with poorly differentiated tumours had worse cancer-specific survival after relapse compared to patients with well or moderately differentiated tumours ($P=0.019$, Mantel–Cox test). In multivariate analysis, both cell proliferation activity and histological grade were independent prognostic factors of cancer-specific survival after relapse. T and M stages, DNA ploidy, age or response to primary therapy did not predict survival after relapse (Table 1).

In this study, representing an aggressive type of prostate cancer, we identified a subgroup of patients (32% of patients), who had an especially poor prognosis after relapse. The median survival time after relapse of this group was only about 7 months. Therefore, high cell proliferation activity does not just seem to describe an aggressive type of disease in local prostate cancer [2], but also in late cancer. These preliminary results seems to indicate that patients with high tumour cell proliferation activity as well as patients with poorly differentiated tumours would need strong intervention, e.g. with chemotherapy, as early as possible, if their survival is to be improved. Also, when clinical trials on secondary treatment are planned, cell proliferation activity should be taken into account as a confounding variable.

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Acknowledgements—We thank Mrs Leena Pankko for technical assistance. This work was supported by the Lahtikari Foundation and by the Finnish Cancer Society.