Prognosis in patients with stage II melanoma is related to features of the primary tumour and of nodal metastases (Balch et al., 1981; Cascinelli et al., 1984; Roses et al., 1985; Koh et al., 1986; Kissin et al., 1987). Survival is inversely related to the number of positive nodes and also decreases when perilymph node invasion is present (Balch et al., 1981; Rayner et al., 1981; Callery et al., 1982; Cascinelli et al., 1984). However, it has also been emphasised that nodal metastases are indicators, not determinants of survival in pathologic stage II melanoma patients (Cady, 1984).

Cell kinetics, studied by different approaches, has emerged as an important indicator of biologic aggressiveness (Bauer et al., 1987; Kallioniemi et al., 1987; Quirke et al., 1987; Griffin et al., 1988; Hall et al., 1988; Bouzubar et al., 1989). In particular, the \(^{3}H\)-thymidine labelling index (\(\text{H-TdR LI}\)) has been shown to be a prognostic factor whose role remains also in the presence of other clinicopathologic prognostic factors (Meyer et al., 1983; Silvestrini et al., 1986, 1989; Chauvel et al., 1988).

Little attention has been given to cell kinetics in malignant melanoma (Shirakawa et al., 1970; Hagemann & Schiffer, 1971; Newburger et al., 1980; Hansson et al., 1982; Costa et al., 1987). The data available on preliminary series of stage II melanoma patients strongly indicate the potential clinical usefulness of cell kinetics as a prognostic marker (Hansson et al., 1982; Costa et al., 1987). However, the relative contribution of cell kinetics in relation to information given by other morphologic and pathologic features has been never analysed.

In this follow-up study, we report on a group of 166 patients with pathologic stage II melanoma of the skin to substantiate, in a larger series with a longer follow-up, our preliminary findings. However, we investigated the possible association of cell kinetics with established clinicopathologic parameters and we estimated the different contribution of all the variables on the clinical course, in terms of relapse-free interval and overall survival, by means of a multiple regression analysis.

The main basic limitation in starting the study was the impossibility to perform the cell kinetics determination on the primary tumour, which is completely reserved for the pathologist for Clark's (1969) and Breslow's (1970) histologic microstaging. This constraint was overcome by using the metastatic nodal lesion. This decision is quite compatible with the microscopic examination of autoradiograms, which allows us accurately to score and consider only tumour cells.
until clinically evaluated relapse or death on 145 patients with an adequate follow-up. The observation times ranged from 1 to 75 months, and the median follow-up was 25 months. First of all, a univariate analysis was carried out to estimate RFS and survival curves relative to the different modalities of each prognostic variable by means of the Kaplan–Meier product-limit method (Kaplan & Meier, 1958). Comparisons among curves were accomplished by resorting to the log rank test (Mantel, 1966). The hazard function was estimated according to the approach proposed by Simes and Zelen (1985). Six-month intervals were chosen to calculate the hazard estimates.

The joint effect of the variables, possibly influencing prognosis, was investigated by a Cox’s multiple regression model (Cox, 1972).

The role of $^3$H-TdR LI was investigated after classifying tumours in two classes with different proliferative rates. the value of 8%, which represents the mean value of the distribution of this variable, was used to discriminate slowly and rapidly proliferating melanomas. Other cut-off levels were tested by means of the Kaplan–Meier product-limit method, and the log rank test was used to assess differences in RFS and survival between subgroups. $\chi^2$ values with one degree of freedom were calculated with the corresponding $P$ values, and the highest $\chi^2$ value was observed at a $^3$H-TdR LI cut-off of 8%.

Results

$^3$H-TdR LI values defined for the overall series of 166 nodal lesions were highly skewed, with a mean value of 8.6% and a range from 0.3 to 31%, in agreement with our previous results (Costa et al., 1987). Cell kinetics was analysed in relation to the clinicopathological features considered prognostic factors in stage II melanoma patients (Table 1). $^3$H-TdR LI was not related to age or sex of the patient, to number of positive nodes, or to type of metastases.

The overall 3-year RFS and survival for the series of 145 patients, for whom cell kinetic and follow-up information was available, were 32% and 59%, respectively. This finding is in agreement with other clinical reports on larger series of stage II melanoma patients (Balch et al., 1981; Cascinelli et al., 1984; Kissin et al., 1987). The analysis as a function of $^3$H-TdR LI showed significant differences in the probability of RFS between patients with slowly and rapidly proliferating tumours (40% vs 22%, $P = 0.007$) (Figure 1a). The survival curves were also significantly different (68% vs 46%, $P = 0.007$) (Figure 1b). Relapse and death hazard over time showed similar patterns for the two kinetic subsets of patients (Figure 2), with a peak of risk from 6 to 12 months from lymphadenectomy. The hazard was higher for patients with rapidly proliferating tumours than for those with slowly proliferating tumours for the whole follow-up period.

Univariate analysis of 3-year RFS and survival as a function of number of positive nodes and type of nodal invasion was performed on the two subsets with less or more than two positive nodes owing to the unbalanced number of cases within the different categories. Patients with partial or massive nodal involvement were considered together. When

![Figure 1](image1.png)

**Figure 1** Clinical outcome as a function of $^3$H-TdR LI of nodal metastases in a series of 145 patients with stage II melanoma of the skin. — — low $^3$H-TdR LI; — — high $^3$H-TdR LI. a, relapse-free survival; b, survival.

![Figure 2](image2.png)

**Figure 2** Estimated hazard function of relapse and death (per 100 persons per 6 months) for patients with slowly (—) and rapidly (—) proliferating tumours.
singly tested, number of positive nodes was associated to clinical outcome. Patients with ≤2 N+ showed a higher probability of RFS (43% vs 13%, P = 0.0004) and survival (66% vs 46%, P = 0.04) than patients with > 2 N+ (Figure 3a,b). Moreover, the probability of RFS at 3 years was significantly lower for patients with extracapsular diffusion of disease (27%) than for patients with intracapsular disease (44%; P = 0.03) (Figure 4a). Within the latter group, a different RFS was observed for patients with partial or massive nodal involvement. No statistically significant difference was observed among the corresponding survival curves. (Figure 4b). Age and sex of patients did not affect RFS or survival.

Multiple regression analysis was carried out to evaluate the joint effect of prognostic factors on RFS and survival. Age and sex were excluded from the evaluation because, by single factor analysis, they were clearly not associated with RFS and survival. The 3H-TdR LI was added to the regression models including the two pathologic variables, i.e. number of positive nodes and type of nodal invasion (Table II). With regard to RFS, the hazard ratio was maximum for number of positive nodes. 3H-TdR LI, as in the univariate analysis, retained its statistical significance and provided a prognostic contribution to the other variables considered in the model (likelihood ratio test: \( \chi^2 = 5.74, 1 \text{ d.f.}, P = 0.017 \)). Type of nodal invasion did not reach the significance level. 3H-TdR LI became the most important prognostic indicator of survival compared to the pathologic variables (likelihood ratio test: \( \chi^2 = 7.23, 1 \text{ d.f.}, P = 0.007 \)).

![Figure 3](image3.png)

**Figure 3** Clinical outcome as a function of number of positive nodes in a series of 145 patients with stage II melanoma of the skin. ---, ≤2 N+; —, > 2 N+. a, relapse-free survival; b, survival.

![Figure 4](image4.png)

**Figure 4** Clinical outcome as a function of type of nodal invasion in a series of 145 patients with stage II melanoma of the skin. ---, intracapsular invasion; —, extracapsular invasion. a, relapse-free survival; b, survival.

### Discussion

Tumour thickness is the most important factor in predicting the risk of nodal metastases in stage I melanoma patients, but it no longer has any prognostic value once nodal metastases have developed. At such a time, in stage II patients, nodal status becomes the most important determinant of evolution, i.e. the second step of melanoma life is mainly conditioned by number of involved nodes and type of nodal metastases (Balch et al., 1981; Rayner et al., 1981; Callery et al., 1982; Cascinelli et al., 1984). This finding has been confirmed in the present series of patients: 3-year RFS and survival were significantly lower for patients with more than two positive nodes and massive invasion, regardless of diffusion beyond the capsule.

As observed for other human tumours (Meyer et al., 1983; Tubiana et al., 1984; Silvestrini et al., 1986, 1989a,b; Chauvel et al., 1988), cell kinetics varies widely from patient to patient, and there is no evident relationship between 3H-TdR LI of metastatic lesions and clinicopathologic features considered to be prognostic factors in stage II melanoma patients.

The present study confirms the previous finding on the value of cell kinetics as a prognostic factor in stage II melanoma patients subjected to nodal dissection (Hansson et al., 1982;
Costa et al., (1987), even after making allowance for all the other known prognostic factors, in fact, multiple regression analysis showed that "T-H-TdR LI retained its importance as an indicator of RFS and became the most important indicator of survival even in the presence of number of involved nodes and type of nodal metastases, at least for the first 3 years after surgery. Our findings, which reproduce those reported for other tumour types (Meyer et al., 1983; Tubiana et al., 1984; Silvestrini et al., 1986, 1989a,b; Chauvel et al., 1988), indicate that, even for stage II melanoma patients, cell kinetics should improve the assessment of prognosis.

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