White Blood Cell Count: A Prognostic Factor and Possible Subset Indicator of Optimal Treatment with Low-Dose Adjuvant Interferon in Primary Melanoma

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ABSTRACT
αIFN has recently been recognized as an adjuvant therapy to surgery in melanoma patients. A major issue is to select patients who will benefit from this therapy and to avoid toxicity in those who will not respond. The aim of this exploratory analysis was to identify the predictive factors of response to αIFN.

The French cooperative group has recently shown that adjuvant therapy of melanoma patients with low-dose αIFN provides a benefit on disease-free interval (DFI). Using this database, predictors of DFI were investigated using Cox models and treatment-covariate interactions were sought.

Gender, age, Breslow thickness, and baseline WBC count, given an αIFN-WBC interaction, were independent predictors of DFI. Baseline WBC count was the only variable for which there was an interaction with αIFN, whatever the Breslow; patients with low WBC count (<6.8 × 10⁹/liter = median) did not benefit from αIFN (HR = 1.27 [95% CI: 0.84–1.91]; P = 0.26) whereas the DFI of patients with high WBC was prolonged (P = 0.0001) with a hazard ratio of 0.50 (95% confidence interval, 0.35–0.71). The estimated values of WBC count for which IFN was significantly superior to no-treatment were those ≥7.2 × 10⁹/liter. The baseline WBC count was correlated to baseline neutrophils but not to Breslow thickness or to time since last melanoma surgery.

αIFN prolonged DFI in patients with a high WBC count but not in those with a low WBC count. The results of this exploratory analysis, if confirmed by other studies, may help to identify patients who are most likely to benefit from αIFN.

INTRODUCTION
It is only recently that αIFN has been recognized as an adjuvant therapy to surgery in patients with high-risk cutaneous melanoma. A 1-year high-dose IFN α-2b regimen has been shown to improve the disease-free and overall survival of AJCC stage IIB and III melanoma patients (1). Furthermore, the beneficial effect on DFI of adjuvant low doses of IFN α-2a in patients with cutaneous melanomas thicker than 1.5 mm without clinically detectable node metastases has recently been shown by our group (2) and confirmed by an ongoing study (3). However, the exact mechanisms by which IFN exerts antitumor effects are not clearly known (4), nor are the characteristics of patients who will respond well defined. Thus, as αIFN is emerging as an adjuvant therapy in high-risk cutaneous melanoma patients, it is becoming critical to identify clinical, immunological, or molecular features that will enable the selection of patients who are likely to benefit from IFN therapy (5), thereby limiting toxicity and impairment of quality of life in patients who will not benefit from IFN.

The aim of this exploratory study is to investigate, in the high-risk primary melanoma patients of the French database (2), the baseline clinical, histological, and biological characteristics that are predictive of relapse and the characteristics that are associated with a beneficial response to IFN.

PATIENTS AND METHODS
Patients. The 489 eligible patients enrolled in a randomized clinical trial, which was recently published by our group, were studied (2). Patients had a high-risk primary cutaneous melanoma (Breslow thickness, ≥1.5 mm) without clinically detectable node metastases; after tumor resection, they were randomized to receive either 3 × 10⁶ IU of IFN three times weekly for 18 months or no treatment. Using a sequential procedure, IFN demonstrated a significant benefit for DFI, which was the primary end point (P = 0.038). After a median follow-up of 5.0 years, 487 patients were evaluable for relapse-free interval. There were 100 relapses among the 244 IFN-treated patients and 119 among the 243 evaluable control patients.

Statistical Methods. The variables that were studied were first those usually recognized as having a prognostic
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Sample.

Cutoffs at 4 mm and 6.80 respectively, and the other continuous variables and, in particular, over two separate intervals with cutoffs at 50 years and 4 mm, gated (7); age and Breslow were coded as continuous variables. The functional form of each continuous variable was investigated (7); age and Breslow were coded as continuous variables over two separate intervals with cutoffs at 50 years and 4 mm, respectively, and the other continuous variables and, in particular, WBC count, were kept as such. For illustrative purposes (Figs. 1–3), Breslow and WBC count were dichotomized with cutoffs at 4 mm and 6.80 $\times 10^9$ liter (median value), respectively.

DFI was the end point. Treatment-covariate interactions were sought. A quantitative interaction arises when there is a variation in the magnitude, but not in the direction, of treatment effects among subsets. Quantitative interactions are said to occur when one treatment is superior for some subsets of patients and the alternative treatment is superior for other subsets (8). The search for treatment-covariate interactions was carried out using the method proposed by Byar and Green (9). The variables that had a prognostic significance in the control group, the IFN group, or both combined were determined (9); DFI was estimated by the Kaplan Meier method (10), and comparisons between groups were made by the log-rank test; the variables that had a prognostic significance were introduced in a Cox model (11), and those that were selected after a step-down procedure with a significance level of 0.05 were considered for the final model. First, the Cox model which predicted relapse and that only in the control group, an increment of 1 $\times 10^9$/liter WBCs being associated with a HR of relapse of 1.23 (i.e., with an increased risk of relapse of 23%).

RESULTS

The clinical and biological variables found to have a prognostic significance for the control group, the IFN group, or both combined are shown in Table 1. In the multivariable analysis, Breslow thickness was a predictive factor of relapse in all three groups. Among the baseline biological variables, only WBC count was predictive of relapse and that only in the control group, the IFN group, or both combined were determined (9); DFI was estimated by the Kaplan Meier method (10), and comparisons between groups were made by the log-rank test; the variables that had a prognostic significance were introduced in a Cox model (11), and those that were selected after a step-down procedure with a significance level of 0.05 were considered for the final model. First, the Cox model which predicted relapse and that only in the control group, an increment of 1 $\times 10^9$/liter WBCs being associated with a HR of relapse of 1.23 (i.e., with an increased risk of relapse of 23%).

The Cox model that predicted DFI when considering interactions of prognostic factors with treatment (Table 2) fits the main effects of gender, age, Breslow, WBC count, and treatment as well as the WBC treatment interaction term. The prognostic value of Breslow thickness and WBC count, using 4 mm, which is the cutoff value between AJCC stage IIA and IIB for Breslow thickness was a predictive factor of relapse in all three groups. Among the baseline biological variables, only WBC count was predictive of relapse and that only in the control group, the IFN group, or both combined were determined (9); DFI was estimated by the Kaplan Meier method (10), and comparisons between groups were made by the log-rank test; the variables that had a prognostic significance were introduced in a Cox model (11), and those that were selected after a step-down procedure with a significance level of 0.05 were considered for the final model. First, the Cox model which predicted relapse and that only in the control group, an increment of 1 $\times 10^9$/liter WBCs being associated with a HR of relapse of 1.23 (i.e., with an increased risk of relapse of 23%).
7.22 IFN was significantly superior to no treatment were those and van Eys (12), the estimated values of WBC count for which are given, the first corresponding to the lower values.

Because age and Breslow are coded as linear variables over two separate intervals (cutoffs at 50 years and 4 mm, respectively), two HRs are given, the first corresponding to the interval with lower values.

A different way of illustrating the WBC-IFN interaction, data not shown). Because age and Breslow are coded as linear variables over two separate intervals (cutoffs at 50 years and 4 mm, respectively), two HRs are given, the first corresponding to the lower values.

Table 1 Prognostic factors of the disease-free interval for the control group, the IFN group, and both combined in univariable and multivariable analysis with estimated HR ratio of relapse

<table>
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<th>Treatment group</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
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<tr>
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<td>Variable</td>
<td>P (log-rank)</td>
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<td>Control (n = 243)</td>
<td>Age (years)**</td>
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<tr>
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<td>Clark</td>
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<td>Platelets</td>
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<td>γGT</td>
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<td>IFN (n = 244)</td>
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<td>Age (years)</td>
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<td>WBC (10^9/L)</td>
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<td>Platelets</td>
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<td></td>
<td>γGT</td>
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<td>Control + IFN (n = 487)</td>
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<td>Age (years)</td>
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<td>WBC (10^9/L)</td>
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<tr>
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<td>WBC treatment interaction</td>
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(a) LRT, likelihood ratio test; Gamma GT, gamma glutamyl transferase.  
(b) Because age and Breslow are coded as linear variables over two separate intervals (cutoffs at 50 years and 4 mm, respectively), two HRs are given, the first corresponding to the lower values.

and van Eys (12), the estimated values of WBC count for which IFN was significantly superior to no treatment were those ≥7.22 × 10^9/liter.

A different way of illustrating the WBC-IFN interaction, whatever the Breslow thickness, is shown in Fig. 3. In control patients, WBC count had a prognostic value (Fig. 3A and Table 1), whereas WBC count had no prognostic value in the IFN group (Fig. 3B and Table 1), the DFI curves of high WBC IFN-treated patients being similar to those of low WBC control patients. Among patients with Breslow thickness <4 mm, IFN improved the DFI of patients with a high baseline WBC count [HR, 0.52 (95% CI, 0.34–0.79); P = 0.002, log-rank test] but not that of patients with a low WBC count [HR, 1.32 (95% CI, 0.82–2.13); P = 0.25]. Among patients with Breslow ≥4 mm, there was a trend toward an increased DFI in high baseline WBC IFN-treated patients compared with control patients [HR, 0.56 (95% CI, 0.29–1.08); P = 0.08], whereas in low baseline WBC counts, IFN did not improve DFI [HR, 0.96 (95% CI, 0.43–2.13); P = 0.91]. When the baseline NC was considered instead of WBC count, similar results were obtained, with the NC being a predictor of DFI (P = 0.0001) and there being a significant interaction between NC and IFN (P = 0.0001). However, when baseline lymphocyte count or eosinophil count were considered, no predictive value of these counts and no significant interaction between them and IFN were found (data not shown).

Baseline WBC count, mean (±SD) 7.2 (±1.96) × 10^9/liter, was positively correlated only with baseline neutrophils (Spearman correlation coefficient, r = +0.91; P = 0.0001), lymphocytes (r = +0.43; P = 0.0001), and platelets (r = +0.26; P = 0.0001) and negatively correlated only with baseline age (r = −0.14; P = 0.01), and bilirubin (r = −0.12; P = 0.01). The baseline WBC count was not correlated with Breslow thickness [median, 2.51 mm (range, 1.5–20.0); r = +0.04; P = 0.32] nor with eosinophils (r = +0.02, P = 0.72). There was no significant difference in WBC count between site, Clark level, or gender subgroups. The baseline WBC count, which was measured at a median of 19 (range, 0–52) days after last melanoma surgery, was not correlated to time since last melanoma surgery (r = −0.06; P = 0.21).

The mean (±SD) change in WBC count at 1 month was,
among patients with WBC counts <6.8 × 10⁹/liter, +0.24 (±1.22) × 10⁹/liter in control patients and −1.22 (±0.95) × 10⁹/liter in IFN-treated patients, and, among patients with WBC ≥6.8 × 10⁹/liter, −0.78 (±2.05) × 10⁹/liter in control patients and −2.96 (±2.32) × 10⁹/liter in IFN-treated patients. Among the 234 evaluable IFN-treated patients [one relapse the first month and nine (4%) missing data], the relative decrease in WBC count (median, −30%; range, −81, +46) was nearly significantly predictive of relapse [HR, 1.011 (95% CI, 0.999–1.022), P = 0.06], patients having greater decreases of WBCs being at lower risk of relapse.

In the final model to predict DFI, significant interactions between WBC count and Breslow thickness and between gender and age were found (Table 3), although the test for heterogeneity (8) of these two interactions was not significant (0.05 < P < 0.10 and 0.10 < P < 0.25 respectively). Regarding the age-gender interaction, among patients younger than 50 years, female patients had a prolonged DFI compared with males (P = 0.003); however, for patients above 50 years, gender had no prognostic value (P = 0.29).

**DISCUSSION**

In this study, gender, age, Breslow thickness, and WBC count, given that there was an interaction between WBC count and IFN, were predictive of relapse. The baseline WBC count was the only clinical, histological, or biological characteristic for which there was an interaction with treatment and which predicted a beneficial response to adjuvant low-dose IFN; patients with low baseline WBC counts experienced no benefit from IFN, whereas the DFI of patients with high baseline WBC counts was highly significantly prolonged in IFN-treated patients compared with control patients, and this, whatever the Breslow thickness. Similar results were found when the baseline NC was considered instead of WBC count.

The prognostic value of Breslow thickness, age, and gender are well known (6). To our knowledge, the prognostic value of clinical and histological factors in primary melanoma has been
extensively studied, whereas the prognostic significance of baseline biological variables has received little attention (6, 14). In our study, the high prognostic value of baseline WBC counts in the no-treatment group, high values being associated with a poor prognosis, was unexpected.

In this study, the baseline WBC count was analyzed as a continuous variable because this coding best modeled the influence of WBC count on DFI. Although the median value of WBC count (6.8 × 10^9/liter) was used as a cutoff value in the figures, the values of baseline WBC counts for which IFN was estimated to be significantly superior to no treatment were those ≥7.2 × 10^9/liter. These values were determined on a population of resected AJCC stage IIA–IIB melanoma patients whose inclusion criteria were, among others, WBC count ≥4.0 × 10^9/liter. However, only very few patients were not included in the study because of WBC counts below 4.0 × 10^9/liter; the WBC count of our study population is, therefore, presumably representative of the WBC counts of patients with primary melanoma thicker than 1.5 mm without clinically detectable node metastases.

To our knowledge, the WBC-IFN interaction has not been reported previously. We believe that this finding merits attention. Indeed, the difference in DFI between the IFN and control group, the high values being associated with a different response to treatment according to different levels of a variable, which was carried out in this study, was not initially planned and, therefore, belongs to exploratory data analysis (17). The method used is one that allows an understanding of the prognostic role of the clinical, histological, and biological variables in the three groups considered (i.e., the IFN group, the control group, and both combined). Exploratory analyses, unlike hypotheses testing, are concerned with the manipulation and summarization of data to make it more comprehensible to the human mind and with discovering patterns that may be concealed in complex data sets (18). Patterns should be sought and examined to see whether they lead to interesting questions; one must bear in mind, however, that the surest way to assess the truth of these patterns is by finding them in other data sets (9).

Our results should be validated in similar groups of patients with low-dose IFN regimens, ideally in a prospective setting. Moreover, they should be sought in different melanoma populations and with different IFN regimens; indeed, because the WBC-IFN interaction is presumably linked to the mechanism of action of IFN, it might not be found in high-dose regimens where different mechanisms of IFN are at work (i.e., immunomodulatory versus cytotoxic effects).

In this exploratory study, the only initial characteristic that predicted a beneficial response to low-dose adjuvant IFN was a high baseline WBC count. The fact that such a simple and rough marker could be related to prognosis and response to IFN may surprise, especially when subtle mechanisms are at work. However, one should remember that simple markers such as sedimentation rate are very useful markers in the management of many inflammatory diseases. If our results are confirmed by other studies, WBC counts may help to identify patients who would benefit from IFN and avoid unnecessary toxicity and quality of life impairment in those who would not respond to IFN.

REFERENCES


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