expression for 96% (BT-474) and 93% (MCF7) of the cell line population with MFI ratios of 46 and 26, respectively. Only BT-474 showed an amplification of the Her/2 neu oncogene by FISH. Comparisons of frequencies were performed using Pearson’s χ2 test. Overall (OS) and disease-free (DFS) survivals were estimated by the Kaplan-Meier product limit method. Curves were compared by the stratified log-rank test.

Fifteen B-ALL patients were found to be positive for surface Her2/neu expression, including 2 children and 13 adults, 11 male and 4 females. The median percentage of Her2/neu positive blasts was 94% (range: 11–99%). The median MFI was 7.7 (range: 3.5–54.5). The incidence of positivity in children was only 3.4% (2/59) compared to 31% in adults (13/41) (p = 0.001). None of the positive B-ALL patients showed gene amplification, as detected by FISH analysis, suggesting that another mechanism is involved, such as transcriptional activation or post-translational modifications. Considering only adult B-ALL patients (n=38, median follow-up: 11 months, range (1-113), in whom the main prognostic parameters and treatment (70% of patients were treated according to or in the GOELAL2 trial) did not differ significantly between Her2/neu positive and negative patients (Table 1), we observed that surface Her2/neu expression was associated with chemoresistance (50% versus 11%, p = 0.03). Furthermore, trends for correlation with refractory disease (41% versus 11%, p = 0.08) and disease relapse (55% versus 36%, p = 0.08) were observed, suggesting that surface Her2/neu expression could be a prognostic marker of poor clinical outcome in ALL. However, overall survival (median 9 months versus 18 months, p = 0.17) and disease-free survival in patients with complete remission (median 11 months versus 39 months, p = 0.27) were similar between the two groups. This may be due to the small number of patients in the series, but also because, in the same proportion of Her2/neu negative patients, some patients received autologous or allogeneic stem cell transplants because of the poor results of the first chemotherapy.

Target-directed signal transduction inhibition therapy using anti Her2/neu monoclonal antibody might be a therapeutic possibility in this selected group of poor-risk adult B-ALL patients. We are currently conducting a phase II trial of efficacy and tolerance of trastuzumab in adult Her2/neu positive relapsed/refractory B-ALL patients.

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References


Malignant Lymphomas

Methylation status of the p15, p16 and MGMT promoter genes in primary cutaneous T-cell lymphomas

p15MKsa, p16MKsa and O′-methylguanine DNA methyltransferase (MGMT) gene hypermethylation was studied in 22 patients with primary cutaneous T-cell lymphomas (CTCL). p15MKsa and p16MKsa inactivation is present in early and advanced disease and seems to be independent of disease stage. MGMT inactivation may play a pathogenetic role in a subset of CTCL.

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Translational repression by hypermethylation of promoter sequences has been postulated as a possible way in which tumor suppressor genes are inactivated in cancer. Inactivation of the INK4 family (composed of p15MKsa, p16MKsa, p18MKsa, and p19MKsa)) may lead this mechanism in several malignant neoplasms. Promoter hypermethylation in p15MKsa and p16MKsa seems to be major mechanism of inactivation in both B- and T-cell hematologic malignancies. These phenomena occur as independent events and seem to occur with a tumor-specific pattern. Homozygous deletions also play a role in 10–40% of the cases depending on the subtype, however, mutations in these genes are rarely detected in leukemias and lymphomas. MGMT is a DNA repair enzyme that protects cells from alkylating agents that frequently target the O6-position of guanine. Methylation-associated silencing of MGMT has been described in many human neoplasms, and may predict good response to alkylating agents. However, MGMT could also play a role in the protection against carcinogenesis and its inactivation has also been implicated in the generation of transition mutations in K-ras and p53 genes.

Twenty-two patients with CTCL were included in the study (9 with patch/plaque mycosis fungoides (MF); 6 with tumoral MF, 1 with Sézary’s syndrome, and 6 with peripheral T-cell lymphoma primarily arising in the skin).

CpG island methylation status was analyzed as previously described. In brief, DNA was modified by sodium bisulfite and a subsequent methylation-specific polymerase chain reaction (PCR) was carried out using primers designed for methylated and unmethylated p15MKsa, p16MKsa and MGMT promoter genes. DNA from normal lymphocytes was used as the negative control for unmethylated alleles and DNA methylated in vitro with the bacterial methyltransferase Sss I was used as the positive control (Figure 1).

Selective hypermethylation of either the p15MKsa or p16MKsa gene occurred in 13 out of 22 cases (59%). Hypermethylation of the p15MKsa gene was detected in 4/6 patients (66%) with tumor-stage MF, and in one patient with plaque stage...
This last patient presented MF associated with lymphpomatoid papulosis, and both lesions showed hypermethylation in the p16 INK4a gene. p15 INK4b was the only gene hypermethylated in the patient with Sézary’s syndrome. Two out of 6 (33%) of the patients with peripheral T-cell lymphoma had p15 INK4b gene inactivation. Conversely, hypermethylation of the p16 INK4a gene was found in 4/9 (44%) patients with plaque stage MF, but in only one case of 6 (16%) with tumor-stage MF. Of the 6 patients with peripheral lymphoma, 3 (50%) showed hypermethylation of the p16 INK4a gene. p15 INK4b methylation was demonstrated in 8 out 22 skin biopsies (36%). One patient with plaque stage MF, 2/6 with tumor-stage MF and 5/6 biopsy specimens (84%) from peripheral T-cell lymphomas showed this alteration (Table 1). Navas et al. detected p16 INK4a alterations (hypermethylation and/or allelic loss) in 7 out of 9 MF tumor-stage lesions. Alterations in p16 INK4a and p15 INK4b were detected in 18% and 5%, respectively, of patients with early MF but in 73% and 27%, respectively, of those with aggressive MF. The authors postulated that p16 INK4a inactivation could be a necessary step for tumor progression in MF, but that additional changes are required to allow or accelerate it.7,8

In contrast, Scarisbrick et al. detected allelic loss on 9p21 in 25% of a large series of CTCL cases. Hypermethylation of the p15 INK4b gene was demonstrated in 29% and 45%. These abnormalities seemed not to be dependent on the stage of the disease. Hypermethylation of the p15 INK4b gene was not identified in any of the cases of Sézary’s syndrome, although 5/12 (42%) patients had hypermethylation of the p15 INK4b gene. Our results regarding p15 INK4b and p16 INK4a gene methylation seem to support these latter results.9

We have demonstrated a high frequency of MGMT hypermethylation (84%) in primary cutaneous peripheral T-cell lymphomas.
lymphoma. As far as we know, this phenomenon has not been previously reported, and the significance of this observation remains elusive. In addition, in our series MGMT hypermethylation does not seem to play a significant role in the response to chemotherapy in CTCL patients. The possibility that MGMT hypermethylation may be a negative prognostic factor identifying a specific subset of lymphomas cannot be completely ruled out.

In conclusion, our results demonstrate that hypermethylation in p15\textsuperscript{ink4a} and p16\textsuperscript{ink4a} promoter genes seems to play a role in the pathogenesis of cutaneous T-cell lymphoma. These phenomena occur independently as tumor-specific events and probably are not dependent on the stage of the disease. MGMT promoter hypermethylation occurs in a significant percentage of T-cell cutaneous lymphomas.

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Key words: methylation status, MGMT, primary cutaneous T-cell lymphoma.

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References


Blood Doping

Longitudinal variation of hemoglobin and reticulocytes in elite rowers

Longitudinal monitoring of athlete’s hemato logic parameters holds considerable promise as a strategy to detect and thereby deter illicit blood doping. This study documents temporal changes of hemoglobin concentration (Hb) and reticulocyte counts in elite rowers. The ‘within subject’ variation in rowers was comparable to that of athletes from other sports. Reticulocyte results were dependent on the type of instrument used.

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The concept of utilizing a Hematologic passport as a tool to highlight athletes who have used illegal blood doping to enhance performance is gaining credibility. Faced with a limited budget, anti-doping authorities must balance the frequency of testing with the scope of hematologic evaluation. Two cornerstones of this approach will be Hb concentration and reticulocyte counts, which can both be measured on portable analyzers relocated to the event venue. Thus samples collected at the time of competition for routine blood screens could also provide a cost-effective and convenient source of Passport data.

A crucial element of the Passport approach is to define in advance normal fluctuations in blood parameters, to enable authorities to discriminate between expected and suspect changes. It is also necessary to recognize whether biological variability is sport-specific or whether such changes are universal across different sports. The first aim of this research was to report the variation of Hb and reticulocytes in rowers, and then to contrast these with data reported previously for athletes from another discipline. A second aim was to verify whether it was tenable to compare hematologic results obtained on different instruments.

Blood samples were obtained from members of the French National Rowing squad (n=83 males, n=31 females). Between two and eight (average 4.8 males and 3.7 females) EDTA blood samples were collected from each rower between July 2001 and March 2004, and were measured on either an ADVIA 120 Hematol ogy Analyzer or a Sysmex Roche XE2100 (instruments were calibrated according to manufacturers’ specifications). To provide heightened resolution, reticulocytes are reported on a square root scale. These results were contrasted with previously reported data comprising longitudinal evaluations from a subset of n=288 male professional football players measured on average 2.9 times between May 1999 and July 2002. Analysis of variance was used to partition the total variation present in each data set into three components: the variation within subjects, the variation between days (within instruments) and the variation between subjects.

Table 1 gives estimates of the variances. In each case the estimate allows for the other two components of variation. All of the between subject and between days variances are significantly greater than zero (F-test, P < 0.001). The estimates show that the within subject variance for both Hb and reticulocytes is comparable among (male) football players and rowers. For Hb the between subject variances for males are comparable (estimates are at least double those of the within subject estimates, although lower for females). In