cytogenetic risk category, type (e.g. primary vs. secondary) and FLT3-status (Table 1). The overall complete remission rate (CR) was 58.2% and median OS was 12.4 months. The presence of RAS mutations had no influence on the rate of complete remissions. This was found throughout different cytogenetic risk and age groups. As expected, the CR rate was lower for patients >60 years and those in the high risk group. In the multivariate analysis including age, disease status, cytogenetics, FLT3- and RAS-status, the last had no independent influence on survival. Overall and disease-free survival was similar for patients with mutated and wild type RAS (Figure 1 A,B). Considering the specific risk stratification in our AML study (low risk=t(8;12)) we reanalyzed the data after regrouping all CBF leukemias (t(8;21)+ inv(16)) into the low risk group. Compared to the original data set no significant differences were found.

As far as we know, this report is the largest study on the prognostic significance of mutations of N- and K-RAS in this disease group. We used a previously published PNA-based PCR technique for the analysis of K-RAS and developed an analogous assay for N-RAS. Since the samples were analyzed in a blinded fashion and randomly taken from a nationwide multicenter prospective trial our results should be representative of AML. The overall prevalence of RAS mutations was, albeit in the upper range, comparable to that in previous studies. We could not confirm a correlation between RAS mutations with any clinical feature, e.g. blast percentage, as reported earlier. Published reports addressing the clinical significance of RAS mutations in patients with acute myeloid leukemia are inconclusive. Whereas some studies demonstrated a beneficial clinical effect of RAS mutations, others reached a different conclusion (e.g. lower CR). Most studies did not that show that patients with RAS mutations had significantly different outcomes.

In conclusion, despite the evidence that activation of the Ras-signaling cascade contributes to the molecular pathogenesis of myeloproliferative disorders, the prognostic value of RAS-mutations seems to be of minor relevance compared to that of age or karyotype.

References


Acute Lymphoblastic Leukemia

Overexpression of Her2/neu is observed in one third of adult acute lymphoblastic leukemia patients and is associated with chemoresistance in these patients

Among 100 patients with acute lymphoblastic leukemia (ALL), 15 B-ALL patients were found positive for surface Her2/neu expression. The incidence in children was only 3.4% compared to 31% in adults (p=0.001). Considering only adult B-ALL patients (n=38), surface Her2/neu expression was associated with chemoresistance (50% versus 11%, p=0.03) suggesting that it could be a prognostic marker of poor clinical outcome in ALL.


Letters to the Editor

The Her2/neu/c-erb-B2 protein is a transmembrane receptor tyrosine kinase related to the epidermal growth factor receptor. Her2/neu is overexpressed on several epithelial tumors and correlated with poor prognosis in some of them, especially breast and ovarian cancers.

Burting et al. reported that about 40% of patients with B-acute lymphoblastic leukemia (ALL) expressed Her2/neu on the surface of their leukemic blasts. More recently, Müller et al. reported that the incidence of patients with Her2/neu-positive ALL was 16% (n=5/31) and demonstrated that Her2/neu-specific autologous cytotoxic T-lymphocytes could be generated in vitro using peptide-pulsed dendritic cells indicating that vaccination strategies targeting Her2/neu could be evaluated in a subset of patients with ALL.

Herceptin® (rhu-mAb-Her2, Trastuzumab, Genentech Inc., San Francisco, CA, USA) is the humanized equivalent of the murine 4D5 monoclonal antibody and is targeted against the
Letters to the Editor

Her2/neu cell-surface receptor, blocking tumor proliferation by inducing antibody-dependent cellular cytotoxicity. The availability of this monoclonal anti-Her2/neu antibody as a therapeutic agent incited us to re-evaluate the incidence of Her2/neu expression in 100 patients with ALL (B-ALL: n=87; T-ALL: n=13), including 93 at diagnosis and 7 at relapse, and 41 adults and 59 children (<18 years). The period of enrollment was between March 1993 and January 2004. After density separation (lymphocyte medium; Eurobio, les Ulis, France) 0.5×10^6 bone marrow mononuclear cells were incubated for 20 minutes at room temperature with anti-CD19 APC (J4.119), anti-CD7 FITC (8H8.1) (Coulter Immunotech, Miami, FL, USA), anti-CD45 PerCP (2D1) and anti-Her2/neu (Neu 24.7) or isotype control PE (BD Biosciences, San José, CA, USA) monoclonal antibodies. After 2 washes, cells were fixed in 1% formaldehyde. Five thousand blasts identified on the CD45/SSC dot plot (Figure 1) were counted per tube, using a flow cytometer (FACS Calibur, BD). The mean fluorescence intensity (MFI) ratio was obtained by dividing the MFI of Her2/neu antigen by the MFI of its isotypic control. The threshold of positivity for Her2/neu expression was defined by a ratio ≥2 (Figure 1). All the patients with Her2/neu expression detected by FACS analysis were analyzed by fluorescent in situ hybridization (FISH) using the Her2/neu DNA probe kit (Vysis, Downers Grove, IL, USA). Two breast tumor cell lines MCF-7 and BT 474 were used as positive controls: MCF-7 and BT474, obtained from the DSMZ (Braunschweig, Germany).

The two control specimens demonstrated surface Her2/neu

Table 1. Characteristics of adult patients with B-ALL according to Her2/neu expression.

<table>
<thead>
<tr>
<th>Patients (n=38)</th>
<th>Her-2/neu positive patients (n=12)</th>
<th>Her-2/neu negative patients (n=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>8/4</td>
<td>14/12</td>
<td>0.3</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>48 (18-83)</td>
<td>46 (19-74)</td>
<td>0.7</td>
</tr>
<tr>
<td>Leukocytes &lt;30,000/mm³: yes/no</td>
<td>5/7</td>
<td>14/12</td>
<td>0.36</td>
</tr>
<tr>
<td>Ph+ ALL: yes/no</td>
<td>6/6</td>
<td>11/15</td>
<td>0.46</td>
</tr>
<tr>
<td>Other cytogenetic abnormalities: yes/no</td>
<td>6/6</td>
<td>10/16</td>
<td>0.37</td>
</tr>
<tr>
<td>Sensitivity to corticosteroid therapy</td>
<td>8/4 (day 1 of first line CT): yes/no</td>
<td>22/4</td>
<td>0.33</td>
</tr>
<tr>
<td>Sensitivity to corticosteroid therapy</td>
<td>6/6 (days 7, 8 or 15 of first line of CT): yes/no</td>
<td>23/3</td>
<td>0.03</td>
</tr>
<tr>
<td>Complete remission 1: yes/no</td>
<td>7/5</td>
<td>23/3</td>
<td>0.08</td>
</tr>
<tr>
<td>GOELAL2 trial: yes/no</td>
<td>7/5</td>
<td>18/8</td>
<td>0.3</td>
</tr>
<tr>
<td>No graft/ autograft/allograft</td>
<td>7/1/4</td>
<td>13/1/2</td>
<td>0.31</td>
</tr>
<tr>
<td>Relapse: yes/no/not evaluable</td>
<td>4/5/3</td>
<td>9/16/1</td>
<td>0.08</td>
</tr>
<tr>
<td>Median OS : months (range)</td>
<td>9 (1-86)</td>
<td>18 (1-113)</td>
<td>0.17</td>
</tr>
<tr>
<td>Median DFS: months (range)</td>
<td>11 (5-24) (n=7)</td>
<td>39 (1-113) (n=23)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

CT: chemotherapy.

Figure 1. FACS analysis. A) Patients with Her2/neu positive B-ALL. In the top row, only a subpopulation of blasts expressed Her2/neu on their surface. (35%, MFI ratio=11). In the 2 bottom rows, all of the blasts expressed Her2/neu on their surface (MIFR=4.2 and 28). B) Breast tumor cell lines MCF-7 and BT 474 in the top row and in the bottom row, respectively.
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/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 B-ALL patients. We are currently conducting a phase II trial of efficacy and tolerance of trastuzumab in adult B-ALL patients. We are currently conducting a phase II trial of efficacy and tolerance of trastuzumab in adult B-ALL patients. We are currently conducting a phase II trial of efficacy and tolerance of trastuzumab in adult B-ALL patients.

Patrice Chevallier,* Nelly Robillard,* Saraya Wuijte-Toumi,† Françoise Méchinaud,* Jean-Luc Harousseau,* Hervé Avet-Loiseau*

*Service d’Hématologie Clinique, Centre Hospitalier Universitaire, 9 quai Moncousu, 44093 Nantes Cedex 01, France; †Laboratoire d’Hématologie, Institut de Biologie, Centre Hospitalier Universitaire, 9 quai Moncousu, 44093 Nantes Cedex 01, France

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Key words: HER2/neu expression, acute lymphoblastic leukemia, FACS analysis.

Correspondence: Patrice Chevallier, Service d’Hématologie Clinique, Centre Hospitalier Universitaire, 9 quai Moncousu, BP1005, 44093 Nantes Cedex 01, France. Phone: international +33.240083264, Fax: international +33.240083250. E-mail: pchevallier@chu-nantes.fr

References


Malignant Lymphomas

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

p15INK4b, p16INK4a, and O’-methylguanine DNA methyltransferase (MGMT) gene hypermethylation was studied in 22 patients with primary cutaneous T-cell lymphomas (CTCL). p15INK4b and p16INK4a 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.

Transcriptional 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 p15INK4b, p16INK4a, p18INK4c, and p19INK4d) seems to be a 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).

CxG 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 p15INK4b, p16INK4a, 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 p15INK4b or p16INK4a gene occurred in 13 out of 22 cases (59%). Hypermethylation of the p15INK4b gene was detected in 4/6 patients (66%) with tumor-stage MF, and in one patient with plaque stage...