stimulating factor, blood and platelet support, and empiric antibiotic treatment, the patient died of pneumonia in September 1996.

No specific association is known between primary myelofibrosis and lymphoma, despite a few reported cases of myelofibrosis complicated by concomitant or subsequent lymphoma. On the other hand, lymphoma is a recognized, albeit uncommon, cause of myelofibrosis. Severe myelofibrosis in the setting of follicular NHL is exceedingly rare, and its occurrence following fludarabine administration suggests an etiopathogenetic link. The role that fludarabine played in our patient's myelofibrosis can only be speculated, but given the short experience with this drug we do not believe physicians to be aware of this potential severe complication of purine analog administration.

Key words
Myelofibrosis, fludarabine, indolent lymphoma

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References

In vitro modulation of bcl-2 protein expression, drug-induced apoptosis and cytotoxicity by interleukin-10 in chronic lymphocytic leukemia

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Interleukin-10 failed to modify either the percentage of bcl-2+ cells or the number of bcl-2 molecules, or to reduce 2-chlorodeoxyadenosine- and fludarabine-induced apoptosis. The cytokine at 0.1 ng/mL induced an increase of cell survival both in the absence or in the presence of 2-chlorodeoxyadenosine, while no difference was appreciated with fludarabine.

Controversial results have been recently reported on interleukin-10 (IL-10) as an apoptosis inducer in chronic lymphocytic leukemia (CLL) cells. In this regard, we would like to report our experience on its effect on 22 previously untreated CLL patients.

IL-10 (Schering-Plough, Milan, Italy) at 0.1 ng/mL induced a statistically significant increase of cell survival, as measured by the MTT assay, compared to cytokine-free cultures (p=0.024). Similarly, IL-10 0.1 ng/mL significantly raised the 2-chlorodeoxyadenosine- (2-CDA) (Leustatin, Ortho Biotech, USA) LD50 values (p=0.009), while no significant difference was appreciated in the fludarabine (FAMP) (Fludara, Schering AR, Germany) group (Figure 1A). IL-10 also failed to reduce significantly the percentage of either 2-CDA- or FAMP-induced apoptotic nuclei (Figure 1B) as evaluated by flow-cytometry analysis. Finally, the percentage of bcl-2+ cells, which significantly reduced after a 4 days of culture, remained unmodified in the presence of IL-10 (Figure 2A). In 11 cases, the number of bcl-2 molecules was also analyzed by DAKO QUIFIKIT assay (Figure 2B). The number of bcl-2 molecules decreased spontaneously after 4 days of culture (14,746 versus 6,689 ABC units, p=0.0044). We failed to document that IL-10 had an effect on reducing bcl-2 antigen density (6,689 versus 6,130 ABC units, p=not significant).

Our data are in line with the results of Jurlander et al. regarding the effect of IL-10 in modulating both cell viability and apoptosis of CLL cells. Our experiments, like those performed by Jurlander, were carried out on freshly isolated cells; this is a key point to explain why our and Jurlander results differ from those of Fluckiger. It is worth noting that other authors demonstrated that IL-10 prevents CLL cells from undergoing apoptosis. In our study in vitro culture of CLL cells significantly reduced both the percentage of cells expressing the bcl-2 protein content and the antigen density. The addition of IL-10, which has been claimed to down-regulate the bcl-2 product in CLL, slightly, but not significantly, lowered both the percentage and the amount of molecules of the bcl-2 positive-cells. Likewise, a more recent report showed that IL-10 enhanced the survival of CLL cells by inhibiting the process of apoptotic cell death, without increasing bcl-2 expression. The finding that IL-10 mRNA expression is firmly associated with non-progressive disease gives a speculative indication for immunotherapy with rh-IL-10 in CLL patients to prevent disease progression. On the other hand, the fact that the growth of the B-1 malignancy, a murine counterpart of CLL, is dependent on autocrine production of IL-
is in line with our results showing a significant increase of cell viability after 4 days of culture in the presence of a low concentration of IL-10.

In conclusion, these data suggest that IL-10 has no in vitro relevance in giving a death signal either by down-regulating bcl-2 cellular content, or by enhancing apoptosis or by improving the effect of either 2-CDA or FAMP in inducing cell cytotoxicity in CLL cells. However, clinical approaches are required to analyze the magnitude of its potential in vivo effect.

Key words
bcl-2 protein, apoptosis, interleukin-10, CLL

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References

Figure 1. A) In vitro effect of several IL-10 concentrations on 2-CDA- and FAMP-LD90 values. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test: the comparison of the mean values was significant only between IL-10=0 and 0.1 ng/mL in the presence of 2-CDA (p=0.009). B) Effect of IL-10 100 ng/mL on flow cytometry analysis of 2-CDA- (1.25 µg/mL) or FAMP- (1 µg/mL) induced apoptotic nuclei on CLL cells after 4 days of incubation. Statistical analysis revealed no significant modification caused by the addition of IL-10.

Figure 2. A) Flow-cytometry evaluation of bcl-2 percentage on freshly isolated cells (day 0), and cells after 4 days of culture with or without IL-10 100 ng/mL. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test: day 0 versus day 4, p=0.0037; day 0 versus day 4 + IL-10, p=0.0006; day 4 versus day 4 + IL-10, p= not significant. B) Flow cytometry determination of bcl-2 antigen density, expressed as antigen binding capacity units, on freshly isolated cells (day 0) and after 4 days of culture without or with IL-10 100 ng/mL. Statistical analysis was performed by Wilcoxon matched pairs signed-ranks test: day 0 versus day 4, p= 0.0044; day 4 vs. day 4 + IL-10, p = not significant.


**Table 1. Stimulation of mononucleated HUCB and PB samples with PHA.**

<table>
<thead>
<tr>
<th>Hours</th>
<th>HUCB</th>
<th>PB</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>CD3+HLA-DR+ cells (%)</td>
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</tr>
<tr>
<td>0</td>
<td>3.6±0.5</td>
<td>3.6±0.9</td>
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<tr>
<td>24</td>
<td>34.6±12.7</td>
<td>38.6±10.3</td>
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<tr>
<td>48</td>
<td>40.3±10.2</td>
<td>42.1±6.5</td>
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</tr>
<tr>
<td>72</td>
<td>58.2±12.1</td>
<td>59.4±5.6</td>
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<tr>
<td>CD3+CD25+ cells (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3.9±0.5</td>
<td>5.1±0.3</td>
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<tr>
<td>24</td>
<td>45.2±9.6</td>
<td>42.4±14.9</td>
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<tr>
<td>48</td>
<td>44.3±15.8</td>
<td>55.8±16.5</td>
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<tr>
<td>72</td>
<td>64.6±7.4</td>
<td>70.1±14.6</td>
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<tr>
<td>S-phase cell number (%)</td>
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<td></td>
<td></td>
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<tr>
<td>0</td>
<td>1.7±1.2</td>
<td>1.4±0.4</td>
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<tr>
<td>72</td>
<td>24±12.8</td>
<td>25±9.8</td>
<td>ns</td>
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</tbody>
</table>

Data are expressed as mean percentage cell number±standard deviations. Statistical comparisons were carried out using Student’s t-test.