'From the cradle to the grave': novel therapeutic approaches to attack the microenvironment in chronic lymphocytic leukemia

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Concluding remarks
One focus of the studies described in this thesis is the mechanism of apoptosis induction by novel drugs that act independently of the p53 response pathway. In chapter 2 we discussed the insights obtained from the studies on these drugs in relation to the current debate regarding the interactions and functional mechanism of the Bcl-2 family members. Our conclusions are summarized in a model (chapter 2)\(^1\).

A second major aspect of this thesis concerned the functional consequences of CD40 triggering and the identification of abnormalities in apoptosis pathways in CLL cells, in relation to drug sensitivity (chapter 4-5-6). In this final chapter the relevance of our findings and the implications with respect to future treatment strategies for CLL are discussed.

CD40L expressed on the surface of fibroblast or epithelial cell lines has been widely used to enhance the \textit{in vitro} survival of CLL cells\(^2\)-\(^9\). We have used this system to study the effects of CD40 ligation on drug sensitivity. The basic premise underlying these studies is that CD40-stimulated peripheral blood (PB) CLL cells are a representative model for CLL cells residing in lymph node (LN) proliferation centers, the site where they receive pro-survival signals from the microenvironment. Thus, a key question is whether the model is indeed appropriate and relevant. To our opinion the answer to this question should be affirmative because of the following arguments.

(1) Histochemical studies have shown that \textit{in vivo} CLL cells may be exposed to CD40L. T lymphocytes have been found interspersed between CLL cells in LN and bone marrow (BM) proliferation centers\(^10\)-\(^11\) and CD40L-positive T lymphocytes have been detected in LN pseudofollicles\(^12\). However, the functional activity of these T cells remains to be proven. It is known that \textit{ex vivo} CD40 stimulation provides significant survival signals to normal tonsil-derived B lymphocytes\(^13\)-\(^14\). Similar experiments with LN-derived CLL cells have to our knowledge not been published, but it appears likely that these would yield comparable results.

(2) We and others have shown remarkable similarities between \textit{in vitro} CD40L stimulated PB CLL cells and \textit{in vivo} LN CLL cells regarding changes in expression pattern of apoptosis regulating proteins. Table 1 summarizes the expression of important members of the Bcl-2 family (Bcl-X\(_L\), Mcl-1, Bid, Bim\(_{el}\) and Noxa), the activated form of the MAP kinase ERK (p-ERK), XIAP and survivin. Both in CD40-triggered PB CLL cells and in LN CLL cells expression of Bcl-X\(_L\), Mcl-1, p-ERK, XIAP, survivin and Bid are increased, whereas Noxa and Bim\(_{el}\) are decreased.

(3) In CD40 stimulated PB CLL cells and in LN CLL cells the expression pattern of these proteins is comparable not only qualitatively but also quantitatively. In our experience, the observed changes compared to PB CLL are of the same order of magnitude. As a corollary, this notion provides an argument against the possibility that
the *in vitro* CD40 system provides unphysiological stimuli. Of course, some caution must be exerted in directly extrapolating the data to the *in vivo* situation because it has to be assumed that *in vivo* CLL cells will receive a variety of signals.

Table 1. Protein expression in LN CLL and *in vitro* CD40L stimulated PB CLL cells is similar.

<table>
<thead>
<tr>
<th>Protein</th>
<th>LN</th>
<th>CD40L</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-X₁</td>
<td>Up</td>
<td>Up</td>
<td>[3, 4, 7, 15]</td>
</tr>
<tr>
<td>Mcl-1</td>
<td>up</td>
<td>Up</td>
<td>[3, 4, 7, 15]</td>
</tr>
<tr>
<td>Bid</td>
<td>up*</td>
<td>Up*</td>
<td>[2, 7]</td>
</tr>
<tr>
<td>BimEL</td>
<td>Down</td>
<td>Down</td>
<td>Chapter 5 and 6</td>
</tr>
<tr>
<td>Noxa</td>
<td>Down</td>
<td>Down</td>
<td>[15]</td>
</tr>
<tr>
<td>p-ERK</td>
<td>Up</td>
<td>Up</td>
<td>Chapter 5 and 6</td>
</tr>
<tr>
<td>XIAP</td>
<td>Up*</td>
<td>Up</td>
<td>[4, 5]</td>
</tr>
<tr>
<td>Survivin</td>
<td>Up</td>
<td>Up</td>
<td>[16]</td>
</tr>
</tbody>
</table>

Protein expression of indicated proteins was evaluated in LN CLL cells and in PB CLL cells after *in vitro* CD40 triggering. Changes as compared to non-stimulated PB CLL cells are indicated by up- or down-regulation.

* (Hallaert, unpublished observations)

These observations have 2 important implications.

1. The proliferation centers in LN, BM and spleen might be considered as ‘sanctuary sites’ where CLL cells are protected from the effects of cytotoxic drugs. Thus, these sites probably are the source of the relentless relapses that characterize the clinical course of CLL, explaining why thus far curative treatment is lacking. Relapses occur even with recently introduced, very potent drug combinations (like FCR) which result in minimal residual disease (MRD) negativity in the PB and BM in a high percentage of patients\(^{17}\). Whereas in the later phases of the disease (acquired) p53 dysfunction is an important cause of therapy resistance, at diagnosis only 10-15% of patients have a 17p deletion\(^{18,19}\). In contrast, the microenvironmentally induced chemo-resistance is of great importance in all phases of CLL.

2. Novel therapies should be tested on CD40L stimulated CLL cells. To date, the potential efficacy of novel cytotoxic agents is usually assessed on PB CLL cells. This disregards the potential protective signals from the microenvironment. As a consequence, various compounds induce significant apoptosis *in vitro*, but the responses *in vivo* remain disappointing.
It can be concluded that the ideal treatment of CLL should at the same time be able to overcome resistance in the sanctuary sites and induce apoptosis independent of p53 function. A promising drug combination in this perspective is the c-Abl kinase inhibitor with proteasome inhibitors (chapter 6). In clinical trials in CLL however, dasatinib and bortezomib administered as single agent yielded disappointing effects, underscoring the need for combination regimens. To explore this, in the near future a clinical trial with dasatinib plus fludarabine will be started in fludarabine refractory CLL.

In the more distant future, the CD40 system might not only be useful for new drug testing in general but may also enable pre-treatment assessment of the potential responsiveness to (combinations of) drugs in individual patients, opening possibilities to attain the important goal of individualized, “tailor made” therapy in CLL.


