chapter 8

Summary

The work described in this thesis can be divided into two parts. The first part focuses on the mechanism of apoptosis induction by novel drugs that act independently of the p53 response pathway (chapter 2-3). The second part focuses on the functional consequences of CD40 stimulation and the understanding of aberrant apoptosis pathways in CLL. These studies were designed to test drug sensitivity and to find future treatment strategies for CLL (chapter 4-5-6).

Chapter 1 introduces the current understanding in therapy, apoptosis and the microenvironment in CLL and provides an outline for the studies presented.

In **chapter 2** we investigated the mechanism underlying apoptosis elicited by the cyclin dependent kinase inhibitor, roscovitine, in CLL. Biochemical analysis showed that the BH3-only protein Noxa was associated with the Bcl-2 homologue Mcl-1 in CLL cells. Furthermore, this apoptosis route required the BH3-only member Bim. Deficiency of these Bcl-2 family proteins, assessed by RNA interference techniques, protected CLL cells from roscovitine induced apoptosis. We concluded that CLL survival depends on the balance between these pro- and anti-apoptotic Bcl-2 family members,and that roscovitine activates a selective apoptosis pathway, described as the Mcl-1/Noxa axis.

In **chapter 3** we focused on the effect of a chemical compound, γ-secretase inhibitor (GSI-1), that is used in the treatment of Alzheimer's disease. We found that GSI-1 was a potent inducer of apoptosis in CLL through efficient blocking of proteasomal activity. Furthermore, inhibition of proteasomal activity triggered the accumulation of the pro-apoptotic molecule Noxa. The role of Noxa was also demonstrated via RNA interference experiments. We suggest that GSI-1 or related compounds may hold promise for therapeutic applications in CLL.

In **chapter 4** we compared apoptosis gene profiles from peripheral blood with lymph node material of CLL patients. A prominent difference between CLL cells in the peripheral blood and the lymph node was the decreased expression of the pro-apoptotic protein Noxa and the increased expression of anti-apoptotic Mcl-1 and Bcl-X_L in the lymph node. This differential expression pattern was also observed by *in vitro* CD40 stimulation of peripheral blood CLL cells. Direct manipulation of Noxa protein levels was achieved by proteasome inhibition (increase in Noxa expression, see chapter 3) in CLL cells and RNA interference techniques (decrease in Noxa expression) in model cell lines. In both experiments, cell viability was directly linked with the levels of Noxa. We concluded that the suppression of Noxa in the lymph

node microenvironment contributes to the resistance of the CLL cells at these sites. The hypothesis is that proliferating CLL cells in the lymph node are protected from apoptosis through the modulation of pro- and anti-apoptotic molecules by CD40 signaling. Once CLL cells leave their lymph node 'cradle', the nurturing CD40 signal is lost and a strong increase in Noxa levels occurs, which corresponds with the high apoptosis rate of peripheral blood-derived CLL *in vitro*. We propose that Noxa is a promising therapeutic target to treat CLL.

In **chapter** 5 we gained more insight in how CLL cells in the lymph node differ from the CLL cells which have moved into the circulation. We mimicked this transit *in vitro* from the lymph node to the peripheral blood by temporary CD40 receptor triggering of CLL cells (CD40 model) and we monitored the expression of apoptosis regulatory genes in relation to sensitivity to various types of drugs during and following CD40 triggering. We showed that CD40 induced changes in the apoptosis gene expression profile and that the observed broad drug resistance was reversible after cessation of CD40 stimulation. In contrast, however, Bim and Mcl-1 protein levels remained unchanged and also roscovitine resistance was sustained. We conclude that this model system to mimic the *in vivo* lymph node setting could help to predict responses of CLL to new drugs.

Chapter 6 aimed to define the molecular basis for the increased drug resistance observed after CD40 stimulation in CLL and searched for novel strategies to circumvent this resistance. As shown in chapters 4 and 5, CD40 triggering resulted in an anti-apoptotic RNA and protein profile and resulted in resistance to various chemotherapeutic drugs. The anti-apoptotic profile of CD40-stimulated CLL cells resembles BCR-Abl-dependent changes seen in Chronic Myeloid Leukemia (CML). This abnormality is characterized by a translocation between one chromosome 9 and one chromosome 22, resulting in the BCR/Abl fusion-gene. This prompted us to use c-Abl inhibitors imatinib and dasatinib, which prevented the entire anti-apoptotic profile of CD40 triggered CLL, and restored drug sensitivity. These effects also occurred in CLL samples with a dysfunctional p53. We concluded that treatment settings, combining c-Abl inhibitors such as imatinib or dasatinib with other chemotherapeutic drugs may be promising for the treatment of CLL.