Chapter 1

Introduction
Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, comprising approximately 30% of all leukemias diagnosed annually. The median age at presentation is 65 years, with an incidence of less than 1 per 100,000 in persons under 40 years of age, rising to 19 (females) and 36 (males) per 100,000 in persons older than 75 years.

The disease is characterized by the accumulation of small monomorphic B lymphocytes in the peripheral blood (PB), bone marrow and lymph nodes (LN). The liver and spleen are typically infiltrated and other extra-nodal sites may occasionally be involved. There has been a longstanding debate as to the normal cellular counterpart(s) of CLL, but based on surface membrane phenotype and gene expression profile, CLL most likely derives from an antigen-experienced B cell.

The clinical course of CLL is very heterogeneous. Approximately 30% of patients has an indolent course and eventually dies from causes unrelated to CLL, whereas 15% dies rapidly (within 2 to 3 years from diagnosis) from CLL and/or treatment related causes. In the remaining portion of patients, the disease has a relatively indolent course during the first 5 to 10 years, followed by a terminal phase marked by considerable morbidity, both from the disease itself and from complications of therapy.

Many disease associated features have been found to be related to adverse outcome. In order to distinguish patients based on their prognosis as to survival, clinical staging systems have been developed by Rai et al, and by Binet et al a few years later. These staging systems are still widely used. However, 30 - 40% of patients with early stage disease (i.e. Rai stage 0/ Binet stage A) experiences rapid clinical progression and ultimately dies from CLL related causes. Because at present the disease is diagnosed more frequently in young and asymptomatic patients, while at the same time therapeutic options expand, accurate risk stratification is highly relevant. Several features intrinsic to the CLL cells have been found to be associated with adverse outcome, among which mutation status of the immunoglobulin heavy chain (IgVH) gene, CD38 expression, ZAP-70 expression and cytogenetic abnormalities, especially those affecting chromosome 17p on which the p53 gene is located (see below).

Although some patients remain asymptomatic, the majority of CLL patients eventually requires treatment. At present, remission rates achieved with conventional (immuno-) chemotherapy are high; however, cures are not obtained and the need for repeated cycles of therapy inevitably results in drug resistance which infers a very poor prognosis.

The origin of drug resistance in CLL
Alkylating drugs and nucleoside-analogs (including fludarabine) are the mainstay of the treatment of CLL. These drugs exert their activity via p53 dependent induction of apoptosis (programmed cell death). Apoptosis is initiated when (in response to cellular stress) anti-apoptotic Bcl-2 family members such as Bcl-2, Bcl-xL, Bfl-1/A1 and Mcl-1 are engaged by pro-apoptotic BH3-only proteins. Such interactions are not promiscuous; only certain protein pairs associate inside cells. Bim and Puma engage all
Bcl-2 family members, whereas Noxa only binds Mcl-1 and Bfl-1/A1, and Bad binds Bcl-2, Bcl-xL and Bcl-w (Figure 1). Binding of BH3-proteins to Bcl-2 family members leads to activation of the effector proteins Bax and Bak and the subsequent cascade of events which will ultimately result in cell death: permeabilization of the mitochondrial membrane, cytochrome C release and activation of effector caspases. We have previously found that p53 dependent death following (fludarabine-) treatment of CLL cells is mediated by transcriptional upregulation of the BH3-only protein Puma. Also the BH3-only protein Noxa was found to be decisive for cell fate in CLL cells, presumably through interaction with its anti-apoptotic counterpart Mcl-1, especially in CLL cells residing in the LN. Although the response to several (novel) drugs involves Noxa, the role and regulation of Noxa in response to drug treatment in CLL are not well known. Noxa is a response gene of p53 in many cell types, but this seems not to be the case in CLL.
Drug resistance often finds its origin in the balance between pro- and apoptotic proteins. Several mechanisms contribute to drug resistance in CLL: (1) in the secondary lymphoid tissue and bone marrow, CLL cells engage in interactions with various cell types which results in proliferative and anti-apoptotic signaling; and (2) the evolution of clones harbouring cytogenetic alterations, of which especially those affecting the p53 response have clinical impact 25;26.

1. Interactions of CLL cells with the microenvironment

In the bone marrow CLL cells mainly interact with mesenchymal stromal cells (MSCs), whereas in secondary lymphoid tissue CLL cells interact with T cells, nurse-like cells (NLCs) and follicular dendritic cells.

Activated CD4+ T cells found in the pseudofollicles in lymphoid tissue of CLL patients express CD154 which engages CD40 expressed on the surface of CLL cells. We have previously demonstrated upregulation of the anti-apoptotic proteins Bcl-xL, Bfl-1/A1, and Mcl-1 upon prolonged CD40 stimulation of PB derived CLL cells in vitro 27, in a similar pattern as was found in lymphoid tissue derived leukemia cells 21. Numbers of activated T cells, both CD4+ and CD8+, are increased in CLL 28-31. Although these expansions have been thought to be driven by a putative tumour-derived antigen, in vitro data do not support this hypothesis, as cytotoxic responses against autologous CLL cells have rarely been observed 30;32;33. We have found that the expansion of CD8+ T cells in CLL is largely confined to CD8+ T cells exhibiting the cytotoxic CD45RA+/−CD27- phenotype and is exclusively seen in CMV-seropositive patients 34. A similar pattern was recently described for the CD4+ compartment 35. The cause of the enhanced T cell activation and whether this finding is unique for CLL is currently not known.

Another cell type abundantly present in the secondary lymphoid tissue of CLL patients is the NLC 36. In vitro, NLCs enhance viability of CLL cells via the secretion of stromal-derived factor-1 (SDF-1)/CXCL12, B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) 37. NLCs also express CD31 (PECAM-1), a member of the Ig family. Recently, CD31 was proposed to function as a ligand for CD38 expressed on CLL cells, resulting in anti-apoptotic and proliferative signaling in CLL cells and thus accounting for the worse prognosis of CD38high CLL 38. Although all these factors have been linked to the survival-supporting property of NLCs, their independent contributions have not yet been properly studied. Yet, in order to develop targeted strategies directed at these interactions, such knowledge is crucial.

Microenvironmental interactions might account for the difference in clinical course between patients with mutated versus unmutated IgVH. Although the gene expression profile of both subgroups is highly similar, a subset of genes is differentially expressed 39;40. Zeta associated protein of 70kDa (ZAP-70) is more frequently expressed in unmutated CLL. ZAP-70 expression allows for enhanced IgM signaling in CLL 41, but it is not known how this affects prognosis. It has been speculated that enhanced NF-κB signaling in ZAP-70+ CLL may play a role in CLL cell survival 42;43. Ligand-receptor interactions, such
as CD40L-CD40 and BAFF and APRIL and their receptors, have been found to increase NF-κB activity in CLL and could therefore account for the difference in biological behaviour between mutated and unmutated CLL.

2. Cytogenetic alterations

Cytogenetic abnormalities can be demonstrated by fluorescence in situ hybridization (FISH) in approximately 80% of patients with CLL. Several recurrent abnormalities have been recognized which relate to prognosis; deletion of 13q14.3 and trisomy 12 are the most prevalent abnormalities and predict a relatively favourable overall survival of 133 and 114 months respectively. Deletions of 11q22-23 and 17p13 are far less prevalent in previously untreated disease; however, these deletions infer a poor prognosis with an overall survival of 79 and 32 months respectively. Loss of function of p53 accounts for the poor prognosis and inferior response to treatment in patients with a deletion of 17p. Chromosome 11q harbours the ATM gene (Ataxia Teleangietasia Mutated), which is also involved in the p53 response. Although, at diagnosis, only 5% of patients with CLL shows deletion of 17p or mutations in the p53 gene, these abnormalities are found in up to 30-40% of relapsed or chemotherapy refractory cases. Whether this results from selection of p53-dysfunctional clones or clonal evolution is subject of investigation.

In recent years, many advances have been made both in upfront treatment and subsequent salvage regimens. At the same time, the standard of supportive care has been improved, allowing CLL patients to live longer in relatively good health. However, with conventional treatment strategies, cures are not obtained, and as the disease evolves, treatment options become increasingly limited by the occurrence of drug resistance. Therefore it is highly necessary to develop therapeutic strategies that circumvent common resistance mechanisms in CLL.
Outline of the thesis

In the first part of this thesis interactions between CLL cells and the microenvironment are studied in order to provide a rationale for further development of targeted strategies for the treatment of CLL. In chapter 2 alterations in the T cell compartment in CLL are compared with those in other B cell malignancies in order to elucidate the mechanisms driving such changes. In chapter 3 the intracellular effects of environmental stimuli (in this case CpG-ODN and CD40-ligand stimulation as a model for the LN environment) are analyzed in both IgVH mutated and unmutated CLL cells, to study whether such stimuli contribute to the difference in clinical course between these subgroups. In chapter 4 we investigate the functional consequences of the interaction of CD38 expressed on CLL cells with CD31 expressed on fibroblasts (as a model for CD31 expressing NLCs in vivo).

The focus of the second part of this thesis is on exploration of p53 independent mechanisms of cell death. Since cisplatinum proved to be highly active in chemoresistant CLL (chapter 5), platinum-based compounds were used to investigate p53 independent death inducing pathways in CLL cells. In chapter 6 the regulation and role of the p53 family member TAp73 in p53 dysfunctional cells is explored. In chapter 7, the efficacy and mechanism of action of cisplatinum is studied in more detail in drug resistant CLL, either due to p53 dysfunction or as a result of microenvironmental stimuli. In this chapter we focus on the cellular redox balance as a possible target to induce p53 independent apoptosis. Lastly, current insights into the optimal treatment of relapsed and chemorefractory CLL will be summarized in chapter 8.
Reference List


37. Nishio M, Endo T, Tsukada N et al. NURSELIKE cells express BAFF and APRIL, which can promote survival of chronic lymphocytic leukemia cells via a paracrine pathway distinct from that of SDF-1alpha. Blood 2005;106:1012-1020.


