Antigen receptor triggering and apoptotic pathways in neoplastic B cells
Mackus, W.J.M.

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CHAPTER 1

General introduction
Chapter 1

B CELL-MEDIATED IMMUNITY

The immune system forms the defense mechanism to eliminate pathogens such as viruses and bacteria from the infected individual. Next to the innate immune system which forms a first line of defense\(^1\), it is composed of the adaptive immune system consisting of T and B lymphocytes which ensure life-long protection against recurrent infection with the same pathogen. B cells detect the presence of a pathogen in the environment via recognition of antigen by the B cell receptor (BCR).

The BCR consists of a membrane-bound Ig molecule composed of two Ig heavy (H) chains and two light (L) chains. Throughout B cell differentiation diversity and specificity of the BCR arises from several recombination processes changing the configuration of the H and L chains. This can give rise to non-functional B cells and also potentially harmful, self-reactive B cells which might lead to auto-immunity\(^2\). Therefore, selection of B cells is essential throughout all stages of B cell development and this is dependent on BCR triggering.

During early B cell development in the bone marrow the H and L chains are constructed from variable, diversity and joining region gene segments via V(D)J-rearrangement\(^3\). First the pre-BCR is formed consisting of a H chain and a surrogate L chain. Pre-B cells that fail to assemble a functional pre-BCR are deleted. Signaling via the pre-BCR is essential for continuation of B cell differentiation since it induces clonal expansion and heavy chain allelic exclusion followed by formation of the L chain\(^4\). This results in a complete BCR (IgM) first expressed on immature B cells which, selected for non-self specificity, differentiate into mature B cells expressing a BCR needed for the continuous delivery of a maintenance signal\(^5\). These IgM\(^+\)IgD\(^+\) naive B cells migrate to secondary lymphoid organs were, upon encounter of antigen along with signals from activated T cells, they start to proliferate (clonal expansion) and form germinal centers which also contain small amounts of T cells and follicular dendritic cells. In the germinal center B cells undergo a number of important changes. Somatic hyper mutation (SHM) introduces point mutations in the Ig variable gene region of both H and L chains to produce IgS with higher affinity for antigen\(^3\). Variation in the effector function of the antibody is generated by switching the completed VDJ gene from the downstream constant (C\(_H\)) \(\mu\) or \(\delta\) gene region segments to either \(\alpha\), \(\gamma\) or \(\varepsilon\) gene region segments (class switch recombination, CSR)\(^6\). Since SHM may change both the affinity and specificity of the BCR, another round of selection is required. B cells expressing a BCR with low affinity for antigen or having obtained specificity for self-antigen are eliminated via programmed cell death, or apoptosis\(^7\). On the other hand, B cells can be positively selected after high affinity binding of antigen (affinity maturation). The processes in the germinal center are dependent on simultaneous cross-linking of the BCR by antigen presented on follicular dendritic cells and ligation of CD40 by T cells. Finally, B cells can differentiate into either long-lived memory cells, that retain the capacity to proliferate and further differentiate upon renewed contact with the same pathogen, or plasma cells, that secrete IgS (antibodies)\(^8\). Antibodies can bind a pathogen resulting in neutralisation, opsonization or complement-activated lysis of that particular pathogen, leading to its elimination.
**APOPTOSIS**

Within the immune system programmed cell death is a central mechanism in maintaining normal lymphocyte homeostasis both during early development and in response to antigenic stimuli when B cells recognize self (negative selection). Programmed cell death, or apoptosis, is a physiological process for the regulated destruction of a cell, first described by Kerr and Wyllie. Important in apoptosis is the participation of a family of cysteine-dependent aspartate-specific proteases, called caspases, which are synthesized as pro-enzymes (zymogens). Initiator caspases are activated in response to apoptotic signals via protein-protein interactions, whereas effector caspases are activated proteolytically by an upstream caspase. This culminates in cleavage of cellular proteins finally resulting in disassembly and removal of the cell.

**The extrinsic and intrinsic apoptosis pathways**

The proapoptotic signals activating caspases can be delivered via two major pathways, the so called extrinsic and intrinsic apoptosis pathways (reviewed in 11;12 (Fig. 1)). The “extrinsic” or death receptor-mediated apoptosis pathway is induced upon activation of trimerized receptors belonging to the TNF-receptor super family by their trimerized ligands. The best characterized example is CD95 (Fas, Apo1). When engaged by its cognate ligand CD95 binds the intracellular adaptor protein FADD (Fas-associated-DD containing protein) which in turn recruits procaspases 8 or 10 to form the death-inducing signaling complex (DISC). Subsequently, procaspase 8 is activated via autoproteolytic cleavage. This may either directly lead to activation of executioner caspases 3, 7, and 6, or induces an amplification loop that relies on caspase 8-mediated cleavage of the pro-apoptotic Bcl-2 family member Bid followed by activation of the mitochondrial apoptosis pathway (see below). Finally cellular substrates are cleaved leading to the execution of cell death. Additional proteins have been found which also complex to the DISC, such as Flip (Flice (caspase 8)-inhibitory protein). The best understood function of Flip is prevention of apoptosis induction via interfering with caspase 8 for its association to the DISC.

The second apoptosis pathway integrates cellular stress signals at the level of the mitochondria and therefore is called the “intrinsic” or mitochondrial apoptosis pathway (Fig. 1). DNA damage, hypoxia, and cytokine withdrawal induce mitochondrial outer membrane permeabilisation and release of mitochondrial proteins such as cytochrome C (cytC), AIF, Smac/Diablo, Omi/HtrA2 and endonuclease G, all regulating apoptotic cell death. When released into the cytoplasm the electron transport chain protein cytC binds to the scaffolding protein apoptotic protease-activating factor-1 (Apaf-1) causing, in the presence of ATP, a conformational change that results in Apaf-1 oligomerization. Procaspase 9 binds to the Apaf-1 oligomers resulting in the formation of a high-molecular mass complex, the apoptosome. This enhances the enzymatic activity of caspase 9 which in turn proteolytically activates caspase 3 resulting in apoptosis.
Chapter 1

Figure 1. Modulation of apoptosis pathways
Apoptosis is mediated via two pathways. The extrinsic pathway is initiated by triggering of death receptors leading to DISC formation and caspase activation. The intrinsic apoptosis pathway is induced by cellular stress signals integrated at the mitochondria leading to the release of mitochondrial proteins and activation of caspases. Both pathways are coupled via the cleavage of Bid which migrates to the mitochondria inducing cytC release. Caspase activation is further controlled either by interactions of Bcl-2 family members at or IAP family members downstream of the mitochondria. For further details see text. Adapted from 20.

The Bcl-2- and IAP-family: regulators of apoptosis
Loss of mitochondrial integrity is essential for induction of apoptosis and is controlled by the B cell lymphoma-2 (Bcl-2)-family of proteins (reviewed in 23 (Fig. 1)). Bcl-2 was first discovered as a result of the chromosomal translocation t(14:18) in B cell lymphoma where its high expression levels conferred resistance to apoptosis. The mammalian Bcl-2-family consists of both pro- and anti-apoptotic proteins which show sequence and structural similarity in the Bcl-2 homology (BH) regions of which four domains have been identified. Bcl-2-family members can be divided in three subfamilies (see also chapter 5, Table 2): (1) the anti-apoptotic Bcl-2-family members including Bcl-2, Bcl-xL, Bcl-w, Boo/Diva/Bcl-b, Mcl1, and A1/Bfl1, (2) the pro-apoptotic Bax-like family members including Bax, Bak, Bclxs, Bok/Mtd and Bcl-Gl, Rambo, and (3) the apoptogenic BH3-only proteins including Bad, Bcl-Gs, Bid, Bik/Nbk, Bim/Bod, Blk, Bmf, Hrk/DP5, Noxa and Puma/Bbc3.

The precise mechanism of how Bcl-2 family members control apoptosis is not fully understood. Apoptotic signals induce a conformational change of Bax-like proteins upon which these translocate from the cytosol into the outer mitochondrial membrane and homodimerize to form pores. Both Bax and Bak are essential for the final execution of apoptosis. Inactivation of either one affected apoptosis only slightly, but inactivation of both genes dramatically impaired apoptosis in many tissues. It is
thought that anti-apoptotic Bcl-2 family members prevent the release of cytC, but it is not precisely known how this is mediated\textsuperscript{23,29}. The BH3-only proteins seem to act as damage sensors and direct antagonist of the anti-apoptotic proteins. Whereas some of the BH3-only proteins are under transcriptional control, most of these pro-apoptotic proteins are present in a dormant form and are activated by posttranslational modifications that result in conformational changes and release from an inactive complex\textsuperscript{30} (Fig. 1).

Downstream of mitochondria, caspase activity can be controlled by inhibitor of apoptosis (IAP) proteins, including XIAP, cIAP1, cIAP2, NAIP, Survivin and Livin (Fig. 1). These IAPs bind with their baculovirus inhibitor repeat (BIR)-domain to the active site of caspases, acting as competitive inhibitors\textsuperscript{31,32}. In general, the anti-apoptotic effect of IAPs is neutralized upon release of the mitochondrial proteins, such as Smac/Diablo and Omi/HtrA2, which can sequester the IAPs\textsuperscript{33,34} and thus add another level in the regulation of apoptosis.

**Apoptosis regulation in B cells**

In B cells apoptosis can be mediated either via cell surface receptor stimulation of CD95 or the BCR. Upon activation B cells upregulate CD95 rendering them potentially vulnerable to CD95-mediated apoptosis. Triggering of CD95 results in elimination of cells, unless they receive appropriate survival signals mediated by antigen-receptor triggering and costimulatory molecules, which allows them to survive and clonally expand\textsuperscript{35}. Recent investigations have shown that BCR-induced cell death involves mitochondrial dysfunction and is dependent on caspase 3 activation\textsuperscript{36-38}. CD40-mediated survival signals are induced via NF-\textkappa B-mediated upregulation of anti-apoptotic proteins such as A1\textsuperscript{39,40}. How the coordinated signals mediated after CD95-, BCR- and CD40-triggering are integrated resulting in either apoptosis or survival of B cells is not completely understood.

**B CELL NEOPLASIA**

All three recombination processes activated during B cell development, V(D)J rearrangements, SHM and CSR, involve the introduction of double-strand DNA breaks which occasionally are aberrantly resolved. This might lead to chromosomal translocations or deletions resulting in the potential activation of oncogenes or loss of activity of tumor suppressor genes. It has been suggested that errors in all three processes may contribute to the pathogenesis of B cell malignancies in various ways\textsuperscript{41}. Most of the chromosomal translocations involved in B cell malignancy coincide with fusion of the IgH locus containing strong promoters, with another gene such as the gene encoding for Bcl-9, t(1:14) in acute lymphoblastic lymphoma; Bcl-10, t(1:14) in MALT-lymphoma; c-MYC, t(8:14) in Burkitt lymphomas; Pax5, t(9:14) in lymphoplasmacytic lymphoma; cyclin D1, t(11:14) in mantle cell lymphoma; and Bcl-2, t(14:18) in follicular lymphoma\textsuperscript{41,42}. SHM can also target non-immunoglobulin loci, such as Bcl-6 which is involved in many cases of diffuse large B-cell lymphoma\textsuperscript{43}, whereas many of the translocations involved in multiple myeloma and sporadic Burkitt lymphoma originate through CSR because the translocation breakpoints occur in IgH switch regions\textsuperscript{44}. 

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B CELL CHRONIC LYMPHOCYTIC LEUKEMIA

B cell chronic lymphocytic leukemia (B-CLL) is defined by an accumulation of small, long-lived B lymphocytes in cell cycle arrest that express CD19, CD20, CD5 and CD23, and low levels of slgM and slgD. The clonal expansion of the leukemic cells is thought to occur by small aggregates of proliferating cells, which form the so-called pseudofollicles, situated in lymph nodes and scattered in the bone marrow. Although in more than 80% of B-CLL cases cytogenetic aberrations are involved, for most of the frequently affected genomic regions in B-CLL the candidate genes responsible for the disease have not been found.

B-CLL is one of the most common types of adult leukemia in the Western countries with an average incidence of 2.7 persons per 100,000 in the United States. B-CLL is generally a disease of the elderly, with a median age at diagnosis exceeding 60 years. The risk of developing CLL increases progressively with age and is two times higher for men than for women. The disease is characterised by lymphocytosis, lymphadenopathy and hepatosplenomegaly. The clinical outcome of B-CLL can be very variable with some patients having a long survival and never requiring therapy, whereas in others the disease pursues an aggressive course that demands intensive treatment. In advanced stages many patients suffer from infections caused by hypogammaglobulinemia and of autoimmune-associated phenomena directed against cells of the hematopoietic system. At present, no curative treatment has been found.

Origin of B-CLL cells

As mentioned above, at the clinical level B-CLL is considered to be a very heterogeneous disease. Recently a basis for this heterogeneity has been found at the cellular and molecular level. Many of the B cell malignancies retain histological, cellular and molecular features related to different stages of B cell development, implying that they may arise from a normal B cell counterpart. Analysis of \( V_H \) gene sequence is increasingly being employed in the investigation of chronic B cell malignancies as it may relate to the clonal history of the cell of origin. According to the \( lgV_H \) mutation status B cell malignancies may be categorized into different stages of B cell development (Fig. 2).

Early studies on small numbers of cases have shown that \( lgV_H \) genes were unmutated in CLL suggesting its naïve nature as also indicated by cell morphology and the common \( lgM^+lgD^+ \) phenotype. This view changed when it was documented that the leukemic cells of ~60% of CLL patients express \( lgV_H \) somatic point mutations with a skewed usage of \( V_H \) gene segments such as \( V_{1-69} \), \( V_{3-23} \), and \( V_{4-34} \). Based on the \( lgV_H \) mutation status, two subgroups of B-CLL patients with a different clinical outcome can be distinguished (see "\( lgV_H \) mutation status") and has lead to the suggestion of both a pre-germinal center and post-germinal center stage of origin.

Using the novel technique of large scale gene expression profiling, information about changes in gene expression of B cells during the germinal center reaction can be obtained. Regardless of the \( lgV_H \) mutation status B-CLL cells express a gene expression profile most similar to those of memory B cells. Moreover, expression of a subset of genes which are modulated upon BCR triggering, "the BCR-activation gene signature", has predominantly been associated with the unmutated \( lgV_H \) status. Although it still cannot be excluded that B-CLL cells can originate from a naïve pre-
Figure 2. Assignment of human B cell malignancies to normal B cell counterparts
Based on IgV_H mutation status B cell tumors are postulated to arise from pre-germinal center, germinal center and post-germinal center B cells. Tumors are indicated within dashed boxes. (ALL, Acute lymphoblastic leukemia; DLBL, diffuse large B cell lymphoma; FL, follicular lymphoma; HCL, hairy cell leukemia, MALT, mucosa-associated lymphoid tissue; MM, multiple myeloma). Adapted from 50.

germinl center B cell precursor, accumulating evidence suggests that B-CLL cells may also originate from antigen-experienced B cells60.

Prognostic markers in B-CLL
The current clinical staging systems of Rai and Binet identify CLL patient groups with different prognosis61,62 and are based on clinical symptoms, physical signs and laboratory values. Nevertheless, these systems fail to predict accurately the course of the disease in individual patients and do not take into account new discoveries about the molecular pathology of this disease. Therefore, there has been a continuous effort to identify other prognostic factors in CLL. The prognostic value of chromosomal abnormalities, IgV_H mutation status and CD38 expression is currently being investigated.

Chromosomal abnormalities
Chromosomal abnormalities have been implicated as prognostic markers in clinical outcome of CLL63. In addition to conventional cytogenetic techniques such as metaphase chromosome analysis, the recent use of fluorescence in situ hybridization analysis has improved the detection of genomic aberrations46,63. In a large single-center study in 82% of B-CLL cases cytogenetic aberrations were identified involving
regions such as 6q21, 11q22-q23, trisomy 12, 13q14.3 and 17p13.1. Patients with a normal karyotype or deletion of chromosome 13q14 have a significantly better prognosis than cases with deletion of 11q (suggested to be associated with the ataxia telangiectasia mutated (ATM) gene) or 17p (associated with the p53-gene). Inactivation of p53 has been associated with an adverse clinical outcome and poor response to therapy. This can be mediated either by p53 mutation or by inactivation of the gene encoding ATM, a kinase that regulates p53 and occurs in 15-25% of B-CLL patients. Thus, chromosomal abnormalities are important predictors of clinical outcome independent from clinical stage.

IgV<sub>H</sub> mutation status
The significance of IgV<sub>H</sub> gene mutation status was reported simultaneously by the groups of Hamblin and Damle. Both groups used homology of ≥98% to the germline sequence to define absence of SHM. Patients with cells expressing unmutated IgV<sub>H</sub> genes showed a distinctly more aggressive course and shorter survival than those which did express somatic mutations. In addition, unmutated IgV<sub>H</sub> patients responded less well to (multiregimen) chemotherapy. Many studies have confirmed the prognostic value of the IgV<sub>H</sub> mutation status in B-CLL. Determination of the IgV<sub>H</sub> mutation status is time consuming and costly. Hence, identification of the IgV<sub>H</sub> mutation status is not widely available in clinical practice. Nevertheless, so far it remains the best marker to assess the prognosis of CLL patients. Thus, finding a surrogate marker for the IgV<sub>H</sub> mutational status in B-CLL is an important priority. CD38 expression, and, recently, ZAP-70 expression have been implicated as possible surrogate markers for IgV<sub>H</sub> mutation status.

CD38 as surrogate marker for IgV<sub>H</sub> mutation status
Human CD38 is a type II transmembrane glycoprotein of ~45 kDa expressed by several cell types, including B cells. The function of CD38 in B cells is not clearly defined, and its expression level varies with the stage of maturation, the type of activation and the milieu in which activation takes place. Damle et al were the first to show that clinical heterogeneity among B-CLL patients can be distinguished based on expression of CD38. Patients expressing CD38 on ≥30% of their leukemic cells experience a worse clinical course and shorter survival than patients expressing CD38 on ≤30% of the CD5<sup>+</sup>CD19<sup>+</sup> B cells. The potential of CD38 expression as an important prognostic factor in B-CLL has been confirmed by others. However, there is a debate as to the appropriate cut-off levels for CD38-positivity (levels of 30%, 20% and 7% have been reported), and the stability of CD38 expression, as it varies under influence of cytokines and during disease progression. Thus, the reliability of CD38 expression as a prognostic marker in CLL needs to be further evaluated.

IgV<sub>H</sub> mutation status has been associated with CD38 expression and chromosomal aberrations in relation to clinical outcome. Cells from patients with the unmutated IgV<sub>H</sub> status more frequently express high levels of CD38 and cytogenetic changes such as 11q22–23 deletion, trisomy 12, and 17p deletion. All these markers associate with poor clinical outcome. On the contrary, leukemic cells expressing mutated IgV<sub>H</sub> genes show low levels of CD38 and the cytogenetic abnormality 13q14 deletion, all associating with a benign clinical course. Although some of the associations have been disputed by others, the prognostic significance in predicting
clinical outcome of each of these markers has been independently recognized in different cohorts of CLL patients15-27.

**ZAP-70 as surrogate marker for IgV<sub>H</sub> mutation status**

In one of the recent gene expression profiling studies the unmutated IgV<sub>H</sub> gene status, unexpectedly correlated strongly with expression of ZAP-7059. In addition, ZAP-70 protein expression correlated with clinical outcome of these patients85. ZAP-70 is a well studied molecule which belongs to the ZAP-70-Syk family of protein tyrosine kinases (PTKs). It has been reported to be expressed exclusively in T cells and natural killer cells and has a critical role in the initiation of T cell signaling86-88. Whether ZAP-70 might serve as a reliable surrogate markers for IgV<sub>H</sub> mutation status in predicting the clinical outcome of the individual B-CLL patient awaits further investigation.

**BCR responsiveness of B-CLL cells**

As outlined above BCR signaling is central to B cell development and the response to antigen. Downstream signaling via the BCR is mediated by the BCR coreceptors, CD79a (Igα) and CD79b (Igβ), which together with the IgH and L chain form the BCR complex. Upon antigen binding to the mlg the src-kinase Lyn is activated which rapidly phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs) present in CD79a and CD79b. These ITAMs serve as docking sites for Src Homology (SH2) domains present in the protein tyrosine kinase (PTK) Syk and the TEC family kinase Btk, facilitating their recruitment and activation89. Syk is a key B cell signaling molecule, since disruption of Syk prevents most downstream BCR signaling90 and leads to a (partial) block in B cell development91,92. In addition to kinases, several phosphatases, linker proteins and the VAV family of RHO GTP-ases are important in regulation of BCR signaling. Upon activation, Syk and Btk are coupled to several downstream signaling pathways crucial for the survival and proliferation of B cells which include the PLCγ, PI3-kinase, RAS-RAF-Erk and NF-κB pathways93.

In the early 1990s studies indicated that B-CLL patients behave differently upon engagement of their BCR thereby distinguishing two subgroups94-97. B-CLL cells either are anergic to stimulation via the BCR or respond similar as normal B cells via mobilisation of intracellular Ca<sup>2+</sup> and phosphorylation of PTKs. Differences in BCR responsiveness in B-CLL cells have been attributed to differences in the constitutive levels96 or phosphorylation of Syk97 and aberrant expression of CD79b98-100. Next to differences in signaling, the functional consequences of BCR triggering of B-CLL cells can be very distinct. BCR crosslinking of leukemic cells has been shown either to induce101 or to prevent apoptosis102. Whereas induction of apoptosis has been correlated with high CD38 expression101, prevention of apoptosis may be related to aberrant expression of CD79b103. The underlying mechanism and how it may be related to other CLL markers such as IgV<sub>H</sub> mutation status is not completely understood.

**Apoptosis dysregulation in B-CLL**

B-CLL represents a typical example of a human malignancy that appears to result primarily from defects in cell death regulation as opposed to cell proliferation. Because B-CLL cells are arrested in the G<sub>0</sub>/G<sub>1</sub>-phase of the cell cycle successful treatment with
cytostatic drugs is hampered as many of these rely on the induction of DNA damage or inhibition of DNA synthesis during cell division\textsuperscript{104}. Thus, although these conventional treatment strategies can induce partial or complete remission they do not cure the disease\textsuperscript{105}.

The underlying mechanisms of apoptosis dysregulation in CLL are not precisely known. B-CLL cells express high levels of Bcl-2\textsuperscript{106,107} which, in contrast to follicular lymphoma's, does not involve t(14:18) translocation\textsuperscript{24}. The high levels of Bcl-2 cannot protect leukemic cells from undergoing spontaneous apoptosis when cultured in vitro. Within 24 hrs of culture around 50% of cultured B-CLL cells undergo spontaneous apoptosis which is in sharp contrast to their extended life span in vivo\textsuperscript{108}. Cell survival of leukemic cells can be influenced by cells from the microenvironment such as bone marrow stromal cells, nurse-like cells, and follicular dendritic cells. These cells enhance survival either via secretion of cytokines and chemokines such as IL-4 or SDF, or via direct cell-cell interactions\textsuperscript{109-111}. Remarkably, B-CLL cells do express CD95 on their membrane but are highly resistant to CD95-mediated apoptosis. Even after further upregulation of CD95 on the leukemic cells by cytokines or CD40 stimulation these cells remain resistant for agonistic anti-CD95 mAbs\textsuperscript{112,113}. Increased levels of anti-apoptotic regulators and inversed ratio of Bcl:Bax have been suggested to block CLL B cell death and have been associated with resistance to cytostatic drugs\textsuperscript{106,107,114,115}. Many of the studies regarding apoptosis regulators in B-CLL have focused on the anti-apoptotic proteins, disregarding the possible involvement of pro-apoptotic proteins. Thus, for a further understanding of the dysregulated apoptosis in B-CLL a comprehensive analysis of both anti- and pro-apoptotic regulators is required.

**SCOPE OF THE THESIS**

This thesis focuses on three major questions: (1) what are the functional consequences of antigen receptor crosslinking of B cells, (2) how can T-B cell interactions influence apoptosis, and, (3) what is the basis for the dysfunctional apoptosis regulation in B-CLL cells?

To investigate the biochemical events upstream and downstream of mitochondrial depolarization resulting in apoptosis of B cells an in vitro model system was used in which signals mediated by CD95, BCR and CD40 triggering are integrated (chapters 2 and 3).

Next to this, apoptosis regulation in leukemic B cells was investigated in cells isolated from B-CLL patients. Expression of the PTK ZAP-70 and the consequences of BCR triggering on spontaneous and drug-induced apoptosis was investigated in relation to IgV\textsubscript{H} mutation status (chapter 4). As apoptosis dysregulation in B-CLL may not be limited to the anti-apoptotic regulators a comprehensive analysis of the expression of both anti- and pro-apoptosis regulators in B-CLL was performed (chapter 5). Cytotoxic effector functions of the expanded T cell pool in B-CLL have been suggested to be possibly directed against the unique antigenic part of the BCR (idiotype) tumor, but convincing evidence is lacking. Thus, we have analysed the antigen-specificity of these T cells (chapter 6).