chapter 1

General introduction

INTRODUCTION

CLL: CLINICAL AND PATHOLOGICAL FEATURES

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by monoclonal small, mature $CD5^+/CD19^+$ B cells which continue to accumulate in the peripheral blood (PB), bone marrow (BM) and lymphoid organs[1]. There is a remarkable clinical variability in patients with CLL[2]. Following diagnosis, some patients have asymptomatic disease that may not progress for many years. Others are diagnosed with advanced-stage disease, or have early-stage disease that progresses rapidly and requires treatment. Clinical symptoms include peripheral blood lymhocytosis, lymphadenopathy and hepatosplenomegaly. When the disease progresses many patients develop hypogammaglobulinemia and neutropenia, resulting in an increased susceptibility to bacterial infections. Furthermore, up to 25% of the patients develop autoimmune cytopenias, mostly autoimmune hemolytic anemia and/or autoimmune thrombocytopenia. The clinical variability is associated with the following molecular markers: cytogenetic abnormalities[3], immunoglobulin V_H gene mutational status[4;5], ZAP-70 expression[6;7] and CD38 expression[8]. How these factors influence the biology of CLL is still subject of intensive research. Immunoglobulin gene mutational status and ZAP-70 expression are associated with the capacity of CLL cells to respond to signals delivered through the B-cell receptor (BCR)[9]. Loss of 17p[10] or 11q[11] may cause dysfunction of p53 DNA damage pathways[12].

Classically, CLL cells were thought to derive from naive B lymphocytes and to behave as inert cells that passively accumulate[13]. However, mRNA studies showed that at the level of gene expression both IgV_H mutated and unmutated CLL cells are most similar to memory B cells[14;15]. Moreover, stable isotope labeling studies with deuterated water (D_2O) have shown that CLL cells divide at a considerable rate, suggesting a more dynamic disease than previously appreciated[16]. Survival and proliferation of the CLL cells are influenced by interactions with non-leukemic cells in the microenvironment of lymph nodes (LN), bone marrow and spleen[1;17;18]. Still, deregulated apoptosis is considered to be a key factor and a major barrier to effective treatment of CLL. Understanding apoptosis pathways and the impact of the microenvironment on these pathways is therefore clinically relevant, because it may open new avenues to effective treatment of CLL.

THERAPY IN CLL

Extensive discussion of the treatment of CLL is beyond the scope of this thesis. Detailed reviews have recently been published[19;20]. Past and current therapeutic approaches to CLL will be summarized here.

About twenty years ago, treatment consisted of alkylating agents, usually chlorambucil. Remission rates were variable (50 – 70%). However, complete remission was rarely obtained[21]. Combination treatment, such as CVP (cyclophosphamide, vincristine and prednisone) and CHOP (cyclophosphamide, doxorubicine, vincristine and prednisone), did not improve overall survival[22]. The introduction of the purine analog fludarabine has brought new impulse to the research of CLL treatments. Clinical trials showed fludarabine to induce higher complete remission rates and longer progression free survival. Again, no survival advantage was achieved[21]. Until recently, the goal of therapy has been palliation with minimal toxicity. Introduction of new therapies and novel combinatorial approaches have initiated a paradigm shift and made potential cure the goal of therapy. Three randomized trials, comparing FC (fludarabine + cyclophosphamide) with fludarabine alone, have been published, all showing higher response rates with prolongation of progression-free survival in the FC arm[23-25]. Nevertheless, thus far no overall survival benefit was obtained. Studies with the most potent combination FC + Rituximab (anti-CD20 chimeric monoclonal antibody) showed high overall and complete remission rate and prolonged progression-free survival, in both previously untreated[26] and relapsed[27] CLL patients. Alemtuzumab (anti-CD52 humanized antibody) has been shown to be effective also in the treatment of p53 dysfunctional CLL patients[28]. Finally, Reduced Intensity allogeneic Stem cell Transplantation (RIST) or 'mini transplants' have broadened the use of allogeneic stem cell transplants in CLL patients. By this approach a long-term relapse-free survival has been achieved [29], but the associated morbidity remains a serious obstacle for wide application[30;31]. Thus, despite recent progress there remains a strong need for novel effective and less toxic treatment options.

APOPTOSIS

Programmed cell death or apoptosis, is essential for tissue homeostasis, and disturbed regulation of this process underlies many diseases, including cancer. Activation of apoptosis is important for the removal of infected, transformed or damaged cells. Apoptotic cells are defined according to morphological characteristics such as cellular

shrinkage, nuclear condensation, membrane blebbing and eventually fragmentation into membrane bound apoptotic bodies[32]. During apoptosis, asymmetry of the cell membrane results in exposure of phosphatidylserine (PS) on the cell surface. PS functions as an 'eat me' signal on apoptotic cells which results in direct recognition, engulfment and degradation by phagocytes[33].

Caspases

Apoptosis involves a family of aspartate-specific cysteine proteases, called caspases[34]. Caspases are synthesized as precursors (pro-caspases), and are converted into mature enzymes upon apoptosis signals. Caspases can be divided into three groups based on their structure and role in apoptosis. First, initiator caspases, consisting of caspase-2, -8, -9 and -10 that cleave inactive pro-forms of effector caspases, leading to activation. Second, effector caspases, consisting of caspase-3, -6 and -7, which in turn cleave other protein substrates within the cell resulting in the apoptotic process[35]. Finally, a third group of caspases is described, caspase-1, -4 and -5, which are not directly involved in apoptosis execution, but play important roles in inflammatory cytokine activation and release[36].

Pathways of apoptosis induction

Two major apoptotic signaling pathways are recognized in mammals: the extrinsic or death receptor mediated pathway and the intrinsic or mitochondrial pathway. Key players in the apoptosis signaling pathway are outlined in **Figure 1**.

The extrinsic pathway is activated upon ligand binding to the tumor necrosis receptor (TNF-R) family members such as Fas (CD95/APO-1), TNF-R and TRAIL-R. This results in receptor trimerization and recruitment of intracellular adaptor proteins, TRADD or FADD, leading to the assembly of the death-inducing signaling complex (DISC)[37;38], and subsequent recruitment and assembly of initiator caspase-8. Caspase-10 can also be recruited to the DISC in a similar manner, but it cannot functionally substitute for caspase-8[39;40]. Subsequently, activated caspase-8 is released into the cytosol where it can activate effector caspases. In addition, caspase-8 can process the BH3-only Bcl-2 family member Bid to the truncated form tBid[41]. Subsequently, tBid can translocate to the mitochondria to exert its pro-apoptotic activity[41;42].

The intrinsic pathway is initiated in response to cellular signals resulting from DNA damage, cell cycle defects, growth factor withdrawal, hypoxia, or other types of severe cell stress and is tightly regulated by Bcl-2 family of proteins. Progression through the

pathway leads to mitochondrial outer membrane permeabilization (MOMP), resulting in cytochrome *c* release and assembly of the apoptosome complex. The apoptosome is a multimeric holoenzyme consisting of apoptotic protease-activating factor 1 (Apaf1), pro-caspase-9 and dATP[43]. Other apoptosis promoting factors are also released from the mitochondria, including Smac/DIABLO, Omi/HtrA2, endonuclease G and apoptosis inducing factor (AIF). Smac/DIABLO and Omi/HtrA2 promote caspase activation by interacting with inhibitors of apoptosis (IAP) family[44;45]. Endonuclease G and AIF translocate to the nucleus and induce DNA degradation[46;47].

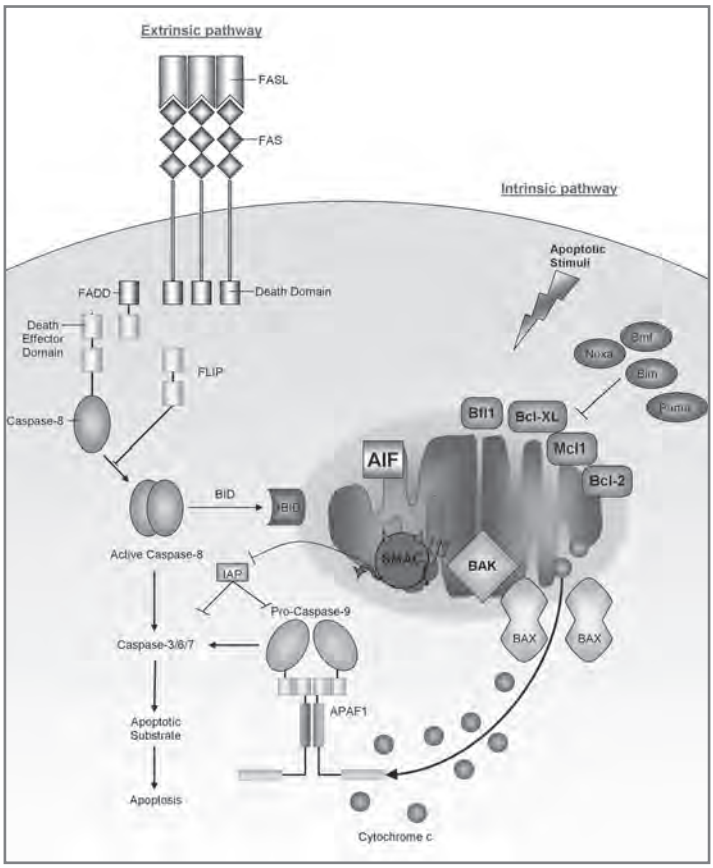

Figure 1. ***See color figures.*** The two main pathways leading to apoptosis. The extrinsic pathway is triggered by ligation of cell surface receptors, such as Fas, resulting in activation of caspase-8. The intrinsic pathway is activated by cytotoxic stimuli, such as DNA damage, which leads to the release of apoptosis promoting factors from the mitochondria. In the cytosol, cytochrome *c* results in the activation of caspase-9. This pathway is regulated by the Bcl-2 family of proteins. Activated caspase-8 and -9 in turn activate effector caspase-3, -6 and -7. Cross-talk between the pathways occurs through Bid, which is cleaved by caspase-8 and then can activate the intrinsic pathway.

Bcl-2 family

The Bcl-2 (B cell lymphoma 2) family in mammals consists of pro- and anti-apoptotic proteins. An overview of the Bcl-2 family protein members is presented in **Table 1**. They all share at least one conserved Bcl-2 homology (BH) domain and are considered to act mainly on the mitochondria[48]. Based on structural and functional features, they can be divided into three subfamilies: the anti-apoptotic subfamily comprising of Bfl-1, Bcl-2, Bcl-W, Bcl-x_L and Mcl-1 (Myeloid-cell leukemia sequence 1) and two other subfamilies which promote cell death: the Bax-like death family and BH3-only family (in Table 1 also several 'BH3-only contenders' are mentioned). Members of the Bax-like death family include Bax (Bcl-2-associated X protein), Bak (Bcl-2-antagonist/killer) and Bok (Bcl-2-related ovarian killer), which contain three BH domains. The BH3-only protein family includes at least 8 members including Bad (Bcl-2-antagonist of cell death), Bik (Bcl-2-interacting killer), Bid (Bcl-2 interacting domain), Hrk (Harakiri, also known as DP5), Bim (Bcl-2-interacting mediator of cell death), Noxa, Puma (p53-upregulated modulator of apoptosis) and Bmf (Bcl-2-modifying factor). They all have only a short BH3 motif. Biochemical studies demonstrated that BH3-only proteins interact selectively and with varying affinities with anti-apoptotic counterparts. Whereas Bim and tBid bind avidly to all the pro-survival proteins, the other BH3-only proteins associate only with selected subsets. For example Noxa binds to Mcl-1 and Bfl-1, while Bad binds to Bcl-2, Bcl-X_L and Bcl-W[49]. For Noxa, however, others reported that Mcl-1 is the only binding partner[50]. Promiscuous binders, like Bim, are much more potent killers than those that cannot engage all the pro-survival proteins[49]. This indicates that the multiple anti-apoptotic proteins all have a different function.

It is the complex interplay between the three Bcl-2 subfamilies that determines the commitment of MOMP and subsequent apoptosis[51]. However, the detailed molecular mechanism remains controversial. The BH3-only proteins act upstream of Bax and Bak, because they cannot induce apoptosis in cells lacking both Bax and Bak[52]. How they induce activation of Bax and Bak is addressed by two distinct models. The *direct activation* model proposes that the BH3-only proteins termed 'activators' (Bid, Bim and perhaps Puma) are capable of binding to and enabling the conformational change and pore formation of Bax/Bak[50;53-57]. Other BH3-only proteins, termed 'sensitizers' (e.g. Noxa and Bad), can displace the activators from anti-apoptotic proteins. In this model, which suggests the existence of a functional hierarchy within the BH3-only subfamily, survival is the default. The *indirect activation* model (also referred as displacement model), on the other hand, suggests that all BH3-only proteins bind to

their specific anti-apoptotic relatives, which are inactivated, thus indirectly enabling the Bax/Bak lethal function. Based on this model, in which death is the default, Bim, Bid and Puma are the most potent apoptosis inducers simply because they are the only ones able to engage all pro-survival proteins[49;54;58;59]. A strong argument for this scenario was made using murine cells without Bim and Bid, and containing reduced Puma. These cells were still capable of undergoing apoptosis[55], something which is hard to reconcile with the direct activation model.

So far most of this work has been done in artificial systems *in vitro* using isolated mitochondria[56], gene ablated murine cells, and transfected or virally transduced cell lines[49]. These approaches leave open the important matter: how these proteins and pathways function in human healthy tissues and primary cancer cells.

Table 1. Overview of Bcl-2 family members and relatives

Prosurvival	Pro-apoptotic		BH3-like contenders
(A) Bcl-2-like	(B) Bax-like	(C) BH3-only	(D)
Bfl-1	Bak	Bad	Bcl-G(S)
Bcl-2	Bax	Bik	BRCC2
Bcl-W	Bok	Bid	MAP-1
Bcl-X_L		Hrk	Mule
Mcl1		Bim	NIP3
		Noxa	Nix
		Puma	Spike
		Bmf	

The Bcl-2 family is subdivided in three main categories: Anti-apoptotic Bcl-2-like proteins **(A)**, pro-apoptotic Bax-like proteins **(B)** and BH3-only proteins **(C)**. Members for which solid evidence has been obtained are reviewed in detail elsewhere[58;60]. In addition, other 'candidates', containing at least a conserved BH3-only or BH3-like domain have been described and are presented in **(D)**. (Adapted from Alves[61])

IAPs

The human IAP family has been implicated in cell division, cell cycle progression, signal transduction[62] and consists of at least 8 members, and includes XIAP, cIAP1, cIAP2, NIAP, and survivin. Overexpression of several IAPs has been detected in various cancers[63]. Of all IAPs, XIAP is most probably the only actual inhibitor of caspases[64]. IAP1 and -2 associate with TNF-receptor family members and recent data have demonstrated that their presence in fact blocks NF-κB signaling. When unleashed, this pathway leads to TNF production which can kill cancer cells in an autocrine fashion[65]. Survivin has been implicated in both apoptosis and cell division, but compelling evidence now points to a specific role in chromosome segregation[66;67].

FLIP

c-FLIP (c-FLICE-inhibitory protein) is a family of alternatively spliced protein variants, and primarily exists as long (c-FLIP$_L$) and short (c-FLIP$_S$) isoforms in human cells. c-FLIP$_L$ is homologous to caspase-8 but cannot become actived. Both FLIP variants can be recruited to the DISC, where they can block pro-caspase-8 activation and protect cells from death receptor mediated apoptosis[68].

APOPTOSIS DEREGULATION IN CLL

Apoptosis regulatory proteins

In CLL several mechanisms contribute to resistance towards apoptosis. First, Bcl-2 is expressed 1.7 – 25-fold higher in CLL than in normal lymphocytes, and CLL cells isolated from patients refractory to standard chemotherapy show an increased Bcl-2/Bax ratio[69;70]. The high expression of Bcl-2 has been postulated to be related to deletions of two Bcl-2 suppressing miRNAs, *miR-15a* and *miR-16-1*, located in a cluster at 13q14.3, which is deleted in ~65% of the CLL patients[71;72]. Furthermore, a polymorphism in the promoter region of the BCL-2 gene (-938C>A) was reportedly associated with inferior clinical course and with increased expression of Bcl-2[73]. However, these initial findings could not be corroborated in two follow-up studies[74;75]. Secondly, high Mcl-1 levels were found to be associated with a failure to achieve complete remission following chemotherapy in CLL[76]. Furthermore, Mcl-1 downregulation correlated with *in vitro* apoptosis induced by various therapeutics in CLL[77-79]. Significant Mcl-1 upregulation and subsequent protection against spontaneous apoptosis was induced upon *in vitro* co-culture of CLL cells with CD40 ligand (CD154) expressed on fibroblasts[80] and with follicular dendritic cells (FDC)[81]. Thirdly, a nucleotide polymorphism, -248G>A, in the 5' promoter region of Bax was found in CLL, causing a reduced protein expression[82]. However, the outcome of the disease did not seem to be influenced by this polymorphism, and, thus the clinical impact was uncertain[83]. Finally, IAPs such as XIAP, cIAP1 and cIAP2 may also be overexpressed in CLL[84]. XIAP inhibitors, which can potentially de-repress caspase activity in malignant cells, are currently viewed as promising novel treatment options[85].

Death receptors

CLL cells express barely detectable levels of Fas on their surface, although transcripts for both Fas and FasL (CD178) are commonly detected. Several stimuli have been shown to up-regulate Fas expression, e.g. IFN and CD40-ligation, although CLL cells

remain resistant to Fas-mediated apoptosis[86]. However, CLL cells become sensitive to Fas-mediated apoptosis upon prolonged stimulation with CD40 ligand, concomitantly with FLIP down-regulation and up-regulation of FADD[87].

Death-inducing receptors for TNF-related apoptosis-inducing ligand (TRAIL), TRAIL-R1, TRAIL-R2, TRAIL-R3 and TRAIL-R4 are expressed on primary CLL cells at a low level. Nevertheless, CLL cells are resistant to TRAIL-induced apoptosis. In correlation with low TRAIL receptor surface expression, DISC formation is hampered and further caspase-8 activation is prevented[88]. Sensitivity to TRAIL was induced when CLL cells were pretreated with nontoxic concentrations of histone deacetylase (HDAC) inhibitors[89]. Furthermore, CD40 ligation induces expression of the pro-apoptotic BH3-only protein Bid and TRAIL-R5. Death receptors CD95 and TRAIL-R5 can act synergistically to induce caspase-dependent apoptosis of CLL cells and Bid can facilitate cross-talk between mitochondrial-dependent and death receptor inducing pathways[90].

p53

p53 plays a protective role in normal somatic tissues by preventing division of damaged cells[91;92]. The locus of the p53 gene is 17p13.1. The p53 protein acts in many cellular processes, including cell-cycle checkpoints, DNA repair, senescence, apoptosis and the surveillance of genomic integrity[93;94]. Stress stimuli such as DNA-damaging drugs rapidly induce a transient increase in p53 protein[95]. Wild type (wt) p53 inhibits cancer development by inducing transcription of a number of target genes involved in cell cycle arrest and apoptosis. A p53-inducible gene involved in cell cycle arrest is p21 (also Cyclin-dependent kinase inhibitor 1A, CDKN1A). On the other hand, p53 can trigger apoptosis via Bax[96], Puma-[97;98] and Noxa-[99] gene transcription.

Deletions of p53 occur in about 10-15% of the CLL cases[10;100]. However, p53 dysfunction can also occur via alternative mechanisms, such as the inactivation of ataxia telangiectasia mutated (ATM) gene[12]. ATM, encoded at chromosome 11q22.3, is a kinase controlling p53 activation in response to DNA double-strand breaks. Dysfunction of p53 by inactivation of ATM accounts for an additional abnormality in 15-20% of the patients[101;102]. In general, defects in p53 function occur in approximately 20% of CLL patients[101]. However, the frequency of p53 dysfunction increases to nearly 50% as the disease progresses following initial therapy[103;104]. CLL patients with p53 dysfunction do not respond to conventional therapy and tend to have a rapidly progressive disease[3].

Many therapeutic strategies require an active p53. Consequently, loss of p53 function results in a selective resistance to DNA-damaging therapies, including alkylating agents and fludarabine[103]. Therefore, an important area of research is devoted to

the identification of new treatment strategies that function independently of the p53 pathway.

ROLE OF THE MICROENVIRONMENT IN CLL

Although CLL cells show characteristics consistent with a defect in programmed cell death and exhibit prolonged survival *in vivo*, during *in vitro* culture CLL cells isolated from peripheral blood can rapidly undergo spontaneous apoptosis[105;106]. This dichotomy highlights the relevance of an *in vivo* microenvironment capable of delivering survival signals. Survival-supporting factors might rescue leukemia cells from cytotoxic therapy[107;18] and this may be the basis for subsequent relapse.

The CLL proliferating compartment is represented by focal aggregates of proliferating lymphocytes that give rise to the so-called pseudofollicles or proliferation centers. In the LN of CLL patients, the pseudo-follicles represent the histological hallmark, as well as in the white pulp of the spleen and in the BM. Immunohistochemistry studies have shown that pseudofollicles are clusters of $CD5^+$ $Ki67^+$ B cells surrounded by new vessels[108;109]. In the LN and BM there are $CD3^+$ T cells, most of them belonging to the $CD4^+$ T helper subset. The $CD4^+$ T cells tend to concentrate around and within the proliferating pseudofollicles and many of them express CD40L implying that they are in an activated state. CLL cells retain the capacity to respond to CD40L, expressed by the $CD4^+$ T cells, thus resulting in their activation[110]. Furthermore, CLL cells purified from LN and PB constitutively express mRNA for T cell attracting chemokines (namely CCL17 and CCL22) and the same holds true for CLL cells stimulated by *in vitro* CD40-crosslinking[110]. These findings indicate that physiological signals in the tumor microenvironment, such as CD40L, give CLL cells chemo-attracting capacity to activated $CD4^+$ T cells, which in turn are able to deliver survival signals to the CLL cells. Other accessory cells, such as bone marrow stromal cells[111], FDCs[81] and so-called nurse-like cells[112] that can be obtained from peripheral blood, may also be involved in cross-talk between malignant cells and the microenvironment. Furthermore, various pro-survival cytokines (IL-2, IL-4, IL-8, TNF-α, IFN-α, IFN-γ and VEGF) and their respective receptors are found on CLL cells (reviewed in Kay 2002[113]). However, they remain unresponsive to most mitogens that induce proliferation of normal B cells.

The pro-survival signaling pathways elicited in CLL appear to depend on both paracrine and autocrine mechanisms. In some cases it is unclear whether the signal comes entirely from the cell in the microenvironment or as a consequence of deregulation in the CLL cells themselves. Alterations in several signaling pathways have been suggested in CLL.

For example, NF-κB is a transcription factor that can promote survival through the

induction of several anti-apoptotic proteins, such as Bfl-1, Bcl-X_L, IAPs and Flip[114]. Higher levels of constitutive NF-κB are seen in unstimulated CLL cells compared to healthy peripheral blood B cells[115]. Engagement with the TNF-superfamily members CD40L, BAFF (B cell-activating factor of tumor necrosis factor (TNF) family, also known as BlyS) and APRIL (A proliferation-inducing ligand), enhances this activity[115;116]. However, whilst it has been shown that APRIL is overexpressed in some cases of CLL[117], the APRIL driving pro-survival signaling is also derived from nurse-like cells in the tumor niche[118]. The activation of the canonical NF-κB pathway also contrasts with the alternative pathway in response to BAFF[116], which also suggests intrinsic deregulation of the responses to certain cytokines. Additionally, recent studies showed an association between the expression of NF-κB subunit Rel A and *in vitro* survival of CLL cells[119].

Also, the PI3K/AKT, Raf/MEK/ERK and JNK/STAT signaling pathways are essential for cell survival, and are frequently deregulated in malignancy[120]. They control expression and function of many proteins, including apoptosis regulatory proteins. Several studies done in CLL suggested that also these pathways may contribute to survival of the malignant cells[121;122;123]. Relatively new in the CLL field is the nonreceptor tyrosine kinase c-Abl. Oncogenic fusion versions of c-Abl drive malignancy in chronic myeloid leukemia, and are the target for successful therapy with kinase inhibitors[120]. A recent study highlighted the importance c-Abl itself in CLL survival[124] and furthermore it was reported that c-Abl becomes active upon CD40 triggering[125]. The relevance of these signaling events for the *in vivo* biology of CLL, and especially in the context of survival niches, is an important aspect that remains to be established.

THERAPEUTIC APROACH TO ATTACK THE MICROENVIRONMENT

As mentioned above, despite improvements in treatment effects for CLL by a combination of chemo- and immuno-therapy, the disease will invariably relapse. Clearly, the clearance of the CLL peripheral blood pool which usually occurs is not sufficient. The likely hypothesis is that the proliferation centers in LN, BM and spleen constitute “germinative” foci, afford resistance to drugs, and will replenish the peripheral blood after a successful cycle of therapy.

Therefore, drugs that target bystander cells and/or their protective effects on CLL cells in the microenvironmental “niche”, would provide more efficacious options to the treatment of CLL patients. Accordingly, the microenvironment may be targeted by interfering with various cytokines or by modulating immune effector cells. Clinical

trials in refractory or relapsed CLL cases have reported encouraging results with immunomodulating agents, i.e. thalidomide and its analog lenalidomide. Although the exact antitumour activity of such compounds remains uncertain, they do not exert a direct cytotoxic effect on CLL cells[126;127]. In addition, therapies aiming to target CLL cells in the protective "niches" by counteracting the pro-survival changes provided at these sites, could be a novel and potentially effective strategy. In this regard, small-molecule BH3 mimetics such as ABT-737 which is a potent and specific Bcl-2/Bcl-X_L/Bcl-W inhibitor are now in preclinical or clinical development[128].

To conclude, CLL cells possess and rely on various different ways to escape apoptosis. Direct cell-to-cell contact between CLL cells and bystander cells creates a microenvironment in which both membrane-bound and soluble factors collaborate in protecting CLL cells from apoptosis. Learning how to manipulate the microenvironment, or to specifically target the leukemic cells residing in these niches, may reveal new strategies for restoring apoptosis sensitivity and improving therapeutic outcome.

SCOPE

The aims of this thesis are:

1. To gain insight into the mechanism of action of potential novel drugs with activity independent of the p53 pathway, with emphasis on effects on apoptosis regulatory molecules.
2. To obtain insight into the molecular basis of apoptosis (dys-) regulation and survival of CLL cells both in peripheral blood and in the lymph node microenvironment in connection with drug sensitivity.

In Chapters 2 and 3 the apoptosis pathway of two p53-independent drugs are investigated; the cyclin dependent kinase (CDK) inhibitor roscovitine (Seliciclib, cyc202), and a novel proteasome inhibitor. In the following section the influence of the microenvironment on CLL survival is addressed. In chapter 4 a comparative study of CLL cells in the peripheral blood and the lymph node compartments with respect to mRNA and protein expression levels of various apoptosis regulatory molecules is presented. In Chapter 5, in order to model the *in vivo* LN setting of CLL, the influence of the CD40-signaling on the expression of apoptosis regulatory proteins is studied, in relation to sensitivity to various current and novel chemotherapeutic drugs. In chapter 6 we describe an approach to overcome CD40L induced drug resistance in CLL using cAbl kinase inhibitors. Finally, in chapter 7 an integrated discussion is presented and future directions are suggested.

REFERENCE LIST

1. Caligaris-Cappio F, Hamblin TJ. B-cell chronic lymphocytic leukemia: a bird of a different feather. J.Clin.Oncol. 1999;17:399-408.

2. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N.Engl.J.Med. 1995;333:1052-1057.

3. Dohner H, Stilgenbauer S, Benner A et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N.Engl.J.Med. 2000;343:1910-1916.

4. Damle RN, Wasil T, Fais F et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999;94:1840-1847.

5. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848-1854.

6. Rassenti LZ, Huynh L, Toy TL et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N.Engl.J.Med. 2004;351:893-901.

7. Wiestner A, Rosenwald A, Barry TS et al. ZAP-70 expression identifies a chronic lymphocytic leukemia subtype with unmutated immunoglobulin genes, inferior clinical outcome, and distinct gene expression profile. Blood 2003;101:4944-4951.

8. Hamblin TJ, Orchard JA, Ibbotson RE et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood 2002;99:1023-1029.

9. Chen L, Widhopf G, Huynh L et al. Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. Blood 2002;100:4609-4614.

10. Dohner H, Fischer K, Bentz M et al. p53 gene deletion predicts for poor survival and non-response to therapy with purine analogs in chronic B-cell leukemias. Blood 1995;85:1580-1589.

11. Dohner H, Stilgenbauer S, James MR et al. 11q deletions identify a new subset of B-cell chronic lymphocytic leukemia characterized by extensive nodal involvement and inferior prognosis. Blood 1997;89:2516-2522.

12. Pettitt AR, Sherrington PD, Stewart G et al. p53 dysfunction in B-cell chronic lymphocytic leukemia: inactivation of ATM as an alternative to TP53 mutation. Blood 2001;98:814-822.

13. Dameshek W. Chronic lymphocytic leukemia: an accumulative disease of immunolgically incompetent lymphocytes. Blood 1967;29:Suppl-84.

14. Klein U, Tu Y, Stolovitzky GA et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J.Exp.Med. 2001;194:1625-1638.

15. Rosenwald A, Alizadeh AA, Widhopf G et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. J.Exp.Med. 2001;194:1639-1647.

16. Messmer BT, Messmer D, Allen SL et al. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. J.Clin. Invest 2005;115:755-764.

17. Caligaris-Cappio F. Cellular interactions, immunodeficiency and autoimmunity in CLL. Hematol.Cell Ther. 2000;42:21-25.

18. Ghia P, Caligaris-Cappio F. The indispensable role of microenvironment in the natural history of low-grade B-cell neoplasms. Adv.Cancer Res. 2000;79:157-173.

19. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N.Engl.J.Med. 2005;352:804-815.

20. Auer RL, Gribben J, Cotter FE. Emerging therapy for chronic lymphocytic leukaemia. Br.J.Haematol. 2007;139:635-644.

21. Rai KR, Peterson BL, Appelbaum FR et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. N.Engl.J.Med. 2000;343:1750-1757.

22. Leporrier M, Chevret S, Cazin B et al. Randomized comparison of fludarabine, CAP, and ChOP in 938 previously untreated stage B and C chronic lymphocytic leukemia patients. Blood 2001;98:2319-2325.

23. Catovsky D, Richards S, Matutes E et al. Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. Lancet 2007;370:230-239.

24. Eichhorst BF, Busch R, Hopfinger G et al. Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 2006;107:885-891.

25. Flinn IW, Neuberg DS, Grever MR et al. Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US Intergroup Trial E2997. J.Clin.Oncol. 2007;25:793-798.

26. Keating MJ, O'Brien S, Albitar M et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J.Clin.Oncol. 2005;23:4079-4088.

27. Wierda W, O'Brien S, Wen S et al. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J.Clin. Oncol. 2005;23:4070-4078.

28. Stilgenbauer S. Advances in the use of alemtuzumab in CLL. Clin.Adv. Hematol.Oncol. 2008;6:23-24.

29. Dreger P, Brand R, Hansz J et al. Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. Leukemia 2003;17:841-848.

30. Gribben JG. Stem-cell transplantation in chronic lymphocytic leukaemia. Best.Pract.Res.Clin.Haematol. 2007;20:513-527.

31. Gine E, Moreno C, Esteve J, Montserrat E. The role of stem-cell transplantation in chronic lymphocytic leukemia risk-adapted therapy. Best. Pract.Res.Clin.Haematol. 2007;20:529-543.

32. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br.J.Cancer 1972;26:239-257.

33. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. Nature 2000;407:784-788.

34. Alnemri ES, Livingston DJ, Nicholson DW et al. Human ICE/CED-3 protease nomenclature. Cell 1996;87:171.

35. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. Nat.Rev.Mol.Cell Biol. 2004;5:897-907.

36. Kuida K, Lippke JA, Ku G et al. Altered cytokine export and apoptosis in mice deficient in interleukin-1 beta converting enzyme. Science 1995;267:2000-2003.

37. Kischkel FC, Hellbardt S, Behrmann I et al. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J. 1995;14:5579-5588.

38. Muzio M, Chinnaiyan AM, Kischkel FC et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex. Cell 1996;85:817-827.

39. Kischkel FC, Lawrence DA, Tinel A et al. Death receptor recruitment of endogenous caspase-10 and apoptosis initiation in the absence of caspase-8. J.Biol.Chem. 2001;276:46639-46646.

40. Sprick MR, Rieser E, Stahl H et al. Caspase-10 is recruited to and activated at the native TRAIL and CD95 death-inducing signalling complexes in a FADD-dependent manner but can not functionally substitute caspase-8. EMBO J. 2002;21:4520-4530.

41. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998;94:491-501.

42. Wei MC, Lindsten T, Mootha VK et al. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. Genes Dev. 2000;14:2060-2071.

43. Li P, Nijhawan D, Budihardjo I et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479-489.

44. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33-42.

45. Suzuki Y, Imai Y, Nakayama H et al. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol.Cell 2001;8:613-621.

46. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 2001;412:95-99.

47. Susin SA, Lorenzo HK, Zamzami N et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999;397:441-446.

48. Tsujimoto Y. Cell death regulation by the Bcl-2 protein family in the mitochondria. J.Cell Physiol 2003;195:158-167.

49. Chen L, Willis SN, Wei A et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol.Cell 2005;17:393-403.

50. Certo M, Del GM, V, Nishino M et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 2006;9:351-365.

51. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science 2004;305:626-629.

52. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Genes Dev. 2001;15:1481-1486.

53. Cartron PF, Gallenne T, Bougras G et al. The first alpha helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and PUMA. Mol.Cell 2004;16:807-818.

54. Kim H, Rafiuddin-Shah M, Tu HC et al. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nat.Cell Biol. 2006;8:1348-1358.

55. Kuwana T, Bouchier-Hayes L, Chipuk JE et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol.Cell 2005;17:525-535.

56. Letai A, Bassik MC, Walensky LD et al. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2002;2:183-192.

57. Walensky LD, Pitter K, Morash J et al. A stapled BID BH3 helix directly binds and activates BAX. Mol.Cell 2006;24:199-210.

58. Willis SN, Chen L, Dewson G et al. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev. 2005;19:1294-1305.

59. Willis SN, Fletcher JI, Kaufmann T et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 2007;315:856-859.

60. Strasser A. The role of BH3-only proteins in the immune system. Nat. Rev.Immunol. 2005;5:189-200.

61. Alves NL, van Lier RA, Eldering E. Withdrawal symptoms on display: Bcl-2 members under investigation. Trends Immunol. 2007;28:26-32.

62. Schimmer AD. Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. Cancer Res. 2004;64:7183-7190.

63. Nachmias B, Ashhab Y, Ben-Yehuda D. The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. Semin.Cancer Biol. 2004;14:231-243.

64. Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. EMBO Rep. 2006;7:988-994.

65. Vince JE, Wong WW, Khan N et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 2007;131:682-693.

66. Stauber RH, Mann W, Knauer SK. Nuclear and cytoplasmic survivin: molecular mechanism, prognostic, and therapeutic potential. Cancer Res. 2007;67:5999-6002.

67. Lens SM, Vader G, Medema RH. The case for Survivin as mitotic regulator. Curr.Opin.Cell Biol. 2006;18:616-622.

68. Scaffidi C, Schmitz I, Krammer PH, Peter ME. The role of c-FLIP in modulation of CD95-induced apoptosis. J.Biol.Chem. 1999;274:1541-1548.

69. Schena M, Larsson LG, Gottardi D et al. Growth- and differentiation-associated expression of bcl-2 in B-chronic lymphocytic leukemia cells. Blood 1992;79:2981-2989.

70. Pepper C, Hoy T, Bentley DP. Bcl-2/Bax ratios in chronic lymphocytic leukaemia and their correlation with in vitro apoptosis and clinical resistance. Br.J.Cancer 1997;76:935-938.

71. Calin GA, Dumitru CD, Shimizu M et al. Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc.Natl.Acad. Sci.U.S.A 2002;99:15524-15529.

72. Cimmino A, Calin GA, Fabbri M et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc.Natl.Acad. Sci.U.S.A 2005;102:13944-13949.

73. Nuckel H, Frey UH, Bau M et al. Association of a novel regulatory polymorphism (-938C>A) in the BCL2 gene promoter with disease progression and survival in chronic lymphocytic leukemia. Blood 2007;109:290-297.

74. Kaderi MA, Norberg M, Murray F et al. The BCL-2 promoter (-938C>A) polymorphism does not predict clinical outcome in chronic lymphocytic leukemia. Leukemia 2008;22:339-343.

75. Majid A, Tsoulakis O, Walewska R et al. BCL2 expression in chronic lymphocytic leukemia: lack of association with the BCL2 938A>C promoter single nucleotide polymorphism. Blood 2008;111:874-877.

76. Kitada S, Andersen J, Akar S et al. Expression of apoptosis-regulating proteins in chronic lymphocytic leukemia: correlations with In vitro and In vivo chemoresponses. Blood 1998;91:3379-3389.

77. Byrd JC, Lin TS, Dalton JT et al. Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Blood 2007;109:399-404.

78. Kitada S, Zapata JM, Andreeff M, Reed JC. Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. Blood 2000;96:393-397.

79. Hahntow IN, Schneller F, Oelsner M et al. Cyclin-dependent kinase inhibitor Roscovitine induces apoptosis in chronic lymphocytic leukemia cells. Leukemia 2004;18:747-755.

80. Kitada S, Zapata JM, Andreeff M, Reed JC. Bryostatin and CD40-ligand enhance apoptosis resistance and induce expression of cell survival genes in B-cell chronic lymphocytic leukaemia. Br.J.Haematol. 1999;106:995-1004.

81. Pedersen IM, Kitada S, Leoni LM et al. Protection of CLL B cells by a follicular dendritic cell line is dependent on induction of Mcl-1. Blood 2002;100:1795-1801.

82. Saxena A, Moshynska O, Sankaran K, Viswanathan S, Sheridan DP. Association of a novel single nucleotide polymorphism, G(-248)A, in the 5'-UTR of BAX gene in chronic lymphocytic leukemia with disease progression and treatment resistance. Cancer Lett. 2002;187:199-205.

83. Skogsberg S, Tobin G, Krober A et al. The G(-248)A polymorphism in the promoter region of the Bax gene does not correlate with prognostic markers or overall survival in chronic lymphocytic leukemia. Leukemia 2006;20:77-81.

84. Munzert G, Kirchner D, Stobbe H et al. Tumor necrosis factor receptor-associated factor 1 gene overexpression in B-cell chronic lymphocytic leukemia: analysis of NF-kappa B/Rel-regulated inhibitors of apoptosis. Blood 2002;100:3749-3756.

85. Schimmer AD, Dalili S, Batey RA, Riedl SJ. Targeting XIAP for the treatment of malignancy. Cell Death.Differ. 2006;13:179-188.

86. Osorio LM, Jondal M, guilar-Santelises M. Regulation of B-CLL apoptosis through membrane receptors and Bcl-2 family proteins. Leuk.Lymphoma 1998;30:247-256.

87. Chu P, Deforce D, Pedersen IM et al. Latent sensitivity to Fas-mediated apoptosis after CD40 ligation may explain activity of CD154 gene therapy in chronic lymphocytic leukemia. Proc. Natl.Acad.Sci.U.S.A 2002;99:3854-3859.

88. MacFarlane M, Harper N, Snowden RT et al. Mechanisms of resistance to TRAIL-induced apoptosis in primary B cell chronic lymphocytic leukaemia. Oncogene 2002;21:6809-6818.

89. Inoue S, MacFarlane M, Harper N et al. Histone deacetylase inhibitors potentiate TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in lymphoid malignancies. Cell Death.Differ. 2004;11 Suppl 2:S193-S206.

90. Dicker F, Kater AP, Fukuda T, Kipps TJ. Fas-ligand (CD178) and TRAIL synergistically induce apoptosis of CD40-activated chronic lymphocytic leukemia B cells. Blood 2005;105:3193-3198.

91. Lane DP. Cancer. p53, guardian of the genome. Nature 1992;358:15-16.

92. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323-331.

93. Evan GI, Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001;411:342-348.

94. Ko LJ, Prives C. p53: puzzle and paradigm. Genes Dev. 1996;10:1054-1072.

95. Giaccia AJ, Kastan MB. The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev. 1998;12:2973-2983.

96. Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. Cell 1995;80:293-299.

97. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. Mol.Cell 2001;7:673-682.

98. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol.Cell 2001;7:683-694.

99. Oda E, Ohki R, Murasawa H et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000;288:1053-1058.

100. Wattel E, Preudhomme C, Hecquet B et al. p53 mutations are associated with resistance to chemotherapy and short survival in hematologic malignancies. Blood 1994;84:3148-3157.

101. Austen B, Powell JE, Alvi A et al. Mutations in the ATM gene lead to impaired overall and treatment-free survival that is independent of IGVH mutation status in patients with B-CLL. Blood 2005;106:3175-3182.

102. Stankovic T, Stewart GS, Fegan C et al. Ataxia telangiectasia mutated-deficient B-cell chronic lymphocytic leukemia occurs in pregerminal center cells and results in defective damage response and unrepaired chromosome damage. Blood 2002;99:300-309.

103. Sturm I, Bosanquet AG, Hermann S et al. Mutation of p53 and consecutive selective drug resistance in B-CLL occurs as a consequence of prior DNA-damaging chemotherapy. Cell Death.Differ. 2003;10:477-484.

104. Lozanski G, Heerema NA, Flinn IW et al. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood 2004;103:3278-3281.

105. Collins RJ, Verschuer LA, Harmon BV et al. Spontaneous programmed death (apoptosis) of B-chronic lymphocytic leukaemia cells following their culture in vitro. Br.J.Haematol. 1989;71:343-350.

106. McConkey DJ, guilar-Santelises M, Hartzell P et al. Induction of DNA fragmentation in chronic B-lymphocytic leukemia cells. J.Immunol. 1991;146:1072-1076.

107. Caligaris-Cappio F. Role of the microenvironment in chronic lymphocytic leukaemia. Br.J.Haematol. 2003;123:380-388.

108. Pileri SA, Ascani S, Sabattini E et al. The pathologist's view point. Part I--indolent lymphomas. Haematologica 2000;85:1291-1307.

109. Pileri SA, Ascani S, Sabattini E et al. The pathologist's view point. Part II --aggressive lymphomas. Haematologica 2000;85:1308-1321.

110. Ghia P, Granziero L, Chilosi M, Caligaris-Cappio F. Chronic B cell malignancies and bone marrow microenvironment. Semin.Cancer Biol. 2002;12:149-155.

111. Lagneaux D, Huhtinen M, Koskinen E, Palmer E. Effect of anti-freeze protein (AFP) on the cooling and freezing of equine embryos as measured by DAPI-staining. Equine Vet.J.Suppl 199785-87.

112. Burger JA, Tsukada N, Burger M et al. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. Blood 2000;96:2655-2663.

113. Kay NE, Hamblin TJ, Jelinek DF et al. Chronic lymphocytic leukemia. Hematology.Am.Soc.Hematol.Educ. Program. 2002193-213.

114. Takada Y, Kobayashi Y, Aggarwal BB. Evodiamine abolishes constitutive and inducible NF-kappaB activation by inhibiting IkappaBalpha kinase activation, thereby suppressing NF-kappaB-regulated antiapoptotic and metastatic gene expression, up-regulating apoptosis, and inhibiting invasion. J.Biol.Chem. 2005;280:17203-17212.

115. Furman RR, Asgary Z, Mascarenhas JO, Liou HC, Schattner EJ. Modulation of NF-kappa B activity and apoptosis in chronic lymphocytic leukemia B cells. J.Immunol. 2000;164:2200-2206.

116. Endo T, Nishio M, Enzler T et al. BAFF and APRIL support chronic lymphocytic leukemia B-cell survival through activation of the canonical NF-kappaB pathway. Blood 2007;109:703-710.

117. Planelles L, Carvalho-Pinto CE, Hardenberg G et al. APRIL promotes B-1 cell-associated neoplasm. Cancer Cell 2004;6:399-408.

118. 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.

119. Hewamana S, Alghazal S, Lin TT et al. The NF-kappaB subunit Rel A is associated with in vitro survival and clinical disease progression in chronic lymphocytic leukemia and represents a promising therapeutic target. Blood 2008;111:4681-4689.

120. Steelman LS, Pohnert SC, Shelton JG et al. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 2004;18:189-218.

121. Ringshausen I, Schneller F, Bogner C et al. Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase Cdelta. Blood 2002;100:3741-3748.

122. Barragan M, Bellosillo B, Campas C et al. Involvement of protein kinase C and phosphatidylinositol 3-kinase pathways in the survival of B-cell chronic lymphocytic leukemia cells. Blood 2002;99:2969-2976.

123. Wickremasinghe RG, Ganeshaguru K, Jones DT et al. Autologous plasma activates Akt/protein kinase B and enhances basal survival and resistance to DNA damage-induced apoptosis in B-chronic lymphocytic leukaemia cells. Br.J.Haematol. 2001;114:608-615.

124. Lin K, Glenn MA, Harris RJ et al. c-Abl expression in chronic lymphocytic leukemia cells: clinical and therapeutic implications. Cancer Res. 2006;66:7801-7809.

125. Dicker F, Kater AP, Prada CE et al. CD154 induces p73 to overcome the resistance to apoptosis of chronic lymphocytic leukemia cells lacking functional p53. Blood 2006;108:3450-3457.

126. Chanan-Khan A, Porter CW. Immunomodulating drugs for chronic lymphocytic leukaemia. Lancet Oncol. 2006;7:480-488.

127. Ferrajoli A, Lee BN, Schlette EJ et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood 2008;111:5291-5297.

128. Oltersdorf T, Elmore SW, Shoemaker AR et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677-681.