

# The Biological Relevance of ZAP-70 in CLL

Valerie Pede, Ans Rombout, Bruno Verhasselt and Jan Philippé  
*Ghent University  
Belgium*

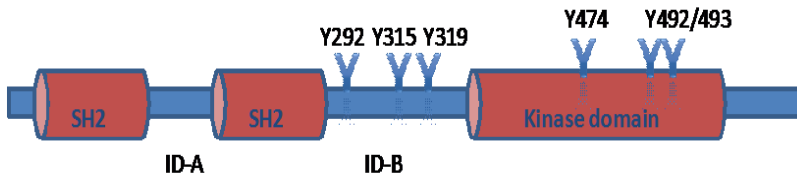
## 1. Introduction

Initially, CLL was considered as a homogeneous disease caused by the accumulation of functionally incompetent B lymphocytes carrying no mutations in the immunoglobulin heavy chain variable (IgV<sub>H</sub>) genes. However, further studies by Schroeder and Dighiero suggested that IgV<sub>H</sub> genes may be mutated in CLL (Schroeder & Dighiero 1994). This report changed the general view of CLL and gained even more significance when the mutational status of the IgV<sub>H</sub> genes was linked to the prognosis of the patients in two independent studies (Damle et al. 1999, Hamblin et al. 1999). These studies showed for the first time that patients with CLL cells expressing unmutated IgV<sub>H</sub> genes presented with a more aggressive disease and shorter survival than those with cells carrying mutated IgV<sub>H</sub> genes. Determination of the mutational status of the IgV<sub>H</sub> genes in CLL patients became of great interest, but even today remains difficult to carry out in most medical centers since the technique is laborious, expensive and time-consuming. Therefore many efforts have been made to identify possible surrogate markers with the same prognostic value as the mutation status. In 2001, two independent groups published their studies in which they compared the gene expression profiles for IgV<sub>H</sub> mutated versus unmutated CLL cells. Because, the description of the two groups of CLL with markedly different prognosis prompted the idea that CLL consisted of two different entities originating from either naive or memory cells, it was unexpected that only a few differentially expressed genes were found (Rosenwald et al. 2001, Klein et al. 2001). Among them, ZAP-70 appeared to be one of the most significant (Rosenwald et al. 2001). Subsequently, the correlation of ZAP-70 expression with the mutational status of the IgV<sub>H</sub> genes was assessed in larger series of CLL patients, where ZAP-70 was mostly found expressed in unmutated CLL (reviewed in (Van Bockstaele et al. 2009)). Further clinical studies revealed that ZAP-70 was also an independent prognostic marker (Bosch et al. 2006). There were several attempts to standardize the determination of ZAP-70 expression by flow cytometry (Crespo et al. 2003, Wang et al. 2011, Van Bockstaele et al. 2006). Although, a successful standardized procedure was put forward by the European Research Initiative on CLL (ERIC), the determination and interpretation of ZAP-70 remains difficult (Letestu et al. 2006) and optimalization is still ongoing.

## 2. Structure of ZAP-70

The zeta-chain associated protein kinase with a molecular weight of 70 kDa, ZAP-70, is a member of the Syk family kinases predominantly involved in T cell receptor (TCR) signaling initiation and subsequent T cell activation (Chan et al. 1992). The ZAP-70 gene is located on

chromosome 2q11.2 and is composed of 14 exons encoding a protein tyrosine kinase (PTK) made out of 619 amino acids building three functional domains; two Src homology 2 (SH<sub>2</sub>) domains arranged in tandem at the amino-terminus and a tyrosine kinase domain at the carboxy-terminus (Figure 1) (Au-Yeung et al. 2009).



ID-A: interdomain A; ID-B: interdomain B; Y: Tyrosine residue

Fig. 1. Zeta-chain-associated protein kinase 70 (ZAP-70) protein structure.

The two SH<sub>2</sub> domains are separated by a linker region known as interdomain A (ID-A) and a linker region known as interdomain B (ID-B) connecting the SH<sub>2</sub> domains to the kinase domain. SH<sub>2</sub> domains are involved in the recruitment of ZAP-70 to phosphorylated immunoreceptor tyrosine-based activation motifs (ITAMs) on the CD3  $\zeta$  chain homodimers and both SH<sub>2</sub> domains are responsible for ZAP-70 dependent signal transduction. The kinase domain of ZAP-70 contains two tyrosine residues, Tyr492 and Tyr493, which are phosphorylated by the Src tyrosine kinase, Lck, or autophosphorylated by ZAP-70 itself, after TCR engagement. Mutation of Tyr493 impairs ZAP-70 kinase activity, revealing a positive regulatory role of this residue, while mutation of Tyr492 increases kinase activity, indicating its inhibitory role (Wange et al. 1995). The tyrosine 474 of ZAP-70 is required for association with the Src Homology 2 domain Containing (Shc) adaptor protein and coupling of the activated TCR to the Ras/Raf/Erk pathways. Although the catalytic activity of ZAP-70 represents its major function, it is likely that interactions of ZAP-70 with other proteins contribute to its role in signal transduction. Interdomain B contains three tyrosines, Tyr292, Tyr315 and Tyr319, also representing important phosphorylation targets (Au-Yeung et al. 2009). These three tyrosines are phosphorylated by one of the Src family kinases, i.e. Lck or Fyn after TCR stimulation in T cells. Each of these binding sites, once phosphorylated, may bind to a different signaling molecule, conferring a role of ZAP-70 in the recruitment of additional signaling molecules to the antigen receptor complex. Tyr292 is able to bind the E3 ligase c-Cbl, which might be important for the turnover of the signaling complex (Rao et al. 2002). This implies a negative regulatory role for this tyrosine that however was recently shown to function also as a binding site for the p85 regulatory subunit of PI3K, indicating that it may also play a positive role in signal transduction. The two other tyrosines, Tyr315 and Tyr319 are positive regulators of ZAP-70 activity. Their phosphorylation is important for ZAP-70 activity, because it appears to prevent ZAP-70 from returning to an auto-inhibited conformation (Au-Yeung et al. 2009). Phosphorylated Tyr315 is a binding site for the SH<sub>2</sub> domain of Vav-1. This plays a key role in activation of the Rho family of GTPases involved in cytoskeleton remodeling after receptor stimulation (Wu et al. 1997, Sanchez-Aguilera et al. 2010). Tyr319 is a binding site for the C terminal SH<sub>2</sub> domain of phospholipase C  $\gamma$  (PLC $\gamma$ ), and phosphorylation of Tyr319 is required for PLC $\gamma$  phosphorylation and subsequent activation of downstream signals, such as calcium mobilization or Il-2 production (Williams et al. 1999).

### 3. Expression of ZAP-70

#### 3.1 Expression of ZAP-70 in T and B cells

For a long time ZAP-70 expression was considered to be restricted to T and NK cells, until it was found to be expressed as well in leukemic cells of CLL patients. Later studies have revealed ZAP-70 expression in a subset of normal tonsillar and splenic B cells (Cutrona et al. 2006, Nolz et al. 2005) and in bone marrow pro-B cells (Crespo et al. 2006, Guillaume et al. 2005). Besides the occurrence of ZAP-70 in a subset of CLL patients it was also found in a number of other human B cell tumors like some cases of mantle cell lymphoma, splenic marginal zone lymphoma, B-ALL and Burkitt lymphoma (Crespo et al. 2006, Guillaume et al. 2005, Admirand et al. 2004, Chiaretti et al. 2006, Scielzo et al. 2006, Sup et al. 2004, Orchard et al. 2005). A correlation was described between maturation of tumor cells and expression of ZAP-70, ZAP-70 being more expressed in the more mature cases with IgM, higher CD20 expression and pre-B rather than pro-B phenotype (Chiaretti et al. 2006).

#### 3.2 Regulation of ZAP-70 expression in CLL cells

In 2005, Corcoran et al. showed that there was an association between the methylation status of the ZAP-70 gene and expression status of ZAP-70 protein (Corcoran et al. 2005). It is well established that abnormal methylation is frequent in malignancy. Hypermethylation of CpG islands within gene promoters results in gene silencing, while hypomethylation may cause genomic instability or the upregulation of gene expression (Herman & Baylin 2003). Specifically, they found that the majority of cases expressing ZAP-70 protein lacked methylation in the intron 1-exon 2 boundary region of the ZAP-70 gene. This region was found unmethylated in circulating ZAP-70 expressing T cells, but methylated in ZAP-70 negative normal B cells. These data give a possible explanation for ZAP-70 overexpression in a subset of CLL patients. But the absence of an association between ZAP-70 expression and methylation status indicates that other factors must play a role.

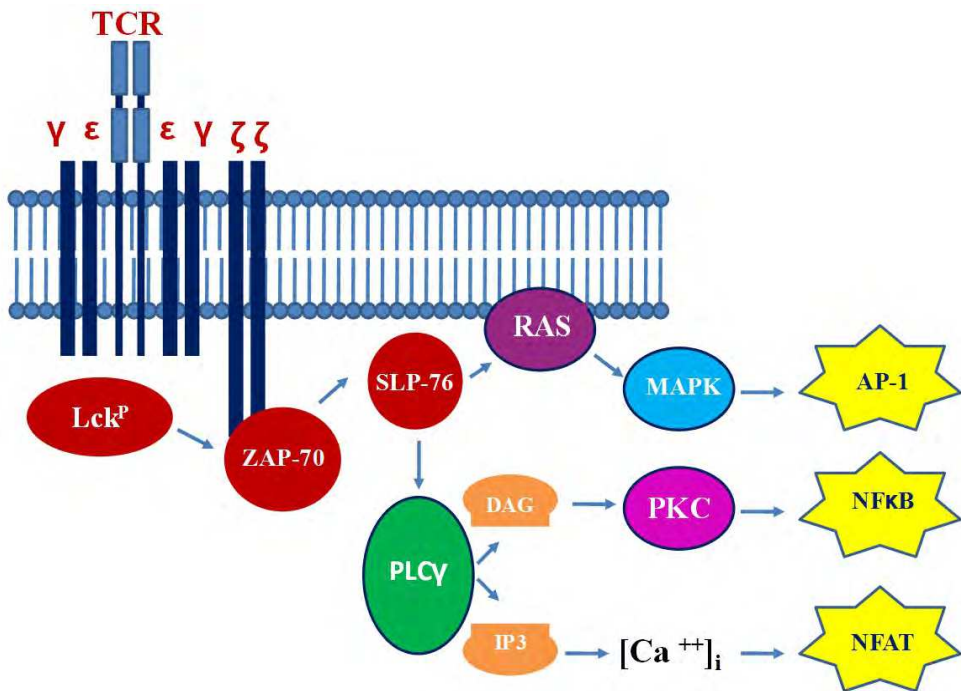
ZAP-70 expression in B cells may also be regulated by the heat shock protein (Hsp)90. Hsp90 is a molecular chaperone that catalyses the conformational maturation of a large number of signaling proteins in cancer that are collectively described as 'clients'. In advanced tumours, it exists in an activated form complexed with other molecular chaperones, whereas in normal tissue it is present in a latent, uncomplexed state (Kamal et al. 2003). Using inhibitors, Castro et al. have demonstrated that ZAP-70 is an Hsp90 client protein in tumour cells, but not in T cells (Castro et al. 2005).

### 4. ZAP-70 in lymphocyte receptor signaling

#### 4.1 ZAP-70 in T cell signaling

Ligation of the TCR triggers a cascade of intracellular signals that culminate in cytokine gene expression, proliferation, and the execution of T cell effector functions. After engagement of the TCR with a peptide bound to a major histocompatibility complex molecule, a signaling cascade is activated by the sequential activation of two families of PTKs. First of all, members of the Src family, Lck and FynT, initiate this process by phosphorylating tyrosine residues at the ITAMs of the CD3  $\zeta$  subunits. Once the ITAMs in the receptor cytoplasmic tails have been phosphorylated, they can recruit the next player in

the signaling cascade, ZAP-70, belonging to the second family of the PTKs, the Syk family. ZAP-70 binds to the double phosphorylated ITAMs via its SH<sub>2</sub> domains with high affinity. Recruitment of ZAP-70 to the ITAMs leads to ZAP-70 itself becoming phosphorylated. The subsequent activation of bound ZAP-70 by phosphorylation leads to three important signaling pathways. ZAP-70 phosphorylates the adaptor proteins linker for activation of T cells (LAT) and SH2-domain-containing leukocyte protein (SLP)-76, which in turn leads to the activation of PLC $\gamma$  and the Rho-GTPase Ras. PLC $\gamma$  cleaves phosphatidylinositol biphosphate (PIP<sub>2</sub>) to yield diacylglycerol (DAG) and inositol triphosphate (IP<sub>3</sub>). IP<sub>3</sub> increases intracellular Ca<sup>2+</sup> concentration, activating the phosphatase calcineurin, and subsequently calcineurin activates a transcription factor, nuclear factor of activated T cells (NFAT). DAG and the increase in Ca<sup>2+</sup> concentration activate protein kinase C (PKC). PKC will in turn activate the transcription factor, nuclear factor kappaB (NF $\kappa$ B). Another pathway involves the Rho-GTPase Ras, which activates a mitogen-activated protein (MAP) kinase cascade. This Ras-induced kinase cascade induces and activates Fos, a component of the activator protein (AP)-1 transcription factor. The three transcription factors NF $\kappa$ B, NFAT and AP-1 act to induce specific gene transcription, leading to cell proliferation and differentiation (Figure 2).



*Schematic presentation of the intracellular signaling pathways initiated by the T cell receptor complex (TCR) leading to activation of three important transcription factors.*

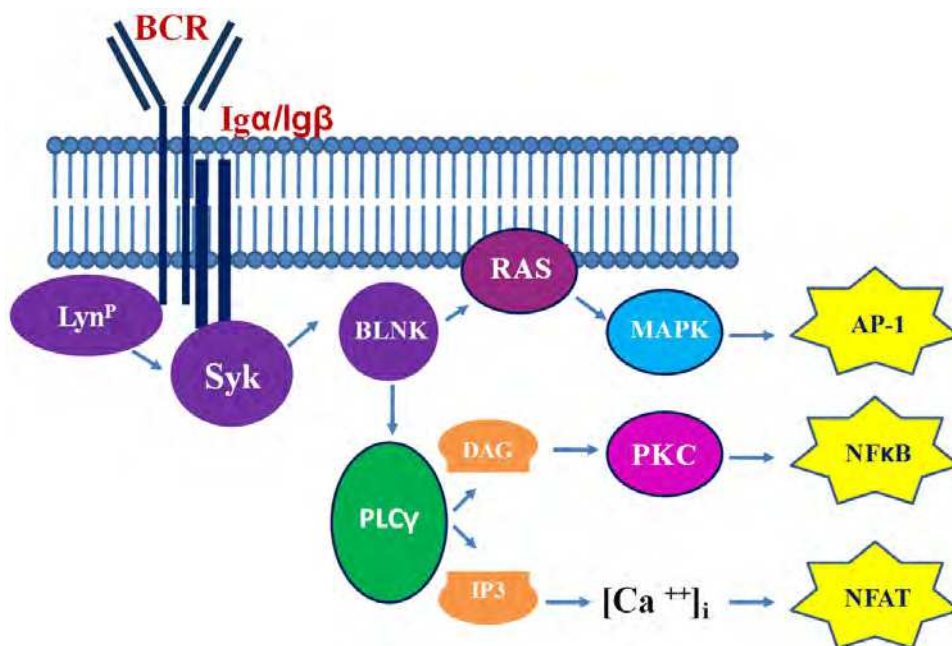
Fig. 2. ZAP-70 in T cell signaling

In addition to its importance in T cell signaling through the TCR, ZAP-70 has also been shown to be required for effective signaling by the chemokine CXCL12 (Ticchioni et al. 2002). CXCL12 is an important chemoattractant for T cells, driving them from the blood to tissue sites where they are likely to encounter antigens. Binding of CXCL12 to its ligand CXCR4 results in phosphorylation of ZAP-70, which is required for activation of downstream proteins, such as ERK and Vav-1, leading to T cell migration.

## 4.2 ZAP-70 in CLL B cell signaling

### 4.2.1 Normal B cell signaling

Signaling in normal B cells occurs after crosslinking of the surface immunoglobulin molecules (Ig $\alpha$  and Ig $\beta$ ) with an antigen. On clustering of the receptors, the receptor-associated Src-family protein tyrosine kinases Blk, FynB and Lyn are activated. These activated kinases phosphorylate the ITAMs in the receptor complex, which bind and activate the cytosolic protein kinase Syk. Subsequently, Syk phosphorylates other targets, including the adaptor protein BLNK, which help to recruit Tec kinases that in turn phosphorylate and activate the enzyme PLC $\gamma$ . Similar to T cell signaling, PLC $\gamma$  together with Ras, leads to activation of the three main signaling pathways to the nucleus: activation of NF $\kappa$ B, NFAT and AP-1, initiating new gene transcription that results in the differentiation, proliferation and effector actions of B cells (Figure 3).



*Schematic presentation of the intracellular signaling pathways initiated by cross-linking of B cell receptors (BCR) by an antigen, leading to activation of three important transcription factors.*

Fig. 3. B cell signaling

#### 4.2.2 Interplay between ZAP-70, Syk and CLL B cells

The role of ZAP-70 in proximal signaling after TCR activation and its homology with Syk, suggest that ZAP-70 may augment signaling through the BCR, thus, providing a biological explanation for the more aggressive clinical outcome of the ZAP-70 positive subgroup of patients (Chen et al. 2002). Nevertheless, the molecular mechanisms underlying the role of ZAP-70 in BCR signaling remain largely unknown. In Figure 4 the interactions between ZAP-70, Syk and BCR signaling is shown.

Studies, performed in the eighties and nineties had indicated that CLL cells varied in their capacity to respond to IgM ligation (Hivroz et al. 1986, Karray et al. 1987). In general, CLL cells were less able to respond to BCR stimulation than normal B cells (Lankester et al. 1995). Furthermore, several investigators found that, upon IgM crosslinking, CLL cells with unmutated Ig genes showed significantly increased levels of tyrosine-phosphorylated proteins, including Syk, compared to CLL cells that expressed mutated Ig receptors (Lanham et al. 2003, Chen et al. 2002). Remarkably, this was not related to differences in the levels of Syk protein found (Semichon et al. 1997).

In 2002, Chen et al. linked this phenomenon to the expression of ZAP-70. They found a greater increase in Syk tyrosine phosphorylation in cells expressing ZAP-70 compared to CLL cells lacking the protein. Consistent with this notion, they found that, besides Syk, also ZAP-70 itself undergoes tyrosine phosphorylation and complexes with the proteins of the BCR complex. At that time, two possible explanations were put forward. ZAP-70 might function to enhance BCR signaling by phosphorylation of specific motifs in CLL cells and thereby lower the threshold for Syk phosphorylation. Alternatively, expression of ZAP-70 in CLL cells might enhance the stability of phosphorylated Syk, allowing accumulation of the functional form of Syk in CLL cells.

In a subsequent study, the same group (Chen et al. 2005) examined samples that expressed unmutated IgV<sub>H</sub> genes without ZAP-70 or samples carrying mutated IgV<sub>H</sub> genes with ZAP-70, allowing them to examine the relative importance of ZAP-70 versus the IgV<sub>H</sub> mutational status in influencing the relative intensity of IgM signaling. A greater Ca<sup>2+</sup> influx was seen in samples expressing ZAP-70 independently of the mutational status. Therefore, it was concluded that the induced increase in phosphorylated forms of Syk, BLNK or PLC $\gamma$  and Ca<sup>2+</sup> influx after IgM ligation appeared more closely associated with the expression of ZAP-70, rather than with the expression of unmutated IgV<sub>H</sub>. However, it could not be excluded that secondary factors other than ZAP-70 might also have been associated with the mutational status of the leukemic cells. To challenge this hypothesis it was required to transduce the CLL cells. Transduction studies revealed that expression of ZAP-70 in initially ZAP-70-negative CLL cells was sufficient to enhance IgM signaling.

#### 4.2.3 ZAP-70 tyrosine phosphorylation

Gobessi et al. explored in 2007 the phosphorylation of ZAP-70 in CLL cells (Gobessi et al. 2007). ZAP-70 was found to be inefficiently activated in CLL and relatively weakly activated compared to Syk. Phosphorylation of Tyr319 and Tyr493 could not be detected. Phosphorylation of these tyrosines are required for the catalytic activation of ZAP-70 (Figure 1). Phosphorylation of the corresponding tyrosines in Syk was readily detectable with the same phospho-specific antibodies (Gobessi et al. 2007). ZAP-70 contains additional sites of

tyrosine phosphorylation (Figure 1) not involved in the regulation of its catalytic activity, but regulating the recruitment of downstream signaling molecules and adaptor proteins. With the use of specific antibodies against Tyr292 constitutive phosphorylation of Tyr292 in CLL B cells was demonstrated. Although, BCR stimulation did not induce a significant change in the level of Tyr292 phosphorylation, suggesting that the interactions mediated by this tyrosine do not require additional phosphorylation. Instead, after IgM ligation an association between c-Cbl and PI3K and ZAP-70 (Gobessi et al. 2007) was observed. Additionally, an association between ZAP-70 and Shc, which requires phosphorylation at Tyr474, was detected. The same authors, in agreement with Chen et al. (2005), reported a stronger and prolonged BCR-induced phosphorylation of Syk, ERK and Akt in ZAP-70 transfected CLL cells.

#### 4.2.4 Molecular interactions of ZAP-70 in CLL and downstream effects

To our knowledge, how ZAP-70 enhances BCR signaling in CLL cells is not known. It is conceivable that the capacity of ZAP-70 to enhance BCR signaling is not dependent upon its kinase activity. Two findings support this hypothesis. First, although Syk and ZAP-70 play similar roles in receptor signaling, Syk has approximately a 100-fold greater kinase activity *in vitro* than does ZAP-70 (Latour et al. 1996). Second, the kinase specific tyrosines are not phosphorylated in CLL cells (Gobessi et al. 2007). To address this, Chen et al. (Chen et al. 2007) transduced ZAP-70 negative cells with an adenovirus containing a ZAP-70 lacking kinase activity. In these cells, similar responses were observed after IgM stimulation compared to CLL cells naturally expressing ZAP-70 or cells being transduced with the wild-type ZAP-70. This unequivocally shows that ZAP-70 doesn't enhance BCR signaling by means of its intrinsic kinase activity.

Subsequently, Chen et al. as well as Gobessi et al. considered the hypothesis that ZAP-70 instead may enhance BCR signaling indirectly by enhancing the stability of activated Syk following ligation of the BCR. It is known that activated ZAP-70 and Syk are targets of the E3 ubiquitin ligase, c-Cbl, which in turn directs polyubiquitination and proteosomal degradation of the activated PTK (Fournel et al. 1996, Lopher et al. 1998, Rao et al. 2002). Possibly, the activated ZAP-70 may compete with activated Syk for binding to c-Cbl and thereby prolong the half-life of activated Syk. However, transduction of CLL cells with an adenovirus encoding a mutant form of ZAP-70 carrying a mutation at position 292, which abrogates the ability of the mutant form to interact with c-Cbl, also enhanced BCR signaling of ZAP-70 negative CLL cells. So, binding of C-cbl to Tyr292 (Gobessi et al. 2007) is unlikely to account for the capacity of ZAP-70 to enhance BCR signaling in CLL B cells.

Another explanation, proposed by both groups, is that ZAP-70 functions as an adaptor protein that facilitates the recruitment of other signaling molecules to the activated BCR. The associations with PI3K and Shc after IgM stimulation are noteworthy in this respect because these proteins are involved in the activation of Akt and ERK, respectively (Gobessi et al. 2007). In addition to this, examination of additional constructs encoding mutant forms of ZAP-70 that lacked a functional SH2 domain revealed that both SH2 domains are required for enhanced IgM signaling. This suggests that docking at the ITAM may be necessary for ZAP-70 to have an effect on CLL Ig receptor signaling (Chen et al. 2007).

The finding that ZAP-70 may enhance IgM signaling by functioning as an adaptor protein is further confirmed by zum Buschenfelde et al. (2010). The interaction of Ig receptors with their ligands has been shown to occur within an organized contact zone known as the immune synapse. Formation of the immune synapse is accompanied by a remodeling of specialized membrane microdomains enriched in sphingolipids and cholesterol known as lipid rafts (Viola & Gupta 2007). In T cells, ZAP-70 is known to be required for clustering signaling molecules into lipid rafts (Blanchard et al. 2002). This group investigated the distribution of ZAP-70 and signaling molecules like PKC- $\beta$ II in lipid raft domains before and after BCR stimulation. They found that ZAP-70 was constitutively expressed in the raft domains. Accordingly, these cells constitutively expressed PKC- $\beta$ II in lipid rafts, whereas the expression of PKC- $\beta$ II was negligible in raft fractions of ZAP-70 negative patients, although total amounts of PKC- $\beta$ II was expressed in the same amounts in whole-cell lysates in both groups. Signaling through the BCR recruited accessory ZAP-70 and PKC- $\beta$ II into lipid raft domains (zum Buschenfelde et al. 2010). These experiments demonstrate that ZAP-70 may function as an adaptor protein in specific lipid raft domains, and therefore recruits specific signaling molecules towards the immune synapse.

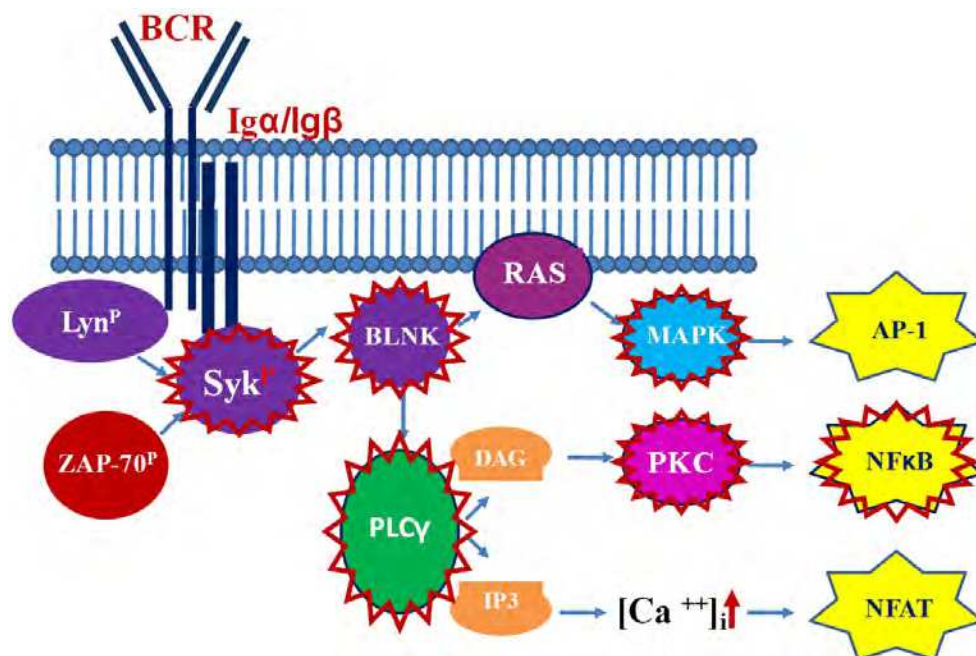
Although reduced BCR internalization by ZAP-70 is never established in CLL cells, it could be a fourth explanation for increasing the magnitude and duration of BCR signaling. Transfection of ZAP-70 in BJAB B cells downmodulates BCR internalization (Gobessi et al. 2007) explaining how ZAP-70 could contribute to the stronger signaling observed in CLL B cells.

As described above, an important mediator of BCR signaling is NF $\kappa$ B, encoded by a family of transcription factors which are key regulators of differentiation and survival in B cells. In humans they include five members: c-Rel, Rel B, p50, p52 and p65 or Rel A. These factors form homo- or heterodimers, which in the resting state are retained in the cytoplasm by binding to the inhibitory I $\kappa$ B proteins. Upon different stimuli, including ligation of BCR, the I $\kappa$ B proteins are phosphorylated by I $\kappa$ B kinases (IKK) and degraded by the proteasome. Consequently, the NF $\kappa$ B dimers become free to translocate to the nucleus and activate the transcription of their target genes. These include antiapoptotic genes (Bcl-2, Mcl-1, Survivin), inflammatory genes (COX-2, MMP-9, VEGF) and genes encoding adhesion molecules, chemokines (IL-1 $\beta$ , IL-6, IL-8) and cell cycle regulatory proteins (Cyclin D1, c-Myc) (Hayden & Ghosh 2008). More generally, NF $\kappa$ B has been implicated in tumorigenesis and survival of a growing list of leukemias and lymphomas (Karin & Lin 2002).

CLL cells have been reported to exhibit high constitutive NF $\kappa$ B activation compared with normal B lymphocytes (Cuni et al. 2004, Furman et al. 2000, Tracey et al. 2005). Although the exact factors responsible for the constitutive expression of NF $\kappa$ B are not fully resolved, many factors, including Akt activation, BCR signaling, CD40 ligation, IL-4 and B cell activating factor (BAFF), have been shown to increase NF $\kappa$ B activity and enhance CLL survival, with members of the Bcl-2 family being principal transcriptional targets (Petlickovski et al. 2005). Hewamana (2008) found an association between ZAP-70 expression and the ability of CLL cells to activate NF $\kappa$ B (Hewamana et al. 2008). Furthermore, they found that the magnitude of the change in NF $\kappa$ B after stimulation with anti-IgM is associated with the suppression of *in vitro* apoptosis. Lopez-Guerra et al. (2009) tested a specific I $\kappa$ B kinase inhibitor, BMS-435541, and found that CLL cells expressing ZAP-



70 are more sensitive to this IKK inhibitor compared to CLL cells without ZAP-70. This supports the hypothesis that there is a functional link between ZAP-70 and NF $\kappa$ B. On the other hand, these results also imply that the therapeutic combination of NF $\kappa$ B inhibitors with other chemotherapeutic drugs, represents a novel strategy especially for the group with high ZAP-70, known to be more resistant to agents currently in use (Lopez-Guerra et al. 2009).



Schematic presentation of the interaction of ZAP-70 into the intracellular signaling pathways occurring in a CLL cell after stimulation of the B cell receptor. Syk, BLNK, PLC $\gamma$ , MAPK, PKC and NF $\kappa$ B (enclosed by a red line) are potentially stimulated by ZAP-70. Also the phosphorylation of Syk (red P) and calcium influx (red arrow) may be augmented by ZAP-70.

Fig. 4. ZAP-70 in CLL signaling in poor prognosis CLL

## 5. Interaction of ZAP-70 with the leukemic microenvironment

In contrast with their long-living capacities in the human body, CLL cells tend to undergo rapid apoptotic cell death when incubated *in vitro*. This has raised the hypothesis of pro-survival environmental factors existing *in vivo*, supporting the CLL cells in their survival and growth (Ghia et al. 2002).

The peripheral blood of CLL patients contains an accumulation of mature B cells that have escaped programmed cell death and have undergone cell cycle arrest in the G<sub>0</sub>/G<sub>1</sub> phase lacking metabolic activity. However, when in *in vivo* experiments patients drank deuterated water (<sup>2</sup>H<sub>2</sub>O) cell generation rates were in the range between 0,1 to 1,75 % of the entire CLL clone per day (Messmer et al. 2005). Considering a CLL clone to contain 10<sup>12</sup>-10<sup>14</sup> cells, an

extensive number of  $10^9$  to  $10^{12}$  cells are produced each day. This implies that, although there is a virtual absence of proliferative cells in peripheral blood, ill-defined areas of proliferation are existing in the bone marrow and affected lymph nodes (Herishanu et al. 2011). Indeed, interactions with stromal cells, or nurse-like cells, or interactions between CD38 and its ligand CD31 rescue CLL cells from apoptosis in vitro and probably do the same in vivo (Chiorazzi et al. 2005), and furthermore enhance proliferation of the cells. Below, several interactions between CLL cells and by-stander cells will be discussed, especially those findings that correlate with the more aggressive nature of the ZAP-70 positive disease.

### 5.1 CD38 and ZAP-70

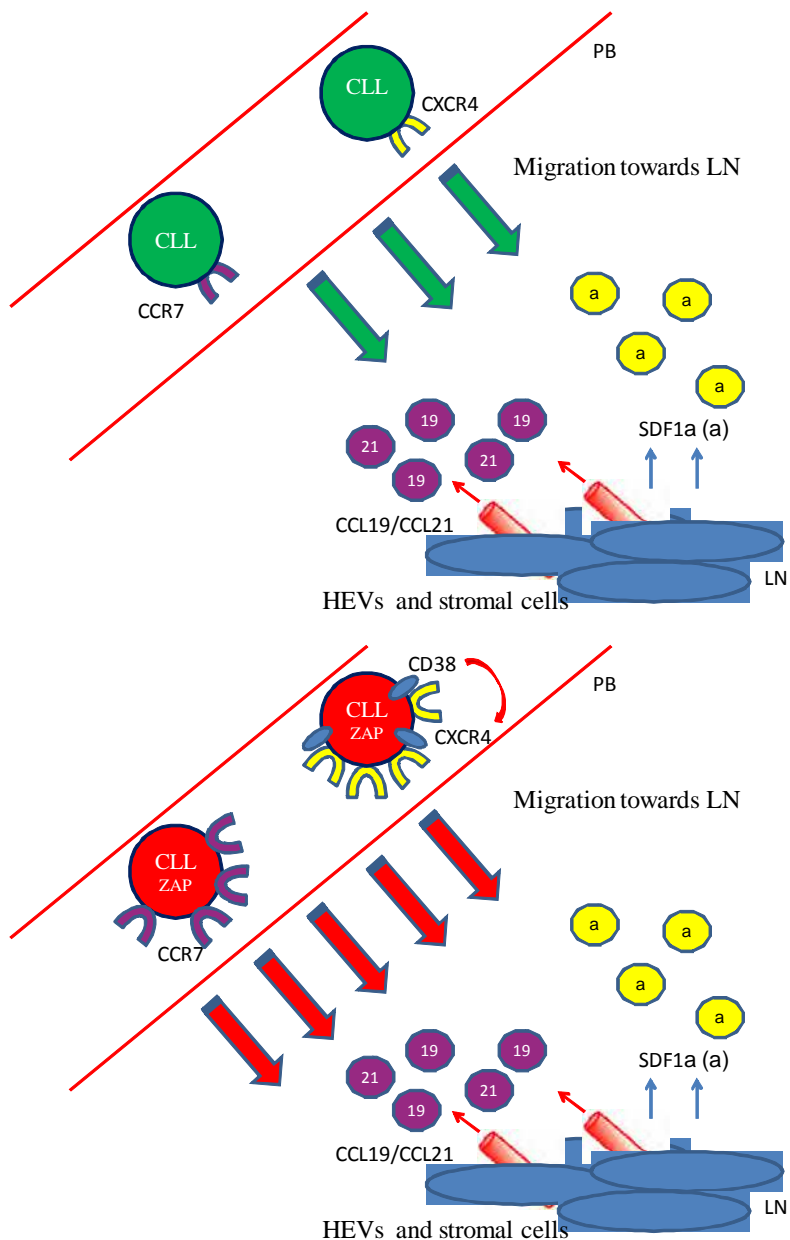
CD38 is a transmembrane glycoprotein that mediates cell-cell interactions (Deaglio et al. 1998). CD38 expression is significantly associated with unmutated IgVH genes in CLL, also with ZAP-70 and subsequently with a more aggressive disease (Damle et al. 1999).

In CLL, chemokines are reported to contribute significantly to the delivery of growth signals to the malignant CLL cells expressing functional receptors. Knowing that ZAP-70 is an essential element in the signaling cascade initiated via CXCR4 in T cells and knowing that the combination of CD38 and ZAP-70 defines a subgroup of patients with the highest migratory potential towards the ligand for CXCR4, SDF1 $\alpha$ , it is tempting to speculate that the CD38-ZAP-70 axis synergizes with the SDF1 $\alpha$ -CXCR4 pathway (Deaglio et al. 2007).

SDF1 $\alpha$  has previously been shown to exert both a chemotactic effect and a prosurvival effect on CLL cells, being a crucial mechanism through which stromal cells or nurse like cells support CLL cells in vitro (Burger et al. 1999, Burger et al. 2000). The finding that CLL cells proliferate at sites where stromal cells are present suggest that SDF1 $\alpha$  is an important factor in CLL pathogenesis. Moreover, several groups confirmed an increased level of CXCR4 on the surface of CLL cells when compared with normal B cells (Richardson et al. 2006, Mohle et al. 1999, Burger et al. 1999). Treatment of CLL cells with SDF1 $\alpha$  resulted in a rapid and sustained ERK activation profile only in the ZAP-70 positive subgroup. Furthermore, treatment with SDF1 $\alpha$  in vitro of ZAP-70 positive but not in ZAP-70 negative cells, resulted in a longer survival. Sustained ERK activation can lead to the initiation of transcription of genes involved in both proliferation and survival (Burger et al. 2000, Xia et al. 1995, Murphy et al. 2002). This could be the explanation for both survival and proliferative advantages seen in ZAP-70 positive cells. These results indicate that ZAP-70 positive cells are more responsive to signals derived from their surrounding environment.

### 5.2 CCR7 and ZAP-70

Several groups found that CCR7 is upregulated on the surface of circulating peripheral blood CLL cells when compared with healthy control peripheral blood B cells (Richardson et al. 2006, Till et al. 2002, Lopez-Giral et al. 2004, Ghobrial et al. 2004). Moreover, Richardson et al. (2006) demonstrates that CCR7 levels are increased in ZAP-70 positive CLL cells when compared with ZAP-70 negative CLL cells. This upregulation in CCR7 confers an increased ability to respond to its ligands, CCL19 and CCL21. Both chemokines are important in both T and B lymphocyte trafficking (Figure 5). Prior studies in B cells (Reif et al. 2002) have shown that antigen engagement upregulates expression of CCR7 and can facilitate the movement of these cells into the lymph nodes and localization to the B/T cells



LN: lymph nodes; HEV: high endothelial venules

Upper: CLL ZAP<sup>-</sup> cells;

Lower: CLL ZAP<sup>+</sup> cells: In ZAP-70 positive cells, increased levels of CCR7 and the interplay between ZAP-70, CD38 and CXCR4 facilitate movement and migratory potential towards lymph nodes.

Fig. 5. Migration of CLL cells towards the lymph nodes

boundary. Since increased CCR7 expression has been documented following antigen contact, this could be reflecting either an increased level of antigen contact, which is likely to be the case for both ZAP-70 positive and ZAP-70 negative CLL B cells, as both cell types have been shown to resemble activated B cells (Damle et al. 2002, Herishanu et al. 2011), or an increased ability to respond to antigen contact. Herishanu et al. (2011) further described the increased proliferative signature of the CLL cells residing in the lymph nodes and bone marrow, due to a more intensive BCR triggering. ZAP-70 helps in a more sustained activation state after BCR triggering. The finding that CCR7 levels are lower in lymph node CLL cells than in peripheral blood CLL cells is suggestive of it being involved in migration to the lymph nodes (Till et al. 2002, Lopez-Giral et al. 2004, Ghobrial et al. 2004).

## 6. Summary

It remains largely unknown why ZAP-70 is or is not expressed in CLL. Hsp90 may be important in this respect. Although the precise molecular mechanism of the role of ZAP-70 in CLL B cells is not yet fully resolved, many hypotheses have been put forward suggesting multiple functions. Strikingly, it is improbable that ZAP-70 exerts its role by its kinase activity. ZAP-70 in CLL may be stabilizing SYK, creating more sustained phosphorylation and in association increasing the proliferative capacity. Moreover, ZAP-70 functions as an adaptor protein that facilitates the recruitment of other signaling molecules to the activated BCR. Also the internalization of the BCR after stimulation may be reduced due to intervention of ZAP-70. An important mechanism explaining prolonged survival of CLL cells has been attributed to its interaction with the microenvironment. In this interaction, ZAP-70 may also be of relevance.

## 7. References

- Admirand, J. H., G. Z. Rassidakis, L. V. Abruzzo, J. R. Valbuena, D. Jones & L. J. Medeiros (2004) Immunohistochemical detection of ZAP-70 in 341 cases of non-Hodgkin and Hodgkin lymphoma. *Mod Pathol*, 17, 954-61.
- Au-Yeung, B. B., S. Deindl, L. Y. Hsu, E. H. Palacios, S. E. Levin, J. Kuriyan & A. Weiss (2009) The structure, regulation, and function of ZAP-70. *Immunological reviews*, 228, 41-57.
- Blanchard, N., V. Di Bartolo & C. Hivroz (2002) In the immune synapse, ZAP-70 controls T cell polarization and recruitment of signaling proteins but not formation of the synaptic pattern. *Immunity*, 17, 389-99.
- Bosch, F., A. Muntanola, E. Gine, A. Carrio, N. Villamor, C. Moreno, M. Crespo & E. Montserrat (2006) Clinical implications of ZAP-70 expression in chronic lymphocytic leukemia. *Cytometry B Clin Cytom*, 70, 214-7.
- Burger, J. A., M. Burger & T. J. Kipps (1999) Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood*, 94, 3658-67.
- Burger, J. A., N. Tsukada, M. Burger, N. J. Zvaifler, M. Dell'Aquila & T. J. Kipps (2000) Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood*, 96, 2655-63.

- Castro, J. E., C. E. Prada, O. Loria, A. Kamal, L. Chen, F. J. Burrows & T. J. Kipps (2005) ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia. *Blood*, 106, 2506-12.
- Chan, A. C., M. Iwashima, C. W. Turck & A. Weiss (1992) ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain. *Cell*, 71, 649-62.
- Chen, L., J. Apgar, L. Huynh, F. Dicker, T. Giago-McGahan, L. Rassenti, A. Weiss & T. J. Kipps (2005) ZAP-70 directly enhances IgM signaling in chronic lymphocytic leukemia. *Blood*, 105, 2036-41.
- Chen, L., L. Huynh, J. Apgar, L. Tang, L. Rassenti, A. Weiss & T. J. Kipps (2007) ZAP-70 enhances IgM signaling independent of its kinase activity in chronic lymphocytic leukemia. *Blood*.
- Chen, L., G. Widhopf, L. Huynh, L. Rassenti, K. R. Rai, A. Weiss & T. J. Kipps (2002) Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. *Blood*, 100, 4609-14.
- Chiaretti, S., A. Guarini, M. S. De Propriis, S. Tavarolo, S. Intoppa, A. Vitale, S. Iacobelli, L. Elia, C. Ariola, J. Ritz & R. Foa (2006) ZAP-70 expression in acute lymphoblastic leukemia: association with the E2A/PBX1 rearrangement and the pre-B stage of differentiation and prognostic implications. *Blood*, 107, 197-204.
- Chiorazzi, N., K. R. Rai & M. Ferrarini (2005) Chronic lymphocytic leukemia. *N Engl J Med*, 352, 804-15.
- Corcoran, M., A. Parker, J. Orchard, Z. Davis, M. Wirtz, O. J. Schmitz & D. Oscier (2005) ZAP-70 methylation status is associated with ZAP-70 expression status in chronic lymphocytic leukemia. *Haematologica*, 90, 1078-88.
- Crespo, M., F. Bosch, N. Villamor, B. Bellosillo, D. Colomer, M. Rozman, S. Marce, A. Lopez-Guillermo, E. Campo & E. Montserrat (2003) ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med*, 348, 1764-75.
- Crespo, M., N. Villamor, E. Gine, A. Muntanola, D. Colomer, T. Marafioti, M. Jones, M. Camos, E. Campo, E. Montserrat & F. Bosch (2006) ZAP-70 expression in normal pro/pre B cells, mature B cells, and in B-cell acute lymphoblastic leukemia. *Clin Cancer Res*, 12, 726-34.
- Cuni, S., P. Perez-Aciego, G. Perez-Chacon, J. A. Vargas, A. Sanchez, F. M. Martin-Saavedra, S. Ballester, J. Garcia-Marco, J. Jorda & A. Durantez (2004) A sustained activation of PI3K/NF-kappa B pathway is critical for the survival of chronic lymphocytic leukemia B cells. *Leukemia*, 18, 1391-1400.
- Cutrona, G., M. Colombo, S. Matis, D. Reverberi, M. Dono, V. Tarantino, N. Chiorazzi & M. Ferrarini (2006) B lymphocytes in humans express ZAP-70 when activated in vivo. *Eur J Immunol*, 36, 558-69.
- Damle, R. N., F. Ghiotto, A. Valetto, E. Albesiano, F. Fais, X. J. Yan, C. P. Sison, S. L. Allen, J. Kolitz, P. Schulman, V. P. Vinciguerra, P. Budde, J. Frey, K. R. Rai, M. Ferrarini & N. Chiorazzi (2002) B-cell chronic lymphocytic leukemia cells express a surface membrane phenotype of activated, antigen-experienced B lymphocytes. *Blood*, 99, 4087-93.

- Damle, R. N., T. Wasil, F. Fais, F. Ghiotto, A. Valetto, S. L. Allen, A. Buchbinder, D. Budman, K. Dittmar, J. Kolitz, S. M. Lichtman, P. Schulman, V. P. Vinciguerra, K. R. Rai, M. Ferrarini & N. Chiorazzi (1999) Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*, 94, 1840-7.
- Deaglio, S., M. Morra, R. Mallone, C. M. Ausiello, E. Prager, G. Garbarino, U. Dianzani, H. Stockinger & F. Malavasi (1998) Human CD38 (ADP-ribosyl cyclase) is a counter-receptor of CD31, an Ig superfamily member. *Journal of Immunology*, 160, 395-402.
- Deaglio, S., T. Vaisitti, S. Aydin, L. Bergui, G. D'Arena, L. Bonello, P. Omede, M. Scatolini, O. Jaksic, G. Chiorino, D. Efremov & F. Malavasi (2007) CD38 and ZAP-70 are functionally linked and mark CLL cells with high migratory potential. *Blood*, 110, 4012-21.
- Fournel, M., D. Davidson, R. Weil & A. Veillette (1996) Association of tyrosine protein kinase Zap-70 with the protooncogene product p120c-cbl in T lymphocytes. *The Journal of experimental medicine*, 183, 301-6.
- Furman, R. R., Z. Asgary, J. O. Mascarenhas, H. C. Liou & E. J. Schattner (2000) Modulation of NF-kappa B activity and apoptosis in chronic lymphocytic leukemia B cells. *Journal of Immunology*, 164, 2200-2206.
- Ghia, P., L. Granziero, M. Chilosi & F. Caligaris-Cappio (2002) Chronic B cell malignancies and bone marrow microenvironment. *Semin Cancer Biol*, 12, 149-55.
- Ghobrial, I. M., N. D. Bone, M. J. Stenson, A. Novak, K. E. Hedin, N. E. Kay & S. M. Ansell (2004) Expression of the chemokine receptors CXCR4 and CCR7 and disease progression in B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma. *Mayo Clinic proceedings. Mayo Clinic*, 79, 318-25.
- Gobessi, S., L. Laurenti, P. G. Longo, S. Sica, G. Leone & D. G. Efremov (2007) ZAP-70 enhances B-cell-receptor signaling despite absent or inefficient tyrosine kinase activation in chronic lymphocytic leukemia and lymphoma B cells. *Blood*, 109, 2032-9.
- Guillaume, N., C. Alleaume, D. Munfus, J. C. Capiod, G. Touati, B. Pautard, B. Desablens, J. J. Lefrere, F. Gouilleux, K. Lassoued & V. Gouilleux-Gruart (2005) ZAP-70 tyrosine kinase is constitutively expressed and phosphorylated in B-lineage acute lymphoblastic leukemia cells. *Haematologica*, 90, 899-905.
- Hamblin, T. J., Z. Davis, A. Gardiner, D. G. Oscier & F. K. Stevenson (1999) Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*, 94, 1848-54.
- Hayden, M. S. & S. Ghosh (2008) Shared principles in NF-kappaB signaling. *Cell*, 132, 344-62.
- Herishanu, Y., P. Perez-Galan, D. Liu, A. Biancotto, S. Pittaluga, B. Vire, F. Gibellini, N. Njuguna, E. Lee, L. Stennett, N. Raghavachari, P. Liu, J. P. McCoy, M. Raffeld, M. Stetler-Stevenson, C. Yuan, R. Sherry, D. C. Arthur, I. Maric, T. White, G. E. Marti, P. Munson, W. H. Wilson & A. Wiestner (2011) The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*, 117, 563-74.
- Herman, J. G. & S. B. Baylin (2003) Gene silencing in cancer in association with promoter hypermethylation. *The New England journal of medicine*, 349, 2042-54.

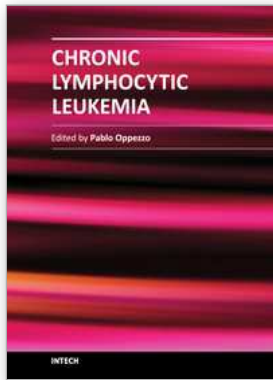
- Hewamana, S., S. Alghazal, T. T. Lin, M. Clement, C. Jenkins, M. L. Guzman, C. T. Jordan, S. Neelakantan, P. A. Crooks, A. K. Burnett, G. Pratt, C. Fegan, C. Rowntree, P. Brennan & C. Pepper (2008) The NF-kappa B subunit Rel A is associated with in vitro survival and clinical disease progression in chronic lymphocytic leukemia and represents a promising therapeutic target. *Blood*, 111, 4681-4689.
- Hivroz, C., C. Grillo-Courvalin, J. C. Brouet & M. Seligmann (1986) Heterogeneity of responsiveness of chronic lymphocytic leukemic B cells to B cell growth factor or interleukin 2. *European journal of immunology*, 16, 1001-4.
- Kamal, A., L. Thao, J. Sensintaffar, L. Zhang, M. F. Boehm, L. C. Fritz & F. J. Burrows (2003) A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. *Nature*, 425, 407-10.
- Karin, M. & A. Lin (2002) NF-kappaB at the crossroads of life and death. *Nature immunology*, 3, 221-7.
- Karray, S., H. Merle-Beral, A. Vazquez, J. P. Gerard, P. Debre & P. Galanaud (1987) Functional heterogeneity of B-CLL lymphocytes: dissociated responsiveness to growth factors and distinct requirements for a first activation signal. *Blood*, 70, 1105-10.
- Klein, U., Y. Tu, G. A. Stolovitzky, M. Mattioli, G. Cattoretti, H. Husson, A. Freedman, G. Inghirami, L. Cro, L. Baldini, A. Neri, A. Califano & R. Dalla-Favera (2001) Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J Exp Med*, 194, 1625-38.
- Lanham, S., T. Hamblin, D. Oscier, R. Ibbotson, F. Stevenson & G. Packham (2003) Differential signaling via surface IgM is associated with V-H gene mutational status and CD38 expression in chronic lymphocytic leukemia. *Blood*, 101, 1087-1093.
- Lankester, A. C., G. M. van Schijndel, C. E. van der Schoot, M. H. van Oers, C. J. van Noesel & R. A. van Lier (1995) Antigen receptor nonresponsiveness in chronic lymphocytic leukemia B cells. *Blood*, 86, 1090-7.
- Latour, S., L. M. Chow & A. Veillette (1996) Differential intrinsic enzymatic activity of Syk and Zap-70 protein-tyrosine kinases. *The Journal of biological chemistry*, 271, 22782-90.
- Letestu, R., A. Rawstron, P. Ghia, N. Villamor, N. Boeckx, S. Boettcher, A. M. Buhl, J. Duerig, R. Ibbotson, A. Kroeber, A. Langerak, M. Le Garff-Tavernier, I. Mockridge, A. Morilla, R. Padmore, L. Rassenti, M. Ritgen, M. Shehata, P. Smolewski, P. Staib, M. Tichioni, C. Walker & F. Ajchenbaum-Cymbalista (2006) Evaluation of ZAP-70 expression by flow cytometry in chronic lymphocytic leukemia: A multicentric international harmonization process. *Cytometry. Part B, Clinical cytometry*, 70, 309-14.
- Lopez-Giral, S., N. E. Quintana, M. Cabrerizo, M. Alfonso-Perez, M. Sala-Valdes, V. G. De Soria, J. M. Fernandez-Ranada, E. Fernandez-Ruiz & C. Munoz (2004) Chemokine receptors that mediate B cell homing to secondary lymphoid tissues are highly expressed in B cell chronic lymphocytic leukemia and non-Hodgkin lymphomas with widespread nodular dissemination. *Journal of leukocyte biology*, 76, 462-71.
- Lopez-Guerra, M., G. Roue, P. Perez-Galan, R. Alonso, N. Villamor, E. Montserrat, E. Campo & D. Colomer (2009) p65 activity and ZAP-70 status predict the sensitivity

- of chronic lymphocytic leukemia cells to the selective IkappaB kinase inhibitor BMS-345541. *Clinical cancer research : an official journal of the American Association for Cancer Research*, 15, 2767-76.
- Lupher, M. L., Jr., N. Rao, N. L. Lill, C. E. Andoniou, S. Miyake, E. A. Clark, B. Druker & H. Band (1998) Cbl-mediated negative regulation of the Syk tyrosine kinase. A critical role for Cbl phosphotyrosine-binding domain binding to Syk phosphotyrosine 323. *The Journal of biological chemistry*, 273, 35273-81.
- Messmer, B. T., D. Messmer, S. L. Allen, J. E. Kolitz, P. Kudalkar, D. Cesar, E. J. Murphy, P. Koduru, M. Ferrarini, S. Zupo, G. Cutrona, R. N. Damle, T. Wasil, K. R. Rai, M. K. Hellerstein & N. Chiorazzi (2005) In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. *The Journal of Clinical Investigation*, 115, 755-764.
- Mohle, R., C. Failenschmid, F. Bautz & L. Kanz (1999) Overexpression of the chemokine receptor CXCR4 in B cell chronic lymphocytic leukemia is associated with increased functional response to stromal cell-derived factor-1 (SDF-1). *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.*, 13, 1954-9.
- Murphy, L. O., S. Smith, R. H. Chen, D. C. Fingar & J. Blenis (2002) Molecular interpretation of ERK signal duration by immediate early gene products. *Nature cell biology*, 4, 556-64.
- Nolz, J. C., R. C. Tschumper, B. T. Pittner, J. R. Darce, N. E. Kay & D. F. Jelinek (2005) ZAP-70 is expressed by a subset of normal human B-lymphocytes displaying an activated phenotype. *Leukemia*, 19, 1018-24.
- Orchard, J., R. Ibbotson, G. Best, A. Parker & D. Oscier (2005) ZAP-70 in B cell malignancies. *Leuk Lymphoma*, 46, 1689-98.
- Petlickovski, A., L. Laurenti, X. P. Li, S. Marietti, P. Chiusolo, S. Sica, G. Leone & D. G. Efremov (2005) Sustained signaling through the B-cell receptor induces Mcl-1 and promotes survival of chronic lymphocytic leukemia B cells. *Blood*, 105, 4820-4827.
- Rao, N., I. Dodge & H. Band (2002) The Cbl family of ubiquitin ligases: critical negative regulators of tyrosine kinase signaling in the immune system. *Journal of leukocyte biology*, 71, 753-63.
- Reif, K., E. H. Ekland, L. Ohl, H. Nakano, M. Lipp, R. Forster & J. G. Cyster (2002) Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. *Nature*, 416, 94-9.
- Richardson, S. J., C. Matthews, M. A. Catherwood, H. D. Alexander, B. S. Carey, J. Farrugia, A. Gardiner, S. Mould, D. Oscier, J. A. Copplestone & A. G. Prentice (2006) ZAP-70 expression is associated with enhanced ability to respond to migratory and survival signals in B-cell chronic lymphocytic leukemia (B-CLL). *Blood*, 107, 3584-92.
- Rosenwald, A., A. A. Alizadeh, G. Widhopf, R. Simon, R. E. Davis, X. Yu, L. Yang, O. K. Pickeral, L. Z. Rassenti, J. Powell, D. Botstein, J. C. Byrd, M. R. Grever, B. D. Cheson, N. Chiorazzi, W. H. Wilson, T. J. Kipps, P. O. Brown & L. M. Staudt (2001) Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med*, 194, 1639-47.



- Sanchez-Aguilera, A., I. Rattmann, D. Z. Drew, L. U. Muller, V. Summey, D. M. Lucas, J. C. Byrd, C. M. Croce, Y. Gu, J. A. Cancelas, P. Johnston, T. Moritz & D. A. Williams (2010) Involvement of RhoH GTPase in the development of B-cell chronic lymphocytic leukemia. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.*, 24, 97-104.
- Schroeder, H. W., Jr. & G. Dighiero (1994) The pathogenesis of chronic lymphocytic leukemia: analysis of the antibody repertoire. *Immunol Today*, 15, 288-94.
- Scielzo, C., A. Camporeale, M. Geuna, M. Alessio, A. Poggi, M. R. Zocchi, M. Chilosi, F. Caligaris-Cappio & P. Ghia (2006) ZAP-70 is expressed by normal and malignant human B-cell subsets of different maturational stage. *Leukemia*, 20, 689-95.
- Semichon, M., H. Merle-Beral, V. Lang & G. Bismuth (1997) Normal Syk protein level but abnormal tyrosine phosphorylation in B-CLL cells. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.*, 11, 1921-8.
- Sup, S. J., R. Domiati-Saad, T. W. Kelley, R. Steinle, X. Zhao & E. D. Hsi (2004) ZAP-70 expression in B-cell hematologic malignancy is not limited to CLL/SLL. *Am J Clin Pathol*, 122, 582-7.
- Ticchioni, M., C. Charvet, N. Noraz, L. Lamy, M. Steinberg, A. Bernard & M. Deckert (2002) Signaling through ZAP-70 is required for CXCL12-mediated T-cell transendothelial migration. *Blood*, 99, 3111-8.
- Till, K. J., K. Lin, M. Zuzel & J. C. Cawley (2002) The chemokine receptor CCR7 and alpha4 integrin are important for migration of chronic lymphocytic leukemia cells into lymph nodes. *Blood*, 99, 2977-84.
- Tracey, L., A. Perez-Rosado, M. J. Artiga, F. I. Camacho, A. Rodriguez, N. Martinez, E. Ruiz-Ballesteros, M. Mollejo, B. Martinez, M. Cuadros, J. F. Garcia, M. Lawler & M. A. Piris (2005) Expression of the NF-kappaB targets BCL2 and BIRC5/Survivin characterizes small B-cell and aggressive B-cell lymphomas, respectively. *The Journal of pathology*, 206, 123-34.
- Van Bockstaele, F., A. Janssens, A. Piette, F. Callewaert, V. Pede, F. Offner, B. Verhasselt & J. Philippe (2006) Kolmogorov-Smirnov statistical test for analysis of ZAP-70 expression in B-CLL, compared with quantitative PCR and IgV(H) mutation status. *Cytometry B Clin Cytom*, 70, 302-8.
- Van Bockstaele, F., B. Verhasselt & J. Philippe (2009) Prognostic markers in chronic lymphocytic leukemia: A comprehensive review. *Blood Reviews*, 23, 25-47.
- Viola, A. & N. Gupta (2007) Tether and trap: regulation of membrane-raft dynamics by actin-binding proteins. *Nature reviews. Immunology*, 7, 889-96.
- Wang, Y. H., L. Fan, W. Xu & J. Y. Li (2011) Detection methods of ZAP-70 in chronic lymphocytic leukemia. *Clinical and experimental medicine*.
- Wange, R. L., R. Guitian, N. Isakov, J. D. Watts, R. Aebersold & L. E. Samelson (1995) Activating and inhibitory mutations in adjacent tyrosines in the kinase domain of ZAP-70. *The Journal of biological chemistry*, 270, 18730-3.
- Williams, B. L., B. J. Irvin, S. L. Sutor, C. C. Chini, E. Yacyszyn, J. Bubeck Wardenburg, M. Dalton, A. C. Chan & R. T. Abraham (1999) Phosphorylation of Tyr319 in ZAP-70 is required for T-cell antigen receptor-dependent phospholipase C-gamma1 and Ras activation. *The EMBO journal*, 18, 1832-44.

- Wu, J., Q. Zhao, T. Kurosaki & A. Weiss (1997) The Vav binding site (Y315) in ZAP-70 is critical for antigen receptor-mediated signal transduction. *The Journal of experimental medicine*, 185, 1877-82.
- Xia, Z., M. Dickens, J. Raingeaud, R. J. Davis & M. E. Greenberg (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science*, 270, 1326-31.
- zum Buschenfelde, C. M., M. Wagner, G. Lutzny, M. Oelsner, Y. Feuerstacke, T. Decker, C. Bogner, C. Peschel & I. Ringshausen (2010) Recruitment of PKC-betaII to lipid rafts mediates apoptosis-resistance in chronic lymphocytic leukemia expressing ZAP-70. *Leukemia : official journal of the Leukemia Society of America, Leukemia Research Fund, U.K.*, 24, 141-52.



## **Chronic Lymphocytic Leukemia**

Edited by Dr. Pablo Oppezzo

ISBN 978-953-307-881-6

Hard cover, 448 pages

**Publisher** InTech

**Published online** 10, February, 2012

**Published in print edition** February, 2012

B-cell chronic lymphocytic leukemia (CLL) is considered a single disease with extremely variable course, and survival rates ranging from months to decades. It is clear that clinical heterogeneity reflects biologic diversity with at least two major subtypes in terms of cellular proliferation, clinical aggressiveness and prognosis. As CLL progresses, abnormal hematopoiesis results in pancytopenia and decreased immunoglobulin production, followed by nonspecific symptoms such as fatigue or malaise. A cure is usually not possible, and delayed treatment (until symptoms develop) is aimed at lengthening life and decreasing symptoms. Researchers are playing a lead role in investigating CLL's cause and the role of genetics in the pathogenesis of this disorder. Research programs are dedicated towards understanding the basic mechanisms underlying CLL with the hope of improving treatment options.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Valerie Pede, Ans Rombout, Bruno Verhasselt and Jan Philippé (2012). The Biological Relevance of ZAP-70 in CLL, Chronic Lymphocytic Leukemia, Dr. Pablo Oppezzo (Ed.), ISBN: 978-953-307-881-6, InTech, Available from: <http://www.intechopen.com/books/chronic-lymphocytic-leukemia/the-biological-relevance-of-zap-70-in-cll>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.