The microenvironment and treatment resistance in chronic lymphocytic leukemia
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Chapter 1

Introduction and scope of the thesis
Introduction

Since the early 1900s, chronic lymphocytic leukemia (CLL) has been recognized as a clinical entity. Nowadays, CLL is the most common adult leukemia in the Western world (1). The incidence of CLL is estimated to be 3.9 per 100,000 people, in men twice as high as in women. The median age at diagnosis is 72 years and CLL is less common among people of African and Asian origin (1). CLL is characterized by an accumulation of CD5+ monoclonal B lymphocytes. Clinical features of the disease are peripheral blood lymphocytosis, hepatosplenomegaly and lymphadenopathy. In later stages of the disease patient may develop increased susceptibility to infections due to neutropenia and hypogammaglobulinemia. In the last decade, it has been shown that CLL is a remarkable heterogeneous disorder (2). Some patients survive for many years without need for treatment, whereas others have a rapidly progressive disease despite aggressive therapy. At present, cures are never obtained due to resistance to (immuno)-chemotherapy. The clinical variability is associated with certain molecular markers, but how these markers influence the biology of CLL is poorly understood. Currently, the hypothesis is that the accumulation of CLL cells is due to impaired apoptosis as well as enhanced proliferation in the protective lymph node microenvironment. The mechanism of dysregulation of apoptotic pathways and the exact influence of non-leukemic cells in the lymph node microenvironment is still a subject of intense research. The aim of this thesis is to investigate which biological and microenvironmental factors are contributing to chemoresistance in CLL and whether it is possible to overcome chemoresistance, in order to develop novel treatment modalities for CLL patients with a rapidly progressive disease.

The normal development of B lymphocytes

In the bone marrow pro-B cells develop into immature B cells. Immunoglobulin gene segments rearrange and code for immunoglobulin molecules that serves as the B cell receptor (BCR) to bind antigen. The BCR is the key molecule for the mature B cell by allowing each individual B cell to recognise a specific antigen. Each B cell displays a distinct BCR that is formed through variable combinations of V, D and J segments of the Ig heavy chain and V and J gene segments of the light chain. Once naïve B-cells have completed maturation they enter the bloodstream and circulate through the peripheral lymphoid tissue. Upon antigen encounter and binding to the BCR, the B cell enters a germinal center (GC) in lymphoid follicles where the cells undergo massive clonal expansion and somatic hypermutation. Somatic hypermutation is the process in which mutations are introduced at a very high rate into the immunoglobulin (Ig) variable region genes to increase affinity of the BCR to antigen (3). B cells with high affinity BCR binding to antigen are positively selected and start to proliferate, whereas B cells which do not bind antigens or only at low affinity are eliminated via apoptosis (4). Many GC B cells also undergo class switch recombination of their Ig heavy chain constant region genes.
Class switch recombination involves replacement only of the constant region of the heavy chain and thereby increase the functional diversity of the immunoglobulin repertoire. Positively selected GC B cells usually undergo multiple rounds of proliferation, mutation and selection until they finally differentiate either into memory B cells or plasma cells and leave the GC. T lymphocytes in the germinal center play an important role in the stimulation and selection process of B cells via CD40-CD40 ligand (CD40L) interaction (4). In the marginal zone of lymphoid tissue, the process of B cell selection occurs in response to bacterial and viral antigens in the absence of T cells (5-7).

The normal counterpart of CLL cells
The surface antigen expression profile of B cells in CLL is defined as CD5, CD19 and CD23 positive. B-CLL cells show reduced levels of surface membrane Ig levels most often IgM or both IgM and IgD (8). There is weak expression of the surface markers FMC7, CD22 and CD79b and CLL cells are negative for cyclin D1 and CD10 (9). CLL can be divided into leukemic cells with (>2%) or without (≤ 2%) somatic hypermutations in the variable regions of the immunoglobulin (Ig) heavy chain (IGHV) genes (10-12). Unmutated CLL patients have a more aggressive course of the disease as compared to mutated CLL patients. In addition, a third minor subset is identified derived from B cells with mutated V genes, but displaying differential IGHV gene patterns (13, 14). Which normal B cell is the cellular counterpart of the CLL clone? At present the answer is unclear. Two different models have been proposed, the single normal counterpart and the multiple normal counterpart model for CLL (15). In the single counterpart model, it is proposed that marginal zone (MZ) B cells, transitional B cells or human B-1-like cells could transform into CLL cells. MZ B cells express BCRs coded by either unmutated or mutated IGHV genes and are polyreactive as well as autoreactive. However, cell surface markers differ between MZ B cells and CLL cells. In mice, B1 cells can give rise to a CLL-like disease in aging mice (16) but until now a human counterpart of the murine B1 cells has not been identified. Finally, transitional B cells, which express CD5 and can express either mutated or unmutated BCRs might serve as the precursors of CLL cells. The main arguments against a transitional B cell origin of CLL cells is the fact that transitional B cells express CD10, do not express CD27, and are not responsive to BAFF. In a multiple counterpart model, it is suggested that mutated CLL cells derive from MZ and/or post GC memory B cells (Figure 1). An argument that CLL cells originate from post GC memory B cells is that one third of the IGHV mutated CLL patients have mutations in Bcl-6, which is the most important regulator in the GC B cell differentiation (17, 18). A small fraction of mutated CLL cells show class switched B cells subsets, mostly IgG1 or IgG3, which are typically GC-associated (19, 20) (Figure 1). The normal counterpart of unmutated CLL cells could be the transitional and/or MZ B cells. An argument that unmutated CLL cells derive from antigen-experienced B cells is that many unmutated CLL cells express poly- and autoreactive antigen receptors and show an activated phenotype.
(21-24). It is unknown whether this activation takes place as part of a T cell independent or T cell dependent immune response. The current view is that the auto-reactive B cells in a T cell independent response may be rescued from apoptosis and acquire characteristics of activated B cells without undergoing a GC reaction and its associated somatic hypermutation (Figure 1) (8).

### Apoptosis regulation

CLL is characterized by an accumulation of monoclonal B cells in primary and secondary lymphoid tissues. Circulating CLL cells are arrested in G0/G1 phase while they possess proliferative capacities in the protective lymph node microenvironment. Apoptosis occurs via either triggering cell surface death receptors (extrinsic pathway) or perturbation of mitochondria following release of cytochrome C and caspase activation (intrinsic pathway) (25, 26) (Figure 2).

Mitochondrial outer membrane permeabilisation (MOMP) is an irreversible process leading to apoptosis via the intrinsic pathway. MOMP is controlled by the different B cell lymphoma 2 (Bcl-2) family members. The Bcl-2 family is characterized by the presence...
of a sequence homology in one to four α-helical Bcl-2 homology (BH) regions, BH1-4. There are three main classes of proteins in the Bcl-2 family, based on the structure and function. The first group are the multidomain anti-apoptotic proteins, Bcl-2, Bcl-X\textsubscript{L}, myeloid cell leukemia sequence 1 (Mcl-1), Bcl-w and Bfl-1 which contain four BH domains (27-30) (Figure 3). Secondly, the multidomain pro-apoptotic proteins consist of three BH domains, which are the Bcl-2-associated X protein (BAX), Bcl-2-antagonist/killer (Bak) and Bcl-2-related ovarian killer (Bok) (31-33) (Figure 3). The third class of Bcl-2 proteins demonstrates homology only in one BH region and are referred to as the BH3 only pro-apoptotic proteins and those proteins were established later (34). This groups
consists of the BH3-interacting domain death agonist (Bid), Bcl-2 antagonist of cell death (Bad), Bcl-2 interacting killer (Bik), Bcl-2-modifying factor (Bmf), Harakiri (Hrk), Noxa and Puma (35, 36) (Figure 3). BH3 only proteins are divided in 2 groups, activators and sensitizers, based on their potency to directly activate Bax and Bak (37-39). Examples of activators are Bim and Bid, whether Puma is an activator remains to be clarified (37-42). The sensitizers of the pro-apoptotic BH3 only proteins bind selectively to anti-apoptotic proteins resulting in oligomerization of the pro-apoptotic proteins Bax and Bak (32, 34, 43) which results in the formation of pores in the mitochondrial outer membrane leading to cytochrome C release (40, 44). Cytochrome C interacts in the cytosol with apoptotic peptidase activating factor 1 (APAF1) and the initiator procaspase 9 and forms the apoptosome. In the apoptosome activated caspase 9 cleaves procaspase 3 into the effector caspase 3 resulting in proteolysis and dysfunction of the cell leading to apoptosis (44, 45). In CLL it has been shown that Bcl-2 levels are high in cells from peripheral blood (46). Furthermore, in CLL cells which are stimulated with CD40L, a model mimicking the protective lymph node environment, also Bcl-XL, Mcl-1 and Bfl-1 levels are strongly increased.

Figure 3. Summary figure of the structures of BCL2 family proteins by group. There are three main classes of proteins in the Bcl-2 family. The first group are the multidomain anti-apoptotic proteins, Bcl-2, Bcl-XL, myeloid cell leukemia sequence 1 (Mcl-1), Bcl-w and Bfl-1 which contain four BH domains. Secondly, the multidomain pro-apoptotic proteins consist of three BH domains, which are the Bcl-2-associated X protein (BAX), Bcl-2-antagonist/killer (Bak) and Bcl-2-related ovarian killer (Bok). The third class of Bcl-2 proteins demonstrates homology only in one BH region and are referred to as the BH3 only pro-apoptotic proteins. This group consists of the BH3-interacting domain death agonist (Bid), Bcl-2 antagonist of cell death (Bad), Bcl-2 interacting killer (Bik), Bcl-2-modifying factor (Bmf), Harakiri (Hrk), Noxa and Puma (35, 36).

Adapted from Letai, Nature Cancer Reviews 2008.
upregulated (47-49). It has to be determined whether downregulation of anti-apoptotic proteins like Bcl-X$_i$ results in enhanced cell death and overcomes drug resistance. The extrinsic apoptosis pathway is triggered via cell surface death receptors. The death receptor family consists of eight members which contain an extracellular domain consisting of up to five cysteine–rich repeats and an intracellular death domain (DD). These eight proteins consist of tumor necrosis factor receptor 1 (TNFR1), CD95/FS-7-associated surface antigen (Fas), death receptor 3 (DR3), tumor necrosis factor (TNF) related apoptosis inducing ligand receptor 1 (TRAILR1)/DR4, TRAILR2/DR5, DR6, ectodermal dysplasia receptor (EDAR) and nerve growth factor receptor (NGFR) (50-53). TNFR1, CD95 and TRAILR1 are the most characterized death receptors. Stimulation of these receptors results in activation of the extrinsic apoptosis pathway via recruitment of caspase 8. Caspase 8, a initiator caspase, cleaves active pro-forms of effector caspases (3, 6 and 7) and leads to apoptosis (54).

An important checkpoint in caspase activation, and thereby regulation of apoptosis via either the intrinsic and extrinsic apoptosis pathway, is formed by inhibitor of apoptosis proteins (IAPs). IAPs are endogenous proteins that block apoptosis pathways by interfering with the activation of effector caspases. The most important IAP family members are: XIAP (55), cIAP1, cIAP2 (56) and Survivin (57). All IAP family members contain at least one baculovirus IAP repeat (BIR) domain. The BIR domain is a 70-80 amino-acid long motif and allows IAPs to bind to caspases (55, 56). XIAP is a direct inhibitor of caspases, whereas cIAP1 and cIAP2 bind to caspases but are not considered to directly inhibit caspases (58, 59). cIAP1 and cIAP2 interfere with caspase ubiquitination and thereby inhibit proteasomal degradation (60). Despite expression of TRAIL receptors on CLL cells, complete resistance has been shown when recombinant TRAIL was used as monotherapy (61). Furthermore, CD95 is strongly upregulated in CD40-stimulated CLL cells, but anti-Fas therapy does not result in apoptosis (62). Combining XIAP inhibitors with extrinsic cell death inducers like TRAIL and CD95 leads to apoptosis in CLL cells (62, 63). Whether cIAP antagonists result in TNFR1 mediated cell death in CLL and synergize with other extrinsic cell death inducers is currently unknown.

Prognostic factors in chronic lymphocytic leukemia

As discussed above, CLL is a disease with a heterogeneous clinical course. Some patients have an indolent course of the disease and eventually die from causes unrelated to CLL, whereas others have a rapidly progressive disease and die within two to three years from diagnosis. In some patients the indolent course of the disease is followed by a progressive phase. The heterogeneity can only partly be explained by differences in clinical stage (64, 65). The Rai and Binet staging systems classify patients according to tumour burden and haematopoietic impairment. This staging system does not fully predict the course of the disease. CLL has been shown to be a biologically heterogeneous entity (8) and many of the biological parameters define prognostic subgroups. The most important
prognostic factors, in addition to the clinical stage and IGHV mutation status (64, 65), are serum markers (for example, thymidine kinase, β2-microglobulin and soluble CD23) (66), specific cell marker expression and genetic parameters. Membrane CD38 and intracellular zeta-associated protein 70 (ZAP-70) are cell markers which are associated with a more aggressive disease (10, 67, 68). CD38 is a surface molecule which plays an important role in the differentiation of B cells. CD38 enhances B cell interactions and augments BCR signalling (69). BCR engagement results in kinase activation of Lyn and Syk which are members of the Src family of protein tyrosine kinases (70). Lyn and Syk induce the key signalling intermediates, such as phospholipase Cγ2 (Plc-γ2) and phosphatidylinositol 3-kinase (PI3K). This results eventually in activation of extracellular signal-regulated kinase (ERK), c-JUN NH2-terminal kinase (JNK) and p38 MAPK, and transcription factors, including nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NF-AT) (70, 71). These pathways play an important role in the immunogenic response of B cells to antigen stimulation (72). Another cell surface marker which relates with a more aggressive disease is ZAP70. In T cells ZAP70 is a protein with a similar function as Lyn and Syk in B cells. It plays an important role in T cell receptor signalling, resulting in activation of NF-κB and leading to T cell proliferation and differentiation (73). Notably, normal B cells do not express ZAP70. However a prognostic subgroup of CLL patients associated with impaired survival shows expression of ZAP70 (74). ZAP70 expression is associated with mutation status, i.e. gene expression studies have shown that IGHV unmutated CLL cells express higher levels of ZAP70 as compared to IGHV mutated CLL cells (75). Chromosomal abnormalities which are associated with an aggressive disease are 11q23 and 17p13 deletions (76-78). The deleted region at the 11q22-23 locus harbours the ataxia telangiectasia-mutated (ATM) gene and mutations of ATM have been shown to be present in a third of cases with 11q23 deletions (79, 80). ATM is a protein kinase involved in the cell’s response to DNA damage. The 17p13 deletion is present only in 3-7% of CLL at diagnosis (76, 81). However, this 17p deletion is found in up to 30 to 40% of relapsed or chemorefractory CLL patients (82). The 17p13 deletion affects tumor suppressor gene (TP)53 and results in a dysfunctional p53 pathway. The 17p13 deletion is associated with short survival and poor response to cytotoxic drugs (81, 83). There is an urgent need to define treatment strategies for patients with a dysfunctional p53 pathway. In conclusion, progression of CLL is remarkably diverse and more insight into the biological characteristics of CLL will lead to the expansion of different treatment strategies for the prognostic subgroups.

Signals from the microenvironment

The current view is that, besides displaying defects in apoptosis, a fraction of leukemic cells does proliferate in the lymph nodes of CLL patients (47). The microenvironment in lymphoid tissue is postulated to play a major role in the survival and accumulation of leukemic cells. This is an important aspect of the disease which might be underscored...
by the fact that leukemic cells die rapidly in vitro (84). Both signals delivered via direct cell-cell contact and soluble factors result in enhanced survival of CLL cells (85). In vitro, it has been shown that cell-cell contact with bone marrow stroma, and not just the soluble factors secreted by stroma, resulted in enhanced survival of CLL cells (84, 86).

Nurse-like cells (NLCs) develop in vitro from CD14+ monocytes by interaction with CLL cells (87). NLCs also express the tumor necrosis factor family members BAFF and APRIL, providing survival signals to CLL cells via corresponding receptors (BCMA, TACI, BAFF-R). CD38 expression allows CLL cells to interact with CD31, the ligand for CD38, expressed by stromal and nurse-like cells. Stimulation of the B-cell antigen receptor (BCR) complex (BCR and CD79a,b) induces downstream signaling by recruitment and activation of Syk. CD40L T cells are preferentially found in CLL pseudofollicles and can interact with CLL cells via CD40. This cross-talk between CLL cells and accessory cells results in enhanced survival and drug resistance of CLL cells.

Figure 4. Cross-talk between CLL cells and the lymph node microenvironment. This figure displays the molecules involved in cross-talk between CLL cells and accessory cells in the lymphoid tissue microenvironments. Contact between CLL cells and nurse-like cells (NLCs) is established and maintained by chemokine receptors and adhesion molecules. NLCs express the chemokines CXCL12 and CXCL13. NLCs also express APRIL and BAFF, providing survival signals to CLL cells via corresponding receptors (BCMA, TACI, BAFF-R). CD38 expression allows CLL cells to interact with CD31, the ligand for CD38, expressed by stromal and nurse-like cells. Stimulation of the B-cell antigen receptor (BCR) complex (BCR and CD79a,b) induces downstream signaling by recruitment and activation of Syk. CD40L T cells are preferentially found in CLL pseudofollicles and can interact with CLL cells via CD40. This cross-talk between CLL cells and accessory cells results in enhanced survival and drug resistance of CLL cells.

Adapted from Burger, Blood, 2009.
Leukemia-1 proto-oncogene (TCL1) TG mice develop a CLL like disease with expansion of the B cell compartment and enlarged spleens (91, 92). Furthermore, in the lymph node microenvironment, formation of proliferation centers of CLL cells (pseudofollicles) is found. These proliferation centers show increased numbers of CD3+ T cells which express CD4 and CD40L (47, 93) (Figure 4). CD40-CD40L interaction results in enhanced survival of CLL cells via NF-κB activation resulting in upregulation of the pro-survival proteins Bfl-1 and Bcl-XL (94). Besides inducing enhanced survival, CD40-CD40L interaction leads to resistance of CLL cells to various cytotoxic drugs such as fludarabine, velcade and roscovitine (94). In vitro stimulation of CLL cells with CD40L results in only minor proliferation. In this thesis, we investigate which cytokines induce proliferation in CD40-stimulated CLL cells.

Murine Models of CLL

Based upon phenotypic characteristics of human CLL cells (CD5+ B cells) a parallel has been drawn with CD5+ B1 cells in mice. The first mouse model for human CLL described was the Eμ-TCL1 transgenic mouse, which develops a clonal CD5+ B lymphoproliferative disease at 12 months of age (95). TCL1 has been shown to be expressed in human CLL and inhibits activator protein 1 (AP-1) activity, an inducer of apoptosis (96, 97) and activates the pro-survival NF-κB pathway (98). Other mouse models for human CLL are the TCL1, APRIL, BAFF/TCL1, SV40 and TRAF2DN/Bcl-2 transgenic mice (92, 99-102). These mice show enlarged spleens, Peyers’ patches and mesenteric lymph nodes with accumulation of monoclonal CD5+ B cells. APRIL and BAFF, as discussed above, are important pro-survival factors produced by NLCs in the protective lymph node microenvironment of CLL patients leading to NF-κB activation. Murine CD5+ B cells are recognized as a distinct B cell subset i.e. B1a B cells. B1a B cells predominate in the peritoneal and pleural cavity and are only present in low numbers in lymph nodes in wild type mice (103). Furthermore, murine B1a B cells produce autoreactive antibodies and function mainly in T cell independent (TI) responses and supply naive hosts with innate protection against bacterial infection (104, 105). Until now it is unclear whether the murine CD5+ B cells are analogous to human CD5+ CLL cells. Human CLL CD5+ expressing B cells are predominantly present in the peripheral blood and in lymphoid organs and are mostly unresponsive to TI stimuli (106). Despite the possible difference in the origin of CD5+ B cells in man versus mouse, APRIL and BAFF/TCL1 mice are a commonly used in vivo model for CLL (91, 92, 99).

Therapeutic regimens in CLL

As discussed above CLL is a very heterogeneous disease with a variable course. Biological markers and new insights into the pathogenesis of the disease lead to better prediction of prognosis and influences therapeutic decisions. In contrast to others leukemias, not every patient with CLL should be treated immediately. No differences in overall survival were
observed comparing early versus late treatment (107). However, trials are performed to assess early treatment of patients with high-risk based on biological and clinical criteria. For several decades monotherapy with alkylating agents such as chlorambucil has been considered the gold standard. Chlorambucil is an oral drug with low toxicity which is an appropriate option for unfit elderly patients. However, chlorambucil can delay disease progression, even though it does not affect overall survival (107). A combination of an alkylating agent and a purine analogue is bendamustine which has recently been approved for CLL treatment. In a randomised phase III trial comparing bendamustine and chlorambucil as a first-line treatment, bendamustine showed significant higher overall response rate (ORR) and longer progression-free survival (PFS) (108). Fludarabine is a purine analogue and is the most extensively studied drug in CLL. Fludarabine was compared to chlorambucil in a randomized phase III trial in elderly patients (median age 70 years) (109). Patients receiving fludarabine achieved higher response rates, but PFS was not prolonged compared to chlorambucil treated patient (109, 110). Combining alkylating agents and purine analogues resulted in improved complete response rates but no effects on OS has been observed (109-111). Based on the results from these studies chlorambucil can still be regarded as an appropriate therapy for the physically unfit elderly CLL patients. However, chlorambucil monotherapy results in low complete response (CR) rates and therefore for younger or physically fit patients other treatment strategies have been shown to be more appropriate (see below). Monotherapy with the anti-CD20 antibody rituximab is less active in CLL compared to other lymphomas. However, combination of rituximab with fludarabine resulted in a better PFS and OS compared to fludarabine treatment alone (112). The combination fludarabine, cyclophosphamide (FC) was compared with FC combined with Rituximab (FCR) and showed that FCR induced significantly more complete remission (CR) than FC (113, 114). Notably, patients with a 11q23 deletion benefited greatly from treatment with fludarabine, cyclophosphamide and Rituximab (115, 116). Furthermore, it was shown that three years after treatment, there was a significantly higher OS in the FCR treated group versus the FC treated group (115). However, the population of this trial is relatively young (median age 60 years) and physically fit and conclusions from this study should not be generalised to unfit and elderly CLL patients.

Alemtuzumab is a fully humanized recombinant monoclonal antibody against the CD52 antigen. Alemtuzumab has been shown to be effective in patients with high-risk genetic markers and p53 mutations (117-119). Maintenance therapy with alemtuzumab seems feasible for CLL patients who have previously been treated with fludarabine but requires close monitoring for infectious agents especially CMV (120).

Despite encouraging results from FCR treatment eventually all CLL patients will relapse. New treatment strategies with small molecules which specifically target signaling pathways and/or anti-apoptotic proteins which play a role in drug resistance are currently in development. ABT-263 is a BH3 mimetic, which is a potent small-molecule antagonists
that binds with high affinity to the anti-apoptotic molecules Bcl-X\textsubscript{L}, Bcl-2 and Bcl-w, but not Mcl-1 or Bfl-1 (121). As recently reviewed, encouraging preliminary data are observed with ABT-263 (122). In a phase I trial in which relapsed of refractory CLL patients were treated with ABT-263 as a single agent, 9 of 26 patients achieved a partial response (35%) of which seven were sustained for more than six months (123). Importantly, responses were also observed in CLL patients with a 17p deletion (123). ABT-263 induces a rapid fall of platelets proportional to dose (123, 124). In current ongoing phase II trials pretreatment of a low dose ABT-263 for 1 week is introduced before dose escalation to the maximal dose in order to stimulate compensatory increased megakaryopoiesis. Clinical trials are currently ongoing in which ABT-263 is combined with standard therapies in order to investigate whether ABT-263 sensitizes CLL cells to cytotoxic drugs.

Scope of the thesis

AIM: to investigate which biological and microenvironmental factors are contributing to proliferation and induction of chemoresistance of CLL cells.

In chapter 2, we study the effects of CD40 and TRL9 stimulation on proliferation and drug resistance in mutated versus unmutated CLL cells. In chapter 3, the effects of activated T cells on CLL cells are investigated focusing on activation, drug resistance and proliferation. In chapter 4, we describe the proliferative effects of IL-21 in combination with CD40-stimulation and we explore whether IL-21 has influence on CD40-induced drug resistance. Chapter 5 and 6 focus on newly designed targeted therapies. In Chapter 5, we study which mechanisms play a role in Compound A resistance in CLL cells. Chapter 6 addresses the role of the Noxa/Mcl-1 balance in CD40-induced ABT-737 resistance. Finally, in chapter 7, we investigate the role of Noxa in the pathobiology of CLL by ablation of Noxa in two commonly used mice models for CLL, APRIL Tg and TCL1 Tg mice.
Reference List


Introduction and scope of the thesis


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