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Chronic lymphocytic leukemia

Studies on cardiovascular disease, biomarkers and subclones

KARIN LARSSON

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Abstract
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The treatment of Chronic lymphocytic leukemia (CLL) is rapidly changing, with targeted therapies, e.g. Bruton's tyrosine kinase inhibitors (BTKi), entering the frontline and relapse setting. However, BTKis have significant adverse effects, most notably cardiovascular disease (CVD). This is of particular concern since the CLL population consists of mainly elderly patients. Data on CVD comorbidity and its possible relation to inflammation in real-world CLL patients is scarce. The aim of this thesis was to study inflammatory biomarkers, the epidemiology of CVD in CLL as well as the occurrence of subclones, in population-based cohorts. In **paper I** we analyzed the plasma/serum concentrations in of 11 biomarkers with known association to inflammation and/or CVD in 139 patients with CLL and 71 healthy age- and sex-matched controls. Eight out of 11 biomarkers were increased among CLL patients compared to controls. In addition, the pattern of biomarker expression differed between patients and controls. In **paper II**, we demonstrated that 32% of all patients diagnosed with CLL in Sweden during 2007-2010 had ≥1 CVD <10 years prior to CLL diagnosis which increased to 37% at start of primary therapy. In **paper III**, a large CLL cohort, n=4261, was studied (all patients diagnosed with CLL during 2007-2015) with the addition of 5 comparators per CLL patient. In line with paper II, a substantial burden of CVD at time of diagnosis was observed, 39% vs 41% among comparators ($p<0.05$). During follow-up, the incidence of CVD was increased among patients with CLL (HR 1.69, 1.61-1.77) compared to comparators, regardless of previous CVD or need of therapy. All-cause mortality (HR 2.29, 2.14-2.14) but not CVD mortality (HR 1.00, 0.89-1.13) was increased among patients with CLL vs comparators. Finally, approximately 50% of patients with CLL were prescribed antihypertensive drugs. In **paper IV**, we studied the occurrence of subclones down to a variant allele frequency (VAF) of 1% using a deep-sequencing NGS panel covering 15 recurrently mutated genes in pretreatment samples from 40 patients with CLL. We found that 48% (n=19/40) exhibited clonal mutations (VAF≥10%,) while 25% (n=10/40) had subclonal mutations (VAF<10%). In 6 out of these 10 patients, subclonal and clonal mutations co-occurred revealing that isolated subclonal mutations were not a frequent event. Mutations were significantly enriched in patients requiring therapy ($p<0.05$). In conclusion, CLL patients have increased blood concentrations of biomarkers associated with low-grade inflammation. However, they do not have an increased risk of CVD at the time of CLL diagnosis. Nonetheless, an increase in CVD during follow-up was observed, the cause of which is currently unknown. Importantly, the CVD burden in CLL is substantial in our population-based studies and of concern in the era of increasing BTKi usage. Finally, subclonal mutations are present among 25% of CLL patients and commonly co-exists with clonal mutations. The prognostic role of these subclonal mutations needs further investigation.

Keywords: : Chronic lymphocytic leukemia, cardiovascular disease, population-based, biomarkers, subclones

Karin Larsson, Department of Medical Sciences, Haematology, Akademiska sjukhuset, Uppsala University, SE-75185 Uppsala, Sweden.

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It always seems impossible until its done.
Nelson Mandela

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Larsson, K., M. Höglund, A. Larsson, M. Thulin & T. Karlsson (2020a) Increased levels of the cardiovascular disease risk biomarkers GDF15 and myostatin in patients with chronic lymphocytic leukaemia. *Growth Factors,* 38**,** 189-196.

II. Larsson, K., M. Mattsson, F. Ebrahim, I. Glimelius & M. Höglund (2020) High prevalence and incidence of cardiovascular disease in chronic lymphocytic leukaemia: a nationwide population-based study. *Br J Haematol*,190, e233-264.

III. Larsson, K., J. Söderling, M. Höglund, I. Glimelius & M. Mattsson (2022) Cardiovascular disease in patients with chronic lymphocytic leukemia: A Swedish nationwide register study with matched comparators. *Am J Hematol,* 97**,** E255-e257.

IV. Larsson, K., Young, E., Höglund, M., Mattsson M,. Rabbani, L & Mansouri, L. Isolated subclonal mutations are not a common feature in untreated chronic lymphocytic leukemia. *Paper in manuscript*.

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Abbreviations

AID	Activation-induced cytidine deaminase
AE	Adverse event
AF	Atrial fibrillation
Allo-HSCT	Allogeneic hematopoietic stem cell transplantation
ATC	Anatomic therapeutic chemical
ATM	Ataxia telangiectasia mutated
B2M	Beta-2-microglobulin
BCL2	B-cell lymphoma 2
BcR	B-cell receptor
BIRC3	Baculoviral IAP repeat containing 3
BR	Bendamustine and rituximab
BRAF	Proto-oncogene B-Raf
BTK/BTKi	Bruton's tyrosine kinase / Bruton's tyrosine kinase inhibitor
CAR-T	Chimeric antigen receptor T-cell
CD	Cluster of differentiation
CDR	Complementary determining region
CH	Clonal hematopoiesis
CIT	Chemoimmunotherapy
CLL	Chronic lymphocytic leukemia
CRP	C-reactive protein
CVD	Cardiovascular disease
CXCR	Chemokine receptor
EGR2	Early growth response 2
EMA	European medicine agency
ERIC	European research initiative on CLL
ERK	Extracellular signal-regulated kinase
FC	Fludarabine and cyclophosphamide
FCR	Fludarabine, cyclophosphamide and rituximab
FDA	Food and drug administration
FISH	Fluorescence *in situ* hybridization
GDF15	Growth differentiation factor 15
GIV	Obinutuzumab, ibrutinib and venetoclax
GV	Obinutuzumab and venetoclax
ICD	International statistical classifications of diseases
Ig	Immunoglobulin

IGHV	Immunoglobulin heavy chain
IL	Interleukin
IPR	Inpatient register
iwCLL	International workshop on CLL
LN	Lymph node
LPL	Lipoprotein lipase
MBL	Monoclonal B-cell lymphocytosis
M-CLL	IGHV mutated CLL
MMP9	Matrix metalloproteinase 9
(u)MRD	(un-)Measurable residual disease
MYD88	Myeloid differentiation primary response 88
NF-κB	Nuclear factor kappa-beta
NFKBIE	Nuclear factor kappa-beta inhibitor epsilon
NGS	Next generation sequencing
NIK	Nuclear factor kappa-beta inducing kinase
NOTCH1	Notch homolog gene 1
ORR	Overall response rate
OS	Overall survival
PCA	Principal component analysis
PFS	Progression free survival
PI3K	Phosphoinositol-3-kinase
PIN	Personal identity number
PLCγ2	Phospholipase C gamma-2
PTX3	Pentraxin 3
RR	Relapse refractory
RV	Rituximab and venetoclax
SCR	Swedish cancer register
SF3B1	Splicing factor 3b subunit 1
SHM	Somatic hypermutation
SLL	Small lymphocytic lymphoma
STAT3	Signal transducer and activator of transcription 3
TLS	Tumor lysis syndrome
TLR	Toll like receptor
TNF/TNFα	Tumor necrosis factor / Tumor necrosis factor alpha
(s)TNFR1	(soluble)Tumor necrosis factor receptor 1
(s)TNRF2	(soluble)Tumor necrosis factor receptor 2
TP53	Tumor protein 53
TTFT	Time to first treatment
U-CAN	Umeå-Uppsala comprehensive cancer consortium
U-CLL	IGHV unmutated CLL
VA	Ventricular arrythmia
VAF	Variant allele frequency
VEGF	Vascular endothelial growth factor
(s)VEGFR1	(soluble) Vascular endothelial growth factor receptor 1

(s)VEGFR2	(soluble) Vascular endothelial growth factor receptor 2
WHO	World health organization
XPO1	Exportin 1

Chronic lymphocytic leukemia

Introduction to the thesis

Chronic lymphocytic leukemia (CLL) is the most common leukemia among adults in the world. At time of diagnosis, the vast majority of patients are asymptomatic and not in need of therapy. Over time, approximately two thirds of all patients require treatment [1]. CLL is currently regarded as an incurable disease.

The relationship between chronic inflammation, cardiovascular disease (CVD) and cancer is well established [2, 3] and there are data describing activated inflammatory pathways in CLL [4, 5] as well as elevated levels of inflammatory markers in serum/plasma before time of CLL diagnosis [6]. Yet, it is unknown whether an underlying CLL clone predisposes to the development of CVD, as has been shown for other clonal hematological disorders [7]. Today, there is an ongoing paradigm shift in CLL treatment, with targeted therapy, such as Bruton's tyrosine kinase inhibitors (BTKi), replacing traditional chemoimmunotherapy (CIT). However, the first-generation BTKi ibrutinib, has been associated with significant cardiovascular side effects in clinical trials, such as atrial fibrillation (AF) and hypertension [8, 9]. Risk factors for cardiovascular side effects are increasing age and preexisting CVD [10-12]. As the median age at diagnosis of CLL is 72 years [1], a large proportion of patients are expected to have CVD comorbidity already at time of CLL diagnosis and thus at therapy initiation. Currently, real-world data on prevalence of CVD prior to the BTKi era are scarce, as well as follow-up data on the risk of developing CVD. There is also a lack of data regarding CVD in CLL patients in relation to the general population.

When treatment is warranted the only predictive markers used today are the presence of unmutated immunoglobulin heavy chain (IGHV) genes, del(17p) and/or Tumor protein 53 *(TP53)* mutation [13, 14]. As few predictive markers are available in clinical routine, new ones are thus warranted, including the possible prognostic and predictive impact of minor CLL clones.

In this context this thesis aims to investigate the occurrence of inflammatory biomarkers linked to CLL, the prevalence of CVD comorbidity, also in relation to the general population, and finally the occurrence of subclones in a real-world CLL cohort.

Epidemiology

CLL is a disease with a described genetic susceptibility [15]. It is more common in Caucasian populations, probably due to genetic rather than environmental factors [16]. First-degree relatives of CLL patients, especially males, have an increased risk of developing CLL themselves. Additionally, familiar CLL debut earlier than sporadic CLL [17-19].

Environmental causes of CLL have been reported, although not with a strong impact on risk. In a pooled analysis of 2444 patients with CLL/ Small lymphocytic lymphoma (SLL) by Slager *et al.*, it was shown that CLL was more common among farmers and hair dressers [18], possibly due to exposure to pecticides and herbicides, as reported earlier for patients with non-Hodgkin lymphoma [20] and exposure to hair products containing solvents, dyes, and ammonia [18]. However, these results regarding environmental risk factors for CLL/SLL are conflicting considering evidence for genetic involvement.

In Sweden, the annual incidence of CLL is approximately 5.3 per 100 000, with a stable frequency during the last decades. The median age at diagnosis is 72 years with a male/female ratio of 1.6:1 [21]. Several studies have demonstrated that men overall have an inferior prognosis compared to women, although the reason for this difference is currently unknown. Due to improved survival in CLL, as well as prolonged survival overall in the total population, the prevalence of CLL has increased overtime [21, 22].

Diagnosis

The diagnosis of CLL is defined by WHO (World health organization) and international workshop on CLL (iwCLL) according to the following criteria:

- ≥ 5 x10^9/L clonal B-lymphocytes in peripheral blood persistent >3 months.
- Morphologically mature lymphocytes with the following phenotype: Cluster of differentiation (CD)5+, CD19+, CD23+, CD20+dim, CD200+, CD10- and the expression of the immunoglobulin light chain on the CLL cell, restricted to either kappa or lambda, i.e., clonal excess, as determined by flow cytometry.

Both of these criteria must be met for the diagnosis of CLL. Notably, bone marrow aspiration or biopsy is not needed for diagnosis, but is recommended before starting therapy [14, 23].

The differential diagnoses to CLL are primarily other non-Hodgkin lymphomas, such as mantle cell lymphoma or splenic marginal zone lymphoma.

Patients with a B-cell clone of CLL phenotype but with a clonal lymphocyte count <5 x10^9/L, are categorized as either SLL or monoclonal B-cell

lymphocytosis (MBL). SLL is manifested as engagement of one or more lymph nodes (LN) or the spleen, and is clinically handled in the same way as CLL [14]. MBL is further divided in low-count and high-count MBL, defined by monoclonal B-cell lymphocytosis $<0.5 \times 10^9$/L or $>0.5 \times 10^9$/L respectively. Nearly all CLL cases are preceded by a high-count MBL [24, 25].

Pathophysiology and immunogenetics

CLL is the disease of morphologically mature, but dysfunctional B-lymphocytes. When and how during B-cell development the malignant transformation occurs has for a long time been an enigma.

In addition to chromosomal aberrations and gene mutations, there is evidence of epigenetic alterations of the CLL genome, with a divergent DNA methylation profile compared to normal B-cells. Increased heterogeneity of DNA methylation in CLL has been associated with transcriptional variation, possibly due to a higher degree of genetic instability in CLL cells with disorded DNA methylation [26, 27]. Furthermore, the CLL cell is dependent on the microenvironment for proliferation and survival, as reflected in the difficulties in culturing CLL cells *in vitro* as they undergo apoptosis under these conditions [28]. For the CLL cell survival, the B-cell receptor (BcR) plays a crucial role.

The B-cell receptor

The interaction between the CLL cell and the microenvironment is mediated through different proteins expressed on the surface of B-cells, of which the BcR is of special importance. This receptor is fundamental in CLL pathophysiology by sending survival and proliferation signals into the cell, influencing gene expression.

The BcR is attached to the cell membrane of the B-cell and consist of two immunoglobulin (IG) heavy (H) and two light (L) chains. These chains are divided into different regions; variable (V), joining (J) and constant (C) regions. In addition, the heavy chains have a diversity (D) region. The variable regions are located outside the cell membrane and form the binding sites for antigens [29].

The first version of the BcR, the pre-BcR, is expressed on pre-B-cells in the bone marrow and consists of two identical heavy chains and two surrogate light chains. Subsequent stepwise rearrangements of the genes coding for the V(D)J segments, results in the BcR, i.e., immunoglobulin M (IgM) which is expressed on immature B-cells. The naïve, mature B-cell is defined by co-expression of IgD, through an alternative splicing of the heavy chain [29].

The mature B-cell migrates from the bone marrow to secondary lymphoid organs, i.e., LNs and spleen, and is presented to antigens in the germinal

centers. If the affinity between the variable region of the IGHV molecule and the antigen is sufficient, the B-cell will undergo a second stage of genetic modification, somatic hypermutation (SHM). During the process of SHM, point mutations are introduced in the variable regions of the IGHV molecule. This is achieved by the enzyme Activation-induced cytidine deaminase (AID). In addition, AID might also induce a class switch recombination of IgM to either IgA, IgG or IgE, expressed by memory B-cells or plasma cells [29, 30]. The purpose of SHM is to select B-cells with high affinity BcRs for further maturation steps resulting in memory B-cells or plasma cells. In the normal B-cell maturation process, B-cells with low affinity BcRs undergo apoptosis [29].

Mutated or unmutated IGHV status

CLL is today separated into two different subgroups depending on the mutational status of the BcR (IGHV status), based on whether the CLL cells have undergone the process of SHM. This separation has proven to have prognostic significance [31, 32]. The IGHV status, i.e. mutated or unmutated, depends on the homology of the IGHV gene in relation to the germline sequence: with <98% homology classified as mutated CLL (M-CLL) and ≥98% sequence homology classified as unmutated CLL (U-CLL) [31]. Approximately 30-40% of all CLL cases are classified as U-CLL and 60-70% as M-CLL [31, 32]. The prognosis of U-CLL and M-CLL differs, where U-CLL has a worse clinical outcome [31, 32] and often co-exists with driver mutations [33].

During the process of SHM, the BcR of M-CLL binds to antigens with high affinity which also means that a limited set of antigens can activate the BcR of M-CLL. U-CLL cells, in contrast, binds with lower affinity to antigens. Following antigen binding to the BcR, downstream signaling in the CLL cell differ; in U-CLL, the BcR shows more responsiveness to activation leading to proliferation. This in contrast to M-CLL, in which anergy, i.e. absence of normal immune response to antigens, is induced [29]. *Figure 1* illustrates the BcR on the surface of the CLL cell and downstream signaling, via Bruton's tyrosine kinase (BTK).

Stereotyped subsets

Approximately 40% of all CLL cases could, in addition to IGHV status, be further divided into subsets, based on having identical or semi-identical complementary determining region 3 (CDR3) of the BcR, so called stereotyped BcRs. It has been proposed that these stereotyped BcRs are the result of specific antigen and/or autoantigen stimulation in CLL [34, 35]. Of special importance is subset #2, which uses IGHV3-21, and is associated with a poor prognosis despite commonly being IGHV hypermutated [36]. Within this subset, del(11q) is often seen, affecting the Ataxia telangiectasia mutated *(ATM)*

gene. Mutations in Splicing factor 3b subunit *(SF3B1)* are also common feature in subset #2 [37]. Subset #8, using IGHV 4-39, has an increased risk of Richter's transformation, i.e. transformation of CLL to diffuse large B-cell lymphoma [38]. Recently, in a study with U-CLL (100% homology) cases, belonging to subset #1 and #8, it was shown that SHM appears also in CDR3, in cases with 100% homology. The immunobiological effects of this needs further investigation [39].

Microenvironment

The microenvironment is crucial for the survival and proliferation of the CLL cell, reflected in the high levels of apoptosis following *in vitro* culturing in a medium supporting B-cells [28]. However, co-culturing CLL cells *in vitro* with various cell lines such as nurse-like cells and bone marrow stromal cells, helps maintain survival [40, 41]. *In vivo* data implies that the proliferation rate is highest in the LNs compared to peripheral blood [42], where the majority of cells remains in a resting phase, indicating that the key feature of CLL is the avoidance of apoptosis rather than cell proliferation [43]. Within LNs, CLL cells proliferate in so-called pseudo follicles, where they interact directly through ligation with stromal cells and other immune cells, such as T-cells and special subsets of macrophages. Data also suggest indirect communication via secreted cytokines, chemokines and extracellular vesicles carrying proteins or microRNAs [44, 45].

The migration of CLL cells between compartments, e.g. blood, bone marrow and LNs, is achieved by upregulation and/or downregulation of genes, often in response to BcR activation. Inhibition or weakening of BcR activation is used in modern CLL targeted therapy, where the BcR signaling is inhibited by BTKi, which blocks the signaling through the BcR downstream due to BTK inhibition [46]. This inhibits cell migration signals mediated through the BcR which repositions CLL cells from proliferation centras in LNs and bone marrow to the blood [47]. Several cytokines with pro-inflammatory effects have been found decreased in plasma following BTKi administration, compared to pretreatment levels [48, 49].

The role of T-cells in the pathophysiology of CLL, is complex. Both T-helper cells (CD4+) and T-cytotoxic cells (CD8+) appear dysfunctional in CLL patients with impaired capacity to communicate with other immune cells and to exert antitumoral properties. Additionally, "exhausted T-cells", i.e. T-cells with signs of chronic activation, as well as regulatory T-cells have been found elevated in CLL patients, further suppressing the antitumor effects that T-cells normally possess [50].

NF-κB pathway

The Nuclear factor kappa beta (NF-κB) family is a subset of transcription factors regulating expression of genes involved in multiple processes such as inflammation, immune response, cell proliferation and survival [2, 51-54]. In B-cells, several upstream signaling cascades operate as either positive or negative regulators, before the transcription factors enter the cell nucleus. Activation of the two NF-κB pathways, the canonical and non-canonical, upon binding of antigens/autoantigens or signaling molecules, e.g. cytokines such as IL-6, supports progression of CLL [55, 56].

In CLL, the canonical pathway is activated from surface receptors, such as the BcR and Toll like receptor (TLR), following stimulation. The non-canonical pathway is activated by cytokines binding to receptors from the Tumor necrosis factors (TNF) superfamily, such as CD40 or TNF receptor 1 (TNFR1) [51]. Characteristically, the activation of the non-canonical pathway is less rapid, but more persistent [53]. A crucial step in the non-canonical pathway is mediated through Nuclear factor kappa beta inducing kinase (NIK), an important negative regulator of this pathway. In addition, crosstalk between the pathways occurs [51, 57]. (Figure 1)

In CLL there are several recurrent gene mutations, i.e. Myeloid differentiation primary response 88 *(MYD88),* Baculoviral IAP repeat containing *(BIRC3)* and Nuclear factor kappa beta inhibitor epsilon *(NFKBIE)*, affecting the downstream NF-κB pathways [52]. In addition, epigenetic alterations affecting molecules involved in the NF-κB pathway have been demonstrated [58].

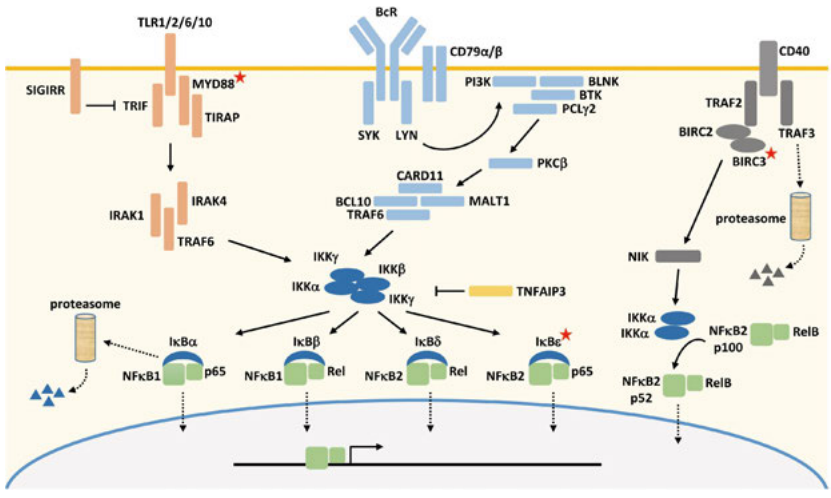

Figure 1. NF-κB pathway and its mediators from cell surface; BcR, TLR and CD40, illustrating their downstream signaling pathways in the CLL cell. A star highlights recurrently mutated genes in CLL affecting the NF-κB pathway. Reprinted from [52], with kind permission from Elsevier.

The genetics of CLL – a model of clonal evolution

About 2000 genes has been found mutated in CLL [33, 59], however only a few have been found to have a prognostic value, and even fewer to have a predictive value [60]. Early events leading to a cancer development, i.e. genetic aberrations found in the majority of the CLL cells, include del(13q) and trisomy 12, which are the two most frequent chromosomal aberrations in CLL [61]. Examples of driver mutations occurring later during disease progression are mutations in the genes Notch homolog gene 1 *(NOTCH1), SF3B1* and *TP53* [33, 60]. Driver mutations have been detected also in MBL. In a targeted gene sequencing study with longitudinal samples from patients with high-count MBL, it was shown that progression from MBL to CLL primarily resulted from growth of existing clones harboring driver mutations, i.e. not through the occurrence of new mutations [62].

Clonal evolution is a well-established phenomenon in tumor biology, including hematological malignancies [63]. Already at diagnosis and prior to start of therapy, CLL patients can have several detectable subclones, usually defined as clones with detectable mutations with variant allele frequency (VAF) <10% [64]. These clones and subclones might co-exist in an equilibrium, or alternatively one clone and/or subclones might proliferate and dominate, often in response to therapy, altering the clonal dynamics [33, 65].

Chromosomal aberrations in CLL

Analyzing the chromosomes of CLL cells using fluorescence *in situ* hybridization (FISH), was a landmark in the prognostication of CLL. The prognostic impact of four chromosomal abnormalities: del(13q), trisomy 12, del(11q) and del(17p) was described in the pivotal study from 2000 by Döhner *et al.* [61], in which the authors presented a hierarchical model based on the prognostic impact of the different aberrations. In total, 82% (268 of 325) of the CLL patients exhibited chromosomal aberrations at time of diagnosis, the most common being del(13q). The median survival differed markedly depending on the detected aberrations with 133 months for del(13q) and only 32 months for patients with del(17p), this in the era of chemotherapy/CIT.

The presence of isolated del(13q) is associated with good prognosis in CLL. Additionally, it is most often present in M-CLL. Del(13q), leads to loss of specific microRNAs who function as suppressors of B-cell lymphoma 2 (BCL2). The final effect is an overexpression of BCL2, with antiapoptotic effects [66].

Trisomy 12 is often associated with U-CLL as well as *NOTCH1* mutations (described below), and is the chromosomal aberration most associated with development of Richter's syndrome [67].

The presence of del(11q) is a poor prognostic marker in CLL. The gene loss due to del(11q) is often *ATM* and/or *BIRC3*, which executes tumor suppressing effects as a regulator of *TP53* [68]. In clinical practice, patients with del(11q) often present with marked lymphadenopathy and progressive disease [69].

Finally, del(17p) affecting the tumor suppressor gene *TP53,* is associated with poor prognosis and described further down in the text.

Complex karyotype, as defined by the presence of ≥3 chromosomal aberrations, is not yet recommended as a standard analysis in CLL [14]. However, the presence of ≥5 chromosomal is associated with poor outcome, regardless of other prognostic markers. More data is needed regarding methods for analysis, the definition of complex karyotype, as well as the impact of involved chromosomes [70].

Recurrent gene mutations

NOTCH1 mutations occur in 10% of patients at the time CLL diagnosis and is associated with inferior clinical outcome [71]. The pathogenic effect is believed to be mediated through altered NOTCH1 signaling which under normal conditions affects cellular processes such as differentiation, proliferation and apoptosis [72]. Eighty percent of patients with *NOTCH1* mutations have unmutated IGHV status and an overrepresentation of Richter's transformation has been observed [71]. *NOTCH1* mutations have also been associated with diminished expression of CD20, hence the additive effect of monoclonal anti-

CD20 antibodies may be impaired [73]. Moreover, *NOTCH1* mutations are overrepresented in patients refractory to chemotherapy [74].

SF3B1 mutations are found in around 9% of the patients at time of CLL diagnosis. Unmutated IGHV status are overrepresented as well as del(11q) [75]. As for *NOTCH1* mutations, *SF3B1* mutations are associated with poor prognosis and refractory CLL [74]. The pathogenic mechanism is believed to affect messenger RNA splicing, as *SF3B1* encodes for a critical component of the spliceosome [76].

TP53 mutations occur in approximately 8% of patients at time of CLL diagnosis, more commonly found in U-CLL, and are enriched over time and following treatment [77, 78]. *TP53* is a tumor suppressor gene located on chromosome 17, encoding a nuclear transcription factor with a pro-apoptotic function. In functional cells, the *TP53* gene is activated in the event of DNA damage leading to cell cycle arrest or if needed, induction of apoptosis [79]. *TP53* appear to have equally poor prognosis independently of clone size, i.e. VAF less or more than 10% [78]. Del(17p) is associated with disease progression and resistance to chemotherapy and CIT [59, 80].

BIRC3 mutations have been detected in approximately 3% of newly diagnosed CLL patients [64]. The gene encoding *BIRC3* is located on chromosome 11, hence del(11q) might include *BIRC3,* which is adjacent to the *ATM* gene. Defective *BIRC3* results in non-degradation of NIK (negative regulator of the non-canonical NF-κB pathway, previously described) leading to NF-κB activation [81]. Clinically, patients with *BIRC3* mutations exhibit a negative prognosis with poor response to CIT [81].

NFKBIE mutations have been detected in several studies and appear more frequent in advanced CLL cases. Recently, in a large-scale study, *NFKBIE* mutations were found in 3% of treatment naïve patients [82]. These mutations affect the NF-κB pathway.

MYD88 mutations are found in <5% of CLL patients at time of diagnosis and occur almost exclusively in M-CLL. The effect is mainly NF-κB activation via the TLR pathway [75, 82, 83].

Early growth response 2 (*EGR2)* mutations are unusual at diagnosis and found in only around 2-4% of patients with CLL, the majority being U-CLL [82, 84]. Frequently, these mutations co-occur with other lesions, such as *ATM*, *TP53* and/or *NOTCH1* mutations. Clinically, *EGR2* mutations exhibits an unfavorable prognosis [84].

Impact of gene mutations status in relation to IGHV status

A large-scale multicenter sequencing study including 4580 treatment naïve CLL patients from the European Research Initiative on CLL (ERIC) in Harmony Alliance was recently conducted to evaluate the occurrence and impact of nine recurrently mutated genes in relation to IGHV status. Among the studied genes, only mutations in *SF3B1* and Exportin 1*(XPO1)* were found as independent markers for outcome, defined as time to first treatment (TTFT), in

both U-CLL and M-CLL. Notably, *TP53* aberrations were independently associated with shorter TTFT in U-CLL patients only [82].

Prognostic role of subclones in CLL?

Today, the detection of minor clones, i.e. subclones, is possible due to the rapid development of next generation sequencing (NGS) techniques. Ultra-deep NGS allows detection of clones at >0.1% VAF. However, it is largely unknown if these minor clones are prognostic and/or predictive in CLL, except for *TP53* mutations, which have been associated with poor clinical outcome even when detected in subclonal size, i.e. VAF <10-12% [78].

In a study from 2016 by Nadeu *et al.* with treatment naïve samples from 406 CLL patients, subclonal mutations (defined as VAF 0.3% to <12%) of *NOTCH1* and *TP53* had a negative impact on TTFT and overall survival (OS), respectively. For *SF3B1*, a negative clinical outcome was confirmed for clonal, but not subclonal, mutations [64]. Following the introduction of new targeted therapies, studies of clonal evolution and dynamics in well-characterized patient cohorts with longitudinal sampling and long-term follow-up are warranted.

Increased inflammation and cardiovascular disease in clonal hematopoiesis and hematological disease

It is well-established that chronic inflammation increases the risk of both cancer and CVD [2, 3]. In the process of atherosclerotic plaque formation, inflammation plays a central role, and its pathogenesis has several similarities with tumor development, such as defect endothelial barrier function and extracellular matrix remodeling, as well as leukocyte infiltration into the vessel wall. Cytokines with pro-inflammatory effects and growth factors, such as Interleukin (IL)-1b, IL-8 and its receptor Chemokine receptor (CXCR) 2, TNFα, and Vascular endothelial growth factor (VEGF), which are involved in cancer development, also act in the formation of atherosclerotic plaques [3, 85, 86] which precedes future CVD events, e.g. stroke and myocardial infarction.

Clonal hematopoiesis and CVD

Clonal hematopoiesis of indeterminate potential, i.e. an abnormal clone of hematopoietic stem cells but without signs of a malignant hematological disease, is a common feature with increasing age [87, 88] and has been associated with and increased risk of CVD [87, 89]. In particular, the risk of coronary heart disease has been shown to be increased among patients carrying mutations in *DNMT3A, ASXL1, JAK2* or *TET2,* where at least *TET2* mutations are believed to play a causal role, possibly due to increased levels of the CXC

chemokines in plasma, e.g. IL-8 [7]. Recently, in a study including 581 patients with a primary ischemic stroke, clonal hematopoiesis (CH) defined as VAF ≥1% was detected among 41% of patients [90]. CH was associated with atherosclerosis in large arteries and a pro-inflammatory profile, e.g. elevated high sensitive C-reactive protein (CRP) and IL-6, in patients with stroke. Larger CH clones, were associated with frequent vascular events and increased all-cause mortality.

CLL and CVD?

It is currently unknown whether clonal conditions of mature B-cells, i.e. MBL or CLL, are associated with the progression of atherosclerosis and CVD. In 2019, Strongman *et al.* found an increase in CVD among patients suffering from cancer, including hematological diseases and CLL/SLL [91]. In addition, a large-scale retrospective study reported an increased frequency of cardiovascular death in patients with non-Hodgkin lymphoma, including CLL/SLL, compared to data on general population in the United States of America [92].

Hyperlipidemia is a well-known risk factor of CVD [93]. Elevated levels of blood lipids have been found in a study analyzing pretreatment samples from 231 CLL patients [94]. Time to primary treatment initiation was longer among patients on statins compared to those not on these drugs, suggesting a role for blood lipids in CLL progression. In line with this, a population-based case-control study of CLL patients found a higher prevalence of dyslipidemia prior to CLL diagnosis, and CLL patients with prescribed drugs decreasing blood lipids appeared to have superior survival [95]. *In vitro,* CLL cells, in contrast to normal B-cells, express Lipoprotein lipase (LPL) on the cell surface, and this expression is associated with increased metabolization of free fatty acids [96]. Furthermore, expression of LPL has been associated with worse clinical outcome [97].

Inflammatory activation in CLL

As described above, CLL cells depend upon their BcR for continuous signaling as well as the cytokine receptors, TLR and CD40. Activation of these receptors, mediated through downstream signaling pathways, leads to activation of NF-κB and Signal transducer and activator of transcription 3 (STAT3), which in turn activates transcription of genes with pro-inflammatory effects such as IL-6 and IL-1 [4, 29]. NF-κB activation is a mediator in inflammation [54] as well as in metabolic disease [98].

TNFα is known to promote inflammation through its receptors TNFR1 and TNFR2, expressed in both plasma membrane bound and soluble (s) forms. Via TNFR2, it can induce NF-κB activation [99]. In 1992, Waage *et al*. reported increased expression of both TNFR1 and TNFR2 on CLL cells and elevated levels of the soluble receptors in serum [100]. More recently, in a German

study of 247 CLL patients, elevated blood levels of sTNFR1 were found, compared to controls [101]. Cardiovascular disease has been associated with increased levels of sTNFR1 and sTNFR2 in a previous Swedish case control study where the investigators reported high levels of sTNFR1 and sTNFR2, which in combination with elevated CRP correlated with an increased risk of myocardial infarction (especially in women) [102].

Beta-2-microglobulin (B2M), is a well-established prognostic marker in CLL and is associated with high tumor burden and poor prognosis [103, 104]. B2M has also been associated with high levels of pro-inflammatory cytokines, such as IL-6, IL-8 and TNFα [105] and with severity in coronary heart disease [106].

To summarize, inflammation may contribute to the development of CLL [4]. A possible association between chronic inflammation and development of CVD in patients with CLL warrants further studies.

Prognostic factors used in clinical routine

Three major discoveries have influenced the current management of CLL in clinical practice; the IGHV status, chromosomal aberrations, including del(17p) detected with FISH [61], and mutations in the *TP53* gene detected with sequencing [77]. Below is a brief summary of current markers in clinical routine.

Beta-2-microglobulin

B2M is a low-weight protein with sequence homology to immunoglobulins. *In vitro* data suggest that CLL cells are able to produce B2M [107]. B2M has been found to correlate with disease stage and prognosis in several studies, thus being an independent prognostic marker in CLL [103]. B2M is also included in the widely used clinical staging system CLL-IPI [108].

IGHV status

IGHV status is a well-established prognostic marker as previously described above [31, 32]. Currently, it is also a predictive marker in CLL therapy. Studies using modern targeted therapies such as BTKi and BCL2 inhibitors have shown these to be more efficient than CIT in patients with U-CLL. Therefore, analysis of the IGHV status is today recommended before initiating therapy. Since the mutational status is stable over time, it only needs to be investigated once [14].

FISH analysis and gene sequencing

Before start of therapy FISH analysis for del(13q), trisomy 12, del(11q) and del(17p) is recommended. In 2010, Zenz *et al.* demonstrated that *TP53* mutations detected with Sanger sequencing (cut-off VAF 10%) had similar inferior prognosis as del(17p) [77], thus sequencing of the *TP53* gene is recommended in absence of del(17p) when analyzed by FISH. In clinical practice, *TP53* is today the only gene recommended for sequencing analysis prior to initiating CLL therapy, including upfront to all following lines [14].

Clinical staging systems

Before the introduction of current/modern laboratory methods for analyzing chromosomal and molecular genetic aberrations, clinical staging systems, mainly the Binet and Rai staging systems (Table 1), have been used to assess the prognosis of CLL. Both of these systems include the hemoglobin and platelet counts, as well as the presence of enlarged LNs, spleen and liver assessed by palpation. The Rai system was originally separating patients into five different risk groups but has been modified and reduced to three groups, in similarity to the Binet staging. In brief, Binet A / Rai 0-I defines early-stage disease whereas Binet C / Rai III+IV defines advanced disease, reflected in shorter median survival [109, 110]. These two systems were published in 1981 and 1975 respectively, i.e. in a treatment era with mainly chemotherapy, and are therefore less relevant today.

Table 1. Staging system in CLL, Binet and Rai.

Clinical staging systems	Risk	Parameters	Survival (years)
Binet stages			
A	Low	Hb and platelets >100, lymphocytosis, <3 areas* of enlarged lymph nodes	>10
B	Intermediate	Hb and platelets >100 lymphocytosis ≥3 areas of enlarged lymph nodes	~7
C	High	Hb +/- platelets <100	<4
Rai stages			
0	Low	Lymphocytosis	>10
I +II	Intermediate	Lymphocytosis + lymphadenopathy +/- spleen +/- liver enlargement	~7
III+IV	High	Hb <110 +/- platelets <100	<4

*Areas defined as palpatory enlargement in 1. Head and neck, 2. Axillar, 3. Inguinal, 4. Spleen, 5 Liver.

Several attempts have been made to modify and improve staging at diagnosis. In 2016 CLL-IPI (international prognostication index) was presented (Table 2). This staging system combines the two previous staging systems with IGHV status, B2M, genetics and age, resulting in five different prognostic groups [108].

Table 2. Staging by CLL-IPI score

CLL-IPI score		
Prognostic factor	**Points**	
Del(17p) +/- TP53 mutation	4	
Unmutated IGHV genes	2	
Beta-2-microglobulin >3.5mg/L	2	
Rai stage I-IV	1	
Age >65 years	1	
Cumulative CLL-IPI score	**Risk category**	**5-year TFS***
0-1	Low	78%
2-3	Intermediate	54%
4-6	High	32%
7-10	Very high	0%

*TFS: Treatment free survival

Diagnostic work-up before start of therapy

In addition to recommendations already mentioned, a bone marrow sample and computer tomography of thorax and abdomen should be obtained to evaluate tumor burden and later on therapy response before start of therapy. If transformation to high malignant lymphoma is suspected, a biopsy from suspected tissue, ideally identified by positron emission tomography scan must be obtained [14].

Treatment

In CLL treatment, as well as for other malignancies, chemotherapy has previously been the main choice of therapy. The introduction of monoclonal antibodies during the late 1990s founded a new entity of regimen, CIT. During the 2010s, targeted therapy has changed the paradigms of CLL therapy.

So far, it has not been shown that patients with CLL can be cured, with the exception of those undergoing allogenic hematopoetic stem cell transplantation (allo-HSCT). Although only a minority, approximately 15% according to a Swedish register report [1], of all CLL patients require therapy at the time of diagnosis, the majority will over time need treatment. Currently, there is no evidence that treatment should be initiated before symptoms or signs of bone marrow suppression, i.e. cytopenias, are present (Table 3). However, most trials investigating the role of therapy in early-stage CLL, are from the "chemotherapy era" [111, 112]. Present recommendations from iwCLL regarding initiation of therapy are shown in Table 3 [14].

Table 3. Indications for start of therapy in CLL.

Clinical indications for treatment initiation
Bone marrow failure with anemia and thrombocytopenia, i.e. Hb <100 mg/L or platelet counts $<100\times10^9$/L.
Splenomegaly* and/or massive LN**
Progressive lymphocytosis or lymphocyte doubling time < 6 months
Steroid refractory autoimmune manifestations of anemia or thrombocytopenia.
Symptomatic or functional extra nodal involvement.
Disease related symptoms: a. Weight loss ≥10% within the previous 6 months. b. Significant fatigue, ECOG-WHO ≥ 2 c. Fevers ≥ 38.0°C and/or night sweats without evidence of infection.

*Spleen ≥6 cm below the left costal margin ** Lymph nodes (LN) with size ≥10 cm

Chemoimmunotherapy

Rituximab, a CD20 monoclonal antibody, was approved by Food and Drug Administration (FDA) in 1997 for treatment of patients with CLL [113]. In the CLL8 trial, the efficacy of fludarabine (a purine analogue)+cyclophosphamide (an alkylating agent)+rituximab (FCR) was compared to fludarabine+cyclophosphamide (FC). The progression-free survival (PFS) was significantly prolonged with FCR; 56.8 months, compared to 32.9 months in the FC arm. At time of publication, median OS was not yet reached for FCR, but 86 months for FC. Long term follow-up data have confirmed long-term remissions, especially evident in M-CLL patients [114].

Following CLL8, the CLL10 trial compared rituximab+bendamustine (BR) with FCR among patients 65 years or younger and in patients older than 65 years. This trial showed overall response rate (ORR) >90% in both arms and age groups. However, PFS for patients ≤65 years receiving BR was 38.5 compared to 53.6 months in the FCR arm. For patients >65 years, BR was shown non-inferior to FCR, probably due to a higher frequency of infectious complications for FCR outweighing its higher efficacy, BR was recommended in elderly patients [115].

CIT, i.e. FCR for patients <65-70 years and BR for patients >65 years, is currently recommended as an alternative for first-line therapy in patients with good markers of prognosis, i.e. mutated IGHV genes, and the absence of del(17p) and /or TP53 mutation, according to Swedish and iwCLL guidelines [14]. However, these recommendations might be revised soon, following the results from the GAIA/CLL13 trial comparing CIT to time-limited, venetoclax-based regimens [116].

Anti-CD20 monoclonal antibodies

CD20 is cell membrane bound molecule expressed on B-cells during the maturation process, but not expressed on early progenitor B-cells or plasma cells. Its function in CLL is not entirely clear [117].

Rituximab is a type I CD20-antibody which binds to and organizes CD20 expressed on B-cells, leading to complement activation through the classical pathway. Additionally, rituximab triggers antibody-dependent T-cell mediated cytotoxicity, resulting in B-cell depletion [118].

In recent years, the second generation CD20-antibody obinutuzumab has been approved. It is a weaker activator of the complement system compared to rituximab. Classified as a type II CD20-antibody it induces direct cell death upon binding to CD20. Its potent and direct effects are reflected by the risk of tumor lysis syndrome (TLS) in patients with a high tumor burden. Obinutuzumab was approved after the publication of the CLL11 trial comparing obinutuzumab+chlorambucil (an alkylating agent), rituximab+chlorambucil and chlorambucil alone, in an elderly and comorbid patient population

[119, 120]. The results were in favor of obinutuzumab+chlorambucil over rituximab+chlorambucil and chlorambucil alone with PFS of 26.7, 16.3, and 11.1 months respectively. OS was significantly prolonged for obinutuzumab+chlorambucil and rituximab+chlorambucil vs chlorambucil alone. No significant difference in OS was seen comparing the obinutuzumab+chlorambucil and rituximab+chlorambucil arms [119, 120]. Thus, the CLL11 study showed an advantage of adding a CD20-antibody to chlorambucil.

BTKi therapy in CLL

In 2014, both FDA and European Medicines Agency (EMA) approved the use of ibrutinib, the first-generation of drugs inhibiting the BTK, for previously treated CLL patients. Ibrutinib is a covalent inhibitor of BTK with short half-time elimination in blood.

Activation of BcR leads to downstream activation of tyrosine kinases, among these BTK and Phosphoinositol-3-kinases (PI3Ks), which in turn activates pathways such as Extracellular signal-regulated kinase (ERK) 1 and 2 and NF-κB [29]. As previously described, inhibiting BTK leads to reduced survival and proliferation signals within the CLL cell. BTK inhibition also leads to mobilization of CLL-cells from LNs and bone marrow into the blood stream, rapidly resulting in lymphocytosis and shrinking of the LNs [29, 48, 49]. However, BTK is also expressed in cells in other organs, such as heart, leading to off-target effects and adverse events (AE) [121].

In the RESONATE trial, ibrutinib was compared to ofatumumab (a type I monoclonal CD20-antibody) in 391 patients with relapse refractory (RR) CLL/SLL of which 57% of the patients had either del(17p) or del(11q). At 12 months, the ORR was significantly higher for patients treated with ibrutinib [122]. The latest update from this study published in 2019, confirmed superiority for ibrutinib after 6 years follow-up with PFS 44.1 vs 8.1 months. Ibrutinib seemed to overcome the negative prognostic effect of genetic high-risk features such as del(17p), *TP53* mutation, del(11q) and/or unmutated IGHV genes [123]. Despite this being a RR CLL cohort, the comparator arm comprised of single ofatumumab, i.e. relatively weak.

The RESONATE-2 trial compared ibrutinib with chlorambucil in 269 treatment naïve patients 65 years or older. The latest follow-up, after 7 years, demonstrated PFS 59% for ibrutinib vs 9% for chlorambucil with 78% OS for ibrutinib. Notably, 24% (n=32) of the patients have discontinued ibrutinib due to AEs, such as AF, and 23% (n=31) had dose reductions due to AEs, after which 90% of the AEs resolved [124].

Following these trials in RR CLL and elderly CLL patients, ibrutinib was studied in the front-line setting vs the established CIT regimens, FCR and BR. The E1912 trial compared ibrutinib+rituximab vs FCR for patients <70 years of age and demonstrated prolonged PFS and OS for the BTKi arm. In a

subgroup analysis of patients with U-CLL, ibrutinib resulted in a superior ORR compared to FCR: 90.7% vs 62.5% at 3 years. Notably, no significant difference was seen between ibrutinib + rituximab and FCR in patients with M-CLL [125]. Thus, FCR remains as an option in these patients.

The ALIANCE trial included 578 patients ≥65 years of age, and, in addition to testing the efficacy compared to BR, it also tried to answer the question whether the addition of rituximab to ibrutinib enhances the effect of treatment. Patients were randomized to ibrutinib monotherapy, ibrutinib+rituximab and BR. PFS at 2 years was 87%, 88% and 74%, for each treatment arm respectively. No significant difference between ibrutinib+rituximab and ibrutinib monotherapy was seen regarding PFS [126].

In the ILLUMINATE trial, elderly, fragile patients treated with obinutuzumab+ibrutinib had superior PFS compared to obinutuzumab+chlorambucil: PFS was 19 months for obinutuzumab+chlorambucil, whereas not reached for obinutuzumab+ibrutinib [127]. The latest published report, at 45 months median follow-up time, PFS was 22 months vs still not reached for obinutuzumab+ibrutinib [128].

In summary, ibrutinib has demonstrated superior or equal efficacy compared to the established CIT regimens in both younger, fit patients and in older, fragile patients. Importantly, ibrutinib is efficient in patients with high-risk genetic markers; unmutated IGHV genes and/or del(17p) and/or *TP53* mutation, confirmed by these trials.

Next generation BTKi

Several new covalent BTKis with higher specificity for BTK as well as non-convalent inhibitors of BTK are currently in trials. The next generation covalent BTKis, acalabrutinib and zanubrutinib, have a higher specificity for BTK than ibrutinib, i.e. fewer inhibiting effects on other tyrosine kinases with less off-target effects. Both these new BTK inhibitors bind to the same epitope on BTK as ibrutinib, namely C481.

ELEVATE-RR, a phase III trial, comparing acalabrutinib to ibrutinib in relapsed CLL (median follow-up 40.9 months) showed non-inferiority with a median PFS of 38.4 months for both acalabrutinib and ibrutinib, OS not yet reached. The non-inferiority of acalabrutinib was consistent in all high-risk groups. The cardiovascular side effects, especially AF and hypertension appeared significantly lower among patients receiving acalabrutinib vs ibrutinib [129].

The phase III ALPINE trial compared zanubrutinib (n=207) to ibrutinib (n=208) in RR CLL. ORR at 15 months was significantly superior for zanubrutinib vs ibrutinib (78.3% vs 69.1%). The incidence of cardiovascular AEs such as AF was 2.5% in the zanubrutinib arm vs 10.1% in the ibrutinib arm. Of note, the ALPINE trial was open-labeled, with risk of confounding the results, as the proportion of discontinuation was higher in the ibrutinib arm (n=50 in the ibrutinib arm compared to n=23 in the zanubrutinib arm) [130].

The non-covalent (i.e. reversible) BTKis, have shown effect in patients progressing on BTKi including those with *C481S* or Phospholipase C gamma-2 *(PLCγ2)* mutations [131]. As they are not dependent of the C481S binding site, they provide a treatment option in patients resistant to acalabrutinib or ibrutinib. Non-covalent BTKis includes the drugs vecabrutinib (SNS-062) [132], pirtobrutinib (LOXO-305) and nemtabrutinib (MK-1026). Pirtobrutinib has shown promising results in the phase I/II BRUIN trial of R/R B-cell malignancies, including SLL/CLL patients (n=170). Pirtobrutinib appears to have a low risk of cardiovascular AEs with n=2/323 patients developing AF and 5% developing hypertension. Fifteen patients had previously stopped treatment with another BTKi due do cardiotoxicity, but none of these 15 patients developed a new CVD while on pirtobrutinib [133]. Phase III trials with pirtobrutinib in CLL are ongoing. Nemtabrutinib is also undergoing clinical trials. In a phase II trial including n=68 patients with CLL/SLL, median of four prior lines of therapy (including 84% with prior BTKi and 63% with C481S mutation), 57.9% demonstrated ORR at follow-up, median time 4.6 months [134]. Next generation BTKis will hopefully play an important role in future CLL therapy.

Resistance to BTKi

Progressive disease during ongoing BTKi therapy is not an uncommon clinical event. Most often mutations are found in the BTK site C481, or downstream in *PLCγ2*, eliminating the inhibitory effect of BTKi. Ibrutinib, acalabrutinib and zanubrutinib are all covalent inhibitors to the BTK. The C481S mutation in BTK prevents ibrutinib from binding to the BTK in an effective manner, resulting in reduced or loss of response. A meta-analysis of four clinical trials evaluating the relapsed patients treated with ibrutinib regimens demonstrated that 85% of the relapsed patients at a median follow-up of 3.4 years had a mutation in either *BTK* C481 or *PLCγ2* [135]. Mutations in *PLCγ2*, occur most often in the sites R665W, L845 and S707Y [135].

A French study investigated mutations in *BTK* and PLC*γ2* among CLL patients treated continuously with ibrutinib for a minimum of three years. *BTK* or *PLCγ2* mutations occurred in 57% and 13% of the patient samples, respectively and were associated with disease progression. Notably, these mutations were detectable by NGS analysis 8.5 months (median time) before progression was clinically evident [136]. However, in the case of Richter's transformation during BTKi therapy, mutations in *BTK* or *PLCγ2* are uncommon events [137].

The non-covalent BTKis are promising in this setting, but resistance to these have also been described. Resistance to pirtobrutinib has been investigated in a study by Wang *et al.* from 2022, investigating CLL patients included in the BRUIN I/II trial. Nine patients progressing on pirtobrutinib were selected for sequencing analysis. Gained mutations (during pirtobrutinib

therapy) in BTK domains V416L, A428D, M437R, T474I and L528W were found (none of these mutations are located at the C481 site) [138].

Today, in the event of progression on BTKi in patients harboring del(11q), del(17p) and/or *TP53* mutations and/or unmutated IGHV genes, the recommendation is to change to another targeted therapy, i.e. BCL2 inhibitor, as described below.

BTKi and adverse events, with focus on CVD

Atrial fibrillation and ventricular arrhythmias

The mechanism by which BTKis increases the risk of AF is not completely understood, but off-target inhibition of cardiac tyrosine kinases has been proposed [121, 139]. A mouse model suggests that inhibition of C-terminal Src (proto-oncogene c-Src) kinase by ibrutinib might be responsible; mice treated with ibrutinib exhibited enlargement of left atrium, inflammation and myocardial fibrosis. Mice treated with acalabrutinib did not develop these symptoms, whereas mice deficient of BTK did [140].

Several studies have identified prior history of CVD, e.g. hypertension, to be a risk factor for new cardiovascular events, such as developing AF during ibrutinib treatment [8, 10, 141]. In a prospective study of 43 CLL patients initiating ibrutinib, 16.3% (n=7) developed AF at a median observation time of eight months. The risk of AF was significantly correlated to previous history of CVD, especially hypertension, and a high atrial diameter assessed by echocardiography [10].

A recent real-world study, compared the risk for new CVDs following therapy with single-agent ibrutinib, intense CIT and non-intense (e.g. monotherapy anti-CD20 antibody+/-chlorambucil) therapy. In conclusion, patients on ibrutinib exhibited an increased risk för new CVD events including AF and hypertension compared to the other arms [142].

An analysis estimating the "AE burden" in the ALLIANCE trial demonstrated a worse AE burden in the BR arm during the first 6 months, compared to ibrutinib. In total, 10% in the BR arm vs 14% in the ibrutinib arm discontinuing therapy due to AEs. Within the ibrutinib arms, the cumulative incidence of grade three (or higher) AF at 36 month was 7.7% [143].

In a meta-analysis of four randomized controlled trials containing ibrutinib and a comparator arm, median time of AF onset following ibrutinib initiation was 2.8 months. All-grade AF was reported in 6% of patients on ibrutinib therapy compared to 2% in the comparator arm. The median treatment time was 13.3 for ibrutinib and 5.8 months for comparators [144]. Two meta-analyzes have shown that for the majority of patients developing AF during ibrutinib therapy, AF seldom leads to discontinuation or dose reductions of BTKi [9, 144].

The incidence of AF (and flutter) during acalabrutinib therapy has been estimated to 5% (n=38) in a pooled analysis of trials with single-agent

acalabrutinib (n=762), with a median time of 17.1 months until onset [145]. In the ELEVATE-TN trial, comparing acalabrutinib+/-obinutuzumab to obinutuzumab+chlorambucil, the incidence of all-grade AF was 3% to 4% in the BTKi arms compared to 1% in the arm without BTKi [146]. *In vivo* studies have not shown evidence for inhibition of C-terminal Src, as for ibrutinib [121], and the mechanisms by which acalabrutinib increases the risk of AF needs further investigation.

Zanubrutinib has so far demonstrated promising data on AF with an incidence of 2% in the phase III ALPINE TN study for CLL/SLL patients described above [130].

Data on AF and the non-covalent BTKi pirtobrutinib is promising following the phase I/II BRUIN study with only 2 out of 323 patients developing AF (not new-onset) [133].

Data on "baseline AF" among real-world CLL patients is scare. A study from the Mayo clinic reported that 6.1% of the CLL patients had a history of AF at time of CLL diagnosis [147]. According to a Swedish register report on AF in the general population, the expected prevalence is 9.7% among individuals 70-80 years old [148]. Considering that the majority of CLL patients initiating therapy are older than 70 years, e.g. 75 years in the Swedish CLL register [1], this is of concern since the use of BTKi increases.

There have been reports of ventricular arrhythmias (VA) and cardiac arrest in patients treated with ibrutinib [149-151]. In one retrospective study, including 582 patients initiated on ibrutinib, the incidence of VAs was increased among ibrutinib patients with a median time to VA of 16 months [149]. The mechanisms behind ibrutinib related VAs are not completely understood but may affect dysregulation of calcium homeostasis in myocardial cells. An *in vivo* study demonstrated that older rats with hypertension were more prone to develop VA following ibrutinib therapy, compared to younger rats (with hypertension) [152]. Recently, reports on VAs and sudden death during acalabrutinib have emerged [153] implying that this might represent a class effect.

Hypertension

New onset or worsening of hypertension is associated with ibrutinib therapy, with a median time of onset of 4-5 months after ibrutinib initiation [151]. The incidence of new all-grade hypertension has been reported in up to 20-30% of all patients on ibrutinib therapy and high-grade hypertension (>160/110 mmHg) in around 5% [139, 154]. The incidence of developing hypertension continues over time [151]. In a study including 562 ibrutinib treated patients, 78% developed new or worsened hypertension during median 30 months follow-up. Furthermore, hypertension was associated with an increased risk of major adverse cardiac events, which decreased if the patients were treated with antihypertensive therapy [141]. Thus, treatment according to guidelines for

blood pressure may be particularly important in patients receiving BTKis [139, 154].

In the pooled analysis of 762 acalabrutinib treated patients, 9% (n=67) exhibited a hypertensive event. Out of these 9%, 64% (n=43) had a previous diagnosis of hypertension. The median time to onset of hypertension (a new event) was 6.5 month from start of therapy. The majority of patients (n=43) had known hypertension. Thirty patients experienced hypertension of grade ≥3 [145].

In the recent phase III ALPINE trial 21.9% were diagnosed with hypertension of any grade in the zanubrutinib arm, compared to 19.8% in the ibrutinib arm, with grade ≥3 14.8% and 11.1% [130], respectively.

The BRUIN phase I/II trial on pirtobrutinib reported a frequency of 5% (n=15) all grade hypertension in the entire study cohort [133].

The mechanisms by which the covalent BTKis increases blood pressure is unknown, but data suggest it to be a class effect. Further data is warranted on non-covalent BTKis to determine their CVD side effects.

Hemorrhages

Hemorrhage is a frequently reported AEs in ibrutinib trials. In a pooled analysis (15 trials with ibrutinib and four randomized controlled trials) of n=1768 ibrutinib treated patients with B-cells malignancies, an incidence of all-grade hemorrhages of approximately 40% and major hemorrhagic events of 4% was reported. Low-grade hemorrhages did not appear to increase the risk of major hemorrhages. Approximately 50% of the patients in the study were also treated with anticoagulants and/or antiplatelet drugs. No significant difference in major hemorrhages between ibrutinib and the comparator arms was seen (after adjustment for a prolonged time of exposure to ibrutinib vs comparator drugs) [155].

A retrospective real-world study of 70 patients treated with ibrutinib found grade 1-2 bleedings in 56% of the patients and 19% with major bleeding. Out of these 19%, 70% were treated with antiplatelet drugs, 17% with anticoagulants and 13% on both antiplatelet and anticoagulants, suggesting that simultaneous ibrutinib and anticoagulant therapy might lead to complications when applied in patients outside of clinical trials, who often suffer from comorbidity and multi-pharmacy [156].

The mechanisms of increased bleeding tendency among patients on ibrutinib is thought to be mediated by inhibition of BTK and Tec kinases in platelets, affecting downstream signaling via glycoprotein VI (a collagen receptor), which is important in platelet aggregation. *In vitro* studies of blood from ibrutinib treated patients demonstrated impaired platelet adhesion on von Willebrand factor and reduced collagen-mediated platelet aggregation. This impaired platelet aggregation correlated with bleeding events and was reversed if ibrutinib was stopped [157]. Due to observed platelet aggregation inhibition,

patients are advised to withhold ibrutinib three days before minor surgery and seven days before major surgery [139].

Acalabrutinib may theoretically have less platelet inhibition effect, due to another profile of kinase inhibition [158]. In the ELEVATE RR, comparing ibrutinib with acalabrutinib in RR CLL, patients in the acalabrutinib arm had a lower frequency of minor bleedings, however major bleedings were similar [129]. The ALPINE trial demonstrated no difference regarding bleedings between the zanubrutinib and ibrutinib arm [130]. Data on Pirtobrutinib in the BRUIN phase I/II trial, demonstrated 5% (n=15) all-grade hemorrhages [133].

Due to the potent platelet inhibition by ibrutinib and in some part also acalabrutinib and zanubrutinib *in vitro* [121], the concomitant use of anticoagulants, which is indicated for patients with AF according to stroke risk as assessed by CHA_2DS_2VASc [159], has clinical implications.

PI3K inhibitors

Similar to BTKi, PI3K inhibitors, inhibit a tyrosine kinase downstream from BcR, the PI3K, resulting in reduced signaling through the BcR. The first drug in this class, idelalisib, was approved in 2014, following trials demonstrating efficacy in combination with anti-CD20 antibodies, in patients with del(17p) and/or *TP53* mutation [160, 161].

Idelalisib inhibits a delta isoform of the catalytic subunit of PI3K, which is mainly expressed in leukocytes. Several serious autoimmune side effects, especially in treatment naïve patients, such as diarrhea and/or colitis, neutropenia and elevation of liver transaminases, have been observed [160, 161]. Due to toxicity concerns, PI3K inhibitors are recommended only as a late-stage alternative in case of RR CLL.

BCL2 inhibitors

BCL2 is an antiapoptotic, mitochondrial protein which is expressed by CLL cells, as well as in other hematopoietic cells [162]. Venetoclax, a BCL2 inhibitor, treatment is associated with a substantial proportion of patients reaching deep remissions and a high number with undetectable measurable residual disease (uMRD). Common side effects are cytopenia and infections. Due to its high efficacy within hours of administration, a ramp-up period and thorough prophylaxis of TLS is recommended at treatment initiation [163].

In 2016, venetoclax was approved by EMA as the first BCL2 inhibitor for treatment of CLL patients with del(17p) or *TP53* mutations with RR disease, or not suitable for BTKi therapy due to intolerability or resistance.

In the MURANO trial, comparing rituximab+venetoclax (RV) with BR in 389 RR CLL patients, ORR was 93.3% vs 67.7% in the BR arm. PFS was not reached in the RV arm, compared to 17 months in the BR arm [164].

In first-line treatment for CLL, GAIA/CLL13, a 4-armed phase III trial (n=926) compared time-limited treatment with rituximab+venetoclax (RV), obinutuzimab+venetoclax (GV), obinutuzumab+ibrutinib+venetoclax (GIV) and FCR/BR. Patients with *TP53* defects were excluded, while 56% of the enrolled patients had U-CLL. First co-primary endpoint regarding uMRD demonstrated superiority of GIV and GV vs FCR/BR (86% vs 52%, respectively) [116]. Data on 3-years PFS (the second co-primary endpoint) demonstrated 80.8% for RV, 87.7% for GV, 90.5% for GIV and 75.5 for FCR/BR. Both GIV and GV were superior to FCR/BR, but not RV, regarding PFS [165].

As for other targeted therapies, resistance to venetoclax can develop during therapy e.g. by *BCL2* mutations, by which the affinity of venetoclax to BCL2 is diminished [166, 167]. The mechanisms by which BCL2 resistance develops are numerous and diverse [168], among these are *BCL2* mutations, upregulation of Myeloid cell leukemia 1 and epigenetic alterations. After venetoclax discontinuation, these epigenetic alterations disappear [169].

Allo-HSCT in CLL

Allogenic hematopoietic stem cell transplantation is currently considered as the only curative therapy for CLL. However, allo-HSCT is associated severe side effects, including transplantation related mortality and chronic graft vs host disease. Due to this toxicity profile as well as the age distribution of CLL patients, allo-HSCT has a limited role in CLL. In addition, due to the emerging, targeted therapies introduced since 2014, the need for allo-HSCT in CLL patients has decreased. It remains as a treatment option in younger, fit patients failing or not tolerating targeted therapies.

The CLL3X trial investigated 100 CLL patients between 2001-2007 of which 90 patients ultimately underwent transplantation. Thirty-nine out of 90 patients suffered CLL relapse during follow-up (at 6-year). Notably, uMRD at 1-year reduces the risk of future CLL recurrence [170].

A European register report, evaluating long-term survival of CLL patients (n=2589) following allo-HSCT, found a 5-year's event-free survival of 28%, OS of 35% and a non-relapse mortality of 40%. Importantly, almost 80% of the patients with event free survival at 5 years, were still alive and event-free at 10 years [171]. In conclusion, for younger, fit patients who cannot tolerate or respond to CIT and/or new, targeted therapies, allo-HSCT may be considered and weighed against the risk of severe chronic graft vs host disease, which in several trials has proven to be substantial (approximately 50%) [172].

CAR-T in CLL

Chimeric antigen receptor T-cell (CAR-T) is a therapy with promising results in various of hematological malignancies, such as myeloma [173], mantle cell lymphoma [174] and large B-cell lymphoma [175]. Results in CLL have so

far been less promising with varying rates of CR [176], partly due to T cell exhaustion [177]. Trials are ongoing for CLL patients, where CAR-T is currently is combined with BTKi due to preclinical effects increasing the antitumor effect [178]. In a study with heavily pretreated patients the ORR at 1 years was 59% [179].

How to treat CLL in 2023?

During the last decade the first-line therapy for CLL has been rapidly shifting from CIT to the use of BTKi or BCL2 inhibitor as single therapy or in combination with CD20-antibodies in patients with U-CLL and/or *TP53* aberrations. Due to the long-term remission on M-CLL +/- del(13q) reported in the CLL8 trial, CIT is still an option for these patients. However, this might change with the arrival of new data from trials such as the GAIA/CLL13, a trial that preliminary demonstrates overall deep responses for venetoclax in combinations with obinutuzumab. Notably, the combination RV was not superior to FCR/BR. Moreover, chemotherapy increases the risk of secondary malignancies. On the other hand, these new targeted of therapies have other side effects, including CVD and TLS risk that should be considered.

I the diagnostic work-up including FISH, IGHV status and NGS for *TP53*, the patients comorbidity should be considered in treatment decisions. Patients with significantly reduced renal function, multi-pharmacy and/or CVD will still be a challenge to handle. Due to the age distribution in CLL, suitable therapy for the very old and fragile patients remains problematic.

Population-based studies in CLL

CLL patients in clinical routine are often elderly and suffer from comorbidity. A Danish register study demonstrated that multi-comorbid patients have an inferior outcome in CLL and are more often left untreated than those without comorbidities [180]. A retrospective study from the German CLL group, analyzing data from the CLL4 and CLL5 trials with a median age of 70 years, demonstrated that patients with >2 comorbidities exhibited a higher risk of CLL death. Moreover, they had an increased risk for dose reduction, with a subsequently poorer disease control and risk of death due to progressive disease [181].

The new targeted therapies have demonstrated promising efficacy in elderly patients, but comorbidity appears equally important to assess from current data. In a retrospective multicenter analysis of 145 CLL patients on ibrutinib, the 2-year event free survival and OS were inferior for patients with substantial comorbidity. They also were at higher risk for ibrutinib discontinuation or dose reduction [182]. Another study with 546 CLL patients, assessed that 41% were off ibrutinib at 17 months (median follow-up time), the

majority due to toxicities (e.g. AF, arthralgia, rash and infections) [183]. Thus, data on comorbidity is important to address, with the purpose to optimize CLL (and CVD) therapy for CLL patients.

In this thesis, data from several Swedish nationwide, population-based health care registers have been used to assess CVD comorbidity. Reporting to these registers is mandatory by law and made possible by the personal identity number (PIN), consisting of 10 digits and assigned to all Swedish citizens since 1947. In Sweden, population statistics has a long tradition dating back to the 16th and 17th century in the local church parishes [184]. Today, the main population registers are The Population Register (in Swedish: Folkbokförings-registret), managed by the Swedish Tax Agency (Skatteverket) and the Total Population Register (Registret över totalbefolkningen) managed by Statistics Sweden (Statistiska centralbyrån), recording data such as birth, death, marital status, level of education, migration (i.e. imigration and/or emigration), all linked to PIN. The records reach almost 100% coverage on birth and death, and 90-95% on migration and emigration [185].

Another factor facilitating register research is the use of a coded system for diagnoses, i.e. the International Statistical Classifications of Diseases and Related Health Problems (ICD), based on WHO definitions [186]. Since 1997, the 10th version, i.e. ICD-10 system with a Swedish adaption (ICD-10-SE), is used in clinical routine.

The Swedish Cancer Register

The Swedish cancer register (SCR) was started in 1958. The register is nationwide and it is mandatory by law to report all diagnosed cancers by clinical, morphological or by other laboratory/pathological methods or by autopsy. The register contains information on clinical as well as tumor specific data but no information on treatments. The latest validation of the register was published in 2009 and evaluated the year 1998. In the study the underreporting of cancer diagnoses to the SCR (when comparing to the Patient register) was estimated to only 3.7%. Notably, underreporting was especially high for hematological (leukemia and lymphoma) diagnoses. Possible causes were lack of a pathological diagnosis and confusion on which clinical institution should report the cancer diagnosis. Finally, underreporting was higher for elderly patients [187].

The Swedish CLL Register

The Swedish CLL register is a nationwide quality register and records data on all Swedish patients diagnosed with CLL. The register was founded in 2007 and holds clinically relevant information; i.e. clinical staging at diagnosis, various clinical parameters, need and choice of therapy and response to therapy. For inclusion, the diagnostic criteria described by iwCLL must be met [14].

SLL is registered in the Swedish lymphoma register which also provides long-term data on survival and therapy.

At diagnosis, the responsible physician reports electronically to the register database. The respective Regional cancer centras, RCC, are responsible for data base management and basic controls of reported data. Reports from the CLL register are published regularly and are available on the website (https://cancercentrum.se).

The CLL register was validated in 2015, with a completeness of 99% compared to the SCR. However, delayed reporting remains a problem [1].

The National Patient Register

This register holds information about all diagnoses, using ICD-coding, in specialized health care and is divided in an inpatient [188] and outpatient part.

The Inpatient register (IPR) (in Swedish: slutenvårdsregistret) commenced in 1964 and became nationwide in 1987, including psychiatric diagnoses. The IPR holds information on all diagnoses (main and secondary diagnoses) from hospital discharges. The register has almost 100% coverage. An external review with the purpose to validate the IPR by Ludvigsson *et al.* in 2011, concluded that 85-95% of the registered diagnoses in the IPR are correct, however the sensitivity has a wide range depending on the diagnosis, e.g. high sensitivity for myocardial infarction (91.5%) vs low sensitivity for hypertension and hyperlipidemias, with only 13.7% and 8.8% respectively, identified in the IPR, when comparing to records in primary health care. A tendency for less accuracy was seen in elderly patients and those with numerous non-related diagnoses [188].

The outpatient register commenced in 1997 with reporting on minor surgery and from 2001, all outpatient specialist care is reported to this register. The coverage has been reported around 80%, explained partly by the fact that private caregivers are not obliged to report to the register, for some counties this is main reason for lack of data [189]. Importantly, data from primary health care in Sweden is not recorded in the Patient register [188].

The Cause of Death Register

In Sweden, the clergies commenced documenting the causes of death in 1751, following a decision from the parliament in 1749. From 1911 until 1993, Statistics Sweden governed the register. Since 1994, the Swedish National Bord of Health and Welfare (in Swedish: Socialstyrelsen), is the responsible authority.

Data from 1952 and onwards are electronically available for research and the register is updated annually. The register uses a universal version of the ICD system, facilitating international cooperation on cause of death statistics.

In Sweden, following death of a person, a certificate on the cause of death must be admitted within three weeks (to the National Board of Health and Welfare). The certificate is submitted by a physician and require a diagnosis for main, i.e. underlying, cause of death and when appropriate, contributory causes/diagnoses [190]. The ICD-10 system holds a definition of what can be classified as the main cause of death [186].

In a report published in 2000 covering 75% of all deaths in Sweden 1995, a concordance between the diagnose(s) reported to the Cause of death register and those in the patient medical records in 83% inhospital and 46% out-hospital deaths was found [191]. In another publication from 2009, further validating a cohort from 1995 (n=1094) regarding causes of death, the overall correctness was 77% (compared to diagnoses registered in the inpatient register). Concordance was better for younger patients and certain diagnoses such as malignancies. Lower accuracy was seen in elderly patients [192].

The Prescribed Drug Register

This register holds information about all prescribed drugs that have been collected at pharmacies in Sweden as well as the prescribing persons' profession and work place. The current register, which uses PIN, was founded in 2005. Drugs are encoded by the Anatomic Therapeutic Chemical (ATC) classification system, intiated by WHO. In Sweden, it is not mandatory by law to denote the diagnosis underlying the prescription, i.e. information from this register does not contain diagnoses. The register is often used in epidemiological research, taking advantage of PIN to link data to other health care registries [193, 194].

Uppsala-Umeå Comprehensive Cancer Consortium

Structured biobanking of tumor samples from real-world patients can be used to better understand the complex biological background of CLL. Uppsala-Umeå Comprehensive Cancer Consortium (U-CAN) [195] is a Swedish biobank project for a wide range of tumor diseases, among these CLL. This biobank is based on longitudinal collection of tumor samples from real-world CLL patients before, under and after therapy, enabling unique opportunities for molecular studies of CLL's biology and genetics. In CLL, samples are collected at diagnosis and before start of first-line therapy. The cohort is divided in two groups: watch and wait and need of therapy. For patients on watch and wait, blood samples are collected annually, for patients requiring therapy the initial schedule was every third month, but has recently been changed to every six months during therapy.

Aims of the thesis

The overall aims of this thesis are to study the relationship between CVD and inflammatory and cardiovascular biomarkers in real-world CLL patients, the epidemiology of CVD in CLL, as well as the occurrence and prognostic impact of subclones harboring specific mutations in CLL.

Paper I
To analyze and study 11 proposed cardiovascular biomarkers, among these GDF15, galectin 3 and PTX3, in serum and plasma samples from a real-world CLL cohort.

Paper II
To study the prevalence of CVD, especially AF and hypertension, at time of CLL diagnosis and start of first-line therapy, in a population-based CLL cohort. Furthermore, to study the cumulative incidence of CVD among CLL patients with and without previous CVD undergoing first-line CLL therapy (mainly chemotherapy or CIT).

Paper III
To study if history of CVD is more common among CLL patients (at time of CLL diagnosis) compared to a matched comparator population, i.e., does CLL predispose for CVD?
To study if CVD is more common among CLL patients following CLL diagnosis and therapy, compared to a matched comparator population.
To study the CVD mortality among CLL patients, in relation to comparators.

Paper IV
To study the occurrence and clinical impact of subclones in a treatment naïve real-world CLL cohort, with focus on 15 recurrently mutated genes.

Patients and methods

Patient sources

In **paper I** patient samples of plasma and serum, from the Uppsala U-CAN CLL cohort was used for the analyzes. The samples, collected at time of U-CAN inclusion and consecutively before start of therapy, were analyzed in 139 CLL patients, the majority with early-stage disease, i.e. Binet A. Seventy-one blood donors comprised the control cohort, matched on age and gender. The median age was 70 years for patients and 67 years for controls.

In **paper II** and **III**, the study cohorts included all Swedish CLL patients diagnosed with CLL during a specific time frame. The patients were identified in the SCR and subsequently in the CLL register (data on treatment and clinical staging) and included in the respective studies. In **paper II** all CLL patients diagnosed with CLL between 2007-2010, n=2078 were included (three patients excluded later on due to loss of follow-up data in the CLL register; n=2075) and in **paper III** all patients diagnosed with CLL during 2007-2015, n=4261. In addition, in paper III, five comparators (n=21 304) per CLL patient were identified from the Total population register, matched on birth year, sex, calendar year and county. Individuals with CLL were excluded from the comparator cohort. Thus, paper III provides a larger CLL cohort and also includes comparators. Regarding the time frames chosen; both begins at 2007, which was the year when the CLL register was started. In **paper II**, we aimed for a 5-year follow-up time for a mainly ibrutinib naïve cohort, to study the incidence of new CVDs during follow-up after initiating chemotherapy or CIT (ibrutinib was only available for compassionate use in Sweden in 2014). In **paper III** we wanted a larger cohort with matched comparators to study the research question; is history of CVD at time of CLL diagnosis more common among CLL patients than comparators?

In **paper IV**, 40 CLL patients from the U-CAN biobank were included. Of these, 32 patients had active disease, i.e. in need of therapy and eight patients were on watch and wait (indolent disease). The median age was 68 years for patients with CLL in need of treatment and 69 for patients on watch and wait. For patients with active CLL, the median time from sample collection (i.e. U-CAN inclusion) to start of first-line therapy was 1.7 years.

Methods

All methods are described in detail in papers I-IV with supplements.

In **paper I,** ELISA sandwich commercial kits were used to analyze 11 proposed CVD risk biomarkers in plasma: Galectin 3, Growth differentiation factor 15 (GDF15), Matrix metalloproteinase 9 (MMP9), Myostatin, Pentraxin 3 (PTX3), sTNFR1, sTNFR2, sVEGFR1, sVEGFR2. For the analysis of CRP and B2M, serum was used. All samples were analyzed according to the manufacturers' protocols and blinded regarding diagnosis. All samples were analyzed in the same run, in order to minimize the risk of variations between plates.

Paper II is a retrospective register study in which we identified all patients with a CLL diagnosis between 2007-2010, n=2078. Utilizing their PIN, we searched the Patient register for CVD diagnoses using the ICD-10 (I00-99) chapter within 10 years before CLL diagnosis. We also identified patients in need of first-line therapy during 2007-2015 in the CLL register, n=828, and performed the same search. Finally, we investigated the incidence (at 3 and 5 years after treatment initiation) of new CVDs among patients without previous CVD, after start of CIT or chemotherapy. Data on cause of death was obtained from the Cause of death register (n=678).

In **paper III** a larger CLL cohort with a matched comparator population was used (described above). This paper contains several analyzes, starting at time of CLL diagnosis.

First, we re-evaluated the CVD burden at time of CLL diagnosis using the same procedure as described for paper II, but added a 3 months lag-time, i.e. all CVD diagnoses within three months before the CLL diagnosis was excluded in the analyzes with lag-time. We also performed an analysis without lag-time. The purpose was to answer to two different questions; 1: Do CLL patients have a higher CVD burden already at time of diagnosis, perhaps indicating that a small CLL clone driven by inflammation predisposes for CVD? This question came up following the results in paper I and II and the current data on activated inflammatory pathways in CLL. 2: What is the true CVD burden at time of CLL diagnosis in a real-world patient cohort? This is a relevant question in the era of BTKi due to the elevated risk of cardiovascular events in patients with preexisting CVD when using BTKis. Finally, as the Patient register do not hold information from the primary care units in Sweden, we postulated that we might have underestimated the proportion of patients with hypertension in paper II. To investigate this further, we used the Prescribed drug register and retrieved data on prescribed drugs for hypertension and anticoagulants/platelet inhibitors one year before time of CLL

diagnosis, with lag-time. Drugs were identified by using the ATC codes covering these drugs.

Secondly, at time of CLL-diagnosis a re-match was performed on previous CVD to match with the comparators. In performing analyzes after CLL diagnosis to study the risk of CVD at follow-up, and to determine if therapy with chemotherapy/CIT increases this risk, patients were divided into four groups, with comparators (described in Statistics further down and in Table 4).

Third, the Cause of death register was used to identify if CLL patients had an increased risk of CVD death.

In **paper IV** we used vital frozen CLL cells from the U-CAN biobank. Upon thawing, tumour fraction was analyzed by flow cytometry. Samples with tumour fractions <90% were enriched using a B-CLL Cell Isolation Kit from Miltenyi Biotec. DNA was extracted using the Allprep DNA/RNA mini kit (Qiagen). Targeted sequencing was performed using the custom Lymphomatic TWIST panel covering 15 genes frequently mutated in CLL: *EGR2, BTK,* F-box/WD repeat-containing protein *(FBXW7), XPO1,* Protection of telomeres 1*(POT1), NOTCH1, BIRC3, PLCγ2,* IKAROS Family Zinc Finger 3 *(IKZF3), TP53, BCL2, Ribosolam Protein S15 (RPS15), SF3B1, MYD88* and *NFKBIE.* The sequencing analyses were performed by the SciLife at Karolinska Institute, Solna. Samples were sequenced aiming for a depth coverage of 20K reads per base sequenced.

Statistical analyzes

All papers include descriptive statistics of the included patient population (and in paper I and III their comparators) with basic variables: median, maximum, minimum values, percentage and numbers. In paper III, interquartile range (IQR) were quoted in supplementals. The statistics applied in the papers can be read in detail in the respective papers and supplementals.

In **paper I**, the results from the ELISA analyzes, i.e. concentrations of the respective biomarkes, underwent statistical analysis with the purpose to investigate differences between the two groups; CLL patients and controls. Adjustments were made for age and gender. To adjust for multiple testing the Benjamini-Hochberg method was used. A principal component analysis (PCA plot) was performed to illustrate patterns in biomarker concentrations between CLL patients and controls. Traditional boxplots for each biomarker were produced to visualize differences between the two groups.

In **paper II**, descriptive statistics to calculate the CVD prevalence within 10 years until the date of CLL diagnosis. During follow-up, the incidence of new

CVD at 3- and 5-years follow-up was calculated using register data. Each CVD was only counted once, i.e. if a patient received a diagnosis of AF, it only counted once during follow-up.

In **paper III**, descriptive statistics was applied to calculate CVD prevalence within 10 years until the date of the CLL diagnosis (as in paper II), with and without a 3 months lag-time. To test for statistical significance in CVD between CLL patients and comparators, a chi-2-test was performed. In analyzes assessing the risk of CVD during follow-up, incidence rates, hazard ratios (HR), mortality rate ratios and cause-specific mortality rates in the presence and absence of competing risks were estimated and calculated. Risks were shown as HR, with/without competing risk regression. A landmark analysis was executed to investigate whether CLL patients with and without CVD, who received therapy or were on watch and wait, had an increased incidence in CVD during follow-up compared to the comparators. In this analysis patients diagnosed with CLL during 2007-2012, having a recorded follow-up 3 years in the CLL register were included. Patients were divided in +/- previous CVD, followed by +/- therapy, generating four cohorts (with comparators matched on CVD), demonstrated in Table 4.

In **Paper IV**, the reads from the NGS sequencing run were processed, quality checked and analyzed using standard pipelines as described in the manuscript. Clonal mutations were defined as VAF $\geq$ 10%, and subclonal <10%. For subclonal hotspot mutations, a VAF down to 1% were applied and for non-hotspot mutations, a VAF down to 5%. Follow-up time was calculated from date of sample collection to 1st of December 2022. A two-sided Chi-2-test was performed to investigate whether mutations were more frequent among patients with active CLL compared to indolent disease.

Table 4. The four CLL cohorts and their comparators in the Landmark analysis, at landmark time (3 years after treatment initiation or CLL diagnosis)

Variable	No history of CVD at time CLL diagnosis			
	In need of CLL therapy at landmark time		Watch and wait at landmark time	
	CLL	Comparators	CLL	Comparators
Numbers (N)	389	1 757	1 187	5 465
Sex				
Men	249 (64%)	1 129 (64%)	673 (57%)	3 108 (57%)
Women	140 (36%)	628 (36%)	514 (43%)	2 357 (43%)
Age				
Median (IQR)	70 (63-79)	70 (63-78)	70 (64-78)	70 (64-77)
Comorbidity within 10 years prior to CLL diagnosis until 3 years after CLL diagnosis				
Any CVD*	136 (35%)	329 (19%)	364 (31%)	951 (17%)
Variable	**History of CVD at time of CLL diagnosis**			
	In need of CLL therapy at landmark time		Watch and wait at landmark time	
	CLL	Comparators	CLL	Comparators
Numbers (N)	149	602	664	2 591
Sex				
Men	103 (69%)	428 (71%)	410 (62%)	1 602 (62%)
Women	46 (31%)	174 (29%)	254 (38%)	989 (38%)
Age				
Median (IQR)	76 (69-82)	75 (70-81)	77 (70-83)	77 (70-82)
Comorbidity within 10 years prior to CLL diagnosis until 3 years after CLL diagnosis				
Any CVD*	147 (99%)	577 (96%)	636 (96%)	2 466 (95%

Ethical considerations

For all papers ethical approvals were obtained, according to the Declaration of Helsinki. Prior to inclusion in the U-CAN project, informed consent was obtained.

Main results and discussion

Paper I

Increased levels of the cardiovascular disease risk biomarkers GDF15 and myostatin in patients with chronic lymphocytic leukemia.

This study was designed as a pilot, with the aim to screen a cohort of real-world CLL patients for biomarkers in plasma/serum associated with CVD and inflammation. The CLL cohort was mainly early-stage disease with Binet A 82% (n=104), Binet B n=13 and Binet C n=10. The pattern of biomarker blood concentrations were very heterogenous among the CLL patients, but were more uniform in the control group. The concentrations for eight of 11 biomarkers differed from controls, e.g. galectin 3, MMP9, myostatin, sTNFR1, sTNRFR2 and sVEGFR2 ($p<0.001$). For CRP, PTX3 and sVEGFR1 no difference was seen.

Our data with increased levels of sTNFR1 and sTNFR2 is in line with a previous study [196]. Elevated sTNFR1 was also found among patients participating in the CLL8 trial and was associated with poor outcome in CLL. All samples analyzed were pretreatment samples (in CLL cohort with advanced disease) [101].

Data on VEGF, sVEGFR1 and sVEGFR2 in serum of 83 CLL treatment naïve patients have been published previously, including a control population, n=20. The majority of patients had early-stage disease, n=50/83, and n=33/83 classified as advanced disease. The median age for CLL patients were 68 years, and for the controls 52 years [197]. The study reported on significant increase in VEGF concentration, when comparing the CLL cohort to controls. The concentration of sVEGFR2 (and VEGF) were found significantly increased in advanced disease stages and the concentration correlated to lymphocytosis. This was not seen for sVEGFR1 [197]. Thus, this study is in line with our results on sVEGFR1, but not regarding sVEGFR2. However, we did not investigate correlation to lymphocytosis or clinical staging.

For several biomarkers, data were available for CLL but analyzed in other compartments; i.e. MMP9 has been found elevated on CLL surface and inside CLL cells [198]. Similarly, two studies have investigated the intracellular expression of galactin 3 [199, 200].

Surprisingly, the levels of CRP and PTX3 did not differ between the two groups, one possible explanation might be that these two biomarkers are too insensitive to measure low-grade inflammation in these early-stage CLL patients.

Finally, elevated levels of GDF15 and myostatin, in CLL are to our knowledge, new findings in CLL. Increased concentrations of GDF15 in plasma/serum have been associated to both CVD and cancer [201]. Increased levels of myostatin have been associated with cancer [202, 203] and with development of atherosclerosis [202, 203].

To conclude, the concentrations of the majority of biomarkers differed significantly between CLL patients and controls in a cohort of mainly early-stage CLL patients. Possibly, this altered biomarker expression in CLL patients reflects a low-grade inflammatory environment for CLL cells *in vivo*. We believe this area of research in CLL needs further investigation, including our new findings of elevated GDF15 and myostatin in CLL, specially due to correlation of these biomarkers with CVD and taken into consideration the association between BTKi and CVD.

Paper II

High prevalence and incidence of cardiovascular disease in chronic lymphocytic leukaemia: a nationwide population-based study.

In this study we describe that 32% (n=675/2075) of CLL patients had a history of CVD <10 years prior to the date of the CLL diagnosis while 37% (n=307/828) had a history of CVD prior to the treatment initiation. At time of CLL diagnosis, 9% were diagnosed with AF and 22% with hypertension. Five years after treatment initiation, 28% of the patients without previous CVD had been diagnosed with a new CVD.

Prior to the BTKi era, population-based data on CVD comorbidity with specified diagnoses, e.g. AF, myocardial infarction and hypertension, among CLL patients were rare. A study from the Mayo clinic reported on AF prevalence of 6.1% at time of CLL diagnosis in a younger CLL cohort with a median age of 65 years [147]. Thus, patients were younger than in our cohort, which possibly explains our higher frequency of 9% at time of CLL diagnosis.

When this paper was published, ibrutinib was the prime BTKi used. The next generation BTKis were used in trials with preliminary reports on lesser CVD toxicity. Later publications have indicated this for acalabrutinib vs ibrutinib regarding AF [129] and for zanubrutinib vs ibrutinib [130]. However, reports currently suggest several AEs of BTKis being class effects, such as hypertension [204] and VAs [153], the latter of concern due to the risk of sudden death following VA.

Due to the selection of more fit patients in clinical trials baseline data on CVD among real-world CLL patients is of importance, as these patients tend to be more fragile than those included in clinical trials. We regarded our main

finding, i.e. a substantial CVD comorbidity among CLL patients already at time of diagnosis and at time of primary treatment initiation, as a concern in the era of BTKis, currently entering the frontline therapy setting.

Caveats in this study are that the Outpatient register used was founded 1997, although not complete until 2001, hence patients had unequal follow-up times, as well as that general practitioners do not report diagnoses to the Patient register. Thus patients with hypertension might be underestimated in this paper. The lack of comparators is also a limitation in this study.

Paper III

Cardiovascular disease in patients with chronic lymphocytic leukemia. A population-based nationwide study from Sweden.

In this paper we describe that, with 3 months lag-time before CLL diagnosis, CLL patients had slightly less CVD than comparators, 39% (n=1945) vs 41% (n=8944) ($p<0.05$). Without lag-time, 46% (n=1945) of the CLL patients had at least one CVD at time of CLL diagnosis compared to 42% (n=8944) among comparators ($p <0.05$). Using data from the Prescribed drug register (with lag-time of 3 months), 52% of the CLL patients and the comparators had a prescription of antihypertensive drugs ($p= 0.42$). As for anticoagulants and/or antiplatelet drugs, 31% of the CLL patients and 33% of the comparators had a prescription ($p<0.05$). During follow-up, all CLL cohorts, displayed a higher incidence rate of CVD, regardless of prior CVD in relation to their comparators. A competing risk regression did not alter this statistical significance.

In the Landmark analysis, we studied the impact of primary therapy with chemotherapy/CIT on the incidence of CVD, depending on prior CVD history among CLL patients, also in relation to their matched comparators. Again, each CLL cohort had a higher incidence of CVD vs comparators. Analyzing CVD as cause of death; all-cause mortality was significantly increased among CLL patients, but not CVD mortality.

From this study we draw the following conclusions. Firstly, CVD does not appear to be more common in CLL patients at time of CLL diagnosis, than among a matched comparator population, based on the analysis with lag-time where CLL patients exhibited a tendency of less CVD. This argues against a higher risk of CVD due to chronic inflammation in patients with CLL. In addition, we found a substantial portion (52%) of CLL patients and comparators having a prescription of antihypertensive drug ($p=0.42$) and approximately 30% with anticoagulants and/or platelet inhibitors. Secondly, the incidence of CVD during follow-up was increased in CLL patients compared to comparators, independently of previous CVD or whether the patient received treatment

or not. Thirdly, all-cause mortality was increased among CLL patients, but not CVD mortality.

These data need comprehensive interpretation due to diversity of the cohorts with and without lag-time. We believe that the analysis without lag-time, with a higher prevalence of CVD in CLL patients, mainly reflects surveillance bias during diagnostic work-up of CLL. We consider it unlikely that early-stage CLL would cause a significant increase in CVD during three months. In addition, in analysis with lag-time, CLL patients appear to have slightly less CVD vs comparators. Another explanation for the increase in CVD in the analysis without lag-time, is registration of other chronic secondary diagnoses, e.g. hypertension in the Patient register following first contact with outpatient specialist health care. Data from the Prescribed drug register might support this theory, though a limitation with this register is the absence of diagnosis, e.g. betablockers might be used as migraine prophylaxis.

The Landmark analysis was performed with relatively small patient cohorts, consequently sound statistical analyzes comparing the CLL cohorts were not possible to perform. Thus, we do not know if chemotherapy / CIT further increases the risk for CVD among CLL patients, compared to CLL patients on watch and wait.

Commenting on the increase in all-cause mortality but not CVD mortality, we believe CLL patients perish from their CLL before they are affected by a lethal CVD event. In line with our results, a Danish register study with matched comparators from 2021, found an inferior survival among CLL patients in relation to comparators, suggesting that CLL patients succumb due to their CLL [180].

To conclude, our data do not support the hypothesis that a minor CLL clone predispose to CVD, at least not in early-stage disease. However, due to the increased CVD incidence during follow-up, we cannot from this study dissect surveillance bias from a “true” increase in CVD associated with CLL over time. Hypothetically, a higher tumor burden might increase low-grade inflammation, with an increasing incidence in CVD during follow-up. Recently it was demonstrated that in CH, the size of the clone had impact for the risk of developing a CVD event [90]. Regardless of what causes the elevated CVD risk during follow-up in CLL patients, we demonstrate that CVD is common and this calls for awareness among CLL patients, especially before initiating BTKi.

Paper IV (in manuscript)

Isolated subclonal mutations are not a common feature in untreated chronic lymphocytic leukemia

We observed clonal mutations in 48% (n=19) of the patients while 14 subclonal mutations were detected in 25% (n=10) of patients. In 6 of 10 patients with subclonal mutations, they co-existed with clonal mutations. Among patients requiring therapy, mutations were significantly more frequent, with 66% (n=21/32) harboring mutations in the genes covered by this NGS panel. In the cohort with patients on watch and wait, 25% (n=2/8) were found to harbor mutations. In both patients from the latter group, clonal and subclonal mutations appeared simultaneously. The most frequently mutated genes (clonal or subclonal) were *SF3B1, NOTCH1*, and *TP53,* in line with previous data [59, 205]. No mutations were found in *EGR2, BTK, FBXW7, PLCγ2* or *XPO1* using the current gene panel design.

We also investigated whether patients requiring early treatment (n=20), defined as start of therapy <24 months from sample collection, had a higher mutational burden compared to patients initiating therapy >24 months (n=12) but did not find any significant differences between the two groups.

In summary, we conclude that isolated subclonal mutations are infrequent events among CLL patients and often co-occurs with clonal mutations. Mutations overall appear to be more common in patients requiring therapy. As a next step we intend to validate all subclonal mutations by ddPCR (digital droplet polymerase chain reaction). We will also analyze IGHV gene status for all patients. This is of particular interest since recent data imply that IGHV status affects clinical outcome of specific mutations [82]. As a later step, validation of our results in a larger independent cohort is desired.

Notably, in this study, no clonal or subclonal mutation in *BTK* or *PLCγ2* were found. In the era of targeted therapies for CLL in primary treatment, the need of longitudinal studies of subclonal evolution before, during and following BTKi and BCL2i treatment is warranted. Preferably in a real-world settings, and for this purpose the U-CAN CLL biobank is ideal.

Concluding remarks

To conclude, our hypothesis, i.e. that low-grade inflammation, perhaps driven by a minor CLL clone, would predispose for CVD events at time of CLL diagnosis is not supported from the work in this thesis. That said, following the results from paper III, we do not know the true cause for increased incidence in CVD during follow-up among CLL patients; is this solely due to surveillance bias or does the CLL clone overtime promote the development of atherosclerosis or is it related to CIT including complications? To study this further, prospective studies of CLL patients with focus on CVD would be preferable. Regardless of the cause, we demonstrate that the CVD burden among real-world CLL patients is substantial and of clinical significance, in the era of BTKi therapy.

The last paper, raise several future perspectives and possibilities. Due to a small cohort, the study needs further evaluation in larger, well-characterized material and with longitudinal samples. As the landscape of treatment is rapidly shifting in CLL, studies of clonal evolution following new targeted therapies, are warranted to predict outcome and risk of relapse. Most studies in this field have been performed in the era of CIT.

The major aim of this thesis has been to perform studies of CLL in a real-world setting by using nationwide registers in Paper II and III with an epidemiological approach, and by using samples from CLL patients to study expression patterns of biomarkes and mutations. Following the complex biology of CLL and what is achieved today regarding therapies, continuous translational research, preferably in real-world patient cohorts, are important. I hope to continue working with CLL as a clinician as well as participating in research collaborations with the aim to improve CLL outcome and if possible, to see patients cured outside the setting of allo-HSCT.

Svensk populärvetenskaplig sammanfattning

Kronisk lymfatisk leukemi (KLL) är den vanligaste typen av leukemi i västvärlden och drabbar framförallt äldre individer, med en medelålder på 72 år vid diagnos i Sverige. Vid diagnos är de flesta (85%) asymtomatiska och inte i behov av någon behandling, men med tiden utvecklar ca 2/3 behandlingsbehov. Mellan åren 2015 och 2017 inleddes ett paradigmskifte inom KLL-behandling innebärande att första linjens behandling med kemoimmunoterapi (CIT) ersattes av Brutons tyrosinkinashämmare (BTK-hämmare). Första generationens BTK-hämmare, främst ibrutinib, har visat sig ha betydande kardiovaskulära biverkningar. Data på förekomsten av hjärtkärlsjukdom (CVD) hos patienter med KLL i populationsbaserad kohort, med eller utan behandling, saknas, liksom biomarkörer för att förutsäga risk för CVD vid KLL. Vi har idag begränsad kunskap om prediktiva markörer vid KLL. Nya molekylärgenetiska tekniker såsom riktad gensekvensering, s.k. next generation sequencing (NGS), har påskyndat upptäckten av genetiska mutationer associerade till prognos och terapiresistent sjukdom, men endast två, IGHV mutationsstatus och påvisande av mutationer i genen *TP53*, används i klinisk praxis och styr val av behandling.

För studier av biomarkörer och subkloner har vi använt oss av blodprover från U-CAN (Uppsala-Umeå Comprehensive Cancer Consortium) Uppsala KLL-biobanken, som samlar in tumörprover (för KLL ett vanligt blodprov) vid diagnos och regelbundet under uppföljning och behandling. Kliniska data har samlats in med hjälp av journalgranskning. **Delprojekt I och IV** bygger på blodprover från U-CANs KLL-kohort (Uppsalakohorten) och den lokala kliniska databasen. I **delarbete II och III** har vi använt oss av nationella kvalitetsregistret för KLL samt flera svenska nationella hälsodataregister såsom Cancerregistret, Patientregistret och Dödsorsaksregistret. I **delarbete III** har vi även använt Läkemedelsregistret samt utnyttjat befolkningsregistret vid Statistiska centralbyrån för urval av matchade kontroller.

En bärande idé i avhandlingsarbetet har varit att så långt möjligt generera ”real-world data” genom att utnyttja våra i det närmaste heltäckande nationella populationsbaserade hälsodataregister.

Syftet med **delarbete I (***Increased levels of the cardiovascular disease risk biomarkers GDF-15 and myostatin in patients with chronic lymphocytic leukemia*) var att analysera blodprover från patienter med KLL med avseende på 11 föreslagna CVD/inflammationsbiomarkörer, däribland GDF15, galectin 3 samt PTX3 där data på KLL-patienter saknas. Skiljer sig koncentrationen i blod av dessa biomarkörer mellan KLL-patienter och friska kontroller? I studien ingick 139 KLL-patienter inkluderade i U-CAN. Blodproverna var tagna vid tidpunkt för U-CAN-inklusion / innan start av behandling och majoriteten var icke-behandlingskrävande vid tidpunkt för provtagning. Som kontroller användes 71 friska bloddonatorer, matchade med avseende på ålder och kön. Åtta av 11 biomarkörer hade signifikant högre koncentration hos KLL-patienterna jämfört med kontroller. Vidare uppvisade KLL-patienterna ett annat mönster av CVD-biomarkörer jämfört med friska kontrollpersoner.

I **delarbete II** (*High prevalence and incidence of cardiovascular disease in chronic lymphocytic leukaemia: a nationwide population-based study*) var syftet dels att studera förekomsten av CVD, speciellt förmaksflimmer, vid tidpunkt för KLL-diagnos och behandlingsstart (i en populationsbaserad KLL-kohort) och dels att kartlägga nyinsjuknande i CVD hos KLL-patienter under aktiv behandling (första linjens terapi). Hur vanligt är det med CVD vid KLL-diagnos respektive behandlingsstart och efter given primärterapi? För att svara på detta har vi via Cancerregistret och KLL-registret identifierat alla patienter som under perioden 2007-2010 fått diagnosen KLL, n=2078 patienter, samt behövt KLL-specifik behandling under åren 2007-2015. Genom att länka KLL-registret till Patientregistret (och Dödsorsaksregistret) fick vi uppgifter om CVD-diagnoser. Av de 2078 KLL-patienterna, så hade 1/3 haft en eller flera hjärtkärlhändelser inom en period av 10 år före och fram till och med datum för KLL-diagnos. Motsvarande siffra vi tidpunkt för start av primärbehandling var 37%. Därtill hade 18% av patienterna utan hjärtkärlsjukdom som behövde behandling för KLL efter fem år fått minst en CVD-diagnos. Således uppvisar KLL-patienter en betydande CVD-samsjuklighet såväl vid tidpunkt för KLL-diagnos som efter start av behandling, och detta i en patientgrupp som inte fått BTKi.

För att svara på om KLL-patienter har en högre CVD-börda än normalbefolkningen gick vi vidare i nästa **delarbete III** (*Cardiovascular disease in patients with chronic lymphocytic leukaemia. A population-based nationwide study from Sweden*) där vi adderade en kontrollpopulation. Våra specifika frågeställningar var 1. Är CVD vanligare hos patienter med nydiagnostiserad KLL, jämfört med en ålders, köns-, och regionmatchad kontrollgrupp? 2. Ökar primärbehandling med CIT risken för nyinsjuknande i CVD? 3. Är nyinsjuknande eller död i CVD vanligare hos KLL-patienter? Slutligen ville vi också titta på läkemedelsbördan, vad gäller läkemedel för hypertoni samt antikoagulantia/blodförtunnande, delvis för att undersöka hur stor andelen med

hypertoni kan tänkas vara. I studien inkluderades alla patienter i Sverige som fått KLL-diagnos 2007-2015, n=4261 (således en större kohort än i projekt II). Alla länkningar mellan register (Cancerregistret, KLL-registret, Patientregistret, Läkemedelsregistret, Dödsorsaksregistret) är gjorda via Statistiska centralbyrån, inklusive med fem matchade kontroller per patient. Vi fann ingen säker skillnad i förekomst av CVD vid tidpunkt för diagnos mellan KLL-patienter och kontroller. KLL-patienter har vid uppföljning, oavsett tidigare CVD och behandling med CIT, en högre CVD-börda än kontroller men ingen ökad dödlighet i CVD. Däremot sågs en ökad dödlighet totalt sett, sannolikt pga KLL-sjukdomen. Hälften av alla KLL-patienter (och kontroller) använder vid tidpunkt för KLL-diagnos ett eller flera antihypertensiva läkemedel och ca 30% har recept på blodförtunnande läkemedel eller antikoagulantia. Utifrån detta drar vi slutsatsen att KLL-patienter uppvisar en betydande CVD-börda vid tidpunkt för KLL-diagnos, men inte högre än matchade kontroller. Däremot är nyinsjuknade i CVD vanligare hos KLL-patienter under uppföljning jämfört med kontrollpopulationen, vilket behöver studeras vidare för att klarlägga om det handlar om s.k. surveillance bias eller KLL-sjukdomen i sig och/eller behandlingen av denna. Att KLL-patienter vid diagnos uppvisar ett annat mönster av CVD biomarkörer i plasma/serum jämfört med normalpersoner (Delarbete I) är ett observandum i ljuset av ovanstående. Detta är av kliniskt intresse att studera vidare med tanke på den ökande användningen av BTKi hos KLL-patienter.

I det **fjärde och sista delarbetet,** som föreligger i manuskriptform (*Isolated subclonal mutations are not a common feature in untreated chronic lymphocytic leukaemia*), vill vi undersöka hur förekomsten av små KLL-kloner ser ut innan start av primärterapi hos en populationsbaserad KLL-kohort och om förekomsten/frånvaron av dessa har klinisk betydelse. I studien har tumörprover (=blodprover) från 40 KLL-patienter (n=32 behandlingskrävande och n=8 med icke-behandlingskrävande sjukdom) använts. Patienterna inkluderades mellan 2010-2016 i Uppsala KLL U-CAN biobanken och tumörprover (blodprover) har analyserats med en NGS-panel för 15 gener med metodik som tillåter s.k. djup gensekvensering. Våra första data visar att förekomsten av subklonala mutationer vid diagnos/innan start av primärbehandling av KLL inte är vanligt, men förekommande. Totalt sågs 27 klonala mutationer hos 19 av 40 patienter, medan 14 subklonala mutationer sågs hos 10 av 40 patienter. Mutationer var vanligare (statistiskt signifikant) hos patienter med behandlingskrävande sjukdom jämfört med icke-behandlingskrävande. Information från denna typ av studier kan ge ökad kunskap om klonala och subklonala genmutationers prognostiska betydelse KLL. Då detta är en pilotstudie behövs en fortsättningsstudie med större kohort och analys av uppföljningsprover.

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References

1. Kimby, E., M. Abramsson, and M. Mattsson, *First report from the Swedish CLL-registry : a nationwide registry with high coverage.* Leukemia and Lymphoma, 2015. **56(8. Supp**(S1): p. 42-42.
2. Grivennikov, S.I., F.R. Greten, and M. Karin, *Immunity, Inflammation, and Cancer*. 2010.
3. Libby, P. and S. Kobold, *Inflammation: a common contributor to cancer, aging, and cardiovascular diseases-expanding the concept of cardio-oncology.* Cardiovasc Res, 2019. **115**(5): p. 824-829.
4. Rozovski, U., M.J. Keating, and Z. Estrov, *Targeting inflammatory pathways in chronic lymphocytic leukemia*. 2013.
5. Schulz, A., et al., *Inflammatory cytokines and signaling pathways are associated with survival of primary chronic lymphocytic leukemia cells in vitro: a dominant role of CCL2.* Haematologica, 2011. **96**(3): p. 408-16.
6. Purdue, M.P., et al., *A prospective study of 67 serum immune and inflammation markers and risk of non-Hodgkin lymphoma.* Blood, 2013.
7. Jaiswal, S., et al., *Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease.* N Engl J Med, 2017. **377**(2): p. 111-121.
8. Brown, J.R., et al., *Characterization of atrial fibrillation adverse events reported in ibrutinib randomized controlled registration trials.* Haematologica, 2017.
9. Caldeira, D., et al., *Ibrutinib increases the risk of hypertension and atrial fibrillation: Systematic review and meta-analysis.* PLoS One, 2019. **14**(2): p. e0211228.
10. Reda, G., et al., *Predictors of atrial fibrillation in ibrutinib-treated CLL patients: a prospective study.* J Hematol Oncol, 2018. **11**(1): p. 79.
11. Brown, J.R., et al., *Characterization of atrial fibrillation adverse events reported in ibrutinib randomized controlled registration trials.* Haematologica, 2017. **102**(10): p. 1796-1805.
12. Byrd, J.C., et al., *Long-term follow-up of the RESONATE phase 3 trial of ibrutinib vs ofatumumab.* Blood, 2019. **133**(19): p. 2031 LP-2042.

13. Hallek, M., *Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment.* Am J Hematol, 2019. **94**(11): p. 1266-1287.
14. Hallek, M., et al., *iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL.* Blood, 2018. **131**(25): p. 2745-2760.
15. Slager, S.L., et al., *Genetic susceptibility to chronic lymphocytic leukemia.* Semin Hematol, 2013. **50**(4): p. 296-302.
16. Yang, S., et al., *Ethnic and geographic diversity of chronic lymphocytic leukaemia.* Leukemia, 2021. **35**(2): p. 433-439.
17. Goldin, L.R., et al., *Elevated risk of chronic lymphocytic leukemia and other indolent non-Hodgkin's lymphomas among relatives of patients with chronic lymphocytic leukemia.* Haematologica, 2009. **94**(5): p. 647-53.
18. Slager, S.L., et al., *Medical history, lifestyle, family history, and occupational risk factors for chronic lymphocytic leukemia/small lymphocytic lymphoma: the InterLymph Non-Hodgkin Lymphoma Subtypes Project.* J Natl Cancer Inst Monogr, 2014. **2014**(48): p. 41-51.
19. Tjønnfjord, G.E., et al., *Familial occurrence of chronic lymphocytic leukaemia in Norway.* Tidsskr Nor Laegeforen, 2012. **132**(18): p. 2060-3.
20. Hohenadel, K., et al., *Exposure to multiple pesticides and risk of non-Hodgkin lymphoma in men from six Canadian provinces.* Int J Environ Res Public Health, 2011. **8**(6): p. 2320-30.
21. Mattsson, M., et al., *Increasing prevalence of chronic lymphocytic leukemia with an estimated future rise: A nationwide population-based study.* Am J Hematol, 2020. **95**(2): p. E36-E38.
22. da Cunha-Bang, C., et al., *Improved survival for patients diagnosed with chronic lymphocytic leukemia in the era of chemo-immunotherapy: a Danish population-based study of 10455 patients.* Blood Cancer J, 2016. **6**(11): p. e499.
23. Swerdlow, S.H., et al., *The 2016 revision of the World Health Organization classification of lymphoid neoplasms.* Blood, 2016. **127**(20): p. 2375-90.
24. Marti, G.E., et al., *Diagnostic criteria for monoclonal B-cell lymphocytosis.* Br J Haematol, 2005. **130**(3): p. 325-32.
25. Strati, P. and T.D. Shanafelt, *Monoclonal B-cell lymphocytosis and early-stage chronic lymphocytic leukemia: diagnosis, natural history, and risk stratification.* Blood, 2015. **126**(4): p. 454-62.
26. Landau, D.A., et al., *Locally disordered methylation forms the basis of intratumor methylome variation in chronic lymphocytic leukemia.* Cancer Cell, 2014. **26**(6): p. 813-825.

27. Mansouri, L., et al., *Epigenetic deregulation in chronic lymphocytic leukemia: Clinical and biological impact.* Semin Cancer Biol, 2018. **51**: p. 1-11.
28. Collins, R.J., et al., *Spontaneous programmed death (apoptosis) of B-chronic lymphocytic leukaemia cells following their culture in vitro.* Br J Haematol, 1989. **71**(3): p. 343-50.
29. Burger, J.A. and A. Wiestner, *Targeting B cell receptor signalling in cancer: preclinical and clinical advances.* Nat Rev Cancer, 2018. **18**(3): p. 148-167.
30. Puente, X.S., P. Jares, and E. Campo, *Chronic lymphocytic leukemia and mantle cell lymphoma: crossroads of genetic and microenvironment interactions.* Blood, 2018. **131**(21): p. 2283-2296.
31. Hamblin, T.J., et al., *Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia.* Blood, 1999. **94**(6): p. 1848-54.
32. Damle, R.N., et al., *Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia.* Blood, 1999. **94**(6): p. 1840-7.
33. Landau, D.A., et al., *Mutations driving CLL and their evolution in progression and relapse.* Nature, 2015. **526**(7574): p. 525-30.
34. Agathangelidis, A., et al., *Higher-order connections between stereotyped subsets: implications for improved patient classification in CLL.* Blood, 2021. **137**(10): p. 1365-1376.
35. Agathangelidis, A., et al., *Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies.* Blood, 2012. **119**(19): p. 4467-4475.
36. Tobin, G., et al., *Somatically mutated Ig V(H)3-21 genes characterize a new subset of chronic lymphocytic leukemia.* Blood, 2002. **99**(6): p. 2262-4.
37. Strefford, J.C., et al., *Distinct patterns of novel gene mutations in poor-prognostic stereotyped subsets of chronic lymphocytic leukemia: the case of SF3B1 and subset #2.* Leukemia, 2013. **27**(11): p. 2196-9.
38. Rossi, D. and G. Gaidano, *Biological and clinical significance of stereotyped B-cell receptors in chronic lymphocytic leukemia.* Haematologica, 2010. **95**(12): p. 1992-5.
39. Sofou, E., et al., *Evidence of somatic hypermutation in the antigen binding sites of patients with CLL harboring IGHV genes with 100% germline identity.* Front Oncol, 2022. **12**: p. 1079772.
40. Burger, J.A., 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**(8): p. 2655-63.

41. Lagneaux, L., et al., *Chronic lymphocytic leukemic B cells but not normal B cells are rescued from apoptosis by contact with normal bone marrow stromal cells.* Blood, 1998. **91**(7): p. 2387-96.
42. Herndon, T.M., et al., *Direct in vivo evidence for increased proliferation of CLL cells in lymph nodes compared to bone marrow and peripheral blood.* Leukemia, 2017. **31**(6): p. 1340-1347.
43. Korz, C., et al., *Evidence for distinct pathomechanisms in B-cell chronic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell cycle and apoptosis-associated genes.* Blood, 2002. **99**(12): p. 4554-61.
44. Stevenson, F.K., F. Forconi, and T.J. Kipps, *Exploring the pathways to chronic lymphocytic leukemia.* Blood, 2021. **138**(10): p. 827-835.
45. Ten Hacken, E. and J.A. Burger, *Microenvironment interactions and B-cell receptor signaling in Chronic Lymphocytic Leukemia: Implications for disease pathogenesis and treatment.* Biochim Biophys Acta, 2016. **1863**(3): p. 401-413.
46. Burger, J.A. and N. Chiorazzi, *B cell receptor signaling in chronic lymphocytic leukemia.* Trends Immunol, 2013. **34**(12): p. 592-601.
47. de Rooij, M.F., et al., *The clinically active BTK inhibitor PCI-32765 targets B-cell receptor- and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia.* Blood, 2012. **119**(11): p. 2590-4.
48. Palma, M., et al., *Ibrutinib induces rapid down-regulation of inflammatory markers and altered transcription of chronic lymphocytic leukaemia-related genes in blood and lymph nodes.* Br J Haematol, 2018. **183**(2): p. 212-224.
49. Niemann, C.U., et al., *Disruption of in vivo Chronic Lymphocytic Leukemia Tumor-Microenvironment Interactions by Ibrutinib--Findings from an Investigator-Initiated Phase II Study.* Clin Cancer Res, 2016. **22**(7): p. 1572-82.
50. Svanberg, R., et al., *Targeting the tumor microenvironment in chronic lymphocytic leukemia.* Haematologica, 2021. **106**(9): p. 2312-2324.
51. Hayden, M.S. and S. Ghosh, *Shared principles in NF-kappaB signaling.* Cell, 2008. **132**(3): p. 344-62.
52. Mansouri, L., et al., *NF-κB activation in chronic lymphocytic leukemia: A point of convergence of external triggers and intrinsic lesions.* Semin Cancer Biol, 2016. **39**: p. 40-8.
53. Sun, S.-C., *The non-canonical NF-κB pathway in immunity and inflammation.* Nature Reviews Immunology, 2017. **17**(9): p. 545-558.
54. DiDonato, J.A., F. Mercurio, and M. Karin, *NF-κB and the link between inflammation and cancer.* Immunological Reviews, 2012. **246**(1): p. 379-400.

55. Oeckinghaus, A., M.S. Hayden, and S. Ghosh, *Crosstalk in NF-κB signaling pathways.* Nature Immunology, 2011. **12**(8): p. 695-708.
56. Rozovski, U., et al., *Activation of the B-cell receptor successively activates NF-κB and STAT3 in chronic lymphocytic leukemia cells.* International Journal of Cancer, 2017. **141**(10): p. 2076-2081.
57. Sun, S.C., *Non-canonical NF-κB signaling pathway.* Cell Res, 2011. **21**(1): p. 71-85.
58. Cahill, N., et al., *450K-array analysis of chronic lymphocytic leukemia cells reveals global DNA methylation to be relatively stable over time and similar in resting and proliferative compartments.* Leukemia, 2013. **27**(1): p. 150-158.
59. Puente, X.S., et al., *Non-coding recurrent mutations in chronic lymphocytic leukaemia.* Nature, 2015. **526**(7574): p. 519-24.
60. Gaidano, G. and D. Rossi, *The mutational landscape of chronic lymphocytic leukemia and its impact on prognosis and treatment.* Hematology Am Soc Hematol Educ Program, 2017. **2017**(1): p. 329-337.
61. Döhner, H., et al., *Genomic aberrations and survival in chronic lymphocytic leukemia.* N Engl J Med, 2000. **343**(26): p. 1910-6.
62. Barrio, S., et al., *Genomic characterization of high-count MBL cases indicates that early detection of driver mutations and subclonal expansion are predictors of adverse clinical outcome.* Leukemia, 2017. **31**(1): p. 170-176.
63. Greaves, M. and C.C. Maley, *Clonal evolution in cancer.* Nature, 2012. **481**(7381): p. 306-13.
64. Nadeu, F., et al., *Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia.* Blood, 2016. **127**(17): p. 2122-30.
65. Gaiti, F., et al., *Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia.* Nature, 2019. **569**(7757): p. 576-580.
66. Pekarsky, Y., V. Balatti, and C.M. Croce, *BCL2 and miR-15/16: from gene discovery to treatment.* Cell Death Differ, 2018. **25**(1): p. 21-26.
67. Autore, F., et al., *Morphological, immunophenotypic, and genetic features of chronic lymphocytic leukemia with trisomy 12: a comprehensive review.* Haematologica, 2018. **103**(6): p. 931-938.
68. Schaffner, C., et al., *Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia.* Blood, 1999. **94**(2): p. 748-53.
69. Fegan, C., et al., *Karyotypic evolution in CLL: identification of a new sub-group of patients with deletions of 11q and advanced or progressive disease.* Leukemia, 1995. **9**(12): p. 2003-8.
70. Baliakas, P., et al., *Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact.* Blood, 2019. **133**(11): p. 1205-1216.

71. Rossi, D., et al., *Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia.* Blood, 2012. **119**(2): p. 521-9.
72. Aster, J.C., W.S. Pear, and S.C. Blacklow, *The Varied Roles of Notch in Cancer.* Annu Rev Pathol, 2017. **12**: p. 245-275.
73. Stilgenbauer, S., et al., *Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial.* Blood, 2014. **123**(21): p. 3247-54.
74. Rossi, D., et al., *The genome of chemorefractory chronic lymphocytic leukemia reveals frequent mutations of NOTCH1 and SF3B1.* Leuk Suppl, 2012. **1**(Suppl 2): p. S26-8.
75. Jeromin, S., et al., *SF3B1 mutations correlated to cytogenetics and mutations in NOTCH1, FBXW7, MYD88, XPO1 and TP53 in 1160 untreated CLL patients.* Leukemia, 2014. **28**(1): p. 108-17.
76. Wan, Y. and C.J. Wu, *SF3B1 mutations in chronic lymphocytic leukemia.* Blood, 2013. **121**(23): p. 4627-34.
77. Zenz, T., et al., *TP53 mutation and survival in chronic lymphocytic leukemia.* J Clin Oncol, 2010. **28**(29): p. 4473-9.
78. Rossi, D., et al., *Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia.* Blood, 2014. **123**(14): p. 2139-47.
79. Ozaki, T. and A. Nakagawara, *Role of p53 in Cell Death and Human Cancers.* Cancers (Basel), 2011. **3**(1): p. 994-1013.
80. Gutierrez, C. and C.J. Wu, *Clonal dynamics in chronic lymphocytic leukemia.* Hematology Am Soc Hematol Educ Program, 2019. **2019**(1): p. 466-475.
81. Diop, F., et al., *Biological and clinical implications of BIRC3 mutations in chronic lymphocytic leukemia.* Haematologica, 2020. **105**(2): p. 448-456.
82. Mansouri, L., et al., *Different prognostic impact of recurrent gene mutations in chronic lymphocytic leukemia depending on IGHV gene somatic hypermutation status: a study by ERIC in HARMONY.* Leukemia, 2023. **37**(2): p. 339-347.
83. Martínez-Trillos, A., et al., *Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome.* Blood, 2014. **123**(24): p. 3790-6.
84. Young, E., et al., *EGR2 mutations define a new clinically aggressive subgroup of chronic lymphocytic leukemia.* Leukemia, 2017.
85. Moore, K.J., F.J. Sheedy, and E.A. Fisher, *Macrophages in atherosclerosis: a dynamic balance.* Nature Reviews Immunology, 2013. **13**(10): p. 709-721.
86. Moriya, J., *Critical roles of inflammation in atherosclerosis.* J Cardiol, 2019. **73**(1): p. 22-27.
87. Jaiswal, S., et al., *Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes.* New England Journal of Medicine, 2014. **371**(26): p. 2488-2498.

88. Xie, M., et al., *Age-related mutations associated with clonal hematopoietic expansion and malignancies.* Nat Med, 2014. **20**(12): p. 1472-8.
89. Jaiswal, S. and B.L. Ebert, *Clonal hematopoiesis in human aging and disease.* Science, 2019. **366**(6465).
90. Arends, C.M., et al., *Associations of clonal hematopoiesis with recurrent vascular events and death in patients with incident ischemic stroke.* Blood, 2023. **141**(7): p. 787-799.
91. Strongman, H., et al., *Medium and long-term risks of specific cardiovascular diseases in survivors of 20 adult cancers: a population-based cohort study using multiple linked UK electronic health records databases.* Lancet, 2019. **394**(10203): p. 1041-1054.
92. Abuamsha, H., A.N. Kadri, and A.V. Hernandez, *Cardiovascular mortality among patients with non-Hodgkin lymphoma: Differences according to lymphoma subtype.* Hematol Oncol, 2019. **37**(3): p. 261-269.
93. Alloubani, A., R. Nimer, and R. Samara, *Relationship between Hyperlipidemia, Cardiovascular Disease and Stroke: A Systematic Review.* Curr Cardiol Rev, 2021. **17**(6): p. e051121189015.
94. Chow, S., R. Buckstein, and D.E. Spaner, *A link between hypercholesterolemia and chronic lymphocytic leukemia.* Leuk Lymphoma, 2016. **57**(4): p. 797-802.
95. Mozessohn, L., et al., *The Association of Dyslipidemia With Chronic Lymphocytic Leukemia: A Population-Based Study.* J Natl Cancer Inst, 2017. **109**(3).
96. Rozovski, U., et al., *Aberrant LPL Expression, Driven by STAT3, Mediates Free Fatty Acid Metabolism in CLL Cells.* Mol Cancer Res, 2015. **13**(5): p. 944-53.
97. Heintel, D., et al., *High expression of lipoprotein lipase in poor risk B-cell chronic lymphocytic leukemia.* Leukemia, 2005. **19**(7): p. 1216-23.
98. Baker, R.G., M.S. Hayden, and S. Ghosh, *NF-κB, inflammation, and metabolic disease.* Cell Metab, 2011. **13**(1): p. 11-22.
99. Yang, S., et al., *Tumor necrosis factor receptor 2 (TNFR2)·interleukin-17 receptor D (IL-17RD) heteromerization reveals a novel mechanism for NF-κB activation.* J Biol Chem, 2015. **290**(2): p. 861-71.
100. Waage, A., et al., *p55 and p75 tumor necrosis factor receptors in patients with chronic lymphocytic leukemia.* Blood, 1992. **80**(10): p. 2577-83.
101. Dürr, C., et al., *Tumor necrosis factor receptor signaling is a driver of chronic lymphocytic leukemia that can be therapeutically targeted by the flavonoid wogonin.* Haematologica, 2018. **103**(4): p. 688-697.

102. Carlsson, A.C., et al., *Levels of soluble tumor necrosis factor receptor 1 and 2, gender, and risk of myocardial infarction in Northern Sweden.* Atherosclerosis, 2018. **272**: p. 41-46.
103. Di Giovanni, S., et al., *Beta-2-microglobulin is a reliable tumor marker in chronic lymphocytic leukemia.* Acta Haematol, 1989. **81**(4): p. 181-5.
104. Hallek, M., et al., *Serum beta(2)-microglobulin and serum thymidine kinase are independent predictors of progression-free survival in chronic lymphocytic leukemia and immunocytoma.* Leuk Lymphoma, 1996. **22**(5-6): p. 439-47.
105. Xie, J. and Q. Yi, *Beta2-microglobulin as a potential initiator of inflammatory responses.* Trends Immunol, 2003. **24**(5): p. 228-9; author reply 229-30.
106. You, L., et al., *High levels of serum β2-microglobulin predict severity of coronary artery disease.* BMC Cardiovasc Disord, 2017. **17**(1): p. 71.
107. Simonsson, B., et al., *Production of beta 2-microglobulin by chronic lymphocytic leukaemia cells in vitro.* Scand J Haematol, 1986. **36**(5): p. 424-9.
108. *An international prognostic index for patients with chronic lymphocytic leukaemia (CLL-IPI): a meta-analysis of individual patient data.* Lancet Oncol, 2016. **17**(6): p. 779-790.
109. Binet, J.L., et al., *A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis.* Cancer, 1981. **48**(1): p. 198-206.
110. Rai, K.R., et al., *Clinical staging of chronic lymphocytic leukemia.* Blood, 1975. **46**(2): p. 219-34.
111. Dighiero, G., et al., *Chlorambucil in indolent chronic lymphocytic leukemia. French Cooperative Group on Chronic Lymphocytic Leukemia.* N Engl J Med, 1998. **338**(21): p. 1506-14.
112. *Chemotherapeutic options in chronic lymphocytic leukemia: a meta-analysis of the randomized trials. CLL Trialists' Collaborative Group.* J Natl Cancer Inst, 1999. **91**(10): p. 861-8.
113. Maloney, D.G., et al., *IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma.* Journal of Clinical Oncology, 1997. **15**(10): p. 3266-3274.
114. Fischer, K., et al., *Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial.* Blood, 2016. **127**(2): p. 208-15.
115. Eichhorst, B., et al., *First-line chemoimmunotherapy with bendamustine and rituximab versus fludarabine, cyclophosphamide, and rituximab in patients with advanced chronic lymphocytic leukaemia (CLL10): an international, open-label, randomised, phase 3, non-inferiority trial.* Lancet Oncol, 2016. **17**(7): p. 928-942.

116. Eichhorst, B., et al., *A Randomized Phase III Study of Venetoclax-Based Time-Limited Combination Treatments (RVe, GVe, GIVe) Vs Standard Chemoimmunotherapy (CIT: FCR/BR) in Frontline Chronic Lymphocytic Leukemia (CLL) of Fit Patients: First Co-Primary Endpoint Analysis of the International Intergroup GAIA (CLL13) Trial.* Blood, 2021. **138**(Supplement 1): p. 71-71.
117. Pierpont, T.M., C.B. Limper, and K.L. Richards, *Past, Present, and Future of Rituximab—The World's First Oncology Monoclonal Antibody Therapy.* Frontiers in Oncology, 2018. **8**(163).
118. Boross, P. and J.H. Leusen, *Mechanisms of action of CD20 antibodies.* Am J Cancer Res, 2012. **2**(6): p. 676-90.
119. Goede, V., et al., *Obinutuzumab as frontline treatment of chronic lymphocytic leukemia: updated results of the CLL11 study.* Leukemia, 2015. **29**(7): p. 1602-4.
120. Goede, V., et al., *Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions.* N Engl J Med, 2014. **370**(12): p. 1101-10.
121. Estupiñán, H.Y., et al., *Comparative Analysis of BTK Inhibitors and Mechanisms Underlying Adverse Effects.* Front Cell Dev Biol, 2021. **9**: p. 630942.
122. Byrd, J.C., et al., *Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia.* N Engl J Med, 2014. **371**(3): p. 213-23.
123. Munir, T., et al., *Final analysis from RESONATE: Up to six years of follow-up on ibrutinib in patients with previously treated chronic lymphocytic leukemia or small lymphocytic lymphoma.* Am J Hematol, 2019. **94**(12): p. 1353-1363.
124. Barr, P.M., et al., *Up to 8-year follow-up from RESONATE-2: first-line ibrutinib treatment for patients with chronic lymphocytic leukemia.* Blood Adv, 2022. **6**(11): p. 3440-3450.
125. Shanafelt, T.D., et al., *Ibrutinib-Rituximab or Chemoimmunotherapy for Chronic Lymphocytic Leukemia.* N Engl J Med, 2019. **381**(5): p. 432-443.
126. Woyach, J.A., et al., *Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL.* N Engl J Med, 2018. **379**(26): p. 2517-2528.
127. Moreno, C., et al., *Ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): a multicentre, randomised, open-label, phase 3 trial.* Lancet Oncol, 2019. **20**(1): p. 43-56.
128. Moreno, C., et al., *First-line treatment of chronic lymphocytic leukemia with ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab: final analysis of the randomized, phase III iLLUMINATE trial.* Haematologica, 2022. **107**(9): p. 2108-2120.

129. Byrd, J.C., et al., *Acalabrutinib Versus Ibrutinib in Previously Treated Chronic Lymphocytic Leukemia: Results of the First Randomized Phase III Trial.* J Clin Oncol, 2021: p. Jco2101210.
130. Brown, J.R., et al., *Zanubrutinib or Ibrutinib in Relapsed or Refractory Chronic Lymphocytic Leukemia.* N Engl J Med, 2023. **388**(4): p. 319-332.
131. Lewis, K.L. and C.Y. Cheah, *Non-Covalent BTK Inhibitors-The New BTKids on the Block for B-Cell Malignancies.* J Pers Med, 2021. **11**(8).
132. Burger, J.A., *Bruton Tyrosine Kinase Inhibitors: Present and Future.* Cancer J, 2019. **25**(6): p. 386-393.
133. Mato, A.R., et al., *Pirtobrutinib in relapsed or refractory B-cell malignancies (BRUIN): a phase 1/2 study.* Lancet, 2021. **397**(10277): p. 892-901.
134. Woyach, J.A., et al., *Preliminary Efficacy and Safety of MK-1026, a Non-Covalent Inhibitor of Wild-Type and C481S Mutated Bruton Tyrosine Kinase, in B-Cell Malignancies: A Phase 2 Dose Expansion Study.* Blood, 2021. **138**(Supplement 1): p. 392-392.
135. Woyach, J.A., et al., *BTK(C481S)-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia.* J Clin Oncol, 2017. **35**(13): p. 1437-1443.
136. Quinquenel, A., et al., *Prevalence of BTK and PLCG2 mutations in a real-life CLL cohort still on ibrutinib after 3 years: a FILO group study.* Blood, 2019. **134**(7): p. 641-644.
137. Hampel, P.J., et al., *BTK and/or PLCG2 Mutations in Patients with Chronic Lymphocytic Leukemia (CLL) Treated with Ibrutinib: Characteristics and Outcomes at the Time of Progression.* Blood, 2019. **134**(Supplement_1): p. 3050-3050.
138. Wang, E., et al., *Mechanisms of Resistance to Noncovalent Bruton's Tyrosine Kinase Inhibitors.* N Engl J Med, 2022. **386**(8): p. 735-743.
139. Stephens, D.M. and J.C. Byrd, *How we manage ibrutinib intolerance and complications in patients with chronic lymphocytic leukemia.* Blood, 2019: p. blood-2018-11-846808.
140. Xiao, L., et al., *Ibrutinib-Mediated Atrial Fibrillation Attributable to Inhibition of C-Terminal Src Kinase.* Circulation, 2020. **142**(25): p. 2443-2455.
141. Dickerson, T., et al., *Hypertension and incident cardiovascular events following ibrutinib initiation.* Blood, 2019. **134**(22): p. 1919-1928.
142. Mato, A., et al., *A real-world study to assess the association of cardiovascular adverse events (CVAEs) with ibrutinib as first-line (1L) treatment for patients with chronic lymphocytic leukaemia (CLL) in the United States.* EJHaem, 2023. **4**(1): p. 135-144.

143. Ruppert, A.S., et al., *Adverse event burden in older patients with CLL receiving bendamustine plus rituximab or ibrutinib regimens: Alliance A041202.* Leukemia, 2021. **35**(10): p. 2854-2861.
144. O'Brien, S., et al., *Safety Analysis of Four Randomized Controlled Studies of Ibrutinib in Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma or Mantle Cell Lymphoma.* Clin Lymphoma Myeloma Leuk, 2018. **18**(10): p. 648-657.e15.
145. Brown, J.R., et al., *Cardiovascular adverse events in patients with chronic lymphocytic leukemia receiving acalabrutinib monotherapy: pooled analysis of 762 patients.* Haematologica, 2021.
146. Sharman, J.P., et al., *ELEVATE TN: Phase 3 Study of Acalabrutinib Combined with Obinutuzumab (O) or Alone Vs O Plus Chlorambucil (Clb) in Patients (Pts) with Treatment-Naive Chronic Lymphocytic Leukemia (CLL).* Blood, 2019. **134**(Supplement_1): p. 31-31.
147. Shanafelt, T.D., et al., *Atrial fibrillation in patients with chronic lymphocytic leukemia (CLL).* Leuk Lymphoma, 2017. **58**(7): p. 1630-1639.
148. *Nationwide report on Atrial fibrillation and risk of stroke*. 2011, SWEDISH AGENCY FOR HEALTH TECHNOLOGY ASSESSMENT AND ASSESSMENT OF SOCIAL SERVICES: https://www.sbu.se/sv/publikationer/SBU-utvarderar/formaksflimmer---forekomst-och-risk-for-stroke/.
149. Guha, A., et al., *Ventricular Arrhythmias Following Ibrutinib Initiation for Lymphoid Malignancies*. 2018.
150. Lampson, B.L., et al., *Ventricular arrhythmias and sudden death in patients taking ibrutinib*. 2017, American Society of Hematology. p. 2581-2584.
151. Salem, J.E., et al., *Cardiovascular Toxicities Associated With Ibrutinib.* J Am Coll Cardiol, 2019. **74**(13): p. 1667-1678.
152. Du, B., et al., *Acute Effects of Ibrutinib on Ventricular Arrhythmia in Spontaneously Hypertensive Rats.* JACC CardioOncol, 2020. **2**(4): p. 614-629.
153. Bhat, S.A., et al., *Ventricular arrhythmias and sudden death events following acalabrutinib initiation.* Blood, 2022. **140**(20): p. 2142-2145.
154. Tang, C.P.S., et al., *Management of cardiovascular complications of bruton tyrosine kinase inhibitors.* Br J Haematol, 2021.
155. Brown, J.R., et al., *Incidence of and risk factors for major haemorrhage in patients treated with ibrutinib: An integrated analysis.* Br J Haematol, 2019. **184**(4): p. 558-569.
156. Mock, J., et al., *Risk of Major Bleeding with Ibrutinib.* Clin Lymphoma Myeloma Leuk, 2018. **18**(11): p. 755-761.

157. Levade, M., et al., *Ibrutinib treatment affects collagen and von Willebrand factor-dependent platelet functions.* Blood, 2014. **124**(26): p. 3991-5.
158. Series, J., et al., *Differences and similarities in the effects of ibrutinib and acalabrutinib on platelet functions.* Haematologica, 2019. **104**(11): p. 2292-2299.
159. Patell, R., et al., *Usefulness of CHADS2 and CHA2DS2-VASc Scores for Stroke Prediction in Patients With Cancer and Atrial Fibrillation.* Am J Cardiol, 2017. **120**(12): p. 2182-2186.
160. Furman, R.R., et al., *Idelalisib and rituximab in relapsed chronic lymphocytic leukemia.* N Engl J Med, 2014. **370**(11): p. 997-1007.
161. O'Brien, S.M., et al., *A phase 2 study of idelalisib plus rituximab in treatment-naïve older patients with chronic lymphocytic leukemia.* Blood, 2015. **126**(25): p. 2686-94.
162. Hanada, M., et al., *bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia.* Blood, 1993. **82**(6): p. 1820-8.
163. Kater, A.P., et al., *Venetoclax Plus Rituximab in Relapsed Chronic Lymphocytic Leukemia: 4-Year Results and Evaluation of Impact of Genomic Complexity and Gene Mutations From the MURANO Phase III Study.* J Clin Oncol, 2020. **38**(34): p. 4042-4054.
164. Seymour, J.F., et al., *Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia.* N Engl J Med, 2018. **378**(12): p. 1107-1120.
165. Eichhorst B., et al., *TIME-LIMITED VENETOCLAX-OBINUTUZUMAB +/- IBRUTINIB IS SUPERIOR TO CHEMOIMMUNOTHERAPY IN FRONTLINE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): PFS CO-PRIMARY ENDPOINT OF THE RANDOMIZED PHASE 3 GAIA/CLL13 TRIAL*, in *EHA 2022*. 2022: Vienna.
166. Eugen, T., et al., *Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia.* Haematologica, 2019. **104**(9): p. e434-e437.
167. Blombery, P., et al., *Acquisition of the Recurrent Gly101Val Mutation in BCL2 Confers Resistance to Venetoclax in Patients with Progressive Chronic Lymphocytic Leukemia.* Cancer Discovery, 2019. **9**(3): p. 342-353.
168. Condoluci, A. and D. Rossi, *Mechanisms of resistance to venetoclax.* Blood, 2022. **140**(20): p. 2094-2096.
169. Thijssen, R., et al., *Single-cell multiomics reveal the scale of multilayered adaptations enabling CLL relapse during venetoclax therapy.* Blood, The Journal of the American Society of Hematology, 2022. **140**(20): p. 2127-2141.

170. Krämer, I., et al., *Allogeneic hematopoietic cell transplantation for high-risk CLL: 10-year follow-up of the GCLLSG CLL3X trial.* Blood, 2017. **130**(12): p. 1477-1480.
171. van Gelder, M., et al., *Long-term survival of patients with CLL after allogeneic transplantation: a report from the European Society for Blood and Marrow Transplantation.* Bone Marrow Transplant, 2017. **52**(3): p. 372-380.
172. Gribben, J.G., *How and when I do allogeneic transplant in CLL.* Blood, 2018. **132**(1): p. 31-39.
173. Manier, S., et al., *Current state and next-generation CAR-T cells in multiple myeloma.* Blood Rev, 2022. **54**: p. 100929.
174. Wang, M., et al., *KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma.* New England Journal of Medicine, 2020. **382**(14): p. 1331-1342.
175. Kamdar, M., et al., *Lisocabtagene maraleucel versus standard of care with salvage chemotherapy followed by autologous stem cell transplantation as second-line treatment in patients with relapsed or refractory large B-cell lymphoma (TRANSFORM): results from an interim analysis of an open-label, randomised, phase 3 trial.* Lancet, 2022. **399**(10343): p. 2294-2308.
176. Todorovic, Z., et al., *CAR T Cell Therapy for Chronic Lymphocytic Leukemia: Successes and Shortcomings.* Curr Oncol, 2022. **29**(5): p. 3647-3657.
177. Ramsay, A.G., et al., *Multiple inhibitory ligands induce impaired T-cell immunologic synapse function in chronic lymphocytic leukemia that can be blocked with lenalidomide: establishing a reversible immune evasion mechanism in human cancer.* Blood, 2012. **120**(7): p. 1412-21.
178. Gill, S., et al., *Anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia.* Blood Advances, 2022. **6**(21): p. 5774-5785.
179. Gauthier, J., et al., *Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure.* Blood, 2020. **135**(19): p. 1650-1660.
180. Rotbain, E.C., et al., *Mapping comorbidity in chronic lymphocytic leukemia: impact of individual comorbidities on treatment, mortality, and causes of death.* Leukemia, 2021. **35**(9): p. 2570-2580.
181. Goede, V., et al., *Interactions between comorbidity and treatment of chronic lymphocytic leukemia: results of German Chronic Lymphocytic Leukemia Study Group trials.* Haematologica, 2014. **99**(6): p. 1095-100.
182. Gordon, M.J., et al., *Comorbidities predict inferior outcomes in chronic lymphocytic leukemia treated with ibrutinib.* Cancer, 2018. **124**(15): p. 3192-3200.

183. Mato, A.R., et al., *Toxicities and outcomes of 616 ibrutinib-treated patients in the United States: a real-world analysis.* Haematologica, 2018. **103**(5): p. 874-879.
184. Ludvigsson, J.F., et al., *The Swedish personal identity number: possibilities and pitfalls in healthcare and medical research.* Eur J Epidemiol, 2009. **24**(11): p. 659-67.
185. Ludvigsson, J.F., et al., *Registers of the Swedish total population and their use in medical research.* Eur J Epidemiol, 2016. **31**(2): p. 125-36.
186. Organization, W.H., *International Statistical Classification of Diseases and related health problems: Alphabetical index*. Vol. 3. 2004: World Health Organization.
187. Barlow, L., et al., *The completeness of the Swedish Cancer Register – a sample survey for year 1998.* Acta Oncologica, 2009. **48**(1): p. 27-33.
188. Ludvigsson, J.F., et al., *External review and validation of the Swedish national inpatient register.* BMC Public Health, 2011. **11**: p. 450.
189. Socialstyrelsen, *Kvalitet och innehåll i patientregistret. Utskrivningar från slutenvården 1964-2007 och besök i speciliserad öppenvård (exklusive primärvårdsbesök) 1997-2007*. 2009.
190. Brooke, H.L., et al., *The Swedish cause of death register.* Eur J Epidemiol, 2017. **32**(9): p. 765-773.
191. Johansson, L.A. and R. Westerling, *Comparing Swedish hospital discharge records with death certificates: implications for mortality statistics.* Int J Epidemiol, 2000. **29**(3): p. 495-502.
192. Johansson, L.A., C. Björkenstam, and R. Westerling, *Unexplained differences between hospital and mortality data indicated mistakes in death certification: an investigation of 1,094 deaths in Sweden during 1995.* J Clin Epidemiol, 2009. **62**(11): p. 1202-9.
193. Wallerstedt, S.M., B. Wettermark, and M. Hoffmann, *The First Decade with the Swedish Prescribed Drug Register - A Systematic Review of the Output in the Scientific Literature.* Basic Clin Pharmacol Toxicol, 2016. **119**(5): p. 464-469.
194. Wettermark, B., et al., *The new Swedish Prescribed Drug Register--opportunities for pharmacoepidemiological research and experience from the first six months.* Pharmacoepidemiol Drug Saf, 2007. **16**(7): p. 726-35.
195. Glimelius, B., et al., *U-CAN: a prospective longitudinal collection of biomaterials and clinical information from adult cancer patients in Sweden.* Acta Oncol, 2018. **57**(2): p. 187-194.
196. Waage, A. and T. Espevik, *TNF receptors in chronic lymphocytic leukemia.* Leukemia & Lymphoma, 1994.

197. Gora-Tybor, J., J.Z. Blonski, and T. Robak, *Circulating vascular endothelial growth factor (VEGF) and its soluble receptors in patients with chronic lymphocytic leukemia.* Eur Cytokine Netw, 2005.
198. Kamiguti, A.S., et al., *The role of matrix metalloproteinase 9 in the pathogenesis of chronic lymphocytic leukaemia.* Br J Haematol, 2004. **125**(2): p. 128-40.
199. Asgarian-Omran, H., et al., *Expression profile of galectin-1 and galectin-3 molecules in different subtypes of chronic lymphocytic leukemia.* Cancer Invest, 2010. **28**(7): p. 717-25.
200. Michalová, Z., et al., *Overexpression of Galectin-3 in Chronic Lymphocytic Leukemia Is Associated With 17p Deletion: A Short Report.* Anticancer Res, 2019. **39**(6): p. 2805-2810.
201. Wollert, K.C., T. Kempf, and L. Wallentin, *Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease.* Clin Chem, 2017. **63**(1): p. 140-151.
202. Costelli, P., et al., *Muscle myostatin signalling is enhanced in experimental cancer cachexia.* Eur J Clin Invest, 2008. **38**(7): p. 531-8.
203. Verzola, D., et al., *Myostatin mediates abdominal aortic atherosclerosis progression by inducing vascular smooth muscle cell dysfunction and monocyte recruitment.* Sci Rep, 2017. **7**: p. 46362.
204. Christensen, B.W., V.G. Zaha, and F.T. Awan, *Cardiotoxicity of BTK inhibitors: ibrutinib and beyond.* Expert Rev Hematol, 2022. **15**(4): p. 321-331.
205. Winkelmann, N., et al., *Low frequency mutations independently predict poor treatment-free survival in early stage chronic lymphocytic leukemia and monoclonal B-cell lymphocytosis.* Haematologica, 2015. **100**(6): p. e237-9.

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